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Tracking environmental trends in the Great Bay Estuarine System: An examination of water quality and nuisance macroalgal blooms

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TRACKING ENVIRONMENTAL TRENDS IN THE GREAT BAY ESTUARINE SYSTEM: AN EXAMINATION OF WATER QUALITY AND NUISANCE MACROALGAL BLOOMS

BY

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DISSERTATION

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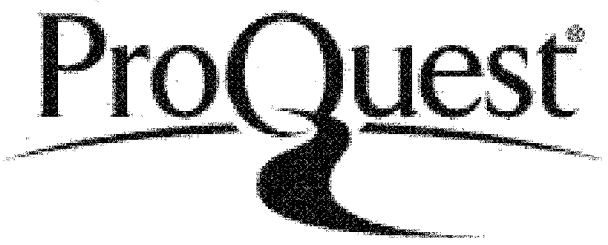

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DEDICATION

To my dear Brita Bug, sorry for all the times I muddied your trunk.

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ABSTRACT

TRACKING ENVIRONMENTAL TRENDS IN THE GREAT BAY ESTUARINE SYSTEM: AN EXAMINATION OF WATER QUALITY AND NUISANCE MACROALGAL BLOOMS

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University of New Hampshire, May, 2012

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. Nitrogen and phosphorus testing revealed that the estuarine system has become eutrophic, and *Ulva* and *Gracilaria* biomass/cover have increased significantly. The percent cover of *Ulva* species, at seasonal maxima, was over 90 times the value recorded in the 1970s at Lubberland Creek, and exceeded 50% cover 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 surpassed *G. tikvahiae* as the dominant *Gracilaria* species in the Great Bay Estuarine System.

Field collections, evaluations of historical herbarium specimens, and molecular investigations (including CO1 gene sequencing) of *Gracilaria vermiculophylla* were used to document its present distribution and approximate dates of introduction within New England. It was found at 18 of 24 Northwest Atlantic sites with existing native *Gracilaria tikvahiae* populations. Presently *G. vermiculophylla* is recorded from Stamford, CT to Greenland, NH. Molecular screening of historical herbarium specimens revealed that *G. vermiculophylla* was collected from five sites in Massachusetts during 2000, while it was first collected in the middle of the Great Bay Estuarine System (Dover Point, NH) during 2003. In Rhode Island, initial specimens were documented during 2007, while those in Connecticut were first confirmed during 2010. As *G. vermiculophylla* has gone primarily undetected in New England since at least 2000, this highlights the difficulty of documenting the arrival and spread of an invasive species that closely resembles a native congener. Hence, DNA sequencing is critical to clarifying the introduction and expansion of such non-native seaweeds.

INTRODUCTION

In the face of increasing worldwide eutrophication of estuarine and coastal systems, the focus of my doctoral work was on measuring related environmental and macroalgal community structure changes in the Great Bay Estuarine System. Chapter I, of this dissertation, describes coastal eutrophication, its causes, prevalence, and impacts. Chapter II outlines the changes in nutrient loads in the Great Bay Estuarine System through the measurement of present day water and algal tissue nitrogen and phosphorus while comparing these data to the findings of previous studies. Chapter III describes the unprecedented peak *Ulva* blooms observed in the 2006 to 2008 ground study. This chapter also identifies the *Ulva* species that were previously undetected in Great Bay and provides evidence for how long each has resided in the system. Chapter IV describes the record blooms of *Gracilaria* observed in the Great Bay during the 2006 to 2008 and fall 2011 studies. This chapter quantifies the abundance of the recently introduced and potentially invasive *Gracilaria vermiculophylla* (Ohmi) Papenfuss. Although the residence time for this species has been brief, it has become the dominant *Gracilaria* species in Great Bay. Chapter V focuses on the current distribution of *Gracilaria vermiculophylla* in New England and provides approximate introduction times for various locations within the region. The final chapter of this dissertation focuses on recovery management efforts in eutrophic coastal systems, including the benefits, difficulties, and expectation setting in such endeavors.

CHAPTER I

EUTROPHICATION AND HARMFUL ALGAL BLOOMS IN COASTAL AND ESTUARINE SYSTEMS: THE CAUSES AND IMPACTS

Eutrophication is described by Schramm and Nienhius (1996) as a natural or anthropogenic nutrient enrichment (predominantly N and P) of an environment that disrupts the flow and cycling of nutrients. Further, hypertrophication is the addition of nutrients to such an extent that detrimental processes will cause potentially irreversible changes to the affected community structure. While high nutrient levels may be caused by natural or human mediated processes (Morand and Briand 1996), and blooms of ephemeral green algae can occur naturally in estuarine environments (Everett 1991), nutrients with anthropogenic origins are a prime factor in system eutrophication (Morand and Briand 1996). Macroalgal blooms are a response to this nutrient abundance (Burrows 1971; Golubic 1970; Lapointe and Bedford 2006; Morand and Briand 1996; McGlathery 2001; Sand-Jensen and Borum 2004; Steffensen 1976).

Many worldwide changes have led to increased eutrophication and subsequent macroalgal blooms in recent years. Since the 1970s, the human population has expanded from 3.7 billion to over 7 billion (Anonymous 2012). In this same timeframe, terrestrial food production has increased from 4.6 billion tons to 6.8 billion tons (Anonymous 2012). To power this increased production, fertilizer use has climbed from 32 million tons N per year to 88 million tons N per year (Smil 2002). Additionally, marine aquaculture production (both plants and animals) increased from 2.2 million tons per year

in the 1970s to nearly 38 million tons per year by 2009 (Anonymous 2012). Nutrient increases have been coupled with the bloom-enhancing warming effects brought about by the world's increased production of CO_2. Emissions of CO_2 have increased from 4.1 gigatonnes (1 Gt = 1 trillion kg) in 1970 to 7.9 Gt in 2005 (Forster et al. 2007).

Increased urbanization and industrialization have been linked to excess nutrient inputs (Deegan et al. 2002; de Jonge et al. 2002), with nutrients added from insufficiently treated municipal waste water, septic systems, agricultural wastes, fertilizer run-off/seepage, and industrial waste. The amount of nutrients contributed by agricultural sources can be quite staggering. For example, farmed livestock in Brittany, France, produce the effluent equivalent of 50 million people, or 15 times the human population of the region (Charlier et al. 2008). Livestock waste in this region is subject to far looser regulation than the human variety, and the majority of this waste is applied directly to the soil, with these nutrient inputs being only slightly diluted before transfer to nearby water sources (Charlier et al. 2008). Agricultural inputs were also the most significant identified source of nitrogen in the blooms observed in the Odense Fjord, Denmark (Frederiksen, 1987) and the Venice Lagoon, Italy (Sfriso et al. 1992). The resulting large influxes of nitrates from agricultural sources and phosphates and ammonia from domestic sources cause overgrowth and proliferation of *Ulva* and *Gracilaria* species (Morand and Briand 1996).

Eutrophication occurs in both estuarine and shallow coastal environments (Duarte 1995; Schories et al. 1997). Moderate to high degrees of eutrophication have been recorded in 67% of the combined surface area of the US estuaries (Boesch 2002). Similar assessments have been made for the estuarine systems in Asia (Boesch 2002) and

Europe (Vidal et al. 1999; Conley et al. 2002; Escaravage et al. 2006). Nitrogen is typically the limiting element in shallow coastal environments (Rosenberg 1985; Nienhuis 1989) and marine systems (Howarth 1988), but a combination of nitrogen and phosphorus has also been shown to be limiting (Gordon et al. 1981; Lapointe 1987). Occasionally, phosphorus is the limiting element in these systems (Lapointe 1987; Peckol et al. 1994). Finally, iron may be a secondary limiting element in some cases (Sequi et al. 1992).

While eutrophic conditions can arise without resulting algal blooms, macroalgal bloom events *cannot* occur without excess nutrients (Morand and Briand 1996). When blooms do occur, there is a positive correlation between the level of eutrophication and the proliferation of macroalgae (Morand and Briand 1996). In most estuarine systems, increasing nitrogen levels have driven bloom events. An example of this found in the microcosm study by Fong et al. (1993), which showed that macroalgal biomass was positively proportional to N availability, but biomass was not correlated to P availability.

Nutrient pollution from anthropogenic sources, such as those mentioned above, causes strong negative effects in estuaries (Morand and Briand 1996), however, systematic responses to eutrophication vary depending on abiotic factors, such as geomorphology and flushing time (de Jonge et al. 2002; Elliot and de Jonge 2002). The size of macroalgal blooms is greatest when the conditions of excess nutrients are combined with other abiotic factors, such as minimal current flow and slow rates of water renewal (Morand and Briand 1996).

Bloom-forming seaweeds thrive in nutrient enriched environments. Greater tolerances for fluctuations of salinity, temperature, and light levels in common bloom-

forming algae such as *Ulva* and *Gracilaria* contribute to their predominance in estuarine habitats (Morand and Briand 1996). Opportunistic species such as *Ulva intestinalis* demonstrate more efficient osmoregulation at lower salinities found in estuarine environments (Black and Weeks 1972), which gives them a distinct advantage. Many opportunistic bloom-forming macroalgae have a thin thallus and therefore a high surface area to volume ratio that facilitates high nutrient uptake (Littler and Littler 1980; Rosenberg and Ramus 1984). Opportunistic species are also better equipped to use alternative nutrient sources. While most macroalgal species take up NH_4^+ preferentially over NO_3^- (Wallentinus 1984), *Ulva* species can utilize both nutrient forms at the same rate (Le Bozec, 1993). Additionally, *Ulva* species can take up nutrients 4-6 times faster than slow growth perennial species (Pedersen and Borum 1997). Even when ambient nitrogen concentrations are low, the addition of phosphorus can, in some cases, stimulate *Ulva* growth, (Steffensen 1976). Finally, thallus fragility, in response to current forces, causes increased opportunities for fragmentation and, consequently, vegetative reproduction in bloom-forming rapidly proliferating species (Morand and Briand 1996).

Opportunistic macroalgal bloom events have become common worldwide in coastal environments and biomass totals range from 0.2 to 400 kg WW m^{-2} (Morand and Briand 1996). While large macroalgal blooms have been reported since the early 1900s (Cotton 1910; Letts and Richard 1911), the number of incidents, regions affected, and incident severity have increased dramatically since the 1960s (Sawyer 1965; Perkins and Abbott 1972; Fahy et al. 1975; Dauer and Conner 1980; Soulsby et al. 1982, 1985; Tubbs and Tubbs 1983; Reise 1983, 1985; Thrush 1986; Frederiksen 1987; Hull, 1987, 1988; O´lafsson 1988; Sundback et al. 1990; Raffaelli et al. 1991, 1998, 1999; Bonsdorff 1992;

Sfriso et al. 1992; Everett 1994; Viaroli 1995; Raffaelli 2000; Österling and Pihl 2001; Franz and Friedman 2002; Auffrey et al. 2004).

Macroalgal blooms can be very extensive and long lasting. Free floating algae are prone to drift together, in estuarine environments, forming multi-layered canopies (Vergara et al. 1998). Spanish coastal blooms of *Ulva* were estimated to be between 250 and 827 g/m^2 DW(Niell et al. 1996). Sfriso et al. (1992) reported *Ulva* biomass levels of 700 to 1400 g/m^2 DW in the Venice Lagoons. On the Swedish west coast, Pihl et al. (1996) found *Ulva* biomass values between 425 and 625 g/m^2 DW. Pregnall and Rudy (1985) measured an Oregon *Ulva* biomass value of 750 g/m^2 DW. The peak abundance of bloom-forming algae can vary by location and year. In temperate environments macroalgal blooms begin in the spring, peak in the summer months, and diminish throughout the fall (Hull 1987). In areas of low hydrodynamic movement, macroalgal blooms can persist for months (Vergara et al. 1998).

The consequences of macroalgal blooms are more varied, indirect, and longer term than those observed in microalgal blooms (Perkins and Abbott 1972; Fahy et al. 1975; Dauer and Conner 1980; Soulsby et al. 1982, 1985; Tubbs and Tubbs 1983; Reise 1983, 1985; Thrush 1986; Hull, 1987, 1988; O´lafsson 1988; Sundback et al. 1990; Raffaelli et al. 1991, 1998, 1999; Bonsdorff 1992; Everett 1994; Viaroli 1995; Raffaelli 2000; Österling and Pihl 2001; Franz and Friedman 2002; Auffrey et al. 2004; Lapointe and Bedford 2006). Macroalgal blooms have led to major structural and compositional changes in estuarine and coastal ecosystems (Rosenberg 1985; Nixon 1995). Winds and currents cause drifting and deposition of algae in concentrated areas, macroalgal mats can develop (Morand and Briand 1996; Vergara et al. 1998). The physical structure of algal

mats has been shown to filter out pelagic larvae, which disrupts the settlement process and can reduce recruitment and colonization of other species (O'lafsson 1988). Abundance and survival of bivalves has been shown to decline in the presence of dense algal mats (Petersen et al. 1994; Norkko and Bonsdorff 1996). The presence of macroalgal mats may cause a decline in the infaunal biodiversity in affected soft-bottomed habitats (Franz and Friedman 2002; Jones and Pinn 2006). In some areas, long-lived habitat-forming species such as *Ascophyllum nodosum* may be out competed in their colonization of rocky substrata by short-lived bloom forming algae (Lobban et al. 1985), making habitat restoration difficult. The night time respiration of large *Ulva* blooms can create daily periods of anoxia (Johnson and Welsh 1985; D'Avanzo and Kremer 1994). Reduction of oxygen in dense *Ulva* beds is harmful and even fatal to shallow mudflat dwelling crabs (Johnson and Welsh 1985). Anoxic conditions caused by macroalgal blooms have also been linked to large fish die offs in Sweden (Rosenberg et al. 1990) and disappearance of macrofauna in the Italy (Ceretti et al. 1985).

Macroalgal bloom events are seasonal in most systems, and bloom die-offs can be more harmful than the grow out phase. Bacterial decomposition of algal mats leads to anoxic conditions in the sediment and water column (Nedergaard et al. 2002), which can lead to the decline of long-lived habitat-forming seagrass and macroalgal species (Borum and Sand-Jenson 1996; Duarte 1995; Valiela et al. 1997; Schramm 1999). Hypoxic conditions can persist for weeks or months (Bolam et al. 2000), often causing the decline or disappearance of resident fauna (Reise 1983; Thorne-Miller et al. 1983; Johnson and Welsh 1985; O'lafsson 1988; Breuer and Schramm 1988; Nienhuis 1992b; Warwick and Clarke 1994; Villano and Warwick 1995; Hansen and Kristensen 1997). Rotting

seaweeds also give off harmful chemicals, such as H_2S, which cause a foul smell that can cause reduced tourism in an affected area. At sufficient concentrations, the chemicals given off by rotting algae have been strong enough to remove lead-based paints from buildings and fences (Sawyer 1965). In several areas, rotting algal blooms have reportedly caused nuisance outbreaks of chironomids, non-biting midges (Buttermore 1977; Orlandini 1988).

In eutrophic environments, species with high nutrient tolerances or affinities survive while less adapted species diminish or disappear (Morand and Briand 1996). In soft bottomed ecosystems, seagrass beds often decline drastically in highly eutrophied systems (den Hartog 1994; Short et al. 1995). Deposits of *Ulva* in Langstone Harbour, England, caused the disappearance of *Zostera* beds due to shading and toxic decay (den Hartog 1994). In Australia's Oyster and Royal Princess Harbours, macroalgal blooms led to an 80-90% decrease in seagrass meadows (Morand and Briand 1996). Such algal blooms were the result of nutrient increases from agricultural runoff and, specifically in the case of Oyster Harbour, leakage from an agricultural fertilizer plant.

Eutrophication and the resulting algal blooms are a worldwide problem. Unfortunately, our New England coastal ecosystems are not immune. The Great Bay Estuarine System watershed has been stressed in multiple ways in recent years. Impermeable surfaces, which lead to higher inputs of nutrient pollutants, have been increasing at a rate of 1,500 acres per year (Anonymous 2009). Over 7.5% of the watershed surface is currently impermeable, and irreversible damage is often observed when levels reach 10% (Anonymous 2009). The 18 wastewater treatment facilities in the

watershed are all contributing nitrogen loads far in excess of EPA regulations (Anonymous 2009). Additionally, over 60% of residents use septic systems, which are only inspected for nutrient release compliance when owners apply for property enhancement permits (Anonymous 2009). Application of chemical fertilizers in the region's urban settings are largely unregulated. The following chapter outlines the degree of eutrophication detected in the Great Bay Estuarine System of New Hampshire and Maine. The resulting blooms of *Ulva* and *Gracilaria* species are reported in Chapters III and IV. The New England distribution of the recently introduced *Gracilaria vermiculophylla* (Ohmi) Papenfuss is described in Chapter V. Restoration and management approaches and considerations are discussed in Chapter VI.

CHAPTER II

ALGAL TISSUE NUTRIENT ANALYSIS AND WATER QUALITY TESTING: ASSESSING THE NUTRIENT STATUS OF THE GREAT BAY ESTUARINE SYSTEM

Introduction

Ecosystem changes have quickened beyond the pace of scientific progress, to the point that anthropogenic forces threaten to cause irreversible damage to ecosystems (Morand and Merceron 2005). Estuarine environments around the world are threatened by anthropogenic inputs of nitrogen and phosphorus. Human terrestrial nitrogen inputs have more than doubled the earth's total natural nitrogen inputs (Vitousek et al. 1997). Excess nitrogen applied to land can accumulate in soil, run off to surface waters, enter and travel through ground waters, or volatilize and reenter the atmosphere (Smith et al. 1999). Agricultural inputs of phosphorus far outweigh phosphorus outputs in production in many locations and are in excess in the soils (Carpenter et al. 1998). Phosphorus leached from soil readily flow through regional watersheds. Both point and non-point sources of nitrogen and phosphorus can have dramatic effects on the receiving bodies of water (Carpenter et al. 1998; Correll 1998). Estuarine habitats receive more nutrient inputs per unit area than any other aquatic ecosystem (Howarth 1993). Such inputs can lead to eutrophic or hypertrophic conditions and ecosystem damage.

The degree of nutrient pollution has been defined for several types of aquatic systems. Marine waters are defined as oligotrophic when total nitrogen (TN) concentrations are < 0.260 mg/L, mesotrophic when between 0.260-0.350 mg/L,

eutrophic from 0.350 to 0.400 mg/L, and hypertrophic above 0.400 mg/L (Håkanson 1994). For total phosphorus (TP), marine waters are considered oligotrophic when phosphorus concentrations are < 0.010 mg/L, mesotrophic when between 0.010-0.030 mg/L, eutrophic from 0.030 to 0.040 mg/L, and hypertrophic above 0.040 mg/L (Håkanson 1994).

In 2000, the Swedish EPA (Smith 2003) published nutrient pollution guidelines for coastal marine waters based upon what they perceived would be pristine conditions. Nitrogen concentrations below 0.252 mg/L were considered very low, those between 0.252 an 0.308 mg/L were considered low, 0.308 to 0.364 mg/L indicated moderate pollution, 0.364 to 0.448 mg/L was classified as high nutrient pollution, and above 0.448 mg/L was considered very high. Phosphorus concentrations below 0.015 mg/L were considered very low, those between 0.015 an 0.019 mg/L were considered low, 0.019 to 0.024 mg/L indicated moderate pollution, 0.024 to 0.031 mg/L was designated as high nutrient pollution, and above 0.031 mg/L was designated as very high.

Eutrophication of estuaries can cause macroalgal blooms (Harlin and Thorne-Miller 1981; Cambridge and McComb 1984; Lapointe and O'Connell 1989; Kinney and Roman 1998). Proliferations of green seaweeds, including *Ulva* spp., are common in highly eutrophic environments in Italy (Sfriso and Marcomini 1996; Tagliapietra et al. 1998), Spain (Hernandez et al. 1997), France (Charlier et al. 2008), Scotland (Raffaelli et al. 1998), Britain (den Hartog 1994) Denmark (Frederiksen 1987), Australia (Morand and Briand 1996), and the US (Sawyer 1965). Opportunistic macroalgae respond positively to nutrient enrichment. In comparing two Massachusetts estuaries, Hauxwell et al. (2000) found that macroalgal biomass was consistently greater in the system with the

higher nutrient load. Further, flat bladed green species, like *Ulva*, have been shown to become more abundant than filamentous red species with increasing nutrients (Karez et al. 2004). The physiological responses of opportunistic macroalgae to nutrient enrichment produces higher tissue concentrations of nitrogen and lower amounts of structural carbon than is found in species like eelgrasses (Enriquez et al. 1993). Decomposition of this algal tissue with high nutrient content occurs far more rapidly than in high carbon plants. An abundance of decomposing algae can cause a rapid release of nitrogenous nutrients that can disrupt ecosystem balance.

Plant yield is limited by elements available in the least quantity relative to the particular organism's growth needs (von Liebig 1855). Nitrogen versus phosphorus limitation is dependent on several factors including the supply rate of each element (Fong et al. 1993). The need for nitrogen and phosphorus can vary by species. In macroalgae, N:P ratios of less than 16:1 generally indicate that nitrogen is limited compared to phosphorus (Redfield 1958). Atomic N:P ratios above 24:1 indicate phosphorus limitation, and ratios between 16:1 and 24:1 indicate an adequate balance between the availability of nitrogen and phosphorus (Björnsäter and Wheeler 1990).

The response of macroalgae to nutrient enrichment can vary by species, nutrient type, nutrient concentration, and other environmental conditions. Harlin and Thorne-Miller (1981) found that additions of ammonium to the water column enhanced the growth of *Ulva* species, causing dense mat formation, while additions of phosphate had no effect on growth. The same study found that neither ammonium nor phosphate additions enhanced growth of the red seaweed *Gracilaria tikvahiae* McLachlan, but each caused tissue reddening (Harlin and Thorne-Miller 1981). Additions of nitrate enhanced

the growth of *Ulva* species, but to a lesser degree than ammonium (Harlin and Thorne-Miller 1981). Nitrate additions caused some growth stimulation in *Gracilaria tikvahiae* and led to tissue reddening (Harlin and Thorne-Miller 1981). In N:P treatments with fixed 15:1 ratios, *Ulva* biomass was greatest when exposed to moderate inputs of both nitrogen and phosphorus (Fong et al. 1993). This same response pattern was seen across the varying treatments at a fixed 30:1 ratio (Fong et al. 1993). Thus, the actual amount of nitrogen and phosphorus present in a system, rather than the N:P ratio may better predict the growth response of opportunistic species.

To assess the nutrient status of an estuarine system, one can test the nutrient levels in the water and in the tissues of resident algal species. Each method has advantages and disadvantages. While collecting water samples is fairly easy, pulses in nutrient inputs and community uptake can cause water column N:P ratios to fluctuate rapidly (Fong et al. 1993), making it difficult to determine long-term trends. A high correlation has been reported between tissue nutrient levels in *Ulva* and long-term ambient nutrient levels (Ho 1987). Consequently, analyzing tissue nutrients has the assumed advantage of providing a more stable picture of site nutrient levels. However, tissue nutrient levels do fluctuate across time. Lapointe and Bedford (2006) observed significant seasonal differences in N:P ratios of algal tissues, with higher means in August (25.4) than October (18.1). In addition collecting enough algal tissues to conduct robust nutrient testing maybe challenging or impossible at some sites and times.

The current study was designed to determine the nutrient status of the Great Bay Estuarine System by measuring total nitrogen (TN) and total phosphorus (TP) in both water and algal tissue from five sites over a two year period beginning in September

2008. Water TN and TP concentrations were also compared to the nutrient pollution categories of Håkanson (1994). The atomic N:P ratios generated from water and tissue samples from each site and time were compared to the nutrient limitation ratios described by Redfield (1958) and Björnsäter and Wheeler (1990). To compare present and historical nutrient enrichment status, tissue nutrient data from the current study were matched to the historical Great Bay *Gracilaria* tissue nutrient data described by Penniman (1983), and compared to the minimal growth requirements outlined by Pedersen and Borum (1997) and Villares and Carballeira (2004). Further, current water nutrient data were compared to the historical water nutrient concentrations for the region, which were outlined by Short (1992) and Jones (2000).

Materials and Methods

Site Descriptions and Sampling Regime

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

Water samples and samples of *Ulva* were collected from each site at low tide on a bi-monthly basis during autumn, spring and summer from September 2008 through July 2010 . Samples of *Gracilaria* were collected from three of the sites (DR, LC and SF) during the first year only. 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

samples were filtered through cellulose membrane filters (Millipore® HAWP 0.45 μm pore) and kept at -20°C until being of analyzed. Temperatures and salinities were enumerated for each site at the time of collection using a floating thermometer and a refractometer, respectively.

Whole thalli of at least 12 specimens of *Ulva* were collected at each site during each visit. The whole thalli at least *Gracilaria* specimens were also collected at the three southern bay sites during year one of the study. They were washed in the field with seawater to remove sediment and detritus, placed in plastic bags, and returned to the laboratory within one hour. In the lab, the samples were gently brushed under running fresh water until clean, then rinsed with distilled water, and dried at 90 °C until a constant weight (up to three days). Dried materials were kept at -20 °C until chemical analysis.

Nutrient Analysis

Tissue total nitrogen and total phosphorus were determined for a subset of *Ulva* and *Gracilaria* specimens. The analyses were done by Penn State's Agricultural Analytical Lab using combustion (Horneck et al. 1998) and dry ash methods (Miller 1998). 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, provided adequate amounts of tissue were available on site. For each species, mean tissue nitrogen and phosphorus levels were compared between sites and between seasons via single-factor analysis of variance (ANOVA) with significance level α=0.05 (Zar 1999), followed with

a Tukey's multiple comparison test. All statistical test analyses were done using Systat 13 (Systat, Inc.)

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 NO_3 and PO_4, yielding results in mg/L.

Results

Great Bay Estuarine System mean water and *Ulva* tissue nitrogen and phosphorus measured during the two year study (Figures 2-5, Appendices A-B) showed no significant differences between the months ($p>0.05$). Mean monthly total nitrogen levels were hypertrophic (TN concentrations from 0.508 to 0.664 mg/L) in all but July 2010 (Figure 2), at which time the water was highly eutrophic (TN concentration 0.398 mg/L). Meanwhile, the Great Bay Estuarine System's mean monthly *Ulva* tissue nitrogen percentages remained between 2.3 and 4.1% (Figure 3), which is above the 2.2% threshold for unlimited growth (Pedersen and Borum 1997). Mean monthly water phosphorus concentrations were between 0.028 and 0.070 mg/L across the Great Bay Estuarine System (Figure 4). Phosphorus concentrations were mesotrophic during one month, March 2010 (0.028 mg/L), and eutrophic in one month, May 2009. In all other months, the phosphorus levels were above the hypertrophic threshold of 0.040 mg/L. *Ulva* tissue mean phosphorus percentages stayed between 0.13 and 0.18% (Figure 5), well above the 0.03% minimum needed for growth (Villares and Carballeira 2004).

The mean total nitrogen of water from each study site was compared (Figure 6). The mean nitrogen values were above Håkanson's (1994) 0.400 mg/L threshold for hypertrophication for each site, except Cedar Point. Total nitrogen mean concentration for Cedar Point was below the eutrophic threshold of 0.350 mg/L.

The mean total nitrogen (TN) from *Ulva* tissue was also compared between sites (Figure 7). There were no significant differences between the sites, but it must be noted that *Ulva* was not available for nutrient testing at Cedar Point during November 2009 and July 2010, months with low tissue nitrogen measures at the other sites. Mean tissue nitrogen for every site was above the 2.2% threshold needed for unlimited *Ulva* growth.

The mean water TP for each site was above the 0.030 mg/L eutrophication threshold, and all sites other than Cedar Point were above the 0.040 mg/L definition of hypertrophy (Figure 8). 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 (0.080 mg/L $\pm$ 0.04 SD).

Ulva tissue was used to track mean phosphorus levels at all sites in the Great Bay Estuarine System (Figure 9). Cedar Point *Ulva* tissue, on average, contained a slightly lower percentage of phosphorus (0.136% $\pm$ 0.036 SD) than any other site ($P<0.01$). Still, mean tissue phosphorus levels were well above the 0.03% minimum required for growth.

No significant differences in N:P ratios for water and *Ulva* tissue samples were found between sites. 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 (Figure 10). Mean water N:P ratios for the two year study ranged between 15.2 $\pm$ 7.9 SD (Cedar Point) and 25.7 $\pm$ 15.4 SD (Lubberland Creek), whereas mean tissue N:P ratios were between 37 $\pm$ 19.7 SD (Wagon

Hill Farm) and 74.1 ± 16.1 SD (Cedar Point). With the exception of Cedar Point's mean water N:P, all of the observed water and tissue summary mean atomic ratios for each site were well above the normal 16:1 Redfield Ratio.

Mean *Ulva* tissue and water N:P ratios were also examined by collection month across the Great Bay Estuarine System (Figure 11). While no significant differences were found in mean N:P ratios over time in water or tissue, the ratios were generally higher in tissue than in water. In the water, N:P ratios ranged from a high of 33.2 ± 15.8 SD in March 2010 to a low of 11.8 ± 4.4 SD in July 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.

Seasonal changes in TN, TP and N:P ratios in water and *Ulva* tissue were examined for each collection site (Table 2, Figures 12 and 13). At Sunset Farm, no significant differences were found between months using the water analyses. Mean monthly water N:P ratios remained between 7.7 ± 0.6 SD and 35.1 ± 17.5 SD during the entire study. Between-month differences in mean N:P ratios were found using the tissue analyses ($P<0.01$), but no clear seasonal trends were evident. The highest mean N:P ratio was observed in November 2009 (65.0 ± 4.4 SD), and the lowest in September 2008 (34.3 ± 1.2 SD), the first month of the study.

The Sunset Farm monthly water nitrogen means were in the hypertrophic range (above 0.400 mg/L) each month of the study (Figure 14). Monthly water phosphorus means were hypertrophic for all but two months (Figure 15), November 2008 and March 2010, in which they were in the eutrophic range (between 0.030 and 0.040 mg/L.

For Depot Road samples, no significant differences in water N:P were found between months (Figure 16). Mean monthly water N:P ratios remained between 12.2 ± 10.2 SD and 39.5 ± 42.0 SD during the course of the study. Monthly means were above the 16:1 Redfield ratio in all but three months (September 2008, March 2009, and July 2010). Significant between-month differences in mean N:P ratios were not found using the tissue analyses (Figure 17). The highest mean N:P ratio was observed in March 2008 (76.6 ± 11.2 SD), and the lowest mean value was in September 2008 (39.7 ± 3.3 SD), the first month of the study.

Total nitrogen in Depot Road site water was oligotrophic (≤0.260 mg/L) during November 2008, May 2009, and July 2010 (Figure 18). Nitrogen concentrations were in the mesotrophic range (0.260 to 0.350 mg/L) September 2008, and hypertrophic (above 0.400 mg/L) during the other 6 measurement periods.

Mean monthly total phosphorus concentrations at the Depot Road site (Figure 19) were in the mesotrophic range (between 0.010 and 0.030 mg/L) during November 2008, May 2009, July 2009, July 2010, eutrophic during September 2008 and March 2009, and hypertrophic during the other four measurement periods.

Water and *Ulva* tissue from the Lubberland Creek site were analyzed for seasonal variation in N:P ratios (Figures 20 and 21). 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. Mean monthly water N:P ratios remained between 12.6 ± 2.3 SD (July 2010) and 52.8 ± 12.8 SD (May 2009). The 16:1 Redfield ratio was exceeded in the water at this site in all but two months (July 2009 and 2010). In

the tissue analyses, the mean N:P ratios remained between 39.8 ± 0.6 SD and 76.8 ± 6.0 SD.

Mean monthly water total nitrogen concentrations at the Lubberland Creek site (Figure 22) were at eutrophic levels during May 2010 and at hypertrophic levels (above 0.400 mg/L) during all other months. The mean monthly water phosphorus concentrations at this site were in the eutrophic range during five months (March, May and November 2009, and March and May 2010). Phosphorus concentrations were in the hypertrophic range (above 0.040 mg/L) during the other measurement periods (Figure 23).

Water and *Ulva* tissue from the Wagon Hill Farm site were analyzed for temporal variation in N:P ratios (Figures 24 and 25). Significant differences ($P<0.01$) were found between the months using both methods of analysis. Peak means in the water N:P ratio occurred in March 2009 and March 2010 with measures above 37 in each case. Overall, lower water N:P ratios were registered at this site, relative to the southern bay sites, with four months below the N:P of 16.

The lone peak in the tissue mean N:P ratio was recorded for November 2008 (85.4 ± 15.8 SD). Otherwise, in the tissue analyses, the mean N:P ratios remained between 19.1 ± 2.5 SD and 38.4 ± 2.0 SD.

Mean monthly water nitrogen concentrations from the Wagon Hill Farm site (Figure 26) were in the oligotrophic range during one month (May 2009) and in the mesotrophic range during three months (November 2009, March 2010, and July 2010). Nitrogen concentrations were in the eutrophic range during July 2009 and May 2010 and in the hypertrophic range during the remaining months.

Mean monthly phosphorus concentrations were in the oligotrophic range during March 2010 and in the mesotrophic range during March 2009 and July 2010 (Figure 27). Phosphorus concentrations were in the eutrophic range during May 2009 and 2010, and July 2010. Relative to phosphorus, hypertrophic conditions were found during two months (November 2008 and 2009).

Water and *Ulva* tissue from the Cedar Point site were analyzed for seasonal variation in N:P ratios (Figures 28 and 29). No significant differences were found between months using either method of analysis. Peaks in mean water N:P ratios were recorded in the fall of both 2008 and 2009 with values above 20. Mean water N:P ratios only exceeded Redfield's 16:1 in three months (September 2008, November 2008, and November '09). The amount of dried *Ulva* tissue biomass necessary for analysis was minimal at Cedar Point during several months of the study, with the N:P ratios of the remaining months varying between 31.7 and 94.3.

The mean monthly water nitrogen concentrations from Cedar Point(Figure 30) were in the oligotrophic range during three months (May 2009, July 2009, and July 2010) and in the mesotrophic range during three other months (March 2009, September 2009, and May 2010). Nitrogen concentrations were in the eutrophic range during March 2010, and in the hypertrophic range during the remaining three measurement periods.

The mean monthly water phosphorus concentrations from the Cedar Point site were in the mesotrophic range during September and November 2008, and July 2009. Phosphorus concentrations were in the eutrophic range during May, September and November 2009, and in March and July 2010 (Figure 31) and hypertrophic during March 2008 and May 2010.

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 3).

Discussion

The Great Bay Estuarine System is currently in a highly nutrient enriched state with excess nitrogen and phosphorus. The mean water nitrogen concentrations across the study sites and times indicated that the bay was highly eutrophic or hypertrophic during the entire two year study. Likewise, mean water phosphorus concentrations were at highly eutrophic or hypertrophic levels during all but one month of the two year study, with values ranging between 0.028 and 0.070 mg/L across the system.

Nitrogen values found in the Great Bay Estuarine System (0.398 to 0.664 mg/L) were comparable to and in some cases higher than those observed in other eutrophic systems during nuisance macroalgal blooms. In the study of water samples linked to the sea lettuce (*Ulva*) blooms in Boston Harbor in the early 1960s, Sawyer (1965) found maximal ammonia nitrogen to be 0.750 mg/L at a station in immediate proximity to the city's sewage outfall pipe. Further inland where the bloom occurred, ammonia levels were mostly between 0.050 and 0.200 mg/L. While total nitrogen water at rural sites around Hong Kong Island was 0.050 mg/L (Ho 1987), in sites near heavily populated areas, where blooms occurred, the nitrogen concentrations were 0.294 mg/L (Ho (1987).

Excessive growth of *Monostroma* was observed in Arcachon Bay, New Zealand, where the nitrogen level was 0.200 mg/L (Le Bozec, 1993).

The mean total phosphorus values in the Great Bay Estuarine System (0.028 to 0.070) were again comparable to those observed in other eutrophic systems during nuisance macroalgal blooms. Phosphorus levels associated with *Ulva* blooms near heavily populated areas around Hong Kong were 0.045 mg/L (Ho 1987).

Meanwhile the Great Bay Estuarine System's mean monthly *Ulva* tissue nitrogen percentages remained between 2.3 and 4.1%, which is above the 2.2% threshold for unlimited growth (Pedersen and Borum 1997). *Ulva* tissue mean phosphorus percentages ranged between 0.13 and 0.18%, which is well above the 0.03% minimum needed for growth (Villares and Carballeira 2004). The critical tissue N and P concentrations, or the concentrations at which maximal growth rate is achieved, for *Ulva rigida* are 20 and 0.25 mg/g DW, or 2% and 0.025% respectively (Lavery and McComb 1991), so for this species, phosphorus limitation in the estuary could be preventing maximal growth.

The *Ulva* tissue values in the Great Bay Estuarine System compare well with those found in other bloom studies. For example, the mean tissue N and P percentages in *Ulva* blooms in Hong Kong were between 2.2-5.2 and 0.08-0.31, respectively (Ho 1987). In that study, *Ulva* tissue nitrogen and phosphorus were 71 and 97% higher, respectively, in urban than in rural sites (Ho 1987). In the heavily eutrophied Venice Lagoon, Italy, Sfriso et al. (1993) observed algal N and P content to be 2.17% and 0.19% of dry weight, respectively. Maximal *Ulva* tissue N percentages in the Veerse Meer Lagoon, the Netherlands, were 5.5 and 5.01% DW in 1992 and 1994 respectively (Malta 2000). Minimal *Ulva* tissue N percentages in the same years were 0.89 and 1.49% DW.

Gracilaria tissue nitrogen and phosphorus were tested over several months in the southern Great Bay sites. Penniman (1983) measured the percent nitrogen and phosphorus in *Gracilaria tikvahiae* specimens collected subtidally near Nannie Island (close to Sunset Farm). The tissue nitrogen values during 1976 and 1977 ranged from 2% to 4.5%, and the phosphorus values ranged from 0.18% to 0.35%. These should be compared to the ranges of 2.5% - 3.6% (TN) and 0.17% - 0.33% (TP) levels observed in the current study. Such stability in the face of increasing nutrient availability could be indicative of a preferred steady state for these organisms. Since *Gracilaria* sp. can grow very rapidly (Lapointe 1987), it is likely that excess available nutrients are directly converted into increased biomass production. Hence, the thalli, or the populations grow via nutrient uptake, but the overall tissue nutrient concentrations remain unchanged.

Hydrodynamic forces and differing source nutrient sources have created a non-uniform nutrient regime within the Great Bay Estuarine System. Although all sites were eutrophic or hypertrophic overall, 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. Such a trend was also revealed in tissue nitrogen analysis, but differences were not significant. In measures of TP, water analysis revealed that Sunset Farm had significantly higher mean values than either of the northern sites, Wagon Hill Farm and Cedar Point. Tissue tests of TP revealed that Cedar Point *Ulva* only had slightly lower levels than those 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 tissues than the water column, which is likely

due to the tendency of *Ulva* to preferentially sequester nitrogen at times of availability (Hanisak, 1983).

Mean monthly nitrogen and phosphorus were averaged among the sites over the study period using both water and *Ulva* tissue analyses. Neither method revealed any significant temporal differences in TN, TP, or atomic N:P ratios, but the trend in water samples had seasonal N:P highs in spring and lows in fall. Mean water TN remained between 0.4 and 0.7 mg/L, or above 9 μM, throughout the study sites. Such a value is in line with the 10 μM nitrogen concentrations reported by Short (1992) and the eight year average 8.8 μM DIN concentration in the 1988-1996 study of Great Bay's Furber Strait (Jones 2000). Such figures are comparable to mean DIN concentrations found in macroagal bloom affected Florida regions, which were 7.36 μM (Lapointe and Bedford 2006). The Great Bay Estuarine System's mean water TP was between 0.028 and 0.07 mg/L, or around 0.5 μM , which was lower than the mean value (0.9 μM) found by Short (1992) and the eight year average value (0.85 μM) for Furber Strait (Jones 2002), but still elevated.

Atomic N:P ratios of water were above the Redfield Ratio of 16:1 (Redfield 1958) at most times and sites (26 out of 47), with water values ranging from 7.3:1 to 52.8:1. The mean water N:P ratio across all times and sites was 20.2:1, which is nearly three times higher than the NOAA 1989 values for the Great Bay Estuarine System which were 7:1 (Short 1992) and marks an overall shift from nitrogen limitation to conditions in which neither phosphorus nor nitrogen is limiting to nuisance macroalgal growth, even at the times of heaviest algal blooms.

