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III. Project Narrative

Introduction: *Purpose of the study and background*

In this project, “**The Eelgrass Resource of Southern New England and New York: Science in Support of Management and Restoration Success,**” we investigated genetic and other eelgrass (*Zostera marina* L.) information to advance both management and restoration science in southern New England and New York coastal waters. The project assessed the genetic diversity of eelgrass across the region and tested it against an experimental factorial design of stress parameters, to yield maps of eelgrass distribution and to discover eelgrass populations that are resilient to various stressors that occur regionally. We created a database of the multiple stressors of eelgrass and learned new information about scientific site selection for eelgrass restoration. Overall, we completed an evaluation of eelgrass genetic diversity and population differentiation across the region, detailed geographic studies of eelgrass genetic diversity and resilience, and conducted experimental testing of plant tolerance to multiple stressors in mesocosm experiments. The project contributes to regional eelgrass habitat sustainability through its insights into eelgrass ecology, genetics, and the conditions that affect eelgrass growth, while also yielding information for improved eelgrass restoration. The results of our study provide information on environmental parameters and stressors to eelgrass, information that both to improves site selection for restoration and identifies potentially resilient populations of eelgrass with adequate genetic diversity to be used as restoration donor sources.

The underlying motivation for this project is the region-wide decline of eelgrass, documented repeatedly over the past decade and more (Short and Short 2003, Waycott et al. 2009). As we show in our results, all eelgrass in the region now experiences some anthropogenic influence. Primarily, eelgrass is negatively impacted by excess nutrient loading and suspended sediments, both of which decrease water clarity, limiting the light these rooted plants receive through the water column. Eelgrass habitat also experiences direct insults from boating, aquaculture practices, dredge and fill operations, and other coastal development. Then there are the wider-scale impacts of atmospheric nitrogen deposition and climate change. In immediate terms, in the area under study, the disposal of sewage treatment plant output and runoff from watershed activities are the two major adverse

impacts to eelgrass. Eelgrass loss correlates closely with coastal human population density and development. Over the past century, 65% of eelgrass in this region of southern New England and New York has been lost (Short and Short 2003). Restoration suffers from both inadequate environmental conditions, primarily coastal water quality, but also from lack of knowledge and lack of political will. The project reported below was done in part to assess the degree to which human-derived nitrogen impacts the remaining eelgrass beds regionally, and also to learn new science about eelgrass, its genetic structure and response to stressors, that may contribute to more successful and economically viable restoration efforts.

Overall Approach: *Field sampling, genetic analysis, and stressor experiments*

Our approach was to determine, based on genetic, population, and plant tolerance studies, eelgrass populations with high resilience to multiple stressors and with high likelihood of restoration success in southern New England and New York coastal waters. We conducted broad-scale field and experimental studies of eelgrass plant responses to multiple stressors regionally. The work had, and our full-scale report (below) has, three parts: *first*, field measurements of plant characteristics and environmental conditions; *second*, broad scale sampling of genetic diversity (testing a number of genetically different populations in an area to understand the resilience of eelgrass in the region) and genotypic diversity of eelgrass populations (determining eelgrass clones across the region having different genetic makeup); and *third*, experimental mesocosm studies of stressor effects (light, temperature and sediment organic matter) and plant tolerance and resilience.

The studies were conducted over two full years and included the contributions of many regional collaborators representing State and Federal agencies and organizations, steady input from The Nature Conservancy, and the cooperation of four principal investigators at the University of New Hampshire. Experimental mesocosms, testing selected eelgrass populations in a factorial design of chosen stressors, were run at the University of New Hampshire and the University of Rhode Island. Genetics analysis was conducted at the University of New Hampshire (UNH) with fragment analysis conducted by the Hubbard Center for Genome Studies at UNH.

First, we identified and mapped the most recent eelgrass distribution information across the region. Then we sampled eelgrass populations regionally at 37 sites to evaluate the genotypic

diversity of eelgrass, and to determine its genetic diversity, discovering its allelic richness, numbers of unique alleles, clonality and level of inbreeding. Finally, we evaluated the resilience of 10 selected and distinct eelgrass populations, chosen for their high levels of genetic diversity, using experimental mesocosm testing of plant tolerance to stressors of light, temperature, and sediment organic content. Initially, we planned to sample eelgrass at 40 sites, then settled on 39 sites for logistical reasons. We found eelgrass at 36 of these locations across New York, Connecticut, Rhode Island and Massachusetts. The total number of sites in our analysis is 37, as it includes one reference site in New Hampshire.

Brief Summary of Our Findings

With eelgrass in crisis across the northeastern U.S., experiencing rapidly declining habitats and increased human stresses, we were charged by The Nature Conservancy to investigate the resilience of eelgrass populations and their genetic diversity in order to create new insights into how preserve, protect and restore these critical coastal habitats. We evaluated genetic differentiation of eelgrass across the region, conducted detail studies of eelgrass genetic diversity and resilience and experimentally tested eelgrass tolerance to multiple stressors. To summarize, we learned that there are three metapopulations of eelgrass across the southern New England and New York region, which are location-based to some degree (New York, Connecticut-Rhode Island, and Massachusetts-New Hampshire). These metapopulations experience gene flow, one (CT-RI) being a mixed population likely with sub-populations, and two (NY and MA-NH) that contain eelgrass populations more resilient to the multiple environmental stressors we tested. The eelgrass populations of southern New England and New York are genetically diverse and have been impacted over time by phenotypic acclimation. Several of the populations tested demonstrated greater resilience to environmental stressors and high potential for use in restoration as well as the ability to withstand stressors associated with climate change; we did not see consistent correlation of genetic diversity and resilience. We found few eelgrass populations with large clones (greater than 2 m diameter), and all populations were relatively genetically diverse, with moderate to low levels of inbreeding. In fact, of the 709 eelgrass plants (or ramets) tested, 688 were unique genets (or clones). The studies of eelgrass response to sediment and temperature stressors in particular led to new understandings of the controls on eelgrass growth and will enhance site selection for eelgrass transplanting and seeding projects. Additionally, our study provided information on

nitrogen, confirming anthropogenic sources of nitrogen as the major stressor of eelgrass in the northeast. The study met and exceeded its objectives; more genetic analyses would further contribute to our understanding. Besides yielding science that will improve restoration success, our results clearly show the need for increased management of nitrogen loading into coastal waters as well as protection of eelgrass throughout the region with the need for additional focus on preserving resilient eelgrass populations with high potential as restoration donor sources.

What We Learned: *Eelgrass genetics*

In this first intensive study of eelgrass genetics region-wide, we sampled 37 separate eelgrass populations and analyzed 7 microsatellites, testing 709 eelgrass plants and finding 688 unique clones. Many of the eelgrass populations showed low inbreeding and none showed very high levels of inbreeding. Overall, the low inbreeding and high allelic richness we found support a region-wide conclusion of rather high genetic diversity with broad gene flow in eelgrass populations of southern New England and New York. We found three metapopulations of eelgrass across the region, location-based to some degree. One was predominantly located in Massachusetts and New Hampshire, with two of the more resilient eelgrass populations found within this metapopulation; one in New York with all but one of the remaining resilient eelgrass populations; and the third metapopulation centered in Connecticut-Rhode Island, but found in all the southern states, which had only one moderately resilient eelgrass population. Overall, the clonal richness of all of the tested eelgrass populations was high, with only eight populations showing significant repeat clones. The finding of high clonal richness implies that the majority of eelgrass populations across the region depend on sexual reproduction for establishment and maintenance of eelgrass meadows rather than extensive vegetative expansion as previously thought. We found no direct correlation between genetic diversity and resilience of the eelgrass populations: some of the more genetically diverse populations of eelgrass did less well in our stressor studies.

What We Learned: *Eelgrass stressor responses*

The responses of eelgrass populations to the stressors in the mesocosms of reduced light, elevated temperature and two levels of sediment organic content clearly distinguished some plant populations as more resilient and better able to survive transplanting and to expand by producing

lateral shoots, which was ultimately our measure of plant growth and success. The ten populations studied were chosen to be geographically representative of the region, but beyond that were selected because in preliminary genetic screening, they demonstrated higher genetic diversity and allele richness, compared to populations of eelgrass from other sites. However, among these ten sites, many differences of response were seen to the stressors in the mesocosm experiments, showing us that different plant populations have abilities to withstand various stressor conditions. So far, we do not have the capacity to determine eelgrass resilience via genetic markers.

Eelgrass grew best under high light conditions with low organic sediment levels in the eutrophic conditions of the mesocosms in New Hampshire with the high-nutrient water from Great Bay. In New Hampshire, the two high sediment organic matter treatments had low and very low success under both light conditions. In Rhode Island mesocosms, with lower levels of water-borne nutrients and high light, eelgrass was the most productive in ambient temperature conditions but with high sediment organic matter levels, while at +2° above ambient temperature, eelgrass production was low and at +4° above ambient, it was very low.

Great South Bay Grass Island, also known as West Fire Island (NY11), and Nannies Island (NH1) did the best in lateral shoot production under all stressors, although the Nannies Island population could not be rated regarding the temperature stressor due to flowering of the plants. The production of lateral shoots in eelgrass is a good measure of “plant success” since it indicates a robust plant that is spreading and able to adapt to local, *in situ* conditions. Independent of temperature and light, Great South Bay eelgrass did better than all other populations, and rated “excellent” in the high sediment organic matter condition when water nitrogen was low. With intermediate production of lateral shoots, eelgrass populations from North Prudence Island (RI2), Southway (MA2), and Ninigret Pond (RI10) only did fair under both light and temperature stressors. All plants did better in the high organic sediments when water nitrogen levels were low. The remaining 5 sites tested in the mesocosms, Shelter Island (NY4), West Falmouth and West Island (MA6 & MA7), and Duck Island and Ram Island (CT7 & CT1), did poorly under all stressor treatments tested, indicating that these eelgrass populations have lower resilience to the kinds of stressors with major adverse effects on eelgrass populations.

The Nannies Island location in Great Bay Estuary, NH was included in the mesocosm studies as a standard reference site, i.e., a site from which eelgrass has been successfully used in many previous mesocosm studies. By including it, we had a reference to earlier research at UNH in which these plants demonstrated their ability to grow under mesocosm conditions. Somewhat to our surprise, as discussed above, the Nannies Island eelgrass plants proved to be one of the most resilient populations studied, along with Great South Bay Grass Island in New York.

Contrary to some assumptions, the genetic diversity of eelgrass populations we studied did not correlate with their ability to thrive under stress – based on number of private alleles, allele frequency and stressor responses in mesocosm studies. Also, with the genetic analyses currently available for eelgrass studies, we found no distinct markers for resilience. However, we did find that our most successful (resilient) eelgrass populations were part of the two most distinct metapopulations from the north (outer Cape Cod and New Hampshire, MA-NH) and the south (south shore of Long Island, NY).

Plants did better with low temperature and high light. In other words, high temperature and low light are significant stressors to eelgrass. In most of our stressor experiments, eelgrass growing in low organic sediments did better, except when the plants were nitrogen limited, in which case higher organic sediment conditions made needed nitrogen available to the plants and thereby contributed positively to lateral shoot production. We scientifically demonstrated these interactive effects of temperature and of light in relation to the stressor effects of sediment organic matter levels. Our work identifies a clear need in eelgrass restoration efforts to understand not only light conditions, but also levels of organic matter in the sediment, and potential temperature stress at a given site.

What We Learned: *The take-away message for managers*

Our findings give managers of coastal habitat improved information on eelgrass health and survival in the face of multiple stressors including temperature, a major climate change variable. We show that site selection can be improved with the now better-defined stressor relationships our study has established. The map of eelgrass genetic diversity for the region and the finding of three metapopulations regionally give new information to managers on how to think about genetic flow

and diversity. We identify specific populations with higher resilience to multiple stressors which, although they need further field testing, have strong potential as donor populations for restoration projects regionally. Some of our specific findings from the mesocosm experiments will allow pairing of resilient donor eelgrass populations with improved site selection (i.e., best possible plants into most suitable locations). We identified unique alleles in populations of eelgrass that should be considered for protection in order not to lose these genetically valuable eelgrass plant populations. We are ready to develop outreach material to make the management-related information accessible to those who plan and carry out restoration.

Implications for Restoration

Restoration of eelgrass is known to be expensive, risky and difficult to achieve. Success rates in the region of our study have varied from high to low, but most managers agree that more knowledge contributing to more assured eelgrass restoration would be useful. We identified two populations of eelgrass that responded more robustly to the stressors in our experiments than others: Nannies Island (NH1) which is genetically linked to eelgrass populations on outer Cape Cod and Great South Bay Grass Island (NY11) which is related to other eelgrass populations along the south shore of Long Island. These two eelgrass populations significantly out-performed all the other populations tested in our study and are now deserving of field testing along with other eelgrass within these metapopulations. We also identified three additional populations which were moderately resilient.

We did show that donor eelgrass resilience varies by population, and therefore genetics does play a role, but we also showed that the eelgrass neutral genetic markers that are currently available do not correlate with eelgrass resilience to stressors. There are presently no genetic characteristics (or markers) for resilience which can be easily employed by managers to select donor eelgrass for restoration. From the current study, it seems clear that the choice of donor plants for restoration projects should come from the metapopulation most closely related to the restoration site. Also, we now know that some eelgrass is indeed more resilient, and therefore using donor plants from known resilient populations (or testing resilience of other populations through test transplant) is important and will pay off. Further, for transplanting in Long Island Sound or Narragansett Bay, the area encompassing of the least robust of the three metapopulations, we recommend testing populations

of eelgrass from the New York (southern) metapopulation, particularly if there are concerns about high temperature stress associated with climate change. In all cases, the geographic extent of resilient populations of eelgrass within the northern and southern metapopulations needs further study, to identify other likely resilient plant sources.

The site selection model currently used in the region to determine the optimal sites for eelgrass planting efforts only considers a sediment grain size cut-off level (silt/clay) to distinguish better and worse (“plant and don’t plant”) sediment conditions. New results from the present study show that the silt/clay sediment level directly relates to levels of sediment organic matter in eelgrass beds, and that high sediment organic matter is a major stressor of eelgrass, except in low-nitrogen environments. Additionally, stress conditions from high sediment organic content are directly correlated with the stresses of light limitation and high temperature via plant ecophysiology and sediment biogeochemistry. These scientific results provide information needed to refine and improve the site selection model, which will improve the success of eelgrass restoration efforts regionally in the future. Transplanting or seeding eelgrass into high organic sediments should only be attempted in very clear, shallow waters with good light conditions. If light conditions are at all impaired, eelgrass planting should be limited to low organic sediments. As a rule of thumb, for successful, rapid eelgrass restoration, more than 50% ambient light reaching the leaves is needed.

Implications for Preservation and Protection

A major outcome of our study is the identification of specific eelgrass populations regionally that contain private alleles in their genetic structure – that is, genetic coding at the basic level that is not shared by other populations. As yet, we do not know the possible value of these private alleles, but as eelgrass regionally is threatened and in decline, it is important to protect areas with unique genetic signatures that could be useful to the overall genetic diversity of the eelgrass resource.

By state, the locations of populations with private alleles are:

NH – Nannies Island in Great Bay

MA – Southway on Monomoy Island, Sage Lot Pond, West Falmouth Harbor,
Nantucket Harbor

RI – Point Judith Pond, Jamestown Island west

CT – Ram Island, Duck Island

NY – Fishers Island, Plum Island, Moriches Bay, Shinnecock Bay east

All of these sites need protection to ensure preservation. This is not to say that other eelgrass does not merit protection!

Additionally, the sites we found with populations of very or moderately resilient eelgrass must be preserved and protected to allow for future testing and, eventually, use in restoration activities. These include Great South Bay Grass Island in New York and Nannies Island in New Hampshire, along with Prudence Island and Ninigret Pond in Rhode Island and Southway on Monomoy Island in Massachusetts. If and when these populations become donor sites for restoration projects, their own sustainability must be assured. It's also important to remember that harvesting seeds as well as plants from donor sites for use in restoration represents the removal of genetic material from the donor bed that may be important to donor population survival and genetic diversity. If anything, our study showed that sexual reproduction (flowering and seeding) in eelgrass across the region is more important than previously thought in maintaining the habitat, including the relatively high genetic diversity (allelic richness) that we discovered.

More Study is Needed: *Where do we go from here?*

In Massachusetts, up-to-date eelgrass distribution information is needed to provide a complete map. In addition, in Narragansett Bay and much of the south shore of Long Island, eelgrass distribution information is a decade old. We recommend encouraging state agencies to maintain eelgrass maps that are less than five years old for management purposes. Current information is particularly important since eelgrass is declining rapidly in many areas and many development decisions now heavily rely on eelgrass status information.

More extensive genetic screening of eelgrass is needed on both populations already tested and other populations occurring within the metapopulation regions we identified as having the most resilient eelgrass (NY and MA-NH). Another priority is to complete the genetic screening on the population samples that were archived in the present study, which will strengthen the genetic results

and provide additional information, better defining the extent of the metapopulations we found. Also, it would be valuable to identify the size of the eelgrass populations with private alleles and the size of the two already-determined most resilient populations.

The next step in the evaluation of eelgrass plant resilience for restoration must be field testing of selected resilient plants as transplant material to evaluate how well these populations do under different, natural field conditions. We recommend a series of common-garden studies regionally, where plants of known, specific genetic characteristics are transplanted together into several environmentally varied locations to see how they respond. Of course, the eelgrass populations we have identified as the most resilient should receive attention and follow-up as the highest priority. Common garden studies as described above are the cornerstone of testing plant and environmental interactions and the best mechanism for distinguishing phenotypic vs. genotypic responses.

Are These Findings Transportable?

Yes: the short answer is yes. The studies designed and presented here represent a rigorous format for other studies in other regions which will likely yield information very useful to resource managers and resource protection efforts. They also point to further work that must be done regionally to insure the preservation of crucial eelgrass genetic resources and resilient plant characteristics. The Nature Conservancy's leadership will be important as the research continues.

INTRODUCTION

The study “The Eelgrass Resource of Southern New England and New York: Science in Support of Management and Restoration Success” was designed to identify whether some specific eelgrass beds across the region (Figure 1) are better donor sites for eelgrass restoration based on investigation of plant genetics and phenotypic characteristics. That is, are some eelgrass populations more resilient and therefore more likely to survive and succeed as transplants when eelgrass restoration work is carried out? The background motivation was two-fold: eelgrass habitat across the region is generally in decline and often in need of restoration and many eelgrass restoration efforts have not been successful, creating the question of how to optimize these efforts. Selection of the best plant material is clearly desirable, although other work has shown that site selection is extremely important.

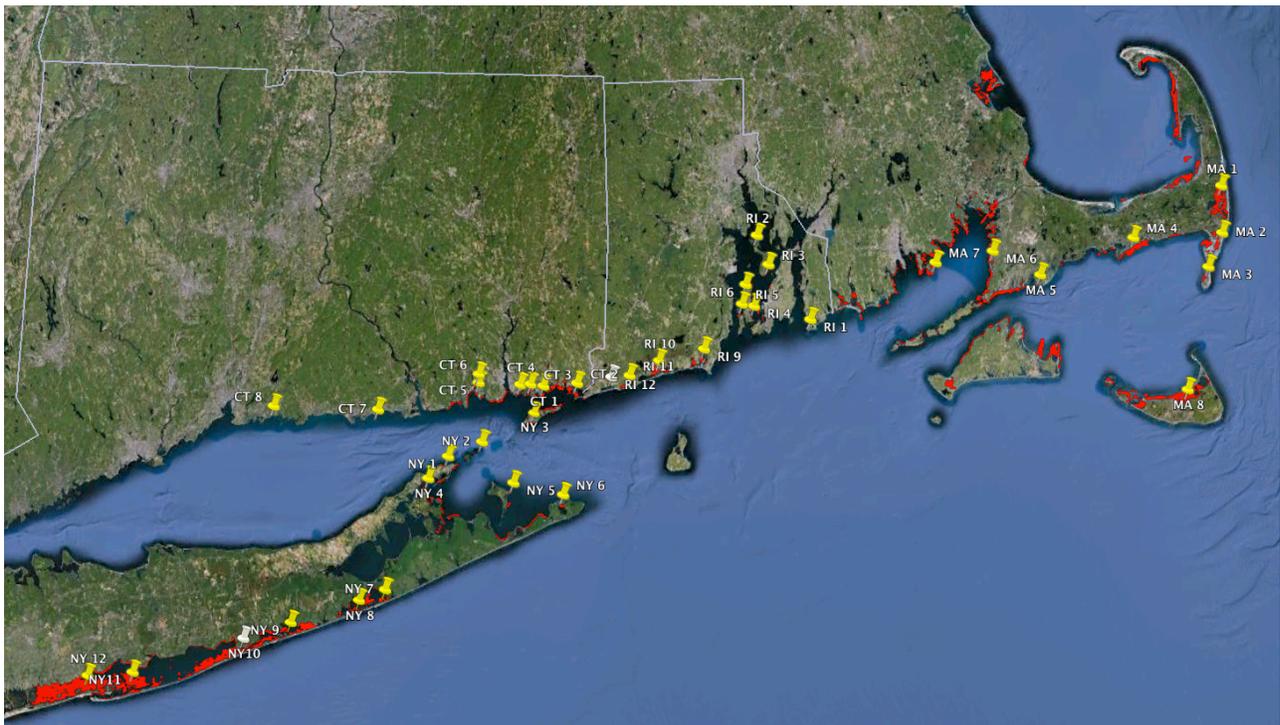


Figure 1. Southern New England and New York eelgrass distribution in red. Sites chosen for the 2010 eelgrass genetic and resilience sampling (pins); sites where eelgrass was collected (yellow pins); and sites where no eelgrass could be found (white pins). New Hampshire site at Nannies Island (NH 1) in the Great Bay Estuary not shown. Google Earth base map.

The approach used was to assess genetic diversity of eelgrass across the southern New England and New York region and test it against an experimental factorial design of potential stress parameters, yielding maps of eelgrass distribution and resilience along with a database of multiple stressors to eelgrass. We evaluated the genetic differentiation of eelgrass populations across the region, made detailed geographic studies of eelgrass genetic diversity and resilience, and conducted experimental testing of plant tolerance to multiple stressors based on field studies, mesocosm experiments, and our collective long-term expertise. The results of our study provide information on environmental parameters and stressors to eelgrass to improve site selection for restoration and also identify resilient populations of eelgrass for restoration or preservation.

IV. Methodology

Objectives and Experimental Design

Our objectives were to conduct a broad scale field and experimental study of eelgrass genetics in combination with eelgrass plant responses to multiple stressors throughout the region. Our efforts included: (1) broad scale sampling of genetic diversity (or allelic diversity) of different populations in an area to understand the potential for genetic resilience of eelgrass in the region (2) field measurements of plant characteristics and environmental conditions; (3) experimental mesocosm studies of stressor effects, plant tolerance and plant resilience to clearly identify the major threats to eelgrass health; and finally, (4) development of final products informing and improving eelgrass management and restoration success, including both site selection for transplanting and selection of optimal plants (donor site selection) when undertaking the major effort of restoration through transplantation or seeding.

Eelgrass Mapping

A composite map of eelgrass for the region was created from various data sources to update as much as possible the 2003 eelgrass distribution map (Green and Short, 2003). In the map (Figure 1), eelgrass distribution data for Long Island Sound, Connecticut and southern Rhode Island are from 2009. Peconic Estuary data are from 2006. The south shore of Long Island data are from 2002, with 2007 data for Moriches and Shinnecock Bays. The data for Narragansett Bay eelgrass distribution are from 2002. Massachusetts eelgrass distribution data are fragmented with many bays mapped in 2010 (the south shore of Cape Cod) but other areas mapped in 2006-07 (Elizabeth Islands) or 2001 (Buzzard's Bay, Martha's Vineyard and Nantucket). All the distribution data for the region were collated in ArcView GIS and transferred to Google Earth. Eelgrass is declining in many of the areas shown in Figure 1 and our collaborators on the project anecdotally noted some eelgrass losses that are not represented on the map, particularly in Great South Bay (LI, NY), Buzzard's Bay and the islands (MA), and the south coast of Rhode Island.

