EXHIBIT 49 (AR K.12)

Tracking environmental trends in the Great Bay Estuarine System through comparisons of historical and present-day green and red algal community structure and nutrient content

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Abstract

Monitoring macroalgae populations is an effective means of detecting long term water quality changes in estuarine systems. To investigate the environmental status of New Hampshire's Great Bay National Estuarine Research Reserve, this study assessed the abundance/distribution of macrophytes, particularly *Gracilaria* and *Ulva* species, relative to eutrophication patterns; compared historical (1970s-1990s) and current algal biomass/cover at several sites; and compared *Ulva* and *Gracilaria* tissue N/P content to ambient and historical levels. *Ulva* and *Gracilaria* biomass/cover have increased significantly at several sites. Cover by *Ulva* species, at seasonal maxima, was over 90 times the value recorded in the 1970s at Lubberland Creek, and exceeded 50% at all sites in the upper estuary. *Gracilaria* cover was greater than 25% at Depot Road in the upper estuary, whereas the historical measure was 1%. Sequencing of ITS2, *rbc*L and CO1 revealed the presence of previously undetected *Ulva* and *Gracilaria* species, including *Gracilaria vermiculophylla* (Ohmi) Papenfuss, an invasive species of Asian origin. *Gracilaria vermiculophylla* has exceeded *G. tikvahiae* as the dominant *Gracilaria* species in Great Bay. Historical voucher specimen screening suggests *G. vermiculophylla* was introduced as recently as 2003. Nitrogen and phosphorus levels are elevated in the estuary. We should expect continued seasonal nuisance algal blooms.

Key words: *Ulva*, *Gracilaria vermiculophylla*, nuisance, invasive, blooms, nutrients, nitrogen, phosphorus, N:P, eutrophication

NERRS GRF focus: nutrient dynamics and/or effects of non-point source pollution and eutrophication

Monitoring water quality parameters through the use of indicator organisms

Introduction

Increased eutrophication or nutrient enrichment within the Great Bay Estuarine System (Jones 2000) appears to be causing enhanced growth of nuisance green tide seaweeds like *Ulva* (Fletcher 1996), which are cosmopolitan, opportunistic, stress-tolerant annuals with broad physiological tolerances (Diaz et al. 2002; Kindig and Littler 1980; Raffaelli et al. 1998; Raven and Taylor 2003; Sawyer 1965). Many of these ulvoid green algae grow in eutrophied and hydrologically variable habitats like those found in some areas of the Great Bay Estuarine System. In summarizing the effects of eutrophication on seaweed populations, Schramm and Nienhuis (1996) outlined three patterns, which we expected to observe within the Great Bay Estuarine System: (1) a decline or disappearance of certain perennial plant communities (eelgrass) that are often replaced by annual, fast growing forms (e.g. folious green algae or filamentous reds); (2) a reduced diversity of associated flora and fauna; and (3) mass developments of short-lived annuals or 'nuisance algae,' such as *Ulva* and *Gracilaria*.

Many Great Bay Estuarine System studies serve as a strong baseline to assess current water quality and green tide problems. Mathieson and Hehre (1986) summarized the species composition, phenology, longevity, and distributional patterns of New Hampshire seaweeds, while Mathieson and Penniman (1986, 1991) summarized analogous studies within the Great Bay Estuarine System. Mathieson and Fralick (1973) compared the seaweed populations from the Merimack River Estuary, MA, which was one of the most polluted rivers in New England (Jerome et al. 1965; Miller et al. 1971), finding a depauperate flora dominated by ulvoid green algae and lower numbers of taxa/sites versus the Great Bay and Hampton-Seabrook Estuarine Systems of New

Hampshire (Maine). Hardwick-Witman and Mathieson (1983) established a series of sites from the outer to inner reaches of the Great Bay System and recorded the dominant benthic plant and animal populations. Chock and Mathieson's Cedar Point study (1976, 1983) provided a detailed quantification of biomass for seaweeds and salt marsh populations within the Great Bay Estuarine System. In the fall of 2007, the gross distribution of macroalgae and eelgrass in the Great Bay system were estimated with hyperspectral imaging (Pe'eri et al., 2008). Historical *Gracilaria* tissue nutrient data were described from Great Bay (Penniman, 1983), and historical water nutrient concentrations for the region were outlined by Short (1992) and Jones (2000).

This study aimed to verify the identity of all of the bloom forming *Gracilaria* and *Ulva* species in Great Bay, and, in the case of newly detected species, to determine approximate introduction dates. We also aimed to assess the abundance and distribution of *Ulva and Gracilaria* within the Great Bay Estuarine System of New Hampshire relative to major patterns of eutrophication, and compare historical and current biomass and percent cover measurements for algal populations at several sites where ecological studies were previously conducted. We aimed to compare total nitrogen and total phosphorus concentrations in *Ulva* and *Gracilaria* tissues to those observed in water analyses for the same sites and collection times. Lastly we wished to compare historical and current water quality measurements throughout the Great Bay Estuarine System to detect if nutrient availability had changed since the baseline studies.

Materials and Methods

Algae sampling was conducted within the intertidal zones at five sites in Great Bay, NH. The sites were designated as Cedar Point, Wagon Hill Farm, Lubberland Creek, Depot Road, and Sunset Farm (Figure 1). At each site and collection time, specimens of all conspicuous macroalgal species were gathered and identified based on morphological characteristics. Voucher specimens were also collected outside of the transect lines for use in the molecular verification of species identity.

Percent cover of component species was measured bi-monthly at the five study sites along four 10 x1 m line transects oriented parallel to shore with elevations of approximately 0.0 m, 0.5m, 1.0 m, and 1.5 m above mean low water. Ten quadrats (0.5 m by 0.5 m) per transect were measured for percent cover using digital photography. Images were analyzed using the point intersect method. For this purpose, 25 randomly distributed dots were drawn on a clear sheet of plastic which was laid over the digital image for manual estimations of cover. Only algal specimens with holdfasts in the quadrats were included, with the exception of the free floating species found in the southern bay. When quadrats contained multiple layers of algae, each tier was assessed individually.

Percent cover data will were arcsine transformed. Analyses of variance, using the General Linear Model in Systat 13, were performed to determine the effects of elevation, time, and site on the abundance of *Ulva* and *Gracilaria* populations. Post-hoc pair-wise comparisons were perfomed using Tukey's test.

Biomass (g dry wt/m^2) of component species was estimated through destructive sampling at each collection month and site along the above transect lines. Within each of the forty quadrats, 10 randomly selected 0.1 m by 0.1 m sections were denuded. All algal and plant materials were removed and placed in plastic bags specifically labeled for the collection month, site, and quadrat. In the laboratory, the algae (and marsh grasses) were sorted, rinsed in freshwater, dried at 90 °C for up to 72 hours, weighed, and converted to g dry weight/ m^2 biomass values.

The results for each species separately and for total measurements of all species combined were analyzed by single-factor analysis of variance (ANOVA) with significance level $\alpha=0.05$ (Zar, 1996), followed with a Tukey's multiple comparison test. Time and site were the only factors considered in ANOVA.

Tissue measurements of total nitrogen and total phosphorus were recorded for a subset of the conspicuous *Ulva* and *Gracilaria* specimens following the methods of Lourenço et al., 2006. The whole thalli of at least 12 specimens were collected independent of size. They were washed in the field with seawater to remove sediment and detritus, placed in plastic bags, returned to the laboratory within one hour. In the lab, the samples were gently brushed under running water, rinsed with distilled water, and dried at 90 °C until a constant weight (up to three days). The dried materials were kept frozen until chemical analysis. Total nitrogen and total phosphorus were determined in algal tissue by Penn State's Agricultural Analytical Lab using the combustion (Horneck et al., 1998) and dry ash (Miller, 1998) methods. Dry tissue material of at least 200 mg was used for each replicate test of total nitrogen percentage. Another 200 mg dry material was used for each test of total phosphorus percentage. For each species and sampling event, at least three independent, from different thalli, measurements of tissue N and P were performed, given adequate amounts of tissue were available on site. The results for each species were analyzed by single-factor analysis of variance (ANOVA) with significance level α=0.05 (Zar, 1996), followed with a Tukey's multiple comparison test. Time and site were the only factors considered in ANOVA.

Surface water total nitrogen and total phosphorus were measured by the University of New Hampshire Water Quality Analysis Lab using an alkaline persulfate digestion followed by colorimetric measurement of NO3 & PO4 yielding results in mg/L. Three 250 ml water samples for dissolved nutrient analyses were taken from 10 cm below the water surface at each study site during each visit. The water samples were filtered through cellulose membrane filters (Millipore® HAWP 0.45 µm pore) and kept at -20°C until the time of analysis. Temperatures and salinities were enumerated for each site at the time of collection.

Molecular Methods

The *Ulva* and *Gracilaria* samples were ground in labeled 1.7 ml microcentifuge tubes using disposable plastic pestles, a pinch of molecular grade sand, and 300 ml of Gentra Puregene® Cell Lysis Solution (D-5002). The DNA was extracted with a Gentra Puregene ® Isolation Kit as per the manufacturer's instructions. Samples were incubated in a 65°C heatblock for one hour inverting 10 times at 30 minutes and cooled to room temperature before 100 µl of Protein Precipitation Solution (Gentra D-5003) was added. Samples were inverted 150 times and chilled at -20°C for 45 minutes before they were centrifuged for 15 minutes at 13,000 rpm. The supernatant was then poured into at new 1.7 ml microcentifuge tube containing 300 µl of 100% isopropanol and inverted 50 times before centrifugation for 10 minutes at 13,000 rpm. The alcohol was decanted and replaced with 300 µl of 70% ethanol before inversion and 5 minutes of centrifugation at

13,000 rpm. The alcohol was decanted, and the sample was air dried for 60 minutes before 50 µl of DNA Hydration Solution (Gentra D-5004) was added. After briefly mixing, the samples were incubated in a 65°C heatblock for one hour and centrifuged for 5 minutes.

Polymerase chain reactions were carried out in 50 µl volumes containing 4 µl extracted DNA, 10 µl Taq buffer (Promega GoTaq® Flexi Green), (0.2 mM) Mg^{2+}, 1 µl dNTPs, 1 µl each (20 mM) primer, and 0.25 µl Taq polymerase (GoTaq® Flexi). The primers used for amplification and sequencing of *Gracilaria* samples were CO1F238 (5' ACA GGA TGA ACA GTK TAT CCY C 3') and CO1R524 (5' CCA CCT GCW GGA TCA AAG A 3'). For *Ulva* samples the primers for amplification and sequencing were ITS2 F5.8S30 (5'-GCA ACG ATG AAG AAC GCA GC-3') ITS2 R ENT26S (5'-GCT TAT TGA TAT GCT TAA GTT CAG CGG GT-3').

The PCR products were separated by electrophoresis on a Cyber-Safe® treated low-melt agarose gel (0.8%) in nTBE Buffer (0.5x). On a UV lightbox, the desired DNA bands were excised using microscope slide covers and transferred to 1.7 ml tubes, incubated at in a 65°C heatblock for five minutes, and then transferred to 37°C heatblock. To each tube, 1.5 µl of agarase (Sigma A6303, 50 units/ml) were added, and the mixture was incubated overnight.

Concentrations of DNA were quantified using an Invitrogen™ Quant-iT™ dsDNA BR Assay Kit (Q32851) and an Invitrogen™ Qubit™ fluorometer (Q32857) as per the manufacturer's instructions, and appropriate volumes of DNA and primers were sent to Hubbard Genomic Center (UNH) for clean-up and sequencing reactions using

Applied Biosystems BigDye Terminator Cycle Sequencing Kits (v1.1 and v3.1). The DNA samples were resolved by capillary electrophoresis on an ABI3130 DNA Analyzer.

Resulting sequences were trimmed in Chromas (version 2.2, Technelysium, Pty. Ltd., Tewantin, Queensland, Australia). Sequence assembly, alignments were made and proofed using Seq Man II (version 7.1 for Windows, DNAStar, Inc., Madison, Wisconsin). Comparative alignments and GenBank searches were performed using MegAlign (version 7.1 for Windows, DNAStar, Inc., Madison, Wisconsin).

Site Descriptions

Five Great Bay Estuarine System study sites were selected based on ease of access and proximity to historical algal community study sites (Figure 1). These sites were Cedar Point (CP), Wagon Hill Farm (WH), Lubberland Creek (LC), Depot Road (DR), and Sunset Farm (SF). These sites varied in substrata, hydrographic regime, and human traffic (Table 1).

Table 1 Great Bay study site descriptions and locations

Abbrev.	Site Name	Coordinates	Habitat Description	Blooming Taxa Found
CP	Cedar Point	43°07'42"N 70°51'13"W	Rocky substrata, dominant cover by *Ascophyllum nodosum* and *Fucus vesiculosus*, strong tidal current	*Ulva rigida, Ulva intestinalis*
WH	Wagon Hill Farm	43°07'27"N 70°52'07"W	Mudflat with mowed grass shoreline, adjacent to a public swimming area, stronger current than found at the southern sites	*Ulva rigida, Ulva intestinalis, Ulva compressa*
LC	Lubberland Creek	43°04'30"N 70°54'12"W	Mudflat between Vol's Island and the mouth of Lubberland Creek that is characterized by low water motion and sizeable fall algal blooms	*Ulva rigida, Gracilaria tikvahiae, G. vermiculophylla*
DR	Depot Road	43°03'22"N 70°53'50"W	Muddy substratum, at the Great Bay Discovery Center boat launch, site characterized by *Ulva* and *Gracilaria* blooms in the fall	*Ulva rigida, Gracilaria tikvahiae, G. vermiculophylla*
SF	Sunset Farm	43°03'24"N 70°50'03"W	Mudflat near public golf course and popular ice fishing access point with dominant fall cover by *Ulva* and *Gracilaria* species	*Ulva rigida, U.compressa, Gracilaria tikvahiae, G. vermiculophylla*

Figure 1 Map of the Great Bay Estuary System, New Hampshire showing the locations of the five study sites. From top center and clockwise: Wagon Hill Farm (WH), Cedar Point (CP), Sunset Farm (SF), Depot Road (DR), and Lubberland Creek (LC)-- satellite image courtesy of Google Maps

The Cedar Point study transects were established on and adjacent to a public boat launch at the northern end of Little Bay (Figure 2). The site's substrata consist of shale scree and metamorphic boulders. Fucoid algae made up the dominant cover year round. The Wagon Hill Farm transects were located on a tidal mudflat near the mouth of the Oyster River (Figure 3). Scattered sticks, logs, shells, rocks, dislocated marsh-grass hummocks and the protected stream-bank provided the only means of attachment for *Ulva* specimens at this site. Tidal currents could be strong. The Lubberland Creek site is located in the southwestern section of Great Bay (Figure 4). The tidal mudflat is home to large blooms of unattached *Ulva* and *Gracilaria* specimens in the fall months. Water motion at this site is minimal. The Depot Road site has a sandy shore leading to an open mudflat. There is a public boat launch here, which is mainly used for kayaks, but a large gundalow has been docked here during the summer months for educational purposes (Figure 5). *Ulva* and *Gracilaria* are the dominant cover species at this site, but their presence is seasonal (fall blooms). Again, most algae here are unattached and water motion is minimal. The Sunset Farm site (Figure 6) is located near the Portsmouth Country Club, a popular golf course. The site experiences fall bloom events comprised of *Ulva* and *Gracilaria* species. Like the other two sites in southern Great Bay, this site is completely covered with snow and ice for several months of the year. In the winter, this is a popular access point for ice-fishermen.

Figure 2 Cedar Point boat launch A) facing south B) facing north with boat launch and retaining wall.

Figure 3 Wagon Hill Farm A) broad view of mudflat with transect line B) *Ulva* specimen found attached to shell.

Figure 4 Lubberland Creek A) west facing, *Ulva* bloom (November 2008) B) east facing, two months earlier (September 2008).

Figure 5 Depot Road A) summer 2009 with gundalow and student group B) quadrat on transect line

Figure 6 Sunset Farm A) *Ulva* and *Gracilaria* bloom (September 2008) B) winter snow and ice cover can last for a few months in southern Great Bay

Results

DNA analysis of blade forming *Ulva* specimens revealed the presence of *Ulva rigida* C. Agardh, and *U. compressa* Linnaeus, but no *U. lactuca* Linnaeus at the study sites. DNA analysis of *Gracilaria* specimens verified the presence of both the native *Gracilaria tikvahiae* McLachlan and the introduced, possibly invasive, *G. vermiculophylla* (Ohmi) Papenfuss at all of the study sites in southern Great Bay.

Molecular screening of Great Bay historical herbarium specimens, demonstrated that *U. rigida* had been present, but misidentified since 1966. The foliose form of *U. compressa* had been present but undetected since 1972. *Ulva pertussa*, an introduced Asian species, which was not found at any of the study sites, but was verified at other Great Bay sites in a concurrent study (Hoffman et al. 2010), was revealed to have been present, yet unidentified in Great Bay since 1967. A sample of *Gracilaria vermiculophylla*, which had been mistakenly identified as *G. tikvahiae* based on morphological features, was collected at Dover Point in Great Bay in 2003.

The mean *Ulva* biomass for each Great Bay study site was determined for the period from September 2008- July 2010 (Figure 7). The differences between sites were statistically significant (P=0.00), with the greatest mean *Ulva* biomass in the southern portion of Great Bay. The Lubberland Creek site had the highest mean *Ulva* biomass (138.2 g dry weight/m^2 +/- 228.9 SD) followed by Sunset Farm (97.1 g dry weight/m^2 +/- 174.6 SD) and Depot Road (79.6 g dry weight/m^2 +/- 102.1 SD). The Wagon Hill Farm site in the northern part of the bay had the lowest mean *Ulva* biomass for the study period (6.8 g dry weight/m^2 +/- 8.7 SD).

The mean *Ulva* biomass for all study sites was determined for each of the ten collection times from September 2008- July 2010 (Figure 8). Significant seasonal variation was observed (P=0.00). Seasonal *Ulva* biomass lows occurred in March of both years following ice out (2.3 g dry weight/m^2 +/- 2.5 SD and 5.8 g dry weight/m^2 +/- 5.7 SD). Biomass levels remained low throughout the spring and summer months, but major blooms occurred in the fall of both years. The greatest yearly mean *Ulva* biomass was observed in November of 2008 and 2009 (227.4 g dry weight/m^2 +/- 299.9 SD and 115.3 g dry weight/m^2 +/- 1.16.6 SD).

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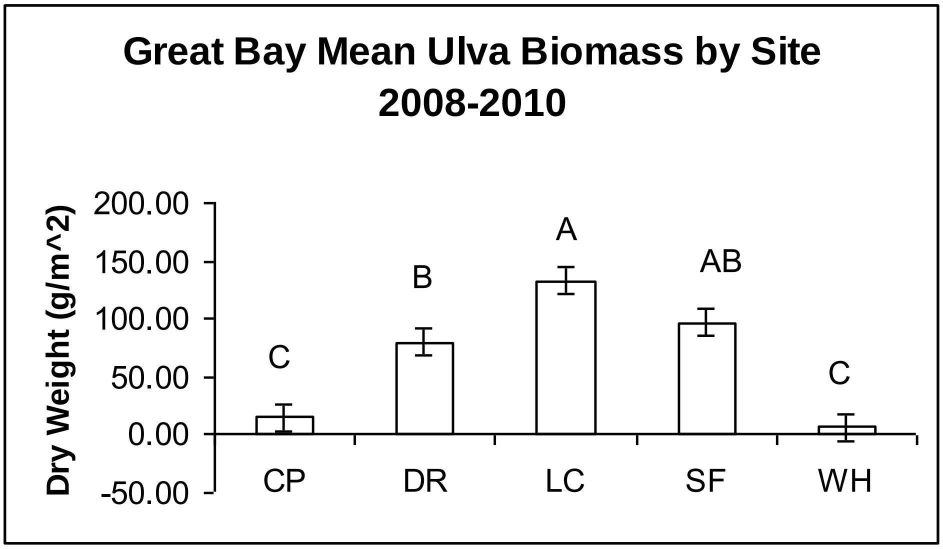

Figure 7 Great Bay *Ulva* mean biomass by site from 2008-2010

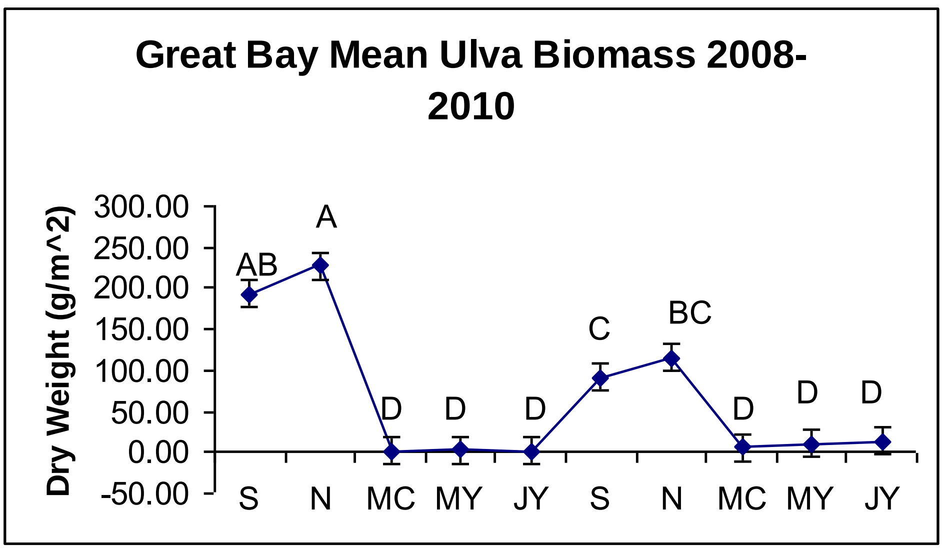

Figure 8 Great Bay *Ulva* monthly mean biomass across five study sites

The mean *Ulva* cover estimates followed the same trends across the sites as were observed for mean biomass (Figure 9), with significant differences between the sites (P=0.00). The greatest mean *Ulva* percent cover for the two year study was observed at the Lubberland Creek site (39.3% +-40.1 SD), followed by the other two sites in southern Great Bay, Depot Road (21.8% +- 32.1 SD) and Sunset Farm (21.0% +- 31.6 SD). Wagon Hill and Cedar Point, the northernmost sites, had the lowest mean *Ulva* cover over the study period (11.2% +- 24.4 SD and 1.3% +- 6.7 SD).