The mean water N:P ratios observed in the Great Bay Estuarine System are comparable to those found in other studies of eutrophic coastal marine habitats. Lapointe and Bedford (2006) observed significant seasonal differences in N:P ratios of algal tissues, with higher means in August (25.4) than in October (18.1). The lowest N:P ratio at any site and time in their study was 12.8, and the highest was 42.4.

In the present study, *Ulva* tissue atomic N:P ratios were from 38.5:1 to 61:1 and the *Gracilaria* atomic N:P ratios from 22:1 to 41:1, which indicate that these organisms were not nitrogen limited, even at peak bloom. According to Björnsäter & Wheeler's (1990) assessment, tissues with N:P ratios of less than 16:1 indicate nitrogen limitation, greater than 24:1 indicate phosphorus limitation, and intermediate values indicate sufficient nitrogen and phosphorus for continued growth. Although the mean *Ulva* tissue N:P ratios observed in the Great Bay Estuarine System 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. Other studies have found that *Ulva* growth may not be nitrogen limited when ambient water N:P ratios fall below 16:1. During a major bloom in the Venice Lagoon, the N:P ratio remained below 10 during the macroalgal growing season (Sfriso et al. 1989). Boynton et al. (1982) found that N:P ratios were below the 16:1 ratio in 22 of 27 estuarine sites at the time of peak algal growth. Waite and Mitchell (1972) concluded that limitation by either nitrogen or phosphorus is unlikely for *Ulva* because growth was stimulated by increases in either nutrient in their study.

In comparing the methods of estimating nutrient regime by site and time, this study found water testing to be slightly superior to tissue testing in its ability to reveal significant differences, although neither method revealed many conspicuous 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 Estuarine System 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. Also, both water and tissues sampling methods demonstrated the excessive nutrient enrichment of the Great Bay Estuarine System.

As the Great Bay Estuarine System has become an ecosystem that can be classified as eutrophic or even hypertrophic, restoration steps must be considered. Non-point nutrient pollution of surface waters can be reduced by lowering nitrogen inputs generated through fossil fuel burning, altering farming practices by limiting chemical fertilizer and manure application to non-excessive levels, and reducing runoff from

farming and industry (Carpenter et al. 1998). It is possible to reverse eutrophication in marine systems through reductions nitrogen and phosphorus inputs, but recovery can take a very long time due to a multitude of reasons (Carpenter et al. 1998). The primary reservoir of long lasting stored excess nutrients in eutrophic systems is the sediment, which is an important source of NH_4 and PO_4 for estuarine macroalgae (Morand and Briand 1996).

Although the restoration battle in the Great Bay Estuarine System may be a long one, it should be waged. Successes have been seen in systems more heavily impacted than our own. Following restoration changes to the Tunis Lagoon, Tunisia, water nitrogen concentration dropped from 4.000 mg/L to 0.400 mg/L, while water phosphorus fell from 0.600 mg/L to 0.020 mg/L (Morand and Merceron 2005). *Ulva* production fell more than three fold in the two years following this change.

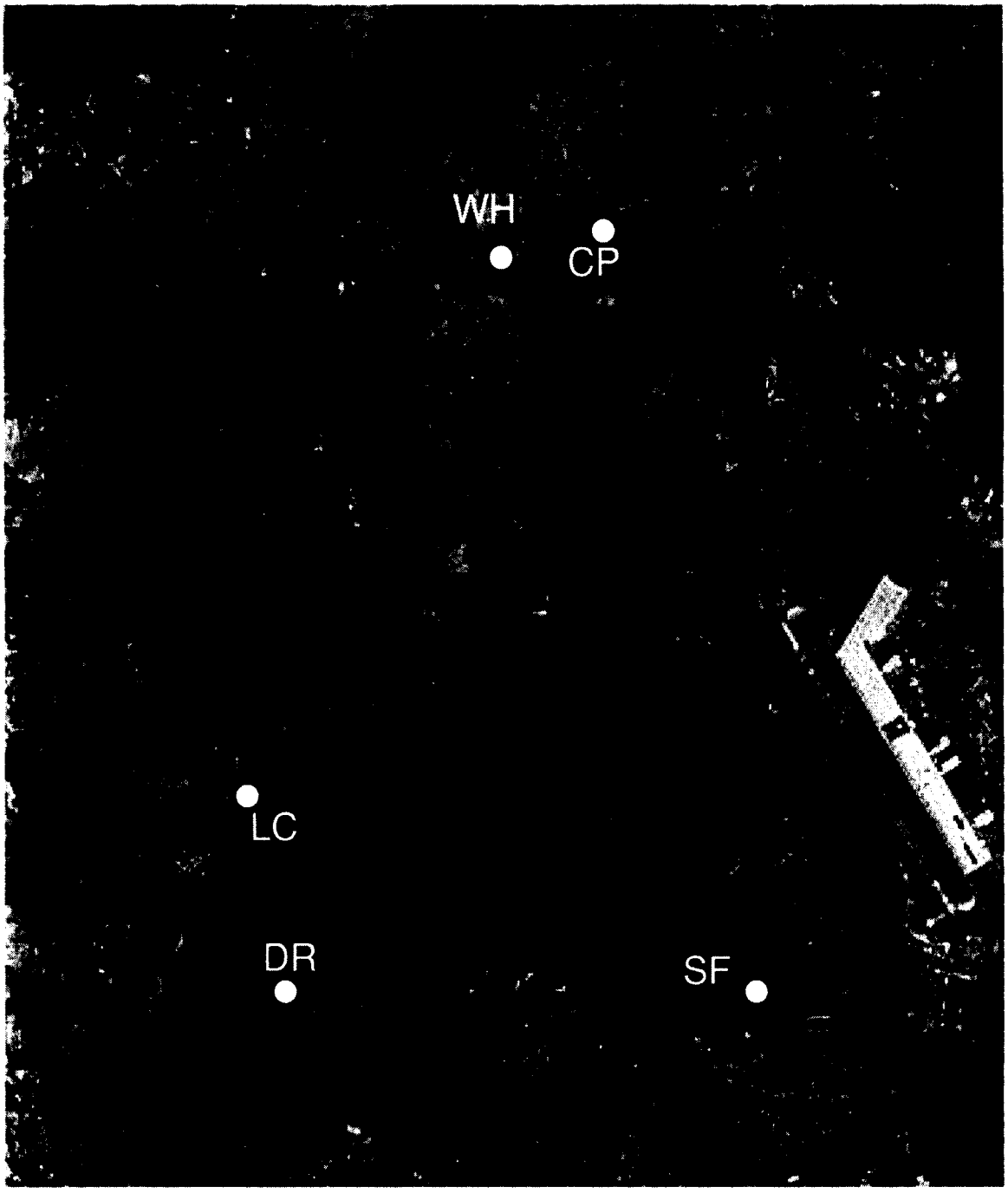

Figure 1 Map of the Great Bay Estuarine System, New Hampshire showing the locations of the five study sites. From top center and clockwise: Wagon Hill Farm (WH), Cedar Point (CP), Sunset Farm (SF), Depot Road (DR), and Lubberland Creek (LC)-- satellite image courtesy of Google Maps.

Table 1 GBES 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*

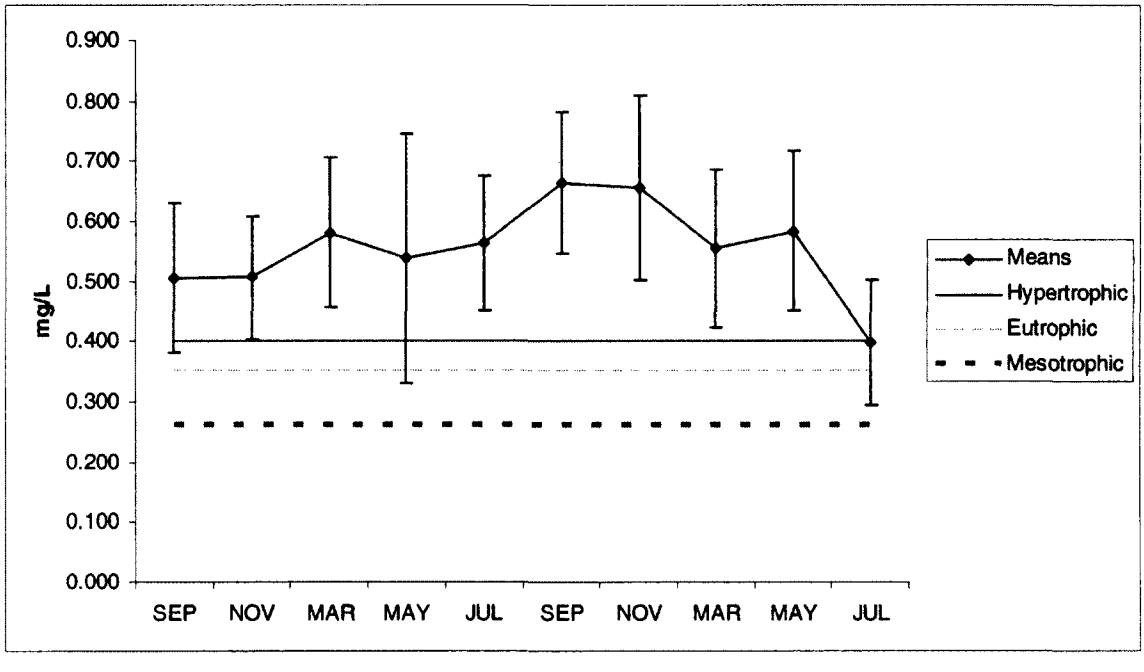

Figure 2 GBES water mean total nitrogen by month (2008-2010) averaged across the five study sites. Error bars represent standard error.

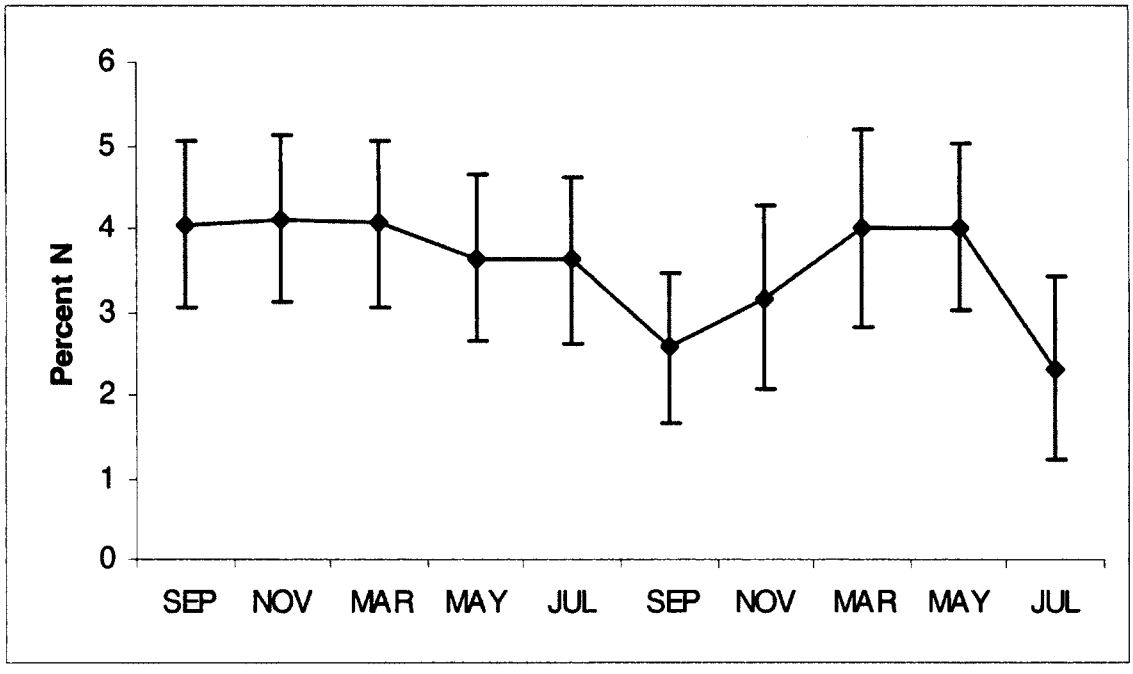

Figure 3 GBES *Ulva* tissue mean total nitrogen as percent dry weight (2008-2010) averaged across the five study sites. Error bars represent standard error.

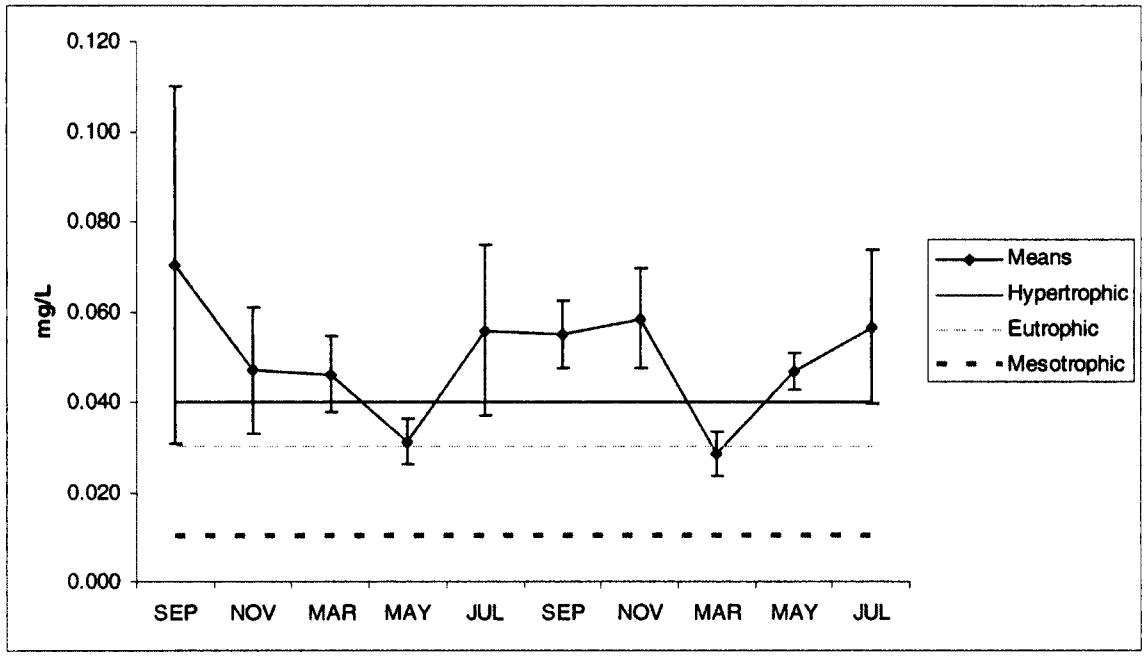

Figure 4 GBES water mean total phosphorus by month (2008-2010) averaged across the five study sites. Error bars represent standard error.

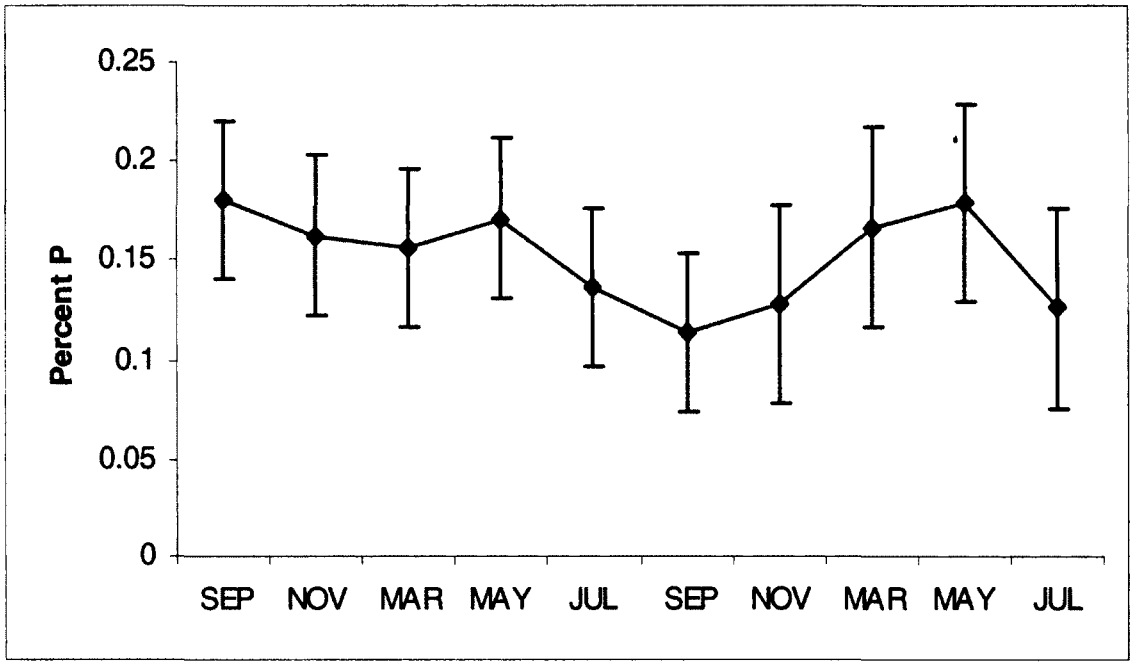

Figure 5 GBES *Ulva* tissue mean total phosphorus as percent dry weight (2008-2010) averaged across the five study sites. Error bars represent standard error.

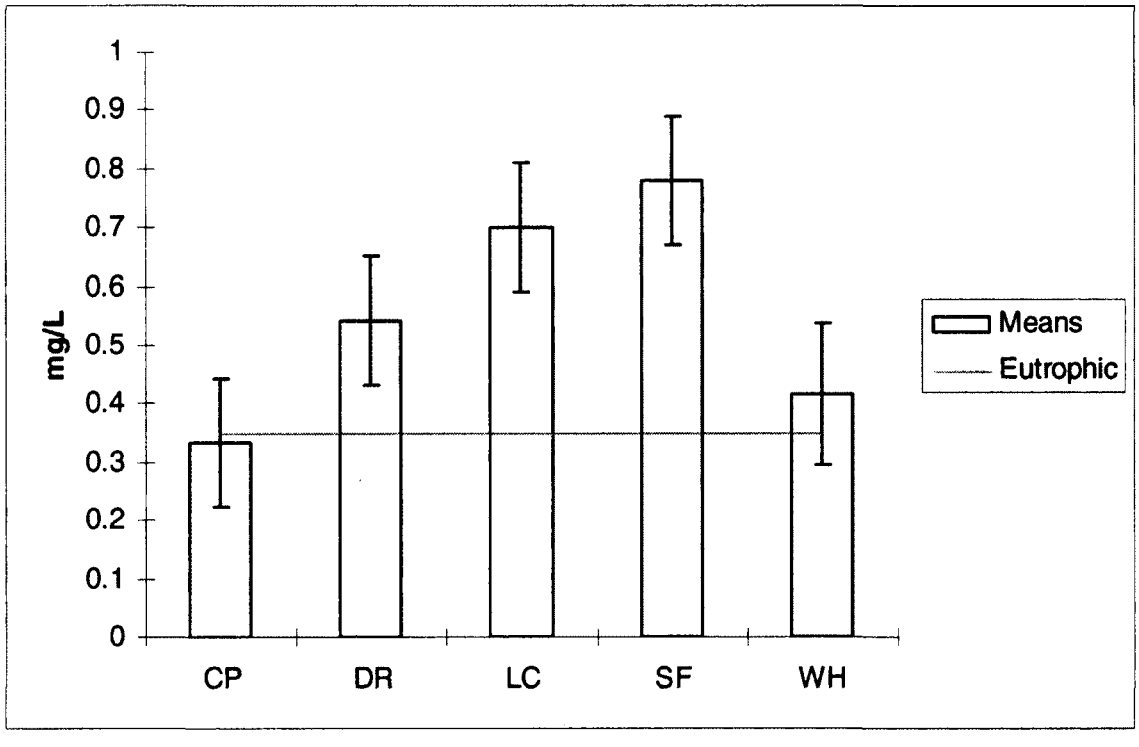

Figure 6 GBES water mean TN by site (2008-2010). Cedar Point (CP), Depot Road (DR), Lubberland Creek (LC), Sunset Farm (SF), Wagon Hill Farm (WH). Error bars represent standard error.

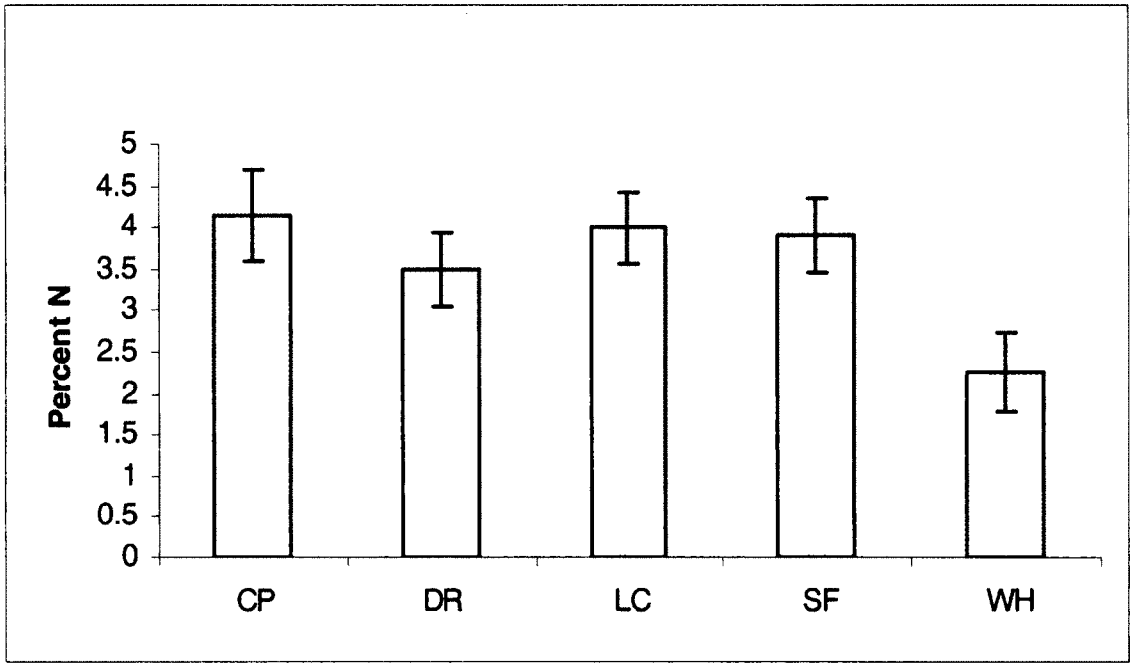

Figure 7 GBES *Ulva* tissue mean nitrogen by site (2008-2010). Cedar Point (CP), Depot Road (DR), Lubberland Creek (LC), Sunset Farm (SF), Wagon Hill Farm (WH). Error bars represent standard error.

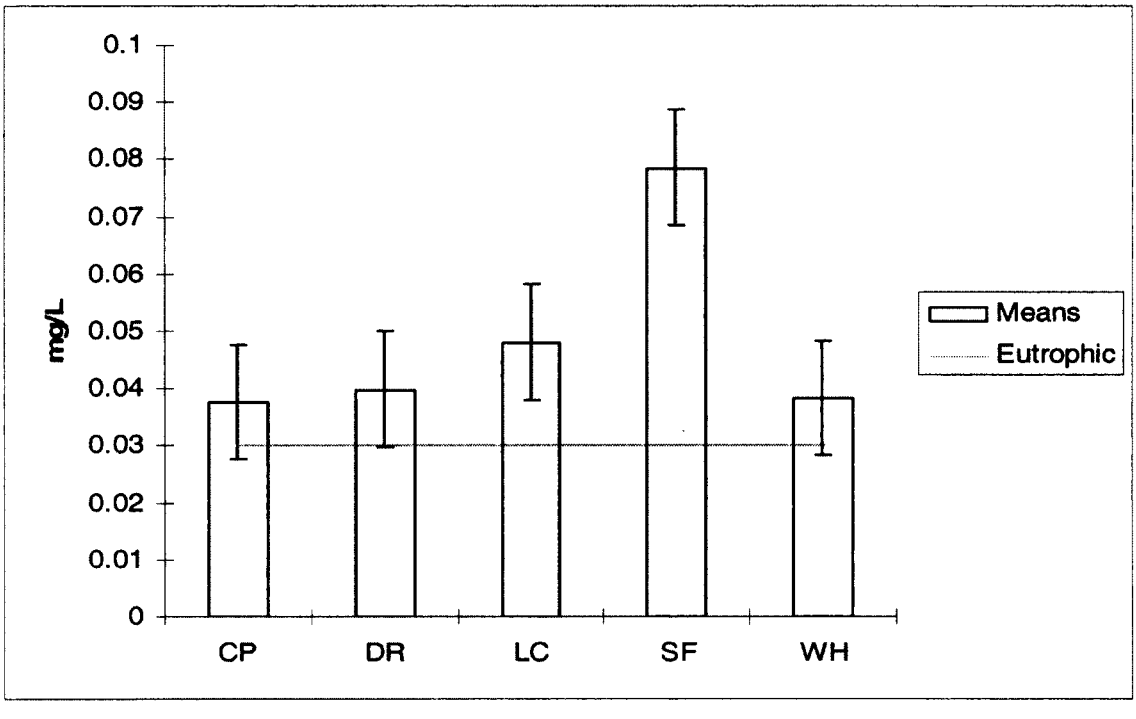

Figure 8 GBES water mean total phosphorus by site (2008-2010). Cedar Point (CP), Depot Road (DR), Lubberland Creek (LC), Sunset Farm (SF), Wagon Hill Farm (WH). Error bars represent standard error.

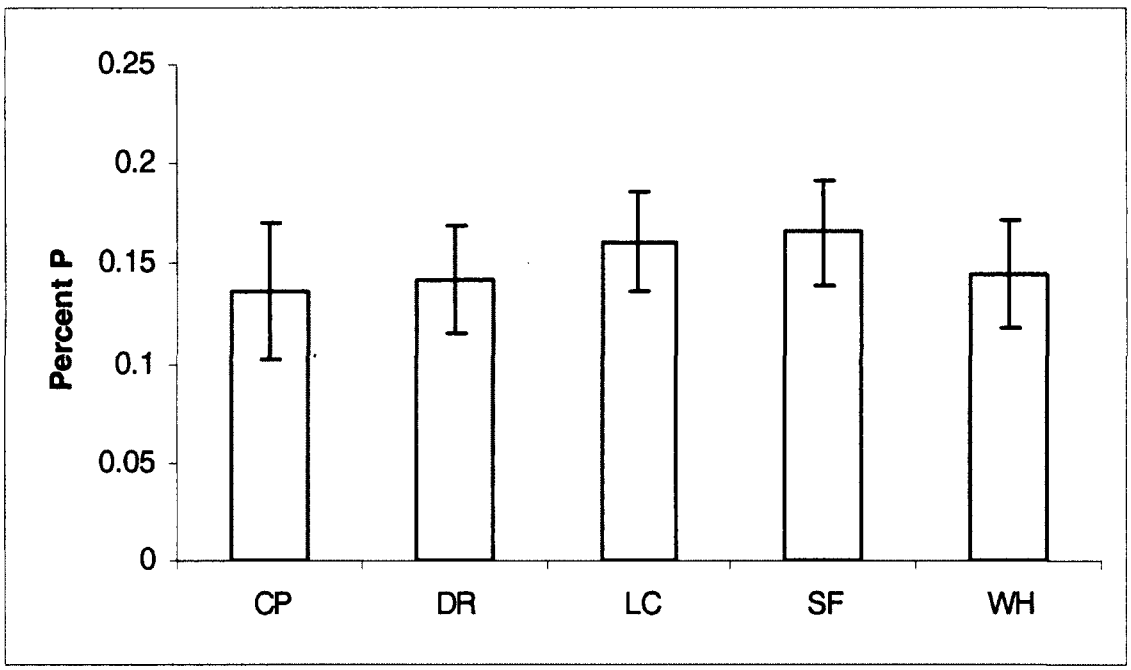

Figure 9 GBES *Ulva* tissue mean total phosphorus by site as percent dry weight (2008-2010). Cedar Point (CP), Depot Road (DR), Lubberland Creek (LC), Sunset Farm (SF), Wagon Hill Farm (WH). Error bars represent standard error.

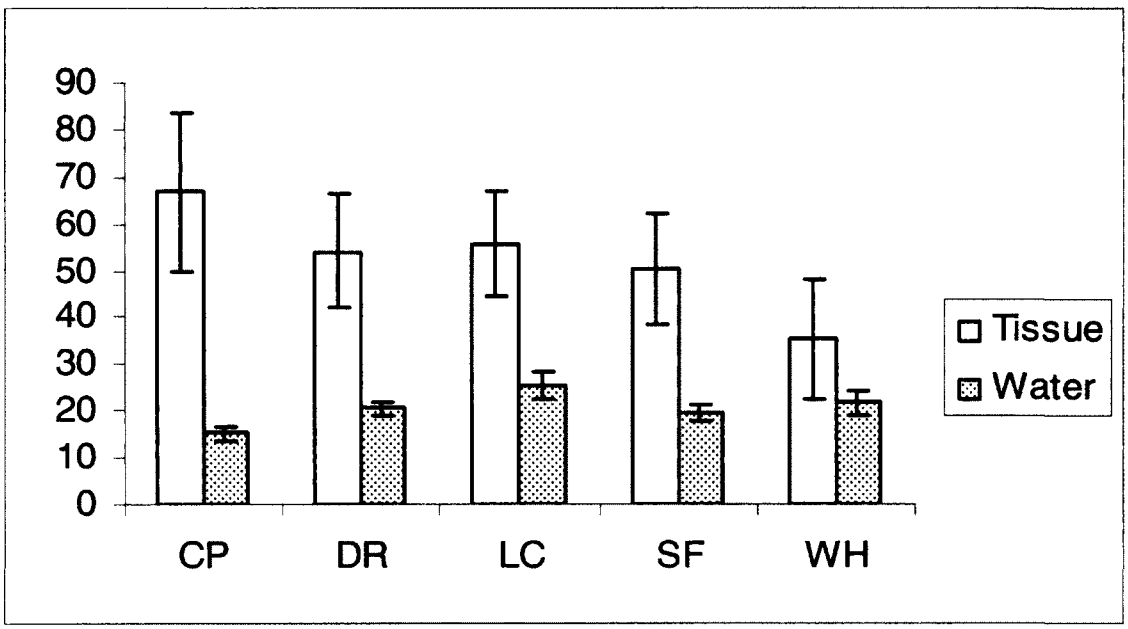

Figure 10 GBES *Ulva* tissue and water mean atomic N:P ratios by site for the two year study period (2008-2010). Cedar Point (CP), Depot Road (DR), Lubberland Creek (LC), Sunset Farm (SF), Wagon Hill Farm (WH). Error bars represent standard error.

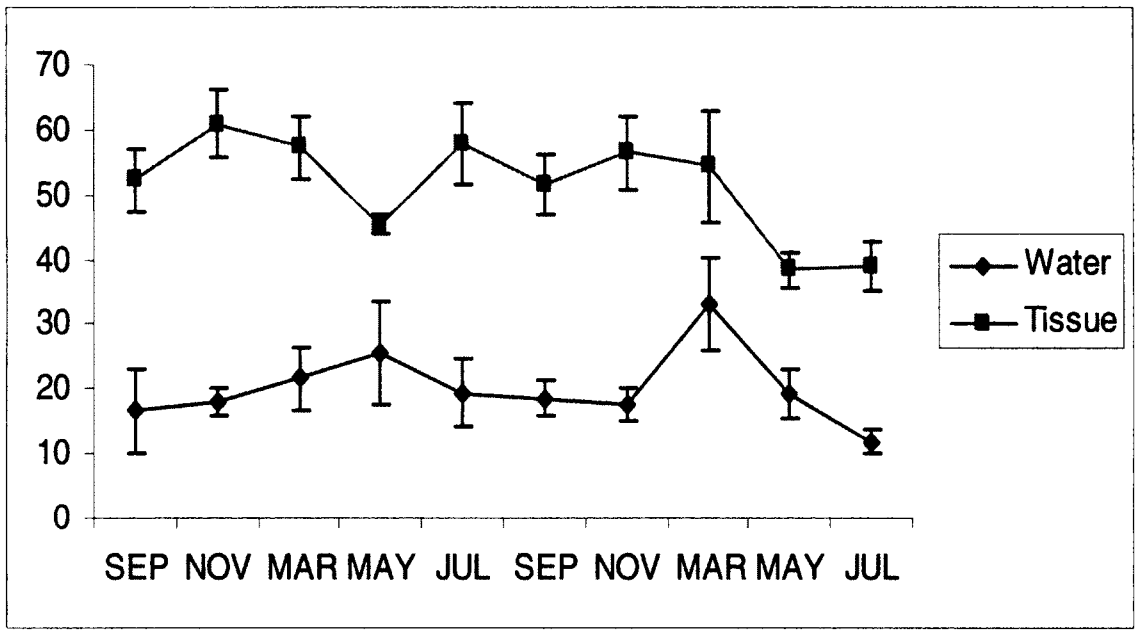

Figure 11 GBES *Ulva* tissue and water monthly atomic N:P ratios averaged across all study sites. Error bars represent standard error.

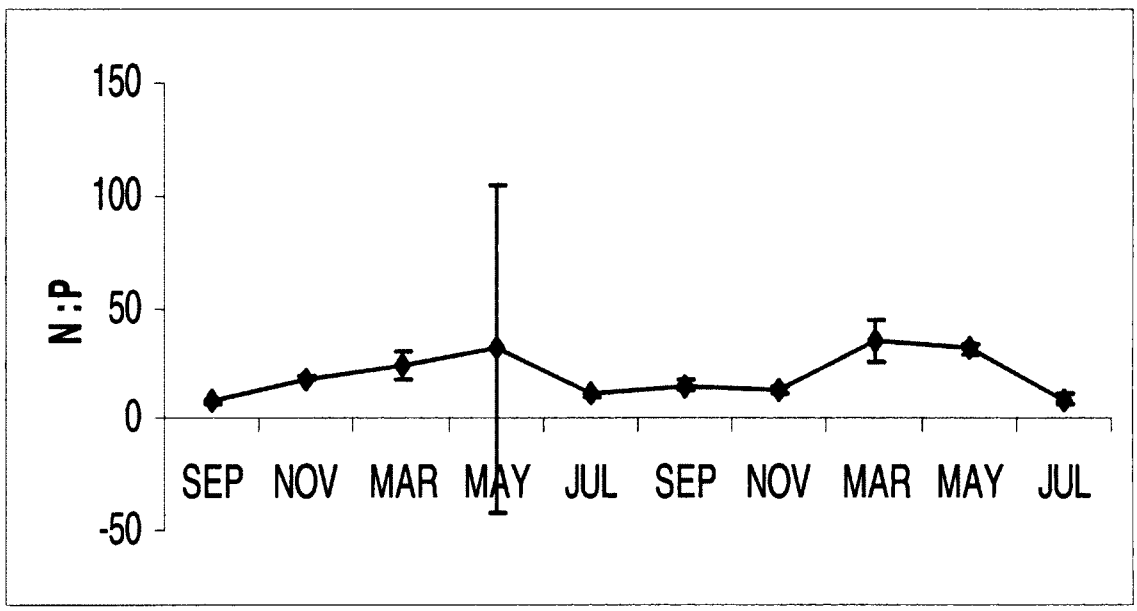

Figure 12 Sunset Farm water mean atomic N:P ratios by month (2008-2010). Error bars represent standard error.

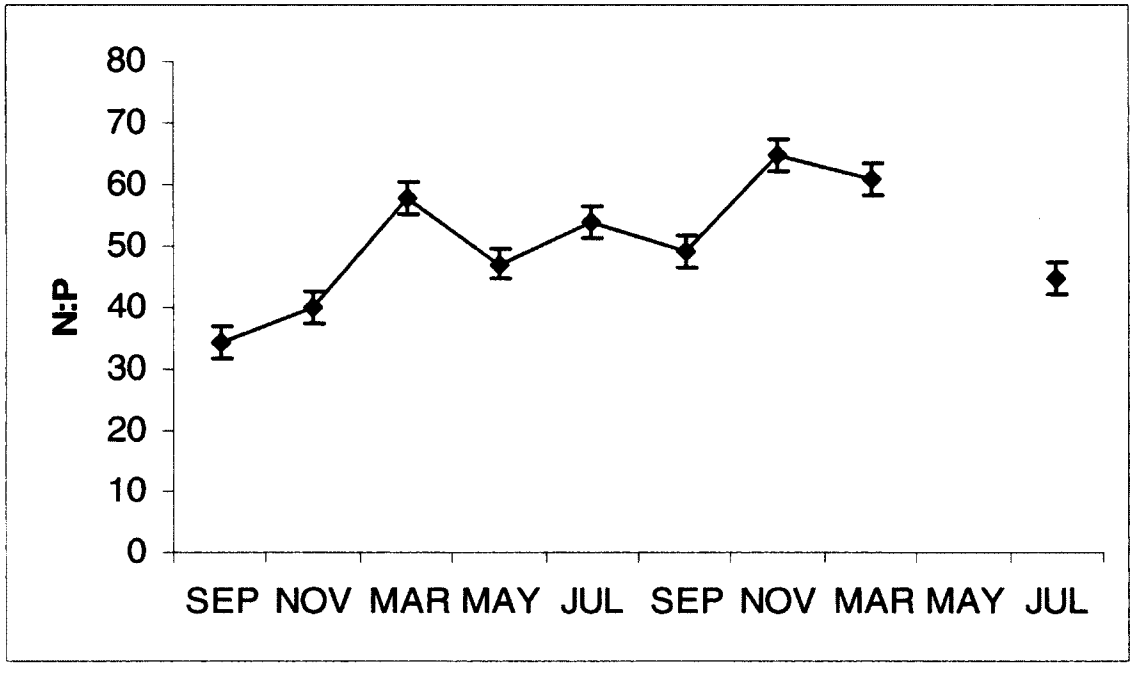

Figure 13 Sunset Farm *Ulva* tissue mean atomic N:P ratios by month (2008-2010). Error bars represent standard error.

Table 2 *Ulva* tissue mean monthly TN and TP from 2008-2010

	Cedar Point		Depot Rd		Lubberland Creek		Sunset Farm		Wagon Hill Farm	
	%N	%P	%N	%P	%N	%P	%N	%P	%N	%P
SEP	4.258	0.125	4.421	0.200	3.802	0.164	3.721	0.232		
NOV	3.879	0.118	4.505	0.151	4.397	0.237	3.862	0.208	3.875	0.098
MAR			4.579	0.129	4.325	0.164	4.615	0.172	2.718	0.161
MAY	4.205	0.185	3.452	0.183	4.112	0.170	3.968	0.180	2.451	0.137
JUL	4.980	0.113	4.103	0.146	3.901	0.156	3.650	0.146	1.498	0.122
SEP	3.320	0.098	2.578	0.117	2.518	0.108	2.633	0.118	1.783	0.130
NOV			3.272	0.132	3.819	0.127	3.472	0.110	2.057	0.160
MAR	4.302				4.985	0.144	4.689	0.165	2.326	0.160
MAY			2.257	0.122	5.470	0.229	5.307		2.724	0.188
JUL			2.333	0.112	2.717	0.122	3.203	0.153	0.976	0.116

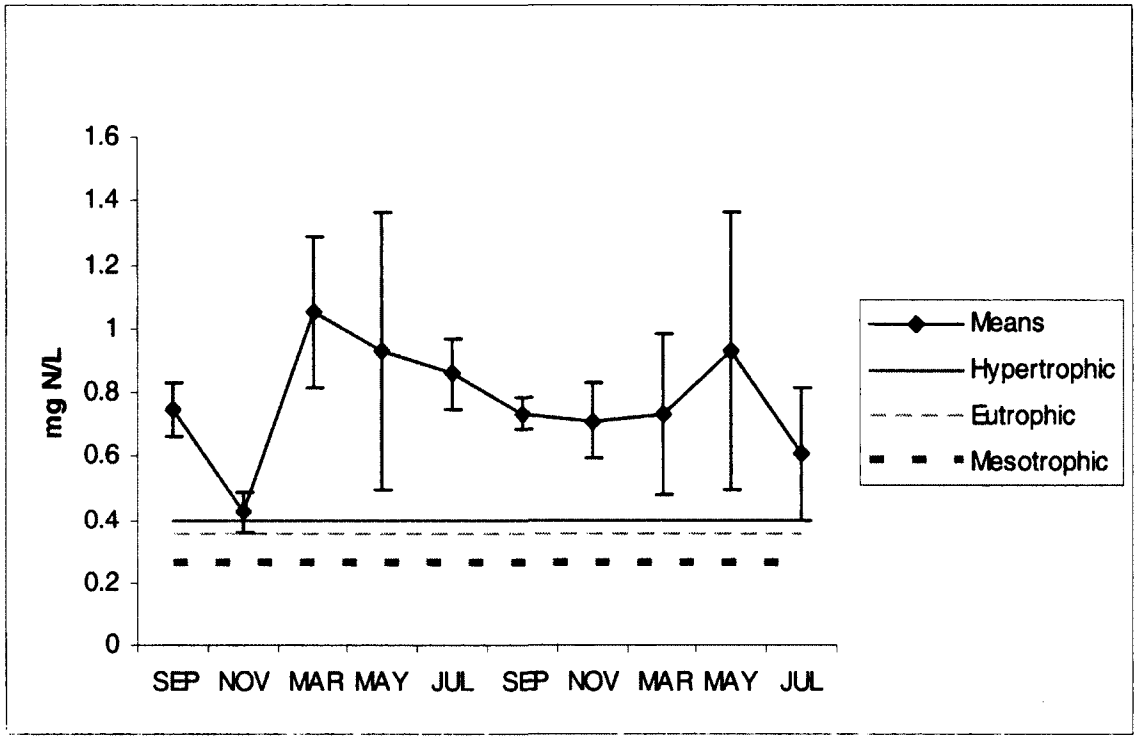

Figure 14 Sunset Farm water mean total nitrogen (2008-2010). Error bars represent standard error.