Field Methods

Our experimental design was established to assess eelgrass populations across southern New England and New York to identify genetic differences and eelgrass resilience. A region-wide sampling (Figure 1) for genetic analysis (metapopulation structure, genetic differentiation of populations, unique alleles) was designed and carried out in 2010; this field assessment also included measurement of plant resilience characteristics and stressor levels. Subsequently, in 2011, we conducted experimental mesocosm manipulations both at the University of New Hampshire and the University of Rhode Island to test the resilience of selected eelgrass plants of genetically differentiated populations (as determined in 2010, Figure 2) to major eelgrass stressors of low light,

high sediment organic matter (elevated sediment nutrients and sediment anoxia with high concentrations of sulfides from decomposition), and elevated temperature. These stressors were chosen for the experimental analysis because they are the primary factors impacting eelgrass growth and survival.



Figure 2. The nine Southern New England and New York sites where eelgrass was collected for the 2011 mesocosm stressor studies. The tenth site at Nannies Island in Great Bay, NH was used as a control.

Detailed methods descriptions are presented as UNH Standard Operating Procedures (SOPs) in Appendix 1 of this report. The decision about where to make field collections of eelgrass was based on our knowledge of eelgrass distribution across the region and the most up-to-date existing

maps of eelgrass distribution in southern New England and New York, along with discussions with project partners and regional seagrass experts, following the methods in the UNH SOPs. We were looking for sites that spanned the region, were representative of coastal conditions including exposure, geomorphology and nutrient levels. Sites were selected at 1 m depth (at low tide) wherever possible and with a balance of sites per state. A site was added in Branford, CT, which represented the westernmost eelgrass growing in Long Island Sound.

Laboratory analysis of eelgrass plant samples from across the region also followed pre-established procedures (SOPs) for determination of eelgrass morphology, nitrogen resources and stressors, and plant population genetic characteristics. The preliminary genetic screening (using the five available microsatellites to compare allelic richness) of all eelgrass collected throughout the region contributed to the determination of populations used for stressor studies of 10 genetically different eelgrass populations. Additional considerations in selecting 10 populations for the stressor studies included detection of unique genetic alleles and geographic distribution across the region. Populations were also selected to represent a range of suspected resilience, based on local eelgrass knowledge. Nannies Island in New Hampshire was included as a reference population representing eelgrass that had been successfully grown in mesocosms in previous years. All the resulting data from the field component of the study were incorporated into an electronic database submitted with this report and available online at [TNC Eelgrass Database](#).

Genetics Analysis Methods

DNAs were extracted from silica dried tissue using the method of Elphinstone et al.2003. Microsatellite genotyping was conducted to compare population structures within and between populations. Seven microsatellite loci (GA12, GA20, GA17D, GA19, GA16, GA2 and GA23), were genotyped; the primers of these loci are known to amplify DNAs from North Atlantic eelgrass

populations (Olsen et al. 2004). Two other loci, used in previous studies, proved difficult to replicate and not to be useful (Reusch and Boström 2011).

Microsatellite loci were genotyped to measure clonal diversity, R (Olsen et al. 2004); estimate allelic richness, \hat{A} (Kalinowski 2005); compare observed and expected heterozygosity, H_o and H_E , respectively (Lewis and Zaykin 2001); measure inbreeding, F_{is} (Lewis and Zaykin 2001) within a population, which could result from a population bottleneck; and finally, determine differentiation between populations, F_{st} (Goudet 2002). In addition, metapopulation structure, i.e., where there is gene flow between populations, was inferred using Bayesian methods as implemented in the program STRUCTURE (V.2.3.3, Pritchard et al. 2000, Falush et al. 2003, Hubisz et al. 2009). Population differentiation, F_{st} , was used in part to select presumed eelgrass clones across the region for subsequent stressor studies.

The microsatellite primers were provided by our colleague Dr. Jeanine Olsen at the Centre for Ecological & Evolutionary Studies, University of Groningen, The Netherlands. Sampling of 12-40 individual plants was conducted for accurate estimation of allelic richness and genetic diversity (van Dijk and van Tussenbroek 2009), looking at the 7 microsatellite loci (genotyping for seven alleles: GA 12, GA 20, GA 17D, GA 19, GA 16, GA2, and GA23) surveyed (Olsen et al. 2004). DNA results created an archival record that could be further analyzed to provide more detail on the genetic profile of eelgrass populations with future funding. All genetic analysis techniques employed were standard methodologies used in previous seagrass genetic screenings (Olsen et al. 2004, van Dijk and van Tussenbroek 2009, Procaccini et al. 2007, Reusch 2002, Reusch et al. 2000); and for genetic data processing, CONVERT (Glaubitz 2004), FSTAT (GENEPOP; Rousset 2008), and Genetic Data Analysis (GDA; Lewis and Zaykin in Weir 1996).

Clonal diversity was initially estimated following Olsen et al. (2004): # of genets divided by # ramets sampled per population and using the method of Arnaud-Haond et al. (2007) to minimize bias in small sample sizes (<20). We used CONVERT (Glaubitz 2004) to prepare Excel data files for various formats used to estimate F statistics. F_{is} is an estimation of inbreeding within an eelgrass population and was tested using standard methods (Lewis and Zaykin 2001). Pairwise F_{st} is a measure of differentiation between any two populations. It is calculated over all seven loci, with permutations to estimate the significance of each measurement (Goudet 1995). Principal component analysis of F_{st} was tested with standard methods (Peakall and Smouse 2006).

UNH Mesocosm Experiment Methods

The objective of the UNH mesocosm experiments (Figure 3) was to evaluate the responses of eelgrass from genetically differentiated populations and environments to reduced light and increased sediment organic content in controlled conditions. We hypothesized that eelgrass from genetically differentiated populations would vary in ability to become established and expand in a new environment in response to the stresses of low light and high sediment organic matter content. We evaluated the survival, productivity, morphology and photosynthetic characteristics of eelgrass from different populations subjected to different stress treatments. Our experimental study was based on previous mesocosm experiments (Short et al. 1995, Ochieng et al. 2010).



Figure 3. UNH (left) and URI (right) mesocosm facilities.

Experimental design

We conducted a common garden experiment in mesocosms to test whether eelgrass plants from genetically differentiated populations vary in ability to become established in a new environment and persist and grow under the stresses of increased sediment organic content and reduced light. Mature vegetative eelgrass ramets (shoots with attached rhizomes) were collected from nine populations growing in shallow water locations during the beginning of June 2011. The donor populations were from southern New England and New York locations, and one from New Hampshire. These 10 populations were selected to represent the range of environmental conditions and genetic diversity present within eelgrass beds of southern New England and New York, based on our preliminary genetic screening results and also to give geographic representation across the four states that were the focus of the study. The New Hampshire site was used as a control because eelgrass from the site had proved successful for mesocosm experiments in the past (Short et al. 1995, Ochieng et al. 2010). Genetic differences were identified using DNA microsatellites developed for *Z. marina* (Reusch et al. 1999, Reusch et al. 2000). Plants were transported to Jackson Estuarine Laboratory, where they were transplanted into twelve 1 m³ outdoor flow-through seawater mesocosms equipped with circulation pumps (Short et al. 1995). Seawater was pumped from the Great Bay Estuary, adjacent to JEL, and gravity fed to each mesocosm.

Mesocosms were filled with sediment 10 cm-deep. Half received low organic matter content sediment (LOM, 1% by dry weight), which served as the control, and the other half high organic content (HOM, 8%, by dry weight). We created these two sediment treatment levels by mixing high organic content (20%) marine mud with very low organic content terrestrial sand (< 1%). A total of 1,800 ramets were transplanted into the mesocosms after being cleaned of epiphytes and pruned to minimize differences in initial plant mass. Specifically, leaves greater than 30 cm were trimmed and

rhizomes were cut to have only one fully developed root node. Plants were held no longer than 72 hours in flow-through seawater tanks before they were planted. Shoots were planted into the mesocosms between 7 and 17 of June 2011. Each mesocosm was sectioned into 10 plots, using garden edging cut to 11 cm sunk vertically into the sediment, creating a barrier that prevented rhizomes from growing into neighboring populations. Fifteen single shoot ramets, with one root node and one shoot each, from each population were planted into one randomly assigned plot in each mesocosm. Neutral density screens, which reduced ambient sunlight by 50%, were added to half the LOM and half the HOM mesocosms on 23 July, at least five weeks after each population had been planted (Figure 4). Each tank was stocked with mud snails (*Ilyanassa obsoleta*) to prevent algae build-up and three fish, sticklebacks (*Apeltes quadracus* and *Pungitius pungitius*) to control amphipod populations in the tanks (Figure 5). The mesocosm experiments were run for a total of 13 weeks, shown in our previous work to be a sufficient time for recovery from transplant shock achieve acclimation, grow roots and leaves in response to the conditions in the tanks, and to yield comparative results (Short et al. 1995, Ochieng et al. 2010).

Environmental measurements

Temperature was recorded continuously in each tank at 30-minute intervals using HOBO Pendant loggers. Salinity was monitored weekly and dissolved oxygen and pH were measured intermittently throughout the experimental period using the hand-held YSI 85 (YSI Inc. Yellow Springs, Ohio). PAR was measured using LICOR 2 π underwater quantum sensors (400-700 nm) (LICOR Inc. Lincoln, Nebraska).



UNH Mesocosm Experiments 2011

Experimental design for testing of different genetic eelgrass populations.
(10 eelgrass shoots / section; 4 treatments; 10 locations; 3 replicates / treatment / location)

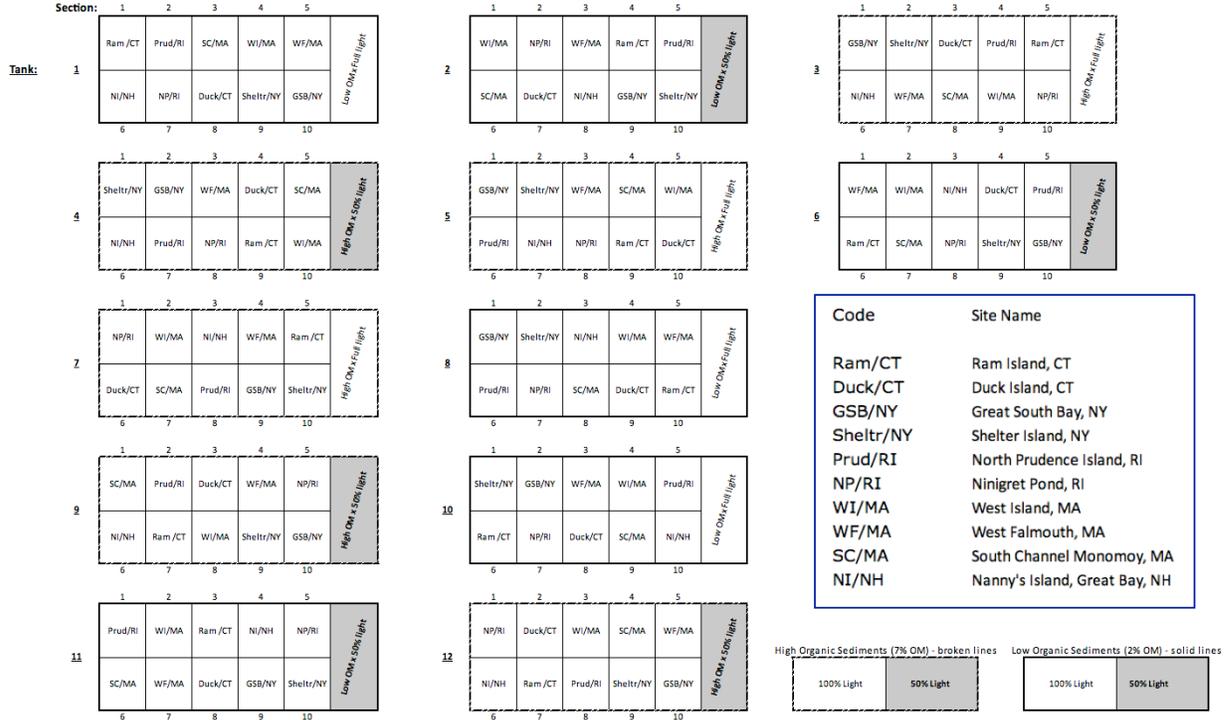


Figure 4. Experimental design of the UNH mesocosm stressor study using eelgrass plants from 10 New England/New York sites.

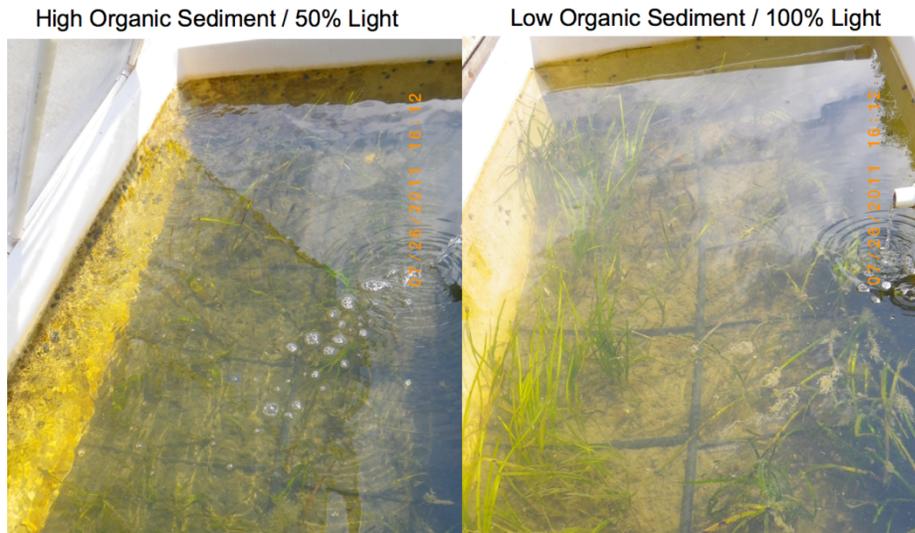


Figure 5. Eelgrass plants in the highest stress (left) and lowest stress (right) mesocosm tanks.

Eelgrass response measurements

Productivity and morphology

Shoot productivity was analyzed by measuring shoot density weekly between weeks 1 and 9, and at the end of the experimental period. Shoot productivity rates (shoot day⁻¹ plot⁻¹) were estimated between weeks 1 to 5 (before light treatments) and week 5 to the end of the study (with the two light treatments) by dividing the number of new shoots produced by the number of days that had past between the two measurements. At the end of the experiment (84 to 101 days of growth in the mesocosms, 45 to 59 days of shading) all plants were uprooted from the mesocosms between the 5th and 19th of September, keeping lateral shoots intact and maintaining the structure of entire ramets. All eelgrass material was brought into the laboratory where ramets and shoots were enumerated and morphology characteristics were measured.

Ramet survival was analyzed by quantifying the number of live ramets in each population and mesocosm. Percent survival was calculated by dividing the number of live ramets by the initial number of ramets planted (15 ramets sub-plot⁻¹). Shoot productivity was measured by dividing the difference between shoot density at the beginning of the shade treatment and the end of the experiment by the number of days between the two shoot density measurements. Rhizome productivity (rhizome plastochrone interval) for the entire experimental period was calculated by dividing the total number rhizome nodes produced in the mesocosms (number of nodes) by the number of days the ramet was in the mesocosm.

Morphological features of three ramets per population per mesocosm were analyzed by measuring total number of shoots, rhizome nodes, total rhizome length for each ramet, shoot morphology of four shoots (the terminal and three laterals which were representative of the

different sized shoots). Shoot morphology was evaluated by measuring total number of leaves per shoot, sheath length, leaf length, and leaf width of the third leaf. Ramet and shoot density and morphological characteristics were used to estimate (or interpolate) total rhizome length, number of nodes and leaf area plot⁻¹. Total rhizome length per plot was calculated by multiplying the average rhizome length per ramet by ramet density. Total number of rhizome nodes was calculated similarly. Total leaf area was calculated by multiplying the mean leaf area per shoot by shoot density. Each of these calculations was performed within each population in each mesocosm.

Photosynthetic characteristics

Photosynthetic characteristics were quantified during the last week of the study. Three measurements of effective quantum yield (Y) in light adapted leaves (second leaf of each of three replicate shoots) and the fraction of photosynthetically active radiation (PAR) absorbed by the leaves (AF) were made per population per mesocosm using the Diving-PAM® (Pulse Amplitude Modulated) fluorometer (Walz, Germany). AF was derived by measuring light transmittance through attached submerged leaves and incident light adjacent to the leaf, then dividing the difference between the measurements by the incident light. Electron transport rates (ETR) were calculated following the equation: $Y \times \text{incident PAR} \times \text{AF} \times 0.5$ (Beer et al. 1998).

URI Mesocosm Experiment Methods

In parallel to the UNH mesocosm experiments, mesocosm experiments at the University of Rhode Island were designed to distinguish plant responses to treatments of elevated sediment organic content and elevated temperature (Figure 3). The URI experiments complemented the UNH light and sediment organic stressor studies by adding temperature as an additional eelgrass

stressor. Also, at URI, the water in the mesocosms was lower-nutrient southern Narragansett Bay water ($< 2 \mu\text{M N}$) in contrast to the high nutrient Great Bay water ($\sim 21 \mu\text{M N}$) supplied to the UNH mesocosms from Great Bay. Overall, the two mesocosm experiments combined to provide a view of stressors on eelgrass including high and low sediment organic matter, reduced light conditions, elevated temperature conditions, and distinct water column nutrient conditions.

Sediment treatments at URI were identical to those at UNH, having high organic matter at 8% and low organic matter at 1%. The temperature treatments consisted of ambient water temperature, as well as 2°C and 4°C above ambient. All mesocosms were set up as $3 \times 2 \times 2$ matrices with 10 eelgrass populations represented in each treatment with adequate replication to insure statistically testable results. At URI, an accidental shut-down of the seawater flow for several days resulted in overheating of the mesocosms that caused eelgrass death. The mesocosm treatments were re-started with new plants collected from seven of the same populations across New England. Natural population densities of local snails and fish were maintained in the tanks to control algae and epiphytes and to mimic bay conditions. Monitoring of shoot density and canopy height was conducted every two weeks along with measures of temperature.

Statistical Methods

The sampling strategy included a series of collections at each of 37 eelgrass populations stretching from Great South Bay along the southern shore of Long Island to Pleasant Bay on the eastern shore of Cape Cod, including one site in Great Bay, New Hampshire. Plants were collected for genetic analysis (50 plants at each location), morphology, wasting disease and leaf chemistry, including the Nutrient Pollution Indicator (12 plants at each location) and sediment (organic matter and texture for 5 samples at each location). Each plant sample was considered an observation, and

linked with morphology and chemistry for 12 observations, and sediment for 5 observations, for each site.

Relationships between leaf morphology and chemistry variables were examined using Pearson correlations with each plant as an observation (n=360). Raw data were used since correlations do not require homogeneity of variance or normality. Data for leaf morphology and chemistry and sediment texture were examined for each site and one-way ANOVAs were run to examine whether differences existed among sites. Residuals were examined to ensure homogeneity of variance and normality. Some variables were log transformed to meet these assumptions of ANOVA. If differences were found important, Tukey's post hoc comparisons ($\alpha = 0.05$) were made to determine whether significant differences occurred between specific populations (sites).

To examine plant-sediment associations, the site means for plant and sediment variables for 36 sites (n=36) were compared using Pearson correlations to develop a correlation matrix. Site means were also used for a discriminant function analysis. Linear combinations of variables were generated to separate 31 eelgrass populations (sites) by state in multi-dimensional space. Results show what variables are most important in distinguishing differences between states, that is, those plant attributes that contribute most to our classifying populations (sites) into their correct states. Another multivariate technique, hierarchical cluster analysis, used the site means to cluster the most similar sites together regardless of state affiliation. In this analysis using Ward's method of clustering, a series of independent variables representing plant morphology, leaf chemistry, etc. was used to find the populations and sites that were most alike.

For the mesocosm studies, means and standard errors for all measured variables were calculated for each population and environmental treatment. Means of productivity and morphological characteristics for each subplot were analyzed using a least squares restricted

maximum likelihood (REML) regression analysis to test the effects of population, sediment and light on response measurements. A post-hoc Tukey-Kramer test was used to compare response variables among populations in each of the four environmental treatments. All statistical analyses were done using JMP (Version 9.0.2, SAS Institute Inc.) with significance determined at the 95% probability level ($p < 0.05$).

V. Results and Discussion

Eelgrass Distribution and Characterization

The distribution of eelgrass across the southern New England and New York region was assembled from available sources for each state using the most up-to-date information possible. The information was combined and entered in ArcView. The resulting composite distribution was imported to Google Earth and plotted (Figure 1). Eelgrass is relatively widely distributed at the extremes of the region in outer Cape Cod and Monomoy Island (MA) and Great South Bay (NY). These are populations growing in quiescent waters protected by high energy barrier beach systems open to the Atlantic Ocean (outer shore of Cape Cod and the Islands and the outer or southern shore of Long Island, NY). The eelgrass distribution map contributed to the decision-making process regarding where to establish the original 39 stations. Some of the Massachusetts data that was available is fairly old and the areas shown greatly exceed current eelgrass distributions. Similar caveats apply in the south short of Long Island and Narragansett Bay. Overall, eelgrass in the region has dramatically decreased from its historical distribution, to the extent of approximately 65% decline (Short and Short 2003). Particular losses are noted in Narragansett Bay, Peconic Bay, and Buzzard's Bay.

Extensive field sampling provided the opportunity to look at the morphological as well as genetic characteristics of eelgrass populations. Leaf length (measured as the length of the 3rd leaf) ranged from less than 25 cm to over 100 cm across the region. The largest leaves were found in eastern Connecticut while the smallest leaves occurred in the southern coastal bays of Long Island (Figure 6). Sheath length, an indicator of plant size that is not impacted by broken or damaged leaves, showed the same patterns found for leaf length (Figure 6).