Seasonal trends in mean *Ulva* cover were observed throughout the study period (Figure 10), with significant differences between fall maxima and spring/summer minima (P=0.00). Peak cover was achieved in November of 2008 and 2009 (38.7% +- 40.6 SD and 31.2% +- 42.6SD). The seasonal mean *Ulva* cover low occurred in July of 2009 (14.5% +- 25.5 SD), whereas the 2010 low, which was significantly lower than the previous year, was observed in March immediately following ice-out (2.9% +- 11.6 SD).

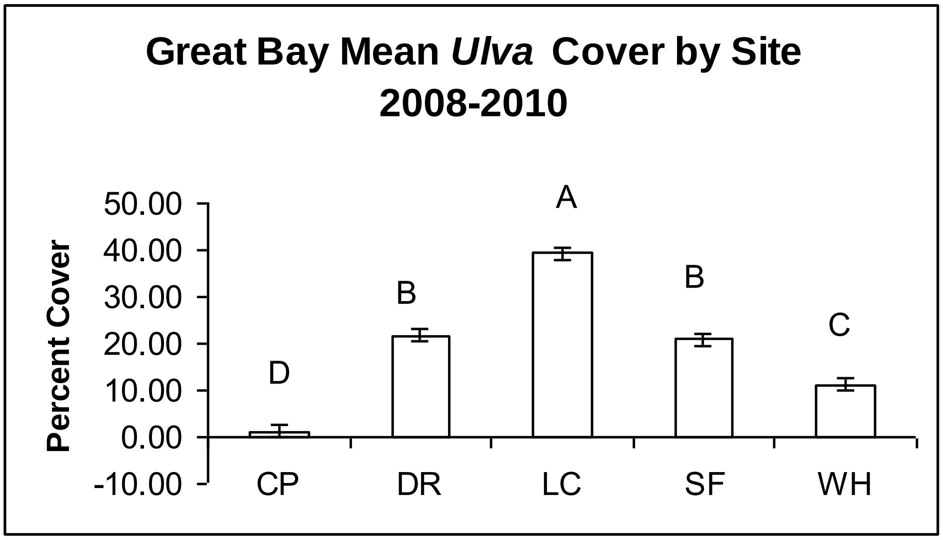

Figure 9 Great Bay *Ulva* mean percent cover by site from 2008-2010

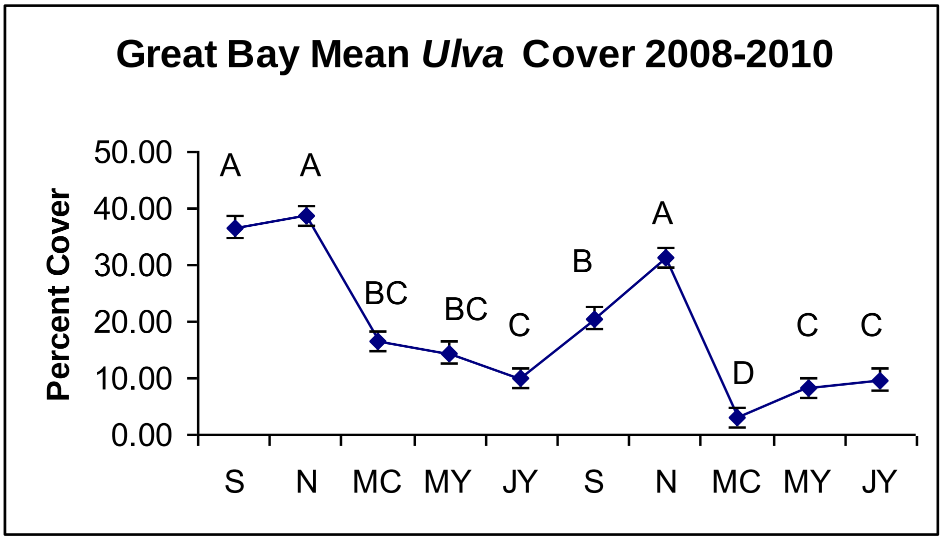

Figure 10 Great Bay *Ulva* monthly mean cover across 5 sites from 2008-2010

The mean *Gracilaria* biomass was tracked across the five study sites from 2008-2010 (Figure 11). Differences were found between the sites (P=0.00), with no *Gracilaria* measured at Wagon Hill Farm and Cedar Point in the northern bay, and significantly more at the sites in the southern bay. Mean *Gracilaria* biomass was the greatest at Depot Road and Sunset Farm (82.8 g dry weight/m^2 +/- 141.7 SD and 72.6 g dry weight/m^2 +/- 109.5 SD respectively). Mean *Gracilaria* biomass at Lubberland Creek was significantly lower (16.2 g dry weight/m^2 +/- 20.7 SD).

Seasonal differences in mean *Gracilaria* biomass were observed through the bay (P=0.00), with the maxima occurring in the fall of both years (Figure 12). Peak *Gracilaria* biomass (245.8 g dry weight/m^2 +/- 195.4 SD) in November 2008 was significantly greater P=0.01) than the peak in November 2009 (122.5 g dry weight/m^2 +/- 130.7 SD). *Gracilaria* biomass minima levels were observed from March ice-out through July of both study years.

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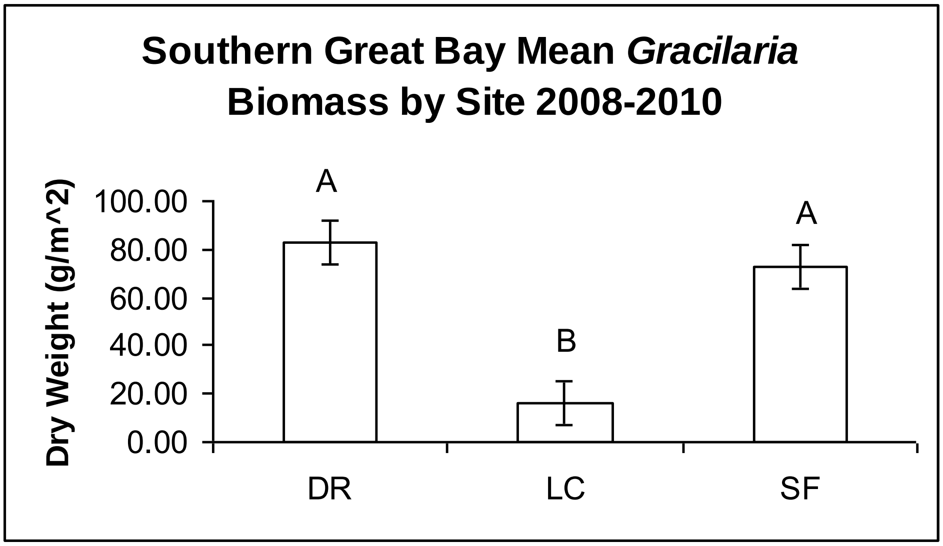

Figure 11 Great Bay *Gracilaria* biomass by site from 2008-2010

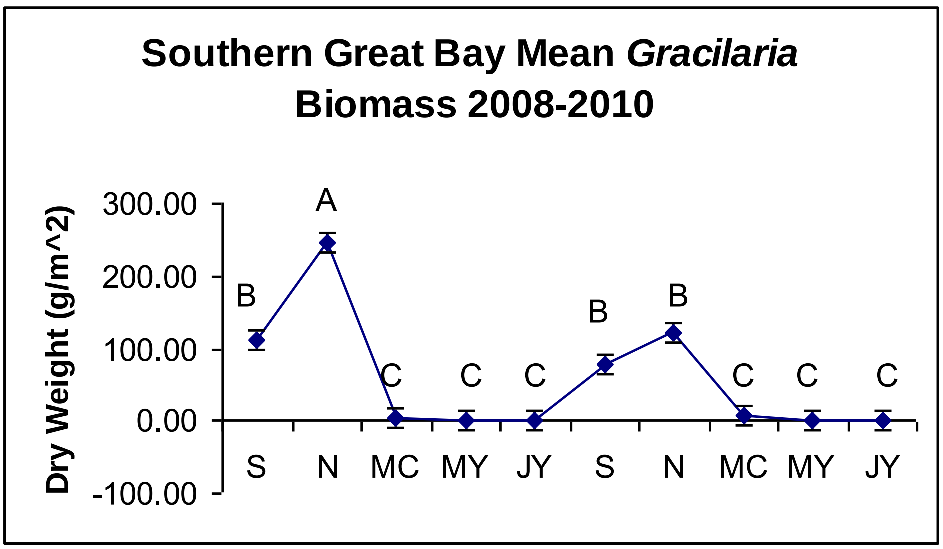

Figure 12 Southern Great Bay *Gracilaria* monthly mean biomass from 2008-2010. The Cedar Point and Wagon Hill Farm sites were not included in these calculations due to absence of organisms.

Mean *Gracilaria* cover results closely followed the trends seen in *Gracilaria* biomass (Figure 13), with highest levels measured at the Sunset Farm and Depot Road sites (15.5%+/- 15.1 SD and 12.4% +/- 12.9 SD respectively). The Lubberland Creek site had significantly lower mean *Gracilaria* cover (4.8% +/- 4.7 SD) during the study period.

Gracilaria cover exhibited a significant (P=0.00) seasonal trend across the Great Bay study sites (Figure 14). Seasonal highs in mean cover were observed in November of 2008 and 2009 (30.9%+/- 18.8 SD and 15.9% +/- 16.5 SD) with the maxima in 2008 being significantly greater (P=0.00, post-hoc). The lowest mean cover values were observed in May of both 2009 and 2010 (2.2%+/- 1.6 SD and 0.3% +/- 0.27 SD), which was later than was seen in the *Ulva* cover seasonal trends.

The mean algal biomass differed across the sites in Great Bay (P=0.00), with Cedar Point (1078.3 g dry weight/m^2 +/- 1070.2 SD) far exceeding the other four sites (Figure 15). The dominant contributors to the mean biomass at Cedar Point were fucoid algal species found in abundance attached to the site's shale substratum.

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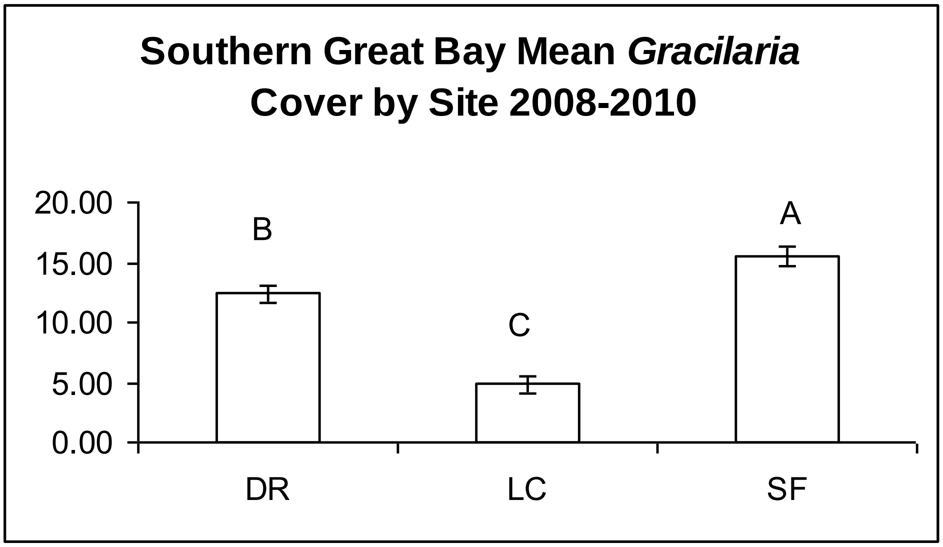

Figure 13 Southern Great Bay *Gracilaria* mean cover by site (2008-2010)

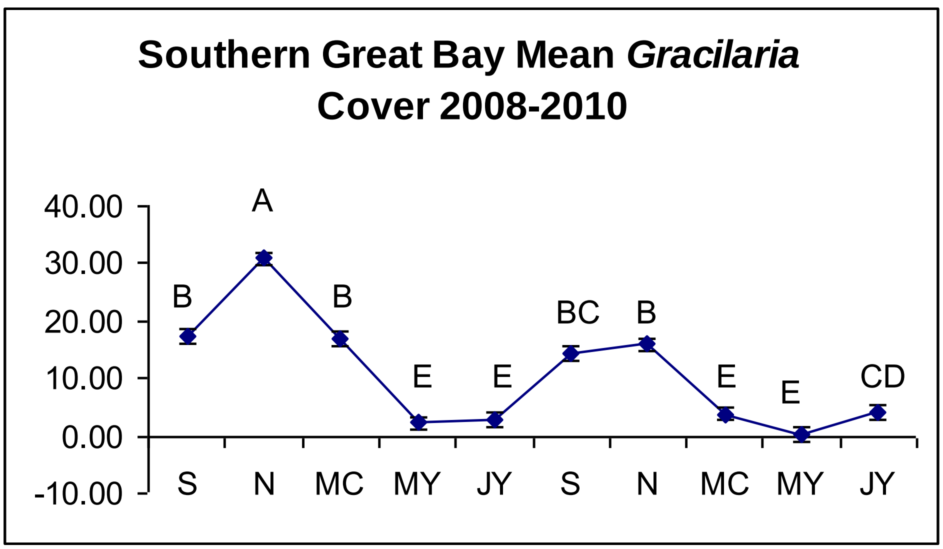

Figure 14 Southern Great Bay monthly mean *Gracilaria* cover (2008-2010)

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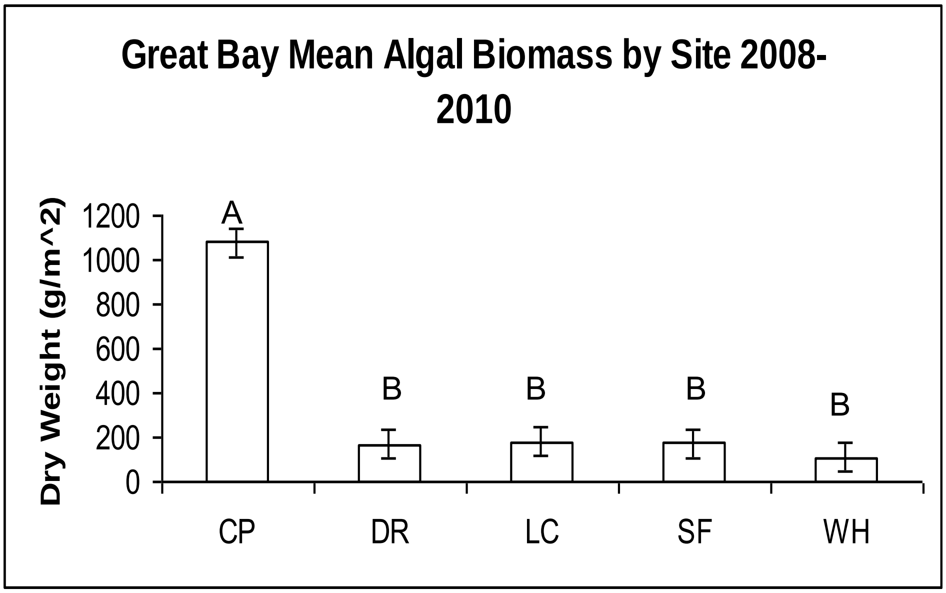

Figure 15 Great Bay algae mean biomass by site (2008-2010)

The mean total nitrogen of water from each Great Bay study site was compared (Figure 16). The only significant difference (P=0.04) observed was between the Sunset Farm site (0.78 mg/L +- 0.19 SD) and the Wagon Hill Farm site (0.42 mg/L +- 0.2 SD).

The mean total nitrogen from *Ulva* tissue was also compared between sites (Figure 17). There were no significant differences found between the sites, but it must be noted that *Ulva* was not available for nutrient tests at Cedar Point during November 2009 and July 2010, months with low tissue nitrogen measures at the other sites.

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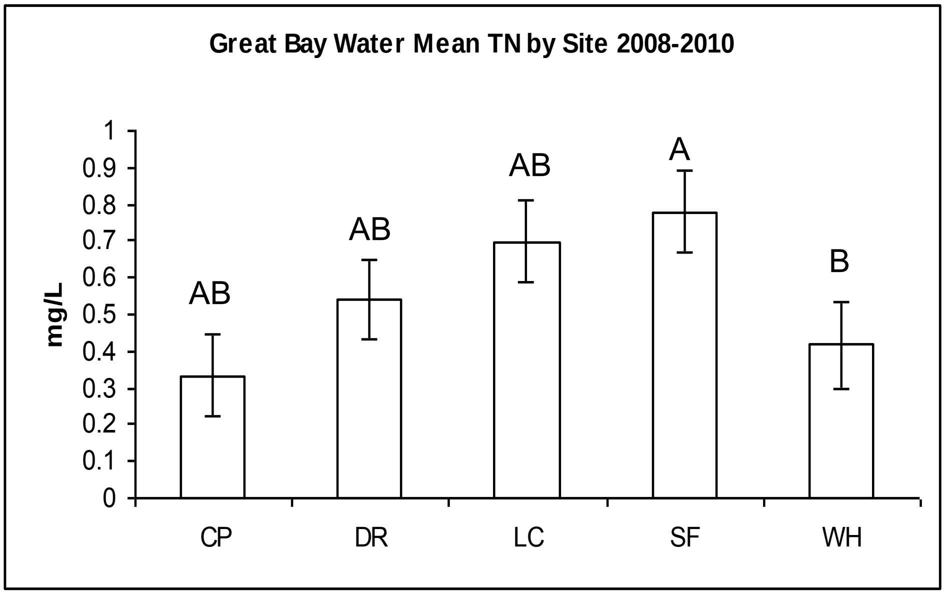

Figure 16 Great Bay water mean TN by site (2008-2010)

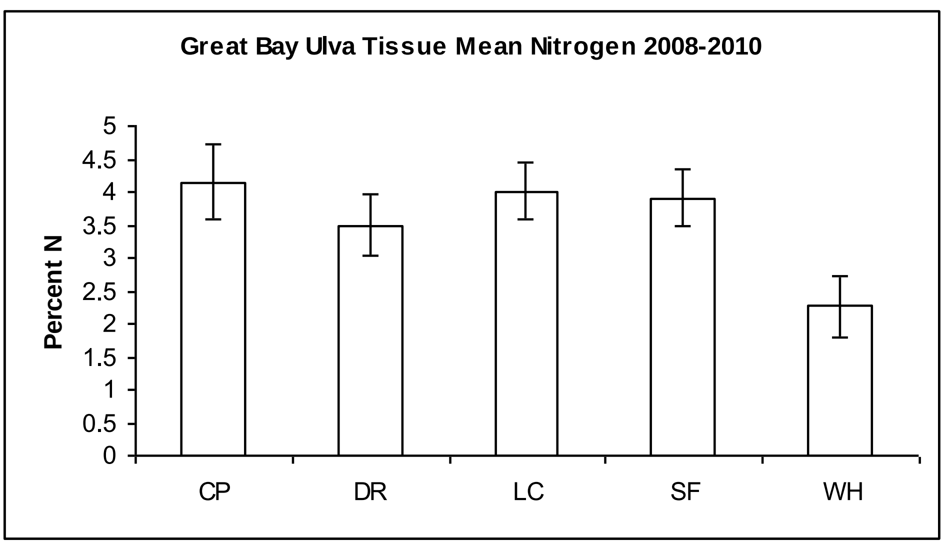

Figure 17 Great Bay *Ulva* tissue mean nitrogen by site (2008-2010)

Great Bay mean water nitrogen and *Ulva* tissue nitrogen were measured throughout the two year study (Figures 18, 19, 20, and 21). No significant differences were found between the months using any of these methods. Great Bay water mean total nitrogen levels remained between 0.39 and 0.66 mg/L throughout the study period. Meanwhile the mean *Ulva* tissue nitrogen percentages remained between 2.3 and 4.1%. Mean water phosphorus concentrations were between 0.028 and 0.07 mg/L across Great Bay, and *Ulva* tissue mean phosphorus percentages stayed between 0.013 and 0.018%.