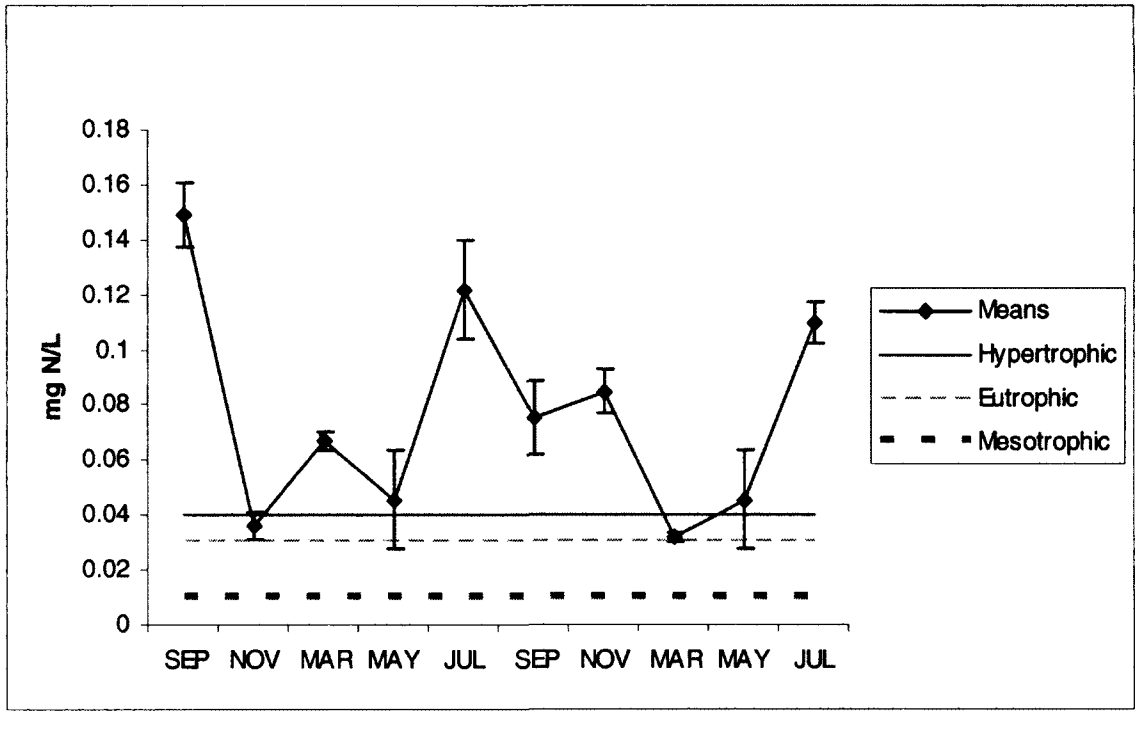

Figure 15 Sunset Farm water mean total phosphorus (2008-2010). Error bars represent standard error.

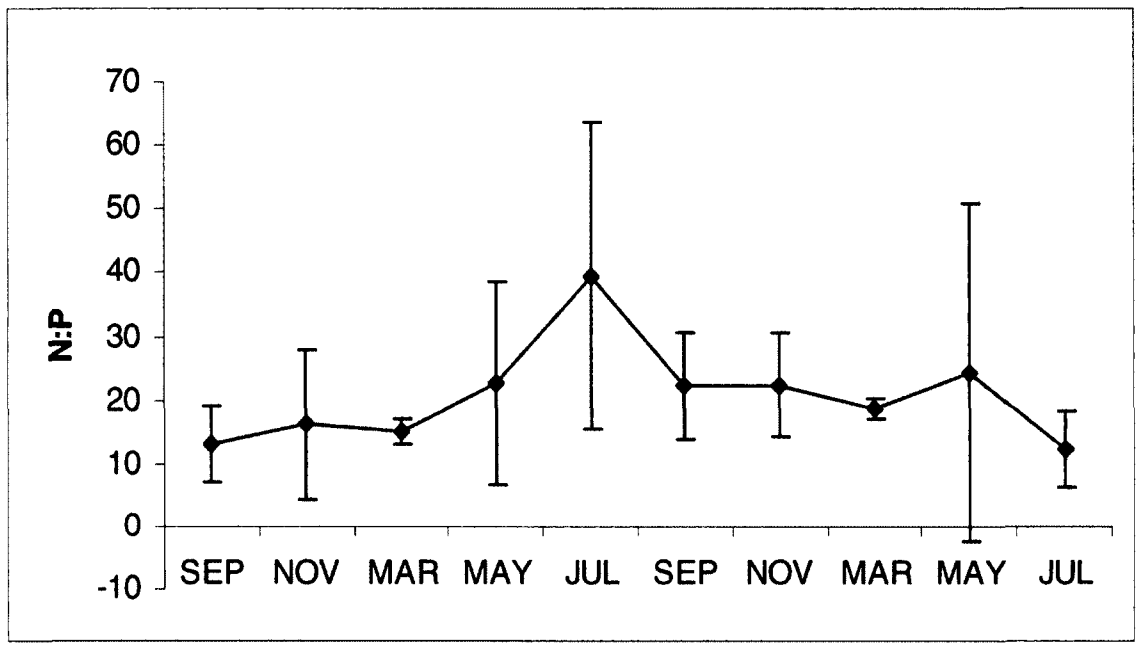

Figure 16 Depot Road water mean atomic N:P ratios by month (2008-2010). Error bars represent standard error.

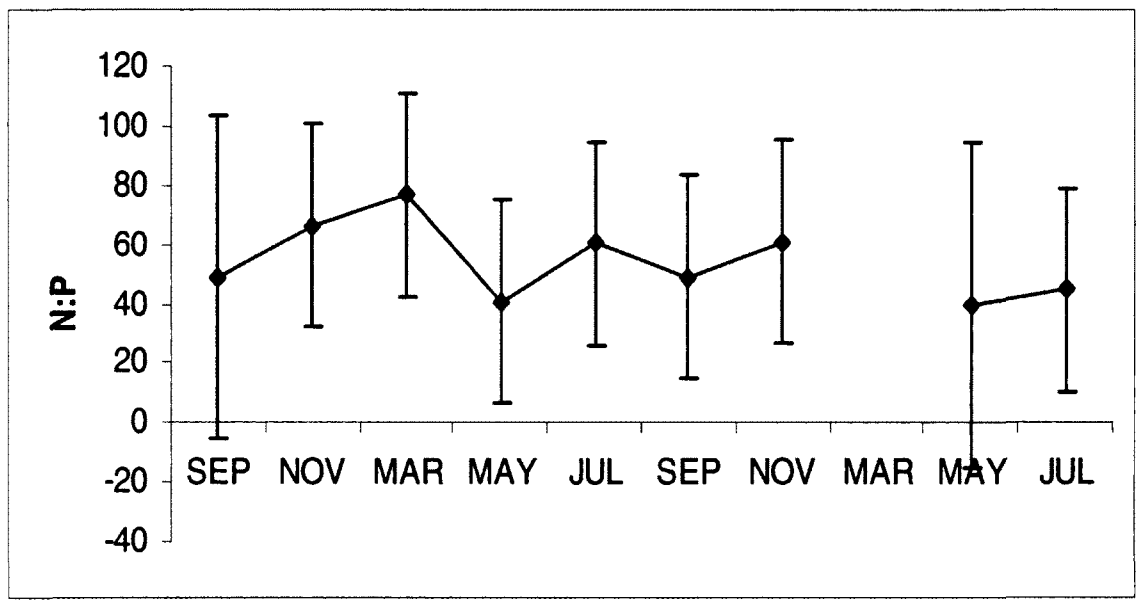

Figure 17 Depot Road *Ulva* tissue mean atomic N:P ratios by month (2008-2010). Error bars represent standard error.

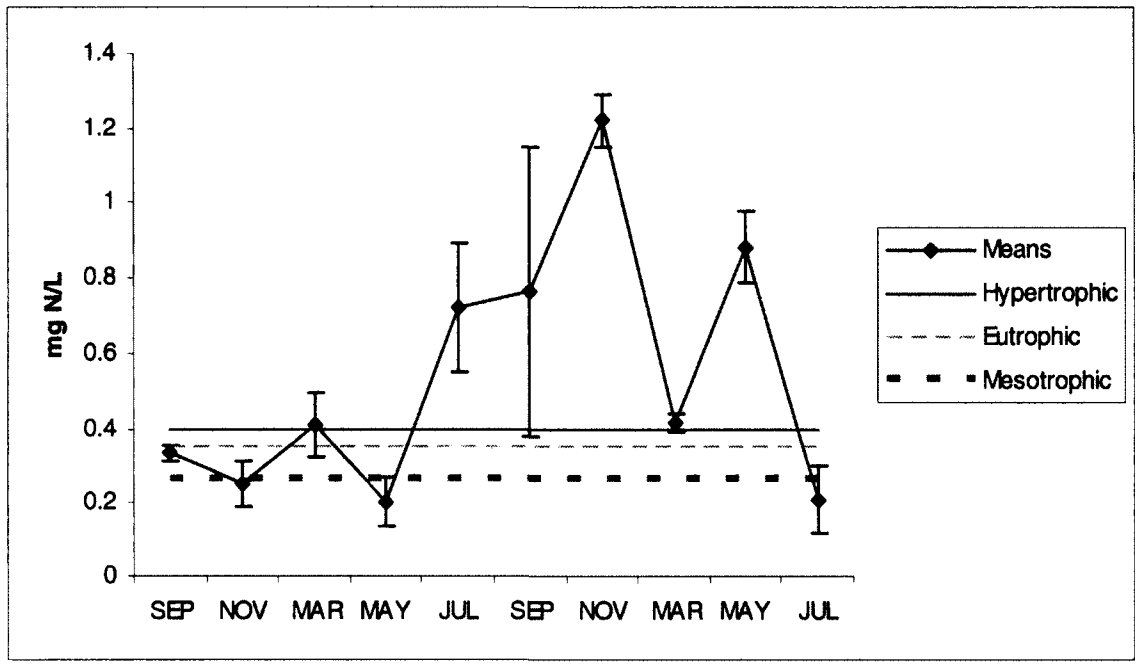

Figure 18 Depot Rd water mean total nitrogen (2008-2010). Error bars represent standard error.

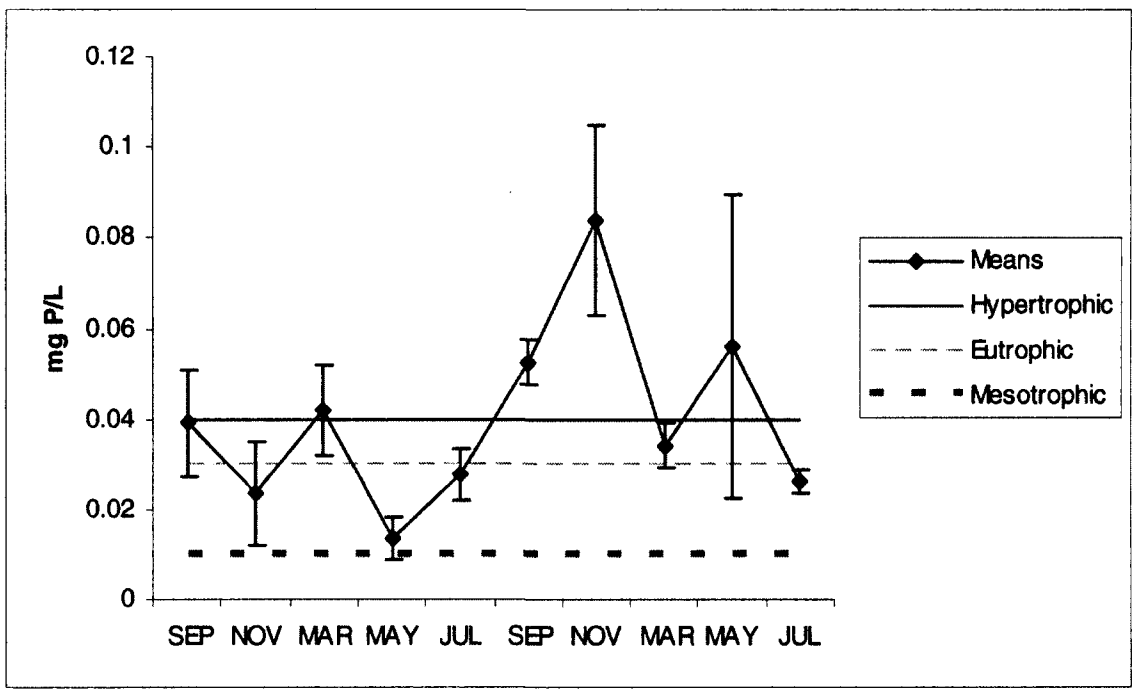

Figure 19 Depot Rd water mean total phosphorus (2008-2010). Error bars represent standard error.

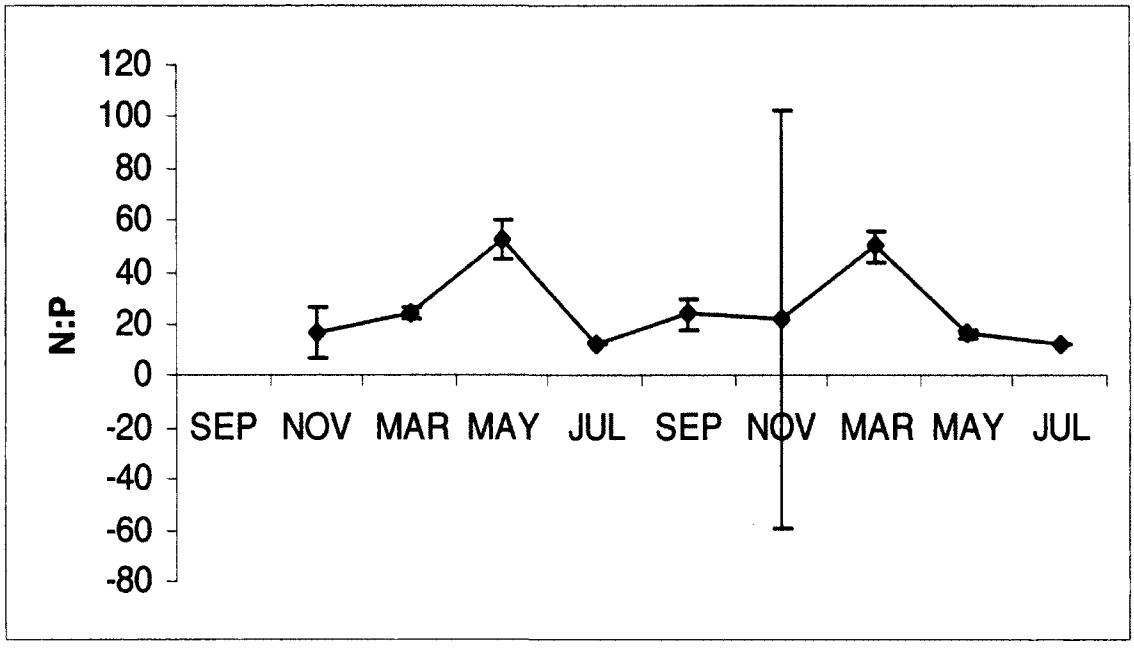

Figure 20 Lubberland Creek water mean monthly atomic N:P ratios (2008-2010). Error bars represent standard error.

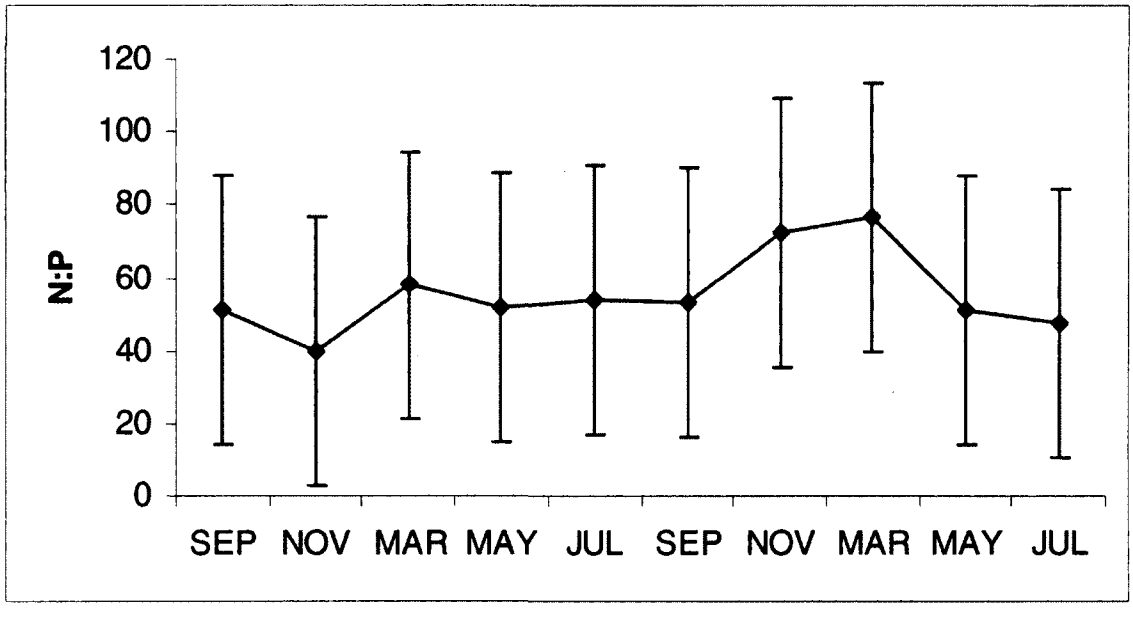

Figure 21 Lubberland Creek *Ulva* tissue mean monthly atomic N:P ratios (2008-2010). Error bars represent standard error.

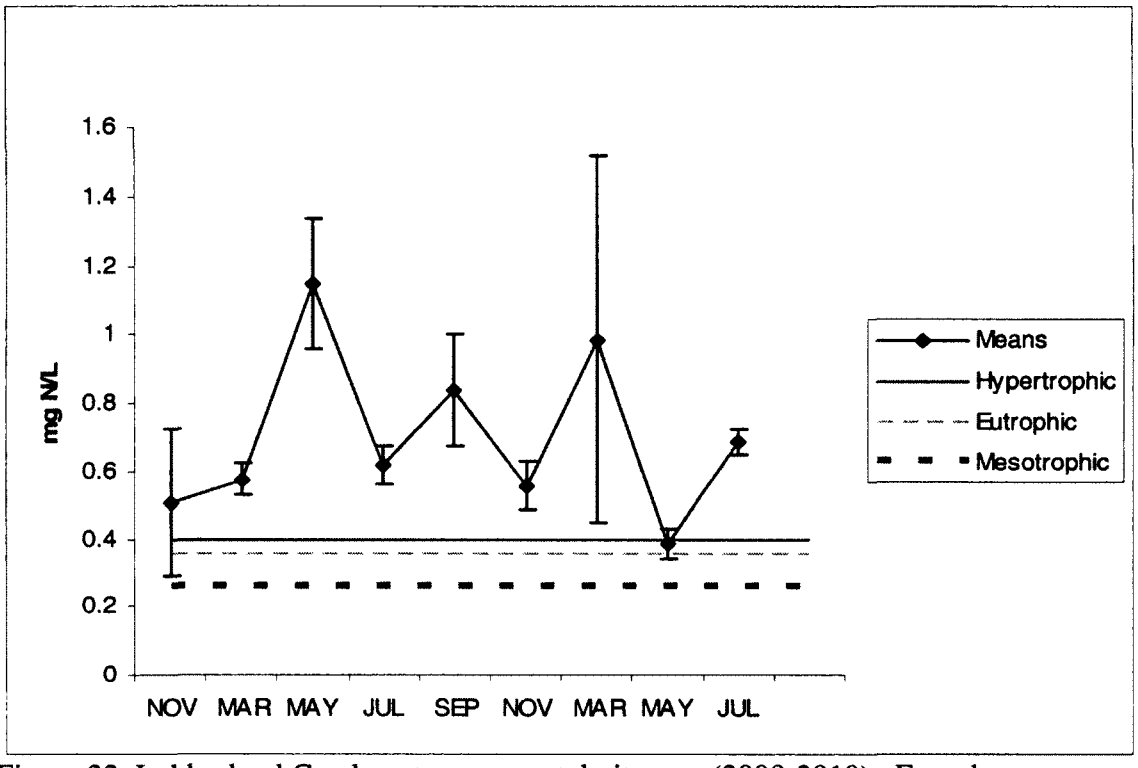

Figure 22 Lubberland Creek water mean total nitrogen (2008-2010). Error bars represent standard error.

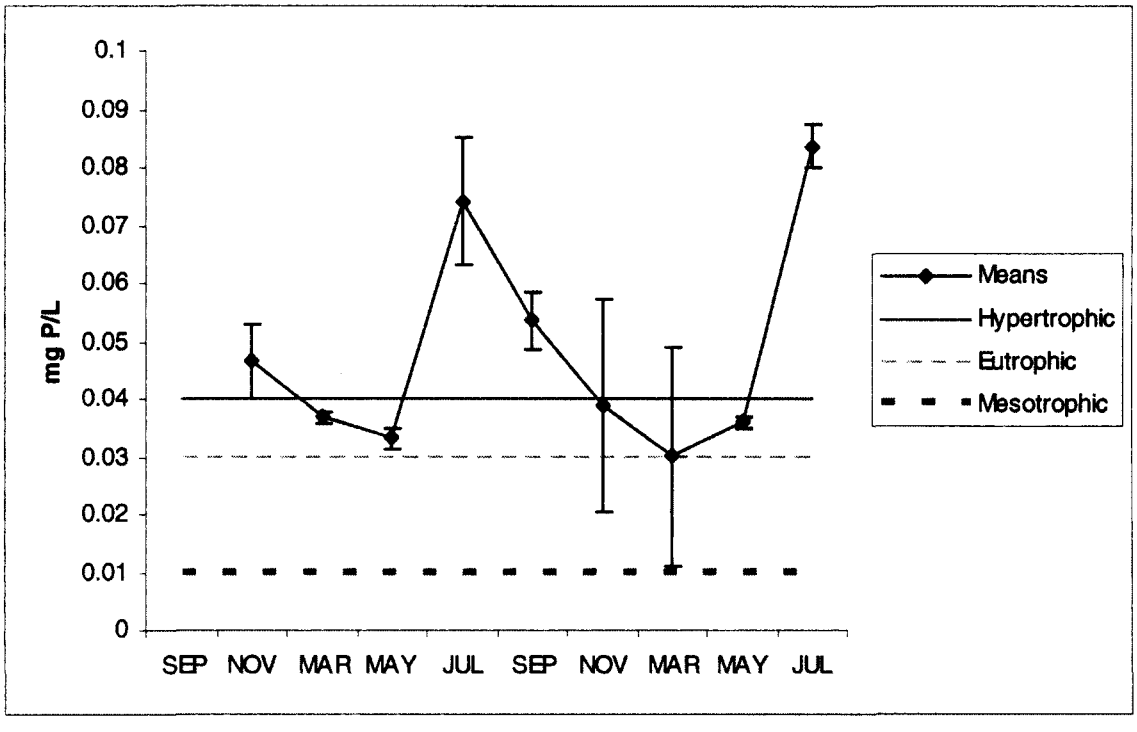

Figure 23 Lubberland Creek water mean total phosphorus (2008-2010). Error bars represent standard error.

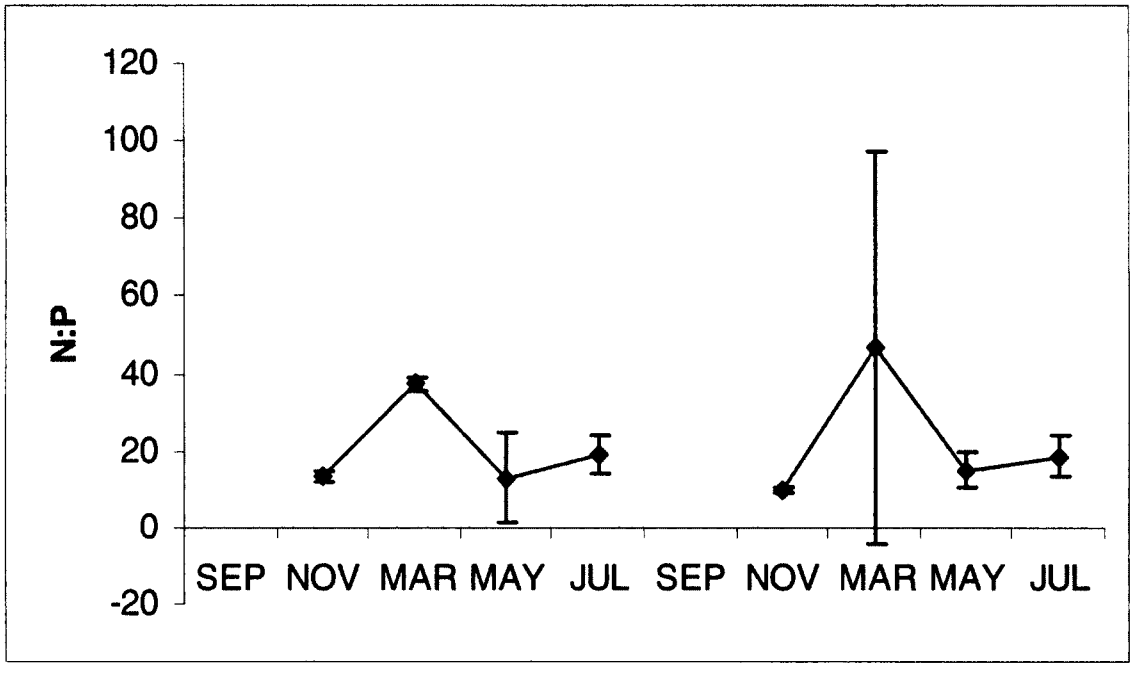

Figure 24 Wagon Hill Farm water mean monthly atomic N:P ratios (2008-2010). Error bars represent standard error.

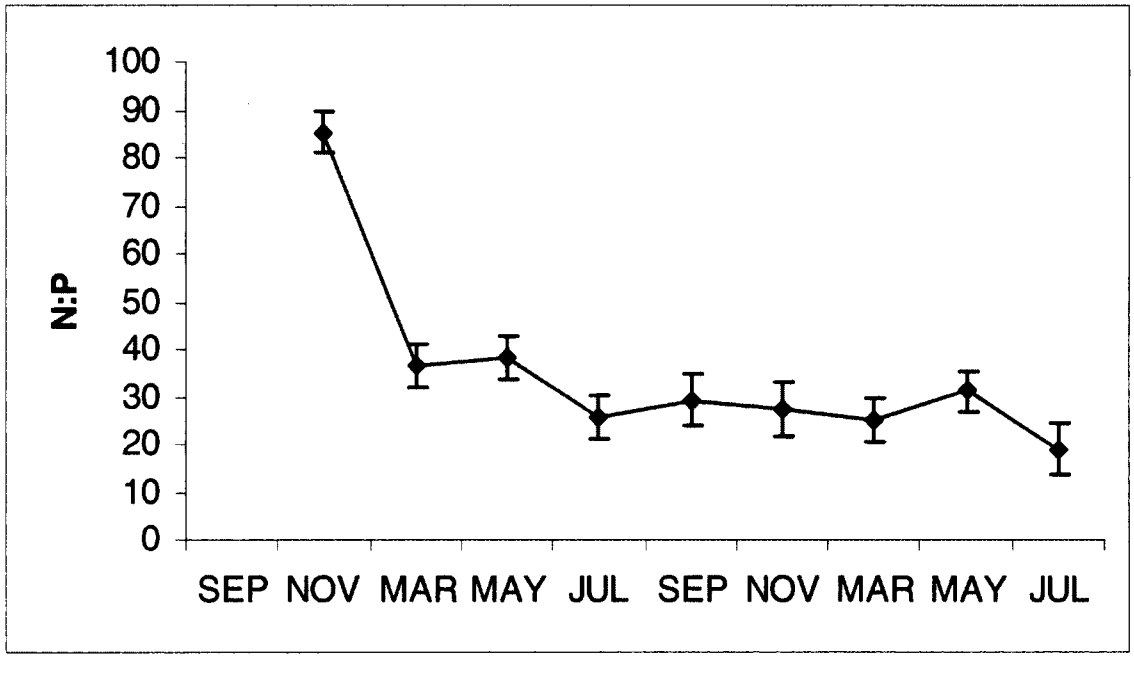

Figure 25 Wagon Hill Farm *Ulva* tissue mean monthly atomic N:P ratios (2008-2010). Error bars represent standard error.

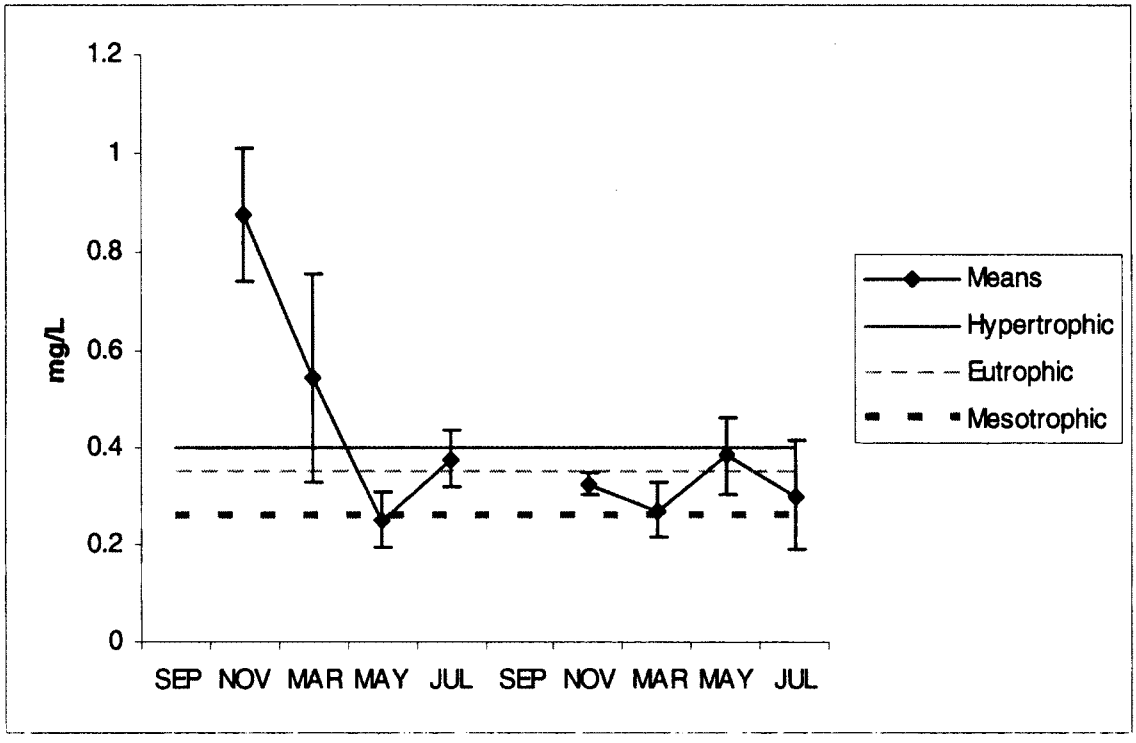

Figure 26 Wagon Hill Farm water mean total nitrogen (2008-2010). Error bars represent standard error.

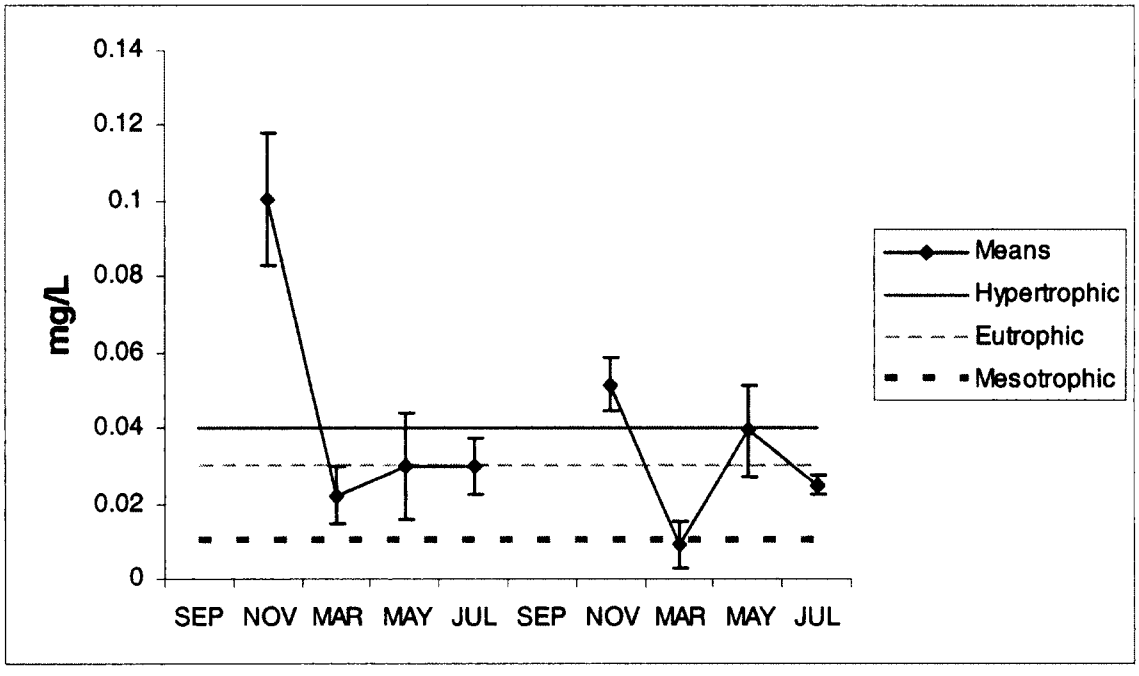

Figure 27 Wagon Hill Farm water mean total phosphorus (2008-2010). Error bars represent standard error.

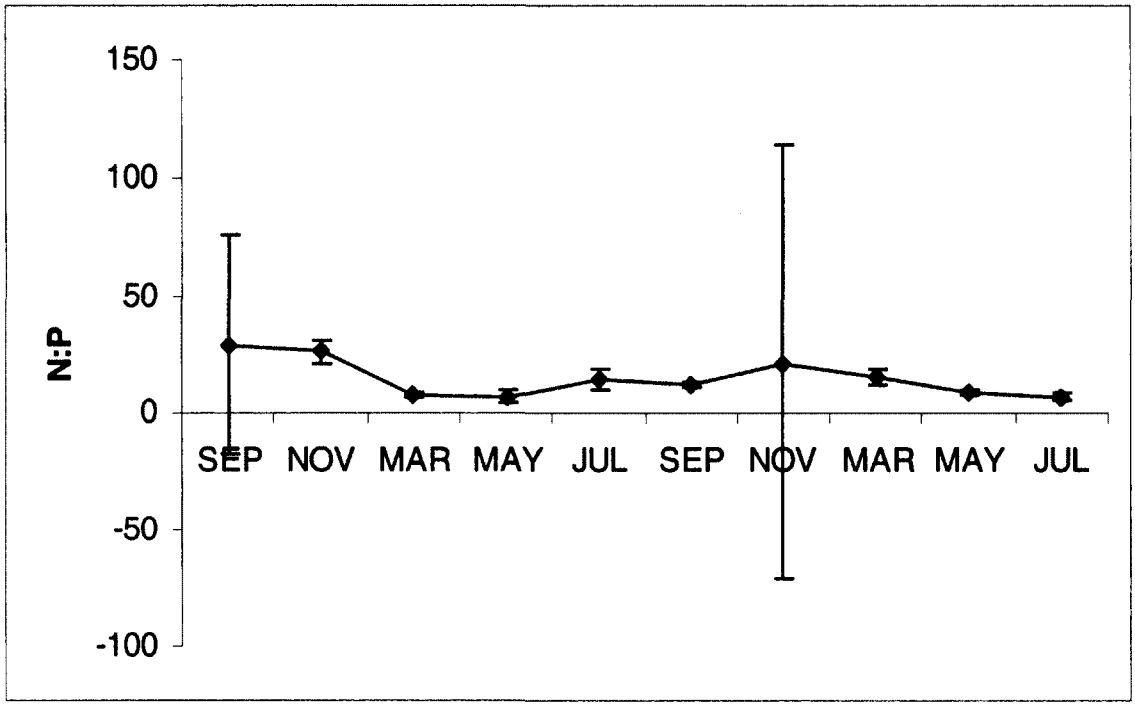

Figure 28 Cedar Point water mean monthly atomic N:P ratios (2008-2010). Error bars represent standard error.

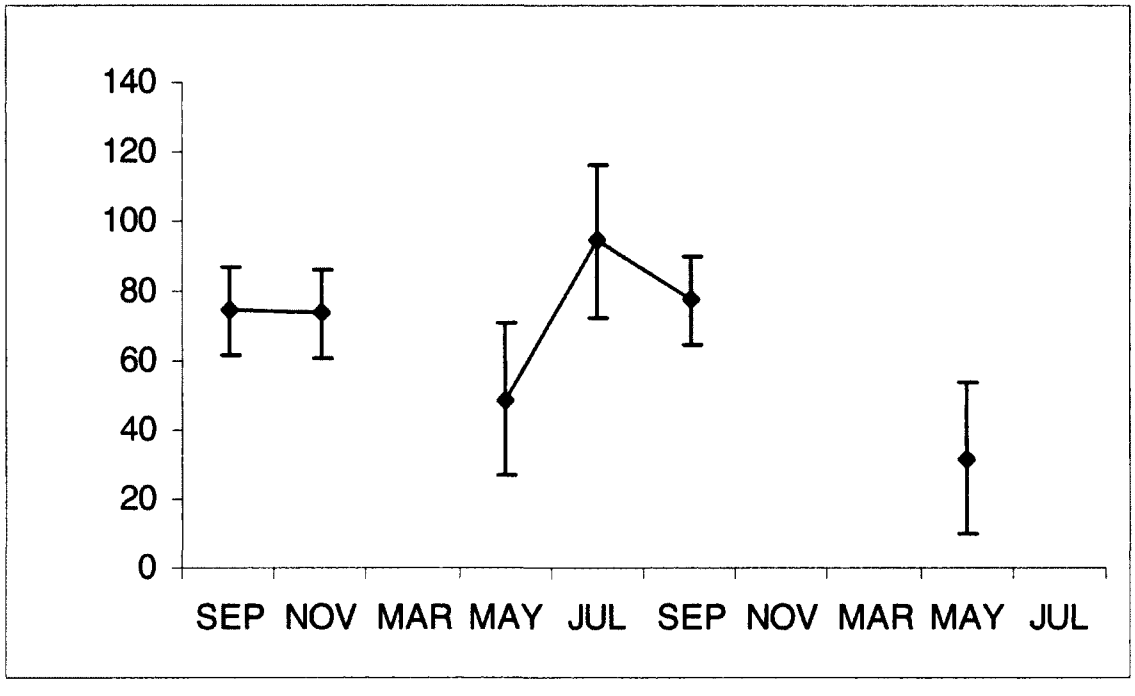

Figure 29 Cedar Point *Ulva* tissue mean monthly atomic N:P ratios (2008-2010). Error bars represent standard error.