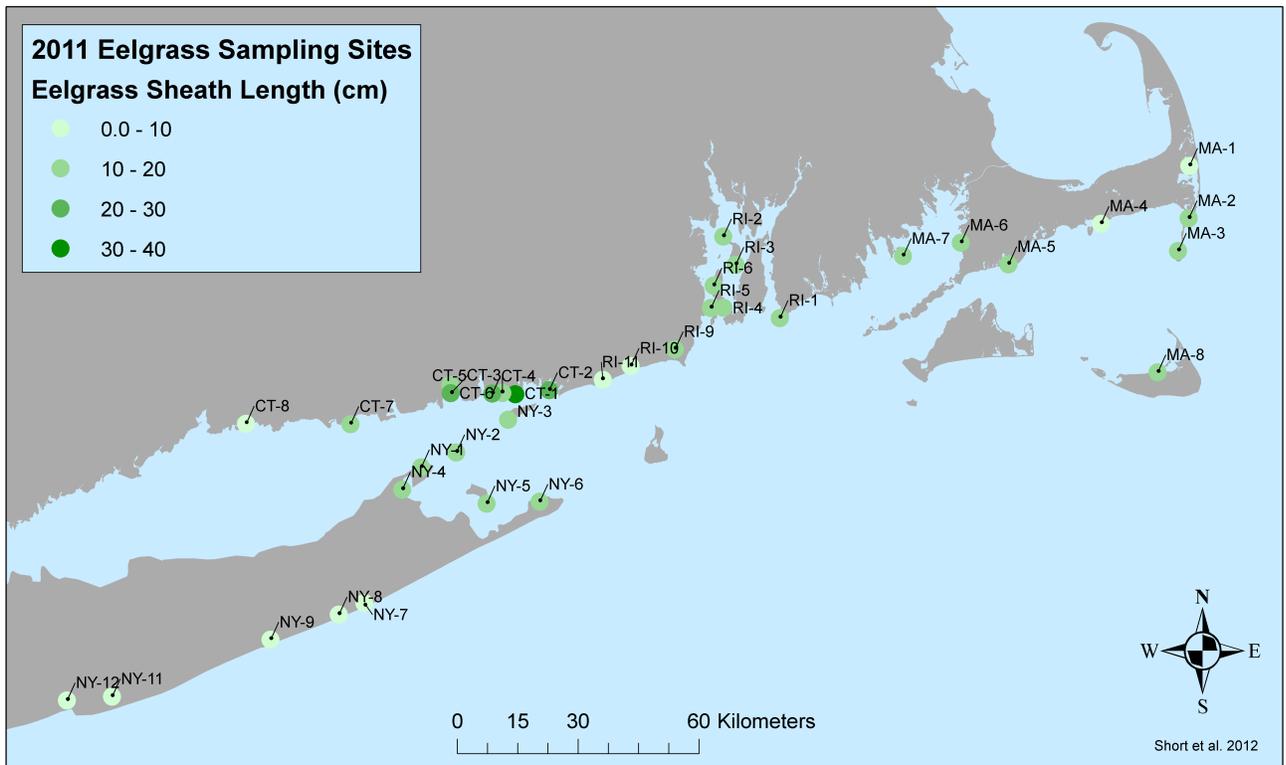
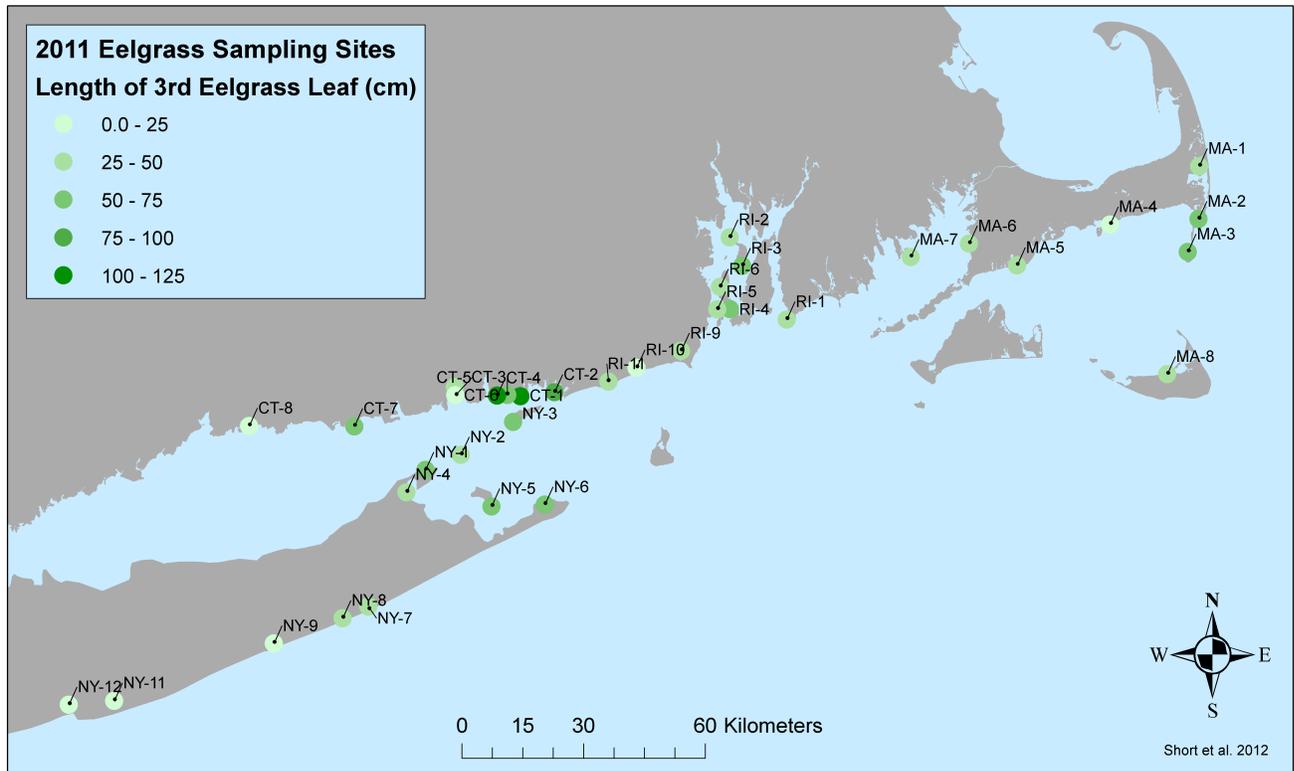


Figure 6. Leaf Length and Sheath Length for eelgrass at sample sites in the region.

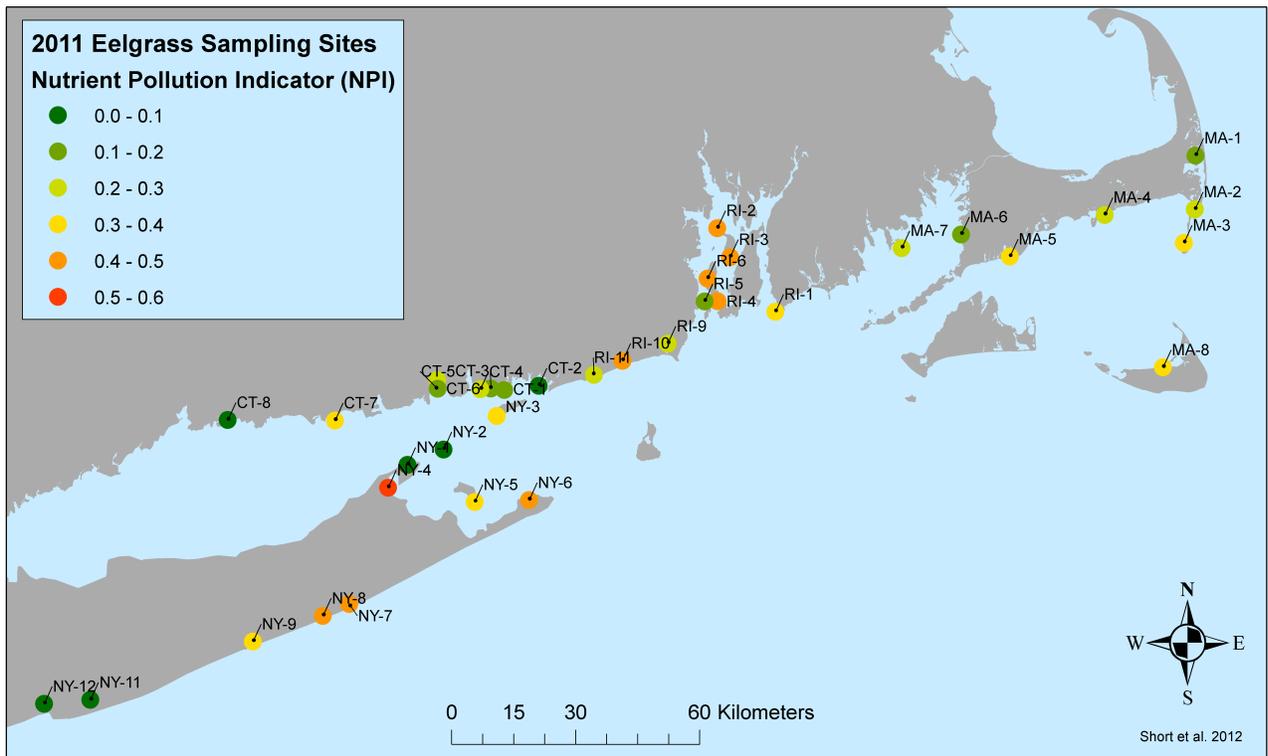
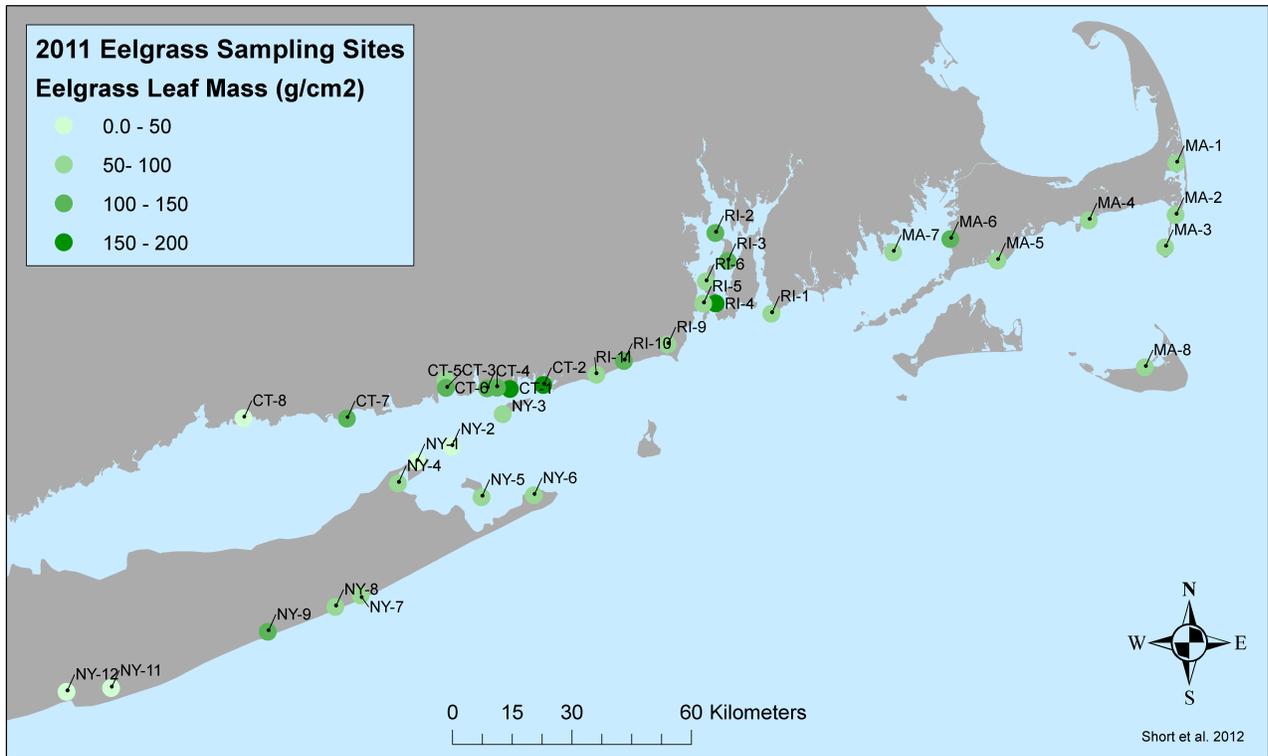


Figure 7. Leaf Mass and NPI for eelgrass at sample sites in the region.

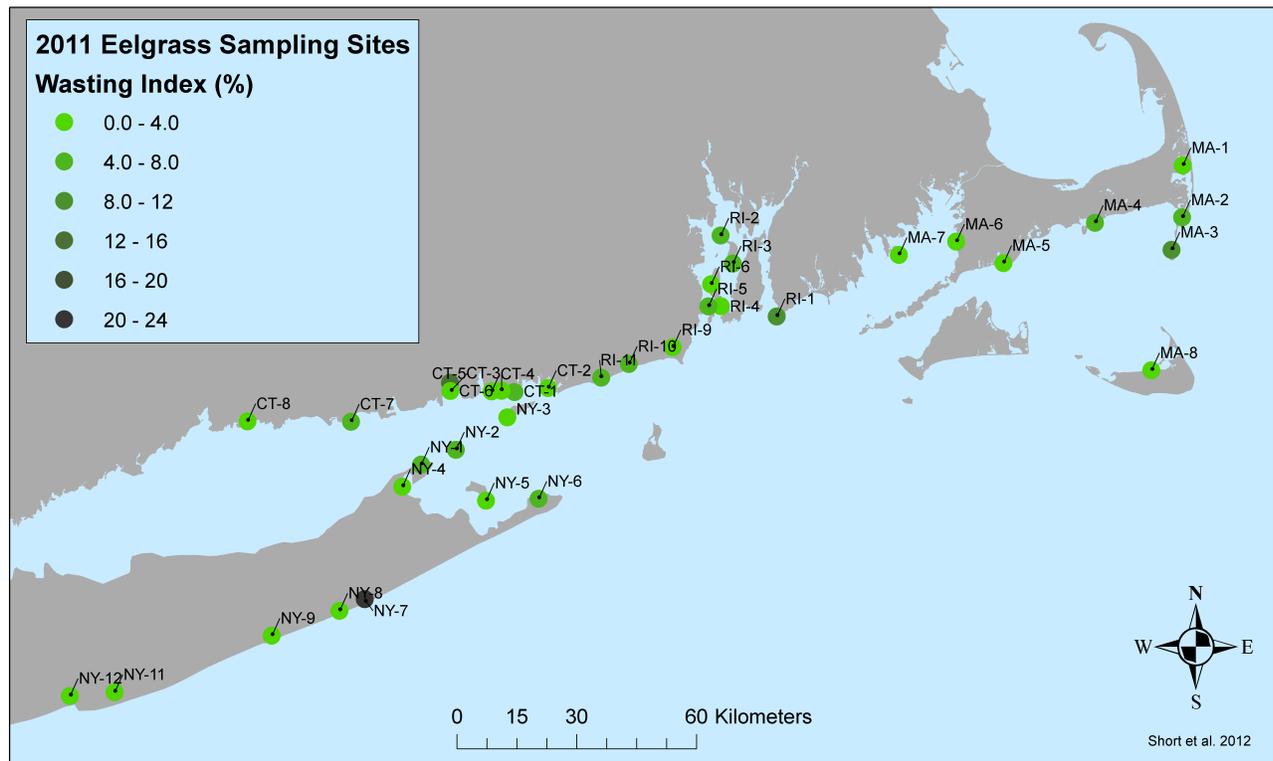
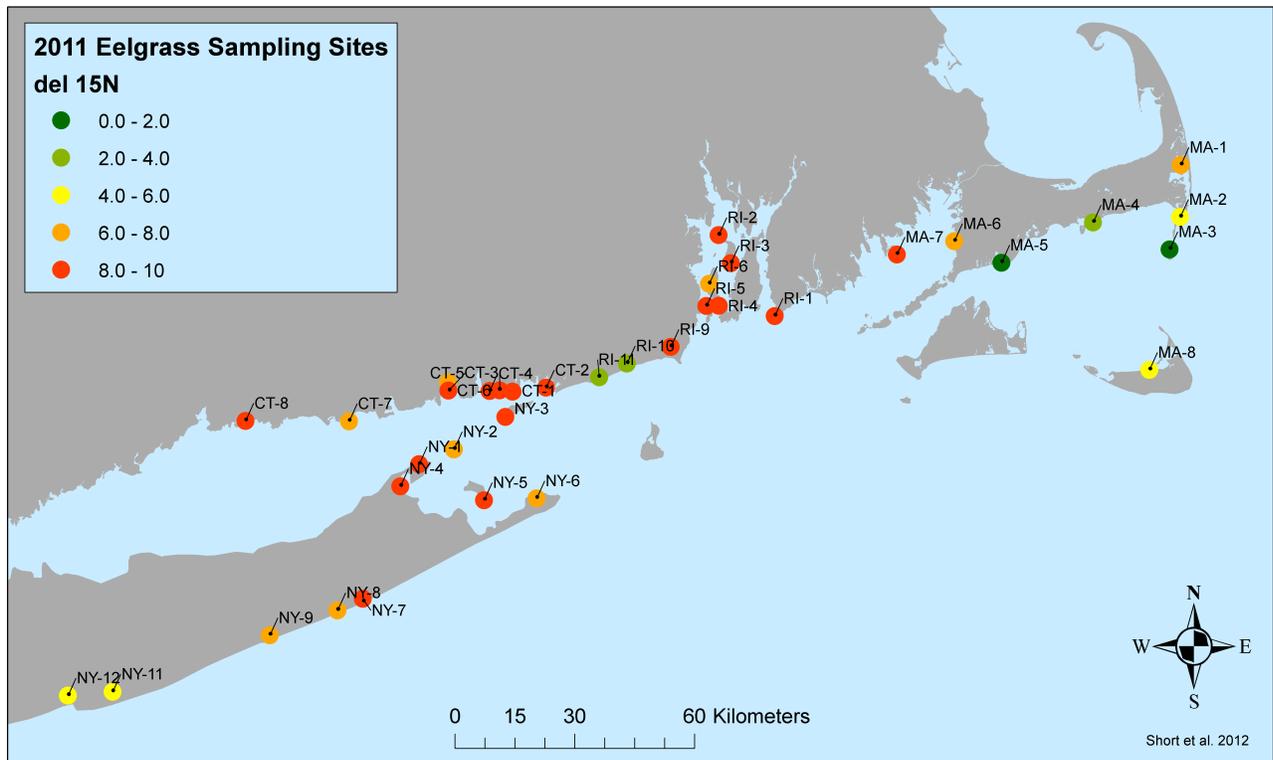


Figure 8. $\delta^{15}N$ and Wasting Index for eelgrass at sample sites in the region.

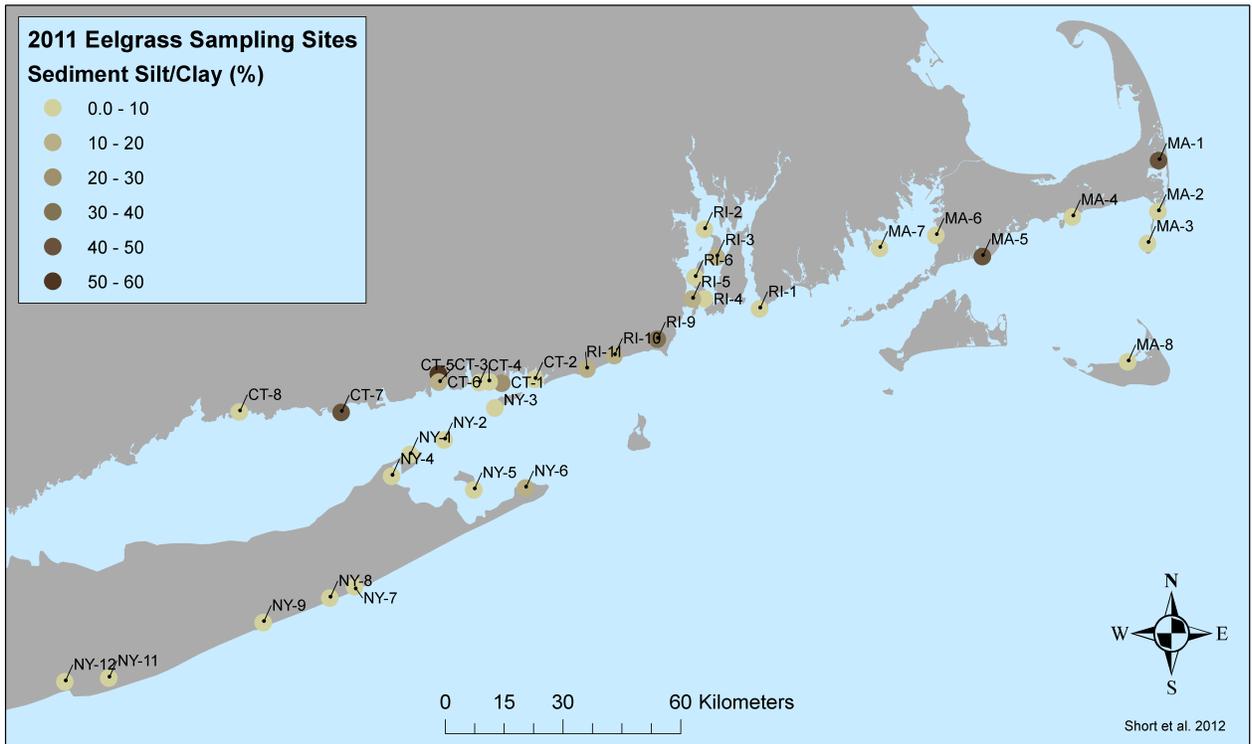
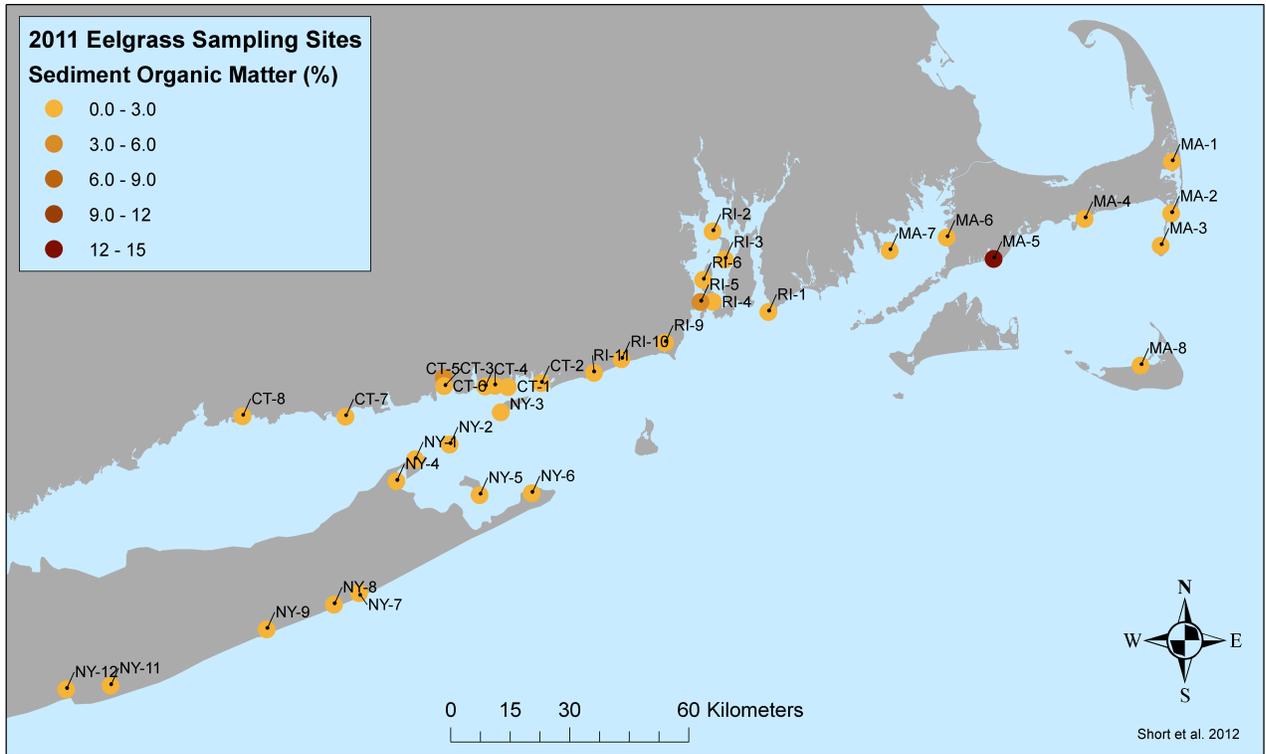


Figure 9. Sediment Organic Matter and % Silt /Clay at sample sites in the region.