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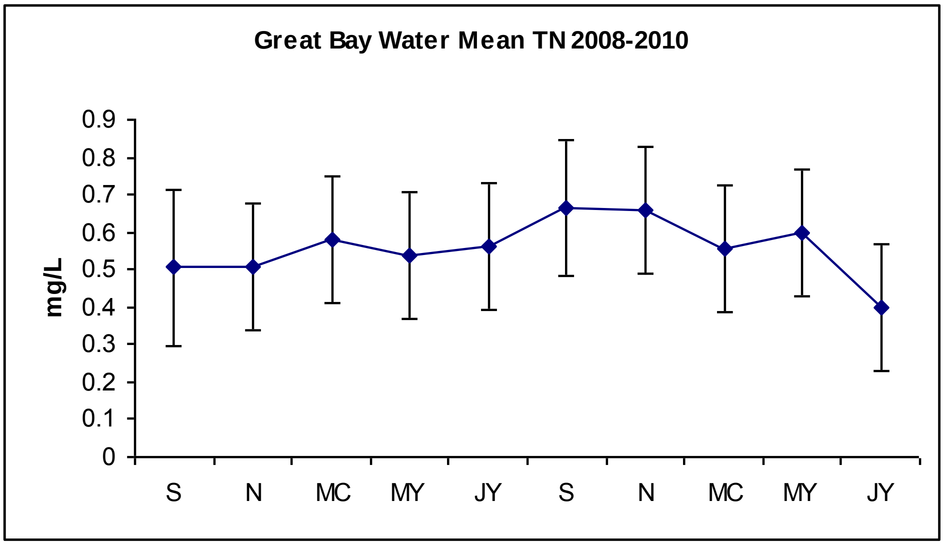

Figure 18 Great Bay water mean total nitrogen by month (2008-2010)

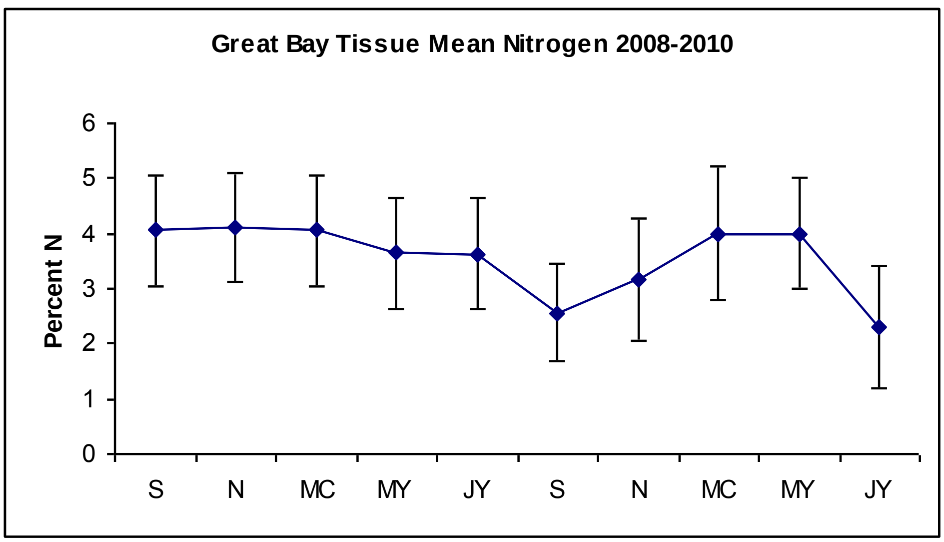

Figure 19 Great Bay *Ulva* tissue mean total nitrogen as percent dry weight (2008-2010)

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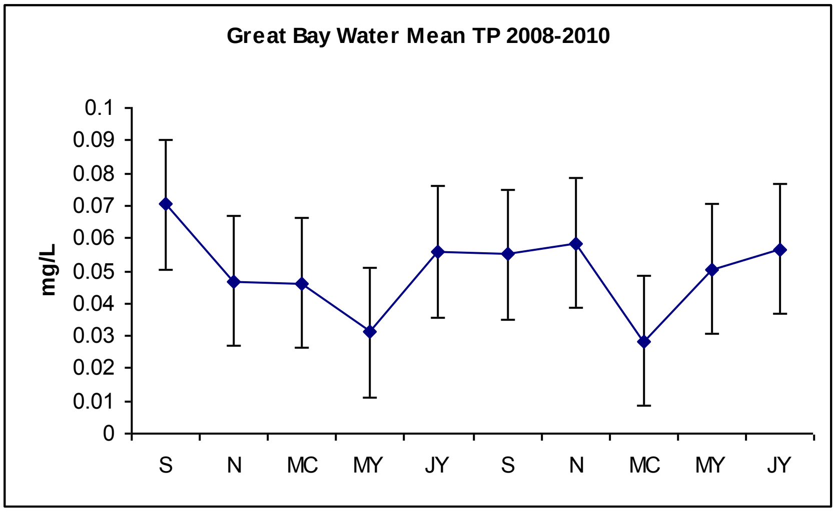

Figure 20 Great Bay water mean total phosphorus by month (2008-2010)

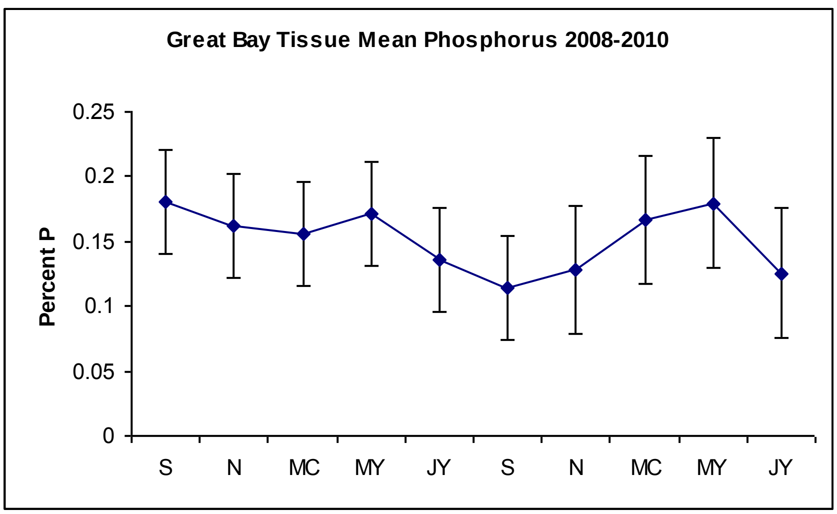

Figure 21 Great Bay *Ulva* tissue mean total phosphorus as percent dry weight (2008-2010)

Great Bay water mean total phosphorus was calculated for each site for the study period (Figure 22). The trend was for higher ambient phosphorus in the southern portion of Great Bay (P=0.01) with the highest mean concentration at Sunset Farm (0.08 mg/L +- 0.04 SD).

Ulva tissue was used to track mean phosphorus levels at all sites in Great Bay (Figure 23). The Cedar Point *Ulva* tissue, on average, contained a slightly lower percent of phosphorus (0.136% +- 0.036 SD) than was found at any other site (P<0.01). This method also revealed a trend toward greater levels of phosphorus in the southern bay.

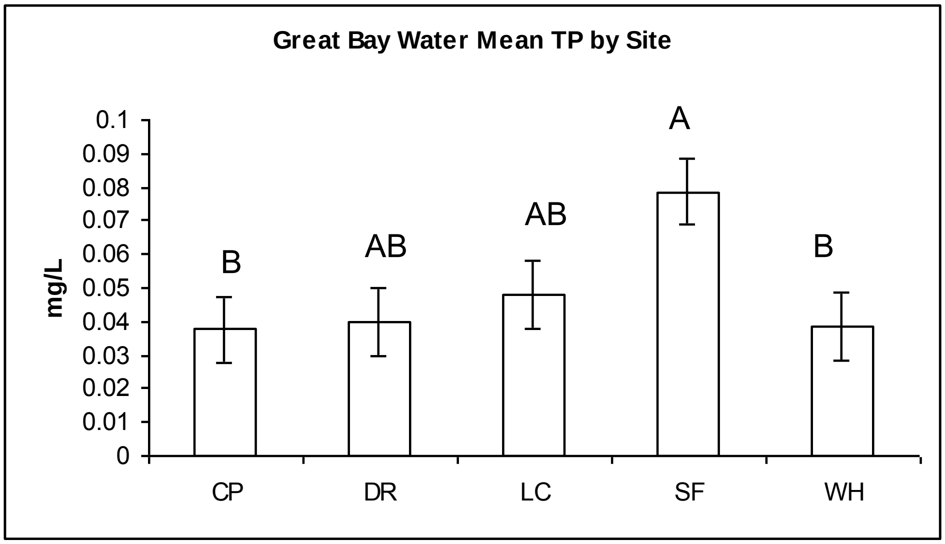

Figure 22 Great Bay water mean total phosphorus by site as percent dry weight (2008-2010)

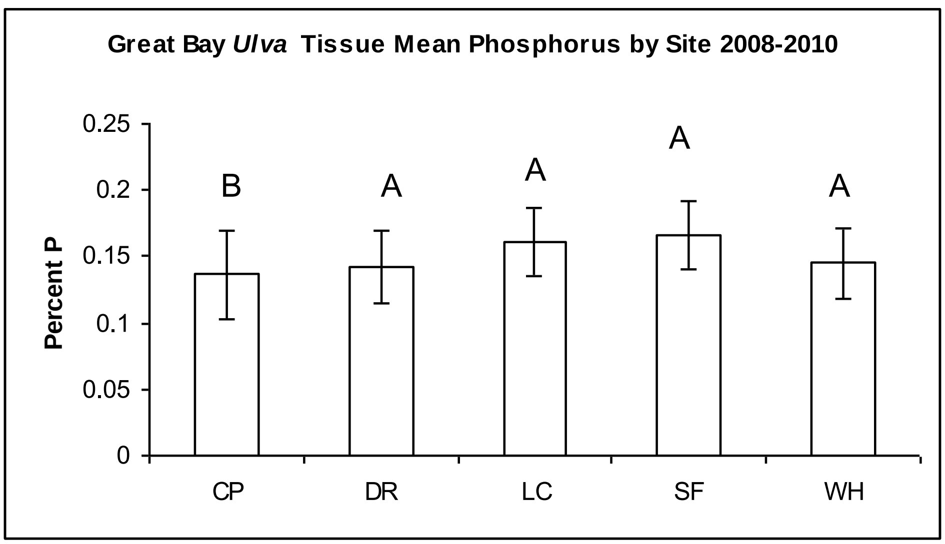

Figure 23 Great Bay *Ulva* tissue mean total phosphorus by site as percent dry weight (2008-2010)

The atomic N:P ratios for Great Bay water and *Ulva* tissue samples were calculated, and mean values per site were determined for the study period (Figure 24). No significant differences were found between sites using either method, and comparisons between the methods showed no significant differences in the N:P ratios by site. However, comparisons of the methods revealed a trend of lower N:P ratios in the water than in the *Ulva* tissues at four of five sites. Mean water N:P ratios for the two year study ranged between 34.2 +- 33.4 SD (Sunset Farm) and 48.8 +- 38.9 SD (Lubberland Creek), whereas mean tissue N:P ratios were between 37 +- 19.7 SD (Wagon Hill Farm) and 74.1 +- 16.1 SD (Cedar Point). All of these means levels by site were well above the normal 16:1 Redfield Ratio.

Ulva tissue mean N:P ratios and water mean N:P ratios were also calculated by collection month across Great Bay (Figure 25). While no significant differences were found in mean N:P ratios over time by either method, the trend was for slightly greater mean ratios from the *Ulva* tissue analysis. In the water tests, N:P ratios ranged from a low of 26.2 +- 7.3 SD in September 2009 to a high of 69.4 +- 65.7 SD in November of the same year. In tissue tests, the N:P ratios ranged from a low of 38.5 +- 9.5 SD in May 2010 to a high of 61 +- 20.4 SD in November 2008.

EXHIBIT 49 (AR K.12)

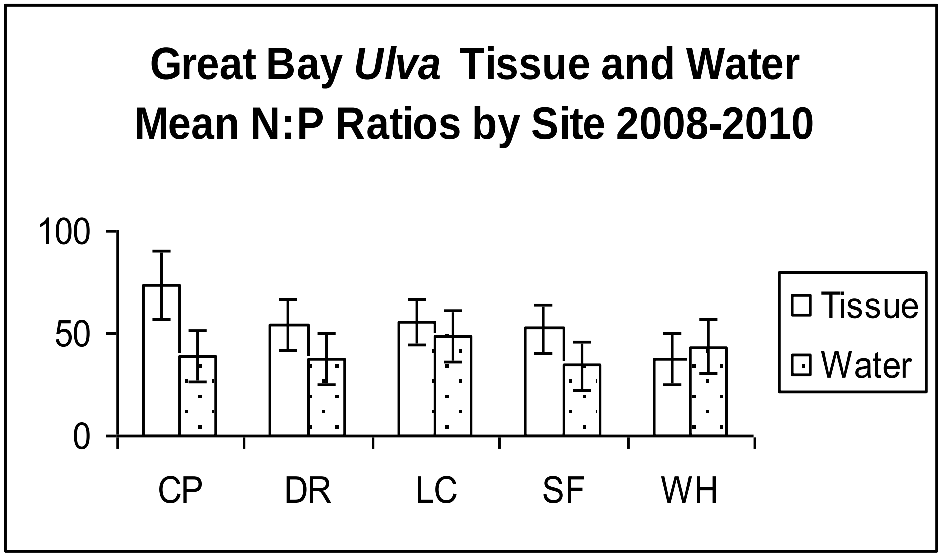

Figure 24 Great Bay *Ulva* tissue and water mean atomic N:P ratios by site for the two year study period (2008-2010)

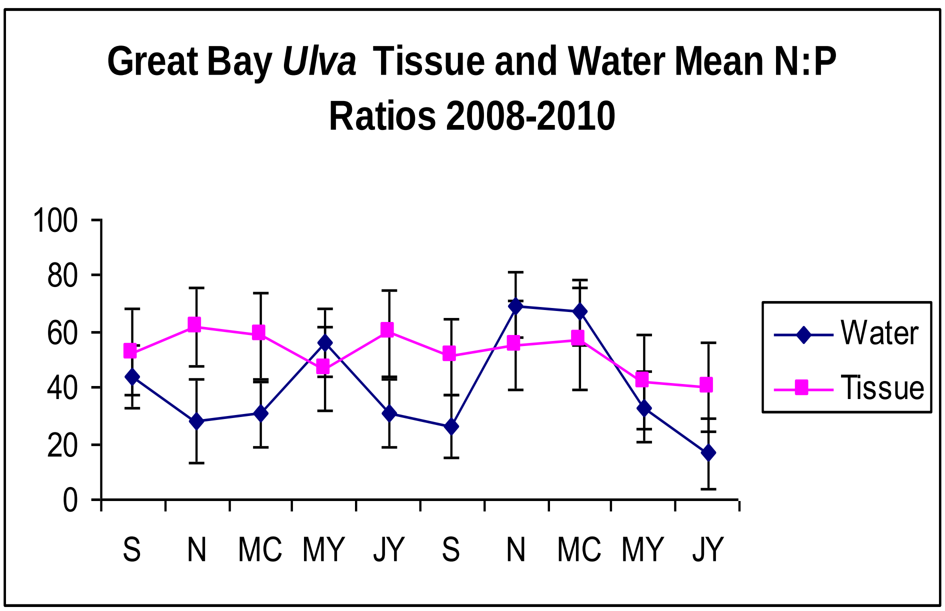

Figure 25 Great Bay *Ulva* tissue and water monthly atomic N:P ratios averaged across all study sites

Within site mean *Ulva* biomass was calculated for each month of the study at the Sunset Farm site (Figure 26). Mean biomass varied with time (P=0.00), with peak levels measured in the fall of both 2008 and 2009. The seasonal maxima achieved in September 2008 was significantly greater (P=0.00) than the maxima observed in November of the following year (547.8 g dry weight/m^2 +- 802.1 SD vs. 124.3 g dry weight/m^2 +- 163.5 SD). Seasonal mean biomass lows occurred both years following ice-out in March, with *Ulva* biomass remaining below 5 g dry weight/m^2 through July of 2009 and below 35 g dry weight/m^2 through July 2010.

The mean *Gracilaria* biomass was also calculated for each collection month at the Sunset Farm site (Figure 27). Seasonal differences were found (P=0.00), with peak biomass in November of both 2008 and 2009 (264.8 g dry weight/m^2 +- 391.9 SD and 273.6 g dry weight/m^2 +- 380.6 SD). As was observed with *Ulva* at this site, there was a pronounced decline in *Gracilaria* mean biomass over the months of ice cover, with seasonal minima levels observed in March 2009 (1.97 g dry weight/m^2 +- 4.1 SD) and May 2010 (0.06 g dry weight/m^2 +- 0.36 SD).

EXHIBIT 49 (AR K.12)

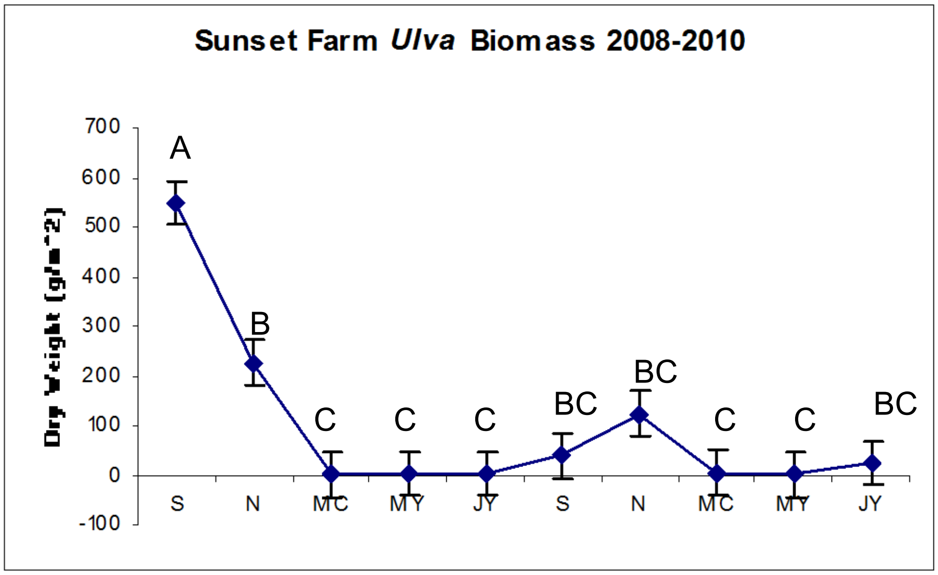

Figure 26 Sunset Farm *Ulva* mean biomass per month (2008-2010)

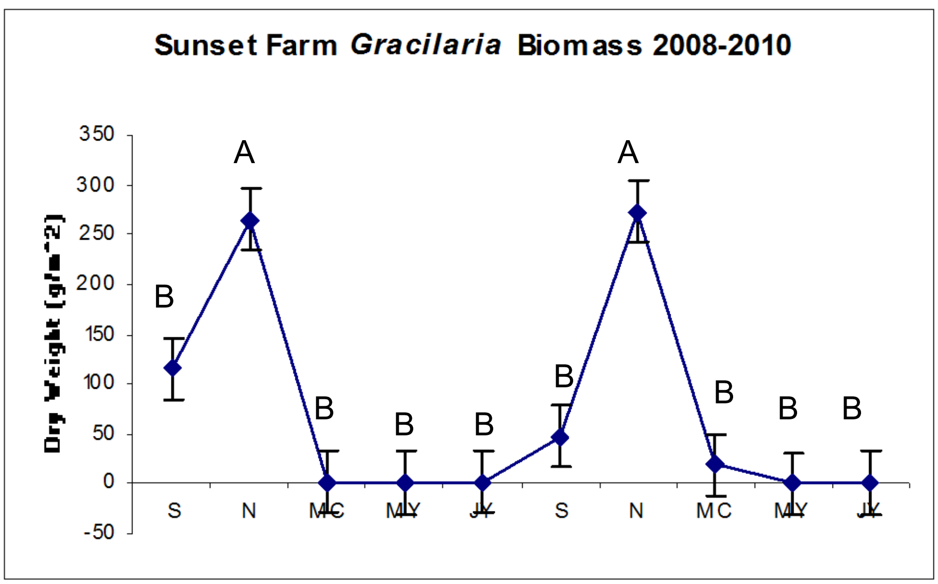

Figure 27 Sunset Farm *Gracilaria* mean biomass per month (2008-2010)

The *Ulva* mean percent cover per month was tracked for Sunset Farm (Figure 28), and significant seasonal differences were found (P=0.00). Cover maxima were observed in November 2008 and November 2009 (59.9% +- 33.1 SD and 45.2% +- 46.1 SD), and seasonal minima were observed in March of both study years (5.2% +- 8.7 SD and 0.7%+- 2.5 SD).

The mean *Gracilaria* cover was also determined by month for the Sunset Farm site (Figure 29). A significant seasonal trend was observed (P=0.00), with peak bloom in November of both years (39.2% +- 35.9 SD and 34.9 +- 37.3 SD). Mean *Gracilaria* cover was lowest in May of 2009 and 2010 (3.1% +- 7.3 SD and 0.6% +- 1.7 SD), which lagged behind March ice-out.

EXHIBIT 49 (AR K.12)

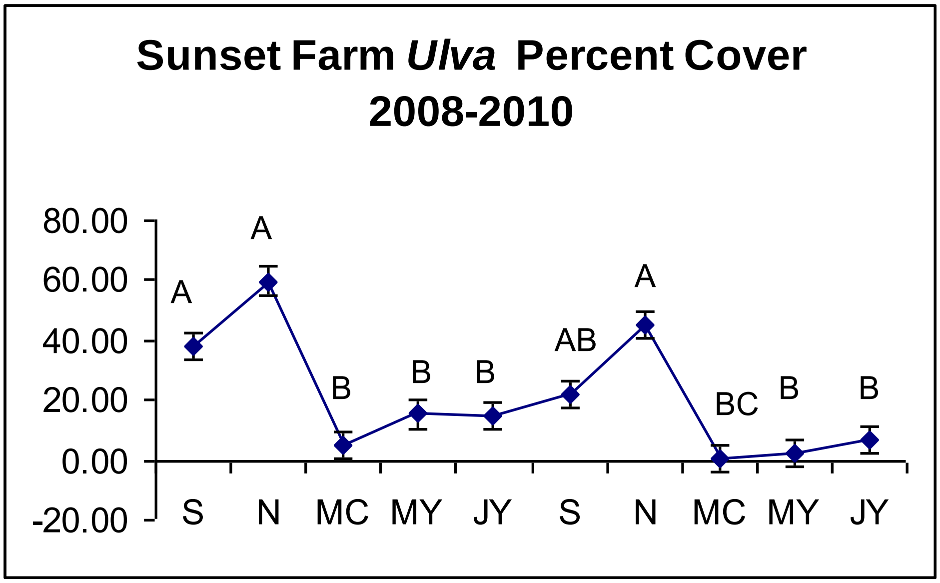

Figure 28 Sunset Farm *Ulva* mean percent cover (non-transformed) 2008-2010

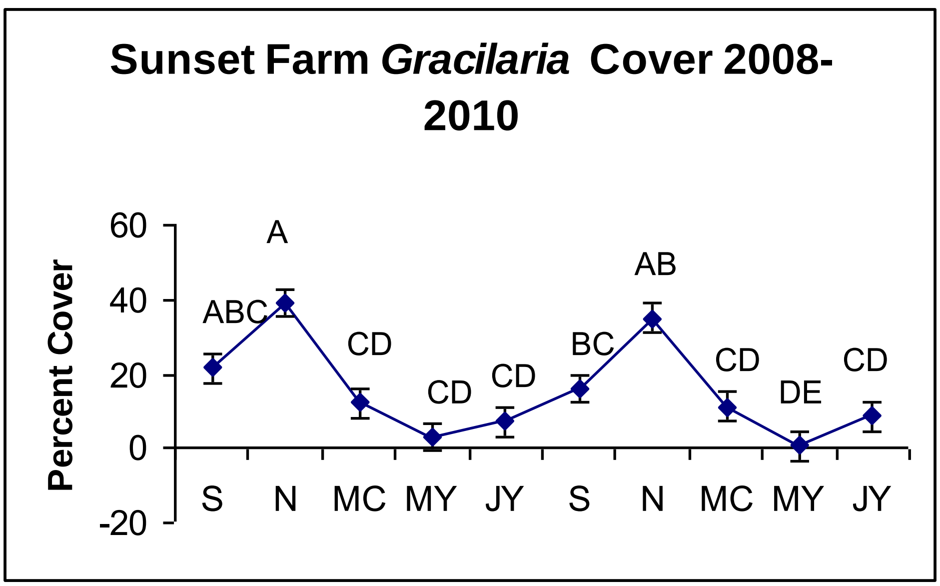

Figure 29 Sunset Farm *Gracilaria* percent cover (non-transformed) 2008-2010

Trends in *Ulva* and *Gracilaria* elevation were examined throughout the study period. Although significant differences were not found in either case, *Ulva* and *Gracilaria* distributions tended to be slightly more concentrated at higher elevations, though the vast majority of the specimens were free floating and able to move with the prevailing water currents.