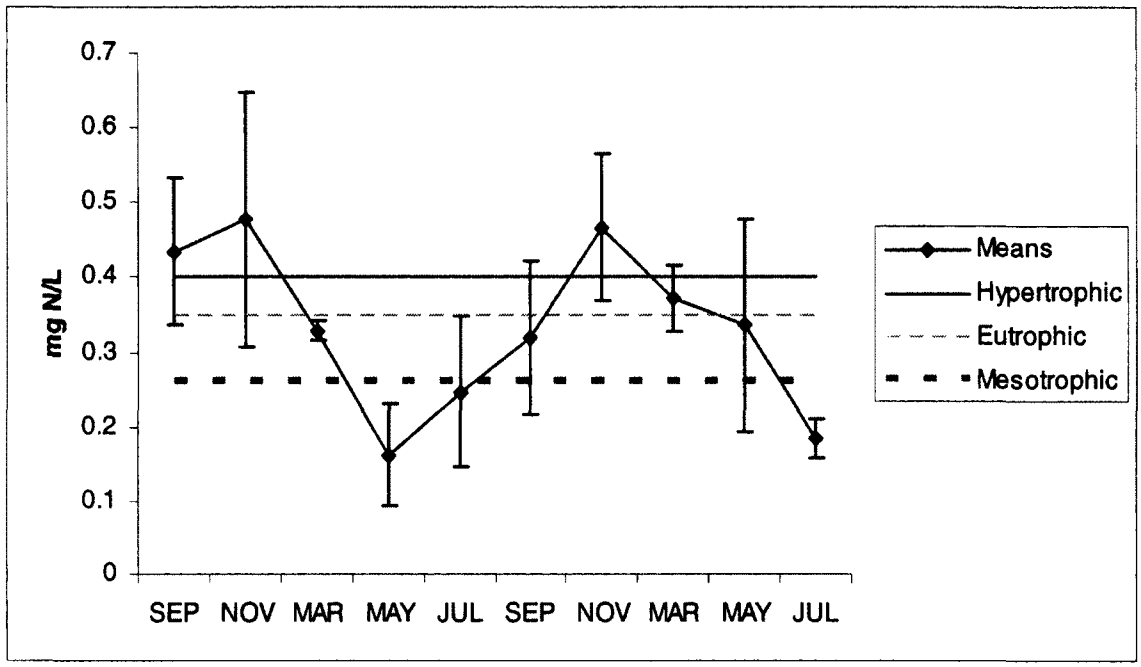

Figure 30 Cedar Point water mean total nitrogen (2008-2010). Error bars represent standard error.

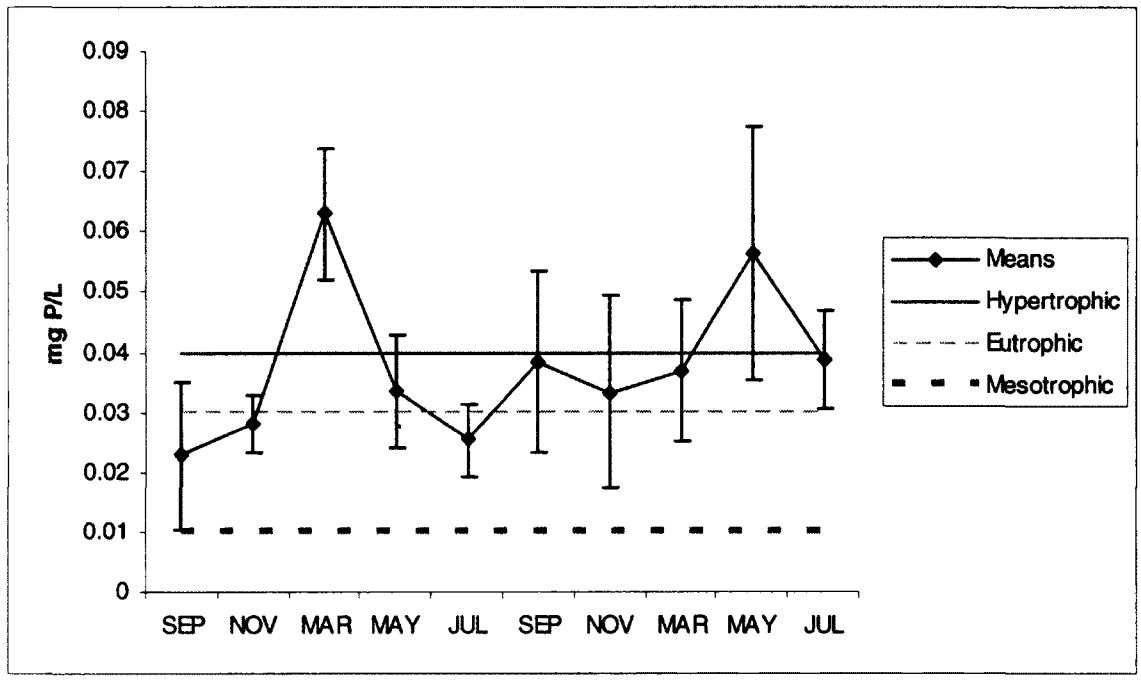

Figure 31 Cedar Point water mean total phosphorus (2008-2010). Error bars represent standard error.

Table 3 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

CHAPTER III

ULVA DISTRIBUTION, DENSITY, AND BIOMASS IN THE GREAT BAY ESTUARINE SYSTEM: AN HISTORICAL COMPARISON

Introduction

Excessive nutrient enrichment within the Great Bay Estuarine System 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 (Sawyer 1965; Kindig and Littler 1980; Raffaelli et al. 1998; Diaz et al. 2002; Raven and Taylor 2003). Many of these ulvoid green algae grow in eutrophied and hydrologically variable habitats like those found within the Great Bay Estuarine System. In summarizing effects of eutrophication on seaweed populations, Schramm and Nienhuis (1996) outlined three patterns that were expected to occur 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. foliose green algae or filamentous red algae); (2) a reduced diversity of associated flora and fauna; and (3) mass blooms of short-lived annuals (*Ulva*) or 'nuisance algae' such as *Gracilaria.*

Many earlier studies of the Great Bay Estuarine System 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 (1976, 1983) and West et al. (2001) provide 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).

The present study aimed to identify of all of the bloom forming *Ulva* species in the Great Bay Estuarine System, and, in the case of newly detected species, to determine approximate introduction dates. The study aimed to assess the abundance and distribution of *Ulva* 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.

Materials and Methods

Algal sampling was conducted within the intertidal zones at five sites in Great Bay Estuarine System, NH (Figure 1 cf. Chapter II). The sites were designated as Cedar Point, Wagon Hill Farm, Lubberland Creek, Depot Road, and Sunset Farm. At each site

and collection time, specimens of all conspicuous macroalgal species were gathered and identified based on morphological characteristics. Voucher specimens were collected outside of the transect lines for use in 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 a 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.

Additionally, an unused series of Kodachrome slides of Brackett's Point quadrats, taken during Hardwick-Whitman and Mathieson's (1983) study, were examined for further historical percent cover measures. The above point intercept methods were used in these analyses.

Percent cover data 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* 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 during 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 date, site, and quadrat number. In the laboratory, the algae (and marsh grasses) were sorted. Tentative identifications of macroalgae were made based upon macroscopic and microscopic characters using keys to the marine algae of the northwestern Atlantic (Villalard- Bohnsack 1995; Sears 2002). Specimens were 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 α=0.05 (Zar, 1996), followed with a Tukey's multiple comparison test. Time and site were the only factors considered in ANOVA.

Molecular Methods

Historical (Appendix E) and freshly collected (Appendix F) *Ulva* 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 a 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 were ITS2 F5.8S30 (5'-GCA ACG ATG AAG AAC GCA GC-3') and ITS2 R ENT26S (5'-GCT TAT TGA TAT GCT TAA GTT CAG CGG GT-3').

The PCR products were separated by electrophoresis on a SYBR®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 cf. Chapter II). These sites were Cedar Point (CP), Wagon Hill Farm (WH), Lubberland Creek (LC), Depot Road (DR), and Sunset Farm (SF). The sites varied in substrata, hydrographic regime, and human traffic (Table 1 cf. Chapter II).

The Cedar Point study transects were established on and adjacent to a public boat launch at the northern end of Little Bay (Figure 32). 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 33). 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 34). The tidal mudflat is home to large blooms of unattached *Ulva* and *Gracilaria* specimens in the fall. 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 is occasionally docked there during the summer months for educational purposes (Figure 35). *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 36) 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 during a typical year. In the winter, it is a popular access point for ice-fishermen.

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 five study sites (Appendix F).

Molecular screening of historical Great Bay Estuarine System herbarium specimens (Appendix E), 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 Estuarine System sites in another study (Hofmann et al. 2010), was revealed to have been present, yet unidentified in the Great Bay Estuarine System since 1967.

The mean *Ulva* biomass for each Great Bay Estuarine System study site was determined from September 2008- July 2010 (Figure 37, Appendix C). Differences between sites were statistically significant ($P<0.01$), 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 $\pm$ 228.9 SD) followed by Sunset Farm (97.1 g dry weight/m^2 $\pm$ 174.6 SD) and Depot Road (79.6 g dry weight/m^2 $\pm$ 102.1 SD). The Wagon Hill Farm site in the Little Bay had the lowest mean *Ulva* biomass for the study period (6.8 g dry weight/m^2 $\pm$ 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 38). Significant seasonal variation was observed ($P<0.01$). Seasonal *Ulva* biomass lows occurred in March of both years following ice out (2.3 g dry weight/m^2 $\pm$ 2.5 SD and 5.8 g dry weight/m^2 $\pm$ 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 $\pm$ 299.9 SD and 115.3 g dry weight/m^2 $\pm$ 116.6 SD).

The mean percent cover of *Ulva* followed the same trends across the sites as were observed for mean biomass (Figure 39, Appendix D), with significant differences between the sites ($P<0.01$). The greatest mean percent cover of *Ulva* during the two year study was observed at Lubberland Creek (39.3% $\pm$40.1 SD), followed by the other two sites in southern Great Bay, Depot Road (21.8% $\pm$ 32.1 SD) and Sunset Farm (21.0% $\pm$ 31.6 SD). Wagon Hill and Cedar Point, the northernmost sites, had the lowest mean *Ulva* cover of 11.2% $\pm$ 24.4 SD and 1.3% $\pm$ 6.7 SD.

Seasonal trends in mean percent cover of *Ulva* were observed throughout the study period (Figure 40), with significant differences between fall maxima and spring/summer minima ($P<0.01$). Peak cover occurred in November of 2008 and 2009

(38.7% ± 40.6 SD and 31.2% ± 42.6SD). The seasonal mean *Ulva* percent 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).

Great Bay Estuarine System mean macroalgal biomass varied by site. The Cedar Point site, though not home to *Ulva* blooms, had the highest total algal biomass, owing to its substantial population of *Ascophyllum nodosum* (Linnaeus) Le Jolis (Figure 41).

Within site mean *Ulva* biomass was calculated for each month of the study at the Sunset Farm site (Figure 42). Mean biomass varied with time ($P<0.01$), with peak levels during the fall of both 2008 and 2009. The seasonal maxima achieved in September 2008 was significantly greater ($P<0.01$) 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 monthly percent cover of *Ulva* was tracked at Sunset Farm (Figure 43), and significant seasonal differences were found ($P<0.01$). 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 in March of both study years (5.2% ± 8.7 SD and 0.7%± 2.5 SD).

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

Within site mean *Ulva* biomass was calculated for each month of the study at the Depot Road site (Figure 44). Mean biomass varied with time ($P<0.01$), with peak levels in the fall of both 2008 and 2009. Peak bloom was observed in November 2008 and November 2009 (170 g dry weight/m^2 $\pm$ 245.8 SD and 272.8 g dry weight/m^2 $\pm$ 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.

Ulva mean percent cover per month was tracked at Depot Road (Figure 45), and significant seasonal differences were found ($P<0.01$). Percent cover was maximal in the fall of both years, September 2008 (55.3% $\pm$ 35.7 SD) and November 2009 (42.8% $\pm$ 46.0 SD), and seasonal minima were observed in May 2009 (14.0% $\pm$ 23.4 SD) and March 2010 (0.1%$\pm$ 0.63 SD).

Trends in *Ulva* elevational distribution were examined throughout the study period. *Ulva* distribution favored mid-low elevations of approximately 0.05 m above mean low water ($P=0.01$). It should be noted that the vast majority of specimens observed at this site were free floating and able to move with prevailing tides.

Mean *Ulva* biomass was calculated for each month of the study at the Lubberland Creek site (Figure 46). Mean biomass varied with time ($P<0.01$), with peak values measured in the fall of both 2008 and 2009. The peak value observed in November 2008 (733.8 g dry weight/m^2 $\pm$ 613.0 SD) was significantly greater ($P<0.01$) than that observed the following November (175.8 g dry weight/m^2 $\pm$ 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.

Ulva mean percent cover per month at Lubberland Creek (Figure 47) showed significant seasonal differences ($P<0.01$). Percent cover was maximal in November of both years (90.1% $\pm$ 18.4 SD and 54.0% $\pm$ 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% $\pm$ 27.9 SD). The seasonal low mean *Ulva* cover for the 2010 season was observed in March (3.1%$\pm$ 6.6 SD).

Ulva elevational distributions were examined throughout the study at the Lubberland Creek site. *Ulva* distribution was even throughout the site. As was true at the other southern Great Bay sites, the vast majority of the specimens located here were free floating and able to move with the water currents.

Mean *Ulva* biomass was estimated each month at the Wagon Hill Farm site (Figure 48). It varied with time ($P<0.01$), with only one distinct peak bloom in May 2010 (29.8 g dry weight/m^2 $\pm$ 64.5 SD). *Ulva* specimens on the transect lines at this site were mostly *Ulva intestinalis*, and they were found almost exclusively growing 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 peat islands. At this Oyster River site, the influence of water motion was greater than was seen at the three sites in southern Great Bay. Also, there was open water at this site throughout the majority of the winter months, but freezing of the mudflats and shoreline was common at low tide on cold days.

Ulva mean percent cover per month at Wagon Hill Farm (Figure 49) showed significant temporal differences ($P<0.01$). Percent cover increased between late fall and late spring during both years of the study. The *Ulva intestinalis* population at this site flourished during the cooler months and died back during the warmer summer periods. The mean *Ulva* cover was greatest in May of 2009 and 2010 (21.4% $\pm$ 31.3 SD and 16.1% $\pm$ 28.1 SD). The seasonal mean *Ulva* cover lows were recorded in July of 2009 and 2010 (2.7% $\pm$ 6.6 SD and 5.9% $\pm$ 14.0 SD).

Ulva elevational distributions were examined throughout the study at the Wagon Hill Farm site. Its 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. The site was comprised of a lower and upper stream bank, to which the bulk of the *Ulva* specimens were attached.

The mean *Ulva* biomass was recorded bi-monthly at Cedar Point site (Figure 50). It varied with time ($P<0.01$), with only one distinct peak occurring in September 2009 (134.3 g dry weight/m^2 $\pm$ 330.1 SD) with this consisting of some large clumps of *Ulva rigida* on lowest transect line. During 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 *Ascophylum nodosum* ecad *scorpiodes* and *Fucus vesiculosus* formed the dominant cover and made up the bulk of the site's algal biomass.

Ulva mean percent cover at Cedar Point (Figure 51) showed significant temporal differences were found ($P<0.01$), which matched those observed for mean *Ulva* biomass. The greatest mean percent cover occurred in September of 2008 (3.0% $\pm$ 6.0 SD) and

2009 (7.3% ± 15.8 SD). These seasonal maxima levels were dwarfed by the blooms observed in southern Great Bay.

Discussion

The molecular verification of the presence of *Ulva rigida, U. pertusa* and blade forms of *U. compressa* in the Great Bay Estuarine System 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 the Great Bay Estuarine System 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 the Great Bay Estuarine System (Reynolds 1971; 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. 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; Hofmann 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 *U. pertusa* and *U. rigida* have

been in the Great Bay Estuarine System since the time of the historical studies, the increases in blooms observed in this study cannot be attributed to species introductions.

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/m^2 DW. The same trend was seen in *Ulva* cover, with mean values of the southern sites between 20% and 40% for the duration of the study. As algal annual production should be estimated to be between 1.5 and 4.5 times the maximum yearly biomass (Sfriso et al. 1993), the total yearly *Ulva* production in the southern sites could be estimated to be between 210 and 630 g/m^2 DW.

Because the *Ulva* species 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 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 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. Such hydrodynamic differences between the northern and southern sites are likely a large factor in the different abundance patterns observed, given the nutrient and temperature regimes were similar in both areas. The drifting of free floating bloom-forming macroalgae is common. For example, *Ulva* species in the Prévost Lagoon (France) have been observed to start growing attached to the substratum,

only to become free floating in the early spring and summer months (Casabianca-Chassany, 1989).

Ulva mean biomass peaked in the fall of both 2008 and 2009, with values being significantly greater than during the spring and summer. The peak bloom for the study occurred in November 2008 with mean biomass values greater than 225 g/m^2 DW and percent cover greater than 38% when all sites were combined. Such a 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/m^2 DW (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/ m^2 DWat Cedar Point, West's (2001) November 1998 max of 41.7 g/m^2 DW, or Hardwick-Witman's (unpublished) September 1978 max cover of 0.6 % at Brackett's Point (based on analysis of quadrat slides taken at the southern estuary site between Depot Road and Sunset Farm).

The yearly differences in peak *Ulva* blooms, observed in this study, can occur for natural abiotic reasons such as between year changes in temperature, nutrient availability, or light conditions. Biotic factors can also be involved. Both abiotic and biotic factors have been attributed to large between year variations seen in other systems. *Ulva* biomass decreased in the Venice Lagoon, Italy, in the early 1990s, and in 1995, its peak biomass was 95% lower than that observed in the 80s. Such a decline did not induce an upswing in the abundance of other species, and it was thought to have been brought on by increased grazer pressure and sediment resuspension (Sfriso and Marcomini, 1996).

The *Ulva* biomass peaks in the Great Bay Estuarine System are comparable to other notable major blooms around the world. In Spain's Palmones River Estuary, *Ulva* biomass was 200 g/m^2 DW (Hernandez et al. 1997). *Ulva* biomass in Italy's Venice Lagoon (central area) it was 0.1-15 kg/m^2 WW (Sfriso and Marcomini 1996) or roughly 10-1,500 g/m^2 DW. In the Palude della Rosa sites within the Venice Lagoon, *Ulva* biomass reached 3.5 kg/m^2 WW (Tagliapietra et al. 1998) or roughly 350 g/m^2 DW. In Italy's Sacca di Goro, the biomass of *Ulva* and *Gracilaria* reach peaks of 500 g/m^2 (Morand and Briand 1996). *Ulva* biomass reached measures of 280 g/m^2 DW in Scotland's Ythan Estuary (Raffaelli et al. 1998). On the Breton coast of France, *Ulva* can reach abundances of 400 kg/m^2 with mat thicknesses of one meter (Briand 1991). In the Langstone Harbour in England, *Ulva* peak biomass was 35 g/m^2 DW (Lowthion et al. 1985), and in the Avon- Heathcote Estuary in Christchurch, New Zealand, *Ulva* blooms peaked at 130 g/m^2 DW (Steffensen 1976). Wet weights of *Ulva* biomass at peak bloom in Massachusetts were 650 g m^{-2}, 370 g/m^2 in Rhode Island, and 6 kg/m^2 in Connecticut (Morand and Briand 1996), while other *Ulva* blooms reached 400g/m^2 DW in Rhode Island (Thorne-Miller et al. 1983) and 500 g/m^2 WW in Connecticut (Welsh et al. 1982). Pregnall and Rudy (1985) measured average dry biomass of *Ulva* in an Oregon coastal region to be 300 g/m^2. In the Veerse Meere Lagoons of the Netherlands, *Ulva* species make up 90% of the peak macroalgae biomass found in the shallows (Nienhuis 1992). At peak production, *Ulva* species in the Prévost Lagoon (France) comprise 95% of the algal biomass in the system (Casabianca-Chassany 1989). In Maine, *Ulva* mats have been measured at thicknesses of 8-10 cm (Vadas and Beal 1987), while in Scotland's Firth of Clyde, *Ulva* mats of 15 to 20 cm thickness were measured (Perkins and Abbott, 1972).

Analysis of the monthly cover of all seaweeds within each site revealed peak *Ulva* blooms of unprecedented size. In the falls of 2008 and 2009 *Ulva* blooms in southern Great Bay dwarfed those observed in previous regional studies, with biomass and percent cover increases being substantial. Lubberland Creek's peak percent *Ulva* cover was 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). While the *Ulva* peaks the following fall were generally smaller, the abundance values still eclipsed those measured in previous studies.

In the northern study sites in the Great Bay Estuarine System, *Ulva* abundance changes since the earlier studies were less pronounced. At Wagon Hill Farm *Ulva* did not exhibit fall peaks, but instead the biomass remained below 5 g/m^2 DW throughout all but the last three months of the study. *Ulva* percent cover estimates at this 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 to those seen for Wagon Hill Farm, with low baseline values of around 5 g dry weight/ m^2 throughout the study. There was one exceptional spike in September 2009 of over 130 g/m^2 DW, which was higher than the max observed in several previous Little Bay studies [124 g/m^2 DW (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/m^2 DW at Cedar Point, and West's (2001) November 1998 max of 41.7 g/m^2 DW 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 because the bulk of the *Ulva* measured at Cedar Point in September 2009 was drift algae that had been recently deposited in the lower intertidal zone and likely washed away with the subsequent tides. As drift algae is often deposited close to its source, this leads one to wonder about the subtidal density of *Ulva* near the Cedar Point site.

In summary, three previously undetected distromatic blade-forming *Ulva* species,*U. rigida, U. pertussa*, and *U. compressa*, have been identified as having been in the Great Bay Estuarine System since 1966, 1967, and 1972, respectively. They have likely been included in subsequent GBES ecological studies under the category '*Ulva lactuca.*' Major increases in both mean and peak *Ulva* biomass and percent cover have occurred in the Great Bay Estuarine System, and these changes coincide with the increased eutrophication by nitrogen and phosphorus during the past two decades. Current nitrogen and phosphorus levels in the system are substantial enough to support even larger *Ulva* blooms than were observed, based on minimum growth requirements (cf. Chapter II). 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.

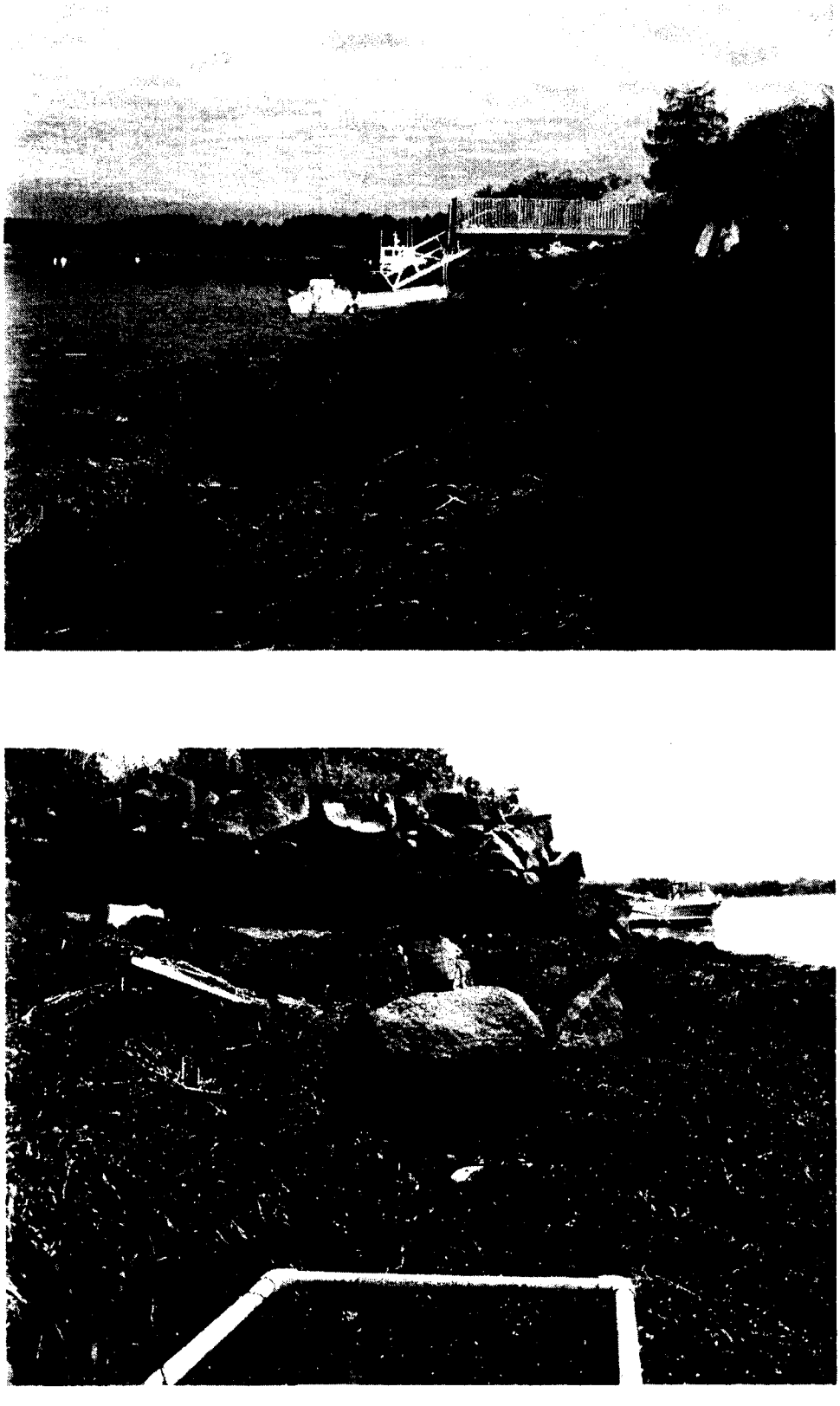

Figure 32 Cedar Point boat launch A) facing south B) facing north with boat launch and retaining wall.

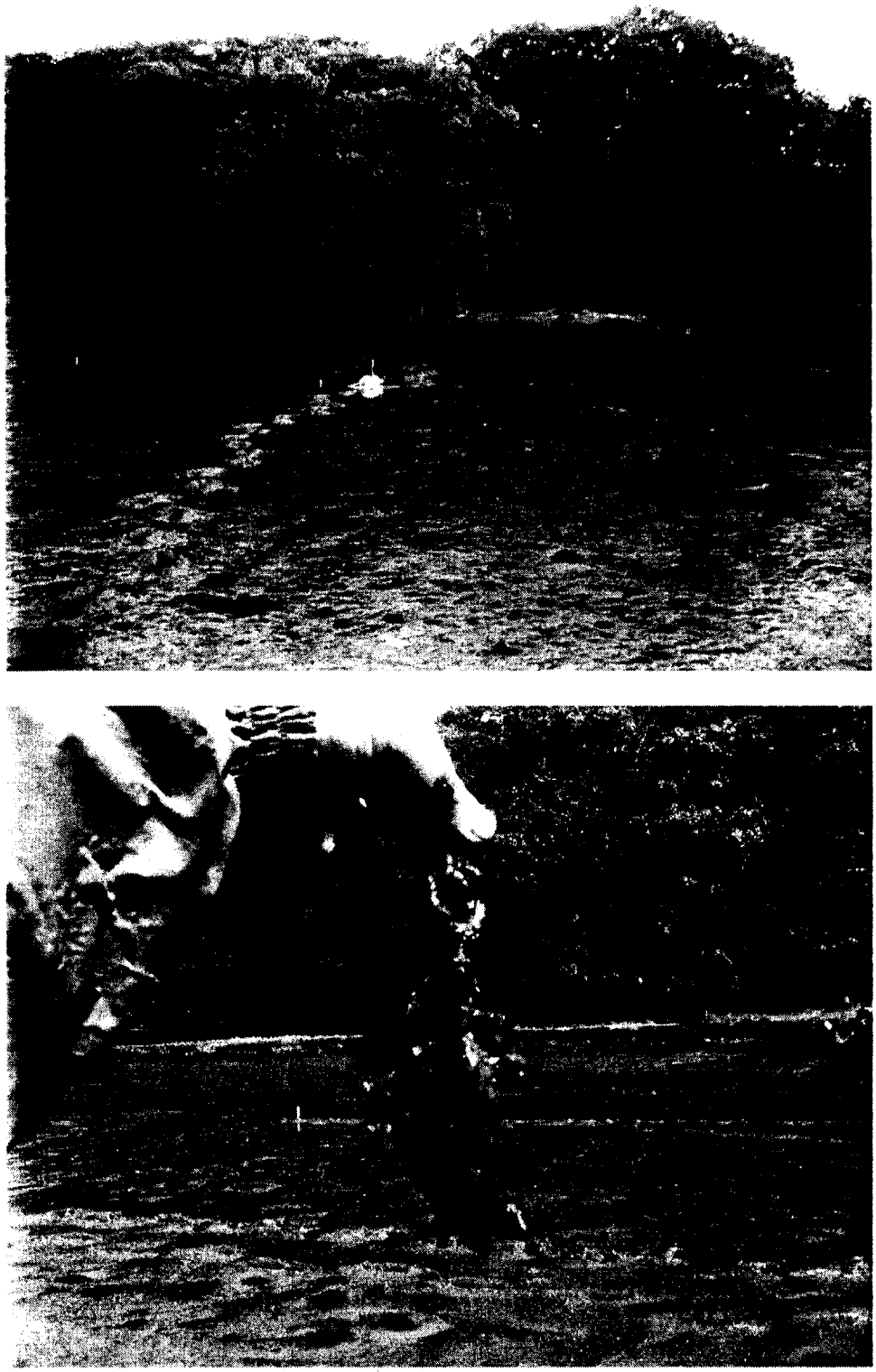

Figure 33 Wagon Hill Farm A) broad view of mudflat with transect line B) *Ulva* specimen found attached to shell.

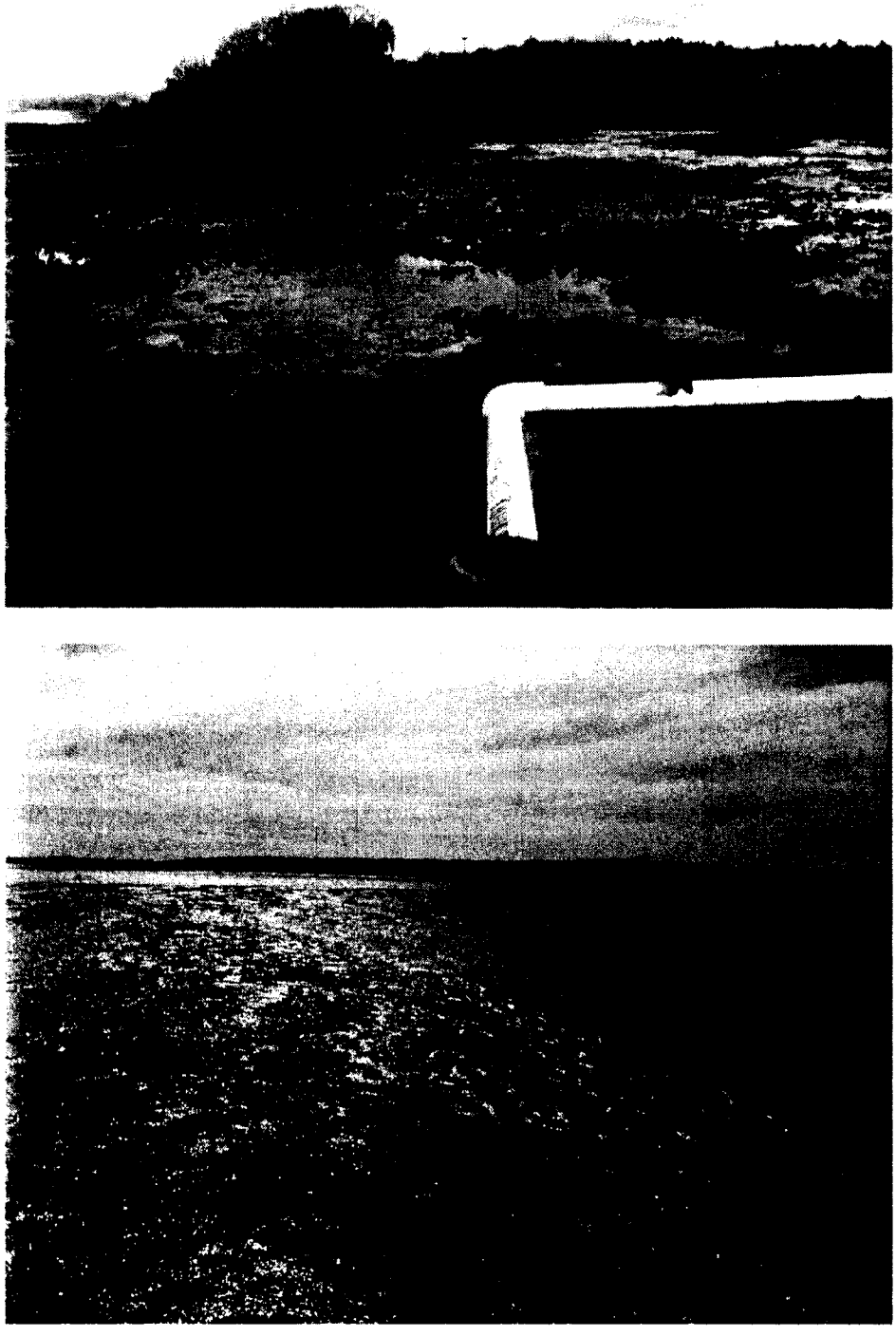

Figure 34 Lubberland Creek A) west facing, *Ulva* bloom (November 2008) B) east facing, two months earlier (September 2008).

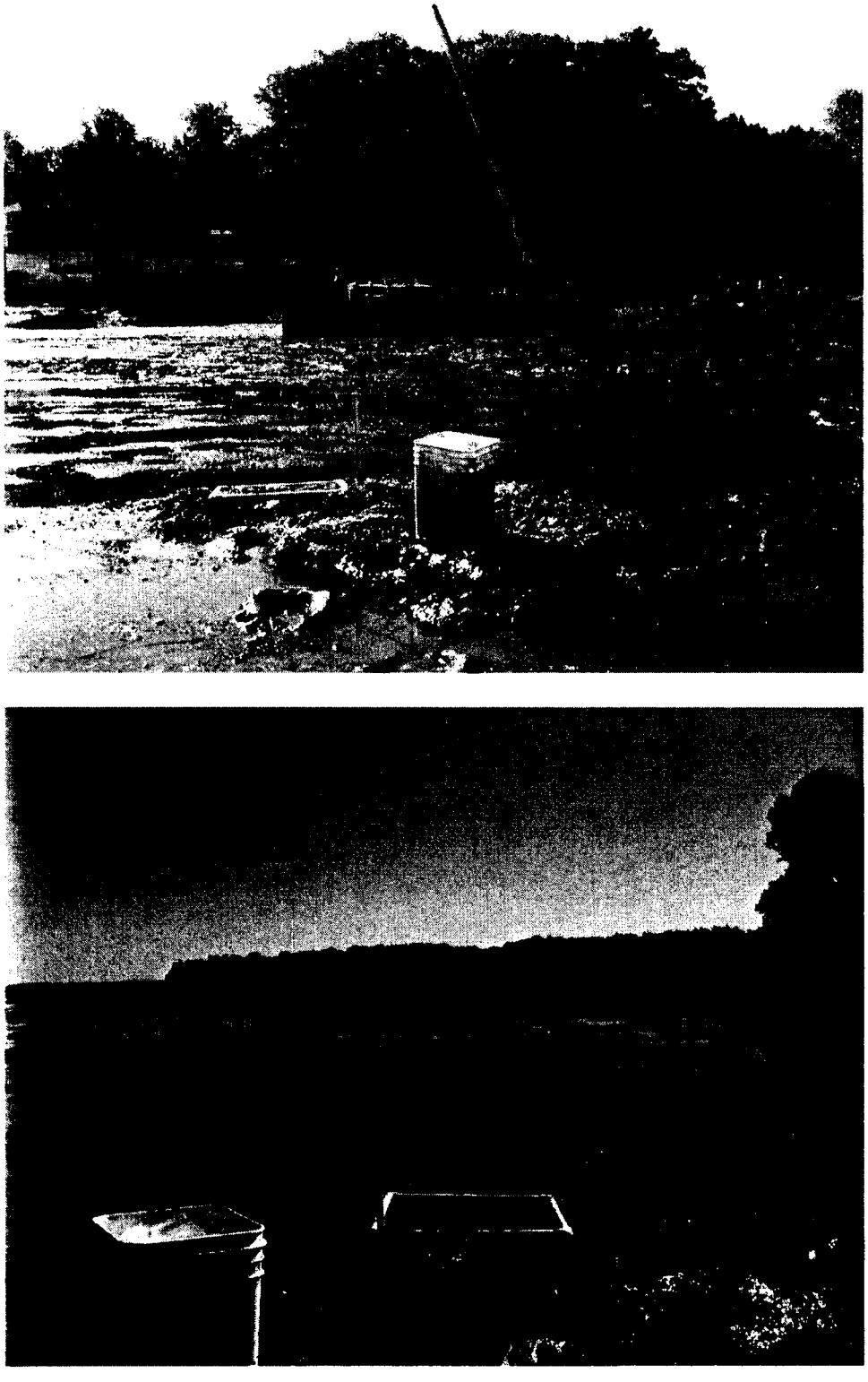

Figure 35 Depot Road A) summer 2009 with gundalow and student group B) quadrat on transect line

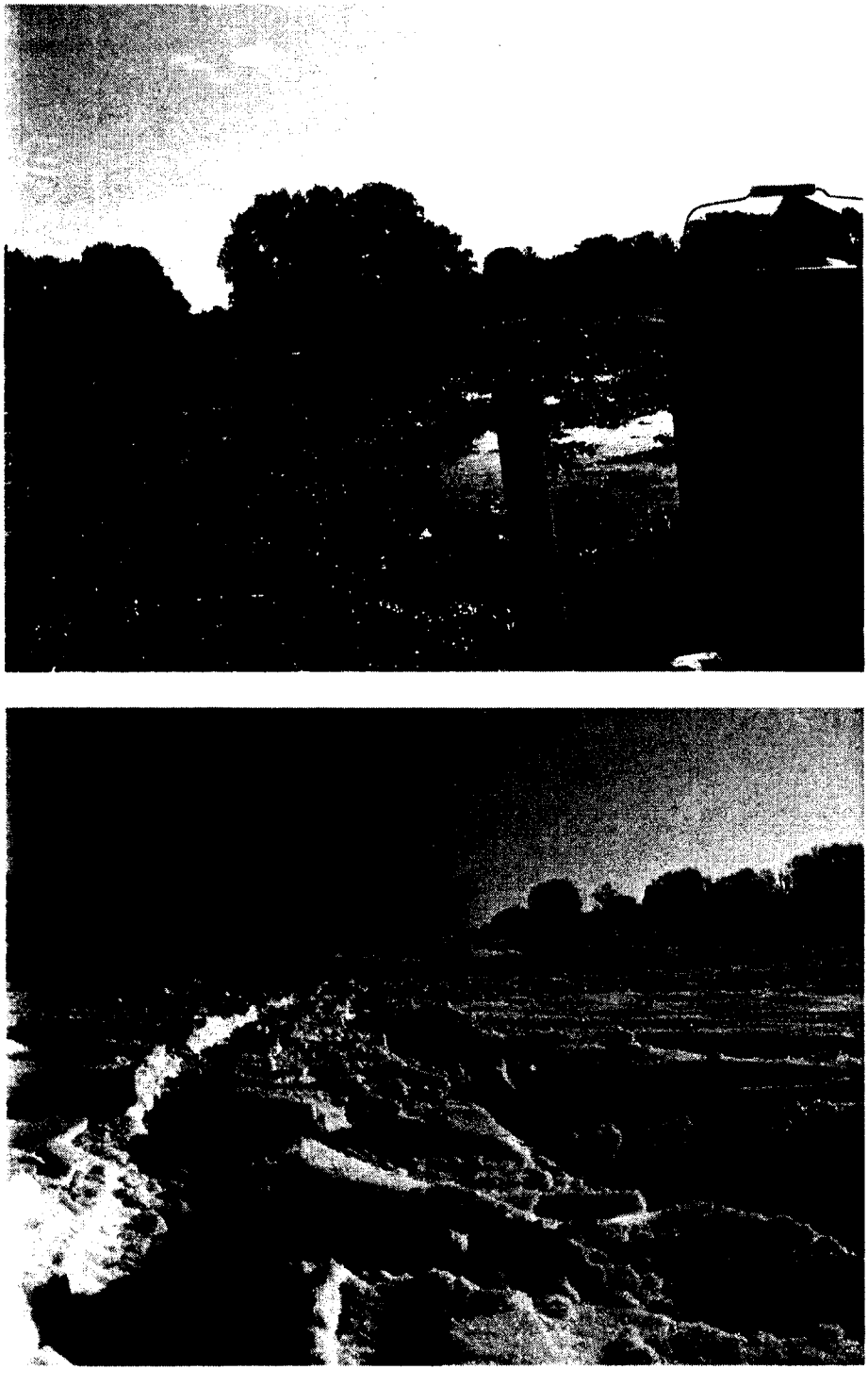

Figure 36 Sunset Farm A) *Ulva* and *Gracilaria* bloom (September 2008) B) winter snow and ice cover can last for a few months in southern Great Bay

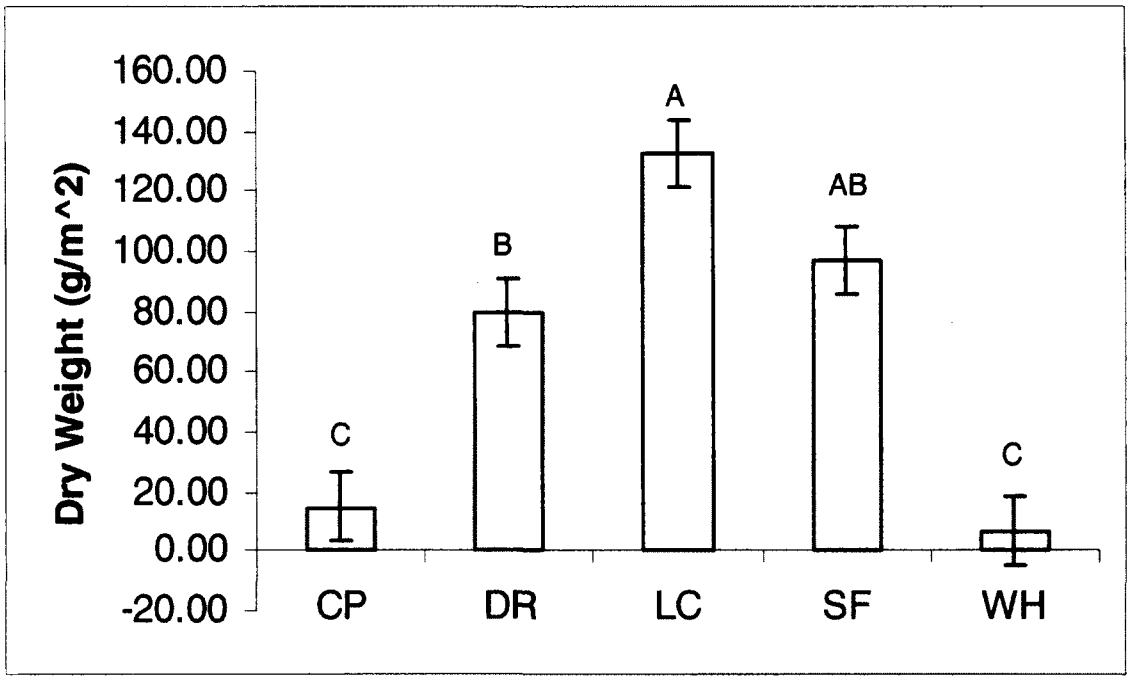

Figure 37 GBES *Ulva* mean biomass by site from 2008-2010. Letter designations denote significant differences (P<0.05). Error bars represent standard error.