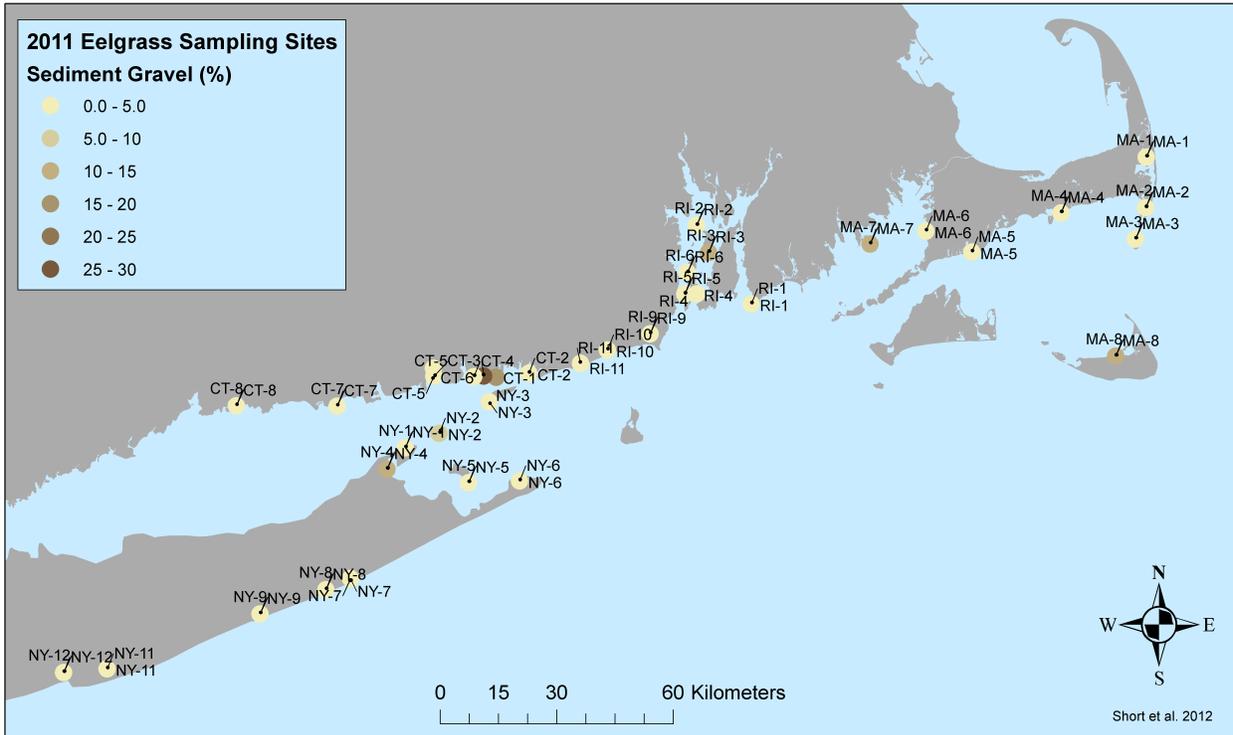
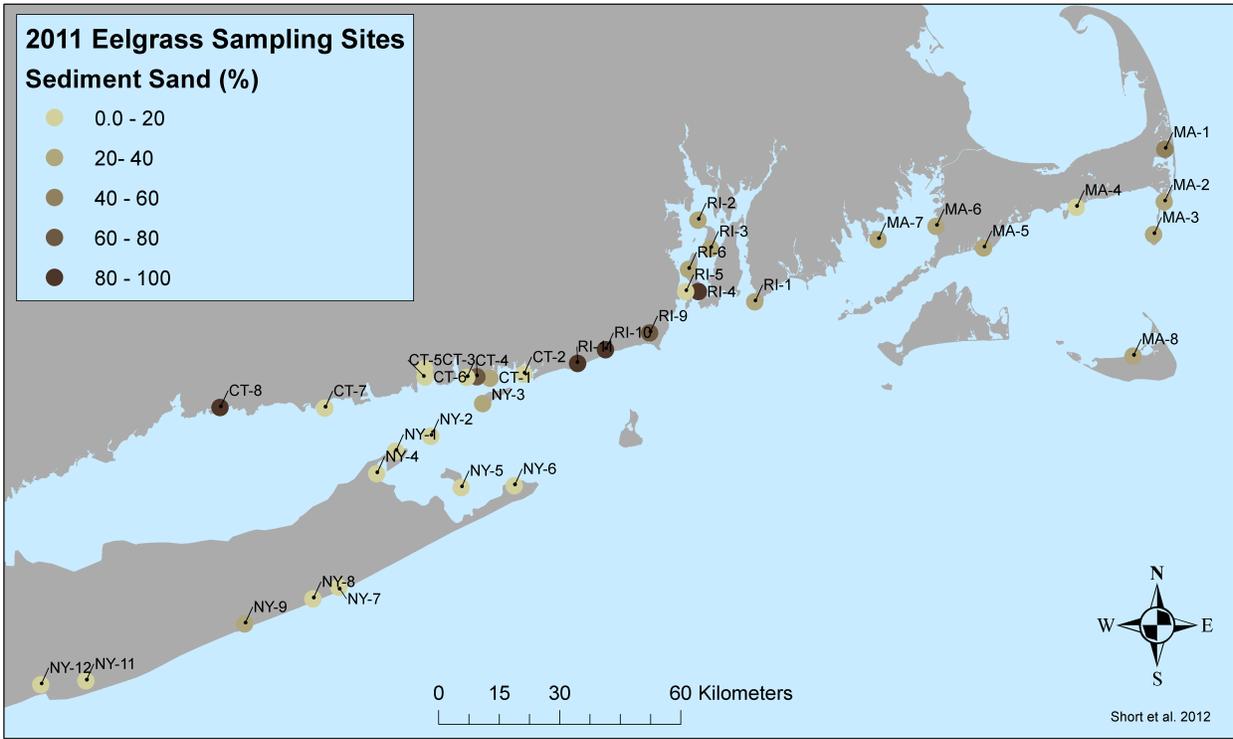


Figure 10. Sediment % Sand and % Gravel at sample sites in the region.

Leaf mass was measured at all sites as an indicator of the weight per cm² of leaf tissue, which is also a component of the Nutrient Pollution Indicator (NPI). The NPI (Lee et al. 2004) provides a view of the nutrient exposure of eelgrass beds in different parts of the coast. The highest NPI values (most exposed to nutrients) were seen in Peconic Bay and Narragansett Bay as well as Shinnecock Bay (Figure 7). Sites in eastern Connecticut showed relatively low NPI as did most of the sites in Massachusetts. NPI values exceeding 0.3 are considered to show signs of eutrophication. In areas like Nantucket Island (MA8) and Waquoit Bay (MA5), the elevated NPI is a result of localized loading conditions. The NPI is a useful eelgrass-based indicator of the nutrient exposure that the eelgrass plants receive as they root in bays in estuaries and take up nitrogen directly from the water column. Direct measurement of water itself for nitrogen level assesses only what nitrogen is left after marine plants have absorbed all they can and is a highly variable and unreliable measure of nitrogen loading.

The measure of $\delta^{15}\text{N}$ in eelgrass blades is indicative of the degree of human-derived nitrogen incorporated into the plant during its growth, reflecting the overall inputs of nitrogen to the area where the plants grow. Higher $\delta^{15}\text{N}$ levels (above 6.0) indicate sewage or other organic waste inputs as the dominant nitrogen source. Lower levels of $\delta^{15}\text{N}$ are indicative of inorganic nitrogen sources like fertilizer or fixed nitrogen from the atmosphere. Narragansett Bay, Long Island Sound, Peconic Bay and the south shore of Long Island all showed evidence of high anthropogenic nitrogen inputs (Figure 8 top); most sites showed some isotopic indication of anthropogenic nitrogen. Waquoit Bay showed high NPI levels and low $\delta^{15}\text{N}$ (indicating a major inorganic nitrogen source) from eelgrass exposed to golf course run-off as the major nitrogen input at the sampling site in Sage Lot Pond (MA).

Wasting disease is a natural stressor of eelgrass which is caused by a slime mold that penetrates cell walls and turns the leaves black, ultimately killing them if extensive (Muehlstein et al. 1991); the Wasting Index, WI, (Figure 8 bottom) quantifies the extent of disease infection in an eelgrass population (Burdick et al. 1993). The site showing the highest WI was a station at Southway, near Monomoy Island in Massachusetts, but these values were still less than 25% infected, suggesting fairly low disease activity throughout the region.

We characterized the sediments at the various sampling sites based on amounts of organic matter, silt/clay fine material, sand and gravel (Figures 9 and 10). The organic matter in the sediments influences both nutrient availability from the sediments and stress levels due to associated decomposition that produces high sulfide and adverse redox conditions for eelgrass growth. The only site showing very high levels of sediment organic matter was in Sage Lot Pond, Waquoit (MA5). Two other sites, Fort Getty, Narragansett Bay (RI5) and Niantic River (CT6) showed organic matter levels higher than 3% which is a level that begins to stress eelgrass in some conditions. Silt/clay was highest in Pleasant Bay, Cape Cod (MA1), Sage Lot Pond (MA5) and Duck Island (CT5) but none of the levels are considered high enough to exceed the site selection model criteria (Short et al. 2002). Sand predominated at most of the sites, typical of southern New England and New York. Gravel content was relatively low except at a few locations in central Connecticut.

Eelgrass plant characteristics were examined for all sites in the study to investigate site differences and patterns of difference (Figure 11). For standardization, the sites were ordered using the NPI, from least to highest NPI value, since the NPI reflects the degree of eutrophication among the sites. The least eutrophied sites were in Connecticut (associated with low organic matter and appreciable gravel content) and the most eutrophied were in New York and New Hampshire.

Eelgrass leaf length and sheath length as well leaf C/N were generally inverse of NPI while $\delta^{15}\text{N}$ showed no relationship to nutrient exposure level (the NPI). Wasting Index was also independent of NPI values. Sediment organic content and salinity as well as sediment % Sand and % Silt/Slay content also showed no patterns relative to NPI. However, % Gravel appears to be inversely related to NPI (Figure 12).

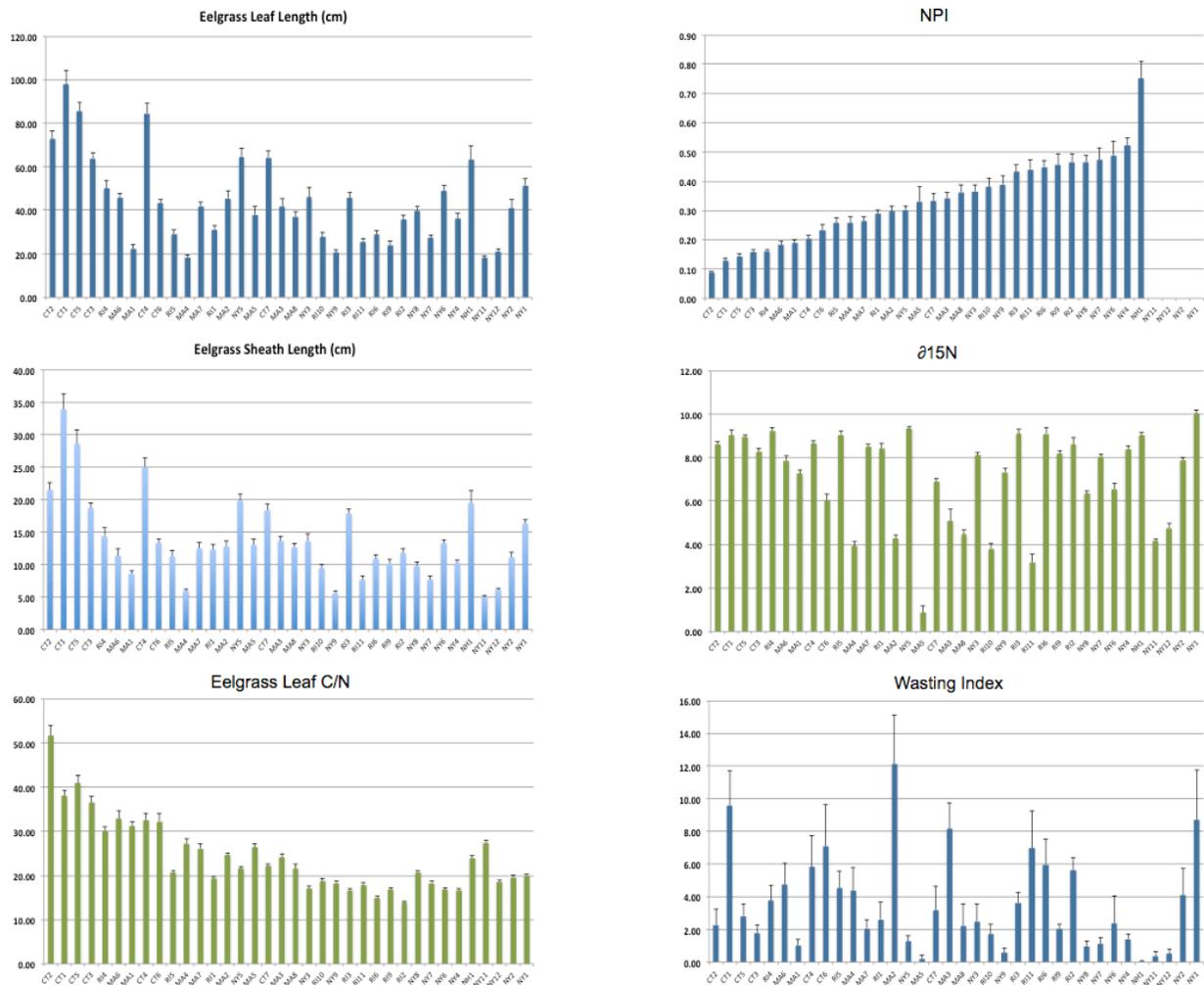


Figure 11. Eelgrass from 37 populations (mean \pm SE) was examined using a one-way ANOVA, revealing differences among populations. Populations are arranged in order of increasing NPI in all graphs: (a) Leaf Length of the number 3 leaf showed significant differences among populations ($r^2=0.80$, $F=44.3$, $P<0.0001$). Longer-leaved plants averaging over 80 cm were found where the NPI was lowest, in eastern Connecticut. However, tall plants were also found in Great Bay (60 cm), where the NPI was greatest (0.75). (b) Sheath Length of eelgrass shoots showed a similar response as leaf length and is a measure directly proportional to eelgrass growth (Gaeckle et al. 2006). (c) Carbon to Nitrogen ratio is an indicator of the nutritional status of the plant and significant differences occurred among populations ($r^2=0.87$, $F=78.6$, $P<0.0001$). Higher C/N ratios were found in populations in eastern Connecticut, where the NPI was low, and generally lower ratios occurred where the NPI was high, indicating greater N content of leaves. The NPI and the C/N ratio were inversely correlated ($r=-0.70$) as was the %N in the leaves ($r=-0.91$). (d) NPI, the eelgrass Nutrient Pollution Indicator, was log transformed to improve normality of residuals and homogeneity of variance and significant differences were found among populations ($r^2=0.82$, $F=50.5$, $P<0.0001$). The highest NPI was found in Great Bay, New Hampshire with several New York and Rhode Island Stations above 0.4, which could indicate eutrophication stresses at these locations (Lee et al. 2004). The lowest levels were found in Connecticut, Massachusetts and some stations in Rhode Island. (e) $\delta^{15}N$, a ratio of nitrogen isotopes in eelgrass leaves, indicated the sources of nitrogen influencing the plants. Human-derived nitrogen sources tend to increase the level of ^{15}N . Significant differences in leaf $\delta^{15}N$ occurred among populations ($r^2=0.90$, $F=103$, $P<0.0001$). Higher $\delta^{15}N$ levels were found in eelgrass off the Connecticut and New York shorelines of Long Island Sound, eastern Long Island and within Narragansett Bay. Lower $\delta^{15}N$ values were found in populations in Western Long Island, Block Island Sound and Nantucket Sound. (f) The Wasting Index was developed (Burdick et al. 1993) to provide information on perhaps the most important natural biological stressor to eelgrass beds, the wasting disease, which nearly wiped out eelgrass along the US eastern seaboard in the 1930s (Short et al. 1987, Muehlstein et al. 1991). Lesions from wasting disease can be found on plant leaves in almost all beds, but only become a threat when their spread exceeds plant growth over several weeks. During our sampling, lesions covered over 10% of leaf area in only one station near Monomoy Island in Massachusetts. Relatively low levels were the norm and so this potential threat was unlikely to have influenced leaf morphology, chemistry or genetic results.

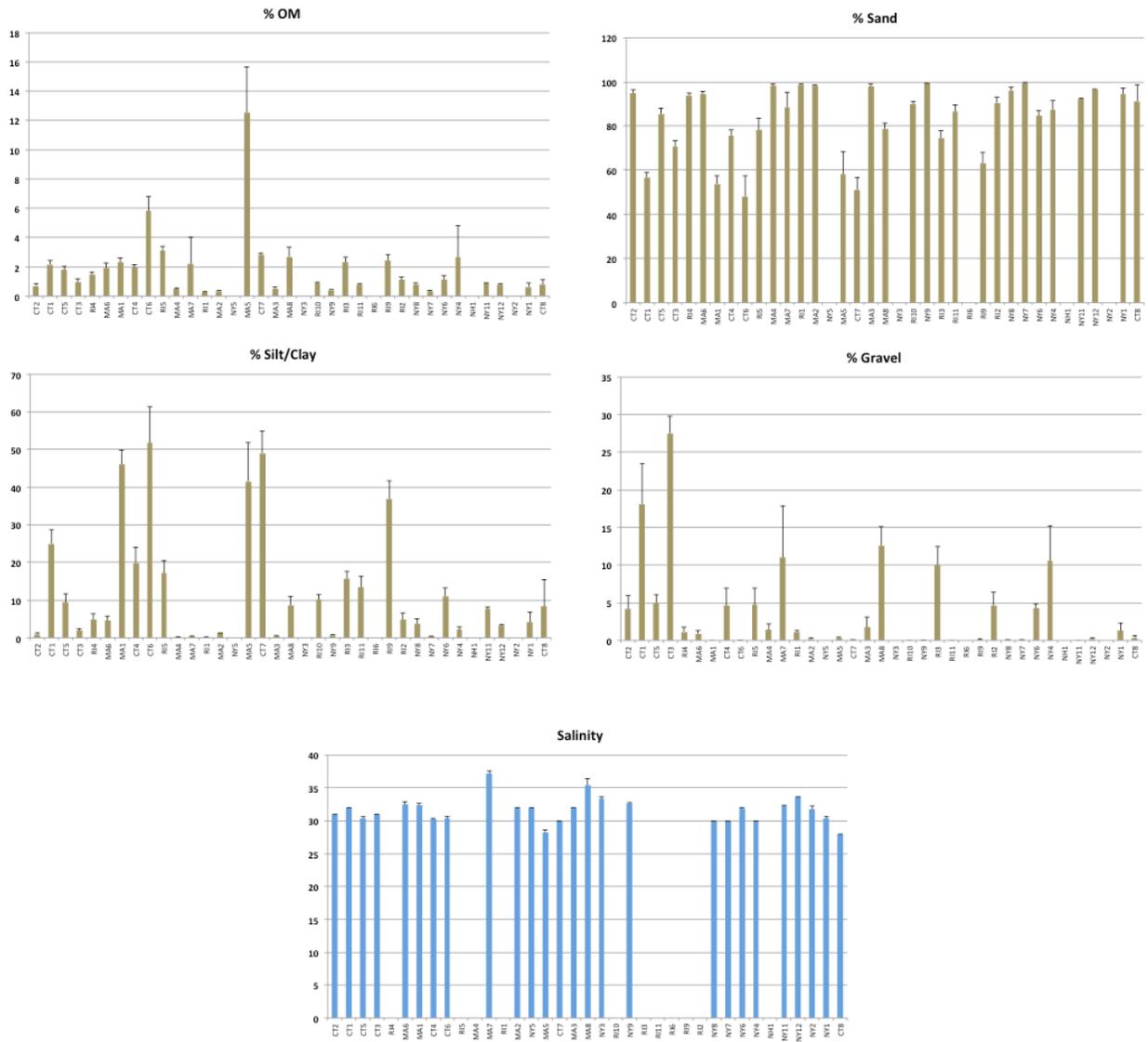


Figure 12. Plots of sediment and salinity data for the eelgrass populations. A one-way ANOVA was used to compare the sediment at the different sites. (a) Sediment Organic Matter was significantly different among stations ($r^2=0.74$, $F=11.0$, $P<0.0001$). Results showed relatively high organic matter ($>10\%$) only in Sage Lot Pond, (MA5). Most sites had 2% or lower organic matter, which would not likely have contributed to stress in populations of eelgrass inhabiting them (see mesocosm results). (b) Silt/Clay (grain size of less than 63μ) content of sediment from the various stations exhibited several significant differences ($r^2=0.89$, $F=33.2$, $P<0.0001$). The two sites with relatively high organic matter content (MA5 and CT6) also had high silt/clay content, revealing the fine nature of the sediment. (c) Sand (between 63μ and 2mm) dominated most sediment samples, but there were differences among sites ($r^2=0.84$, $F=21.4$, $P<0.0001$), with contributions at several stations from coarser gravel (CT1, CT3) and finer silt/clay (MA1, MA5, CT6, CT7 and RI9). (d) Gravel (sediment larger than 2mm) in the samples averaged from none to just under 30% and there was considerable variability between sediment samples within sites; nevertheless significant differences among sites were revealed by the ANOVA ($r^2=0.79$, $F=15.2$, $P<0.0001$). Two of the sites in Connecticut with the lowest NPI also had higher gravel content in the sediment (18-27%). (e) Salinity at all sites was fairly saline, with all averaging at or over 30 ppt, except for two sites (Sage Lot Pond, MA and Hotchkiss Cove, CT).

A complete correlation analysis of relationships between all the eelgrass and environmental data, based on site means (n=36), highlights a number of parameters that are significantly correlated (Table 1). Particularly, Sheath Length is correlated with many other plant characters, as well as being positively correlated with % Gravel and negatively correlated with % Sand. As expected, sediment % OM (organic matter) is significantly correlated negatively to % Sand and positively to % Silt/Clay. Using the more detailed data from individual sample observations of plants alone (n=360), the relation between % C in eelgrass leaf tissue and both leaf % N and leaf length are revealed (Table 2).

Analysis of the eelgrass parameters using discriminant function analysis separates known categories (in our case, states) using a set of variables that describe the characteristics of eelgrass populations (e.g., % N). The program creates canonical functions using linear combinations of the chosen variables, then plots the site scores on a graph defined by the two most important canonical functions along with 50% contours of the means for the state eelgrass populations (our categories) (Figure 13). The discriminant analysis classified 84% of the data from 31 sites into the correct states. The first Canonical Function (x-axis) separates Connecticut from Massachusetts, New York and Rhode Island, and the second (y-axis) further separates Massachusetts from the other 3 states. Separations are dominated in Canonical 1 by Leaf Mass and C/N Ratio (carbon nutrition). In Canonical 2, separations are achieved by differences in %N and $\delta^{15}\text{N}$ (nitrogen nutrition) and also by # Leaves and Length of 3rd Leaf (plant morphology). This discriminant analysis indicates that the factors influencing eelgrass parameters differentiated by state, and are related to latitude/longitude as well as proximity to nutrient sources.

Table 1. Correlation matrix of eelgrass and environmental parameters based on site means (n=36). Red numbers are highly significant ($r^2 > 0.44$, $\alpha = 0.01$); orange numbers are also significant ($r^2 > 0.33$, $\alpha = 0.05$). Positive numbers indicate positive correlations (e.g., eelgrass leaf length increases as the carbon-to-nitrogen ratio increases). Sheath Length (cm) is a relative measure of mean growth rate; # Leaves is the mean number of leaves per shoot; Leaf Length (cm) is the mean length of the #2 leaf from each shoot; Leaf 15N is the mean $\delta^{15}N$ value; Leaf N% is the mean amount of nitrogen in leaves by weight; Leaf 13C is the mean $\delta^{13}C$ value; Leaf C% is the mean amount of carbon in leaves by weight; Leaf C/N is the mean carbon-to-nitrogen ratio by weight of leaves; Leaf Mass is the mean weight of a square centimeter of eelgrass leaf; Leaf NPI is the mean value for the Nutrient Pollution Indicator; WI is the Wasting Index, a measure of the amount of wasting disease infection in a leaf; Sediment %OM is the mean organic matter content of sediments at the sampling site; % Gravel is the mean weight of the gravel portion of the sediments; % Sand is the mean weight of the sand fraction of the sediments; % Silt/Clay is the mean weight of the silt/clay fraction of the sediments; and finally, Salinity is the water salinity in parts per thousand at the site at time of collection.