Water and *Ulva* tissue from the Sunset Farm site were analyzed for seasonal variation in N:P ratios (Figures 30 and 31, Table 2). No significant differences were found between the months using the water analyses, but the trend was for higher N:P ratios in the spring months (March-May) of both years. Monthly mean water N:P ratios remained between 10.7 +- 0.83 SD and 123.2 +-176.1 SD during the course of the study. Between-month differences in mean N:P ratios were found using the tissue analyses (P=0.00), but seasonal trends were unclear. The highest mean N:P ratio was observed in November 2009 (65.0 +- 4.4 SD), and the lowest mean N:P observation was September 2008 (34.3 +- 1.2 SD), the first month of the study.

EXHIBIT 49 (AR K.12)

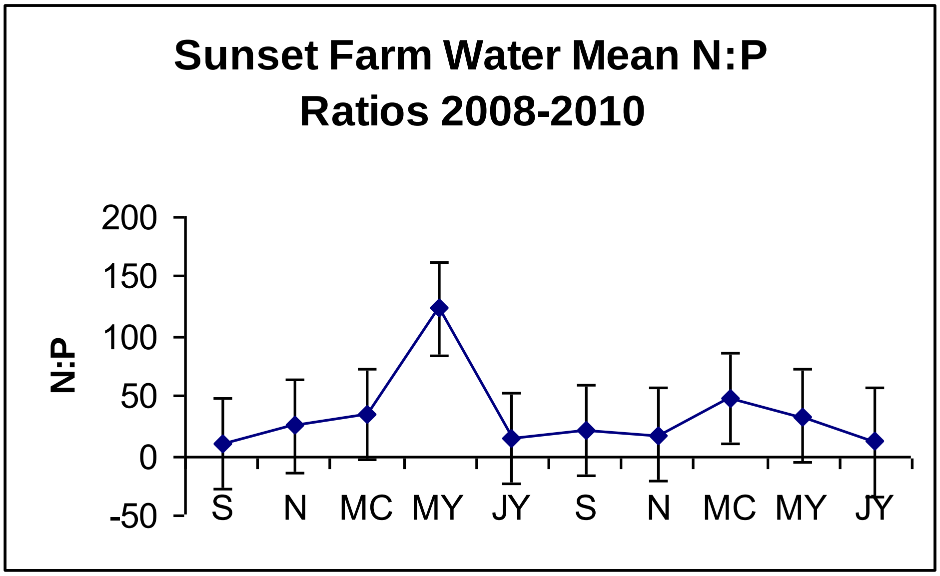

Figure 30 Sunset Farm water mean atomic N:P ratios by month (2008-2010)

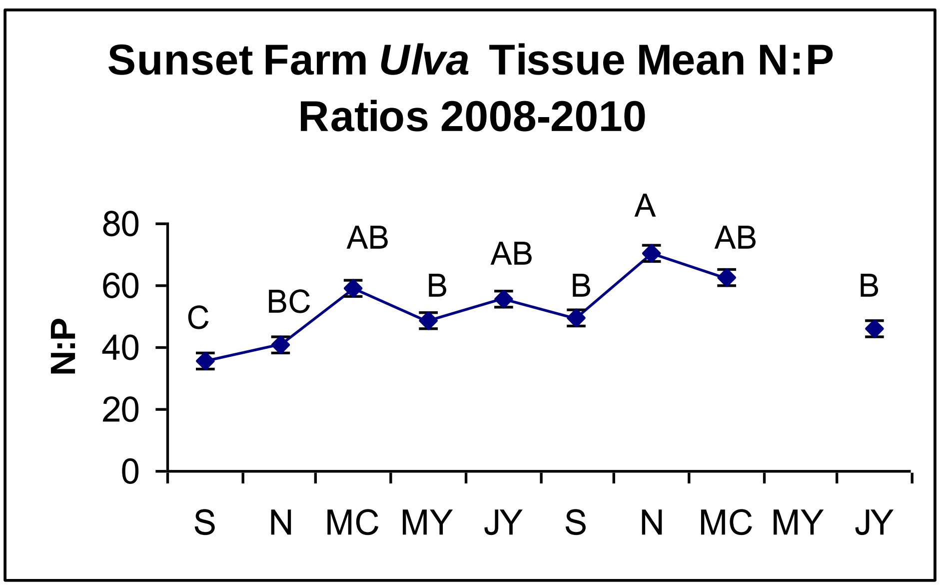

Figure 31 Sunset Farm *Ulva* tissue mean atomic N:P ratios by month (2008-2010)

EXHIBIT 49 (AR K.12)

Table 2 Sunset Farm water and *Ulva* tissue monthly mean TN and TP

Sunset Farm Monthly Mean Values 2008-2010				
	Water TN (mg/L)	**Water TP (mg/L)**	**Ulva Tissue %N**	**Ulva tissue %P**
S	0.748	0.149	3.721	0.232
N	0.426	0.036	3.862	0.208
MC	1.050	0.067	4.615	0.172
MY	0.932	0.046	3.968	0.180
JY	0.858	0.122	3.650	0.146
S	0.734	0.075	2.633	0.118
N	0.710	0.085	3.472	0.110
MC	0.732	0.032	4.689	0.165
MY	0.932	0.046	5.307	
JY	0.608	0.109	3.203	0.153

Within site mean *Ulva* biomass was calculated for each month of the study at the Depot Road site (Figure 32). Mean biomass varied with time (P=0.00), with peak levels measured in the fall of both 2008 and 2009. The peak bloom was observed in November 2008 and November 2009 (170 g dry weight/m^2 +- 245.8 SD and 272.8 g dry weight/m^2 +- 443 SD). Seasonal mean biomass lows were pronounced and occurred both years following ice-out in March, with *Ulva* biomass remaining below 6 g dry weight/m^2 through July of 2009 and below 12 g dry weight/m^2 through July 2010.

The mean *Gracilaria* biomass was also calculated for each collection month at the Depot Road site (Figure 33). Seasonal differences were found (P=0.00), with peak mean biomass in November 2008 and September 2009 (431.1g dry weight/m^2 +- 774.3 SD and 158.8 g dry weight/m^2 +- 383.0 SD). As was observed with *Ulva* at this site, there was a pronounced decline in *Gracilaria* mean biomass over the months of ice cover, with seasonal minima means remaining below 6.3 g dry weight/m^2 from March through July 2009 and below 0.25 g dry weight/m^2 during the same period the following year.

EXHIBIT 49 (AR K.12)

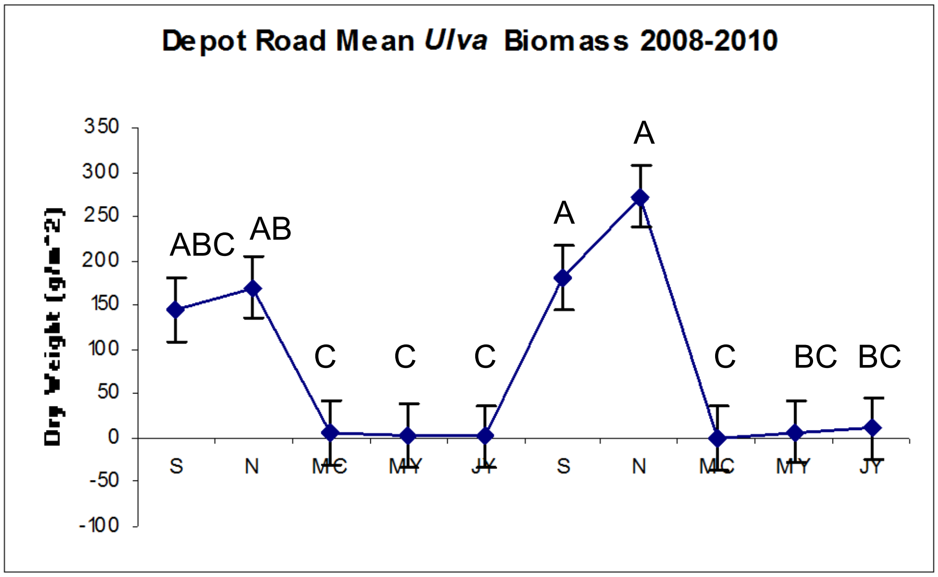

Figure 32 Depot Road *Ulva* monthly mean biomass (2008-2010)

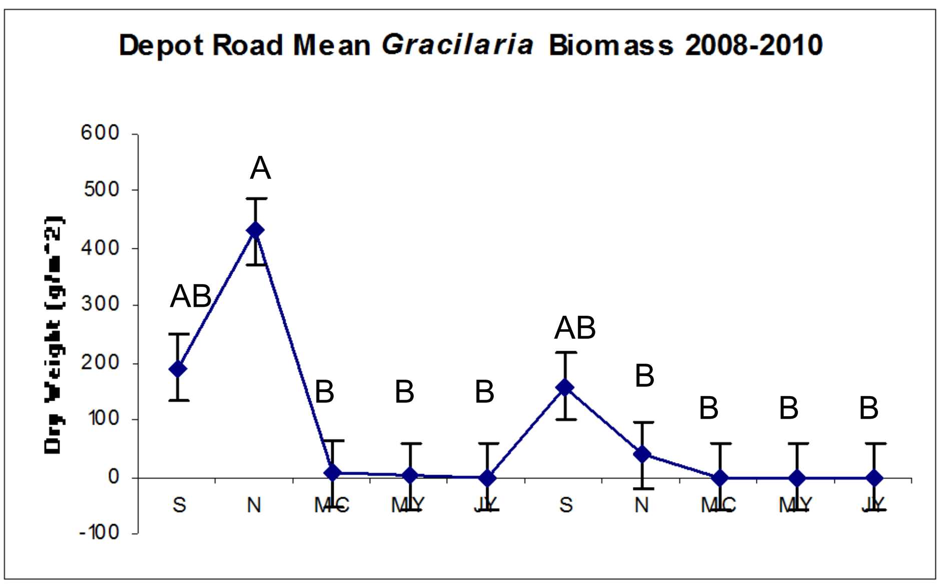

Figure 33 Depot Road *Gracilaria* monthly mean biomass (2008-2010)

The *Ulva* mean percent cover per month was tracked for Depot Road (Figure 34), and significant seasonal differences were found (P=0.00). Cover maxima were observed in the fall of both years, September 2008 and November 2009 (55.3% +- 35.7 SD and 42.8% +- 46.0 SD), and seasonal minima were observed in May 2009 and March 2010 (14.0% +- 23.4 SD and 0.1%+- 0.63 SD).

The mean *Gracilaria* cover was also determined by month for the Depot Road site (Figure 35). A significant seasonal trend was observed (P=0.00), with peak bloom in November 2008 and September 2009 (44.1% +- 33.7 SD and 14.8% +- 25.7 SD). The peak bloom in 2008 was significantly greater than in 2009 (P<0.01). Mean *Gracilaria* cover was lowest in May of 2009 and March of 2010 (3.2% +- 10.5 SD and 0% +- 0 SD). The 2009 low lagged two months behind the thawing of the site's ice cover. *Gracilaria* specimens were present at the site in March and May of 2010, but none were within the study's transect lines.

EXHIBIT 49 (AR K.12)

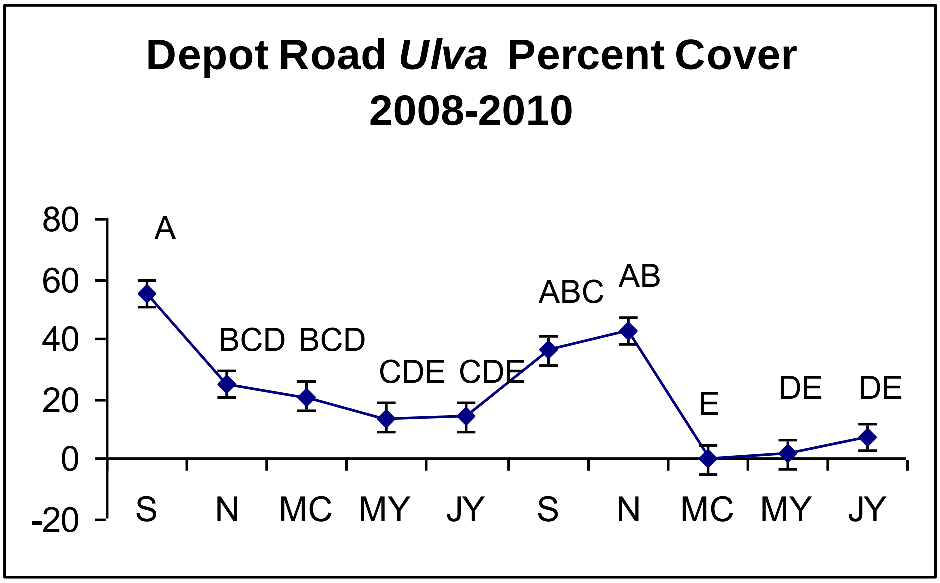

Figure 34 Depot Road *Ulva* monthly mean percent cover (non-transformed)

2008-2010

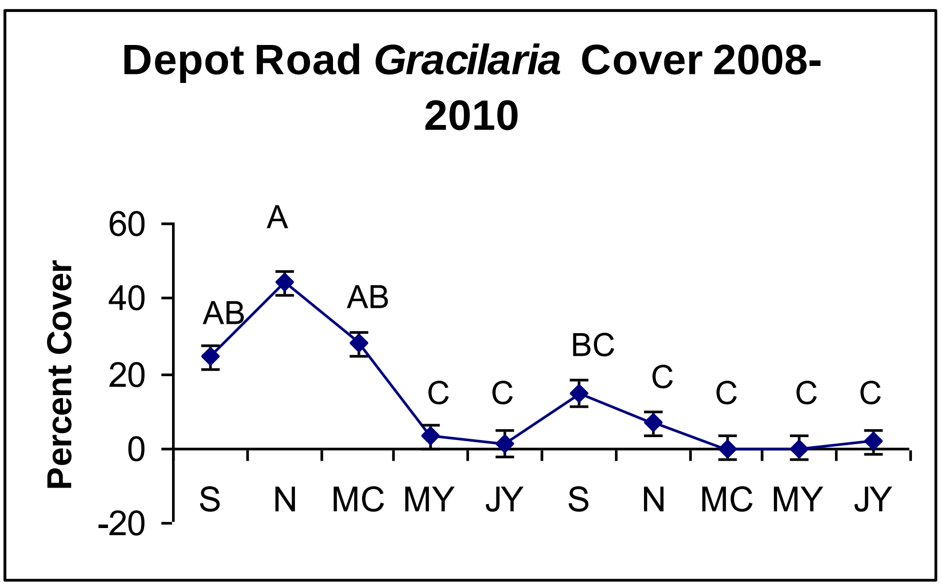

Figure 35 Depot Road *Gracilaria* mean percent cover (non-transformed)

2008-2010

Trends in *Ulva* and *Gracilaria* elevational distribution were examined throughout the study period. *Ulva* distribution favored mid-low elevations of approximately 0.05 m above mean low water (P=0.01). No significant differences were found in *Gracilaria* distributions, but the organisms tended to be slightly more concentrated at the lower elevations. It should be noted that the vast majority of the specimens observed at this site were free floating and able to move with the prevailing water currents.

Water and *Ulva* tissue from the Depot Road site were analyzed for seasonal variation in N:P ratios (Figures 36 and 37, Table 3). No significant differences were found between the months using the water analyses, but the trend was for higher N:P ratios in the spring months (March-May) of both years. Monthly mean water N:P ratios remained between 18.3 +- 14.3 SD and 71.4 +-64.6 SD during the course of the study. Between-month differences in mean N:P ratios were not found using the tissue analyses. The highest mean N:P ratio was observed in March 2008 (76.6 +- 11.2 SD), and the lowest mean N:P observation was September 2008 (39.7 +- 3.3 SD), the first month of the study.

EXHIBIT 49 (AR K.12)

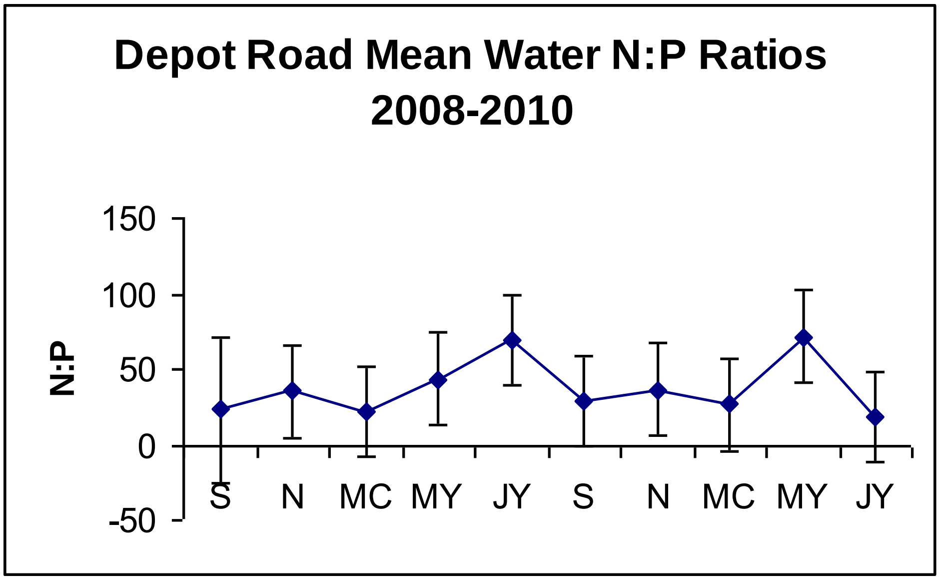

Figure 36 Depot Road water mean atomic N:P ratios by month (2008-2010)

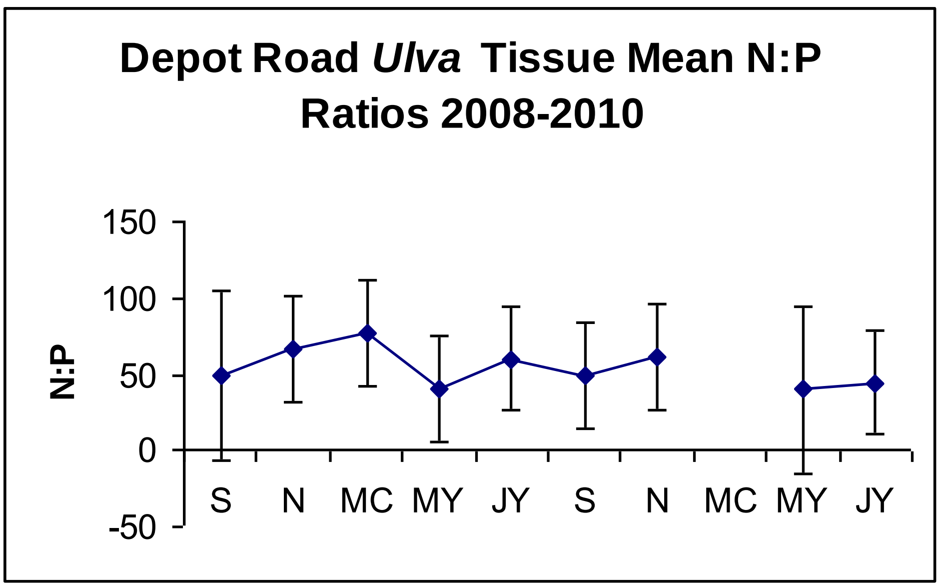

Figure 37 Depot Road *Ulva* tissue mean atomic N:P ratios by month (2008-2010)

EXHIBIT 49 (AR K.12)

Table 3 Depot Road water and *Ulva* tissue monthly mean TN and TP

Depot Road Monthly Mean Values 2008-2010				
	Water TN (mg/L)	**Water TP (mg/L)**	**Ulva Tissue %N**	**Ulva tissue %P**
S	0.335	0.039	4.421	0.200
N	0.249	0.024	4.505	0.151
MC	0.411	0.042	4.579	0.129
MY	0.200	0.014	3.452	0.183
JY	0.720	0.028	4.103	0.146
S	0.765	0.053	2.578	0.117
N	1.221	0.084	3.272	0.132
MC	0.414	0.034		
MY	0.883	0.056	2.257	0.122
JY	0.207	0.026	2.333	0.112

Mean *Ulva* biomass was calculated for each month of the study at the Lubberland Creek site (Figure 38). Mean biomass varied with time (P=0.00), with peak levels measured in the fall of both 2008 and 2009. The peak bloom observed in November 2008 (733.8 g dry weight/m^2 +- 613.0 SD) was significantly greater (P=0.00) than that observed the following November (175.8 g dry weight/m^2 +- 211.5 SD and). Seasonal mean biomass lows were pronounced and occurred both years following ice-out in March, with *Ulva* biomass remaining below 5 g dry weight/m^2 through July of 2009 and below 12 g dry weight/m^2 through May 2010.

The mean *Gracilaria* biomass was determined for each collection month at the Lubberland Creek site (Figure 39). Seasonal differences were found (P=0.00), with peak mean biomass in November 2008 and 2009 (41.7g dry weight/m^2 +- 71.3 SD and 55.9 g dry weight/m^2 +- 110.9 SD). As was observed with *Ulva* at this site, there was a marked decline in *Gracilaria* mean biomass over the months of ice cover, with seasonal low means remaining below 0.9 g dry weight/m^2 from March through July 2009 and below 5.7 g dry weight/m^2 during the same period the following year.