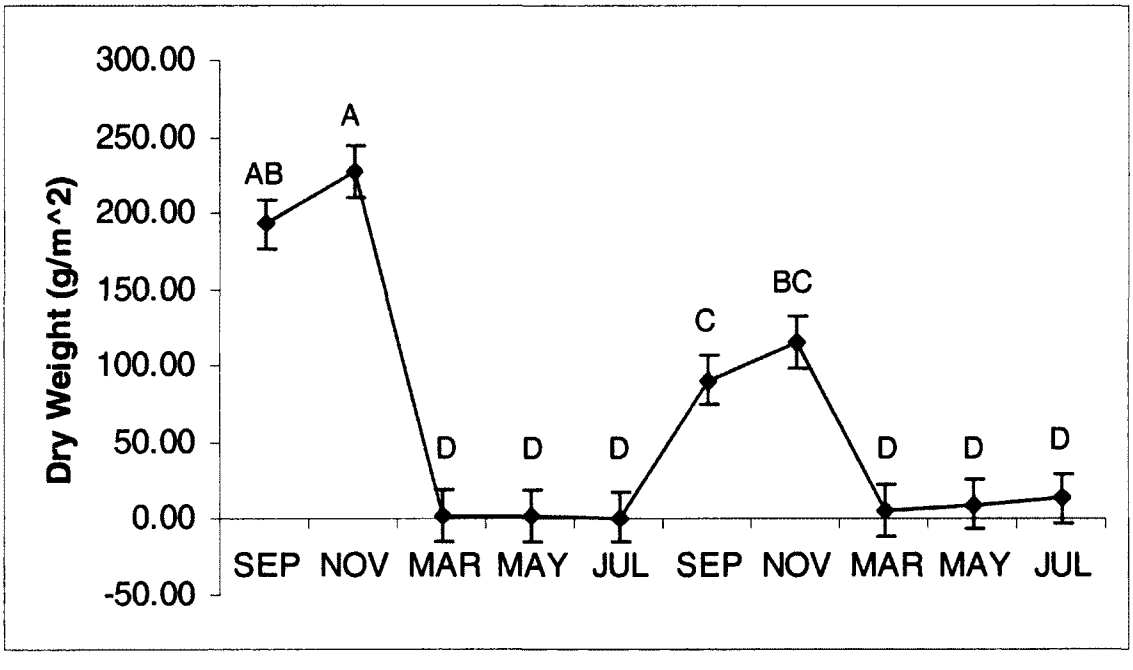

Figure 38 GBES *Ulva* mean monthly biomass across five study sites (2008-2010). Letter designations denote significant differences (P<0.05). Error bars represent standard error.

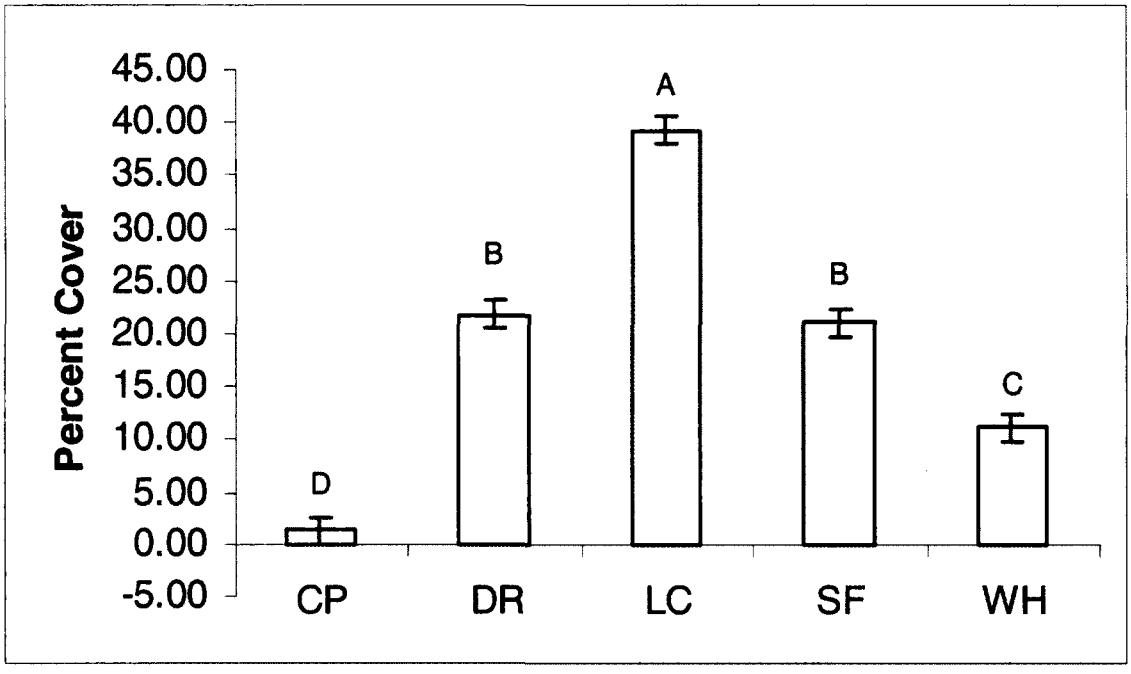

Figure 39 GBES *Ulva* mean percent cover by site from 2008-2010. Letter designations denote significant differences (P<0.05). Error bars represent standard error.

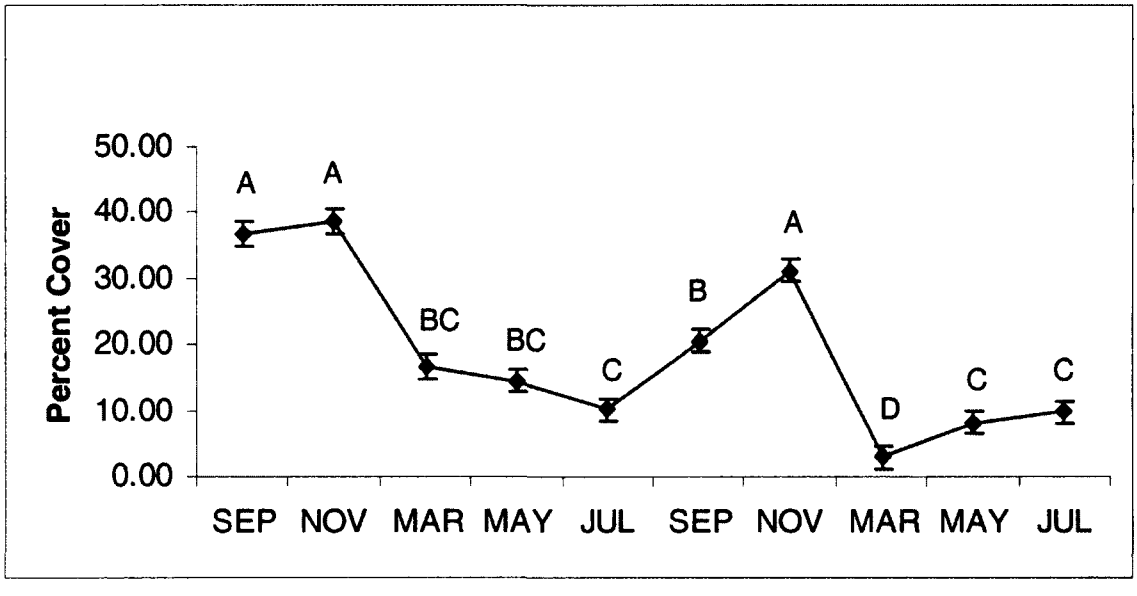

Figure 40 GBES *Ulva* mean monthly cover across 5 sites from 2008-2010. Letter designations denote significant differences (P<0.05). Error bars represent standard error.

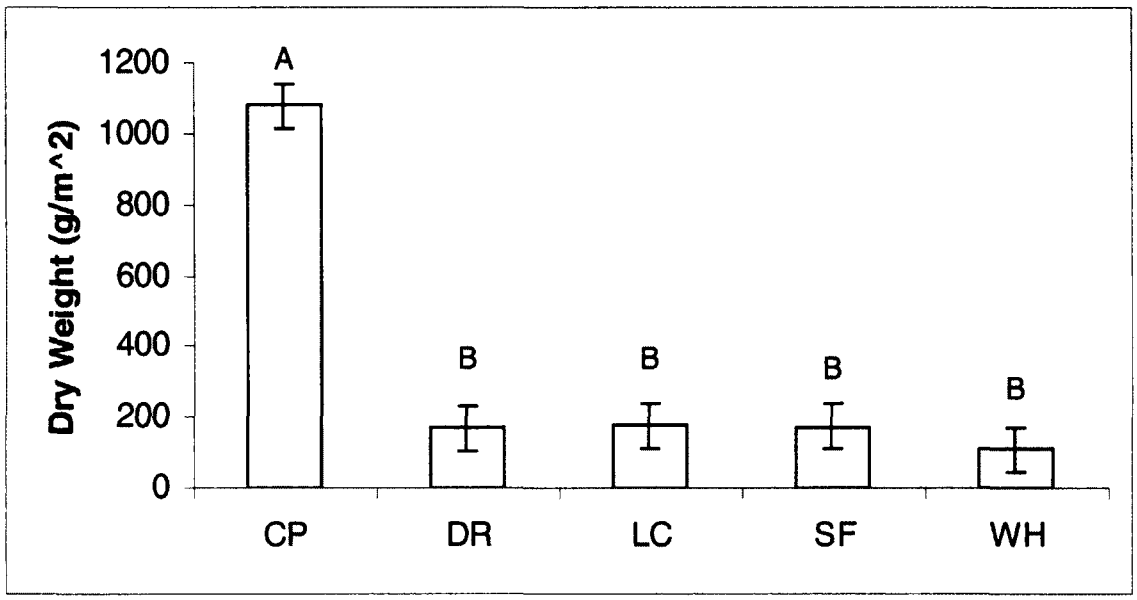

Figure 41 GBES algae mean biomass by site (2008-2010). Letter designations denote significant differences ($P<0.05$). Error bars represent standard error.

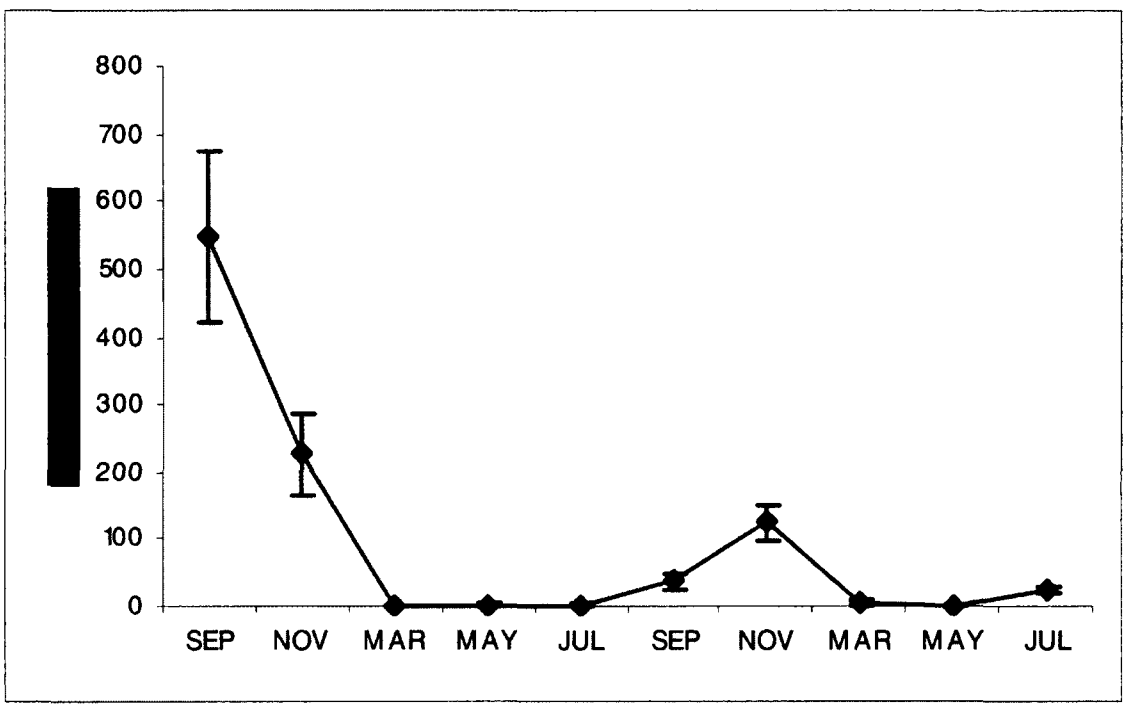

Figure 42 Sunset Farm *Ulva* mean biomass per month (2008-2010). Error bars represent standard error.

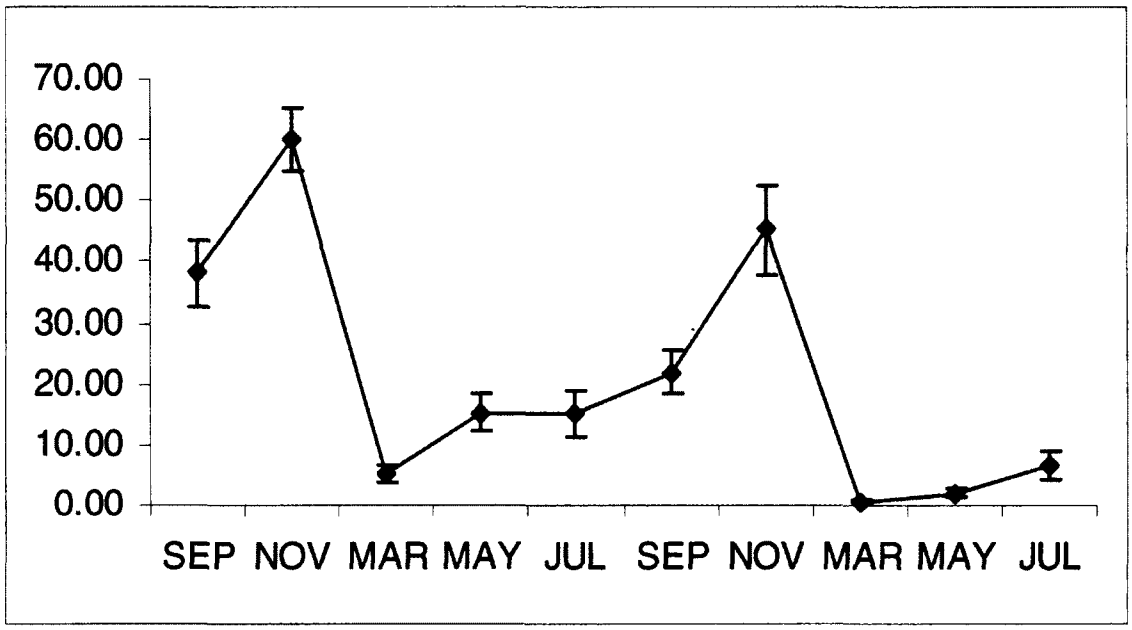

Figure 43 Sunset Farm *Ulva* mean percent cover (non-transformed) 2008-2010. Error bars represent standard error.

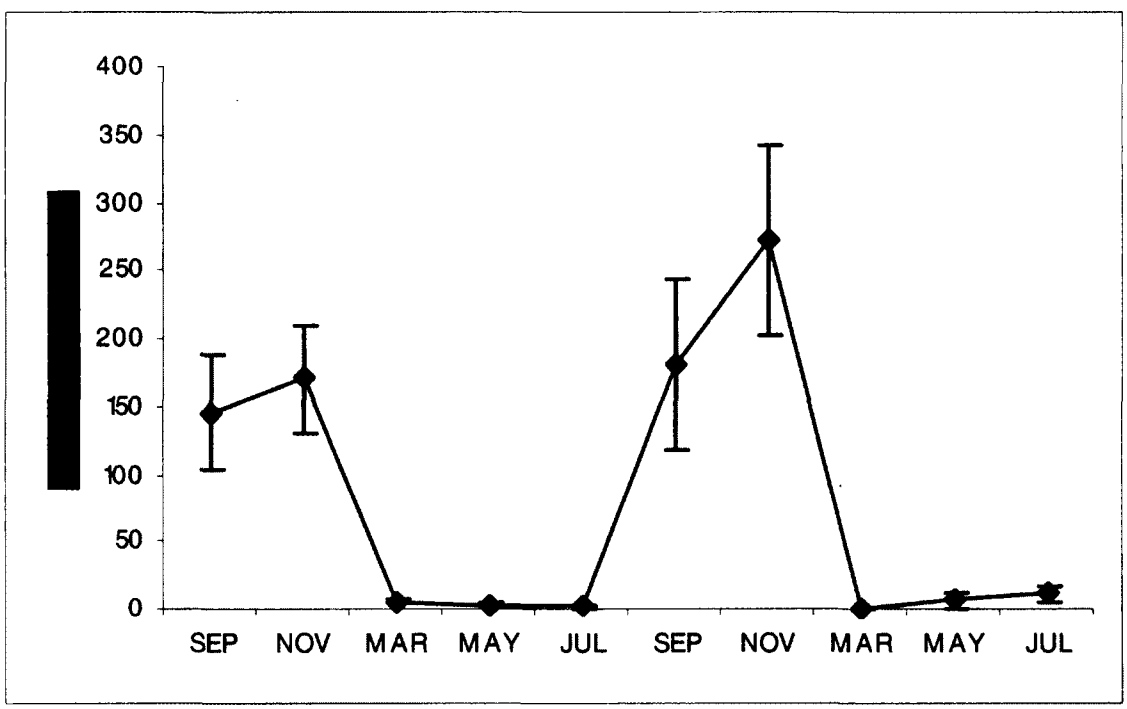

Figure 44 Depot Road *Ulva* mean monthly biomass (2008-2010). Error bars represent standard error.

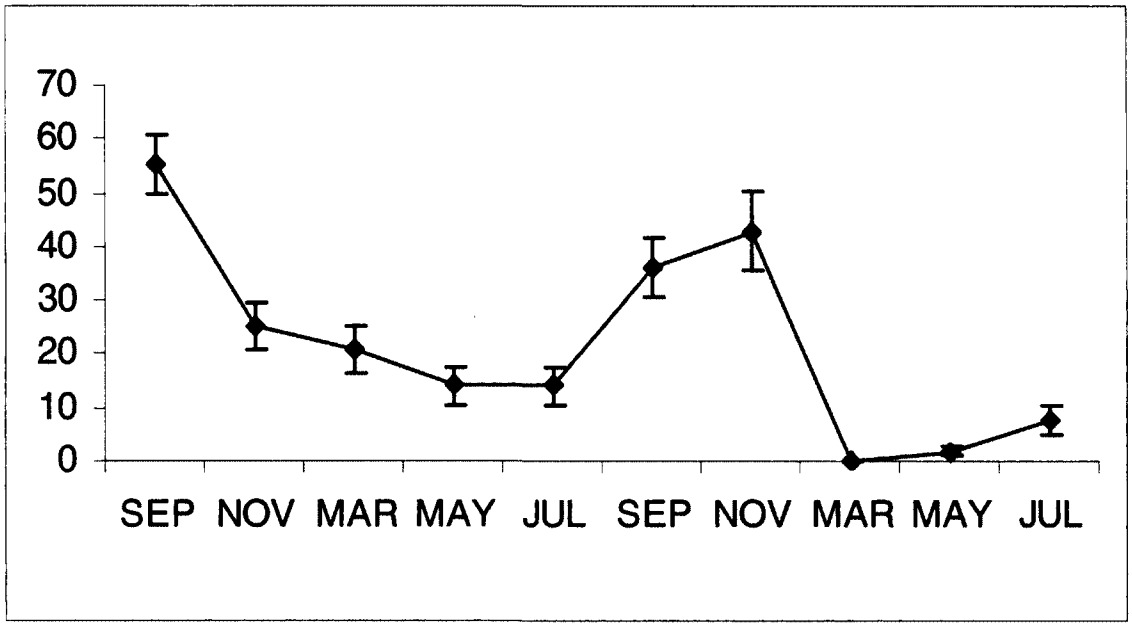

Figure 45 Depot Road *Ulva* mean monthly percent cover (non-transformed) 2008-2010. Error bars represent standard error.

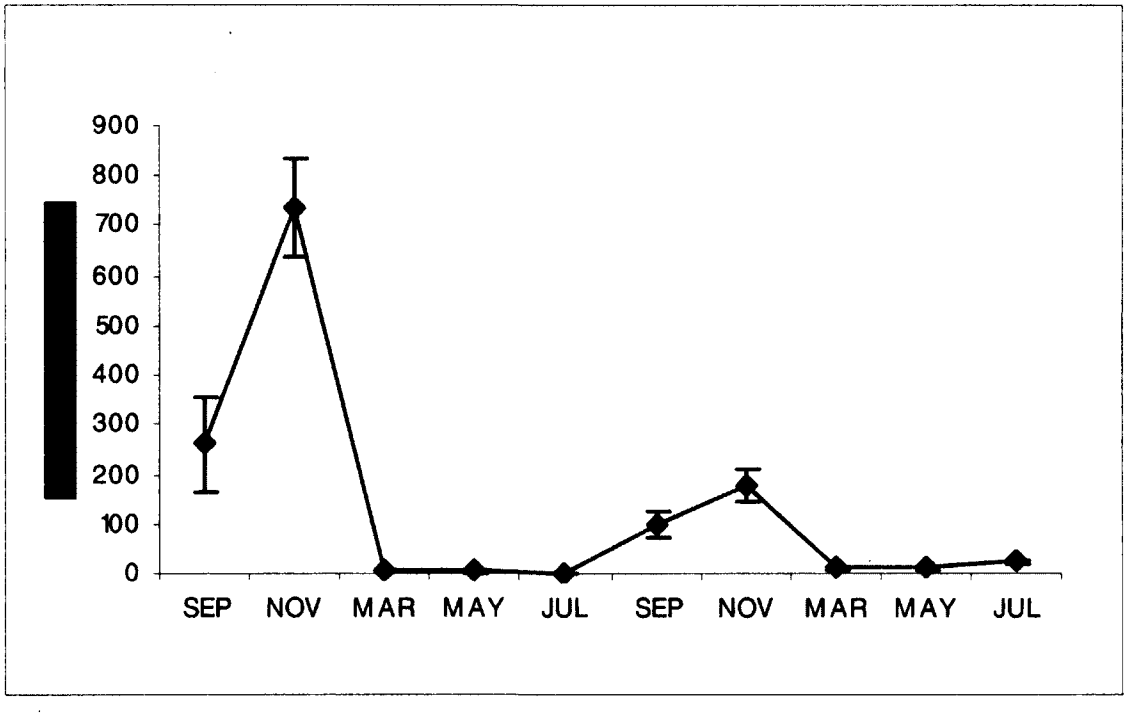

Figure 46 Lubberland Creek *Ulva* mean monthly biomass (2008-2010). Error bars represent standard error.

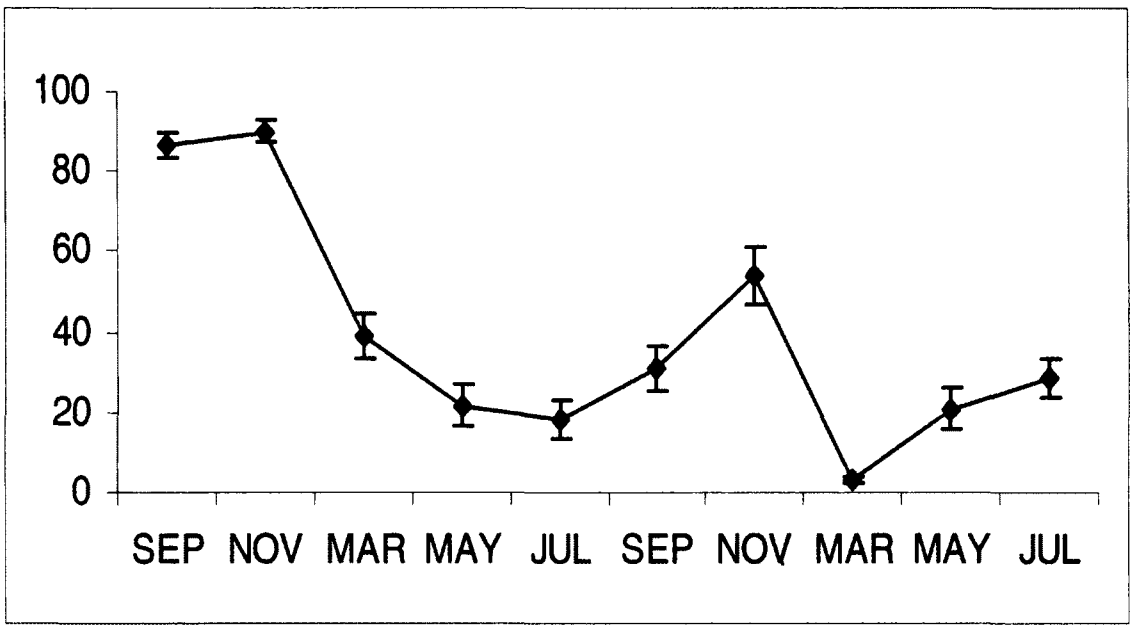

Figure 47 Lubberland Creek *Ulva* mean monthly percent cover (non-transformed) 2008-2010). Error bars represent standard error.

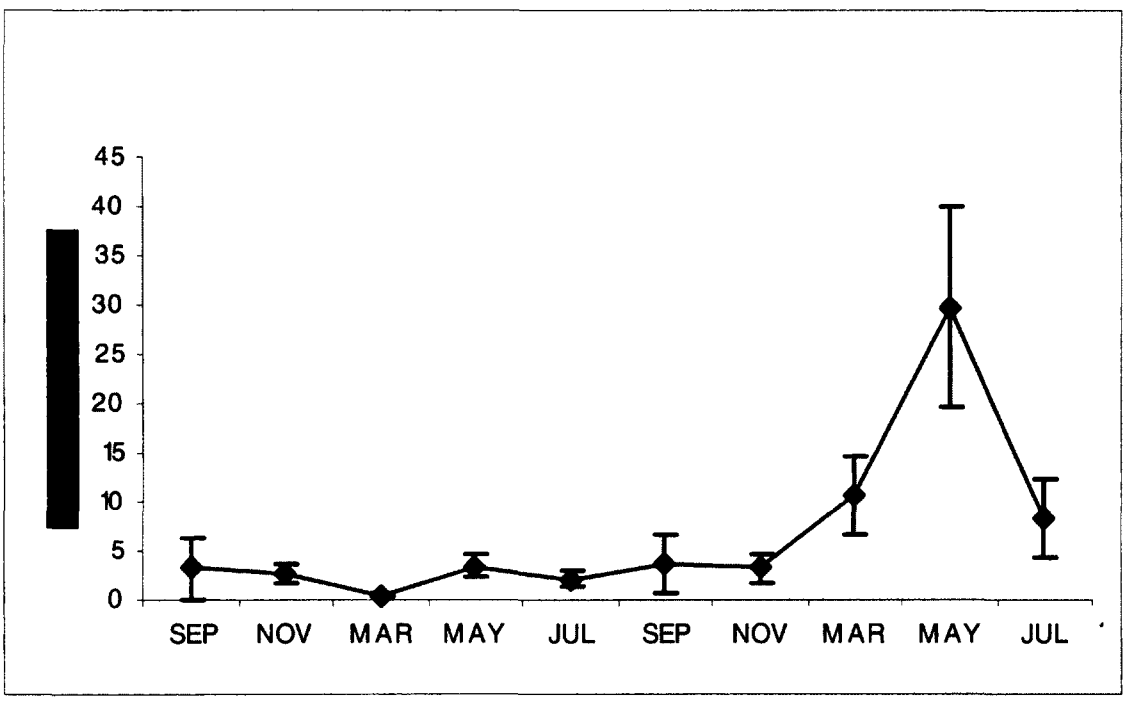

Figure 48 Wagon Hill Farm *Ulva* mean monthly biomass (2008-2010). Error bars represent standard error.

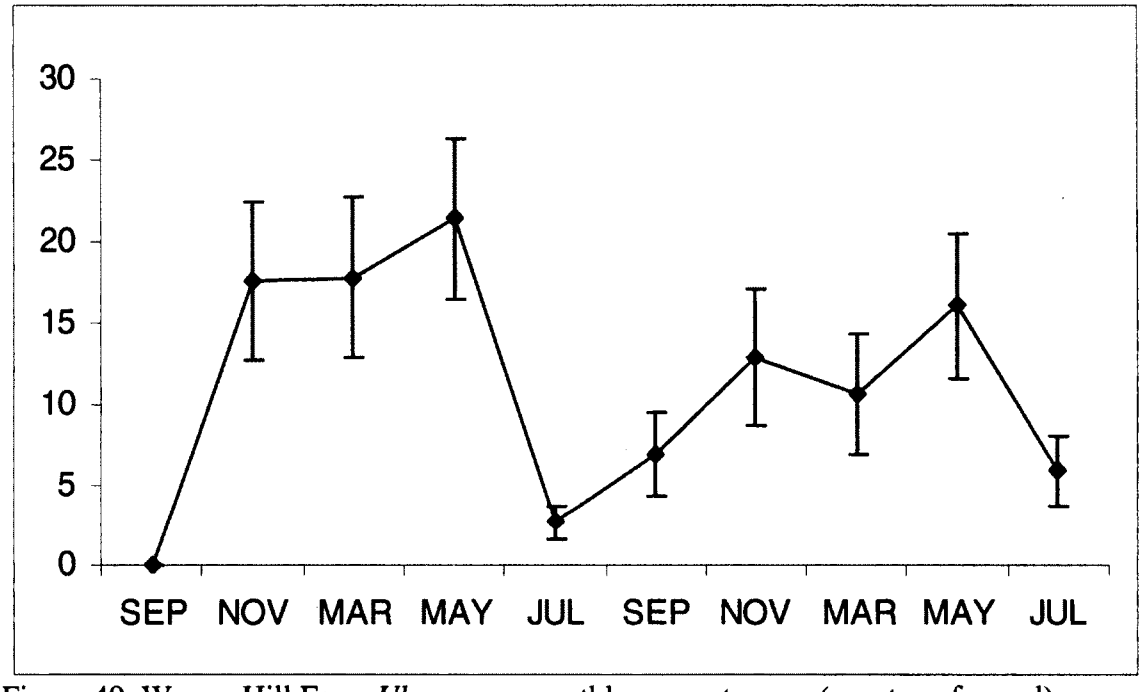

Figure 49 Wagon Hill Farm *Ulva* mean monthly percent cover (non-transformed) 2008-2010. Error bars represent standard error.

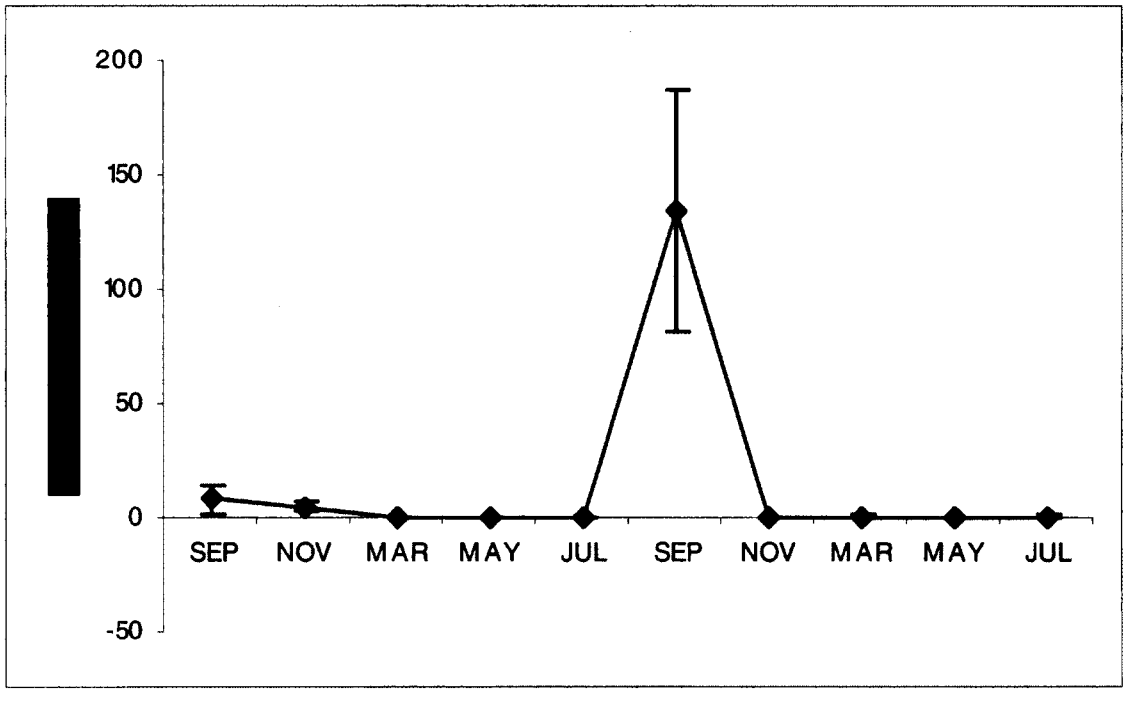

Figure 50 Cedar Point *Ulva* mean monthly biomass (2008-2010). Error bars represent standard error.

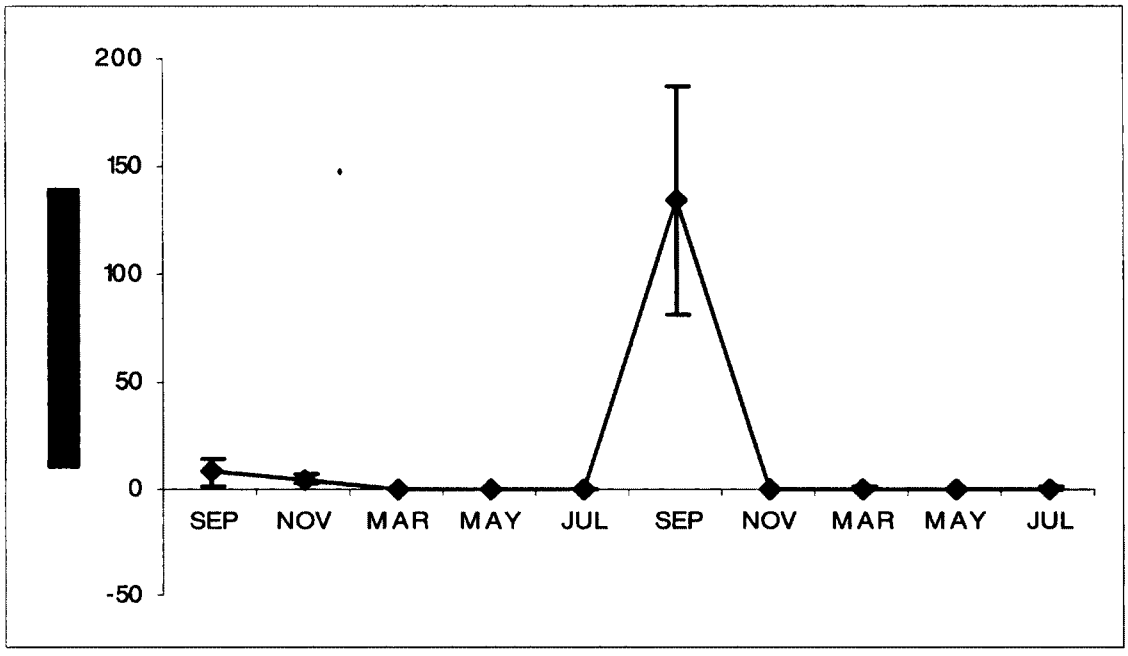

Figure 51 Cedar Point *Ulva* tissue mean monthly percent cover (non-transformed) 2008-2010. Error bars represent standard error.

CHAPTER IV

GRACILARIA VERMICULOPHYLLA INTRODUCTION, PREVALENCE, AND IMPACT WITHIN THE GREAT BAY ESTUARINE SYSTEM

Introduction

The recent discovery of an introduced Asian red algae, *Gracilaria vermiculophylla* (Ohmi) Papenfuss, in the Great Bay Estuarine System is alarming, because it has been shown to grow rapidly, often causing environmental and economic problems in affected regions (Bellorin et al. 2004; Rueness 2005; Nyberg 2007). Its blooms have been quite extensive worldwide (Bellorin et al. 2004; Rueness 2005; Freshwater et al. 2006; Thomsen et al. 2006)

Gracilaria species are adapted to thrive in nutrient enriched conditions, and blooms have been widely documented. In the embayment of Sacca di Goro, Italy, biomass of *Gracilaria* species reached peaks of 500 g/m^2 (Morand and Briand 1996). In Rhode Island, *Gracilaria* biomass has been measured at 250 g/m^2 DW, (Thorne-Miller et al. 1983) and 500 g/m^2 WW in Connecticut (Welsh et al. 1982). Virnstein and Carbonara (1985) measured the dry weight of *Gracilaria* species to be 15 kg/m^2 in an accumulation area within Florida's Indian River Lagoon, with average spring values of 400 g/m^2 WW. In Spain's Tancada Lagoon, *Gracilaria* biomass reached measures of 200 g/m^2 DW (Menéndez and Comin 2000). In Hog Island Bay, Virginia, USA, *G. vermiculophylla* was the dominant seaweed from 1998 through 2002, making up 74% of the total biomass across all study sites and seasons (Thomsen et al. 2006). Further, it has been

demonstrated that *Gracilaria vermiculophylla* congeners can double in biomass in less than three days given appropriate temperatures, irradiance, or nutrients (Lapointe et al. 1984).