Correlations of Site Means (36)	Sheath Length Mean	#Leaves	Leaf Length	Leaf 15N Mean	Leaf N% Mean	Leaf 13C Mean	Leaf C% Mean	Leaf C/N Mean	Leaf Mass /Area
Sheath Length	1.0000								
#Leaves	0.3487	1.0000							
Leaf Length	0.9670	0.2806	1.0000						
Leaf 15N Mean	0.4601	0.1148	0.4419	1.0000					
Leaf N% Mean	-0.4992	-0.1304	-0.5474	0.0361	1.0000				
Leaf 13C Mean	-0.0328	0.2189	-0.0083	-0.1326	0.0871	1.0000			
Leaf C% Mean	-0.2511	-0.1586	-0.2853	0.1231	0.3219	-0.1721	1.0000		
Leaf C/N	0.5675	0.0950	0.6030	0.0948	-0.9248	-0.1230	-0.0910	1.0000	
Leaf Mass/Area	0.3058	-0.2548	0.2546	0.3617	-0.3386	-0.1361	0.3620	0.5241	1.0000
NPI Mean	-0.3901	0.1877	-0.3871	-0.0836	0.7280	0.0733	0.0109	-0.7650	-0.8072
WI Mean	0.2804	0.1037	0.2561	0.0395	-0.1061	0.2753	-0.3270	0.0882	0.1732
Sediment % OM	0.0975	0.0731	0.0468	-0.3430	-0.1642	-0.2484	-0.0666	0.0909	-0.1452
% Gravel	0.4989	-0.0179	0.4702	0.3329	-0.2411	-0.1063	-0.3136	0.2751	0.2254
Sand	-0.3636	-0.0520	-0.2853	0.0034	0.2377	0.1629	0.1202	-0.1958	-0.0295
Silt/Clay	0.1698	0.0600	0.1017	-0.1365	-0.1450	-0.1229	0.0033	0.0888	-0.0602
Salinity	-0.2100	0.0462	-0.2374	0.0478	0.0912	0.2696	-0.1086	-0.1347	0.1074

continued

	Leaf NPI	WI	Sediment % OM	% Gravel	Sand	Silt/Clay	Salinity
NPI Mean	1.0000						
WI Mean	-0.2736	1.0000					
% OM	-0.0476	-0.1497	1.0000				
% Gravel	-0.2432	0.0258	-0.0208	1.0000			
Sand	0.1895	-0.0127	-0.6262	-0.2349	1.0000		
Silt/Clay	-0.0928	0.0026	0.6441	-0.1611	-0.9214	1.0000	
Salinity	-0.0764	-0.0589	-0.3395	0.2272	0.2345	-0.3285	1.0000

Table 2. Correlation matrix of eelgrass parameters based on site observations (n=360) and measured in the units indicated. Red numbers are highly significant ($r^2 > 0.34$); orange numbers are also significant ($r^2 > 0.21$). Positive numbers indicate positive correlations. Sheath Length (cm) is a relative measure of growth rate; # Leaves is the number of leaves per shoot; Leaf Length (cm) is the length of the #3 leaf from each shoot; Leaf 15N is the $\delta^{15}\text{N}$ value; Leaf N% is the amount of nitrogen in leaves by weight; Leaf 13C is the $\delta^{13}\text{C}$ value; Leaf C% is the amount of carbon in leaves by weight; Leaf C/N is the carbon-to-nitrogen ratio by weight of leaves; Leaf Mass is the average weight of a square centimeter of eelgrass leaf; Leaf NPI is the value for the Nutrient Pollution Indicator; WI is the Wasting Index, a measure of the amount of wasting disease infection in the shoot.

Correlations of observations	15N	N%	13C	C%	C/N	Leaf Mass /Area	NPI	#Leaves	WI	Avg Leaf Length
15N	1.0000									
N%	0.0327	1.0000								
13C	-0.1120	0.0489	1.0000							
C%	0.1050	0.2669	-0.0753	1.0000						
C/N	0.0849	-0.9053	-0.0900	-0.0276	1.0000					
LeafMass/Area	0.2681	-0.3187	-0.0034	0.2560	0.4546	1.0000				
NPI	-0.0663	0.6963	-0.0342	0.0179	-0.6882	-0.7863	1.0000			
#Leaves	0.0766	-0.0792	0.1569	-0.0830	0.0426	-0.0913	0.0855	1.0000		
WI	0.0441	-0.0668	0.0798	-0.1691	0.0508	0.0543	-0.1242	0.0227	1.0000	
AvgLeafLength	0.3832	-0.4789	0.0407	-0.2276	0.5149	0.1844	-0.2894	0.1136	0.1565	1.0000

Using discriminant function analysis, with fewer plant parameters but including % Silt/Clay, provides a different perspective on the separation of populations by state. A combination of variables used was: # Leaves, Leaf Length (Lnth 3rd leaf), $\delta^{15}\text{N}$, %N, C/N, and %Silt/Clay of the sediment. The first Canonical Function (x-axis) separates Connecticut and Massachusetts from New York and Rhode Island, and the second (y-axis) further separates Massachusetts from the other 3 states (Figure 14). New York and Rhode Island are superimposed on the two-dimensional graph, but clearly separate on the third function (see 3-D, bottom Figure 14). The discriminant analysis classified 100% of the sites into the correct states, based on prior probabilities (each state had data for 8 populations/sites except CT with data for 7 sites). Canonical Function #1 indicated eelgrass beds in Connecticut generally had higher % Silt/Clay in sediments that supported taller plants with high C/N ratios. Massachusetts plants generally had more leaves with lower leaf %N content and higher C/N ratios. Plants collected from New York and Rhode Island sites generally had greater ^{15}N (from human sources), with moderately high %N. Plants from New York sites tended to have fewer leaves than Rhode Island eelgrass. Compared with New York, Rhode Island plants tended to have relatively greater %C in their leaves.

General differences between states carried over into a cluster analysis (Wards Method) using the variables: # Leaves, 3rd Leaf Length, $\delta^{15}\text{N}$, %N, C/N, Leaf Mass and NPI (Figure 15). In the dendrogram one can see that in the red cluster, 4 of the 7 Connecticut populations appear together; these plants generally had the longest leaves and highest C/N ratios. The teal green sites have common values of high NPI and high ^{15}N (exposure to elevated nitrogen, generally from human sources), these being the Long Island Sound, Narragansett Bay, and South Shore Long Island eelgrass populations. The similarities within the individual eelgrass population groups are shown in the Parallel Plots (Figure 15 bottom). At a higher level (level 3) the groups divide into 3

clusters: the red, green-blue-brown, and teal-purple, typified by low NPI and long leaves, by low NPI and small leaves, and by high NPI and low C/N, respectively. The PCA and cluster analyses of eelgrass plant characteristics provide a unique opportunity to view comparative conditions between states and a geographic continuum of eelgrass populations which separate by plant characteristics, nitrogen loading conditions, and nitrogen source.

Overall, the eelgrass populations in the southern New England and New York region separate by state to some degree based on plant characteristics and plant responses to the environment. Specific eelgrass characteristics, level of eutrophication and exposure to anthropogenic nitrogen sources are the predominant elements that contribute to plant morphological distinctions across the states, besides genetics. New York eelgrass generally has small leaves, and is exposed to nitrogen from anthropogenic sources. Connecticut eelgrass has large plants with fairly low nitrogen exposure, most of which is anthropogenic. In Rhode Island the eelgrass is intermediate in size, with more leaves per shoot and, at least within Narragansett Bay, relatively high anthropogenic nitrogen exposure. Massachusetts eelgrass is moderate in size, with low nitrogen exposure at several sites, from atmospheric or inorganic fertilizer sources; other sites had clear anthropogenic nitrogen signals.

Wasting disease is endemic to eelgrass and was seen throughout the region. We do not as yet know the underlying cause of the 1930s outbreak, beyond knowing the disease organism (Muehlstein et al. 1990), but at present, wasting disease is relatively quiescent in southern New England and New York. We saw evidence of it at most sampling areas but with low levels of infection, mostly in the oldest leaves on a shoot. At these levels, it poses no threat to eelgrass populations. Wasting disease needs high salinity water to thrive; we sampled mostly high salinity areas and did not see severe wasting disease. In the 1930s, it was the brackish-water reserves of

eelgrass that acted as refugia for the plants, which in these less saline areas survived the wasting disease epidemic and persisted to re-populate the devastated coastal areas. Today, many of the brackish-water areas that formerly sustained eelgrass are too polluted for it to grow, depriving us of eelgrass reserves in the event of another wasting disease epidemic.

Examination of eelgrass flowering was not included in our study because phenology (timing of plant reproduction) varies widely across the region. We saw that many areas had extensive flowering and seed production and the genetics analyses we performed support the idea that sexual reproduction is a critical aspect of eelgrass persistence regionally. There is a tendency to think of eelgrass as having primarily vegetative growth and expansion; our findings clearly show that eelgrass populations are composed primarily of small clones (less than 2 m in diameter) at most locations which employ both sexual reproduction and vegetative expansion in their life strategy.

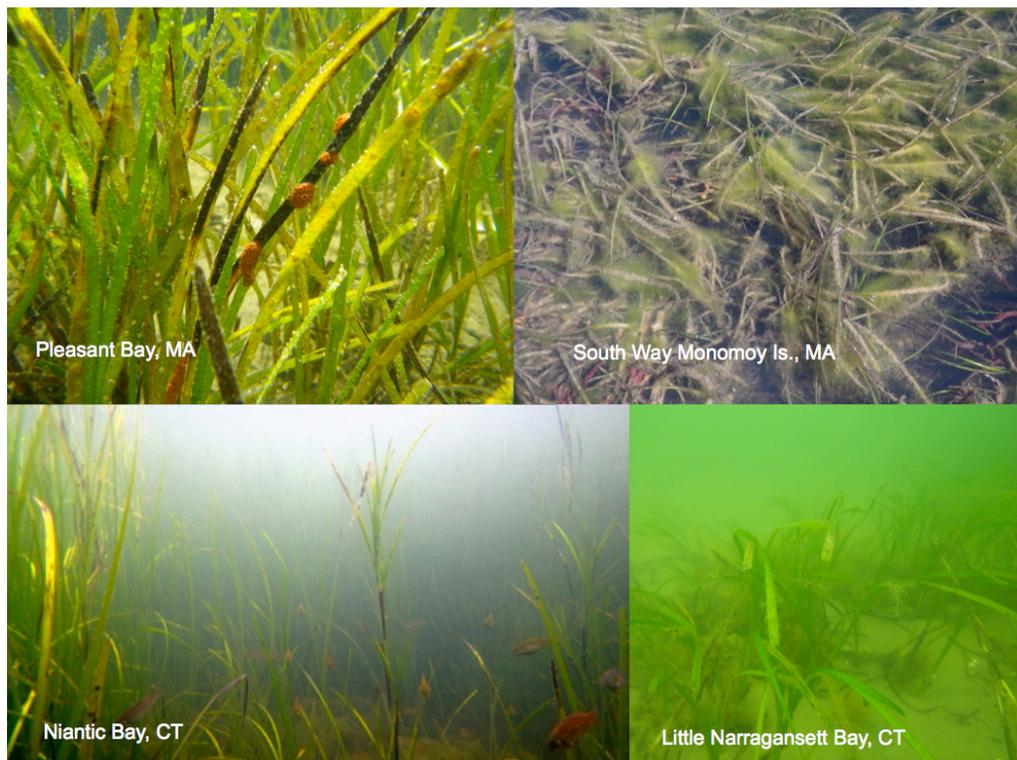
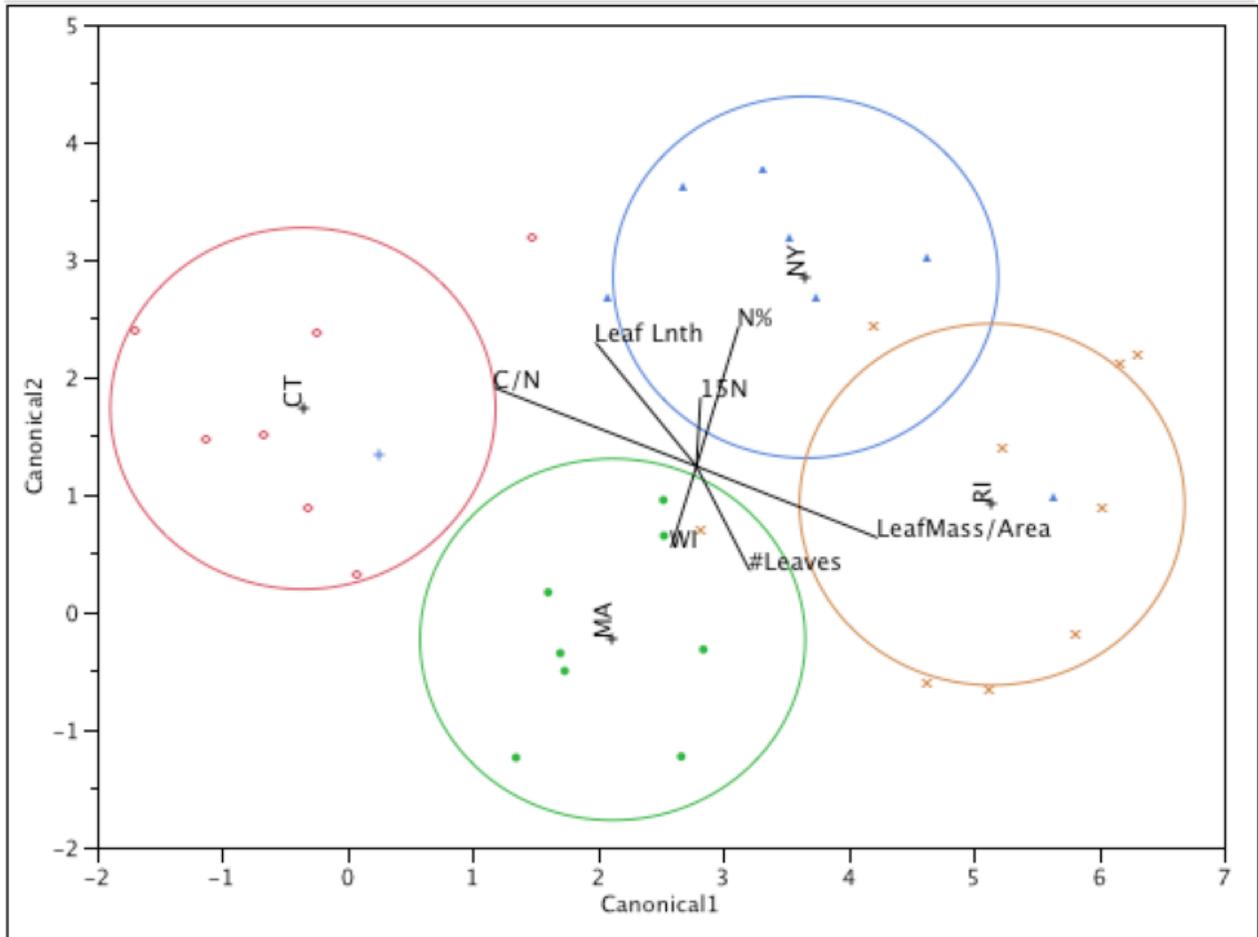


Photo 1: A wide range of eelgrass conditions were found across the region.

The Nutrient Pollution Indicator was used in our study as an indicator of the nitrogen exposure of plants in our populations (Photo 1). The range of NPI results was remarkable for the steady gradient (Figure 11) which shows that some locations are relatively low in nitrogen while others are strongly influenced by nitrogen sources in the watershed. Among the most impacted locations were Great Bay, NH, a known eutrophic estuary, along with Narragansett Bay (all sites) and parts of Peconic Bay on Long Island as well as areas on the south shore Long Island (Figure 7). Massachusetts and Connecticut yielded fairly low NPI values in general; these values probably reflect sites with high ocean flushing rather than a lack of anthropogenic nitrogen loading to coastal waters.

The field study provided both an ecological and a geographic view of eelgrass status across the region, yielding a context for interpretation of both the eelgrass resilience studies and the genetics analysis. This unique view of eelgrass conditions and status for an entire geographic region as a “snapshot” gives added quality assurance which, in combination with the regional mapping, provides managers a context in which to see the status of their own areas of concern.

Figure 13. Discriminant function analysis of eelgrass parameters from the region showing separation by state, in this case including the parameters of %N, C/N Ratio and Leaf Mass, which increase the separation of the eelgrass parameters in the two dimensional view. Here, separations are dominated by carbon nutrition (C/N and Leaf Mass) in Canonical 1 and by nitrogen nutrition (%N and ^{15}N) as well as morphology (# Leaves and 3rd Leaf Length) in Canonical 2. This discriminant analysis suggests that the factors influencing eelgrass parameters may differ by state, and are related to latitude/longitude as well as proximity to nutrient sources.



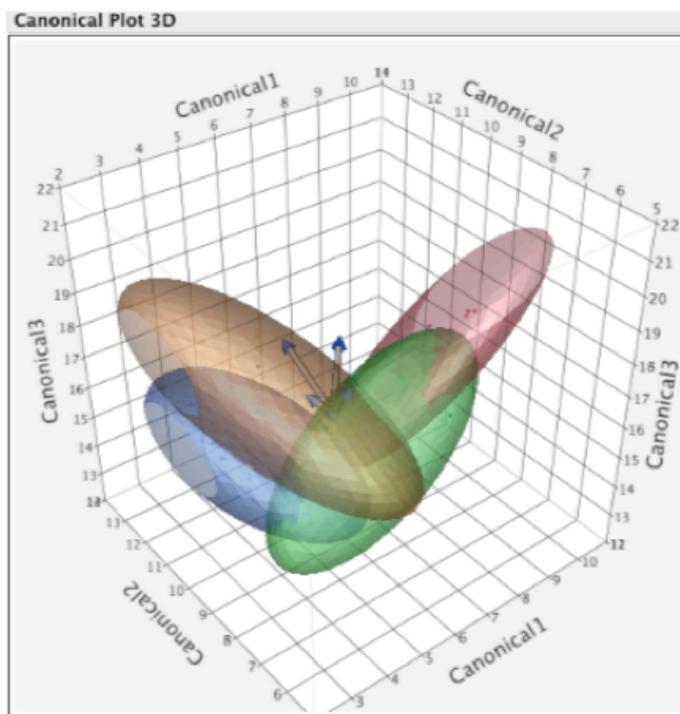
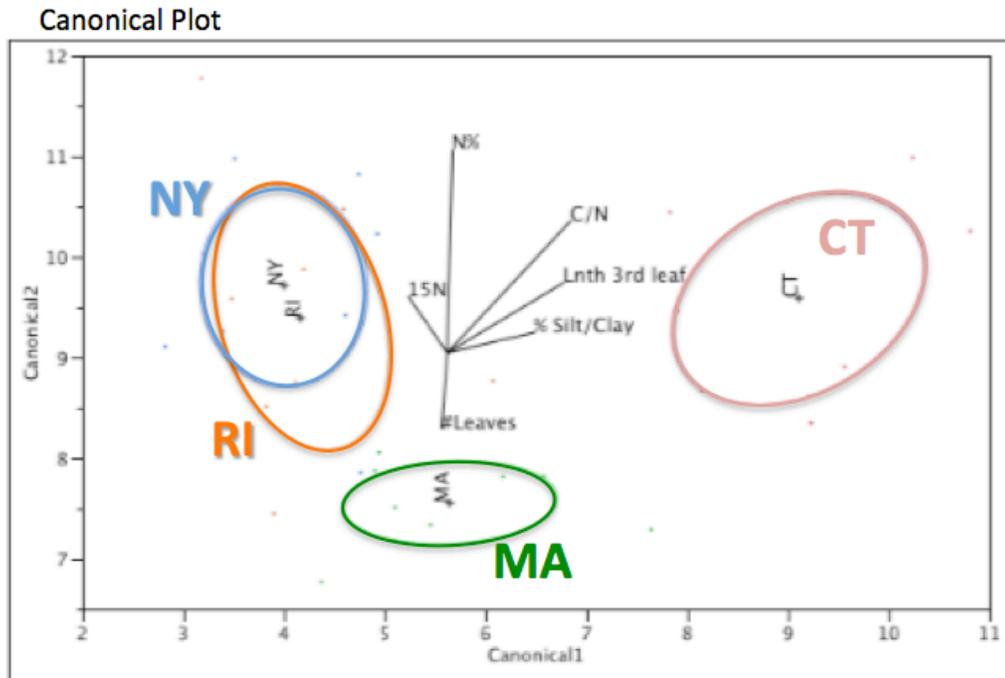
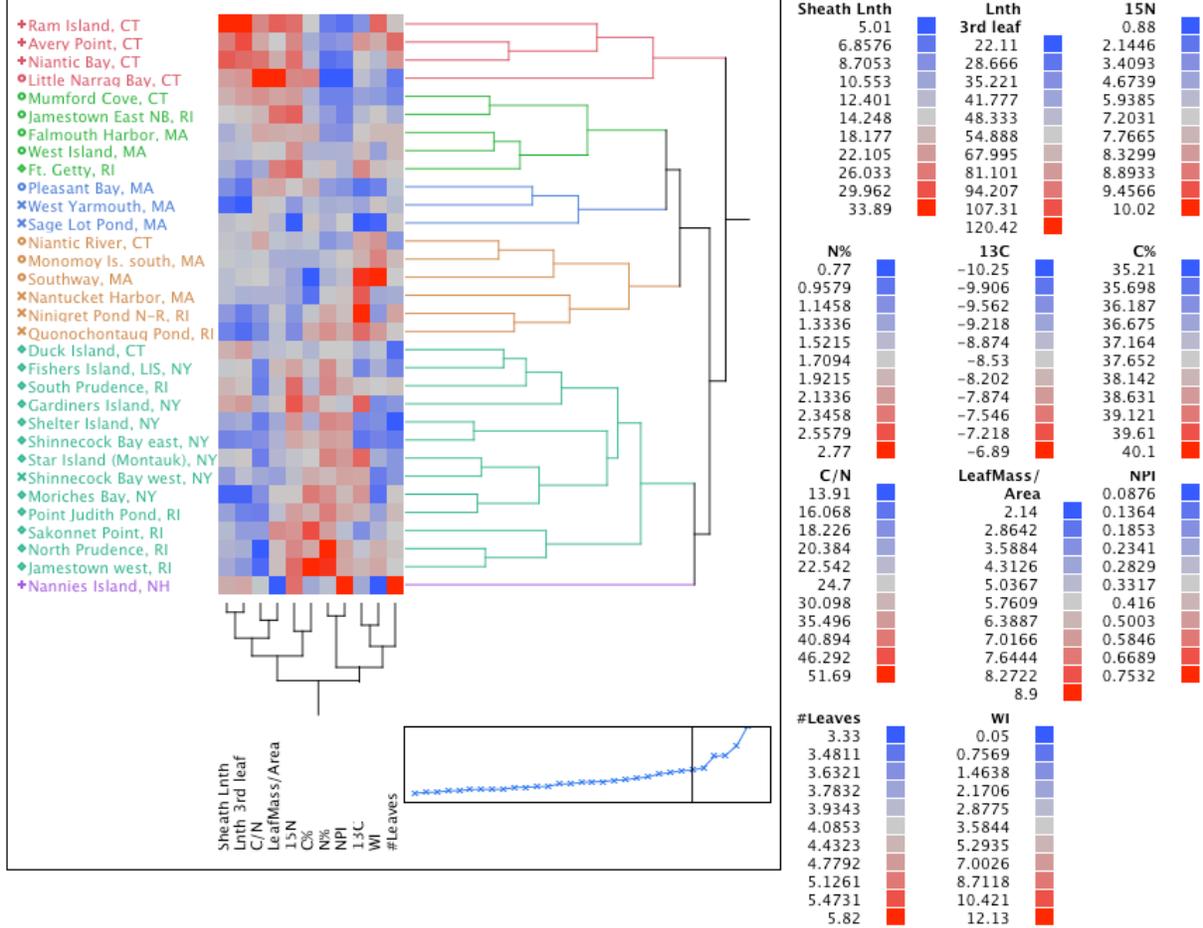


Figure 14. Discriminant function analysis of eelgrass parameters from the region, showing separation by state, based on several eelgrass parameters. The same results are shown in two dimensions (top) and again in three dimensions (bottom). Separations are dominated by Leaf Length and Sediment Silt/Clay in Canonical 1 and by # Leaves and N% in Canonical 2. The apparent overlap of New York and Rhode Island in the two-dimensional plot is shown in three dimensions to be a distinct separation based on differences in leaf chemistry ($\delta^{15}\text{N}$ and C/N). The discriminant analysis indicates that the factors influencing eelgrass meadow characteristics can be used to differentiate populations based on state.