EXHIBIT 49 (AR K.12)

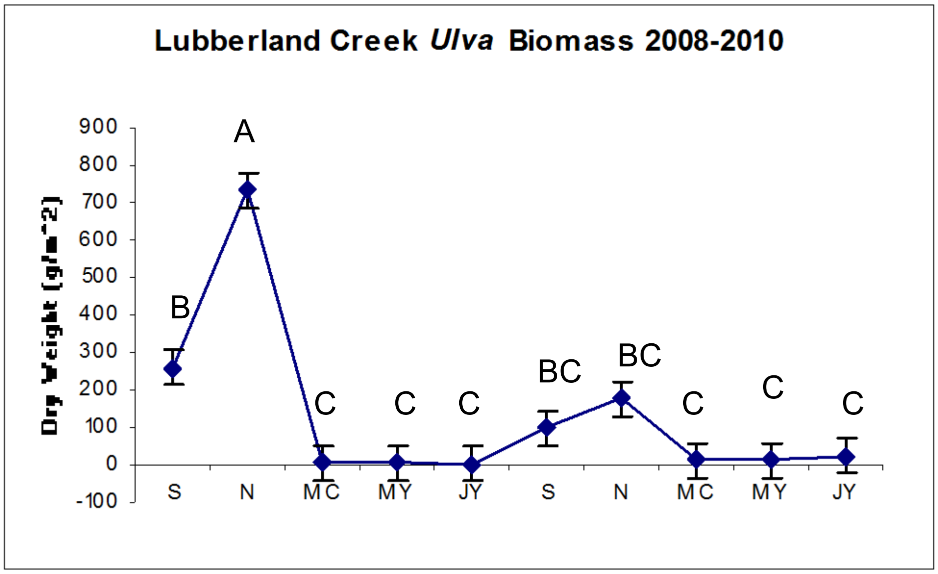

Figure 38 Lubberland Creek *Ulva* mean monthly biomass (2008-2010)

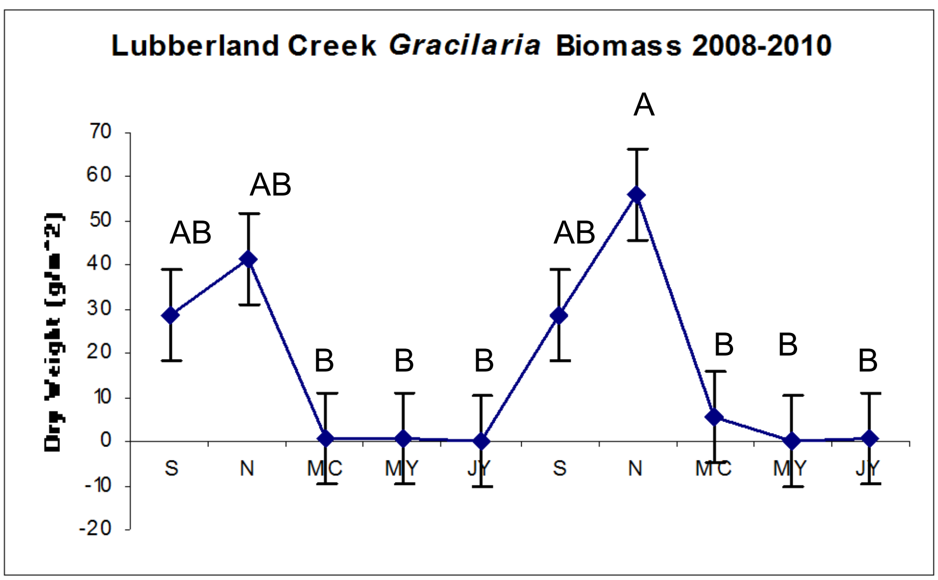

Figure 39 Lubberland Creek *Gracilaria* monthly mean biomass (2008-2010)

The *Ulva* mean percent cover per month was determined at the Lubberland Creek site (Figure 40), and significant seasonal differences were found (P=0.00). Cover maxima was observed in November of both years (90.1% +- 18.4 SD and 54.0% +- 46.0SD). During the November 2008 bloom, the mudflats at this site were almost entirely covered by *Ulva* tissues several layers thick. After the abundant bloom of 2008, the seasonal *Ulva* cover minimum was not observed the following year until July (18.3% +- 27.9 SD). The seasonal low mean *Ulva* cover for the 2010 season was observed in March (3.1%+- 6.6 SD).

The mean *Gracilaria* cover was also determined by month for the Lubberland Creek site (Figure 41). A significant seasonal trend was observed (P=0.00), with peak cover observed in March 2009 and September 2009 (10.8% +- 18.6 SD and 12% +- 22.4 SD). Mean *Gracilaria* cover was lowest in July of 2009 and May of 2010 (0.4% +- 1.5 SD and 0.3% +- 1.4 SD).

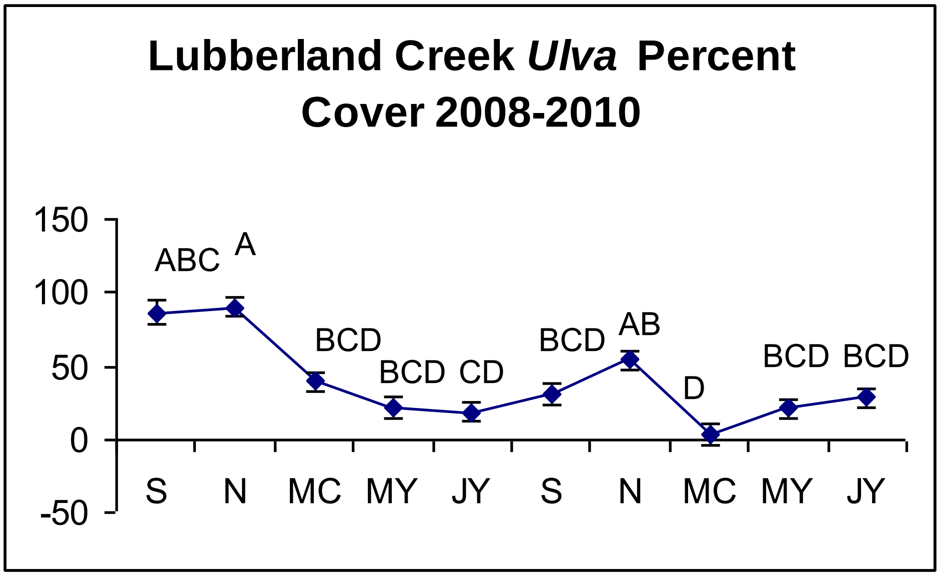

Figure 40 Lubberland Creek *Ulva* monthly mean percent cover (non-transformed) 2008-2010)

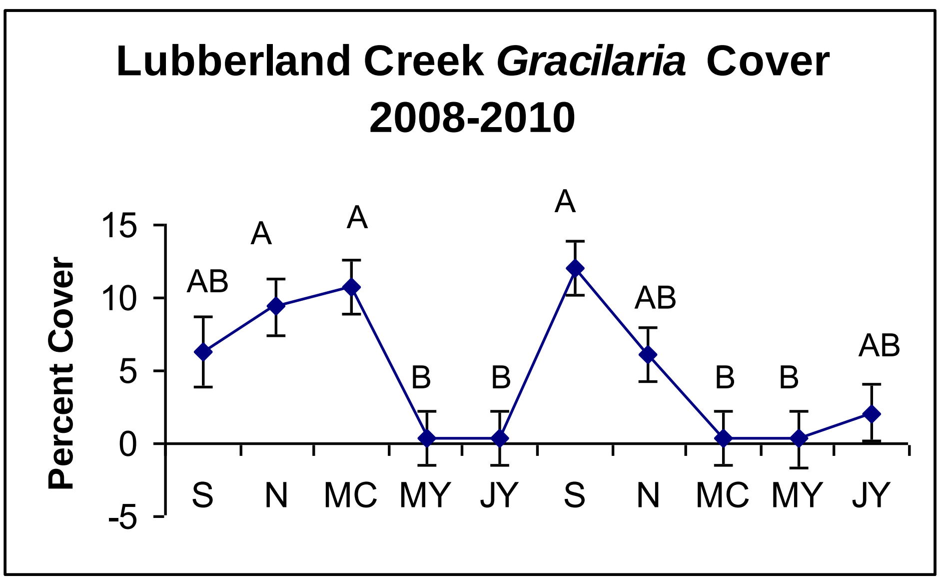

Figure 41 Lubberland Creek *Gracilaria* monthly mean percent cover (non-transformed) 2008-2010)

Ulva and *Gracilaria* elevational distribution were examined throughout the study at the Lubberland Creek site. *Ulva* distribution was even throughout the site. *Gracilaria* distributions were slightly more concentrated at the highest elevations (P=0.05), especially at the marsh-grass/open-mudflat boundary. As was true at the other southern Great Bay sites, the vast majority of the specimens located at this site were free floating and able to move with the prevailing water currents.

Water and *Ulva* tissue from the Lubberland Creek site were analyzed for seasonal variation in N:P ratios (Figures 42 and 43, Table 4). No significant differences were found between the months using the either method of analysis. The trend was for more fluctuation in the water N:P ratios across the months, whereas *Ulva* tissue means were fairly constant through the study period. Monthly mean water N:P ratios remained between 17 +- 0.4 SD and 35 +- 14.9 with the occasional spike into the 70s and above. In the tissue analyses, the mean N:P ratios remained between 39.8 +- 0.6 SD and 76.8 +- 6.0 SD.

EXHIBIT 49 (AR K.12)

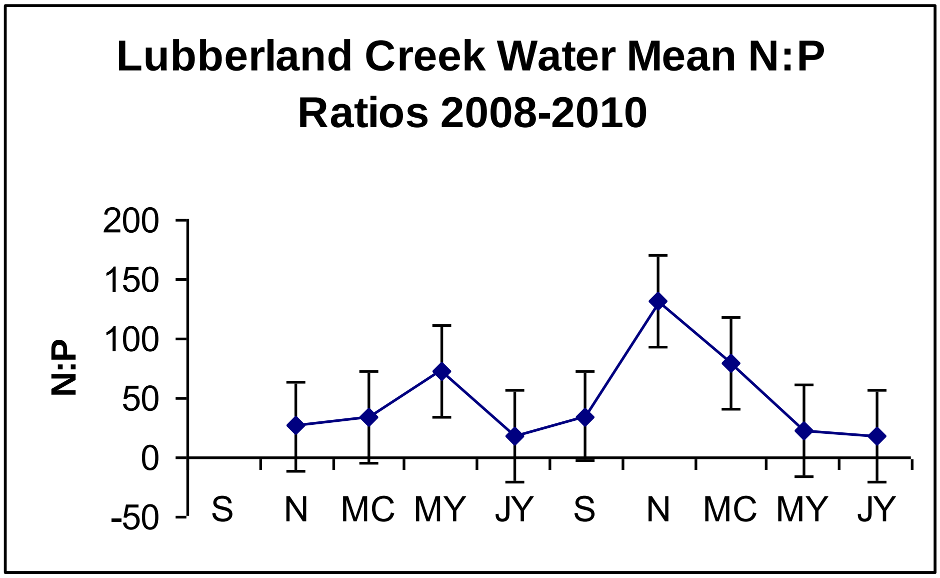

Figure 42 Lubberland Creek water monthly mean atomic N:P ratios (2008-2010)

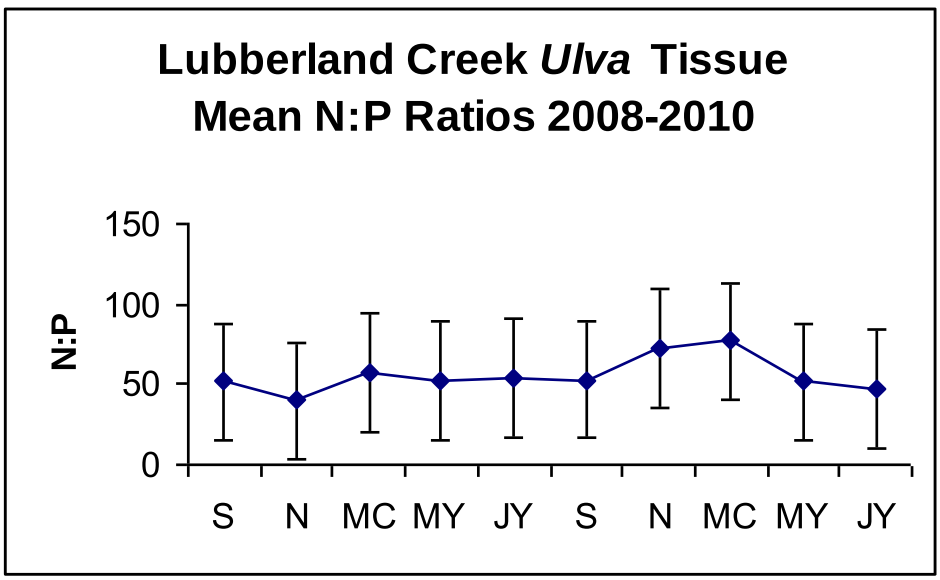

Figure 43 Lubberland Creek *Ulva* tissue monthly mean atomic N:P ratios (2008-2010)

EXHIBIT 49 (AR K.12)

Table 4 Lubberland Creek water and *Ulva* tissue monthly mean TN and TP

Lubberland Creek Monthly Mean Values 2008-2010				
	Water TN (mg/L)	**Water TP (mg/L)**	**Ulva Tissue %N**	**Ulva tissue %P**
S			3.802	0.164
N	0.506	0.047	4.397	0.237
MC	0.576	0.037	4.325	0.164
MY	1.146	0.033	4.112	0.170
JY	0.616	0.074	3.901	0.156
S	0.839	0.054	2.518	0.108
N	0.556	0.039	3.819	0.127
MC	0.982	0.030	4.985	0.144
MY	0.383	0.036	5.470	0.229
JY	0.688	0.084	2.717	0.122

Mean *Ulva* biomass was estimated each month of the study at the Wagon Hill Farm site (Figure 44). Mean biomass varied with time (P=0.00), with only one distinct peak bloom observed in May 2010 (29.8 g dry weight/m^2 +- 64.5 SD). The *Ulva* specimens on the transect lines at this site were mostly of the species *Ulva intestinalis*, and they were found almost exclusively attached to the mud on the site's upper bank. No free floating blade forming specimens were found at this site. When present, these organisms were attached to shells, fucoid algae, sticks, logs, and displaced hummocks of marsh grass. As this site is located on the Oyster River, the influence of water motion was greater than was seen at the three sites in southern Great Bay. Also, there is open water at this site throughout the majority of the winter months, but freezing of the mudflats and shoreline is common at low tide on cold days.

No *Gracilaria* specimens were found at the Wagon Hill Farm site at any time between September 2008 and July 2010.

The *Ulva* mean percent cover per month was determined at the Wagon Hill Farm site (Figure 45), and significant temporal differences were found (P=0.00). Cover expansion occurred between late fall and late spring during both years of the study at this site. The *Ulva intestinalis* population at this site flourished during the cooler months and died back over the warm summer periods. Mean *Ulva* cover was greatest in May of 2009 and 2010 (21.4% +- 31.3 SD and 16.1% +- 28.1 SD). The seasonal mean *Ulva* cover lows were recorded in July of 2009 and 2010 (2.7% +- 6.6 SD and 5.9% +- 14.0 SD).

EXHIBIT 49 (AR K.12)

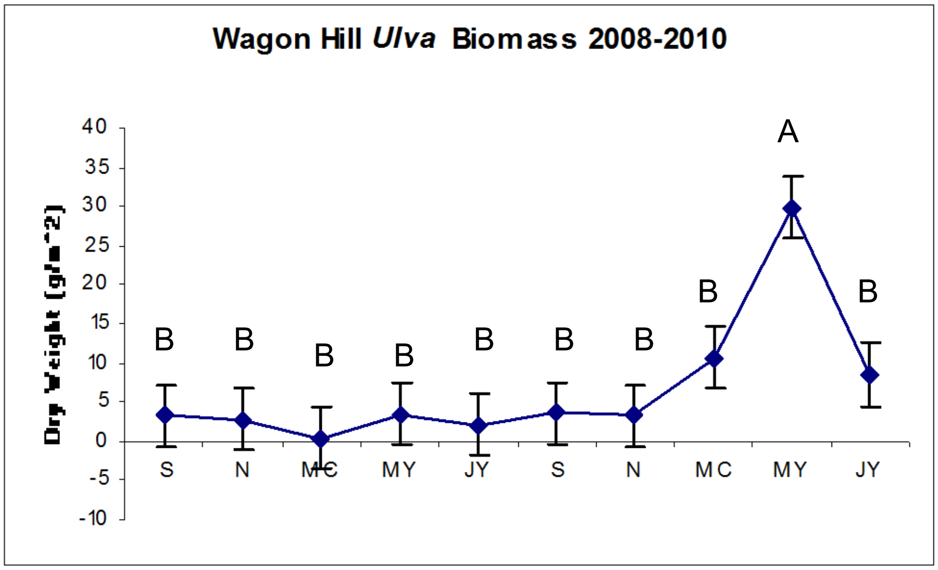

Figure 44 Wagon Hill Farm *Ulva* monthly mean biomass (2008-2010)

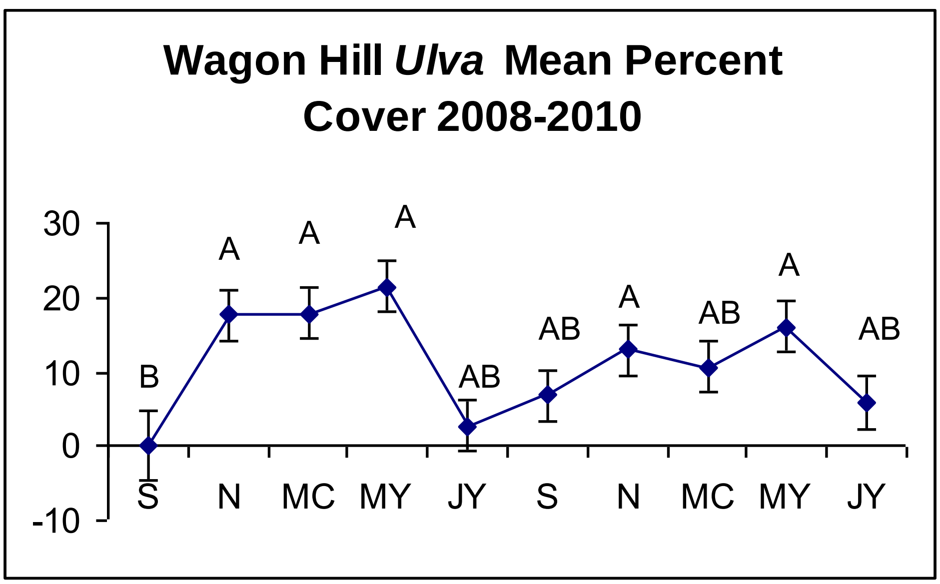

Figure 45 Wagon Hill Farm *Ulva* monthly mean percent cover (non-transformed) 2008-2010

Ulva elevational distributions were examined throughout the study at the Wagon Hill Farm site. *Ulva* distribution was concentrated at the mid-high to high elevations (P=0.01), which were approximately 1.0 and 1.5 m above mean low water. This region of the site was comprised of the lower and upper stream bank, to which the bulk of the *Ulva* specimens were attached.

Water and *Ulva* tissue from the Wagon Hill Farm site were analyzed for temporal variation in N:P ratios (Figures 46 and 47, Table 5). Significant differences (P<0.01) were found between the months using both method of analysis, but the lone peak mean in the water N:P ratio occurred in March 2010, whereas the lone peak in the tissue mean N:P ratio was recorded for November 2008 (156.0 +- 123.1 SD and 85.4 +- 15.8 SD respectively). Beyond the anomalous spikes, the trend was a stable throughout the study period in both the water and *Ulva* tissue N:P ratios. Monthly mean water N:P ratios remained between 14 +- 2.4 SD and 51 +- 4.3 SD. In the tissue analyses, the mean N:P ratios remained between 19.1 +- 2.5 SD and 38.4 +- 2.0 SD.