As *G. vermiculophylla* has spread, it has been deemed an invasive species in many parts of the world (Bellorin et al. 2004; Rueness 2005; Nyberg 2007). Using assessment criteria involving 13 parameters, Nyberg (2007) determined that *G. vermiculophylla* was the most invasive red algal species in Europe, with the highest scores in transportation probability, survival time out of water, reproductive mode and morphology. *Gracilaria vermiculophylla*, even at low density, was shown to have a negative impact on long term habitat forming species such as *Zostera marina* (Nyberg 2007). In the Carolinas, *G. vermiculophylla* has negatively affected fishing operations and industries reliant on natural coolant water intake (Freshwater et al. 2006).

The present study set out to compare blooms of *Gracilaria* in Great Bay relative to historical data. I also wished to determine if *G. vermiculophylla* was present in the system, and if so, determine its approximate introduction date and the extent of its introduction. To quantify blooms, both percent cover estimation and destructive biomass sampling were employed. Molecular identification of *Gracilaria* specimens to species, was done by DNA sequencing and RFLP analyses.

Materials and Methods

Algal sampling was conducted within the intertidal zones at five sites in the Great Bay Estuarine System, NH. The sites were designated as Cedar Point, Wagon Hill Farm, Lubberland Creek, Depot Road, and Sunset Farm. *Gracilaria* collection techniques,

percent cover analyses and biomass measures followed the methods described in Chapter III.

Molecular Methods

Historical (Appendix E) and freshly collected (Appendix F) *Gracilaria* samples were prepared for molecular analyses using the methods described in Chapter III. The DNA sequencing techniques matched those described in Chapter III, with the exception of the primer pairs used. The primers used for amplification and sequencing of *Gracilaria* samples were CO1F328 (5' ACA GGA TGA ACA GTK TAT CCY C 3') and CO1R634 (5' CCA CCT GCW GGA TCA AAG A 3').

RFLP analyses (Appendix G) used DNA samples amplified using the above primers. Following PCR, samples (10 µl) were digested with 1 µl DpnII enzyme (New England Biolabs) in 2 µl DpnII buffer solution and dH_2O (7 µl). The reactions were incubated at 37°C for 60 minutes. The enzyme was deactivated through a 20 minute incubation at 65°C. Resulting samples were run on a 2% analytical grade agarose gel (Promega) stained with SYBR®safe (Invitrogen). Electrophosis occurred in a 0.5 TBE bath at 5V per cm until band separation was clearly visible under UV light. Fragment sizes were compared to those on a 50 bp DNA ladder (New England Biolabs).

The DpnII enzyme, which was derived from an *Escherichia coli* strain carrying *Diplococcus pneumonia* G41 (S. Lacks), was chosen following COI sequence comparisons using New England Biolabs' online program NEBcutterV2.0. Analysis revealed that the COI regions of each of this study's *Gracilaria* species could be cut at two unique places by the DpnII enzyme, which cleaves at the 5' end of 5' GATC 3'

recognition sites. In the native *Gracilaria tikvahiae* McLachlan, this cleavage creates segment lengths of 231 bp and 62 bp, while the introduced *Gracilaria vermiculophylla* digestion produces 189 bp and 104 bp segments (Figure 52).

Results

DNA analysis of *Gracilaria* specimens verified the presence of both the native *Gracilaria tikvahiae* McLachlan and the introduced, possibly invasive, *G. vermiculophylla* at all of the study sites in southern Great Bay (Appendix F). During the 2008 to 2010 study, both *Gracilaria tikvahiae* and *G. vermiculophylla* were lumped together in analyses of biomass and percent cover due to the impossibility of identification through morphological methods.

The mean *Gracilaria* biomass was tracked across the five study sites from 2008-2010 (Figure 53). Differences were found between the sites ($P<0.01$), with no *Gracilaria* measured at Wagon Hill Farm and Cedar Point in Little Bay, and a significantly higher amount at the southern Great Bay sites. Mean *Gracilaria* biomass was greatest at Depot Road (82.8 g dry weight/m^2 $\pm$ 141.7 SD) and Sunset Farm (72.6 g dry weight/m^2 $\pm$ 109.5 SD), while it was significantly lower at Lubberland Creek(16.2 g dry weight/m^2 $\pm$ 20.7 SD).

Seasonal differences in mean *Gracilaria* biomass were observed throughout Great Bay ($P<0.01$), with maxima occurring during the fall of both years (Figure 54). The peak *Gracilaria* biomass (245.8 g dry weight/m^2 $\pm$ 195.4 SD) during November 2008 was significantly greater $P=0.01$) than the peak in November 2009 (122.5 g dry weight/m^2 $\pm$

130.7 SD). Minima levels of *Gracilaria* biomass were observed from March (ice-out) through July of both study years.

Mean *Gracilaria* cover results closely followed the trends seen in *Gracilaria* biomass (Figure 55), with highest levels measured at the Sunset Farm (15.5% ± 15.1 SD) and Depot Road (12.4% ± 12.9 SD). The Lubberland Creek site had significantly lower mean *Gracilaria* cover (4.8% ± 4.7 SD) during the study period.

Gracilaria cover exhibited a significant ($P<0.01$) seasonal trend across the Great Bay study sites (Figure 56). Seasonal highs in mean cover were observed in November of 2008 (30.9% ± 18.8 SD) and 2009 (15.9% ± 16.5 SD) with the maxima in 2008 being significantly greater ($P<0.01$, post-hoc). The lowest mean cover values were observed in May of both 2009 (2.2%± 1.6 SD) and 2010 (0.3% ± 0.27 SD), which was later than that seen for *Ulva* percent cover patterns.

Mean algal biomass differed across sites within the Great Bay Estuarine System ($P<0.01$), with Cedar Point's values (1078.3 g dry weight/m^2 ± 1070.2 SD) far exceeding the other four sites (Figure 57). The major contributors to the mean biomass at Cedar Point were the attached fucoid algae species found growing attached to the site's shale substratum.

The mean *Gracilaria* biomass was also calculated for each collection month at the Sunset Farm site (Figure 58). Seasonal differences were found ($P<0.01$), with peak biomass in November of both 2008 (264.8 g dry weight/m^2 ± 391.9 SD) and 2009 (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 during months of ice cover, with

seasonal minima levels observed in March 2009 (1.97 g dry weight/m^2 $\pm$ 4.1 SD) and May 2010 (0.06 g dry weight/m^2 $\pm$ 0.36 SD).

The mean monthly *Gracilaria* cover was also determined by month for the Sunset Farm (Figure 59) showed a significant seasonal trend ($P<0.01$), with peak bloom in November of both years (39.2% $\pm$ 35.9 SD and 34.9 $\pm$ 37.3 SD). Mean *Gracilaria* percent cover was lowest in May of 2009 (3.1% $\pm$ 7.3 SD) and 2010 (0.6% $\pm$ 1.7 SD), which lagged behind the March ice-out.

Trends in *Gracilaria* elevation were examined throughout the study period. Although no significant differences were found, *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 tides.

Mean monthly *Gracilaria* biomass from Depot Road (Figure 60) showed significant seasonal differences ($P<0.01$), with peak values during November 2008 (431.1g dry weight/m^2 $\pm$ 774.3 SD) and September 2009 (158.8 g dry weight/m^2 $\pm$ 383.0 SD). As was observed with *Ulva* at this site, there was a pronounced decline in *Gracilaria* mean biomass during periods 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.

The mean *Gracilaria* percent cover at Depot Road (Figure 61) revealed a significant seasonal trend ($P<0.01$), with peak bloom in November 2008 (44.1% $\pm$ 33.7 SD) and September 2009 (14.8% $\pm$ 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 (3.2% $\pm$ 10.5 SD) and March of 2010 (0% $\pm$ 0 SD). The 2009 low lagged two months behind

the thawing of the site's ice cover. Although *Gracilaria* specimens were present in March and May of 2010, none were within the study's transect lines.

Trends in *Gracilaria* elevational distribution were examined throughout the study period. No significant differences were found in *Gracilaria* distributions, but they tended to be slightly more concentrated at the lower elevations. The majority of the specimens observed at this site were free floating and able to move with the prevailing water currents.

The mean monthly *Gracilaria* biomass at Lubberland Creek (Figure 62) varied seasonally ($P<0.01$), with peak values in November 2008 (41.7g dry weight/m^2 $\pm$ 71.3 SD) and 2009 (55.9 g dry weight/m^2 $\pm$ 110.9 SD). As observed with *Ulva* population at this site, there was a marked decline in *Gracilaria* mean biomass during the months of ice cover, with seasonal lows 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.

Mean monthly *Gracilaria* percent cover at Lubberland Creek (Figure 63) showed significant seasonal trend ($P<0.01$), with peak values in March 2009 (10.8% $\pm$ 18.6 SD) and September 2009 (12% $\pm$ 22.4 SD). Mean *Gracilaria* cover was lowest in July of 2009 (0.4% $\pm$ 1.5 SD) and May of 2010 (0.3% $\pm$ 1.4 SD).

Gracilaria elevational distribution was examined throughout the study at the Lubberland Creek site. Its distributions were slightly more concentrated at the highest elevations ($P=0.05$), especially at the marsh-grass/open-mudflat boundary. Like at the other southern Great Bay sites, the majority of specimens here were free floating and able to move with the tides.

No *Gracilaria* specimens were found at the Wagon Hill Farm site at any time between September 2008 and July 2010. While few specimens were found adrift at Cedar Point during the final collection in July 2010, no *Gracilaria* specimens were observed within the intertidal zone at this site at any other time during the entire study.

The RFLP study from September and November of 2011 revealed the relative abundance of *Gracilaria vermiculophylla* and *G. tikvahiae* at the three southern bay sites (Figure 64, Appendix G). Of the *Gracilaria* collected in September 2011, *G. vermiculophylla* comprised 100% of the specimens from both Lubberland Creek (n=34) and Sunset Farm (n=32). At the Depot Rd site, 72% of the *Gracilaria* specimens collected were *G. vermiculophylla* (n=26), and 28% were the native *G. tikvahiae* (n=10). In November 2011, again 100% of the *Gracilaria* collected from both Lubberland Creek and Sunset Farm were verified *G. vermiculophylla* specimens (n=28 and n=27 respectively). At Depot, 59% of the *Gracilaria* collected was *G. vermiculophylla* (n=17) and 41% was *G. tikvahiae* (n=12). Overall, *G. vermiculophylla* comprised 88% of the Great Bay *Gracilaria* specimens identified via RFLP in 2011.

Gracilaria percent cover was also determined for the three southern bay sites in September and November of 2011. The total *Gracilaria* coverage for the Lubberland Creek site in September 2011 was 19.5% $\pm$ 5.6 SE and 38.0% $\pm$ 6.6 SE in November 2011. At Depot Rd, the total *Gracilaria* coverage was 40.5% $\pm$ 6.8 SE and 77.0% $\pm$ 6.5 SE during September and November respectively. At the Sunset Farm site, *Gracilaria* cover was 53.8% $\pm$ 6.7 SE in September and 30.7% $\pm$ 6.4 SE in November.

Extrapolating from the findings of the RFLP identification study, percent cover of *Gracilaria vermiculophylla* was estimated for each of the study sites and times (Figure

65). *Gracilaria vermiculophylla* comprised 100% of the *Gracilaria* specimens observed in the cover studies at both Lubberland Creek and Sunset Farm in September and November of 2011. Therefore, *G. vermiculophylla* coverage at Lubberland Creek was 19.5% ± 5.6 SE and 38.0% ± 6.6 SE, while at Sunset Farm, during those two months the percent cover of *G. vermiculophylla* was 53.8% ± 6.7 SE and 30.7% ± 6.4 SE, respectively. The *Gracilaria* observed at the Depot Rd site was a mixture of both native *G. tikvahiae* and introduced *G. vermiculophylla.* In September 2011, the *G. tikvahiae* percent cover was 11.3% ± 1.9 SE, and the *G. vermiculophylla* cover was 29.3% ± 4.9 SE. In November 2011, *G. tikvahiae* percent cover was 31.9% ± 2.7 SE, and *G. vermiculophylla* was 45.1% ± 3.8 SE.

Discussion

The Great Bay Estuarine System 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 (Appendix E). This represents 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 the Great Bay Estuarine System 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). Such a problem is compounded at sites that are known to support both species, which is the case for the three southern Great Bay sites observed here. Because of excessive costs and efforts mentioned previously for *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 Hardwick-Witman and Mathieson (1983) study 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 global warming and increased availability of nutrients.

The combined *Gracilaria* 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 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 (being > 70 g dry weight/ m^2 and >12% during the entire study period). Such values far exceeded even the single month maxima values observed by Harwick-Witman (1983) in which max biomass 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 by partial burial in mud coupled with low site hydrodynamics. The temperature and nutrient regimes of the northern sites appear to be suitable to support *Gracilaria* growth, but its 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 biomass and percent cover of *Ulva* than Depot Road and Sunset Farm, whereas Lubberland Creek had the opposite pattern for *Gracilaria* growth. Such a pattern is likely a function of *Ulva* overgrowth at Lubberland Creek during the fall of 2008. Due its large bladed morphology, *Ulva* 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 major decrease in the *Gracilaria* bloom. Lower growth at this critical time can have carry-over effects during subsequent years, as spring and summer populations build from the individuals that survived the long winter months of snow and ice cover.

Gracilaria monthly mean biomass and percent 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 exceeded 40% and 250 g dry weight/ m^2 in November 2008. Again, these values far exceeded any single site values 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 over the past three decades.

Although concentrations of nitrogen have increased since these earlier 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% - 4.5%, and phosphorus values ranged from 0.18% - 0.35%, compared to the ranges of 2.5% - 3.6% TN and 0.17% - 0.33% TP observed in the current study. Such stability in the face of increasing nutrient availability could be indicative of a preferred steady state for these organisms. Because *Gracilaria* can grow very rapidly, it is possible that excess 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.

Analysis of monthly percent cover within each site revealed peak *Gracilaria* blooms of unprecedented size. *Gracilaria* abundance increases were staggering, with Lubberland Creek's cover exceeding 10%. This was more than ten times the maxima observed by any previous intertidal study (Hardwick-Witman unpublished; Hardwick-Witman and Mathieson 1983) in the Great Bay Estuarine System. 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 by Harwick-Witman (unpublished) and Harwick-Witman and Mathieson (1983). While the *Gracilaria* peak the following fall was smaller in general, the abundance values still eclipsed those measured in previous studies.

Although *G. vermiculophylla* has not been reported as a nuisance to any maritime industry in New England, the abundance of the species in some locations could cause ecological harm. In the Great Bay Estuarine System, where the introduction occurred around 2003, the species accounted for approximately half of the record high *Gracilaria* cover and biomass observations during the falls of 2008 and 2009, and 88% of the current *Gracilaria* cover in the southern bay. Introduced species, adapted to thrive in nutrient enriched environments can alter the composition of local vegetation (Morand and Merceron 2005). Overgrowth of the species can have deleterious effects, including enhanced shading, disruption of the nutrient balance, altered hydrodynamics, and anoxic or hypoxic conditions following die-back. Any of these factors can cause decreased species richness, including the loss of long-lived habitat-forming species, such as eelgrass.

In an assessment of the invasiveness of *G. vermiculophylla* in North Carolina, Freshwater et al. (2006) found the species met six of Chapman and Carlton's (1991) ten qualifying characteristics including the appearance in local regions where it was previously not found, its association with human mechanisms of dispersal (boat entanglement), relatively restricted distribution on the continent compared with native species (at the time, the invasion appeared to be geographically limited), disjunct populations, insufficient active dispersal mechanism to account for the expanded distribution, and exotic evolutionary origin. With the extent of the *G. vermiculophylla* distribution in New England (cf. Chapter V), a seventh criterion can be added to the list: initial expansion following introduction. In a decade, *G. vermiculophylla* has invaded and become established in disconnected lagoons and estuaries across thousands of

kilometers of the US Atlantic coastline, rivaling the distribution of the native *G. tikvahiae*, which is found continuously along the Western Atlantic coast of North America from southern Mexico to southern Canada (Gurgel et al. 2004).

Based on observations from other parts of the world, *Gracilaria vermiculophylla* is particularly well suited to thrive in New England estuarine environments. In Virginia lagoons, the species demonstrated excessive growth in shallow, nutrient-rich, muddy bottomed locations with limited water flow. Thomsen et al. (2007) found that, when artificial panels were provided, *G. vermiculophylla* had the highest level of recruitment, with twice the percentage cover of oysters and more than five times that of the second most abundant macroalgal species, making it a formidable competitor in its new environment and making its permanency in the system more likely. *Gracilaria vermiculophylla* has also demonstrated higher growth in enhanced nutrient treatments (Thomsen and McGlathery 2007), which is important since many estuarine environments in New England are eutrophied, including the Great Bay system (cf. Chapter II). Further, Thomsen and McGlathery (2007) found that *G. vermiculophylla* growth, in the presence of grazers, increased with the increased excreted nitrogen. *Gracilaria vermiculophylla* has a strong tolerance for low salinity conditions (Nyberg 2007), giving the species an advantage in these brackish environments where there is a lower intensity of competition for space and resources than is often found on the open coast (Weinberger et al. 2008).

Gracilaria vermiculophylla is a hardy species, able to survive long periods of burial and total darkness (Thomsen and McGlathery 2007; Nyberg and Wallentinus 2009). In light limited conditions, which are found in the layered blooms of Great Bay, members of the *Gracilaria* genus increase production of phycoerythrin (Lapointe 1981),

which aids in photosynthesis and acts as a N storage pool to allow growth when ambient N concentrations decrease. The hardiness of *G. vermiculophylla* leaves them particularly well suited to survive sedimentation, cold temperatures, and periodic ice and snow cover found in New England estuaries during long winter periods. While biomass and cover were greatly reduced in Great Bay *G. vermiculophylla* populations during these months of cold and darkness (caused by ice and snow cover), the populations were able rebound during the subsequent spring and summer seasons, indicating a high probability of permanence in the system.

The initial introduction vector for the New England *G. vermiculophylla* is not certain, but speculation in other parts of the world has suggested accidental arrival through shellfish mariculture. Thomsen et al. (2007) deduced that the introduction to Europe and the western Atlantic was through attachment to transplanted oysters, due to the proximity of population to oyster farms, but they reasoned that further distribution within the various regions was probably facilitated by entanglement with boat screws, fishing nets and trawls, and other extensions of smaller boats, for which they had seen many examples. In Brittany, France, *G. vermiculophylla* was again thought to have been introduced by the culturing of oysters imported from Asia (Rueness 2005). Likewise, Thomsen and McGlathery (2007) believed transplanted oysters were the vector its introduction in Virginia. It is plausible that the plant's introduction to New England was facilitated by hitch-hiking on imported shells, as maricultural operations can be found throughout the region. It is also likely that much of the subsequent dispersal within New England has been facilitated by entanglement with traveling fishing and recreational boats. As *G. vermiculophylla* is negatively buoyant (Thomsen et al. 2006) and lacks

motile gametes, natural long distance dispersal is unlikely. Given this fact, officials in Maine, the only New England state currently free of *G. vermiculophylla* invasion, may wish to regulate and/or educate the boaters traveling between their state and estuarine environments within the rest of the region in order to prevent or delay initial inoculation.

Host community and environmental characteristics are of great importance in successful invasions. Valentine et al. (2007) outlined conditions that encouraged the introduction and successful colonization of invasive species, including disturbance in the receiving community through nutrient, substrata, or water temperature disruption, high levels of other invaders, and macroalgal removal through grazing or disease. When seagrass or macroalgal cover is low, resistance to invasion is weakened (Cecchereli and Cinelli 1999). As the human population in New England has increased and the anthropogenic pressures have expanded, the above disturbances have been seen in the region's estuarine environments (Jerome et al. 1965; Miller et al. 1971; Mathieson and Fralick 1973; Burdick et al. 2006), leaving vulnerable many locations for *G. vermiculophylla* invasion. Although sites in New Hampshire mark the current northern limit for *G. vermiculophylla* in the western Atlantic, environments with suitable abiotic factors such as temperature, salinity, hydrological regime, substrata, and light conditions occur farther north in Maine. Given the hardiness of the species, the plentiful vectors of transport, and the rapid spread thus far, it is likely the range of *G. vermiculophylla* will continue to expand northwards.

Few studies have examined sites prior to, or in the early stages of, invasion (Schaffelke and Hewitt 2007). As this kind of study can provide valuable information on the impact of introduced species, researchers should document baseline community data

for New England estuaries that currently host no *G. vermiculophylla.* As it has been observed that aliens often remain background species with little impact before expanding to the point of becoming invasive (Stockwell et al. 2003), similar community studies should be undertaken in New England locations with small *G. vermiculophylla* populations.

While those species that are labeled invasive are harmful by definition, not all introduced species are considered to be invasive. Species that have been labeled invasive in one region can have positive impacts in another. Loose lying specimens of *G. vermiculophylla* were collected in the southern study sites. Since these organisms can thrive without the need for solid attachment, they are able to occupy zones that other algal species cannot. In open tidal mudflats, *G. vermiculophylla* can provide otherwise-absent three dimensional structures that serve as protection for small fish, and an attachment substratum for many epiphytic algal species (Norkko et al. 2000; Thomsen et al. 2006; Wallentinus and Nyberg 2007). Therefore the presence of large amounts of loose-lying *G. vermiculophylla* can have some positive effects on the ecological structure of invaded sites.

Introduced species can also have a positive economic effect on invaded region. For example, *Gracilaria vermiculophylla* has shown great potential as a bioremediator in integrated aquaculture systems due to its ability to effectively reduce nutrients while growing rapidly to produce commercially valuable products (Wallentinus and Nyberg 2007, Abreu et al. 2011a,b,c). Due to its potential accidental, the use of *G. vermiculophylla* should be avoided in non-impacted areas, but the aquacultural industry should embrace the species as a valuable bioremediator in thoroughly affected regions.

In summary, the recently introduced and potentially invasive species, *Gracilaria vermiculophylla* was discovered in the Great Bay Estuarine System. Large increases in both its mean and peak biomass and percent cover have occurred. These changes are associated with eutrophic and hypertrophic water nitrogen and phosphorus concentrations recently observed. The increases in nuisance algal blooms are likely the result of excess nutrient loading in the Bay, and, in the case of *Gracilaria vermiculophylla*, may also be a symptom of a harmful invasion. Current nitrogen and phosphorus levels in the system are high enough to support even larger *Gracilaria* blooms than observed here, 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.

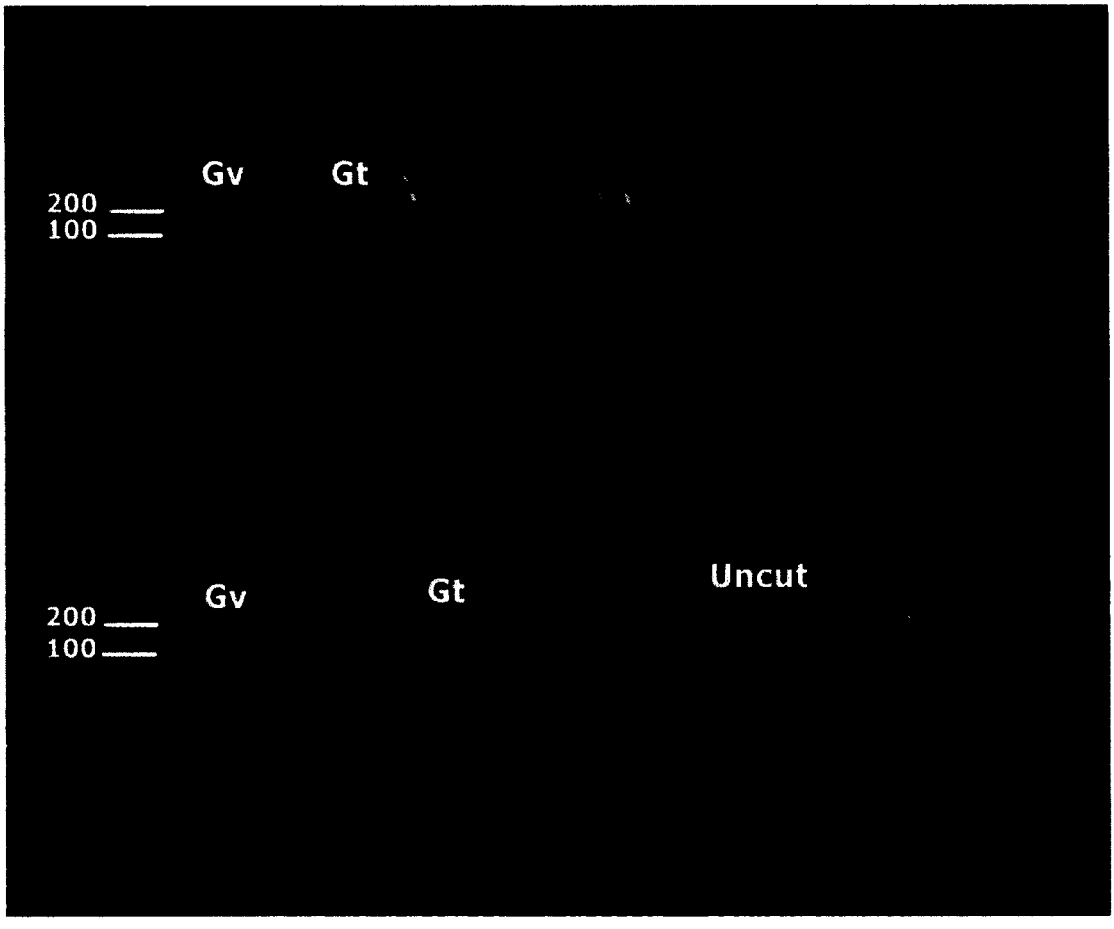

Figure 52 Digest of 3' end of COI gene revealing the cut pattern for *G. vermiculophylla* (Gv) and *G. tikvahiae* (Gt) using the DpnII restriction enzyme. This cleavage creates segment lengths of 231 bp and 62 bp in the amplified 3' CO1 region of *G. tikvahiae*, while the digestion of this region of the introduced *Gracilaria vermiculophylla* produces 189 bp and 104 bp segments.

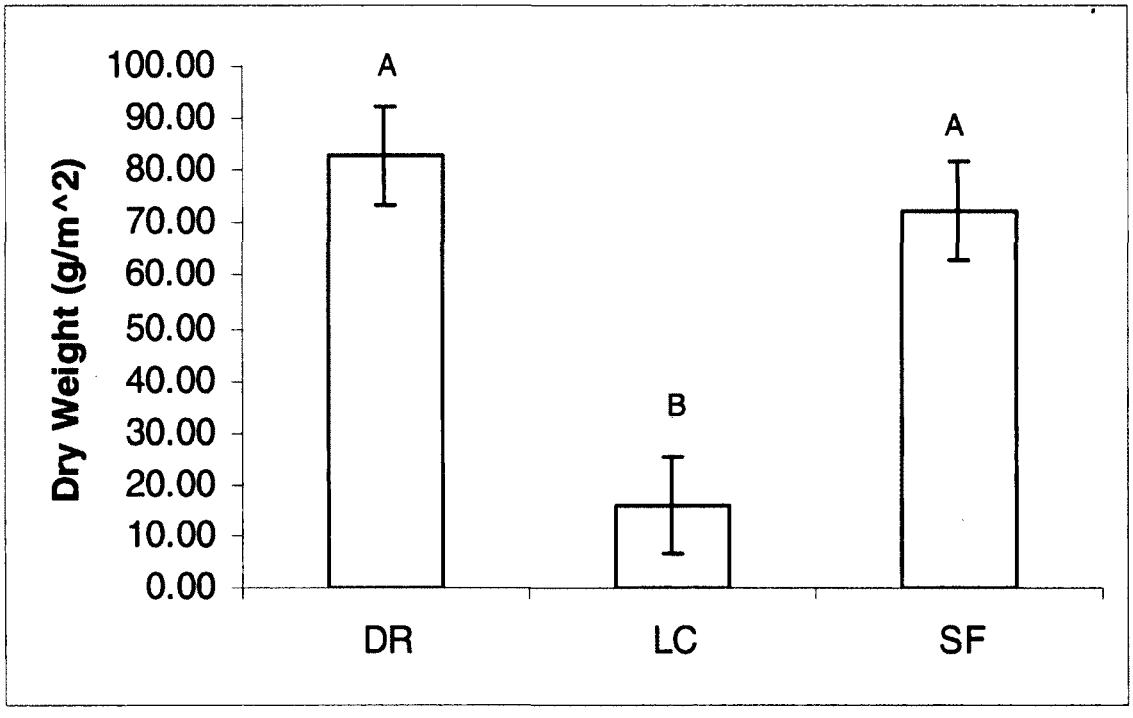

Figure 53 Great Bay *Gracilaria* biomass by site from 2008-2010. Letter designations denote significant differences (P<0.05). Error bars represent standard error.

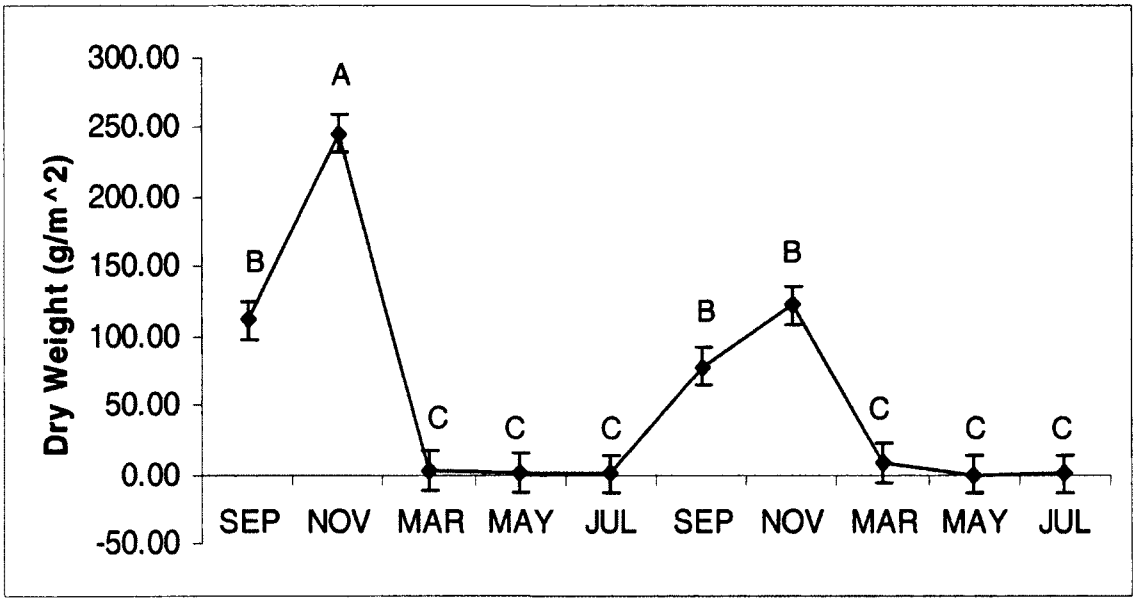

Figure 54 Southern Great Bay *Gracilaria* monthly mean biomass from 2008-2010. The Cedar Point and Wagon Hill Farm sites were not included in these calculations due to absence of organisms. Letter designations denote significant differences (P<0.05). Error bars represent standard error.

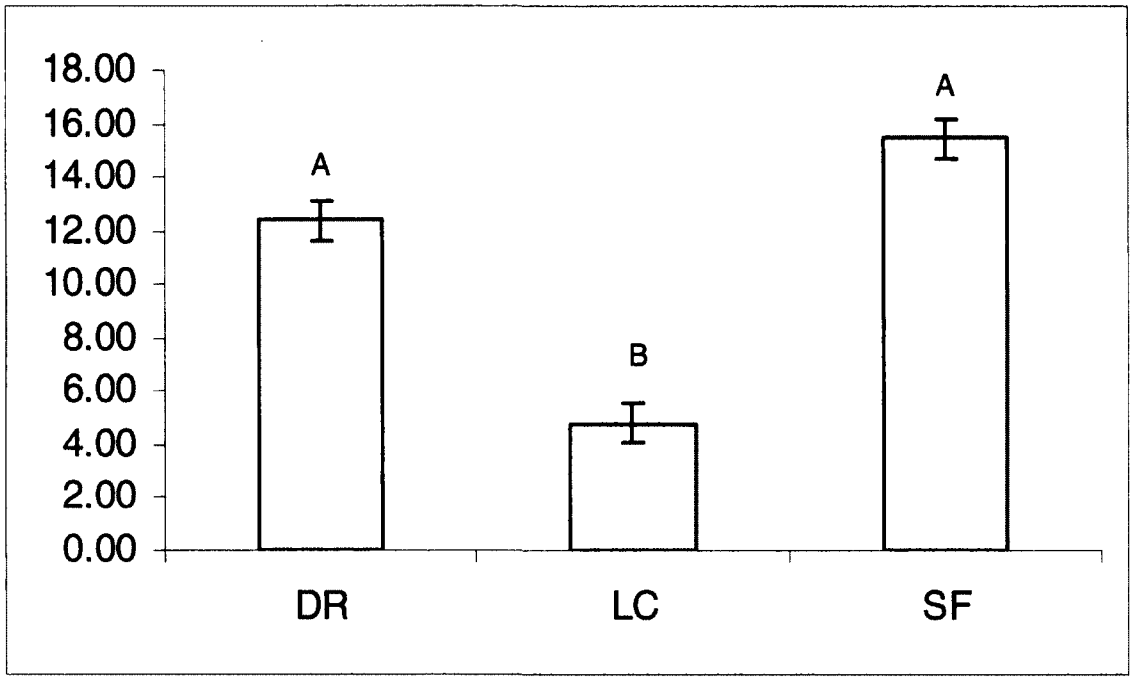

Figure 55 Southern Great Bay *Gracilaria* mean cover by site (2008-2010). Letter designations denote significant differences (P<0.05). Error bars represent standard error.

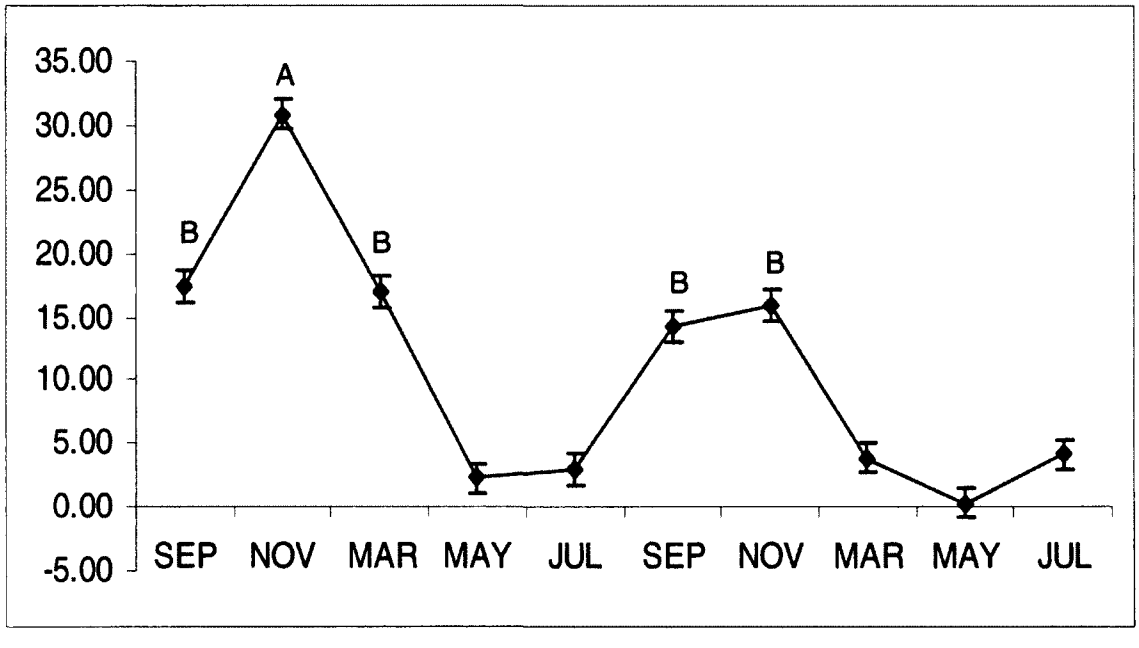

Figure 56 Southern Great Bay mean monthly *Gracilaria* cover (2008-2010). Letter designations denote significant differences (P<0.05). Error bars represent standard error.

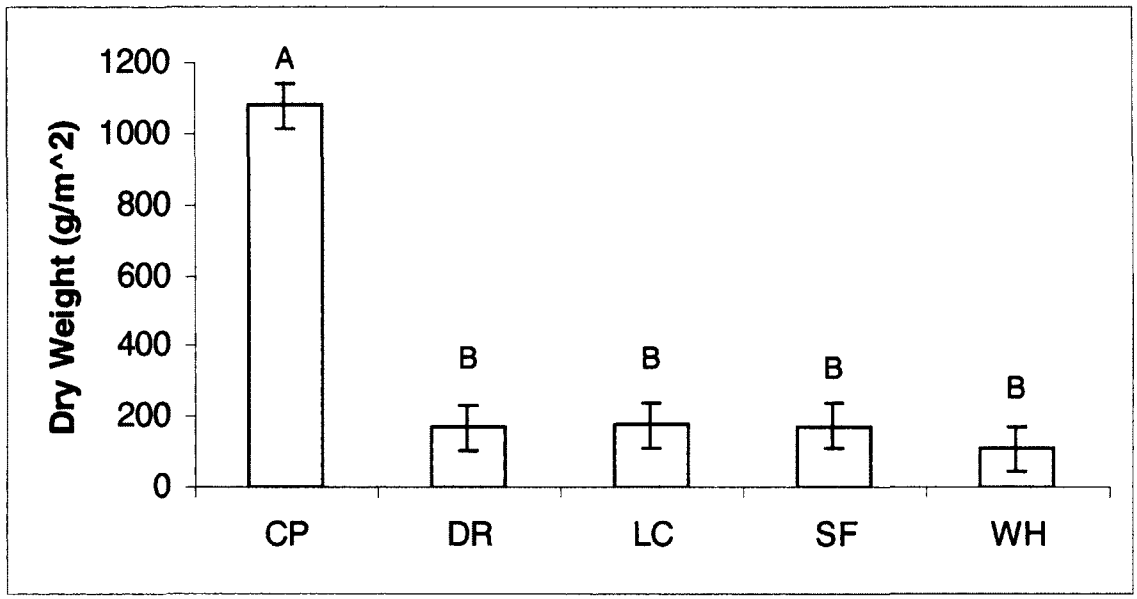

Figure 57 GBES algae mean biomass by site (2008-2010). Letter designations denote significant differences ($P<0.05$). Error bars represent standard error.

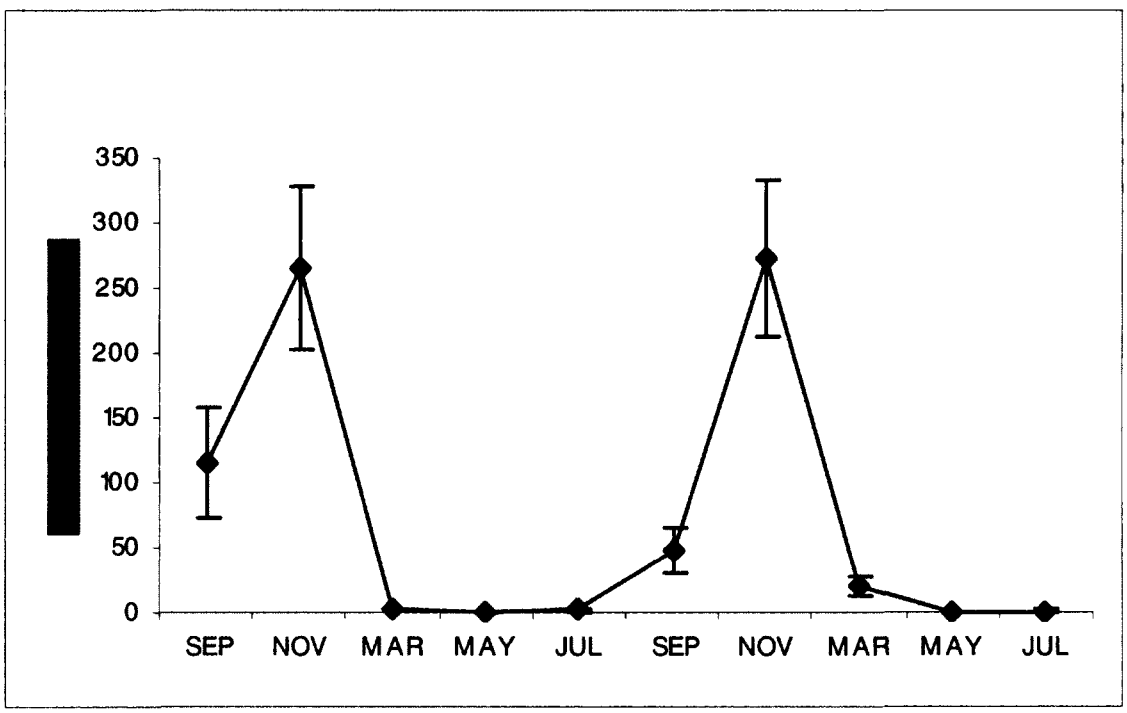

Figure 58 Sunset Farm *Gracilaria* mean biomass per month (2008-2010). Error bars represent standard error.