Hierarchical Clustering

Method = Ward

Dendrogram



Parallel Plot

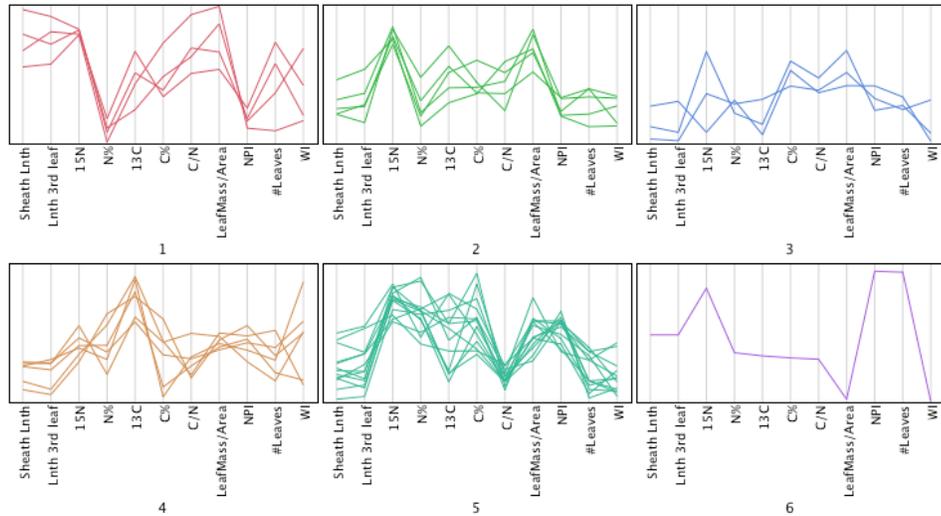


Figure 15. Hierarchical cluster analysis of eelgrass parameters from the region. The dendrogram shows six clusters of sites arranged by station number. Bottom: all eelgrass variables are plotted for each cluster.

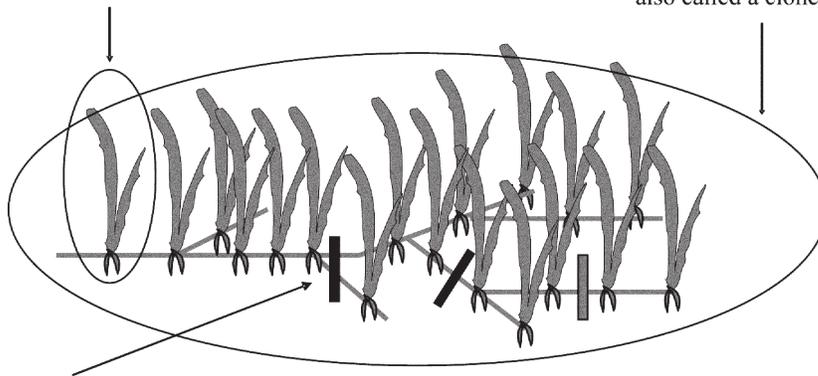
Eelgrass Genetics

A major focus of the study was to assess the genetic variation of eelgrass across the region, determining both the major populations of eelgrass and the genetic diversity of those populations. To do so, it was crucial to be clear about the terminology and definitions when considering genetic difference. We adhere to the following language from Procaccini et al. (2007), which makes a clear distinction between genotypic diversity (number of clones, or genets, within a population) and genetic diversity (diversity of alleles; Figure 16). The underlying assumption of our work was that more genetic diversity in a plant population (i.e., more alleles) should result in plants with more alleles in sequences that code for adaptive responses to stressors and therefore endow greater resilience. In other words these populations have inherently a greater flexibility of response to various stressors because each different allele (i.e., genetic diversity) creates more possibilities of various responses that may be used by the plant to overcome stressor challenges.

A genetic survey of eelgrass across the region was conducted to determine both genotypic and genetic diversity as well as to look for unique (or private) alleles. In addition to populations that have high numbers of alleles, populations that have unique alleles not found in other populations may also have unique alleles that actually code for adaptive responses and thus possess inherent capacities to deal with plant stressors.

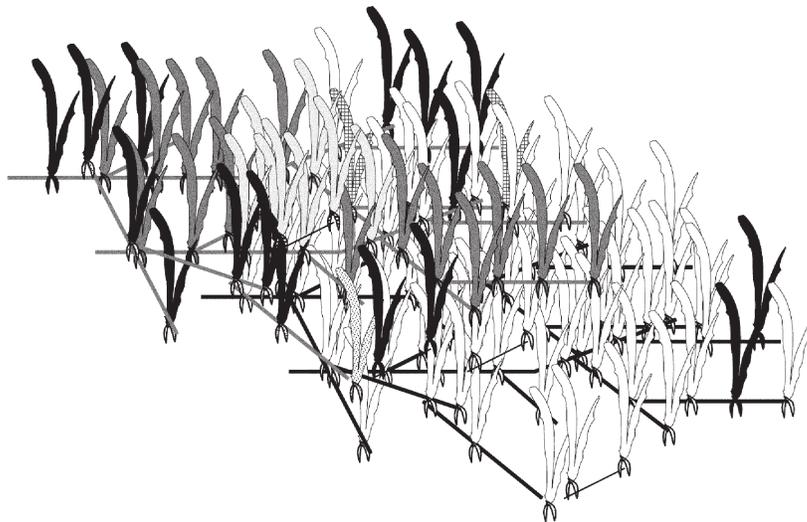
Morphological “individual” = ramet
(also called a module)

Genetic “individual” = genet
(which consists of many ramets;
also called a clone)



Breaks in the rhizome over time make it impossible to empirically identify a genet. A genet is also referred to as a clone which may continue to grow by vegetative spread over many years.

Clonal diversity is thus equal to genotypic diversity, whereas genetic diversity refers to the allelic diversity. In the above example we have one genotype (or one clone).



Each pattern represents a different genet. These genotypes or clones can only be identified using highly polymorphic molecular markers (e.g. microsatellite loci). This example shows intermingling of the different clones. Each clone can have a different genetic (allelic) diversity.

Figure 16. From Procaccini et al. (2007)

We sampled 37 separate eelgrass populations and analyzed 7 microsatellites. Originally we proposed 9 analysis of microsatellites, but a recent publication indicates that two of these may not suitable for population studies; seven microsatellites are sufficient for the assignment of a clonal

lineage (Reusch and Bostrom 2011). We tested 709 eelgrass plants (ramets) and found 688 unique genets (or clones). That is to say, the majority of the eelgrass we sampled represented a unique clone, indicating overall that there are no vast clonal eelgrass colonies across the region and that clonal, or genotypic, diversity in the region is high.

Some sites had much higher allelic richness (Table 3), or genetic diversity. Nannies Island in New Hampshire (NH1), Shelter Island (NY4) and Great South Bay west (NY12) had the highest allelic richness, with several other sites in the high range (above 3.0). The Great South Bay West (NY12) allelic richness is probably the result of small sample size ($n=16$), as is its highest inbreeding (F_{is}) coefficient. Many eelgrass populations showed low inbreeding and none showed very high levels of inbreeding. Overall, the low inbreeding and high allelic richness we found supports a region-wide conclusion of rather high genetic diversity in eelgrass populations of southern New England and New York.

Observed heterozygosity (H_o) is another measure of genetic diversity (Table 3). It ranges from 0 (all loci homozygous) to 1 (all loci heterozygous). Homozygous loci are the result of inbreeding, while heterozygous loci result from genetic exchange with other populations. Nannies Island, Pleasant Bay, Shelter Island, Shinnecock Bay east and Star Island have the highest genetic diversity ($H_o > 0.50$). The populations that have the highest H_e (expected heterozygosity) tend to have the lowest F_{is} , indicating high levels of sexual reproduction. F_{is} measures the partitioning of genetic diversity within a population, as opposed to F_{st} which measures genetic diversity between populations. F_{is} ranges from -1 (all individuals heterozygous) to 1 (no heterozygotes).

Table 3. Site comparison of allelic richness (\hat{A} , where the # of alleles is averaged for each population and normalized to size of smallest population that is being compared 6 individuals), number of private alleles, expected heterozygosity (H_e), observed heterozygosity (H_o), and inbreeding. Site RI10 was not included due to inadequate sample size # once clones were deleted. See Figure 19b for regional inbreeding distribution.

		Total N	Allelic Richness	Private Alleles	H_e	H_o	Inbreeding (Fis)
Nannies Island NH	NH_1	35	3.04	4(84)	0.599321	0.570804	0.048307
Pleasant Bay, MA	MA_1	44	2.97		0.580269	0.551797	0.049612
Monomoy Island South, MA	MA_3	37	2.74		0.524124	0.480585	0.084093
Southway, Monomoy, MA	MA_2	34	2.52	1(84)	0.453048	0.366605	0.193144
West Yarmouth, MA	MA_4	8	1.85		0.308333	0.285714	0.078189
Sage Lot Pond, MA	MA_5	32	2.67	1(84)	0.515782	0.350374	0.324211
Falmouth Harbor, MA	MA_6	35	2.48	3 (84)	0.468844	0.305886	0.350921
West Island, MA	MA_7	24	2.25		0.369453	0.255623	0.312859
Nantucket Harbor, MA	MA_8	32	3.09	2(84)	0.611477	0.453677	0.261375
Fishers Island, NY	NY_3	29	2.89	2(84)	0.608867	0.424349	0.306849
Milford Point, eastern LIS, NY	NY_1	6	1.99		0.391342	0.266667	0.339336
Shelter Island, NY	NY_4	17	3.22		0.675834	0.537815	0.209266
Shinnecock Bay west, NY	NY_8	9	3.1		0.628875	0.503968	0.209215
Shinnecock Bay east, NY	NY_7	18	3	1(84)	0.619048	0.468254	0.24897
South Prudence Narr. Bay, RI	RI_3	22	2.92		0.620833	0.405844	0.351603
Point Judith Pond, RI	RI_9	32	2.81	2(84)	0.557981	0.332949	0.407209
North Prudence, RI	RI_2	31	2.27		0.411649	0.313364	0.241777
Jamestown West, RI	RI_6	27	2.59	1 (84)	0.469375	0.364418	0.228938
Ft. Getty, RI	RI_5	18	2.35		0.446993	0.272642	0.397091
Sakonnet Point, RI	RI_1	26	2.76		0.495619	0.369231	0.258757
Little Narragansett Bay, CT	CT_2	10	2.53		0.44816	0.347619	0.233559
Ram Island, CT	CT_1	27	2.54	1 (84)	0.430662	0.356798	0.174324
Avery Point, CT	CT_4	6	2.66		0.497835	0.347619	0.324107
Niantic Bay, CT	CT_5	11	2.45		0.473609	0.293506	0.392049
Niantic River, CT	CT_6	8	2.49		0.466758	0.433673	0.074526
Duck Island, CT	CT_7	12	2.44	1(84)	0.437203	0.349567	0.207435
Plum Island east end, NY	NY_2	11	2.61	1(84)	0.530612	0.415584	0.225182
Moriches Bay, NY	NY_9	31	2.69	1(84)	0.527634	0.36998	0.302294
Gardiners Island, NY	NY_5	9	2.22		0.417367	0.412698	0.011876
Star Island (Montauk), NY	NY_6	9	2.94		0.572829	0.515873	0.11101
GSB Grass Island, NY	NY_11	16	2.9		0.605991	0.482143	0.209756
GSB (western), NY	NY_12	6	3.13		0.651515	0.333333	0.512195

High Richness

High Inbreeding

Low Richness

Low Inbreeding

Using the STRUCTURE analysis (Pritchard et al. 2000), we identified three metapopulations in the eelgrass of southern New England and New York (Figure 17a, b). Examining these three metapopulations, we found metapopulation 2 (predominantly green) is distinctive with a clear dominance of green elements (specific genetic loci). Metapopulations 1

(red), the largest, and 3 (blue) are not as well resolved, having more plants from the other groups. Several sites are a mix of red and green with no clear dominant group (red-green hatching above the site codes, Figure 17a). The green metapopulation includes eelgrass from Massachusetts and the one New Hampshire site.

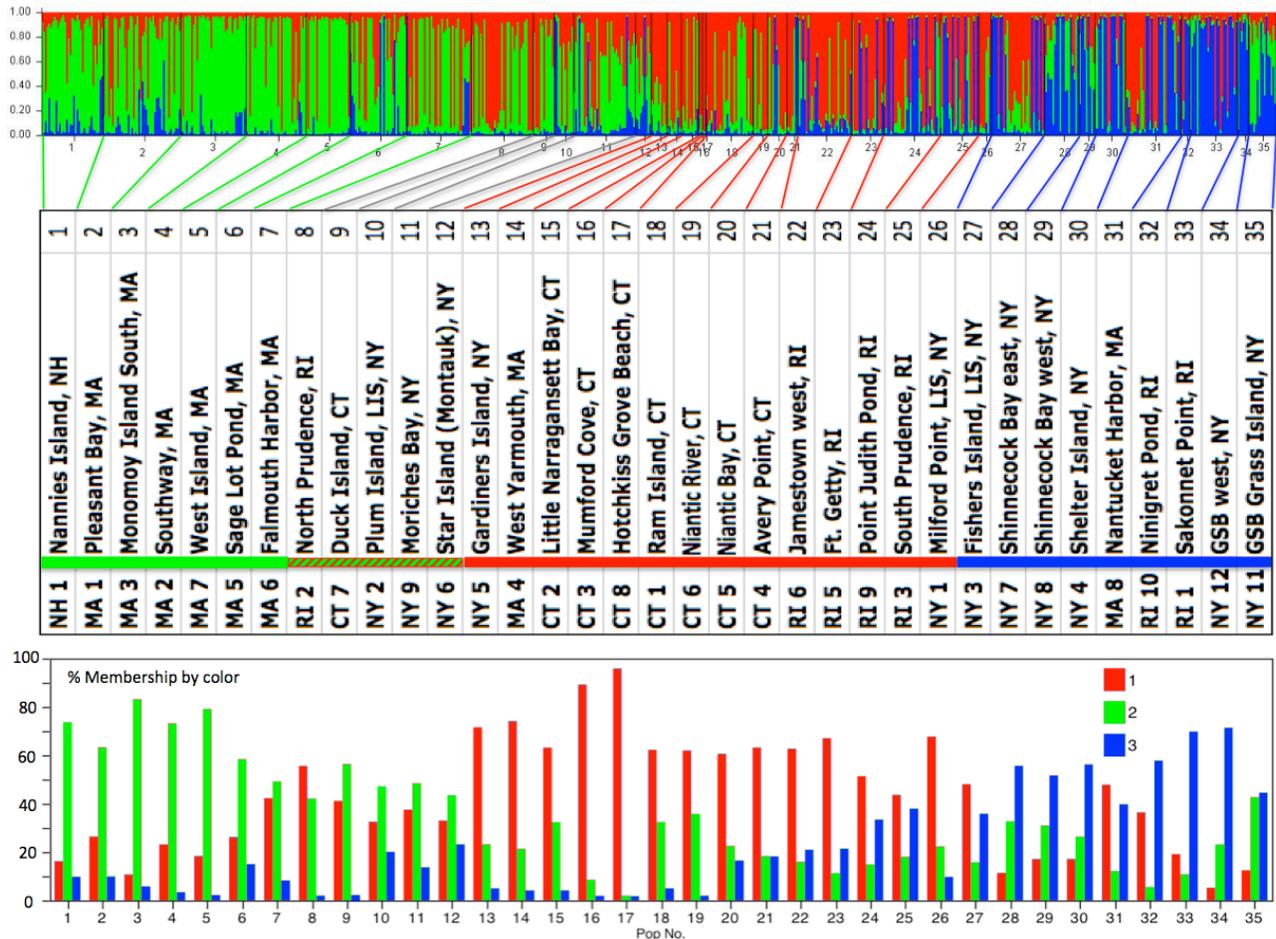


Figure 17a. STRUCTURE analysis of the eelgrass genetic loci represented by the color bars in the top graph for all plant samples in the study, indicating 3 metapopulations, each represented by predominance of one of the three colors (lower color bar graph). Each vertical row of color in the top bar represents an individual genetic sample run for the indicated sample station (1 – 35) with 2 sites lacking sufficient loci. The sites are arranged according to metapopulation and then grouped by location.

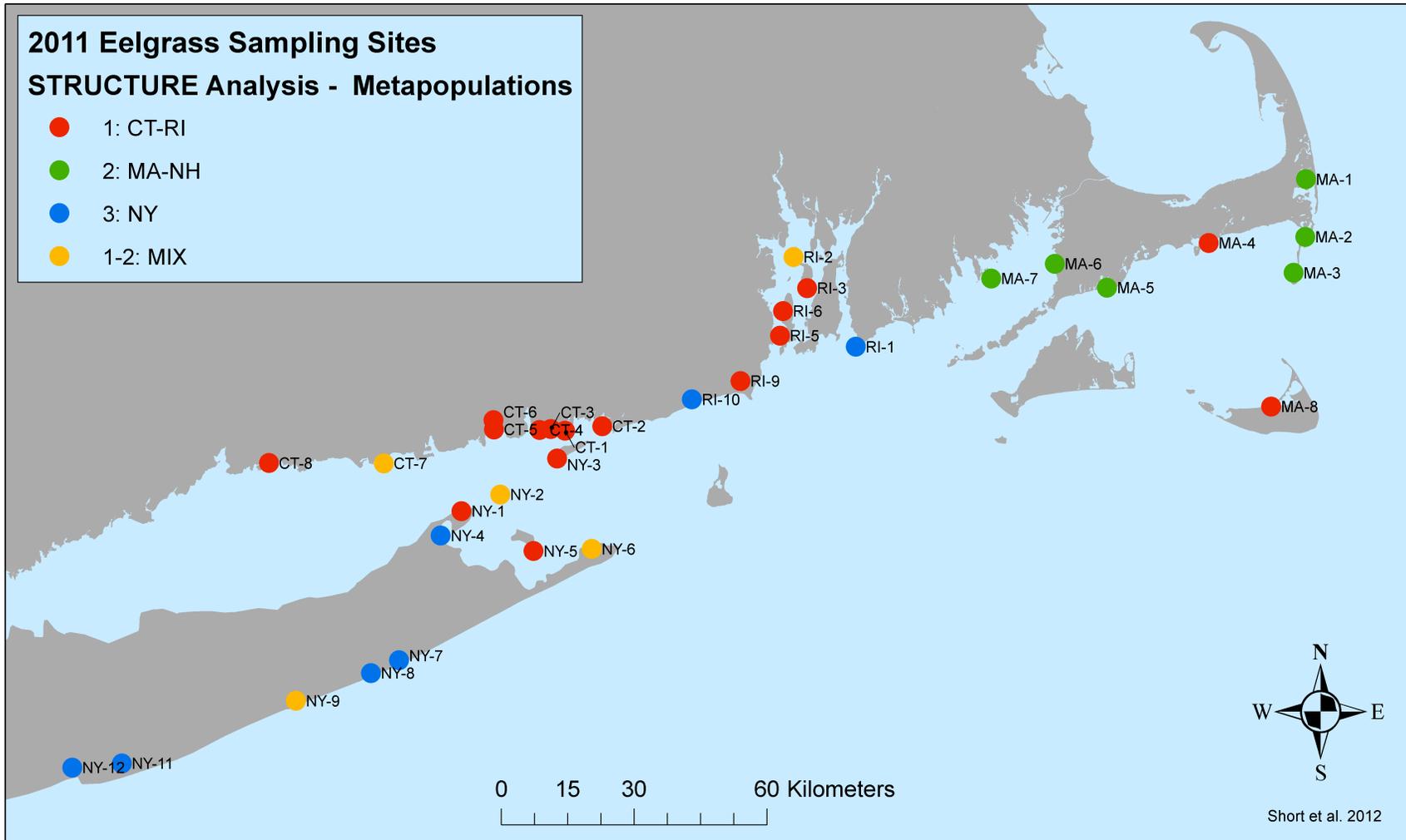


Figure 17b. STRUCTURE analysis as a basis for mapping eelgrass metapopulations in the sampling area.

The red metapopulation is predominantly from Connecticut and Rhode Island eelgrass populations, with two New York and two Massachusetts sites. The blue metapopulation is dominated by New York with two Rhode Island sites. The metapopulations are somewhat centered geographically (MA-NH, CT-RI, and NY) but overlap is evident, indicating gene flow between states.

A critical finding of our study is the identification of the three metapopulations which indicate three primary historical source populations of eelgrass in this region, one likely from the north (#2, green), a southern historic population deriving from southern Long Island (#3, blue), and the third population center (#1, red) that may have multiple sub-populations and is centrally located in Connecticut and Rhode Island. The identification of these three metapopulations and the STRUCTURE analysis allows us to identify each of the eelgrass populations sampled within groups of similar genetic structure (which we call metapopulations) providing us with insights into the historic sources of eelgrass and to some degree, the gene flow across the region. Despite the three metapopulations that we discovered dominating the broader geographic scale and the implications for significant gene flow, the genetic makeup of the eelgrass at the various sites of our study indicates that populations are genetically quite different even though there is obvious gene flow. The presence of metapopulations should not be used to infer that the region is populated by large eelgrass clones.

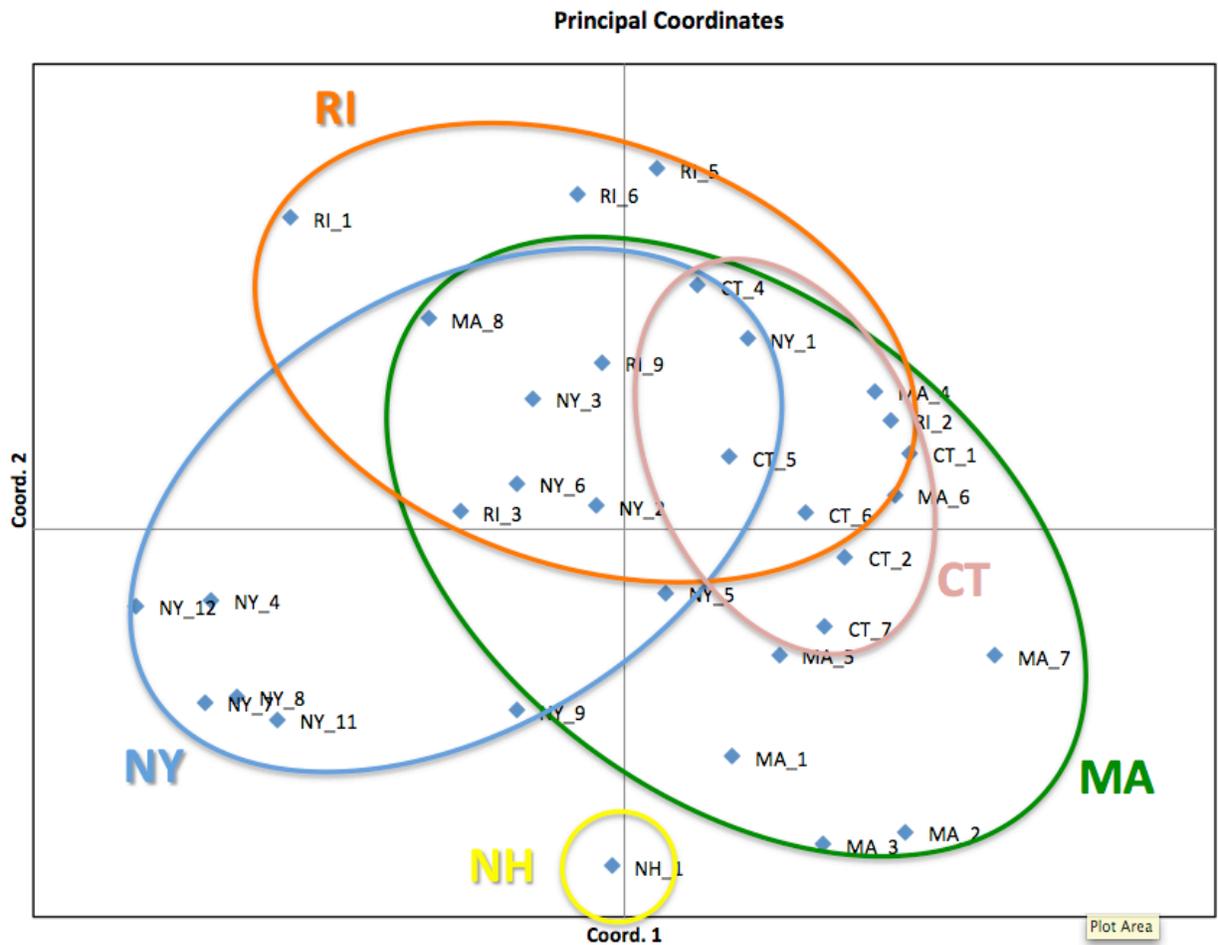


Figure 18a. Principal Coordinates Analysis (PCA) of eelgrass genetic data from across the region.