EXHIBIT 49 (AR K.12)

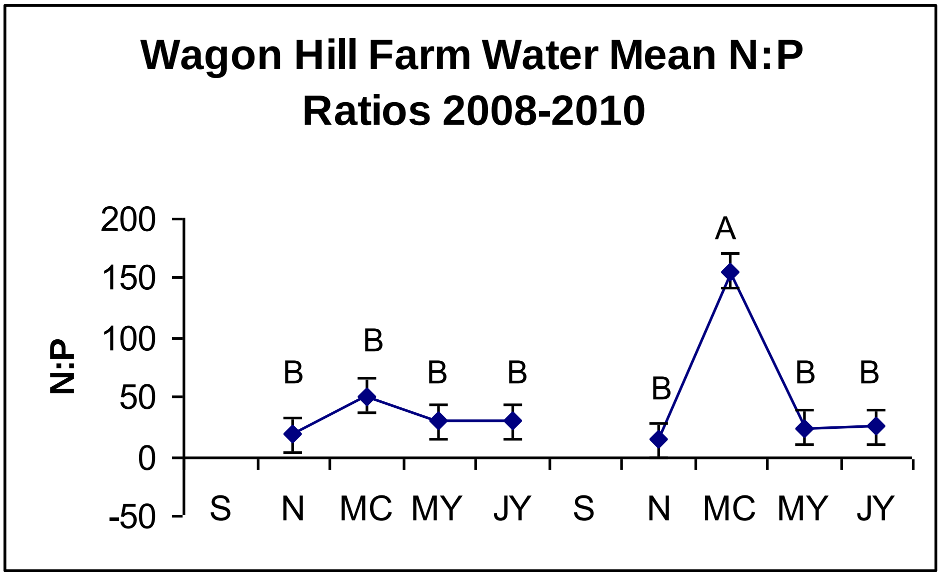

Figure 46 Wagon Hill Farm water monthly mean atomic N:P ratios (2008-2010)

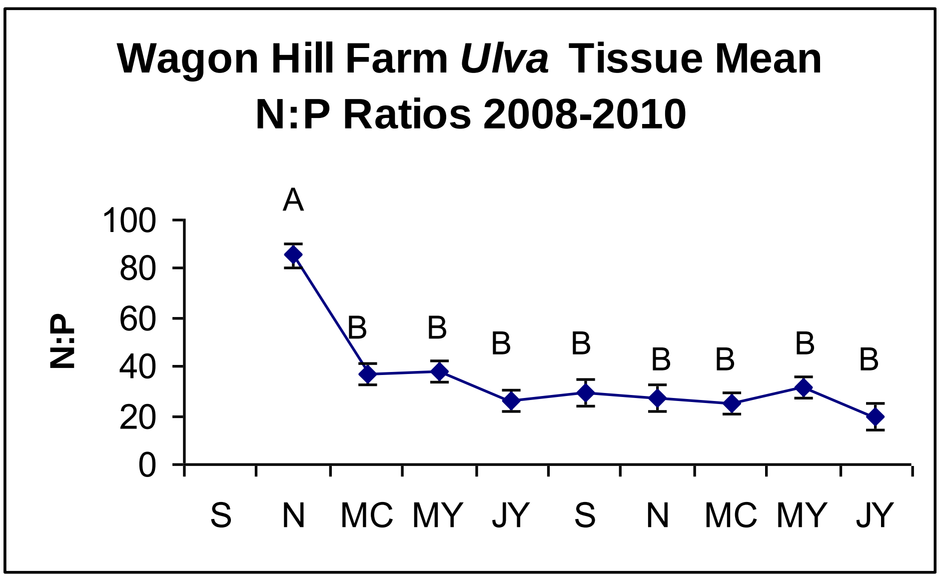

Figure 47 Wagon Hill Farm *Ulva* tissue monthly mean atomic N:P ratios (2008-2010)

EXHIBIT 49 (AR K.12)

Table 5 Wagon Hill Farm water and *Ulva* tissue monthly mean TN and TP

Wagon Hill Farm Monthly Mean Values 2008-2010				
	Water TN (mg/L)	**Water TP (mg/L)**	**Ulva Tissue %N**	**Ulva tissue %P**
S				
N	0.873	0.100	3.875	0.098
MC	0.542	0.022	2.718	0.161
MY	0.253	0.030	2.451	0.137
JY	0.378	0.030	1.498	0.122
S			1.783	0.130
N	0.329	0.051	2.057	0.160
MC	0.273	0.009	2.326	0.160
MY	0.386	0.039	2.724	0.188
JY	0.303	0.025	0.976	0.116

Mean *Ulva* biomass was recorded bi-monthly at the Cedar Point site (Figure 48). Mean biomass varied with time (P=0.00), with only one distinct peak documented in September 2009 (134.3 g dry weight/m^2 +- 330.1 SD), which consisted of some large clumps of *Ulva rigida* on lowest transect line. In most other months, *Ulva intestinalis* was the dominant *Ulva* species at this site, as it grew on the small bare rocks in the active path of the boat launch. Throughout the rest of the site, *Ascophylum nodosum* and *Fucus vesiculosus* formed the dominant cover and made up the bulk of the site's algal biomass

A few *Gracilaria* specimens were found in the drift at the Cedar Point site during the final collection in July 2010. No *Gracilaria* specimens were observed in the intertidal zone at this site at any other time during the study.

The *Ulva* mean percent cover was estimated at the Cedar Point site throughout the study (Figure 49). Significant temporal differences were found (P=0.00), which matched those observed for mean *Ulva* biomass. The greatest mean cover occurred in September of 2008 and 2009 (3.0% +- 6.0 SD and 7.3% +- 15.8 SD). These seasonal maxima levels were dwarfed by the blooms observed in southern Great Bay during this study.

EXHIBIT 49 (AR K.12)

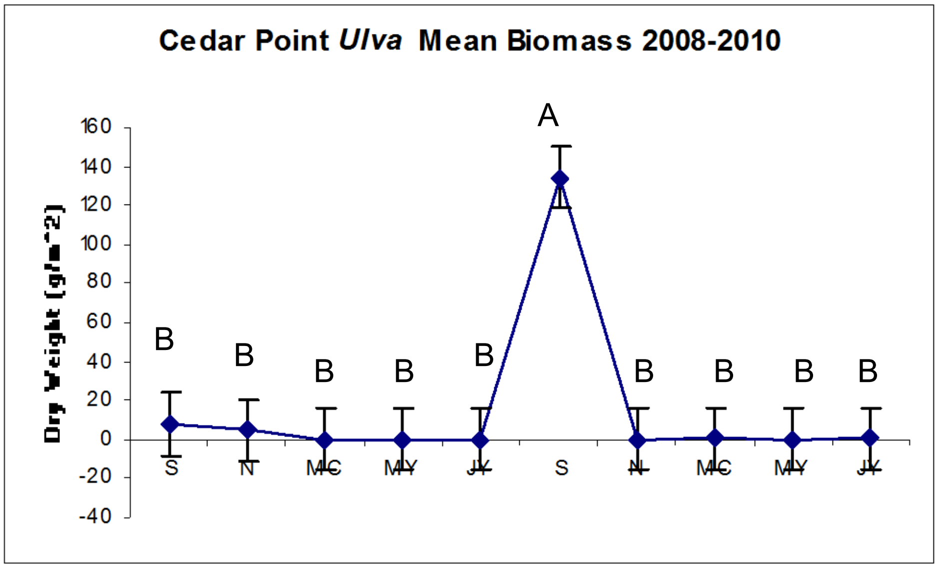

Figure 48 Cedar Point *Ulva* monthly mean biomass (2008-2010)

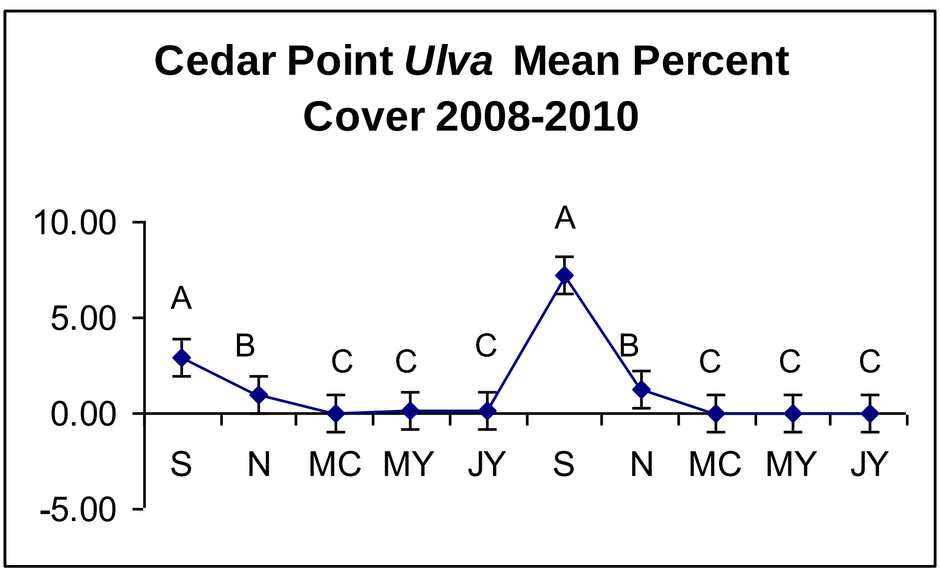

Figure 49 Cedar Point *Ulva* tissue monthly mean percent cover (non-transformed) 2008-2010

Water and *Ulva* tissue from the Cedar Point site were analyzed for seasonal variation in N:P ratios (Figures 50 and 51, Table 6). No significant differences were found between the months using either method of analysis. Other than the highs recorded in September 2008 and November 2009 (97.6 +- 112.9 SD and 146.7 +- 223.7 SD respectively), mean water N:P ratios remained between (10.8 +- 3.9 SD and 34.6 +- 11.4). The amount of dried *Ulva* tissue biomass necessary for analysis was difficult to find at Cedar Point for many months of the study. For the few months that enough material could be gathered, the N:P ratios remained between 31.7 and 94.3.

Gracilaria tissue samples collected in southern Great Bay during year one of the study were analyzed for %N and %P contents, and these results were used to calculate atomic N:P ratios. Comparisons between the tissue nutrients of *Gracilaria* and *Ulva* collected at the same sites and times suggest that *Gracilaria* tissues contained lower concentrations of nitrogen and higher concentrations of phosphorus, which led to lower N:P ratios (Table 7).

EXHIBIT 49 (AR K.12)

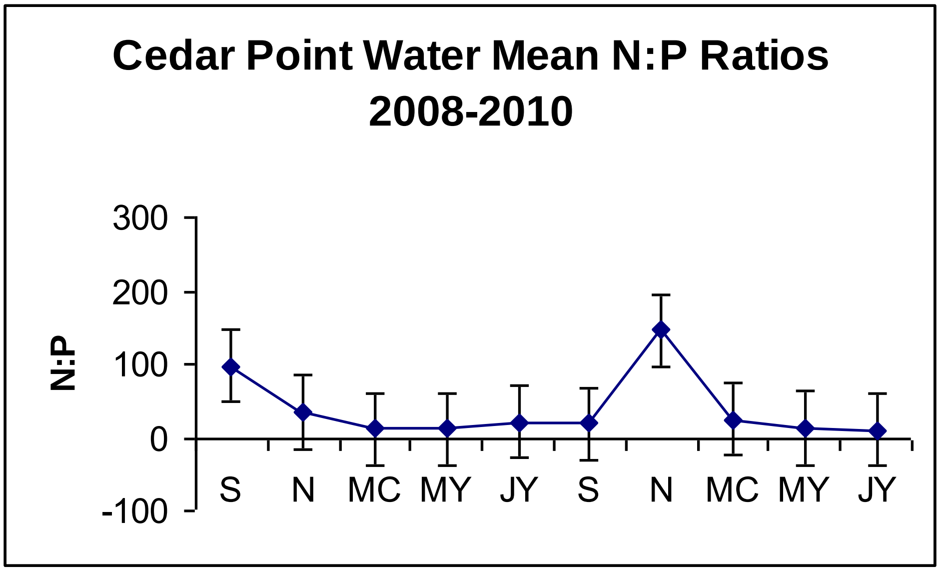

Figure 50 Cedar Point water monthly mean atomic N:P ratios (2008-2010)

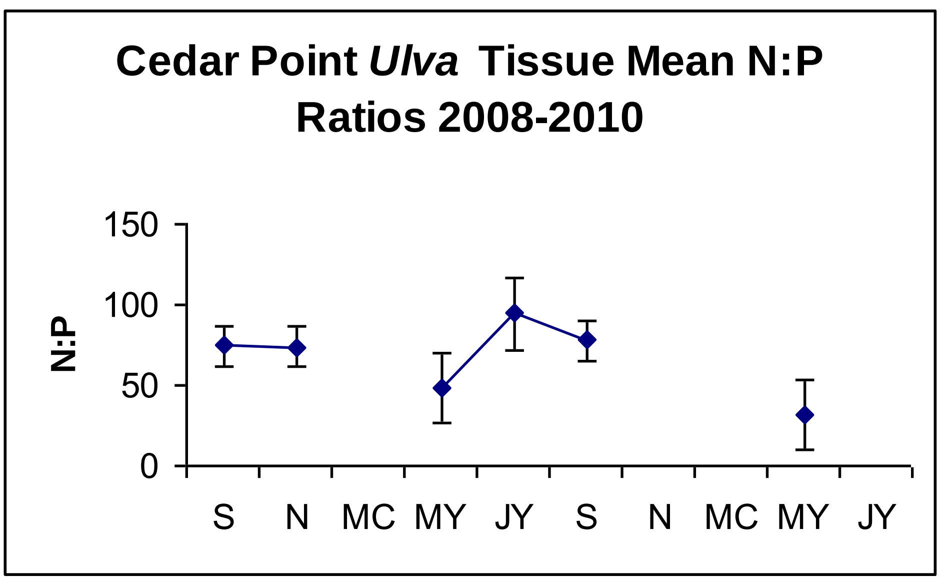

Figure 51 Cedar Point *Ulva* tissue monthly mean atomic N:P ratios (2008-2010)

Table 6 Cedar Point water and *Ulva* tissue monthly mean TN and TP

Cedar Point Monthly Mean Values 2008-2010				
	Water TN (mg/L)	Water TP (mg/L)	Ulva Tissue %N	Ulva tissue %P
S	0.435	0.023	4.258	0.125
N	0.478	0.028	3.879	0.118
MC	0.329	0.063		
MY	0.162	0.033	4.205	0.185
JY	0.247	0.025	4.980	0.113
S	0.318	0.038	3.320	0.098
N	0.467	0.033		
MC	0.372	0.037	4.302	
MY	0.335	0.057		
JY	0.184	0.039		

Table 7 Comparison of mean atomic N:P ratios, %N, and %P from analyses of *Gracilaria* and *Ulva* tissue samples from southern Great Bay (2008-2009)

***Gracilaria* tissue analyses**									
	Atomic N:P			%N			%P		
	DR	LC	SF	DR	LC	SF	DR	LC	SF
S	32.10		16.45	3.12	2.36	2.55	0.21		0.33
N	22.25	15.19	20.24	2.65	2.50	3.01	0.25	0.35	0.34
MC	39.52	25.89	32.37	2.96	2.99	3.08	0.17	0.26	0.20
MY	41.61	41.63	46.62	3.72	3.59	3.65	0.19	0.18	0.17
JY	24.35	39.24		3.28	3.73		0.29	0.20	
***Ulva* tissue analyses**									
	Atomic N:P			%N			%P		
	DR	LC	SF	DR	LC	SF	DR	LC	SF
S	49.13	51.44	34.32	4.42	3.80	3.72	0.20	0.16	0.23
N	66.21	39.76	39.90	4.50	4.40	3.86	0.15	0.24	0.21
MC	76.61	57.89	57.80	4.58	4.33	4.61	0.13	0.16	0.17
MY	40.78	51.82	47.17	3.45	4.11	3.97	0.18	0.17	0.18
JY	60.23	54.26	53.72	4.10	3.90	3.65	0.15	0.16	0.15

Discussion

The molecular verification of the presence of *Ulva rigida*, *U. pertusa* and blade forms of *U. compressa* in Great Bay dating back to the 1960s and 1970s was surprising. Due to confounding morphological plasticity of organisms in the *Ulva* genus, and the previous absence of DNA sequencing technologies, these species went undetected in Great Bay for around 40 years. In all previous ecological studies, the *U. lactuca* identity had been assigned to the distromatic blade-forming *Ulva* specimens observed in Great Bay (Reynolds, 1965; Chock and Mathieson, 1983; Hardwick-Witman and Mathieson 1983; Mathieson and Hehre, 1986; Mathieson and Penniman, 1986; West, 2001). It is likely that historically reported *U. lactuca* biomass and cover statistics actually represent values for multiple *Ulva* species combined. It is also possible that, in some instances, *U. lactuca* was not present when such measurements were taken.

The difficulty in distinguishing distromatic blade-forming *Ulva* species persists today (Blomster et al., 1999; Malta et al., 1999; Tan et al., 1999; Hoffman et al., 2010). To ensure certainty in percent cover and biomass estimates by species, an exhaustive, and very costly amount of molecular analysis would be needed, which was beyond the scope of the current study. As a result, current biomass and cover data have been lumped under the heading of *Ulva* for comparison to the historical figures, which likely also represented suites of *Ulva* species. Because the recently discovered *Ulva* species have been in Great Bay since the time of the historical studies, the increases in blooms observed in this study cannot be attributed to species introductions. In *Gracilaria* figures, the story is different.

The introduction of *G. vermiculophylla*, an Asian species known to be harmfully invasive in other regions of the world (Freshwater et al., 2006; Thomsen et al. 2007), appears to have occurred within the last decade, with the oldest known specimen for the region dating to a 2003 collection from Dover Point. This is the northernmost record of the species in the Northwestern Atlantic, with the nearest known population more than 100 miles to the south in Rhode Island. Screening of *G. tikvahiae* labeled specimens collected in Great Bay between 2002 and 1967 revealed only the native species, which strongly suggests that any historical *G. tikvahiae* biomass, cover, and tissue nutrient data are truly measures for that species.

Although *G. vermiculophylla* and *G. tikvahiae* can be differentiated using traditional morphological techniques, the high degree of morphological plasticity in these organisms makes these methods unreliable for the bulk of specimens collected in the field (Thomsen et al. 2007). This problem is compounded at sites that are known to support both species, which is the case for the three southern Great Bay sites observed in this study. Because of the cost and effort mentioned previously with *Ulva*, only a small subset of *Gracilaria* specimens collected in this study were screened for molecular identification, and all metrics for the two species were combined under the heading *Gracilaria*. Since more than half of the specimens screened in the current study were *G. vermiculophylla*, increases in *Gracilaria* biomass and cover since the baseline studies are certainly influenced by the presence of the newly introduced species, which has been shown to grow rapidly and has become a nuisance in other parts of the world (Freshwater et al., 2006; Thomsen et al. 2007),. Of course, increases in *Gracilaria* abundance may also represent changes brought about by abiotic factors such as warming and the increased availability of nutrients.

Over the course of the two year study, *Ulva* biomass, which was a combination of the biomass of *Ulva compressa*, *U. rigida*, and *U. intestinalis*, was greater in the southern Great Bay study sites (Lubberland Creek, Depot Road, and Sunset Farm), with means between 75 and 140 g dry weight/m^2. This same trend was seen in *Ulva* cover, with mean values of the southern sites between 20% and 40% for the duration of the study. Because the *Ulva* observed in this study were mostly free-floating (not attached to the substratum by a holdfast), the southern sites (Sunset Farm, Depot Road, and Lubberland Creek), with less energetic hydrodynamics, provided better protection for these organisms and allowed for longer residence times than were possible at the more energetic northern sites. If the organisms were physically held in place in the southern sites, it was often by partial burial in the sediments. At the northern sites (Cedar Point and Wagon Hill Farm), nearly all *Ulva* specimens were attached by holdfasts to sticks, shells, stones, or other algal species. Presumably, unattached specimens would have been routinely flushed from these sites. These hydrodynamic differences between the northern and southern sites are likely a large factor in the abundance differences observed, given the nutrient and temperature regimes were similar in both areas.

Ulva mean biomass peaked in the fall of both 2008 and 2009 with values significantly greater than were seen in the spring and summer. The peak bloom for the study occurred in November 2008 with mean biomass values greater than 225 g dry weight/ m^2 and cover greater than 38% when all sites were combined. This peak is well above the maximum historical measures for intertidal *Ulva* from any one site including Reynolds's (1971) October, 1967 max of 124 g dry weight/ m^2 (converted from damp/dry weight per 557 in^2) at Dover Point, Hardwick-Witman and Mathieson's (1983) fall 1979 max of < 1% cover at Lubberland Creek and 0% at Wagon Hill Farm, Chock and Mathieson's (1983) November 1972 max of 60 g dry weight/ m^2 at Cedar Point, West's (2001) November 1998 max of 41.7 g dry weight/ m^2, or Hardwick-Witman's (unpublished) September 1978 max cover of 0.6 % at Brackett's Point (southern estuary site between Depot Road and Sunset Farm).

Gracilaria (a combination of the native *Gracilaria tikvahiae* and the recently introduced *G. vermiculophylla*) biomass and percent cover, were tracked at all five sites during the two year study. *Gracilaria* was all but absent at the northern two sites, but was found throughout the year at the three southern study sites with mean biomass and cover values highest at the Depot Road and Sunset Farm study sites (over 70 g dry weight/ m^2 and over 12% average over the entire study period). These values far exceeded even the single month maxima values observed in the above studies (max biomass and cover in historical studies never exceeded 1 g dry weight/ m^2 or 1% cover per m^2).

Again, the bulk of the *Gracilaria* specimens observed in this study were unattached and held in residence at a given site only by means partial burial in the mud coupled with low site hydrodynamics. The temperature and nutrient regimes of the northern sites appear to be suitable to support *Gracilaria* growth, but growth may be restricted by the limited suitable substrata for attachment, coupled with the more energetic water motion at these sites.

In the southern bay, there was an inverse relationship between the prevalence of *Ulva* and *Gracilaria*. Lubberland Creek had significantly higher mean *Ulva* biomass and percent cover than Depot Road and Sunset Farm, whereas Lubberland Creek had significantly lower *Gracilaria* biomass and percent cover than the other two sites. This is likely a function of *Ulva* overgrowth that was seen at Lubberland Creek in the fall of 2008. Due its large bladed morphology, *Ulva* it can easily shade out other species, such as *Gracilaria* in major bloom events. The physical effects of 90% *Ulva* cover observed at the Lubberland Creek site in November 2008 could have caused a decrease in the *Gracilaria* bloom at that site at that time. Lower growth at this critical time can have carry-over effects in subsequent years, as spring and summer populations build from the individuals that survive the long winter months of snow and ice cover.

Gracilaria monthly mean biomass and cover trends in the southern bay followed those seen in *Ulva*, with peaks observed in November 2008 and 2009. The mean cover and biomass across the three southern sites exceed 40% and 250 g dry weight/ m^2 in November 2008. Again, these values far exceeded any single site *Gracilaria* maxima recorded by Harwick-Witman and Mathieson (1983) or Hardwick-Witman (unpublished, 1978), and further demonstrates that nuisance algal species growth has increased markedly in the Great Bay Estuarine System since the time of the baseline studies.

Mean total nitrogen and mean total phosphorus were recorded for the water and *Ulva* tissues from each collection site and time during the two year study. Significant between site differences were observed using the water nitrogen analysis, which revealed that Wagon Hill Farm had lower TN than Sunset Farm when values were averaged across the entire study time. This trend was also revealed in the tissue nitrogen analysis, but that difference was not significant. In measures of TP, water analysis revealed that Sunset Farm had significantly higher mean values than were seen at either of the northern sites, Wagon Hill Farm and Cedar Point. Tissue tests of TP only revealed that Cedar Point

Ulva had slightly lower levels than were found at any other study site. Atomic N:P ratios generated from both water and tissue testing revealed no significant differences between site or nutrient evaluation method. However, the mean N:P ratios were generally higher in the tissues than in the water column, which is likely due to the tendency of *Ulva* to preferentially sequester nitrogen at times of availability (Hanisak, 1983).