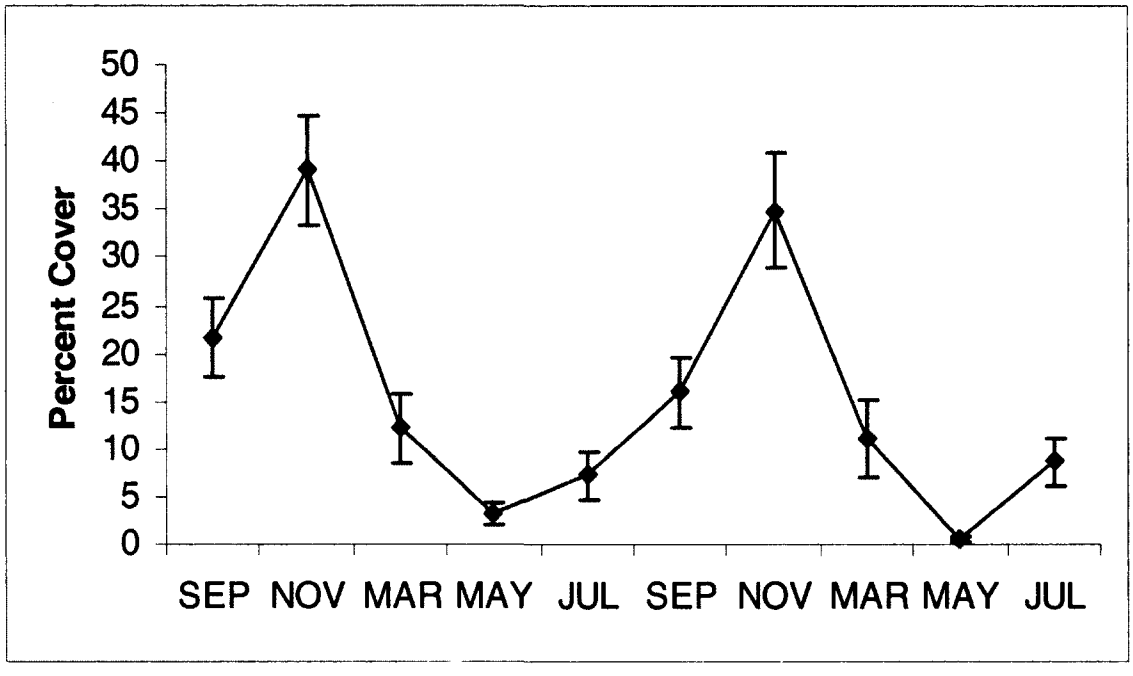

Figure 59 Sunset Farm *Gracilaria* mean percent cover (non-transformed) 2008-2010. Error bars represent standard error.

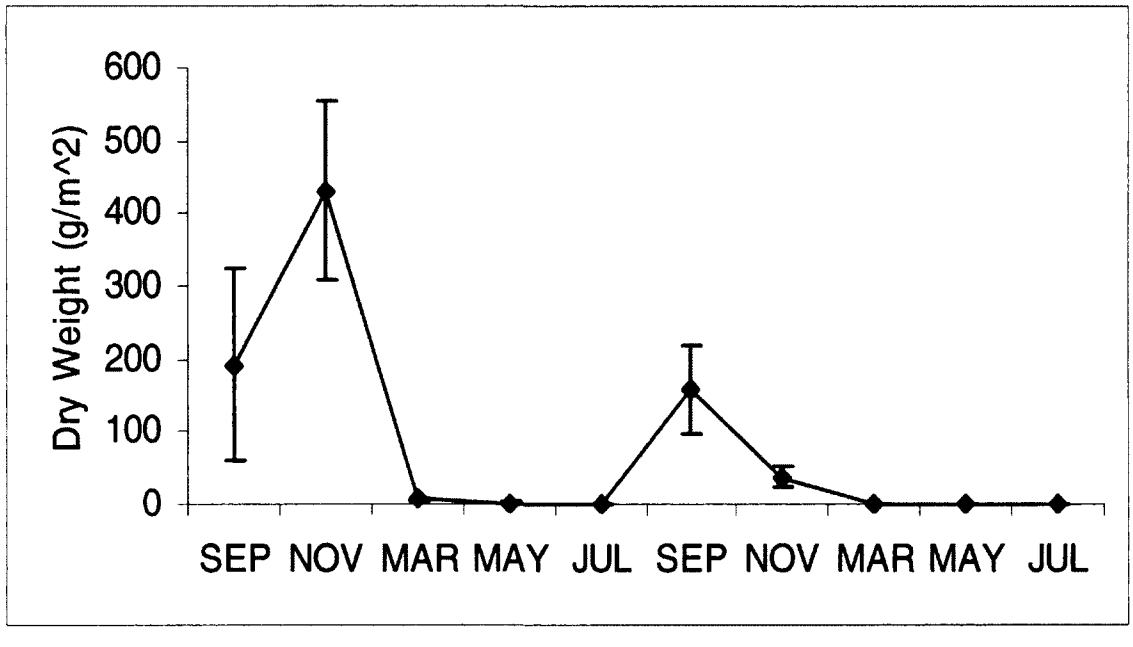

Figure 60 Depot Road *Gracilaria* mean monthly biomass (2008-2010). Error bars represent standard error.

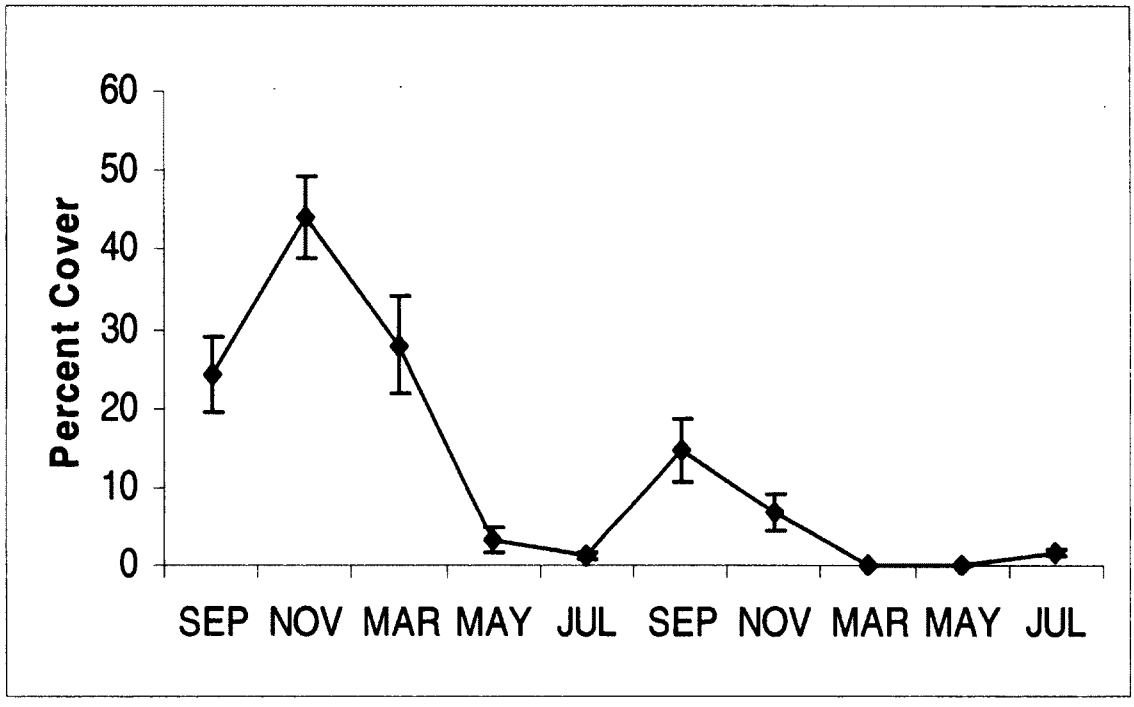

Figure 61 Depot Road *Gracilaria* mean percent cover (non-transformed) 2008-2010. Error bars represent standard error.

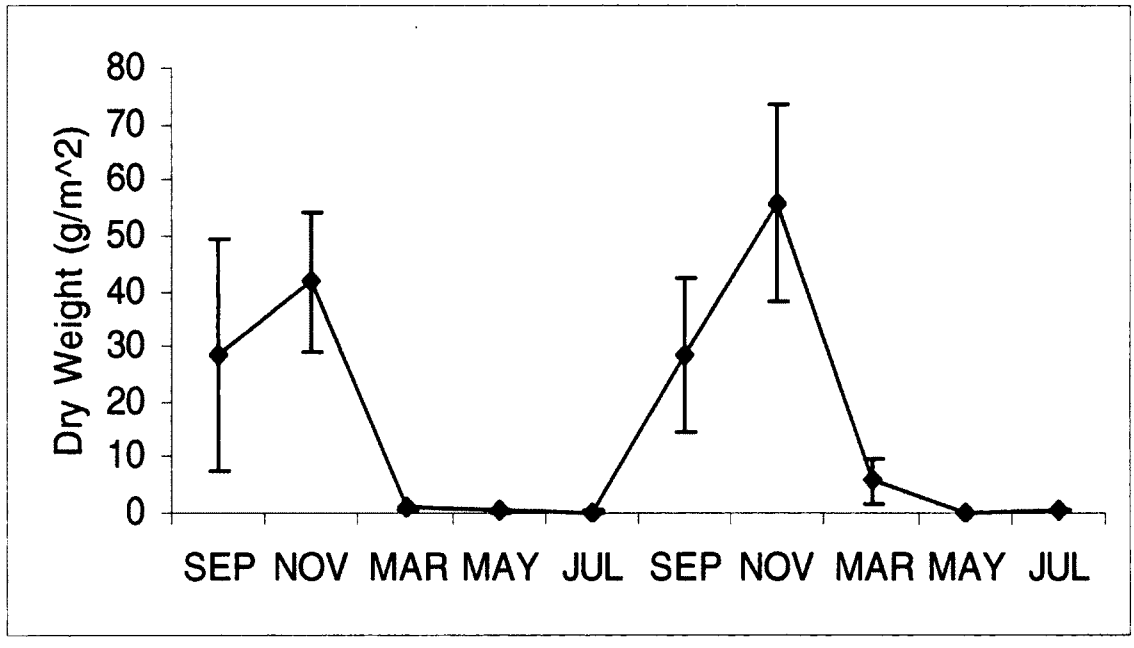

Figure 62 Lubberland Creek *Gracilaria* mean monthly biomass (2008-2010). Error bars represent standard error.

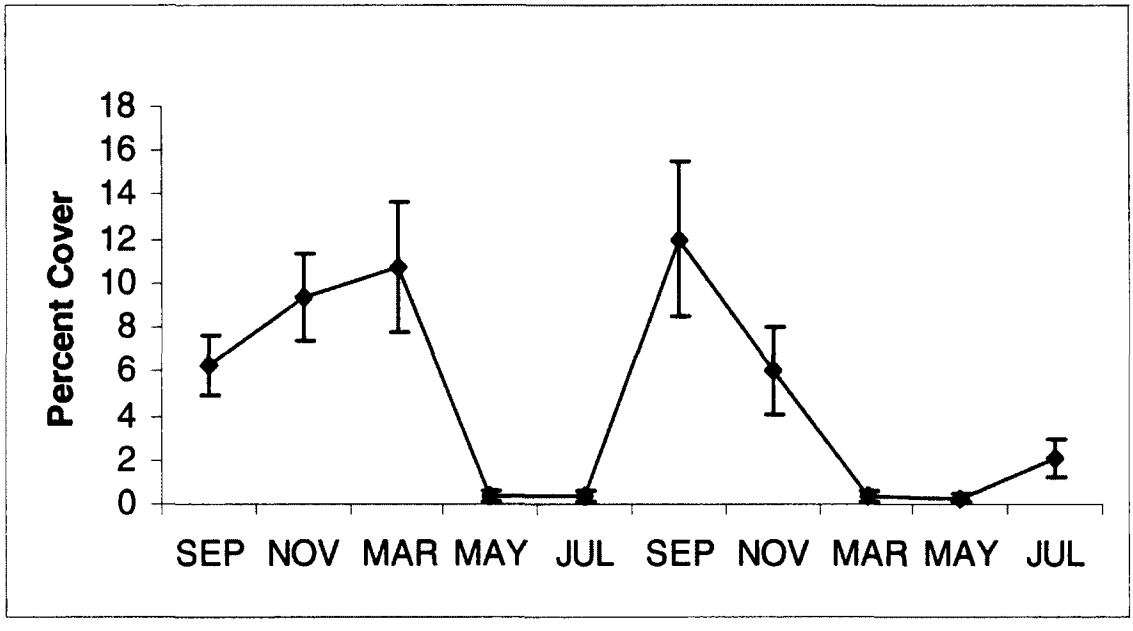

Figure 63 Lubberland Creek *Gracilaria* mean monthly percent cover (non-transformed) 2008-2010). Error bars represent standard error.

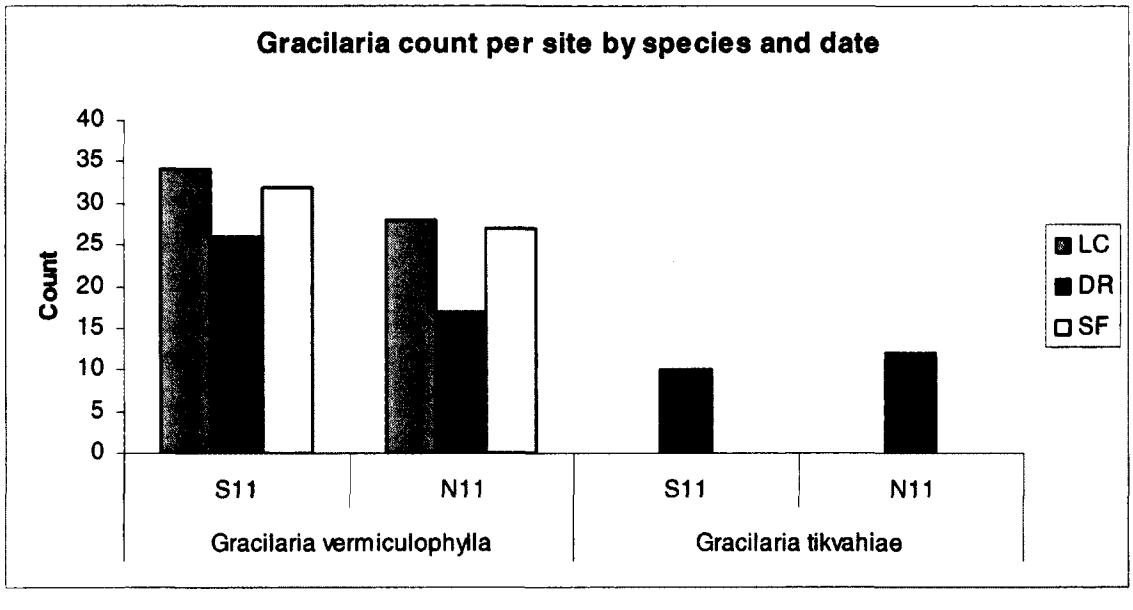

Figure 64 Number of specimens of each *Gracilaria* species verified from each study site in September and November of 2011. Identities were assigned through RFLP analyses.

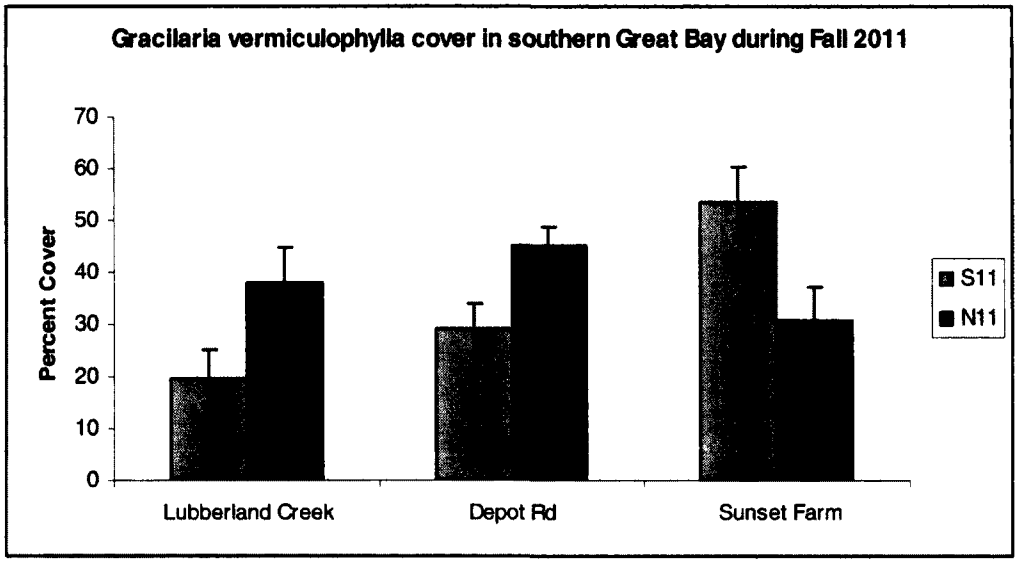

Figure 65 *Gracilaria vermiculophylla* mean percent cover during September and November 2011. Error bars represent standard error.

CHAPTER V

INTRODUCTION AND DISTRIBUTION OF *GRACILARIA VERMICULOPHYLLA* (RHODOPHYTA, GRACILARIALES) IN NEW ENGLAND, USA

Introduction

Currently there are more than 120 known introduced seaweeds worldwide, with many of these causing environmental and economic harm (Mathieson et al. 2008; Nyberg and Wallentinus 2005). Twenty-four introduced seaweeds are known from the Northwest Atlantic, including three green, four brown, and 17 red algae (Hofmann et al. 2010; Mathieson et al. 2008a,b; Schneider 2010; Thornber et al. 2009); many of these species were previously overlooked due to morphological similarities to native species. One such example is the native *Gracilaria tikvahiae* McLachlan and the morphologically similar congener *G. vermiculophylla* (Ohmi) Papenfuss, which has recently and rapidly spread around the globe (Rueness 2005), with introductions in the Eastern Pacific (Bellorin et al. 2004; Saunders 2009), Northeastern Atlantic including Denmark, Sweden (Nyberg 2007), Germany (Thomsen et al. 2007; Weinberger et al. 2008), and France (Rueness 2005), plus the Mid-Atlantic coast of North America (Freshwater et al. 2006; Thomsen et al. 2006) and New England (Mathieson et al. 2008; Saunders 2009; Schneider 2010; Thornber et al. 2009).

The speed of dispersal and subsequent success of *Gracilaria vermiculophylla* in these regions is notable. In the Northeastern Atlantic, it was first detected in the Göteborg archipelago, Sweden during 2003, and in two years it spread to over 30 sites with a

distributional range of 150 km (Nyberg 2007). A few years after its introduction to Germany and Denmark, it became the most abundant seaweed in many soft-bottom estuarine sites, particularly those with low salinities (Thomsen et al. 2007). A half decade after its introduction in France, *G. vermiculophylla* was present in most estuaries of the Brittany and Iberian coasts, often forming extensive unialgal entangled mats (Abreu 2011; Rueness 2005; Saunders 2009). In the southeastern Gulf of California, it was frequently observed forming expansive blooms in coastal lagoons (Pinion-Gimate et al. 2009). In the Mid-Atlantic coast of North America, it was first discovered in North Carolina in 2000, and by 2002 its extensive growth on gill nets and trawls hindered commercial fishing and fouled intake screens at the Brunswick Nuclear Plant (Freshwater et al. 2006). In Hog Island Bay, Virginia, USA, *G. vermiculophylla* was the dominant seaweed from 1998 through 2002, making up 74% of the total biomass across all study sites and seasons (Thomsen et al. 2006). It has continued to have significant impacts on saltmarsh habitat complexity, species richness and abundance, nutrient availability, productivity, and trophic interactions at several Virginia sites (Thomsen et al. 2009). The first published records of *G. vermiculophylla* from New England were from Narragansett Bay, Rhode Island (Schneider 2010; Thornber et al. 2009), but these accounts gave no details regarding its regional distribution or initial dates of introduction. Hence, the present study has attempted to clarify this information for this invasion.

As with many successful invaders, *Gracilaria vermiculophylla* has broad tolerances to temperatures, salinities (Abreu 2011; Nyberg 2007), nutrients, sediment burial, and grazing (Abreu et al. 2011a,b; Thomsen and McGlathery 2007). It also grows extensively from fragments (Abreu et al. 2011a,b; Nyberg and Wallentinus 2009), and is

capable of successfully colonizing and expanding in regions where its entire life history may not be expressed. Nyberg and Wallentinus (2009) also found that it could survive 175 days in moist conditions under total darkness, resuming exponential growth following a return to normal light, salinity, and immersion conditions. Hence, the species is well suited for long distance transport in ballast water, on ship hulls, or ship decks and can survive long term burial in estuarine environments (Nyberg and Wallentinus 2009).

Detection of *Gracilaria vermiculophylla* may be difficult because it is morphologically very similar to other species like *Gracilaria tikvahiae*. *Gracilaria* species have highly plastic morphologies and exhibit subtly distinguishing vegetative features when growing under ideal conditions (Gurgel et al. 2004; Rueness 2005; Saunders 2009). In the absence of sexual characteristics, which is common in *Gracilaria* (Rueness 2005), molecular analysis is the most useful method of species identification (Saunders 2009; Thomsen et al., 2006). In order to determine the current distribution and approximate introduction times of *G. vermiculophylla* in New England I used a three pronged approach, involving field sampling, investigations of historical collections, and molecular identifications of specimens by sequencing of the CO1 gene.

Materials And Methods

I surveyed 24 New England estuarine sites having known *Gracilaria* populations, with these ranging from western Connecticut to mid-coastal Maine (Table 4; Figure 66). In addition, three other Maine sites (Wilbur Neck, Pembroke; Little Augusta River, Whiting; Winslow Park, South Freeport) and one additional Massachusetts site (Damon's Point, Greenbush) with no previous *Gracilaria* records were also surveyed. The sites were either evaluated on foot at low tide or by snorkeling at mid tide. Depending on local

abundance, between five and 15 *Gracilaria* thalli/site were collected for morphological and/or molecular analysis.

In the field, all collected specimens were initially rinsed *in situ* to remove sediments and then placed in labeled zip-lock bags for transfer to the lab where they were floated in seawater, pressed as voucher specimens, and deposited in the Hodgdon Herbarium (NHA) at the University of New Hampshire, or in the herbaria at the Universities of New Brunswick (UNB) or Rhode Island (KIRI).

Gracilaria specimens collected from Connecticut, Rhode Island, Massachusetts, New Hampshire, and Maine between 1966 and 2011 were molecularly screened to confirm their species identifications and potential dates of introduction using the methods for DNA extraction, amplification, purification, sequencing, alignment, and GenBank comparison as outlined previously in Chapter IV. Representative voucher specimen sequences were deposited in GenBank (accession numbers JQ675682-JQ675712, JQ699274-699286, and JQ716364-JQ716366).

The segment of DNA amplified was 307 bp in length extending from position 328 of CO1. The 307 bp 3' end of CO1 was used for our identifications because it amplified more readily in historical collections than did the 5' end of the CO1 gene, and it revealed clear differences between all *Gracilaria* species with reference GenBank sequences. Within this region of CO1, a 13% (41 bp) divergence was evident between *G. tikvahiae* and *G. vermiculophylla* (Nettleton, pers. obs.).

Results

Based upon DNA identifications of the CO1 gene (matched to GenBank accessions FJ499599-FJ499628), the presence of *Gracilaria vermiculophylla* was

confirmed at 18 of the 24 New England sites (75%) known to have *Gracilaria* populations, with these ranging from Stamford, CT to Greenland, NH (Table 4, Figure 66). Such results expand the documented range of this introduction by over 200 km north, 100 km east, and 150 km west. No *G. vermiculophylla* populations were found at any of the five Maine sites where *G. tikvahiae* was previously recorded, while no *Gracilaria* was found at three other Maine sites and one Massachusetts site where such populations were previously unknown (cf. Materials and Methods). Mixed populations of *G. tikvahiae* and G. *vermiculophylla* were found at four New Hampshire sites, as well as in Potter Pond, South Kingston, RI, and Holly Pond, Stamford, CT. In the eight Massachusetts sites surveyed during March 2011, only *G. vermiculophylla* populations were found. The sequenced CO1 region revealed no genetic differences between or within *G. vermiculophylla* populations.

Gracilaria vermiculophylla was primarily found in estuarine sites having muddy or fine sandy bottoms. New Hampshire specimens were almost exclusively loose-lying or partially buried in sediment, whereas those from Massachusetts, Rhode Island, and Connecticut were typically attached to shells, small rocks, and other hard surfaces, with only occasional drifting specimens. Triphasic life history patterns (i.e. male, female and tetrasporic) of Rhode Island *Gracilaria vermiculophylla* populations have been confirmed by Thornber (unpubl. obs.), which suggests that the plant exhibits both sporic and vegetative fragmentation as a means of reproduction.

Molecular screening of historical collections showed the initial occurrence of *Gracilaria vermiculophylla* during 2000 at five Massachusetts sites. In New Hampshire, its first occurrence was during 2003 from Dover Point within the middle of the Great Bay

Estuarine System. An initial confirmation of *G. vermiculophylla* was made from Rhode Island during 2007 (Saunders 2009; Thornber et al. 2009), while it was not found in Connecticut prior to 2010.

Discussion

Based upon molecular evaluations of historical collections, *Gracilaria vermiculophylla* has existed primarily undetected in New England since at least 2000, several years prior to the first published records (Schneider 2010; Thornber et al. 2009). Such findings confirm the difficulty of documenting the arrival and spread of an invasive species that closely resembles a native congener (e.g. *Gracilaria tikvahiae*). To complicate matters, both species can survive year round within estuarine low intertidal/subtidal habitats, and they often grow together. For example, in Great Bay, New Hampshire, I frequently found vegetative specimens of both species within a single 0.25 m^2 quadrat frame. As such, species determinations in the field are impossible, and DNA sequencing is essential for their identification, like other cryptic introduced species in New England (Hofmann et al. 2010).

Since its initial collection in Virginia during 1998 (Thomsen et al. 2006) *Gracilaria vermiculophylla* has invaded and become established in disconnected estuaries across thousands of kilometers along the US Atlantic coastline. Its distribution now rivals *G. tikvahiae* in the western North Atlantic, which occurs in estuarine environments from southern Mexico to the Canadian Maritime Provinces (Gurgel et al. 2004).

Although *Gracilaria vermiculophylla* has not been reported as a nuisance to any maritime industries in New England, its wide distribution (over 500 km) and abundance in some locations could cause ecological problems. Even at low density, it has a negative impact on eelgrass or *Zostera marina* L. (Nyberg 2007; Wallentinus and Nyberg 2007), a species that is an important food source, nutrient cycler, and habitat for various invertebrates and small juvenile fish. *Zostera* populations in the Great Bay Estuarine System, NH/ME, Narragansett Bay, RI, and Long Island Sound, CT have been threatened for several decades (Oviatt 2004; Short 1992; Yarish 2006), and the inevitable spread of *G. vermiculophylla* within these systems could hinder their recovery.

Table 4 Historical/present day *Gracilaria* collections at 24 sites ranging from mid-coastal Maine to Stamford, Connecticut. GT and GV represent *G. tikvahiae* and *G. vermiculophylla* respectively.

Site	Location	Latitude °N	Longitude °W	Year Collected	Species	Herbarium Acc.
1	Oyster Creek, Salt Bay, Damariscotta, ME	44°03'28"	69°30'34"	2010	GT	NHA554802
2	Salt Bay, Damariscotta, ME	44°03'00"	69°51'40"	2010	GT	NHA554806-7
3	Upper New Meadows River, Bath, ME	43°55'50"	69°31'41"	2010	GT	NHA554805
4	Pennellville Landing, Brunswick, ME	43°51'18"	69°57'39"	2010	GT	NHA554803-4
5	Merepoint boat launch, Brunswick, ME	43°49'42"	70°00'59"	2010	GT	NHA554809
6	Wharton Point, Maquoit Bay, Brunswick, ME	43°52'01"	69°59'33"	2010	GT	NHA554801
7	Dover Point, Great Bay, Durham, NH	43°07'15"	70°49'35"	2003	GV, GT	NHA554808
8	Sunset Farm, Great Bay, Greenland, NH	43°03'24"	70°50'03"	2008-2010	GV, GT	NHA524468-9
9	Depot Road, Great Bay, Greenland, NH	43°03'22"	70°53'50"	2008-2011	GV, GT	NHA554795
10	Lubberland Creek, Great Bay, Newmarket, NH	43°04'30"	70°54'12"	2008-2012	GV, GT	NHA524474
11	Mattakeeset Ct Town Landing, Duxbury, MA	42°02'22"	70°40'11"	2000/2011	GV	NHA554785, NHA554798
12	Bluefish River, Shipyard Center, Duxbury, MA	42°02'47"	70°40'18"	2000/2011	GV	NHA554787-8, NHA554799
13	Ellisville Harbor State Park, Plymouth, MA	41°50'28"	70°32'07"	2000	GV	NHA554796
14	Indian Trail Rd, Barnstable, MA	41°42'35"	70°17'02"	2011	GV	NHA554789
15	Millway Beach, Barnstable, MA	41°42'34"	70°17'59"	2011	GV	NHA554793-4
16	Provincetown Harbor, MA	42°02'58"	70°11'09"	2000	GV	NHA554786
17	Capt. Nathaniel Wixon Dock, W. Harwick, MA	41°39'29"	70°06'55"	2011	GV	NHA554792
18	Lewis Pond, Sea Gull Beach, W. Yarmouth, MA	41°38'12"	70°13'40"	2000/2011	GV	NHA554797, NHA554790
19	Goddard State Park, Warwick, RI	41°40'03"	71°25'52"	2007	GV	NHA556929-30
20	Budlong Farm, Warwick, RI	41°41'10"	71°25'24"	2007	GV	NHA556931-3
21	Bass Rock, Narragansett, RI	41°24'18"	71°27'27"	2007	GV	NHA556985
22	Potter Pond, South Kingston, RI	41°22'56"	71°32'04"	2009	GV, GT	NHA556934
23	Seaside Beach, Bridgeport, CT	41°09'10"	73°12'39"	2010	GV	GWS022482-3
24	Holly Pond, Cove Island State Park, Stamford, CT	41°02'57"	73°30'08"	2010	GV, GT	NHA 524712

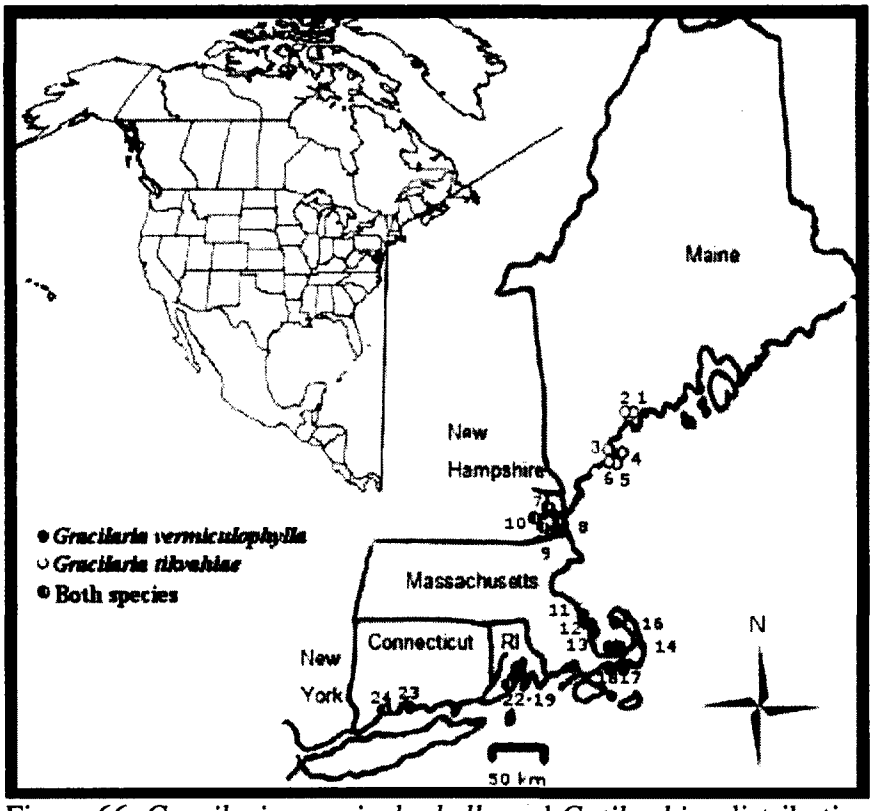

Figure 66. *Gracilaria vermiculophylla* and *G. tikvahiae* distribution in New England. The site numbers correspond to those given in Table 4.

CHAPTER VI

RECOVERY MANAGEMENT EFFORTS IN EUTROPHIC COASTAL SYSTEMS: THE BENEFITS, DIFFICULTIES, AND EXPECTATION SETTING

The Great Bay Estuarine system is currently highly enriched with nutrients. Such eutrophication alters ecosystems, leading to a reduction in species diversity, often of both long lived habitat forming seagrasses and macroalgae, which in turn causes faunal loss of diversity (Thorne-Miller et al. 1983; Breuer and Schramm 1988; Nienhuis 1992b; Villano and Warwick 1995). Disturbed ecosystems are more susceptible to invasion (Cecchereli and Cinelli 1999; Valentine et al. 2007). Unprecedented blooms of nuisance macroalgae, including the invasive *Gracilaria vermiculophylla* (Ohmi) Papenfuss, have been recorded in Great Bay Estuarine System in recent years (cf. Chapters III and IV), and, without coordinated intervention, further ecosystem change is likely.

While the effects of eutrophication and subsequent macroalgal blooms are overwhelmingly negative, some bloom events have had positive effects in their environment. For example, by winning the competition for nutrients, macroalgal bloom-forming species have controlled harmful phytoplankton blooms (Sfriso et al. 1992), and have greatly reduced red tides (Tang et al. 2003). While the opposite is true for most infaunal species, well-segmented worms, Oligiochaetes, can remain at high densities below algal mats (Thiel and Watling 1998), possibly due to the provided cover that serves to protect them from predators (Norkko 1998). Blooms can have a positive effect on grazers (McGlathery 1995), due to the influx of food sources and protective habitat.

High densities of small animals have been found in certain *Gracilaria* beds (Virnstein and Carbonara 1985), at least temporarily increasing biodiversity and population loads. The algal biomass itself can be a positive for an over enriched system, as the bloom-forming organisms absorb excess nutrients (Brault 1983). But unless the nutrient-capturing organisms are removed, the excess nutrients will cycle back into the system.

Although excess nitrogen and phosphorus have been known to play the key role in creating harmful macroalgal blooms, such as the ones observed in the Great Bay Estuarine System, ineffective management of affected regions has been the norm (de Jonge et al. 2002; Morand and Merceron 2004). Part of the problem is that it is difficult to recognize that a system has become eutrophic. The excess nutrients themselves are invisible to the eye and only detectable through water sampling methods unlikely to be performed without cause. Certain macroalgal species can increase the odds of early detection. *Ulva* is a good bioindicator of water quality, as its increased growth is correlated to pollution by coastal nutrient inputs (Levine and Wilce 1980; Ho 1987). When masses of these indicator species are seen, the odds are good that the environment is over enriched.

While adequate grazer pressure may neutralize *Ulva* growth even when the availability of nutrients is increased (Karez et al. 2004), system restoration requires that the problematic excess nutrients be removed from the environment rather allowing them to cycle back in. Physical removal of macroalgal blooms has been carried out in several places in order to reduce the negative environmental impacts of the algal mats themselves and to remove and reuse the stored nutrients (Orlandini 1988; Morand et al. 1991; Cuomo et al. 1993; Sfriso et al. 1993). In Brittany, excess seaweeds have been collected for

many uses including fertilizers for fruit and vegetable growth and livestock feed (Morand et al. 1991). Harvested seaweeds were considered for years for use as an alternative energy source in Brittany, but costs were prohibitive for use on a large scale. That is the production of one therm (100,000 BTU) from seaweed biomass ranged from 10-18 Euro cents versus 5-7 Euro cents for the same energy output from petroleum products (Charlier et al. 2008). In the Venice Lagoon in the late 1980s, 6,000 - 7,000 tons of *Gracilaria* were removed yearly and sold to industry for approximately $420,000 USD (Orlandini 1988). In this same lagoon, ~50,000 m^{-3} of *Ulva* were collected by special harvesting boats each year in the late 1980s and early 1990s (Cuomo et al. 1993). Reaping machines were also employed in the Venice Lagoon with a yearly estimated *Ulva* extraction of 100,000 tons WW (Sfriso et al. 1993).

Extraction of seaweeds from bloom affected regions comes with both environmental and economic problems. Removing seaweed from mudflats causes extensive loss of sediment, due to the low selectivity of mechanized methods (Brault and Golven 1983). Additionally, vehicular and human traffic, used during the algal removal process, damage the superficial sediment layer, which has negative consequences for the resident flora. Accidental removal of sediment stabilizing organisms can also lead to beach erosion, as was observed in the Peel Inlet (Atkins et al. 1993). Removal is also expensive (Briand 1989; Atkins et al. 1993), with annual costs of $161,000 USD (Peel Inlet, Australia) to $200,000 USD (Bays of Saint-Brieuc and Lannion, France).

Nutrient input reduction is the best strategy for successful management and restoration of eutrophic systems. Regulation of agricultural practices, both in fertilizer application management and livestock effluent treatment can lead to great reductions in N

and P inputs. Renovating and upgrading private and municipal sewage wastewater treatment facilities and practices can greatly reduce the release of nutrient pollution. Limiting lawn fertilizer use and industrial inputs aids system health. Through nutrient abatement programs and physical watershed alteration, some degree of restoration is possible. Following a major *Ulva* bloom, restoration efforts involving water flow redirection in a Tunisian lagoon caused a decrease in N inputs from 4000 µg/L to 400 µg/L and P inputs from 600 µg/L to 20 µg/L (Morand and Briand 1996). Using reduced chlorophyll a readings as a tested measure of restoration response (Cloern 2001), reductions in nutrient imputs have improved system health in Tampa Bay, FL (Greening and Janicki 2006) and in the Potomac and Patuxent Rivers, VA (Kemp et al. 2005).

Complete restoration to pre-enrichment conditions may prove difficult or impossible. Reducing nutrients doesn't always lead to the predicted outcomes (Philippart and Cadée 2000; Colijn and Cadée 2003). Duarte et al. (2009) found that nutrient abatement restoration efforts in four heavily studied eutrophic environments (Odense Fjord, Denmark; Gulf of Riga, Latvia/Estonia; Marsdiep, The Netherlands; Helgoland, Germany) were unable to return system conditions to those found prior to the nutrient enrichments that began between the 1960s and 1980s. Years after nutrient levels were reduced in these systems, chlorophyll a or diatom biomass remained at levels associated with their eutrophic peaks.

Lack of complete restoration, however, does not mean these nutrient reduction efforts were a failure. Abating nutrient inputs halted the steady deterioration of these systems rather than fully reversing the course of the decline (Duarte et al. 2009). Considering the multiple change-inducing pressures on a given disturbed ecosystem

(over-fishing, warming, increased atmospheric CO_2, marine acidification, invasion, and eutrophication) complete environmental restoration should not be the anticipated result of addressing only one of the causative factors. Yet, from a managerial and preservation standpoint, preventing excess nutrient inputs is a vital step in arresting deterioration in eutrophic coastal systems. Such efforts in Great Bay should be undertaken posthaste, with the understanding that complete system restoration, to pre-eutrophic conditions, should not be considered a likely final outcome.