A Principal Coordinates Analysis (PCA) analysis was done to examine the genetics of eelgrass at our various sites based on the seven loci and their allelic composition. We grouped the findings by state to look at the affiliation of eelgrass genetics and found that the grouping by state shows considerable overlap except for New Hampshire. This is in contrast to the ability to distinguish the populations' home states seen in the analysis based on eelgrass plant parameters (Figure 13). The PCA indicates that genetic differences in eelgrass populations are driven by factors other than those affecting plant characteristics which showed greater separation between the states. The analysis also suggests that there is a group of sites in the upper right quadrant of the coordinate plot with populations from several states showing commonality.

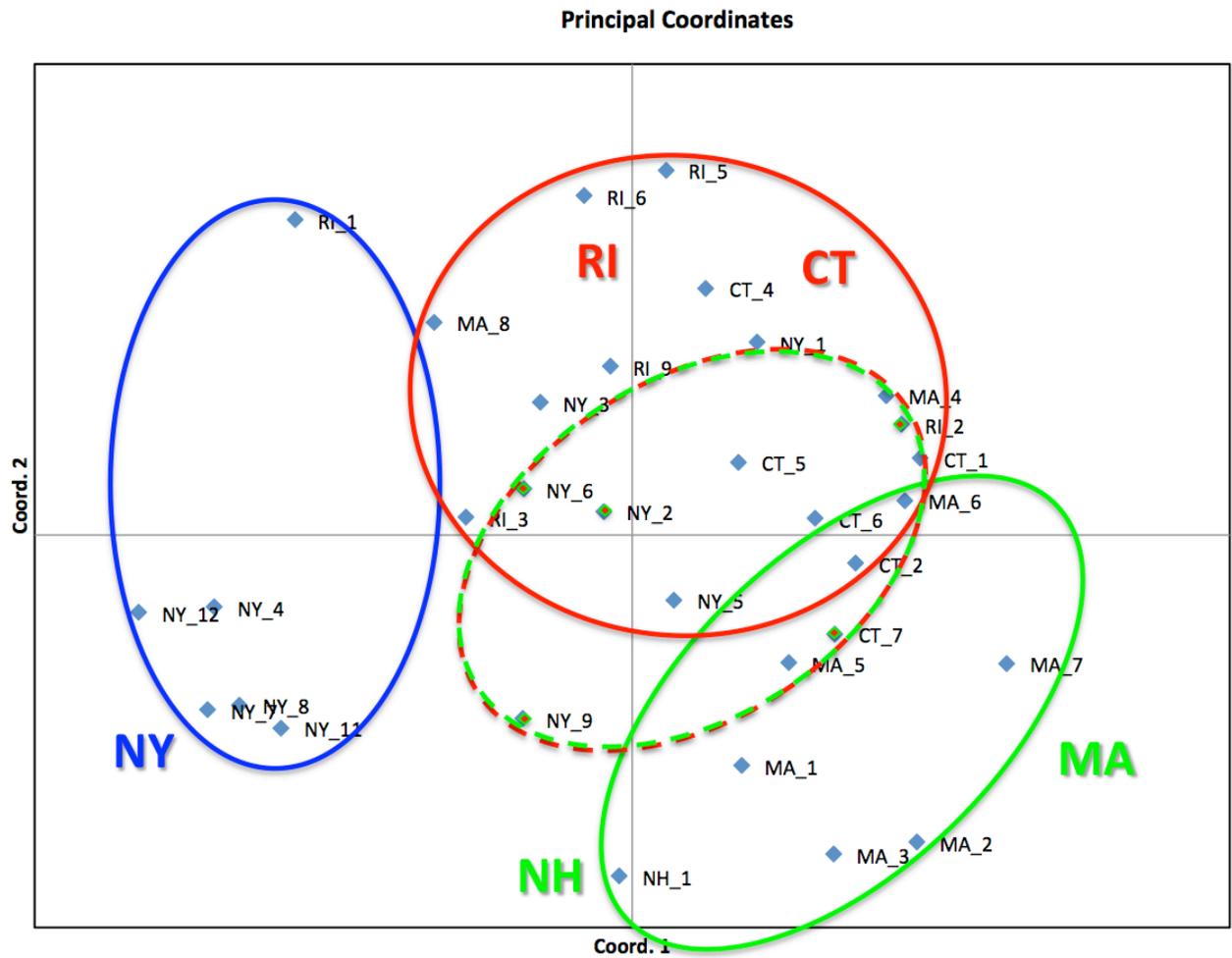


Figure 18b. Principal Coordinates Analysis of eelgrass genetic data from across the region, showing the three metapopulations depicted by the STRUCTURE analysis as well as the overlap (red-green) populations.

In another investigation of the PCA analysis, we grouped the sites by their STRUCTURE results (Figure 18b), applying the three major metapopulations and including an area of red-green overlap, with mixed population genetic structure. In general, New York, with some sites from Rhode Island and Massachusetts (blue), and Massachusetts-New Hampshire (green) show metapopulations with the most clearly defined genetic structure. The red metapopulation is a mix of sites in Connecticut and Rhode Island with some from Massachusetts and New York. These results support the interpretation of the southern metapopulation (blue) shown in the lower left quadrant, the northern metapopulation (green) shown in the lower right quadrant, and the central population (red) being less determinant but clearly in the upper half of the second coordinate. Thus, the STRUCTURE analysis (Figure 17) and the PCA (Figure 18b) support a comparable interpretation.

We investigated the clonal diversity and clonal richness of the eelgrass populations across the southern New England and New York region to develop a sense of the overall clonality in the region, otherwise known as genotypic diversity (Table 4 and Figure 19a). Of the 35 sites analyzed, 17 had repeat genets, while only 12 had repeat genets within a population, the latter indicating samples from the same clone (or genet). Seven of the 27 genets (Table 4) were detected in two (blue text) or three (red text) different sites, indicating genet transfer between location, by inter-site transfer of plants through drift, bird migration or human transplanting. The site with the lowest Clonal Richness (i.e., greatest number of clones) is RI2, North Prudence Island, RI. The majority of our sites, which were sampled with our standard protocol of 2 m between samples, showed no evidence of large clones. The exception was the North Prudence Island population, which showed 6 large clones (Table 4, top) that were greater than 2 m in diameter. This population is the northernmost eelgrass in Narragansett Bay and given the tidal circulation, would likely have little opportunity to receive pollen from other eelgrass populations. The Jamestown west (RI6)

population, in the middle of Narragansett Bay, also shows the presence of several large clones likely due to its being a fairly new population that has developed since management activities have decreased nitrogen inputs to Narragansett Bay. Eleven sites show overlapping clones, suggesting transport of material between sites by currents, boats, or birds, or possibly by human transplanting activity. Overall, the Clonal Richness for all of our eelgrass populations was high with only eight populations showing richness values less than 1 (Table 4, lower). That is, the majority of eelgrass populations across the region exhibit low inbreeding (Figure 19b) and depend on sexual reproduction for establishment and maintenance of eelgrass meadows rather than vegetative expansion alone.

Assessing eelgrass population genetics using a pairwise F_{st} test reveals many of our sampled populations across the region are significantly differentiated (Table 5). For example, the populations in Massachusetts are significantly different than those in Rhode Island or those in southern Long Island, NY. Also the highly clonal population at the North Prudence Island, RI site is significantly differentiated from its next-closest site at the south end of Prudence Island and is differentiated from the New York sites. Milford Point in eastern Long Island Sound is significantly differentiated from the sites on outer Cape Cod. Many of these differences relate to the water transport distance between sites (Table 5, lower panel). In studies of population genetics, transport distance between sites is often used to relate allelic richness to genetic flow between populations in a geographic context.

The genetics investigations revealed much new information about eelgrass across the study region. Most populations are genetically distinct, with fairly high clonal diversity (genotypic diversity) and few unique or private, alleles. There are three metapopulations in the region with geographical bases. With this information, we then analyzed the response of chosen eelgrass

populations to stressors imposed in mesocosm experiments to see if any of the eelgrass populations responded with more resilience to the commonly encountered stressors of sediment enrichment, low light and high temperature. Our investigation represents an initial survey of eelgrass genetics in southern New England and New York, but more sampling will give a more complete view.

Particularly, now that we see the metapopulation structure, further studies can be focused on specific and known sub-areas within the region. We need to determine the exact extent of the metapopulations and of the resilient populations within them, thereby refining tools for better management, protection and restoration of the eelgrass resource.

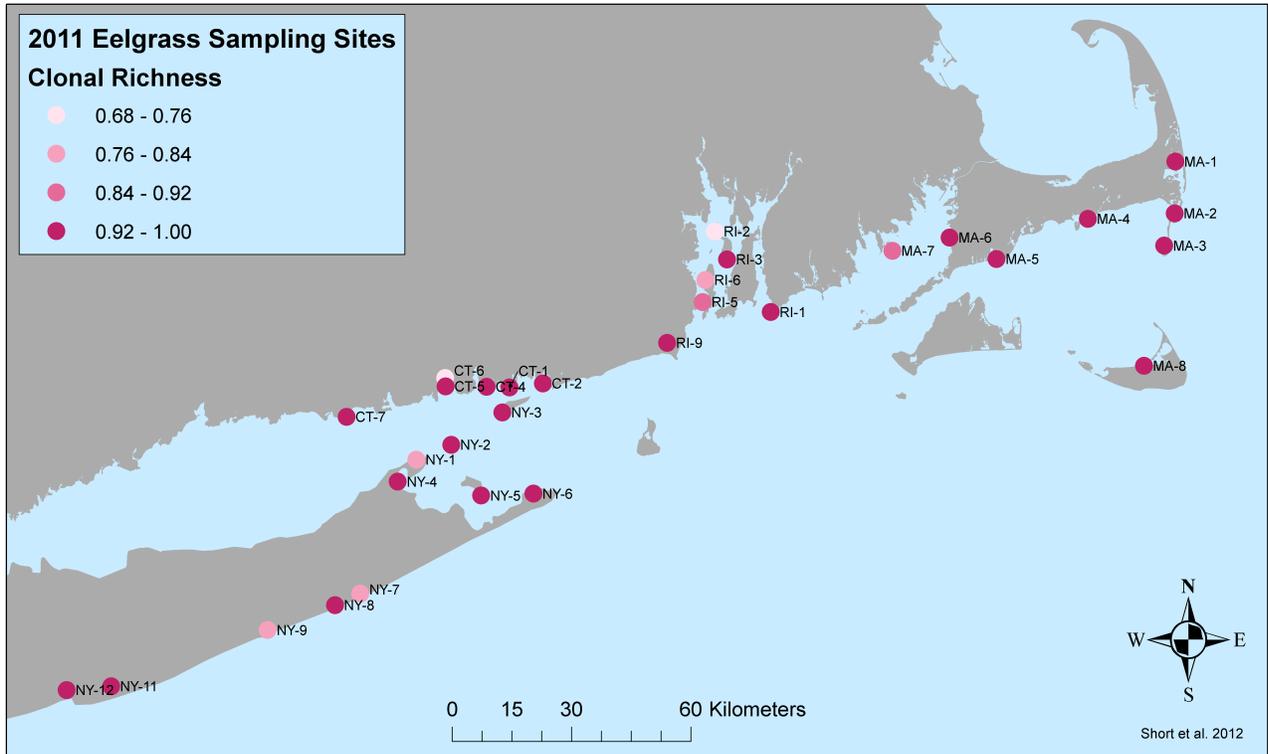


Figure 19a. Regional map of Clonal Richness based on data in Table 4.

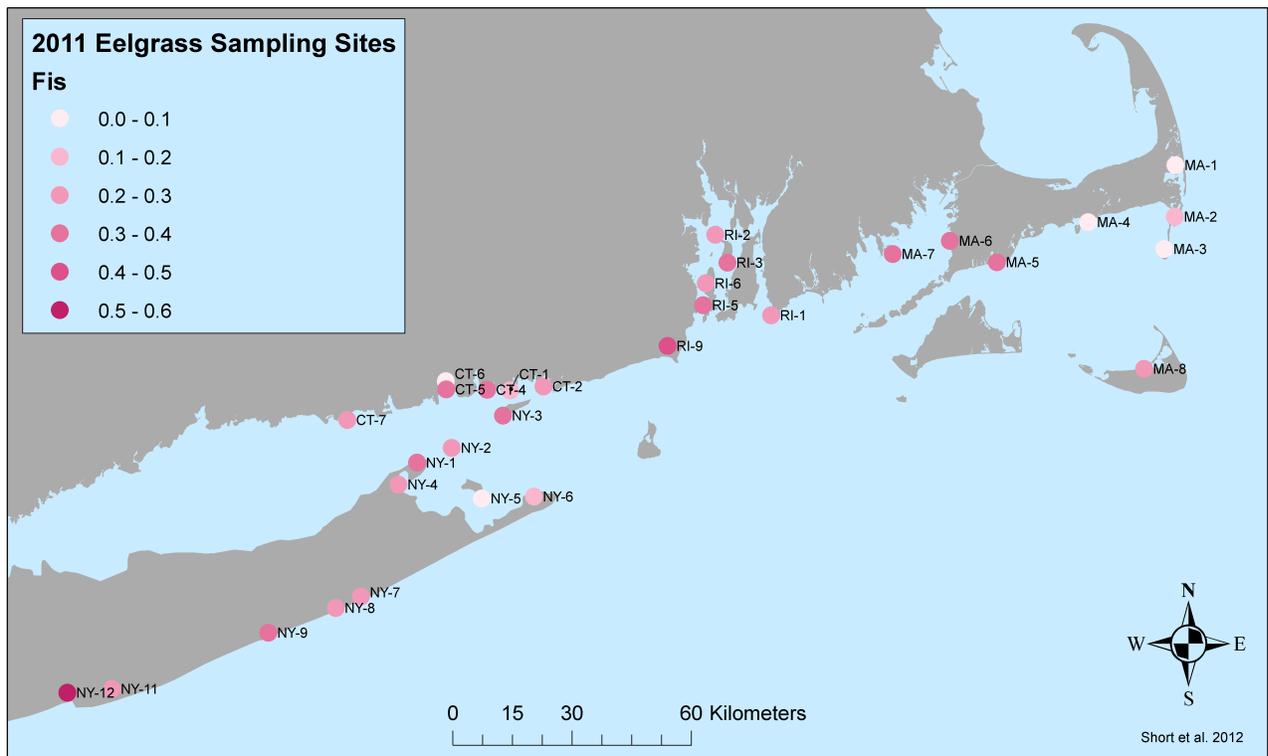


Figure 19b. Regional map of F_{is} (inbreeding) based on data in Table 3.

Table 4. The 17 eelgrass populations where repeat genets were detected (leftmost vertical column), Clonal Diversity, and Clonal Richness (R). See definitions below.

pop	N	C0 01	C0 02	C0 03	C0 04	C0 05	C0 06	C0 07	C0 08	C0 09	C0 10	C0 11	C0 12	C0 13	C0 14	C0 15	C0 16	C0 17	C0 18	C0 19	C0 20	C0 21	C0 22	C0 23	C0 24	C0 25	C0 26	C0 27	No. of different genets
CT_1	27																									1		1	
CT_6	8										2						1												2
MA_1	44																										1		1
MA_2	34															1									1		1		3
MA_3	37																										1		1
MA_4	8																								1				1
MA_6	37		2																		2					1			2
MA_7	25														2							2				1	1		3
NY_1	7													2															1
NY_3	30											2															1		2
NY_7	20					2	2																						2
NY_9	35									2	2																	3	3
RI_1	27	2																											1
RI_2	35											3							2	2		2	2	1					6
RI_5	18															1								1	1				3
RI_6	31			2	3									2															3
RI_9	32																								1				1
		1	1	1	1	1	1	1	1	1	1	1	1	1	1	2	2	1	1	1	1	1	1	2	2	3	2	3	1

Pop	Tot. No. of Repeat Genets (overall)	No. repeat genets within pop	Est. Clonal Diversity	Clonal richness R
CT_1	1	0	1.00	1.00
CT_6	3	2	0.75	0.71
MA_1	1	0	1.00	1.00
MA_2	3	2	0.94	0.94
MA_3	1	0	1.00	1.00
MA_4	1	0	1.00	1.00
MA_6	4	3	0.92	0.92
MA_7	4	3	0.88	0.88
NY_1	2	1	0.86	0.83
NY_3	3	2	0.93	0.93
NY_7	4	3	0.85	0.84
NY_9	7	6	0.83	0.82
RI_1	2	1	0.96	0.96
RI_2	12	11	0.69	0.68
RI_5	3	2	0.89	0.88
RI_6	7	6	0.81	0.80
RI_9	1	0	1.00	1.00

pop = Sample locations where repeat genets were detected.
N = sample size
C001-C027 = Genet (or Clone) ID's for all repeat genets.
No. of different genets (or Genet frequency) = the number of sample populations in which a repeat genet was detected. If genet frequency (upper table, bottom row) exceeded 1, then that genet was detected in more than one population.
Tot. No. of Repeat Genets = The number of times one of the repeat genets was detected in a population.
No. repeat genets within pop = The number of times the same genet was detected within a population, not including genets that repeated in another population.
Est. Clonal Diversity = (N - No. repeat genets within pop)/N. If Estimated Clonal Diversity = 1, then are no repeat genets within the population, but the population has an identical genet match in another population. Genets that repeated among populations are in blue text.
Clonal richness (R) = (N - No. of different genets)/N. Sites with the highest clonal richness have more genetic diversity.
Shaded rows indicate populations having the highest genet frequency, with RI 2 having 6.

Table 5. Eelgrass population genetic pairwise F_{st} matrix. Significant values are bold; (see below). Pairwise F_{st} is a measure of genetic differentiation between any two populations. Here, it is calculated over all seven loci, with permutations to estimate the significance of each measurement. * is significant at the $P < 0.05$ level. NA means the program did not calculate a P value, probably because one locus for the population was too sparse. Sewall Wright's rule of thumb is that $F_{st} < 0.05$ is not differentiated (white), $F_{st} > 0.05 < 0.15$ is moderately differentiated (pink) and $F_{st} > 0.15$ is significantly differentiated (red). Lower half of the matrix gives the distance measures between all sites based on current and flow patterns.

		Nannies Island, NH	Pleasant Bay, MA	Monomoy Island South, MA	Southway, Monomoy, MA	West Yarmouth, MA	Sage Lot Pond, MA	Falmouth Harbor, MA	West Island, MA	Nantucket Harbor, MA	Fishers Island, NY	Milford Point, LIS, NY	Shelter Island, NY	Shinnecock Bay west, NY	Shinnecock Bay east, NY	South Prudence, RI	Point Judith Pond, RI	North Prudence, RI	Jamestown West, RI	Ft. Getty, RI	Sakonnet Point, RI	Little Narragansett Bay, CT	Ram Island, CT	Avery Point, CT	Niantic Bay, CT	Niantic River, CT	Duck Island, CT	Plum Island east end, NY	Moriches Bay, NY	Star Island, NY	GSB Grass Island, NY	GSB west, NY
		NH_1	MA_1	MA_3	MA_2	MA_4	MA_5	MA_6	MA_7	MA_8	NY_3	NY_1	NY_4	NY_8	NY_7	RI_3	RI_9	RI_2	RI_6	RI_5	RI_1	CT_2	CT_1	CT_4	CT_5	CT_6	CT_7	NY_2	NY_9	NY_6	NY_11	NY_12
Nannies Island, NH	NH_1	0	0.036	0.066	0.103	0.158	0.075	0.138	0.161	0.153	0.124	0.219	0.124	0.114	0.113	0.083	0.119	0.167	0.228	0.209	0.264	0.094	0.156	0.133	0.125	0.105	0.127	0.115	0.083	0.154	0.112	0.168
Pleasant Bay, MA	MA_1	241	0	0.026	0.036	0.093	0.058	0.090	0.102	0.123	0.097	0.169	0.134	0.131	0.128	0.077	0.078	0.096	0.151	0.151	0.225	0.054	0.098	0.080	0.080	0.049	0.092	0.080	0.077	0.119	0.108	0.187
Monomoy South, MA	MA_3	263	31	0	0.020	0.141	0.046	0.083	0.071	0.174	0.125	0.194	0.179	0.197	0.189	0.122	0.133	0.148	0.213	0.219	0.279	0.083	0.113	0.135	0.134	0.104	0.104	0.109	0.144	0.142	0.166	0.225
Southway, Monomoy, MA	MA_2	272	22	9	0	0.155	0.091	0.105	0.117	0.218	0.170	0.231	0.252	0.252	0.248	0.173	0.151	0.144	0.227	0.234	0.322	0.117	0.129	0.166	0.139	0.112	0.140	0.181	0.165	0.218	0.203	0.288
West Yarmouth, MA	MA_4	303	62	31	23	0	0.085	0.070	0.126	0.101	0.120	0.247	0.224	0.252	0.234	0.118	0.054	0.063	0.119	0.073	0.220	0.090	0.105	0.015	0.102	0.084	0.140	0.107	0.157	0.184	0.220	0.339
Sage Lot Pond, MA	MA_5	314	73	42	51	26	0	0.030	0.054	0.119	0.082	0.150	0.150	0.152	0.164	0.095	0.084	0.090	0.162	0.140	0.206	0.052	0.072	0.068	0.108	0.088	0.080	0.064	0.142	0.096	0.137	0.181
Falmouth Harbor, MA	MA_6	343	102	71	80	55	29	0	0.068	0.128	0.090	0.122	0.198	0.245	0.239	0.129	0.090	0.062	0.155	0.109	0.243	0.086	0.077	0.034	0.088	0.080	0.124	0.086	0.184	0.110	0.206	0.233
West Island, MA	MA_7	358	117	86	95	70	44	15	0	0.194	0.160	0.202	0.271	0.277	0.263	0.188	0.145	0.098	0.207	0.200	0.302	0.073	0.077	0.131	0.160	0.113	0.069	0.091	0.195	0.130	0.239	0.338
Nantucket Harbor, MA	MA_8	307	66	35	40	39	46	75	126	0	0.041	0.156	0.058	0.115	0.098	0.033	0.022	0.145	0.062	0.054	0.049	0.117	0.143	0.008	0.094	0.109	0.150	0.057	0.129	0.062	0.109	0.108
Fishers Island, NY	NY_3	542	301	270	236	254	228	199	184	170	0	0.058	0.072	0.112	0.114	0.011	0.024	0.123	0.072	0.074	0.109	0.071	0.081	0.012	0.052	0.063	0.122	0.060	0.117	0.065	0.110	0.086
Milford Point, LIS, NY	NY_1	557	316	285	251	269	243	214	199	195	25	0	0.230	0.211	0.251	0.126	0.106	0.152	0.142	0.120	0.242	0.068	0.040	0.074	0.108	0.068	0.139	0.136	0.210	0.143	0.234	0.225
Shelter Island, NY	NY_4	573	332	301	268	285	259	230	215	197	31	16	0	0.071	0.046	0.045	0.104	0.242	0.175	0.184	0.134	0.178	0.231	0.112	0.158	0.173	0.216	0.093	0.130	0.102	0.057	0.022
Shinnecock Bay west, NY	NY_8	549	308	247	235	223	205	197	182	217	94	104	106	0	0	0.076	0.107	0.230	0.178	0.192	0.161	0.140	0.193	0.144	0.158	0.133	0.148	0.121	0.069	0.133	0.002	0.066
Shinnecock Bay, NY	NY_7	542	301	240	228	216	198	190	175	210	87	97	99	7	0	0.074	0.112	0.234	0.177	0.203	0.145	0.176	0.226	0.143	0.161	0.146	0.168	0.103	0.059	0.100	0.004	0.071
South Prudence, RI	RI_3	423	182	151	118	135	109	80	65	132	106	121	137	167	160	0	0.014	0.156	0.110	0.104	0.130	0.070	0.112	0.016	0.042	0.064	0.122	0.076	0.070	0.099	0.093	0.075
Point Judith Pond, RI	RI_9	475	234	202	169	186	160	131	117	113	67	82	98	128	121	39	0	0.087	0.049	0.037	0.102	0.047	0.068	-0.033	0.002	0.027	0.091	0.067	0.086	0.094	0.112	0.128
North Prudence, RI	RI_2	435	194	162	129	146	120	91	77	143	118	133	149	178	171	11	50	0	0.127	0.058	0.239	0.096	0.070	0.060	0.087	0.040	0.111	0.098	0.136	0.116	0.181	0.292
Jamestown West, RI	RI_6	446	205	174	141	158	132	103	88	142	96	111	127	156	149	10	29	22	0	0.051	0.067	0.125	0.114	0.026	0.119	0.110	0.163	0.107	0.169	0.132	0.162	0.218
Ft. Getty, RI	RI_5	440	199	168	134	152	126	97	82	128	90	105	121	150	143	17	22	28	6.2	0	0.117	0.122	0.099	0.004	0.091	0.080	0.163	0.087	0.159	0.130	0.179	0.218
Sakonnet Point, RI	RI_1	393	152	121	88	105	79	50	35	102	101	116	132	147	140	30	33	41	40	26	0	0.229	0.230	0.124	0.222	0.227	0.245	0.145	0.227	0.127	0.159	0.166
Little Nar. Bay, CT	CT_2	513	272	240	207	224	198	169	155	151	29	44	60	123	116	77	38	88	67	60	71	0	-0.004	0.019	0.043	-0.002	0.015	0.064	0.107	0.118	0.169	0.229
Ram Island, CT	CT_1	525	284	252	219	236	210	181	167	163	17	32	48	111	104	89	50	100	79	72	83	12	0	0.044	0.061	0.016	0.037	0.098	0.144	0.147	0.198	0.252
Avery Point, CT	CT_4	531	290	259	226	243	217	188	173	169	10	25	42	104	97	96	57	107	85	79	90	19	7	0	-0.015	-0.002	0.081	0.041	0.105	0.077	0.146	0.164
Niantic Bay, CT	CT_5	546	305	274	241	258	232	203	188	184	17	20	31	111	104	111	72	122	100	94	105	34	22	15	0	-0.020	0.081	0.122	0.076	0.140	0.149	0.183
Niantic River, CT	CT_6	548	307	276	243	260	234	205	190	186	19	22	33	113	106	113	74	124	102	96	107	36	24	17	2	0	0.020	0.078	0.043	0.106	0.131	0.220
Duck Island, CT	CT_7	559	318	287	254	271	245	216	201	197	39	21	36	133	126	123	84	135	113	107	118	46	34	28	13	15	0	0.107	0.105	0.118	0.157	0.262
Plum Island east end, NY	NY_2	557	316	285	252	269	243	214	199	181	15	10	16	96	89	121	82	133	111	105	116	44	32	26	15	17	27	0	0.104	0.024	0.105	0.148
Moriches Bay, NY	NY_9	567	326	265	253	241	223	215	200	235	112	122	124	18	25	185	146	196	174	168	165	141	129	122	129	131	151	114	0	0.143	0.050	0.183
Star Island, NY	NY_6	477	236	175	163	151	133	125	110	165	22	32	34	72	65	94	55	106	84	78	75	51	39	32	39	41	61	24	90	0	0.113	0.144
GSB Grass Island, NY	NY_11	609	368	307	295	283	265	257	242	277	154	164	166	60	67	226	187	238	216	210	207	183	171	164	171	173	193	156	42	132	0	0.073
GSB west, NY	NY_12	620	379	318	306	294	276	268	253	288	165	175	177	71	78	238	199	249	227	221	218	194	182	175	182	184	204	167	53	143	11	0