Monthly mean nitrogen and phosphorus were averaged among the sites over the course of the study period using both the water and *Ulva* tissue analyses. Neither method revealed any significant temporal differences in TN, TP, or atomic N:P ratios. Mean water TN remained between 0.4 and 0.7 mg/L, or above 25 µM, throughout the study sites. This value is more than twice the 10 µM nitrogen concentrations observed by Short (1992). The mean tissue TN remained between 2.3 and 4.1%, which is above the 2.2% required for unlimited growth in *Ulva lactuca* (Pedersen et al. 1997), which has been considered to be representative of the genera (Hernandez et al. 2005). Mean water TP was between 0.028 and 0.07 mg/L, or slightly higher than 0.9 µM , which was the mean value found by Short (1992), and tissue P percentages were between 0.13 to 0.18%, which were well above the 0.03% minimum growth requirement for *Ulva rigida* (Villares and Carballeira 2004).

Atomic N:P ratios of water were always above the Redfield Ratio of 16:1, with water values ranging from 26.2:1 to 69.4:1, much higher than the NOAA 1989 values for Great Bay which were 7:1 (Short, 1992). The current high N:P ratios indicate that growth is certainly not nitrogen limited, even at the times of heaviest algal blooms. The *Ulva* tissue atomic N:P ratios from 38.5:1 to 61:1 and the *Gracilaria* atomic N:P rations from 22:1 to 41:1 further indicate these organisms are not nitrogen limited, even at peak bloom.

According to Björnsäter & Wheeler's (1990) assessment, tissues with N:P ratios of greater than 16:1 indicate nitrogen limitation, 16:1< N:P < 24:1 indicate sufficient nitrogen and phosphorus for continued growth, and N:P > 24:1 indicate phosphorus limitation. Although the *Ulva* tissue N:P ratios observed in Great Bay were greater than 24:1, I would be reluctant to classify the organisms as phosphorus limited given that tissue phosphorus percentages were far above those needed for growth, and given the fact that bloom events seemed limited not by nutrient availability, but rather by the seasonal effects of diminishing daylight hours, decreased temperature, and, in the southern bay, winter over-icing.

Although concentrations of nitrogen have increased dramatically since the baseline studies, the tissue concentrations in *Gracilaria* specimens have remained relatively stable. Penniman (1983) measured the percent of nitrogen and phosphorus in *Gracilaria tikvahiae* specimens collected subtidally near Nannie Island (close to Sunset Farm). The tissue nitrogen values in 1976 and 1977 ranged from 2% to 4.5%, and the phosphorus values ranged from 0.18% to 0.35%, compared to the ranges of 2.5% to 3.6% (TN) and 0.17% to 0.33% (TP) observed in the current study. Such stability in the face of widely increasing nutrient availability could be indicative of a preferred steady state for these organisms. Because *Gracilaria* can grow very rapidly, it is likely that excess available nutrients are directly converted into increased biomass production. The thalli, or the populations grow via nutrient uptake, but the overall tissue nutrient concentrations remain unchanged.

In comparing the methods of estimating nutrient regime by site and time, this study found water testing to be slightly superior in its ability to reveal significant differences, though neither method revealed many such differences. One major advantage with water testing is that water is obviously always available at a study site. Its presence does not fluctuate with the seasons, as does that of ephemeral algal species. Although water nutrient concentrations have been shown to fluctuate dramatically over short periods of time (Loder et al., 1983), this was not observed in the Great Bay monthly mean values estimated in this study. It was expected that the tissue values would be significantly more stable over time, but this was not the case. Furthermore, acquiring adequate amounts of dried *Ulva* tissue (at least 1.2 g dry weight) at each site and collection time proved an impossibility, which led to smaller sample sizes and fewer nutrient measurements than was desired. For future marine studies, which aim to measure nutrient regimes across various sites over time, I would recommend researchers not rely solely on algal tissues for these analyses, and if funding were to allow for only one approach, I would recommend water nutrient analyses. But both methods are valuable, for, with both data sets, comparisons can be made to a wider range of ecological studies.

Analysis of the monthly cover of all seaweeds within each site revealed peak *Ulva* and *Gracilaria* blooms of unprecedented sizes. The fall 2008 and 2009 *Ulva* blooms in southern Great Bay dwarfed those observed in previous regional studies. In both biomass and cover, the increase was substantial. Lubberland Creek's peak *Ulva* cover of more than 90 times greater than that observed for the same site by Harwick-Witman and Mathieson (1983), while the *Ulva* cover at Depot Road (55%) and Sunset Farm (59%)

were far greater than the maximum (<1%) observed at Brackett's Point (between the sites) in 1978 (Hardwick-Witman, unpublished). The *Gracilaria* abundance increases were similarly staggering with Lubberland Creek's cover exceeding 10%, which was more than ten times the maxima observed by any previous intertidal study in Great Bay. At Depot Road and Sunset Farm, the cover values were 44% and 39%, which dwarfed the less than 1% *Gracilaria* cover observed at both Brackett's Point and Lubberland Creek in the previously mentioned studies (Harwick-Witman and Mathieson, 1983; Harwick-Witman, unpublished). While the *Ulva* and *Gracilaria* peaks the following fall were smaller in general, the abundance values still eclipsed those measured in previous studies.

In the northern study sites, *Ulva* abundance changes since the baseline studies were less pronounced. The biomass of *Ulva* at Wagon Hill Farm did not exhibit fall peaks, but instead the biomass remained below 5 g dry weight/ m^2 throughout all but the last three months of the study. *Ulva* cover estimates at the site in all but the first month were between 2% and 21% and were always higher than the < 1% observed by Harwick-Witman and Mathieson (1983).

Ulva biomass trends at Cedar Point were similar, with low baseline values of around 5 g dry weight/ m^2 throughout the study, with the exception of the spike in September 2009 of over 130 g dry weight/ m^2, which was higher than the max 124 g dry weight/ m^2 (converted from damp/dry weight per 557 in^2) Reynold's (1971) October, 1967 observation at Cedar Point, and Chock and Mathieson's (1983) November 1972 max of 60 g dry weight/ m^2 at Cedar Point, and West's (2001) November 1998 max of 41.7 g dry weight/ m^2 at Dover Point. Although this anomalous spike was larger than the values observed at max in the previous studies, this peak should probably be dismissed. The bulk of the *Ulva* measured at Cedar Point in September 2009 was drift algal that had been recently deposited in the lower intertidal zone and likely washed away with the subsequent tides. However, drift algal is often deposited close to its source, which leads one to wonder about the subtidal density of *Ulva* near the Cedar Point site.

In summary, one recently introduced and potentially invasive species, *Gracilaria vermiculophylla* was discovered in Great Bay. Three previously undetected distromatic blade-forming *Ulva* species,*U. rigida*, *U. pertussa* , and *U. compressa*, have been identified as having been in the bay since 1966, 1967, and 1972, respectively, and have likely been included in subsequent Great Bay ecological studies under the category '*Ulva lactuca*.' Great increases in both mean and peak *Ulva* and *Gracilaria* biomass and percent cover have occurred in the Great Bay Estuarine System. These changes coincide with increases in water nitrogen levels observed over the past two decades. The increases in nuisance algal blooms are likely the result of increased nutrient loading in the bay, and, in the case of *Gracilaria vermiculophylla*, may also be a symptom of a harmful invasion. Current nitrogen levels in the system are substantial enough to support even larger *Ulva* and *Gracilaria* blooms than were observed in this study, based on minimum growth requirements. If efforts are not made to reduce nutrient inputs, such harmful algal blooms, and their related side effects of hypoxia and habitat alteration, should be expected in the Great Bay Estuarine System for the foreseeable future.

EXHIBIT 49 (AR K.12)

Acknowledgments

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Literature Cited

Björnsäter BR, Wheeler PA. 1990. Effect of nitrogen and phosphorus supply on growth and tissue composition of *Ulva fenestrata* and *Enteromorpha intestinalis* (Ulvales, Chlorophyta). J. Phycol. 26: 603–611.

Blomster, J., C.A. Maggs, and M.J. Stanhope 1999. Extensive intraspecific morphological variation in Enteromorpha muscoides (Chlorophyta) revealed in molecular analysis. J. Phycol., 35: 575–586.

Burdick, D. M., R. Grizzle, A. C. Mathieson and L. G. Ward. 2006. Restoration of South Mill Pond, Portsmouth, New Hampshire. Jackson Estuarine Laboratory Report.

Chock, J. S. and A. C. Mathieson. 1976. Ecological studies of the salt marsh ecad *scorpiodes* (Hornemann) Hauck of *Ascophyllum nodosum* (L.) Le Jolis. J. Exp. Mar. Biol. Ecol. 23: 171-190.

Chock, J. S. and A. C. Mathieson. 1983. Variations of New England estuarine seaweed biomass. Bot. Mar. 26: 87-97.

Cotton, A. D. 1910. On the growth of *Ulva latissima* in water polluted by sewage. Bull. Misc. Inform. Roy. Bot. Gard. Kew. pp. 15-19.

Diaz, P., J. J. Lopez Gappa, and M. L. Piriz. 2002. Symptoms of eutrophication in intertidal macroalgal assemblages of Nuevo Gulf (Patagonia Argentina). Bot. Mar. 45: 267-273.

Fletcher, R. L. 1996. The occurrence of 'green tides'- a review, pp. 1-43. In; W. Schramm and P. H. Nienhuis, eds., Ecological Studies, Vol. 123, Marine Benthic Vegetation: Recent Changes and the Effects of Eutrophication, Springer-Verlag, Berlin.

Freshwater, D.W, F. Montgomery, J.K. Greene, R.M. Hamner, M. Williams, and P. E. Whitfield (2006) Distribution and identification of an invasive Gracilaria species that is hampering commercial fishing operations in southeastern North Carolina, USA. Biological Invasions 8:631-637.

Hanisak M.D. (1983) Nitrogen relationships of marine macroalgae. In E.J. Carpenter and D.G. Capone. Nitrogen in the Marine Environment. Academic Press. NY, NY 699-730.

Hardwick-Witman, M. N. and A. C. Mathieson. 1983. Intertidal macro-algae and macro-invertebrates: seasonal and spatial abundance patterns along an estuarine gradient. Est. Coast. And Shelf Sci. 16: 113-129.

Hernandez I, MA Fernandez-Engo, JL Perez-Llorens, JJ Vergara. 2005. Integrated outdoor culture of two estuarine macroalgae as biofilters for dissolved nutrients from *Sparus aurata*. J Appl Phycol; 17:557-67.

Hofmann, L.C., J.C. Nettleton, C.D. Neefus, and A.C. Mathieson. 2010. Cryptic diversity of *Ulva* (Ulvales, Chlorophyta) in the Great Bay Estuarine System (Atlantic USA): introduced and indigenous distromatic species. Eur. J. Phycol. 45(3): 230 239.

Horneck, D.A. and R.O. Miller. 1998. Determination of total nitrogen in plant tissue. In Y.P. Kalra (ed.) Handbook and Reference Methods for Plant Analysis. CRC Press, New York.

Jerome, W.C., A. P. Chesmore, C. O. Anderson, J. and F. Grice. 1965. A study of marine resources of the Merrimack river Estuary. Monograph Ser. No. 1, Div. Mar. Fish., Dept. of Natural Res., The Comm. of Mass.

Jones, S. H. 2000. A technical characterization of estuarine and coastal New Hampshire. New Hampshire Estuaries Project, Portsmouth, NH, 273 pp.

Kindig, A.C., M.M. Littler. 1980. Growth and primary productivity of marine macrophytes exposed to domestic sewage effluents. Mar. Environ. Res. 3:81-100.

Kinne, O. 1970. Temperature, pp. 407-514. In: O. Kinne ed., Marine Ecology, Vol. 1, Part 1. Wiley-Interscience, New York.

Loder, T.C., J.A. Love, J.P. Kim, and C.G. Wheat. 1983. Nutrient and hydrographic data for the Great Bay Estuarine System, New Hampshire- Maine, Part II, January 1976- June 1978. UNH Marine Program Publication. University of New Hampshire, Durham. 149 pp.

Lourenço, S.O., E. Barbarino, A. Nascimento, J.N.P. Freitas, and G.S. Diniz. 2006. Tissue Nitrogen and phosphorus in seaweeds in a tropical eutrophic environment: What a long-term study tells us. Journal of Applied Phycology (18): 389-398.

Lüning, K. 1984. Temperature tolerance and biogeography of seaweeds: the marine algal flora of Helgoland (North Sea) as an example. Helgol. Wiss. Meeresunters. 38: 305-317.

Lüning, K. 1990. Seaweeds Their Environment, Biogeography, and Ecophysiology. John Wiley and Sons, Inc., New York.

Malta, E.J., S.G.A. Draisma, & P. Kamermans. 1999. Free-floating Ulva in the southwest Netherlands: species or morphotypes? A morphological, molecular and ecological comparison. Eur. J. Phycol., 34: 443–454.

Mathieson, A. C., and E. J. Hehre. 1986. A synopsis of New Hampshire seaweeds. Rhodora 88: 1-139.

Mathieson, A. C., and C. A. Penniman. 1986. Species composition and seasonality of New England seaweeds along and open coastal-estuarine gradient. Bot. Mar. 29: 161-176.

Mathieson, A. C., and C. A. Penniman. 1991. Floristic patterns and numerical classification of New England estuarine and open coastal seaweed populations. Nova Hedwigia 52: 453-485.

Mathieson, A. C., and R. A. Fralick. 1973. Benthic algae and vascular plants of the lower Merrimack River and adjacent shoreline. Rhodora 75: 52-64.

Miller, B., and D. Normandeau, G. Piehler, P. Hall, A. Mathieson, F. Fralick, D. Turgeon, P. Mahoney, and W. Owen. 1971. Ecological study of Merrimack River Estuary- Massachusetts, presented to the U. S. Army, Corps of Engineers by Normandeau Associates, Inc. and Viast Inc.

Miller, Robert O. 1998. High-Temperature Oxidation: Dry Ashing. In Y.P. Kalra (ed.) Handbook and Reference Methods for Plant Analysis. CRC Press, New York.

Pedersen M F & J. Borum 1997. Nutrient control of estuarine macroalgae: growth strategy and the balance between nitrogen requirements and uptake. Mar Ecol Prog Ser161: 155-63.

Penniman C.A. (1983) Ecology of *Gracilaria tikvahiae* McLachlan (Gigartinales, Rhodophyta) in the Great Bay Estuary. University of New Hampshire. 267 pp.

Pe'eri, S., J. R. Morrison, F. Short, A. Mathieson, A. Brook, and P. Trowbridge. 2008. Macroalgae and eelgrass mapping in Great Bay Estuary using AISA hyperspectral imaging. Final Report to the New Hampshire Estuaries Project.

Rafaelli, D., J. A. Raven, and L. J. Poole. 1998. Ecological impact of mass blooms of benthic algae. Mar. Biol. Oceanogr. Ann. Rev. 36: 97-125.

Raven, J. A., and R. Taylor. 2003. Macroalgal growth in nutrient-enriched estuaries: a biogeochemical and evolutionary perspective. Water, Air, and Soil Pollution 3: 7 26.

Sawyer, C. M. 1965. The sea lettuce problem in Boston Harbor. J. Water Poll. Cont. Fed. 37: 1122-1133.

Schramm, W. P. H. Nienhuis (editors). 1996. Ecological Studies, Vol. 123, Marine Benthic Vegetation: Recent Changes and the Effects of Eutrophication, Springer Verlag, Berlin.

Short, F.T. (1992) The ecology of the Great Bay Estuary, New Hampshire and Maine: An estuarine profile and bibliography. The Jackson Estuarine Laboratory, University of New Hampshire. pp 221.

Tan, I.H., Blomster, J., Hansen, G., Leskinen, E., Maggs, C.A., Mann, D.G., Sluiman, H.J. & Stanhope, M.J. (1999). Molecular phylogenetic evidence for a reversible morphogenetic switch controlling the gross morphology of two common genera of green seaweeds, Ulva and Enteromorpha. Mol. Biol. Evol., 16: 1011–1018.

Thomsen, M.S., P.A. Staehr, C.D. Nyberg, S. Schwaerter, D. Krausse-Jensen, B.R. Silliman (2007) *Gracilaria vermiculophylla* (Ohmi) Papenfuss, 1967 (Rhodophyta, Gracilariaceae) in northern Europe, with emphasis on Danish conditions, and what to expect in the future. Aquatic Invasions 2(2):83-94.

Villares R, A Carballeira (2004) Nutrient limitation in Macroalgae (Ulva and Enteromorpha) from the Rias Baixas (NW Spain). Mar Ecol 25(3): 225-43.

West A.L (2001) Molecular and ecological studies of New Hampshire species of *Porphyra* (Rhodophyta, Bangiales). Univeristy of New Hampshire Thesis.

Zar, J. H (1998) Biostatistical Analysis. 4 Edition. Prentice Hall. 929 pp.

EXHIBIT 49 (AR K.12)

Index I- Biomass Data (g dry weight/m2) +-SD, n=40

Cedar Point Biomass (g dry weight/m^2) 2008-2010

	Ulva	*Gracilaria*	*Polysiphonia stricta*	*Ascophyllum nodosum*	*Fucus vesiculosus*	*Chondrus crispus*	*Ulva intestinalis*	*Zostera marina*
S	7.93+-37.1	0+-0	0.05+-0.22	1187.03+-2165.1	66.85+-231.2	0+-0	0+-0	2.88+-6.85
N	5.03+-11.15	0+-0	2.23+-7.57	3522.5+-4658.09	23.28+-100.87	0+-0	0+-0	2.85+-13.58
MC	0.003+-0.016	0+-0	0.003+-0.016	114.03+-70.43	1.97+-7.35	0+-0	0+-0	0.59+-3.62
MY	0.07+-0.24	0+-0	0.13+-0.37	65.0+-123.29	20.01+-100.91	0.03+-0.16	0.03+-0.21	0+-0
JY	0+-0	0.05+-0.12	0.13+-0.51	118.06+-122.44	1.92+-0.51	0.08+-0.51	0+-0	0.12+-0.22
S	134.3+-330.1	0+-0	1.5+-4.46	407.34+-546.6	60.71+-159.87	8.4+-38.42	0+-0	3.47+-6.28
N	0.19+-1.15	0+-0	0.04+-0.24	1971.4+-6588.2	22.42+-79.36	0+-0	0+-0	0.05+-0.33
MC	0.49+-2.39	0+-0	0.01+-0.02	1463.7+-1814.5	48.7+-161.2	0+-0	0+-0	0+-0
MY	0.34+-2.17	0+-0	0.06+-0.19	678.6+-1189.39	75.86+-183.5	0+-0	0+-0	0.3+-0.9
JY	0.49+-1.95	0.05+-0.3	0.03+-0.09	896.3+-996.6	20.2+-68.7	0.012+-0.08	0+-0	0.9+-2.4

EXHIBIT 49 (AR K.12)

Wagon Hill Farm Biomass (g dry weight/m^2) 2008-2010

	Ulva	*Gracilaria*	*Ascophyllum nodosum*	*Fucus vesiculosus*	*Ahnfeltia plicata*	*Ulva intestinalis*	*Zostera marina*
S	3.25+-20.6	0+-0	0+-0	0+-0	0+-0	0+-0	0+-0
N	2.75+-5.9	0+-0	352.9+-1520.8	216.2+-718.9	0+-0	0.35+-1.1	0+-0
MC	0+-0	0+-0	0+-0	0+-0	0+-0	0.39+-0.9	0+-0
MY	0.013+-0.06	0+-0	2.6+-15.3	2.97+-14.05	0+-0	3.5+-7.3	0.07+-0.25
JY	0+-0	0+-0	7.9+-27.1	6.9+-17.98	0+-0	2.14+-5.8	0+-0
S	0.05+-0.32	0+-0	52.4+-329.3	23.9+-140.2	0+-0	3.6+-18.8	0+-0
N	0.72+-3.1	0+-0	45.17+-285.7	141.7+-841.5	0+-0	2.5+-9.2	1.31+-6.6
MC	0.5+-1.6	0+-0	0+-0	15.7+-71.1	0.05+-0.32	10.18+-26.2	0+-0
MY	6.04+-23.3	0+-0	5.7+-34.6	15.1+-94.7	0+-0	23.8+-57.9	0+-0
JY	5..2+-17.9	0+-0	125.5+-366.7	17.3+-83.1	0+-0	3.2+-18.8	0+-0

EXHIBIT 49 (AR K.12)

Lubberland Creek Biomass (g dry weight/m^2) 2008-2010

	Ulva	*Gracilaria*	*Polysiphonia stricta*	*Ascophyllum nodosum*	*Fucus vesiculosus*	*Ulva intestinalis*	*Zostera marina*
S	260.4+-608.8	28.4+-133.1	0+-0	0+-0	0+-0	0+-0	1.08+-5.7
N	733.8+-613.0	41.7+-79.4	0+-0	0+-0	241.1+-1524.5	0+-0	2.7+-3.8
MC	4.5+-4.7	0.84+-2.5	0+-0	1.4+-8.6	1.8+-5.9	0+-0	1.2+-3.0
MY	4.3+-7.2	0.43+-1.7	0+-0	0+-0	3.7+-11.7	0.03+-0.19	0.13+-0.5
JY	1.7+-3.0	0.19+-0.66	0+-0	0+-0	2.5+-9.8	0+-0	0.29+-1.8
S	98.76+-180.8	28.5+-88.5	0+-0	0+-0	0+-0	0+-0	3.2+-8.6
N	175.8+-211.5	55.85+-110.9	0.35+-1.16	6.2+-39.0	0+-0	0+-0	4.18+-9.2
MC	12.4+-23.3	5.7+-25.7	0+-0	0+-0	0+-0	0+-0	22.2+-51.4
MY	12.2+-21.5	0.12+-0.48	0+-0	0+-0	0+-0	0+-0	0.01+-0.04
JY	24.16+-34.0	0.47+-0.93	0+-0	0+-0	0+-0	0+-0	0.18+-0.3

EXHIBIT 49 (AR K.12)