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APPENDIX A

Water Total Nitrogen and Total Phosphorus

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

APPENDIX B

Ulva tissue nitrogen and phosphorus

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

	A	B	C	Mean	SD
SEP	4.435	4.16	4.179	4.258	0.154
NOV	4.314	3.132	4.192	3.879	0.65
MAR					
MAY	4.205			4.205	
JUL	4.98			4.98	
SEP	2.992	3.387	3.582	3.32	0.301
NOV					
MAR					
MAY	2.638	5.966	4.139	4.248	1.666
JUL					

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

	A	B	C	Mean	SD
SEP	0.149	0.124	0.102	0.125	0.023
NOV	0.092	0.134	0.128	0.118	0.023
MAR					
MAY	0.185			0.185	
JUL	0.113			0.113	
SEP	0.131	0.086	0.078	0.098	0.029
NOV					
MAR					
MAY	0.178			0.178	
JUL					

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

	A	B	C	Mean	SD
SEP					
NOV	4.286	4.222	3.116	3.875	0.658
MAR	2.499	2.927	2.726	2.718	0.214
MAY	2.168	2.598	2.587	2.451	0.245
JUL	1.668	0.666	2.161	1.498	0.761
SEP	1.611	1.955		1.783	0.243
NOV	2.191	1.924		2.057	0.188
MAR	1.616	1.995	3.366	2.326	0.921
MAY	2.418	2.847	2.906	2.724	0.266
JUL	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
SEP					
NOV	0.089	0.12	0.086	0.098	0.019
MAR	0.174	0.147	0.16	0.161	0.014
MAY	0.114	0.15	0.148	0.137	0.02
JUL	0.133	0.108	0.125	0.122	0.013
SEP	0.122	0.137		0.13	0.011
NOV	0.165	0.156		0.16	0.006
MAR	0.157	0.162	0.262	0.194	0.059
MAY	0.192	0.186	0.185	0.188	0.004
JUL	0.115		0.116	0.116	5E-04

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

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

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

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

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

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

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

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

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

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

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

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

APPENDIX C

Monthly biomass by site (g dry weight/m^2) +-SD, n=40

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

	Foliose *Ulva*	*Gracilaria*	*Polysiphonia stricta*	*Ascophyllum nodosum*	*Fucus vesiculosus* + ecad *scorpiodes*	*Chondrus crispus*	*Ulva intest & prolifera*	*Zostera marina*
SEP	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
NOV	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
MAR	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
MAY	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
JUL	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
SEP	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
NOV	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
MAR	0.49+-2.39	0+-0	0.01+-0.02	1463.7+-1814.5	48.7+-161.2	0+-0	0+-0	0+-0
MAY	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
JUL	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

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

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

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

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

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

	Foliose *Ulva*	*Gracilaria*	*Polysiphonia stricta*	*Ascophyllum nodosum*	*Fucus vesiculosus*	*Ceramium rubrum*	*Ahnfeltia plicata*	*Ulva intest & prolifera*	*Zostera marina*
SEP	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
NOV	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
MAR	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
MAY	2.8+-5.7	1.5+-5.5	0+-0	0+-0	0+-0	0+-0	0+-0	0+-0	0.01+-0.05
JUL	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
SEP	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
NOV	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
MAR	0+-0	0+-0	0+-0	0+-0	0+-0	0+-0	0+-0	0+-0	0+-0
MAY	6.6+-38.0	0+-0	0+-0	0+-0	0+-0	0+-0	0+-0	0+-0	0.1+-0.6
JUL	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

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

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

APPENDIX D

Monthly percent cover by site (+- SD, n=40)

Cedar Point Percent Cover 2008-2010

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

Wagon Hill Farm Percent Cover 2008-2010

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

Lubberland Creek Percent Cover 2008-2010

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

Depot Road Percent Cover 2008-2010

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

Sunset Farm Percent Cover 2008-2010

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

APPENDIX E

Herbarium collections identified through DNA sequencing of the designated gene. CO1 is the cytochrome c oxidase 1 region of the mitochondrial genome. ITS2 is the internal transcribed spacer 2 region of the nucleus. All sequences were matched to sample sequences found on GenBank.

Acc #	Site	Date	Collector	Species	Confirmation
70056	Mere Point, Brunswick, ME	11/1/1999	Mathieson, A.	*G. tikvahiae*	Sequenced CO1
78278	Dover Point, NH	10/18/2005	Mathieson, A.	*G. tikvahiae*	Sequenced CO1
78247	Dover Point, NH	11/12/2003	Johnson, K.	*G.vermiculophylla*	Sequenced CO1
15551	Dover Point, NH	5/29/1996	Reynolds, N.B	*G. tikvahiae*	Sequenced CO1
57001	Meduncook River, ME	6/17/1995	Mathieson, A.	*G. tikvahiae*	Sequenced CO1
58130	Glidden PT, Damariscotta, ME	6/16/1995	Mathieson, A., E. Hehre	*G. tikvahiae*	Sequenced CO1
54075	Oyster River, NH	9/21/1994	Mathieson, A.	*G. tikvahiae*	Sequenced CO1
53058	Salt Bay, Nobleboro, ME	8/11/1994	Mathieson, A., E. Hehre	*G. tikvahiae*	Sequenced CO1

63876	Dover Point, NH	9/1/1993	Gerwick, J.	*G. tikvahiae*	Sequenced CO1
50299	Welsh Cove, Great Bay, NH	2/24/1993	Mathieson, A.	*G. tikvahiae*	Sequenced CO1
48948	Damariscotta River, ME	7/4/1985	Penniman, C.	*G. tikvahiae*	Sequenced CO1
48284	Dover Point, NH	9/4/1983	Turgeon, L.	*G. tikvahiae*	Sequenced CO1
35218	Oyster River, NH	8/8/1977	Fullerton, P.	*G. tikvahiae*	Sequenced CO1
35822	Moody PT, NH	8/3/1977	Costa, M., P. Fullerton	*G. tikvahiae*	Sequenced CO1
27963	Goat Island, Great Bay, NH	6/12/1976	Mathieson, A.	*G. tikvahiae*	Sequenced CO1
24068	Dover Point, NH	8/21/1975	Norall, T. & M. Josselyn	*G. tikvahiae*	Sequenced CO1
23944	Sullivan bridge, GBES, NH	7/11/1975	Mathieson, A.	*G. tikvahiae*	Sequenced CO1
18191	Welsh Cove, Great Bay, NH	8/24/1972	Hutchinson, B.	*G. tikvahiae*	Sequenced CO1
10183A	Weeks PT, Great Bay, NH	8/9/1967	Mathieson, A.	*G. tikvahiae*	Sequenced CO1
748	Sandy Point, Greenland, NH	7/20/1966	Mathieson, A.	*G. tikvahiae*	Sequenced CO1

4535	Weeks PT, Great Bay, NH	4/23/1966	Searles, M.P.	*G. tikvahiae*	Sequenced CO1
75757	Ellisville Harbor State Park, MA	11/18/2000	Mathieson, A.C.	*G. vermiculophylla*	Sequenced CO1
72810	Duxbury Marsh, Duxbury, MA	12/2/2000	Mathieson, A.C.	*G. vermiculophylla*	Sequenced CO1
72561	Duxbury Public Pier, Duxbury, MA	8/9/2000	Mathieson, A.C.	*G. vermiculophylla*	Sequenced CO1
73768	West Yarmouth, MA: Seagull Beach	11/4/2000	Mathieson, A.C.	*G. vermiculophylla*	Sequenced CO1
72355	Bourne, MA: MAMaritime Acad.	8/11/2000	Mathieson, A.C.	*G. tikvahiae*	Sequenced CO1
73062	Provincetown Harbor, MA	11/4/2000	Mathieson, A.C.	*G. vermiculophylla*	Sequenced CO1
23781	Sullivan Bridge- Piling 4E, GBES, NH	7/18/1975	Mathieson, A.	*U. rigida*	Sequenced ITS
15269	Dover Point, NH	2/14/1969	Reynolds, N.B	*U. pertusa*	Sequenced ITS
8407	Fort Stark, New Castle, NH	4/20/1967	Mathieson, Hehre, Conway	*U. pertusa*	Sequenced ITS
78229	Rye Harbor, Rye, NH	1/25/2003	Carrington & Glieco	*U. pertusa*	Sequenced ITS
48077	Seabrook, NH station 17 offshore	9/12/1982	Scott, H.	*U. lactuca*	Sequenced ITS

36242	Oyster River, Durham, NH	10/25/1977	Costa, M.	*U. compressa*	Sequenced ITS
24989	Bunker Creek, Durham, NH	9/12/1968	Mathieson, A.	*U. pertusa*	Sequenced ITS
19518A	Little Bay Site, Durham, NH	8/24/1972	Hutchinson, B.	*U. compressa*	Sequenced ITS
4352	Woodman Pt., Newington, NH	8/19/1966	Conway, Shipman	*U. rigida*	Sequenced ITS

APPENDIX F

Doctoral study collections identified through DNA sequencing of the designated gene. CO1 is the cytochrome c oxidase 1 region of the mitochondrial genome. ITS2 is the internal transcribed spacer 2 region of the nucleus. All sequences were matched to sample sequences found on GenBank.

Acc #		Date	Location	Collector	Species	Confirmation
NHA	524468	11/15/2008	Sunset Farm, GBES, NH	JCN	*G. vermiculophylla*	Sequenced CO1
NHA	524469	11/15/2008	Sunset Farm, GBES, NH	JCN	*G. vermiculophylla*	Sequenced CO1
NHA	524474	11/17/2008	Lubberland Creek, GBES, NH	JCN	*G. vermiculophylla*	Sequenced CO1
NHA	524473	5/28/2009	Sunset Farm, GBES, NH	JCN	*G. vermiculophylla*	Sequenced CO1
NHA	524472	5/28/2009	Sunset Farm, GBES, NH	JCN	*G. vermiculophylla*	Sequenced CO1
NHA	524471	5/28/2009	Sunset Farm, GBES, NH	JCN	*G. vermiculophylla*	Sequenced CO1
NHA	524470	5/28/2009	Sunset Farm, GBES, NH	JCN	*G. tikvahiae*	Sequenced CO1
NHA	524478	5/28/2009	Depot Rd, GBES, NH	JCN	*G. tikvahiae*	Sequenced CO1
NHA	524479	5/28/2009	Depot Rd, GBES, NH	JCN	*G. tikvahiae*	Sequenced CO1
NHA	524480	5/28/2009	Depot Rd, GBES, NH	JCN	*G. tikvahiae*	Sequenced CO1
NHA	524476	5/27/2009	Lubberland Creek, GBES, NH	JCN	*G. tikvahiae*	Sequenced CO1
NHA	524475	5/27/2009	Lubberland Creek, GBES, NH	JCN	*G. vermiculophylla*	Sequenced CO1
NHA	524714	7/28/2009	Depot Rd, GBES, NH	JCN	*G. tikvahiae*	Sequenced CO1
NHA	554795A	7/28/2009	Depot Rd, GBES, NH	JCN	*G. tikvahiae*	Sequenced CO1
NHA	554795B	7/28/2009	Depot Rd, GBES, NH	JCN	*G. vermiculophylla*	Sequenced CO1
NHA	554793A	3/17/2011	Barnstable, MA, Millway Beach	JCN TSB	*G. vermiculophylla*	Sequenced CO1

NHA	554793E	3/17/2011	Barnstable, MA, Millway Beach	JCN TSB	*G. vermiculophylla*	Sequenced CO1
NHA	554794D	3/17/2011	Barnstable, MA, Millway Beach	JCN TSB	*G. vermiculophylla*	Sequenced CO1
NHA	554792A	3/17/2011	W. Harwick, MA Near Herring mouth	JCN TSB	*G. vermiculophylla*	Sequenced CO1
NHA	554792E	3/17/2011	W. Harwick, MA Near Herring mouth	JCN TSB	*G. vermiculophylla*	Sequenced CO1
NHA	554790A	3/17/2011	W. Yarmouth, MA Lewis Pond	JCN TSB	*G. vermiculophylla*	Sequenced CO1
NHA	554789C	3/17/2011	Barnstable, MA, near Audobon	JCN TSB	*G. vermiculophylla*	Sequenced CO1
NHA	554789D	3/17/2011	Barnstable, MA, near Audobon	JCN TSB	*G. vermiculophylla*	Sequenced CO1
NHA	554799C	3/23/2011	Duxbury, MA, public shipyard	JCN	*G. vermiculophylla*	Sequenced CO1
NHA	554799D	3/23/2011	Duxbury, MA, public shipyard	JCN	*G. vermiculophylla*	Sequenced CO1
NHA	554788B	3/23/2011	Duxbury, MA, public shipyard	JCN	*G. vermiculophylla*	Sequenced CO1
NHA	554798A	3/23/2011	Duxbury, MA, town landing	JCN	*G. vermiculophylla*	Sequenced CO1
NHA	554798D	3/24/2011	Duxbury, MA, town landing	JCN	*G. vermiculophylla*	Sequenced CO1
NHA	524449	9/30/2008	Sunset Farm, GBES, NH	JCN	*U. rigida*	Sequenced ITS2
NHA	524450	9/30/2008	Sunset Farm, GBES, NH	JCN	*U. compressa*	Sequenced ITS2
NHA	524451	9/30/2008	Sunset Farm, GBES, NH	JCN	*U. compressa*	Sequenced ITS2
NHA	524438	9/30/2008	Depot Rd, GBES, NH	JCN	*U. rigida*	Sequenced ITS2
NHA	524439	9/30/2008	Depot Rd, GBES, NH	JCN	*U. rigida*	Sequenced ITS2
NHA	524423	11/14/2008	Cedar Pt, GBES, NH	JCN	*U. rigida*	Sequenced ITS2
NHA	524429	11/15/2008	Bunker Creek, GBES, NH	JCN	*U. compressa*	Sequenced ITS2
NHA	524430	11/16/2008	Wagon Hill Farm, GBES, NH	JCN	*U. compressa*	Sequenced ITS2
NHA	524431	11/16/2008	Wagon Hill Farm, GBES, NH	JCN	*U. compressa*	Sequenced ITS2
NHA	524426	11/16/2008	Cedar Pt, GBES, NH	JCN	*U. rigida*	Sequenced ITS2
NHA	524426	11/16/2008	Cedar Pt, GBES, NH	JCN	*U. rigida*	Sequenced ITS2

NHA	524460	11/17/2008	Lubberland Creek, GBES, NH	JCN	*U. rigida*	Sequenced ITS2
NHA	524462	11/17/2008	Lubberland Creek, GBES, NH	JCN	*U. rigida*	Sequenced ITS2
NHA	524463	11/17/2008	Lubberland Creek, GBES, NH	JCN	*U. rigida*	Sequenced ITS2
NHA	524464	11/17/2008	Lubberland Creek, GBES, NH	JCN	*U. rigida*	Sequenced ITS2
NHA	556589A	5/15/2008	Charlestown Breachway, RI	JCN	*U. compressa*	Sequenced ITS2
	556589B	5/15/2008	Charlestown Breachway, RI	JCN	*U. compressa*	Sequenced ITS2
NHA	556591A	5/15/2008	New Haven, CT	JCN	*U. compressa*	Sequenced ITS2
	556591B	5/15/2008	New Haven, CT	JCN	*U. compressa*	Sequenced ITS2
	556591C	5/15/2008	New Haven, CT	JCN	*U. compressa*	Sequenced ITS2
NHA	556592	5/15/2008	Black Point, Narragansett, RI	JCN	*U. lactuca*	Sequenced ITS2
NHA	556593	5/15/2008	Black Point, Narragansett, RI	JCN	*U. prolifera*	Sequenced ITS2
NHA	556594	5/15/2008	Rocky Neck SP, RI	JCN	*U. lactua*	Sequenced ITS2
NHA	556595	5/15/2008	Falmouth Heights, MA	JCN	*U. prolifera*	Sequenced ITS2
NHA	524742	7/28/2009	Depot Rd, GBES, NH	JCN	*U. compressa*	Sequenced ITS2
NHA	524738A	7/28/2009	Depot Rd, GBES, NH	JCN	*U. rigida*	Sequenced ITS2
	524738B	7/28/2009	Depot Rd, GBES, NH	JCN	*U. compressa*	Sequenced ITS2
NHA	554842	11/20/2009	New Meadows River, ME	JCN	*U. prolifera*	Sequenced ITS2
NHA	554843	11/20/2009	Wharton Pt., ME	JCN	*U. rigida*	Sequenced ITS2
NHA	556568	11/20/2009	Mere Pt., ME	JCN	*U. compressa*	Sequenced ITS2
NHA	556569	11/20/2009	Mere Neck, ME	JCN	*U. intestinalis*	Sequenced ITS2
NHA	556570	11/20/2009	South Freeport, ME	JCN	*U. prolifera*	Sequenced ITS2
NHA	556572	11/20/2009	Motel East, Eastport, ME	JCN	*U. intestinalis*	Sequenced ITS2
NHA	556572	11/20/2009	Motel East, Eastport, ME	JCN	*U. prolifera*	Sequenced ITS2

NHA	556573	11/202009	Motel East, Eastport, ME	JCN	*U. lactuca*	Sequenced ITS2
NHA	556573	11/202009	Motel East, Eastport, ME	JCN	*U. prolifera*	Sequenced ITS2
NHA	556574	11/22/2009	Little Augusta, Whiting, ME	JCN	*U. rigida*	Sequenced ITS2
NHA	556575	11/22/2009	Little Augusta, Whiting, ME	JCN	*U. prolifera*	Sequenced ITS2
NHA	556576	11/22/2009	Wilber Neck, Eastport, ME	JCN	*U. prolifera*	Sequenced ITS2
NHA	556578	11/22/2009	Wilber Neck, Eastport, ME	JCN	*U. intestinalis*	Sequenced ITS2
NHA	554822	12/14/2009	C. Challenger hull at CML, NH	JCN	*U. lactua*	Sequenced ITS2
NHA	554823	12/14/2009	C. Challenger hull at CML, NH	JCN	*U. lactua*	Sequenced ITS2
NHA	554824	12/14/2009	C. Challenger hull at CML, NH	JCN	*U. lactua*	Sequenced ITS2

APPENDIX G

Restriction Fragment Length Polymorphism identifications. Restriction digests of the 3' end of CO1 using the DpnII enzyme revealed clear band size differences between species.

Sample ID	Accession #	Collected	Location	Collector	Species ID	Confirmation
JCN760A	NHA 556612	28-Sep-11	Lubberland Creek	JCN	*G. vermiculophylla*	Verified by RFLP
JCN760B		28-Sep-11	Lubberland Creek	JCN	*G. vermiculophylla*	Verified by RFLP
JCN760C		28-Sep-11	Lubberland Creek	JCN	*G. vermiculophylla*	Verified by RFLP
JCN760D		28-Sep-11	Lubberland Creek	JCN	*G. vermiculophylla*	Verified by RFLP
JCN761A	NHA 556613	28-Sep-11	Lubberland Creek	JCN	*G. vermiculophylla*	Verified by RFLP
JCN761B		28-Sep-11	Lubberland Creek	JCN	*G. vermiculophylla*	Verified by RFLP
JCN761C		28-Sep-11	Lubberland Creek	JCN	*G. vermiculophylla*	Verified by RFLP
JCN761D		28-Sep-11	Lubberland Creek	JCN	*G. vermiculophylla*	Verified by RFLP
JCN762A	NHA 556614	28-Sep-11	Lubberland Creek	JCN	*G. vermiculophylla*	Verified by RFLP
JCN762B		28-Sep-11	Lubberland Creek	JCN	*G. vermiculophylla*	Verified by RFLP
JCN762C		28-Sep-11	Lubberland Creek	JCN	*G. vermiculophylla*	Verified by RFLP
JCN762D		28-Sep-11	Lubberland Creek	JCN	*G. vermiculophylla*	Verified by RFLP
JCN763A	NHA 556615	28-Sep-11	Lubberland Creek	JCN	*G. vermiculophylla*	Verified by RFLP
JCN763B		28-Sep-11	Lubberland Creek	JCN	*G. vermiculophylla*	Verified by RFLP
JCN763C		28-Sep-11	Lubberland Creek	JCN	*G. vermiculophylla*	Verified by RFLP
JCN763D		28-Sep-11	Lubberland Creek	JCN	*G. vermiculophylla*	Verified by RFLP
JCN764A	NHA 556616	28-Sep-11	Lubberland Creek	JCN	*G. vermiculophylla*	Verified by RFLP
JCN764B		28-Sep-11	Lubberland Creek	JCN	*G. vermiculophylla*	Verified by RFLP
JCN764C		28-Sep-11	Lubberland Creek	JCN	*G. vermiculophylla*	Verified by RFLP
JCN765A	NHA 556617	28-Sep-11	Lubberland Creek	JCN	*G. vermiculophylla*	Verified by RFLP
JCN765A		28-Sep-11	Lubberland Creek	JCN	*G. vermiculophylla*	Verified by RFLP
JCN765A		28-Sep-11	Lubberland Creek	JCN	*G. vermiculophylla*	Verified by RFLP

JCN766A	NHA 556618	28-Sep-11	Lubberland Creek	JCN	*G. vermiculophylla*	Verified by RFLP
JCN766B		28-Sep-11	Lubberland Creek	JCN	*G. vermiculophylla*	Verified by RFLP
JCN766C		28-Sep-11	Lubberland Creek	JCN	*G. vermiculophylla*	Verified by RFLP
JCN767A	NHA 556619	28-Sep-11	Lubberland Creek	JCN	*G. vermiculophylla*	Verified by RFLP
JCN767B		28-Sep-11	Lubberland Creek	JCN	*G. vermiculophylla*	Verified by RFLP
JCN767C		28-Sep-11	Lubberland Creek	JCN	*G. vermiculophylla*	Verified by RFLP
JCN768A	NHA 556620	28-Sep-11	Lubberland Creek	JCN	*G. vermiculophylla*	Verified by RFLP
JCN768B		28-Sep-11	Lubberland Creek	JCN	*G. vermiculophylla*	Verified by RFLP
JCN768C		28-Sep-11	Lubberland Creek	JCN	*G. vermiculophylla*	Verified by RFLP
JCN769A	NHA 556621	28-Sep-11	Lubberland Creek	JCN	*G. vermiculophylla*	Verified by RFLP
JCN769B		28-Sep-11	Lubberland Creek	JCN	*G. vermiculophylla*	Verified by RFLP
JCN769C		28-Sep-11	Lubberland Creek	JCN	*G. vermiculophylla*	Verified by RFLP
JCN770A	NHA 556622	28-Sep-11	Depot Rd	JCN	*G. vermiculophylla*	Verified by RFLP
JCN770B		28-Sep-11	Depot Rd	JCN	*G. vermiculophylla*	Verified by RFLP
JCN770C		28-Sep-11	Depot Rd	JCN	*G. vermiculophylla*	Verified by RFLP
JCN770D		28-Sep-11	Depot Rd	JCN	*G. vermiculophylla*	Verified by RFLP
JCN771A	NHA 556623	28-Sep-11	Depot Rd	JCN	*G. vermiculophylla*	Verified by RFLP
JCN771B		28-Sep-11	Depot Rd	JCN	*G. vermiculophylla*	Verified by RFLP
JCN771C		28-Sep-11	Depot Rd	JCN	*G. tikvahiae*	Verified by RFLP
JCN771D		28-Sep-11	Depot Rd	JCN	*G. tikvahiae*	Verified by RFLP
JCN772A	NHA 556624	28-Sep-11	Depot Rd	JCN	*G. vermiculophylla*	Verified by RFLP
JCN772B		28-Sep-11	Depot Rd	JCN	*G. tikvahiae*	Verified by RFLP
JCN772C		28-Sep-11	Depot Rd	JCN	*G. vermiculophylla*	Verified by RFLP
JCN772D		28-Sep-11	Depot Rd	JCN	*G. vermiculophylla*	Verified by RFLP
JCN773A	NHA 556625	28-Sep-11	Depot Rd	JCN	*G. vermiculophylla*	Verified by RFLP
JCN773B		28-Sep-11	Depot Rd	JCN	*G. vermiculophylla*	Verified by RFLP
JCN773C		28-Sep-11	Depot Rd	JCN	*G. vermiculophylla*	Verified by RFLP

JCN774A	NHA 556626	28-Sep-11	Depot Rd	JCN	*G. vermiculophylla*	Verified by RFLP
JCN774B		28-Sep-11	Depot Rd	JCN	*G. vermiculophylla*	Verified by RFLP
JCN774C		28-Sep-11	Depot Rd	JCN	*G. tikvahiae*	Verified by RFLP
JCN774D		28-Sep-11	Depot Rd	JCN	*G. tikvahiae*	Verified by RFLP
JCN775A	NHA 556627	28-Sep-11	Depot Rd	JCN	*G. vermiculophylla*	Verified by RFLP
JCN775B		28-Sep-11	Depot Rd	JCN	*G. vermiculophylla*	Verified by RFLP
JCN777C		28-Sep-11	Depot Rd	JCN	*G. tikvahiae*	Verified by RFLP
JCN776A	NHA 556628	28-Sep-11	Depot Rd	JCN	*G. vermiculophylla*	Verified by RFLP
JCN776B		28-Sep-11	Depot Rd	JCN	No Band	
JCN776B2		28-Sep-11	Depot Rd	JCN	*G. vermiculophylla*	Verified by RFLP
JCN777A	NHA 556629	28-Sep-11	Depot Rd	JCN	*G. tikvahiae*	Verified by RFLP
JCN777B		28-Sep-11	Depot Rd	JCN	*G. vermiculophylla*	Verified by RFLP
JCN777C		28-Sep-11	Depot Rd	JCN	*G. vermiculophylla*	Verified by RFLP
JCN778A	NHA 556630	28-Sep-11	Depot Rd	JCN	*G. vermiculophylla*	Verified by RFLP
JCN778A2		28-Sep-11	Depot Rd	JCN	*G. vermiculophylla*	Verified by RFLP
JCN778B		28-Sep-11	Depot Rd	JCN	*G. vermiculophylla*	Verified by RFLP
JCN778B2		28-Sep-11	Depot Rd	JCN	*G. vermiculophylla*	Verified by RFLP
JCN778C		28-Sep-11	Depot Rd	JCN	*G. vermiculophylla*	Verified by RFLP
JCN779A	NHA 556925	28-Sep-11	Depot Rd	JCN	*G. tikvahiae*	Verified by RFLP
JCN779B		28-Sep-11	Depot Rd	JCN	*G. tikvahiae*	Verified by RFLP
JCN779C		28-Sep-11	Depot Rd	JCN	*G. tikvahiae*	Verified by RFLP
JCN779D		28-Sep-11	Depot Rd	JCN	*G. vermiculophylla*	Verified by RFLP
JCN780A	NHA 556631	28-Sep-11	Sunset Farm	JCN	*G. vermiculophylla*	Verified by RFLP
JCN780B		28-Sep-11	Sunset Farm	JCN	*G. vermiculophylla*	Verified by RFLP
JCN780C		28-Sep-11	Sunset Farm	JCN	*G. vermiculophylla*	Verified by RFLP
JCN780D		28-Sep-11	Sunset Farm	JCN	*G. vermiculophylla*	Verified by RFLP
JCN781A	NHA 556632	28-Sep-11	Sunset Farm	JCN	*G. vermiculophylla*	Verified by RFLP

JCN781B		28-Sep-11	Sunset Farm	JCN	*G. vermiculophylla*	Verified by RFLP
JCN781C		28-Sep-11	Sunset Farm	JCN	*G. vermiculophylla*	Verified by RFLP
JCN781D		28-Sep-11	Sunset Farm	JCN	*G. vermiculophylla*	Verified by RFLP
JCN782A	NHA 556897	28-Sep-11	Sunset Farm	JCN	*G. vermiculophylla*	Verified by RFLP
JCN782B		28-Sep-11	Sunset Farm	JCN	*G. vermiculophylla*	Verified by RFLP
JCN782C		28-Sep-11	Sunset Farm	JCN	*G. vermiculophylla*	Verified by RFLP
JCN782D		28-Sep-11	Sunset Farm	JCN	*G. vermiculophylla*	Verified by RFLP
JCN783B	NHA 556898	28-Sep-11	Sunset Farm	JCN	*G. vermiculophylla*	Verified by RFLP
JCN783C		28-Sep-11	Sunset Farm	JCN	*G. vermiculophylla*	Verified by RFLP
JCN783D		28-Sep-11	Sunset Farm	JCN	*G. vermiculophylla*	Verified by RFLP
JCN784A	NHA 556899	28-Sep-11	Sunset Farm	JCN	*G. vermiculophylla*	Verified by RFLP
JCN784B		28-Sep-11	Sunset Farm	JCN	*G. vermiculophylla*	Verified by RFLP
JCN784C		28-Sep-11	Sunset Farm	JCN	*G. vermiculophylla*	Verified by RFLP
JCN784D		28-Sep-11	Sunset Farm	JCN	*G. vermiculophylla*	Verified by RFLP
JCN785A	NHA 556900	28-Sep-11	Sunset Farm	JCN	*G. vermiculophylla*	Verified by RFLP
JCN785B		28-Sep-11	Sunset Farm	JCN	*G. vermiculophylla*	Verified by RFLP
JCN785C		28-Sep-11	Sunset Farm	JCN	*G. vermiculophylla*	Verified by RFLP
JCN785D		28-Sep-11	Sunset Farm	JCN	*G. vermiculophylla*	Verified by RFLP
JCN786A	NHA 556901	28-Sep-11	Sunset Farm	JCN	*G. vermiculophylla*	Verified by RFLP
JCN786B		28-Sep-11	Sunset Farm	JCN	*G. vermiculophylla*	Verified by RFLP
JCN786C		28-Sep-11	Sunset Farm	JCN	*G. vermiculophylla*	Verified by RFLP
JCN787A	NHA 556902	28-Sep-11	Sunset Farm	JCN	*G. vermiculophylla*	Verified by RFLP
JCN787B		28-Sep-11	Sunset Farm	JCN	*G. vermiculophylla*	Verified by RFLP
JCN787C		28-Sep-11	Sunset Farm	JCN	*G. vermiculophylla*	Verified by RFLP
JCN788A	NHA 556903	28-Sep-11	Sunset Farm	JCN	*G. vermiculophylla*	Verified by RFLP
JCN788B		28-Sep-11	Sunset Farm	JCN	*G. vermiculophylla*	Verified by RFLP
JCN788C		28-Sep-11	Sunset Farm	JCN	*G. vermiculophylla*	Verified by RFLP

JCN789A	NHA 556911	28-Nov-11	Sunset Farm	JCN	*G. vermiculophylla*	Verified by RFLP
JCN789B		28-Nov-11	Sunset Farm	JCN	*G. vermiculophylla*	Verified by RFLP
JCN789C		28-Nov-11	Sunset Farm	JCN	*G. vermiculophylla*	Verified by RFLP
JCN789D		28-Nov-11	Sunset Farm	JCN	*G. vermiculophylla*	Verified by RFLP
JCN789E		28-Nov-11	Sunset Farm	JCN	*G. vermiculophylla*	Verified by RFLP
JCN790A	NHA 556912	28-Nov-11	Sunset Farm	JCN	*G. vermiculophylla*	Verified by RFLP
JCN790B		28-Nov-11	Sunset Farm	JCN	*G. vermiculophylla*	Verified by RFLP
JCN790C		28-Nov-11	Sunset Farm	JCN	*G. vermiculophylla*	Verified by RFLP
JCN790D		28-Nov-11	Sunset Farm	JCN	*G. vermiculophylla*	Verified by RFLP
JCN790E		28-Nov-11	Sunset Farm	JCN	*G. vermiculophylla*	Verified by RFLP
JCN790F		28-Nov-11	Sunset Farm	JCN	*G. vermiculophylla*	Verified by RFLP
JCN791A	NHA 556913	28-Nov-11	Sunset Farm	JCN	*G. vermiculophylla*	Verified by RFLP
JCN791B		28-Nov-11	Sunset Farm	JCN	*G. vermiculophylla*	Verified by RFLP
JCN791C		28-Nov-11	Sunset Farm	JCN	*G. vermiculophylla*	Verified by RFLP
JCN791D		28-Nov-11	Sunset Farm	JCN	*G. vermiculophylla*	Verified by RFLP
JCN791E		28-Nov-11	Sunset Farm	JCN	*G. vermiculophylla*	Verified by RFLP
JCN791F		28-Nov-11	Sunset Farm	JCN	*G. vermiculophylla*	Verified by RFLP
JCN791G		28-Nov-11	Sunset Farm	JCN	*G. vermiculophylla*	Verified by RFLP
JCN792A	NHA 556904	28-Nov-11	Depot Rd	JCN	*G. vermiculophylla*	Verified by RFLP
JCN792B		28-Nov-11	Depot Rd	JCN	*G. tikvahiae*	Verified by RFLP
JCN792C		28-Nov-11	Depot Rd	JCN	*G. vermiculophylla*	Verified by RFLP
JCN792D		28-Nov-11	Depot Rd	JCN	*G. tikvahiae*	Verified by RFLP
JCN792E		28-Nov-11	Depot Rd	JCN	*G. vermiculophylla*	Verified by RFLP
JCN793A	NHA 556905	28-Nov-11	Depot Rd	JCN	*G. tikvahiae*	Verified by RFLP
JCN793B		28-Nov-11	Depot Rd	JCN	*G. vermiculophylla*	Verified by RFLP
JCN793C		28-Nov-11	Depot Rd	JCN	*G. vermiculophylla*	Verified by RFLP
JCN793D		28-Nov-11	Depot Rd	JCN	*G. vermiculophylla*	Verified by RFLP

JCN794A	NHA 556906	28-Nov-11	Depot Rd	JCN	*G. vermiculophylla*	Verified by RFLP
JCN794B		28-Nov-11	Depot Rd	JCN	*G. vermiculophylla*	Verified by RFLP
JCN794C		28-Nov-11	Depot Rd	JCN	*G. tikvahiae*	Verified by RFLP
JCN794D		28-Nov-11	Depot Rd	JCN	*G. vermiculophylla*	Verified by RFLP
JCN795A	NHA 556907	28-Nov-11	Depot Rd	JCN	*G. tikvahiae*	Verified by RFLP
JCN795B		28-Nov-11	Depot Rd	JCN	*G. vermiculophylla*	Verified by RFLP
JCN795C		28-Nov-11	Depot Rd	JCN	*G. tikvahiae*	Verified by RFLP
JCN795D		28-Nov-11	Depot Rd	JCN	*G. vermiculophylla*	Verified by RFLP
JCN795E		28-Nov-11	Depot Rd	JCN	*G. tikvahiae*	Verified by RFLP
JCN796A	NHA 556908	28-Nov-11	Depot Rd	JCN	*G. tikvahiae*	Verified by RFLP
JCN796B		28-Nov-11	Depot Rd	JCN	*G. vermiculophylla*	Verified by RFLP
JCN796C		28-Nov-11	Depot Rd	JCN	*G. vermiculophylla*	Verified by RFLP
JCN796D		28-Nov-11	Depot Rd	JCN	*G. vermiculophylla*	Verified by RFLP
JCN797A	NHA 556909	28-Nov-11	Depot Rd	JCN	*G. tikvahiae*	Verified by RFLP
JCN797B		28-Nov-11	Depot Rd	JCN	No Band	
JCN797C		28-Nov-11	Depot Rd	JCN	*G. tikvahiae*	Verified by RFLP
JCN797D		28-Nov-11	Depot Rd	JCN	*G. tikvahiae*	Verified by RFLP
JCN797E		28-Nov-11	Depot Rd	JCN	*G. vermiculophylla*	Verified by RFLP
JCN798A	NHA 556910	28-Nov-11	Depot Rd	JCN	*G. tikvahiae*	Verified by RFLP
JCN798B		28-Nov-11	Depot Rd	JCN	*G. vermiculophylla*	Verified by RFLP
JCN798C		28-Nov-11	Depot Rd	JCN	*G. vermiculophylla*	Verified by RFLP
JCN799B	NHA 556914	28-Nov-11	Sunset Farm	JCN	*G. vermiculophylla*	Verified by RFLP
JCN799F		28-Nov-11	Sunset Farm	JCN	*G. vermiculophylla*	Verified by RFLP
JCN800A	NHA 556915	28-Nov-11	Sunset Farm	JCN	*G. vermiculophylla*	Verified by RFLP
JCN800B		28-Nov-11	Sunset Farm	JCN	*G. vermiculophylla*	Verified by RFLP
JCN800C		28-Nov-11	Sunset Farm	JCN	*G. vermiculophylla*	Verified by RFLP
JCN800D		28-Nov-11	Sunset Farm	JCN	*G. vermiculophylla*	Verified by RFLP

JCN800E		28-Nov-11	Sunset Farm	JCN	*G. vermiculophylla*	Verified by RFLP
JCN800F		28-Nov-11	Sunset Farm	JCN	*G. vermiculophylla*	Verified by RFLP
JCN802A	NHA 556916	28-Nov-11	Sunset Farm	JCN	No Band	
JCN802B		28-Nov-11	Sunset Farm	JCN	*G. vermiculophylla*	Verified by RFLP
JCN802C		28-Nov-11	Sunset Farm	JCN	*G. vermiculophylla*	Verified by RFLP
JCN803A	NHA 556917	28-Nov-11	Lubberland Creek	JCN	No Band	
JCN803B		28-Nov-11	Lubberland Creek	JCN	*G. vermiculophylla*	Verified by RFLP
JCN804A	NHA 556918	28-Nov-11	Lubberland Creek	JCN	*G. vermiculophylla*	Verified by RFLP
JCN804B		28-Nov-11	Lubberland Creek	JCN	*G. vermiculophylla*	Verified by RFLP
JCN804C		28-Nov-11	Lubberland Creek	JCN	*G. vermiculophylla*	Verified by RFLP
JCN804D		28-Nov-11	Lubberland Creek	JCN	*G. vermiculophylla*	Verified by RFLP
JCN804E		28-Nov-11	Lubberland Creek	JCN	*G. vermiculophylla*	Verified by RFLP
JCN805B	NHA 556919	28-Nov-11	Lubberland Creek	JCN	No Band	
JCN805C		28-Nov-11	Lubberland Creek	JCN	*G. vermiculophylla*	Verified by RFLP
JCN805D		28-Nov-11	Lubberland Creek	JCN	*G. vermiculophylla*	Verified by RFLP
JCN806A	NHA 556920	28-Nov-11	Lubberland Creek	JCN	*G. vermiculophylla*	Verified by RFLP
JCN806B		28-Nov-11	Lubberland Creek	JCN	*G. vermiculophylla*	Verified by RFLP
JCN806C		28-Nov-11	Lubberland Creek	JCN	*G. vermiculophylla*	Verified by RFLP
JCN806D		28-Nov-11	Lubberland Creek	JCN	*G. vermiculophylla*	Verified by RFLP
JCN806E		28-Nov-11	Lubberland Creek	JCN	*G. vermiculophylla*	Verified by RFLP
JCN807A	NHA 556921	28-Nov-11	Lubberland Creek	JCN	*G. vermiculophylla*	Verified by RFLP
JCN807B		28-Nov-11	Lubberland Creek	JCN	*G. vermiculophylla*	Verified by RFLP
JCN807C		28-Nov-11	Lubberland Creek	JCN	*G. vermiculophylla*	Verified by RFLP
JCN807D		28-Nov-11	Lubberland Creek	JCN	*G. vermiculophylla*	Verified by RFLP
JCN808A	NHA 556922	28-Nov-11	Lubberland Creek	JCN	*G. vermiculophylla*	Verified by RFLP
JCN808B		28-Nov-11	Lubberland Creek	JCN	*G. vermiculophylla*	Verified by RFLP
JCN808C		28-Nov-11	Lubberland Creek	JCN	*G. vermiculophylla*	Verified by RFLP

JCN808D		28-Nov-11	Lubberland Creek	JCN	*G. vermiculophylla*	Verified by RFLP
JCN808E		28-Nov-11	Lubberland Creek	JCN	*G. vermiculophylla*	Verified by RFLP
JCN809A	NHA 556923	28-Nov-11	Lubberland Creek	JCN	*G. vermiculophylla*	Verified by RFLP
JCN809B		28-Nov-11	Lubberland Creek	JCN	*G. vermiculophylla*	Verified by RFLP
JCN809C		28-Nov-11	Lubberland Creek	JCN	*G. vermiculophylla*	Verified by RFLP
JCN809D		28-Nov-11	Lubberland Creek	JCN	*G. vermiculophylla*	Verified by RFLP
JCN809E		28-Nov-11	Lubberland Creek	JCN	*G. vermiculophylla*	Verified by RFLP
JCN810A	NHA 556924	28-Nov-11	Lubberland Creek	JCN	*G. vermiculophylla*	Verified by RFLP