Depot Road Biomass (g dry weight/m^2) 2008-2010

	Ulva	*Gracilaria*	*Polysiphonia stricta*	*Ascophyllum nodosum*	*Fucus vesiculosus*	*Ceramium rubrum*	*Ahnfeltia plicata*	*Ulva intestinalis*	*Zostera marina*
S	144.8+-266.5	191.6+-833.1	2.5+-7.4	1.6+-10.3	15.9+-78.9	0.15+-0.58	0+-0	0+-0	4.4+-6.3
N	170+-245.8	431.1+-774.2	0+-0	0+-0	0+-0	0.28+-1.01	0+-0	0+-0	4.4+-7.2
MC	5.35+-7.7	6.3+-11.3	0+-0	0.6+-4.1	0.12+-0.76		0.01+-0.02	0+-0	1.8+-3.5
MY	2.8+-5.7	1.5+-5.5	0+-0	0+-0	0+-0	0+-0	0+-0	0+-0	0.01+-0.05
JY	1.76+-4.7	0.06+-0.14	0.01+-0.03	0+-0	0+-0	0+-0	0+-0	0+-0	0.05+-0.18
S	180.98+-391.5	158.8+-383.0	0+-0	0+-0	0+-0	0+-0	0+-0	1.15+-4.2	26.4+-111.5
N	272.8+-443.0	38.4+-93.1	0.2+-1.2	0+-0	0.03+-0.16	0+-0	0+-0	0+-0	8.9+-12.0
MC	0+-0	0+-0	0+-0	0+-0	0+-0	0+-0	0+-0	0+-0	0+-0
MY	6.6+-38.0	0+-0	0+-0	0+-0	0+-0	0+-0	0+-0	0+-0	0.1+-0.6
JY	11.3+-41.0	0.23+-1.0	0+-0	0+-0	0+-0	0+-0	0+-0	0.6+-4.1	0.16+-0.8

EXHIBIT 49 (AR K.12)

Sunset Farm Biomass (g dry weight/m^2) 2008-2010

	Ulva	*Gracilaria*	*Polysiphonia stricta*	*Ascophyllum nodosum*	*Fucus vesiculosus*	*Chondrus crispus*	*Zostera marina*
S	547.8+-802.1	115.3+-266.2	0+-0	0+-0	0+-0	0+-0	0.9+-1.7
N	225.6+-377	264.8+-391.9	0+-0	24.0+-151.8	0+-0	0+-0	1.85+-2.7
MC	1.3+-2.5	2.0+-4.1	0+-0	0.2+-0.9	0+-0	0+-0	0.2+-0.5
MY	2.1+-3.8	0.7+-2.5	0.01+-0.02	0+-0	0+-0	0+-0	0+-0
JY	1.9+-4.2	2.1+-5.5	0.01+-0.02	0+-0	0+-0	0+-0	0+-0
S	38.0+-72.5	47.5+-113.3	0.2+-0.8	0+-0	0+-0	0+-0	1.8+-5.6
N	124.3+-163.4	273.1+-380.6	0.02+-0.4	0+-0	0+-0	0.14+-0.9	5.9+-10.7
MC	5.2+-18.8	19.15+-47.2	0+-0	0+-0	0+-0	0.003+-0.02	3.2+-7.0
MY	0.6+-3.0	0.06+-0.4	0+-0	0+-0	13.5+-70.5	0+-0	0.4+-1.5
JY	24.2+-34.0	1.09+-3.9	0+-0	0+-0	0+-0	0+-0	0.7+-4.1

EXHIBIT 49 (AR K.12)

<u>Index II- Percent Cover</u> +- SD, n=40

Cedar Point Percent Cover 2008-2010

	Ulva	*Gracilaria*	*Ascophyllum nodosum*	*Fucus vesiculosus*	*Chondrus crispus*	*Ulva intestinalis*	*Zostera marina*
S	2.95+-6.03	0+-0	80.25+-17.7	0.55+-2.78	0.5+-2.2	0+-0	0.7+-1.57
N	1+-3.4	0+-0	74.9+-20.3	1.6+-7.12	0+-0	0+-0	0.1+-0.63
MC	0+-0	0+-0	82.2+-14.8	0+-0	0+-0	0+-0	0+-0
MY	0.1+-0.6	0+-0	62.6+-27.9	5.0+-16.8	0+-0	0+-0	0.1+-0.6
JY	0.1+-0.6	0+-0	68.7+-22.5	2.2+-6.3	0.2+-1.3	1.3+-8.2	0+-0
S	7.3+-15.8	0+-0	68.4+-28.0	1.3+-3.8	0+-0	0.1+-0.6	0+-0
N	1.2+-5.9	0+-0	69.3+-22.7	1.9+-10.9	0+-0	0+-0	0+-0
MC	0+-0	0+-0	68.7+-28.4	3.0+-15.9	0+-0	0+-0	0.2+-.9
MY	0+-0	0+-0	54.4+-26.1	6.6+-12.5	0+-0	0+-0	2.6+-5.0
JY	0+-0	0+-0	56.2+-21.(	2.3+-5.3	0+-0	0+-0	3.3+-6.1

EXHIBIT 49 (AR K.12)

Wagon Hill Farm Percent Cover 2008-2010

	Ulva	*Gracilaria*	*Ascophyllum nodosum*	*Fucus vesiculosus*	*Ulva intestinalis*	*Zostera marina*
S	0+-0	0+-0	0+-0	0+-0	0+-0	0+-0
N	17.6+-30.4	0+-0	14.2+-33.6	2.0+-9.2	0+-0	12.3+-24.8
MC	17.8+-30.9	0+-0	4.5+-16.7	1.3+-7.6	0+-0	4.2+-11.9
MY	0.1+-0.6	0+-0	10.9+-26.5	0.9+-2.5	21.3+-31.1	0+-0
JY	0.1+-0.6	0+-0	8.8+-24.0	1.5+-6.2	2.6+-6.6	0+-0
S	0+-0	0+-0	8.6+-20.4	2.8+-9.5	6.9+-16.2	0+-0
N	0+-0	0+-0	7.8+-22.7	0+-0	12.9+-26.0	0+-0
MC	0.2+-).9	0+-0	2.8+-8.3	2.1+-6.7	10.5+-23.6	0+-0
MY	0+-0	0+-0	8.2+-22.0	0.9+-2.3	16.1+-28.1	0+-0
JY	0+-0	0+-0	2.3+-5.7	2.9+-8.4	5.9+-14.0	0.4+-1.5

EXHIBIT 49 (AR K.12)

Lubberland Creek Percent Cover 2008-2010

	Ulva	*Gracilaria*	*Fucus vesiculosus*	*Zostera marina*
S	86.7+-14.3	6.3+-6.4	0+-0	0+-0
N	90.1+-18.4	9.4+-12.7	0+-0	0+-0
MC	39.1+-35.4	10.75+-18.6	4.3+-12.5	1.9+-4.8
MY	21.8+-32.9	0.4+-1.5	2.3+-8.2	0+-0
JY	18.3+-27.9	0.4+-1.5	0+-0	1.0+-3.7
S	30.6+-35.1	12+-22.4	0+-0	3.6+-9.2
N	54+-46.0	6.1+-12.5	0+-0	0.4+-2.0
MC	3.1+-6.6	0.4+-1.5	0.9+-4.6	31.5+-36.3
MY	20.8+-32.5	0.3+-1.4	2.4+-8.7	0+-0
JY	28.6+-31.4	2.1+-5.4	1.2+-5.1	2.9+-5.7

EXHIBIT 49 (AR K.12)

Depot Road Percent Cover 2008-2010

	Ulva	*Gracilaria*	*Ascophyllum nodosum*	*Fucus vesiculosus*	*Zostera marina*
S	55.3+-35.7	24.3+-29.6	0+-0	0+-0	2.5+-3.2
N	25.1+-28.0	44.1+-33.7	0+-0	0+-0	0.1+-0.6
MC	20.9+-27.2	27.9+-38.8	0.1+-0.6	0+-0	1.9+-3.9
MY	14.0+-23.4	3.2+-10.5	0+-0	0+-0	0+-0
JY	14.1+-22.2	1.2+-3.6	0+-0	0.2+-1.3	0.8+-5.1
S	36.3+-34.5	14.8+-25.7	0+-0	0+-0	5.9+-16.6
N	42.8+-46.0	6.7+-15.8	0+-0	0+-0	9.2+-22.9
MC	0.1+-0.6	0+-0	0+-0	0.6+-3.2	0+-0
MY	1.7+-5.0	0+-0	0+-0	0.5+-3.2	0.2+-0.9
JY	7.6+-17.7	1.6+-3.6	0+-0	0+-0	0.2+-0.9

EXHIBIT 49 (AR K.12)

Sunset Farm Percent Cover 2008-2010

	Ulva	*Gracilaria*	*Fucus vesiculosus*	*Zostera marina*
S	38.1+-35.9	21.7+-26.4	0+-0	0.7+-1.4
N	59.9+-33.1	39.2+-35.9	0+-0	0+-0
MC	5.2+-8.7	12.2+-23.2	1.4+-8.9	0.7+-4.4
MY	15.3+-19.7	3.1+-7.3	0+-0	2.1+-7.1
JY	15.2+-24.6	7.2+-15.2	0+-0	2.4+-10.0
S	21.9+-22.3	16.0+-22.7	0+-0	3.7+-14.9
N	45.2+-46.1	34.9+-37.3	0+-0	3.4+-15.7
MC	0.7+-2.5	11.2+-25.9	0.1+-0.6	2.0+-5.7
MY	2.1+-4.7	0.6+-1.7	0+-0	4.7+-17.8
JY	6.6+-14.6	8.7+-15.6	0+-0	0+-0

EXHIBIT 49 (AR K.12)

Index III- Water Nutrients 2008-2010

Cedar Point Water Total Phosphorus (mg/L) 2008-2010

	A	B	C	Mean	SD
S	0.0033	0.0195	0.0457	0.0228	0.0214
N	0.0201	0.0274	0.0365	0.028	0.0082
MC	0.0582	0.047	0.0837	0.063	0.0188
MY	0.0147	0.0405	0.0451	0.0334	0.0164
JY	0.0299	0.0328	0.0134	0.0254	0.0105
S	0.0208	0.0258	0.0685	0.0384	0.0262
N	0.0035	0.0377	0.0589	0.0333	0.0279
MC	0.0603	0.027	0.0231	0.0368	0.0205
MY	0.0388	0.0323	0.0984	0.0565	0.0364
JY	0.03	0.031	0.055	0.0387	0.0142

Cedar Point Water Total Nitrogen (mg/L) 2008-2010

	A	B	C	Mean	SD
S	0.351	0.3211	0.6319	0.4347	0.1714
N	0.2604	0.3619	0.8122	0.4782	0.2937
MC	0.352	0.3063	0.3285	0.3289	0.0229
MY	0.1174	0.0705	0.2976	0.1618	0.1199
JY	0.4473	0.1531	0.1412	0.2472	0.1734
S	0.1723	0.2682	0.5149	0.3185	0.1767
N	0.6592	0.4016	0.3398	0.4669	0.1694
MC	0.4622	0.3228	0.331	0.372	0.0782
MY	0.1716	0.217	0.6176	0.3354	0.2454
JY	0.1326	0.2199	0.2005	0.1843	0.0458

Wagon Hill Water Total Phosphorus (mg/L) 2008-2010

	A	B	C	Mean	SD
S					
N	0.0997	0.1311	0.0701	0.1003	0.0305
MC	0.0127	0.0166	0.0374	0.0222	0.0133
MY	0.009	0.0574	0.0234	0.0299	0.0248
JY	0.0158	0.0387	0.0357	0.03	0.0125
S					
N	0.065	0.0479	0.0416	0.0515	0.0121
MC	0.0211	0.0046	0.0012	0.009	0.0107
MY	0.0541	0.0157	0.048	0.0393	0.0206
JY	0.022	0.03	0.023	0.025	0.0044

Wagon Hill Water Total Nitrogen (mg/L) 2008-2010

	A	B	C	Mean	SD
S					
N	1.0541	0.9592	0.6061	0.8731	0.2361
MC	0.3032	0.36	0.9633	0.5422	0.3658
MY	0.2631	0.3442	0.1524	0.2532	0.0963
JY	0.3203	0.4949	0.3178	0.3776	0.1016
S					
N	0.3433	0.3574	0.2856	0.3288	0.0381
MC	0.3344	0.3269	0.1563	0.2725	0.1007
MY	0.5354	0.2733	0.3497	0.3861	0.1348
JY	0.2785	0.5083	0.1236	0.3035	0.1936

Lubberland Creek Water Total Phosphorus (mg/L) 2008-2010

	A	B	C	Mean	SD
S					
N	0.0379	0.0425	0.0594	0.0466	0.0113
MC	0.0384	0.0367	0.0352	0.0368	0.0016
MY	0.0355	0.0344	0.0298	0.0332	0.003
JY	0.0946	0.0569	0.0705	0.074	0.0191
S	0.0509	0.0627	0.047	0.0535	0.0082
N	0.004	0.0667	0.0455	0.0387	0.0319
MC	0.0679	0.0127	0.0096	0.03	0.0328
MY	0.0369	0.0369	0.0338	0.0359	0.0018
JY	0.088	0.087	0.076	0.0837	0.0067

Lubberland Creek Water Total Nitrogen (mg/L) 2008-2010

	A	B	C	Mean	SD
S					
N	0.9336	0.3414	0.244	0.5064	0.3732
MC	0.495	0.6607	0.571	0.5755	0.083
MY	1.0659	1.5104	0.8619	1.1461	0.3316
JY	0.7217	0.5245	0.6014	0.6159	0.0994
S	1.0672	0.5247	0.9251	0.839	0.2813
N	0.6675	0.5794	0.4222	0.5563	0.1243
MC	2.0496	0.476	0.4213	0.9823	0.9247
MY	0.3057	0.4541	0.3897	0.3831	0.0744
JY	0.7191	0.7309	0.6138	0.6879	0.0645

EXHIBIT 49 (AR K.12)

Depot Road Water Total Phosphorus (mg/L) 2008-2010

	A	B	C	Mean	SD
S	0.0423	0.0573	0.0176	0.0391	0.02
N	0.0454	0.0191	0.0063	0.0236	0.02
MC	0.0238	0.0589	0.043	0.0419	0.0176
MY	0.0092	0.0234	0.0083	0.0136	0.0085
JY	0.0338	0.0162	0.0338	0.0279	0.0102
S	0.046	0.0492	0.0627	0.0526	0.0089
N	0.1224	0.0498	0.0796	0.0839	0.0365
MC	0.0307	0.0438	0.0279	0.0342	0.0085
MY	0.1219	0.0357	0.0108	0.0561	0.0583
JY	0.022	0.025	0.031	0.026	0.0046

Depot Road Water Total Nitrogen (mg/L) 2008-2010

	A	B	C	Mean	SD
S	0.3	0.3778	0.326	0.3346	0.0396
N	0.3717	0.1786	0.198	0.2494	0.1063
MC	0.2951	0.5823	0.3544	0.4106	0.1516
MY	0.1443	0.1211	0.336	0.2005	0.1179
JY	0.6944	1.0302	0.4354	0.72	0.2982
S	0.3967	0.365	1.5324	0.7647	0.665
N	1.1408	1.3585	1.1639	1.2211	0.1195
MC	0.4144	0.4545	0.3726	0.4138	0.041
MY	1.0555	0.8717	0.7212	0.8828	0.1674
JY	0.1958	0.375	0.0515	0.2074	0.1621

Sunset Farm Water Total Phosphorus (mg/L) 2008-2010

	A	B	C	Mean	SD
S	0.1329	0.1431	0.1709	0.149	0.0197
N	0.0454	0.0273	0.0358	0.0362	0.0091
MC	0.0602	0.0697	0.0709	0.0669	0.0058
MY	0.0622	0.0099	0.0647	0.0456	0.0309
JY	0.0862	0.1433	0.1358	0.1218	0.031
S	0.0876	0.0898	0.0481	0.0752	0.0234
N	0.0701	0.0956	0.0891	0.085	0.0132
MC	0.0321	0.0289	0.0349	0.032	0.003
MY	0.0553	0.0776	0.0623	0.0651	0.0114
JY	0.099	0.124	0.105	0.1093	0.0131

Sunset Farm Water Total Nitrogen (mg/L) 2008-2010

	A	B	C	Mean	SD
S	0.6039	0.746	0.8933	0.7478	0.1447
N	0.5129	0.299	0.4657	0.4259	0.1124
MC	1.5171	0.8392	0.7948	1.0504	0.4048
MY	1.2139	1.5049	0.0769	0.9319	0.7546
JY	0.6473	1.0299	0.8983	0.8585	0.1944
S	0.7727	0.7948	0.6354	0.7343	0.0864
N	0.4755	0.8104	0.8444	0.7101	0.2039
MC	0.4515	0.5086	1.2373	0.7325	0.4381
MY	0.9407	0.9285	1.1083	0.9925	0.1005
JY	0.5944	0.9772	0.2511	0.6076	0.3632

Index IV- *Ulva* tissue percent total nitrogen and phosphorus

Cedar Point *Ulva* tissue Total Nitrogen Percent 2008-2010

	A	B	C	Mean	SD
S	4.435	4.16	4.179	4.258	0.154
N	4.314	3.132	4.192	3.879	0.65
MC					
MY	4.205			4.205	
JY	4.98			4.98	
S	2.992	3.387	3.582	3.32	0.301
N					
MC					
MY	2.638	5.966	4.139	4.248	1.666
JY					

Cedar Point *Ulva* tissue Total Phosphorus Percent 2008-2010

	A	B	C	Mean	SD
S	0.149	0.124	0.102	0.125	0.023
N	0.092	0.134	0.128	0.118	0.023
MC					
MY	0.185			0.185	
JY	0.113			0.113	
S	0.131	0.086	0.078	0.098	0.029
N					
MC					
MY	0.178			0.178	
JY					

Wagon Hill Farm *Ulva* tissue Total Nitrogen Percent 2008-2010

	A	B	C	Mean	SD
S					
N	4.286	4.222	3.116	3.875	0.658
MC	2.499	2.927	2.726	2.718	0.214
MY	2.168	2.598	2.587	2.451	0.245
JY	1.668	0.666	2.161	1.498	0.761
S	1.611	1.955		1.783	0.243
N	2.191	1.924		2.057	0.188
MC	1.616	1.995	3.366	2.326	0.921
MY	2.418	2.847	2.906	2.724	0.266
JY	0.933	0.868	1.128	0.976	0.135

Wagon Hill Farm *Ulva* tissue Total Phosphorus Percent 2008-2010

	A	B	C	Mean	SD
S					
N	0.089	0.12	0.086	0.098	0.019
MC	0.174	0.147	0.16	0.161	0.014
MY	0.114	0.15	0.148	0.137	0.02
JY	0.133	0.108	0.125	0.122	0.013
S	0.122	0.137		0.13	0.011
N	0.165	0.156		0.16	0.006
MC	0.157	0.162	0.262	0.194	0.059
MY	0.192	0.186	0.185	0.188	0.004
JY	0.115		0.116	0.116	5E-04

Lubberland Creek *Ulva* tissue Total Nitrogen Percent 2008-2010

	A	B	C	Mean	SD
S	3.519	3.898	3.988	3.802	0.249
N	4.57	4.306	4.316	4.397	0.149
MC	3.736	4.605	4.635	4.325	0.511
MY	3.98	4.108	4.249	4.112	0.134
JY	3.906	3.888	3.91	3.901	0.012
S	2.581	2.462	2.511	2.518	0.06
N	3.925	3.251	4.281	3.819	0.523
MC	5.105	5.079	4.772	4.985	0.185
MY	5.887	5.014	5.509	5.47	0.437
JY	2.798	2.671	2.683	2.717	0.07

Lubberland Creek *Ulva* tissue Total Phosphorus Percent 2008-2010

	A	B	C	Mean	SD
S	0.203	0.133	0.157	0.164	0.035
N	0.245	0.229	0.236	0.237	0.008
MC	0.175	0.136	0.179	0.164	0.024
MY	0.166	0.166	0.178	0.17	0.007
JY	0.171	0.16	0.136	0.156	0.018
S	0.135	0.114	0.075	0.108	0.03
N	0.102	0.153	0.103	0.119	0.029
MC	0.134	0.155	0.13	0.14	0.013
MY	0.255	0.195	0.238	0.229	0.031
JY	0.128	0.116	0.123	0.122	0.006

EXHIBIT 49 (AR K.12)

Depot Road *Ulva* tissue Total Nitrogen Percent 2008-2010

	A	B	C	Mean	SD
S	3.649	4.766	4.847	4.421	0.669
N	4.325	4.605	4.585	4.505	0.156
MC	4.785	4.324	4.628	4.579	0.234
MY	3.823	3.773	2.76	3.452	0.6
JY	3.951	4.127	4.23	4.103	0.141
S	1.991	2.918	2.825	2.578	0.51
N	3.376	3.47	2.969	3.272	0.266
MC					
MY	2.419	2.135	2.215	2.257	0.146
JY	2.362	2.347	2.288	2.333	0.039

Depot Road *Ulva* tissue Total Phosphorus Percent 2008-2010

	A	B	C	Mean	SD
S	0.23	0.211	0.16	0.2	0.036
N	0.12	0.193	0.14	0.151	0.038
MC	0.136	0.14	0.112	0.129	0.015
MY	0.18	0.175	0.192	0.183	0.008
JY	0.145	0.15	0.144	0.146	0.003
S	0.139	0.105	0.107	0.117	0.019
N	0.116	0.149	0.09	0.118	0.029
MC					
MY	0.124	0.11	0.132	0.122	0.011
JY	0.114	0.114	0.108	0.112	0.004

Sunset Farm *Ulva* tissue Total Nitrogen Percent 2008-2010

	A	B	C	Mean	SD
S	3.679	3.543	3.942	3.721	0.203
N	4.04	3.017	4.53	3.862	0.772
MC	4.653	4.702	4.49	4.615	0.111
MY	4.074	3.594	4.235	3.968	0.333
JY	3.446	3.628	3.876	3.65	0.216
S	2.865	2.603	2.433	2.633	0.217
N	3.451	3.248	3.718	3.472	0.236
MC	4.657	4.847	4.564	4.689	0.144
MY	5.307			5.307	
JY	3.195	3.127	3.286	3.203	0.08

Sunset Farm *Ulva* tissue Total Phosphorus Percent 2008-2010

	A	B	C	Mean	SD
S	0.229	0.229	0.238	0.232	0.005
N	0.221	0.158	0.245	0.208	0.045
MC	0.178	0.159	0.177	0.172	0.011
MY	0.194	0.167	0.18	0.18	0.014
JY	0.145	0.144	0.148	0.146	0.002
S	0.113	0.102	0.137	0.118	0.018
N	0.106	0.113	0.125	0.115	0.01
MC	0.147	0.184	0.17	0.167	0.018
MY					
JY	0.152	0.152	0.156	0.153	0.002