Mesocosm Stressor Studies

UNH Eelgrass Mesocosm Results

Eelgrass Survival

Ramet survival was higher in Low Organic Matter (LOM) compared with High Organic Matter (HOM) treatments (81% vs. 42%; $p < 0.0001$). Survival also varied among populations ($p < 0.0001$), but not always similarly within different sediment treatments (sediment x population $p < 0.0001$). Overall, Prudence Island had the highest survival rate (79%), although it was not significantly different from plants from Great South Bay, Nannies Island, Southway, Ninigret Pond or Shelter Island (in descending order of percent ramet survival). Ram Island had the lowest overall survival (27%). Within the HOM treatment, Prudence and Great South Bay had the highest survival (Tukey's test $p < 0.05$; 70% and 66% respectively). Survival was slightly lower in reduced light ($p = 0.0421$), but showed no higher order interactions with sediment or population (Fig 20).

Eelgrass productivity and morphology

Shoot productivity (number shoots produce day⁻¹ sub-plot⁻¹) between the initiation of the light treatment and end of the study, was higher in LOM ($p = 0.0003$) and full light ($p = 0.0033$) (Figure 21a) and varied among populations (Figure 21 (b) shoot density by week, (a) factorial treatment and population) with a significant interactive effect of sediment x population ($p < 0.0001$). Shoot productivity was highest overall and in HOM in Great South Bay (1.76 overall and 1.13 HOM shoots⁻¹ day sub-plot⁻¹) and lowest overall in Ram Island, which was not significantly different from West Island (-0.12 and 0.19 shoots⁻¹ day sub-plot⁻¹) (Tukey's test $p < 0.05$). Nannies and Prudence had the second highest shoot productive rate over all in HOM (1.38 and 1.13 overall; 1.07 and 1.04 HOM shoots⁻¹ day sub-plot⁻¹) and in HOM.

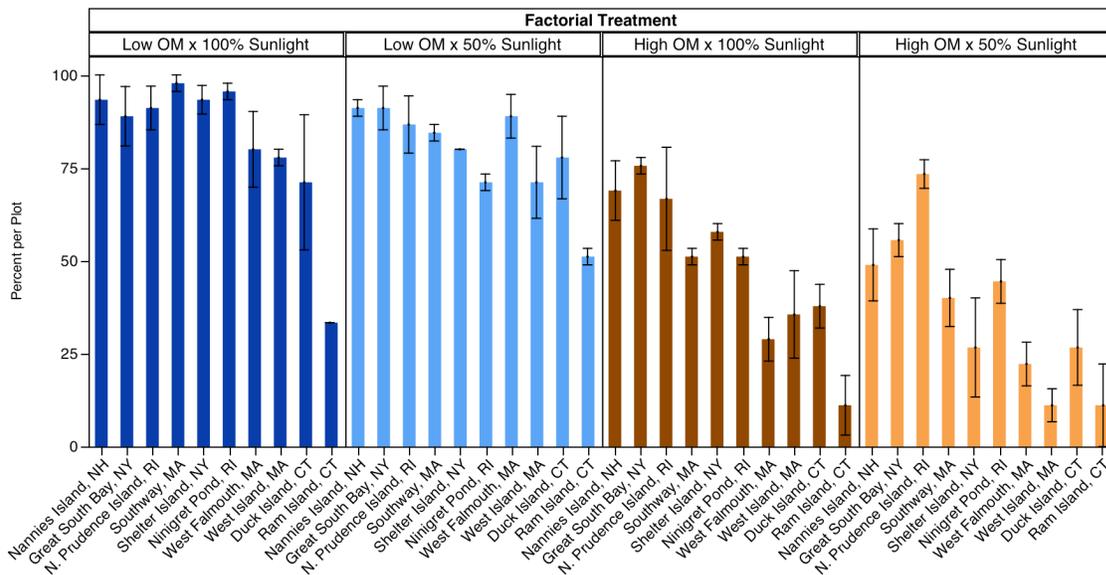
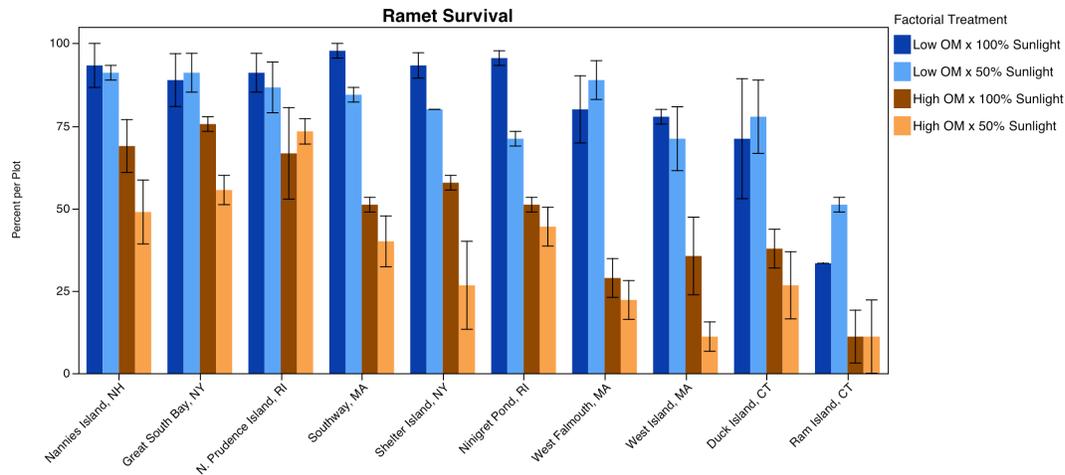


Figure 20. Ramet (eelgrass plant with attached shoots, roots, and rhizome) survival (percent of initial number of ramets planted still living plot⁻¹) among *Z. marina* from ten genetically differentiated populations following a 13-week common-garden mesocosm experiment in which sediment organic matter content and sunlight were manipulated. Means plus or minus standard error for populations within an environmental treatment (n = 3). Plot size = 0.125 m². Results of eelgrass ramet survival for the light and organic matter treatment factorial are shown. The top panel shows treatments grouped by population; the lower panel shows the same data grouped by treatment.

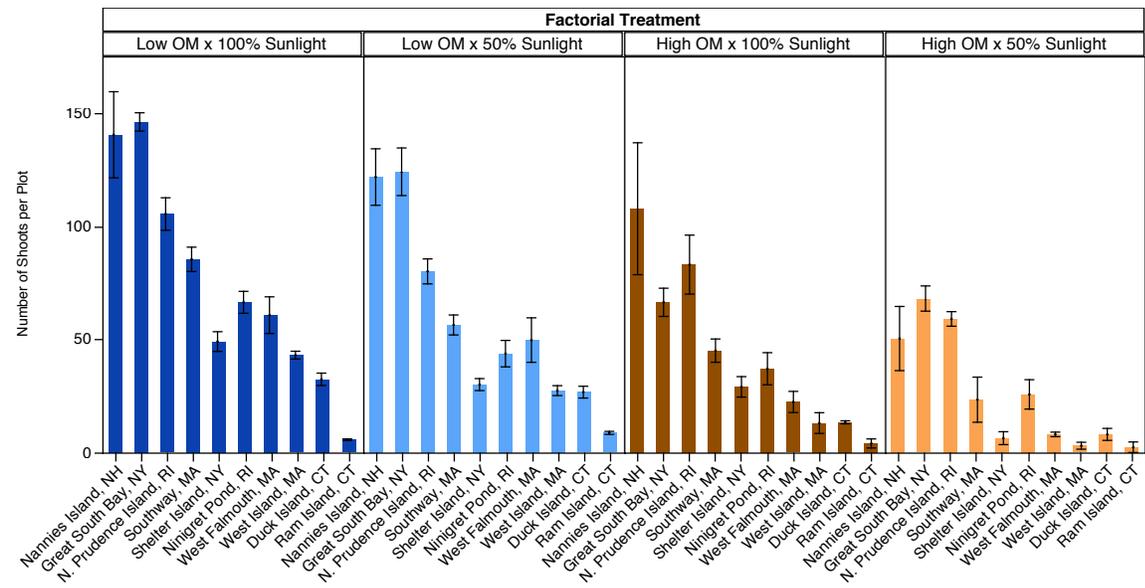
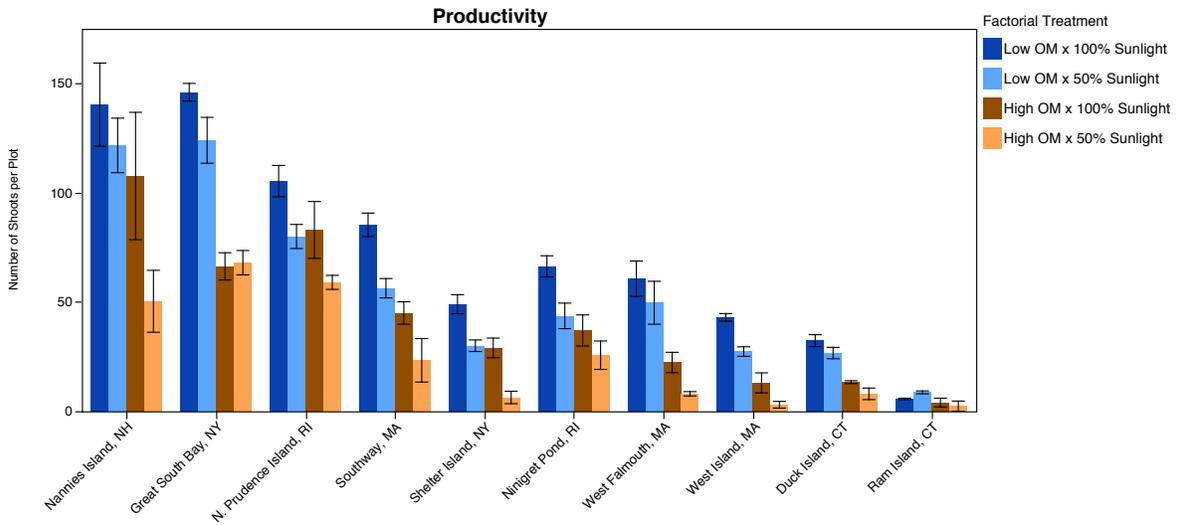


Figure 21a: Productivity (shoots produced day⁻¹ plot⁻¹) variation among *Z. marina* from ten genetically differentiated populations following a 13-week common-garden mesocosm experiment in which sediment organic matter content and sunlight were manipulated. Means plus or minus standard error for populations within an environmental treatment (n = 3). Plot size = 0.125 m². Results of productivity for the light and organic matter treatment factorial are shown. The top panel shows treatments grouped by population; the lower panel shows the same data grouped by treatment.

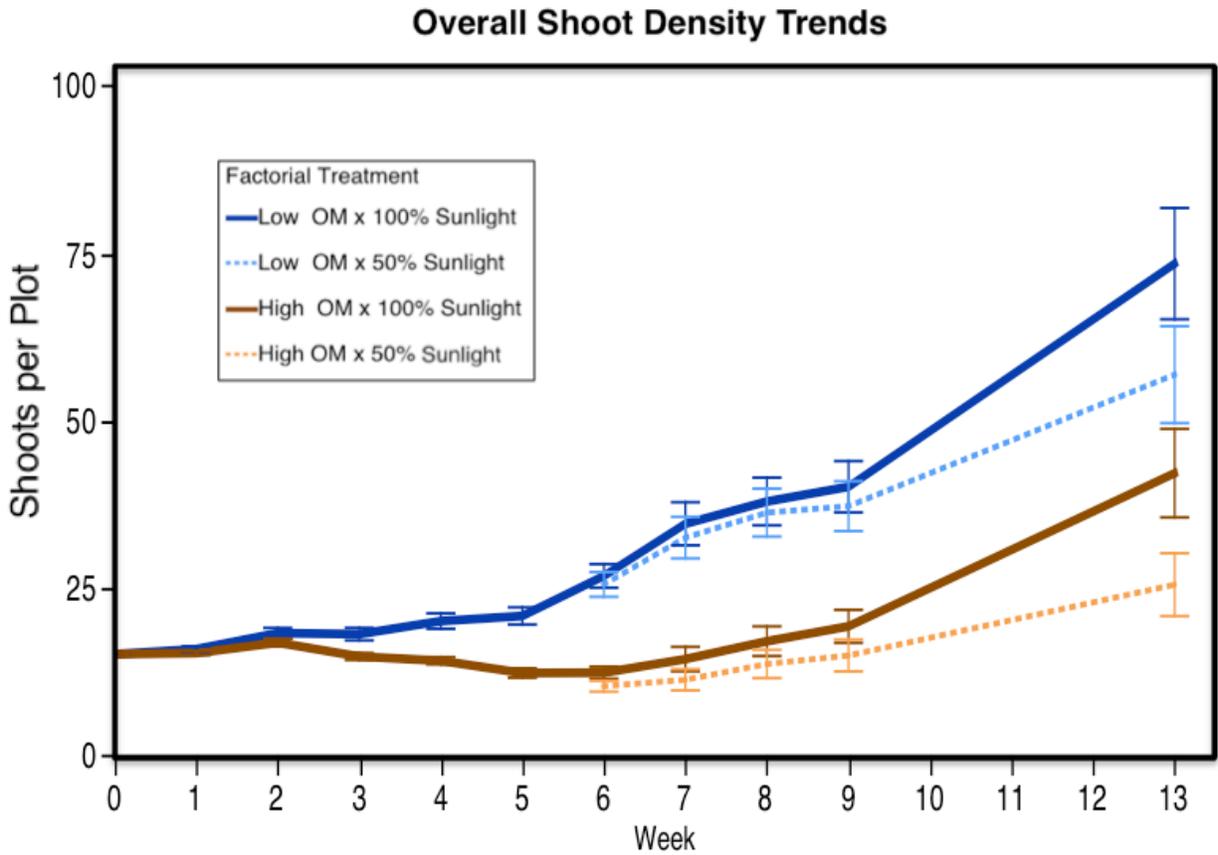


Figure 21b. Overall combined eelgrass shoot density from all 10 populations studied, measured per plot in the UNH mesocosm experiments over 13 weeks, showing the effects of reduced light (solid vs. dotted lines) and elevated sediment organic matter (low organic matter (OM) with blue lines, high organic matter with brown lines). Eelgrass growing in these experiments had relatively high water column nitrogen ($\sim 20\mu\text{M}$) resulting from the water source in the Great Bay Estuary. Eelgrass growing in the Low OM sediments had greater shoot production and higher survival rates than eelgrass in the High OM sediments which were stressed by eutrophic conditions. By week 13, the eelgrass growing in 100% light had significantly higher shoot production than that growing at 50% light in both the Low and High OM sediment treatments.

Shoot Density Trends

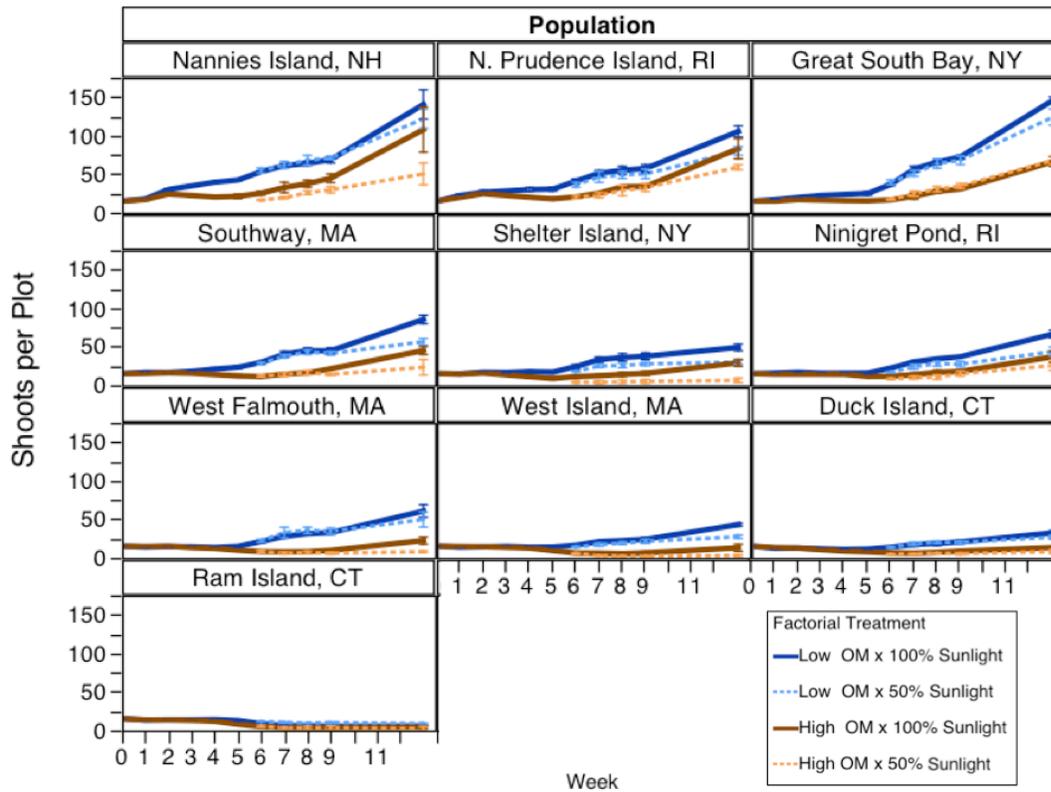


Figure 21c. Eelgrass shoot density for the 10 populations studied over 13 weeks, showing the effects of reduced light (solid vs. dotted lines) and elevated sediment organic matter (low organic matter (OM) with blue lines, high organic matter with brown lines). Eelgrass populations growing in these experiments had relatively high water column nitrogen (~20 μM) resulting from the water source in the Great Bay Estuary. Three eelgrass source populations (Nannies Island, NH; North Prudence Island, RI; and Great South Bay, NY) showed significantly greater shoot density than the three poorest performing sites (West Island, MA; Duck Island, CT; and Ram Island, CT). The results indicate that some plant populations outperform others either because of genetic differences or phenotype differences in the populations.

Rhizome plastochrone interval (nodes produced $\text{day}^{-1} \text{ramet}^{-1}$) varied among populations ($p < 0.0001$), and between sediment ($p = 0.0014$) and light ($p = 0.0005$) treatments with more nodes produced in LOM and full light. There was no significant interaction among any of the treatments. Rhizome elongation (cm produced $\text{day}^{-1} \text{ramet}^{-1}$) also varied among populations ($p < 0.0001$) and was more significantly affected by sediment and light ($p < 0.0001$). Rhizomes were longer in LOM and full light. Rhizome length also varied among populations between sediment treatments (sediment x population ($p < 0.0001$)) (Figure 22). Overall, rhizome plastochrone interval and elongation rates were greatest in Nannies Island (Tukey's test $p < 0.05$). Prudence Island had the second highest rhizome elongation rate overall, although plastochrone interval was not significantly different from Great South Bay, Ninigret or Southway.

Aboveground morphology including number of shoots per ramet (Figure 23), shoot density per plot (Figure 24), leaf length (Figure 25), width (Figure 26) and number of rhizome nodes produced ramet^{-1} (Figure 27), varied among environmental treatments and populations. Number of shoots per ramet was lower in reduced light ($p = 0.006$) and varied among populations ($p < 0.0001$) but sediment did not have an effect. Shoot density was higher in LOW (65.1 LOM, 33.7 HOM shoots sub-plot^{-1}), full light (57.7 full light, 41.1 50% light shoots sub-plot^{-1}) and varied among populations ($p < 0.0001$). Overall Nannies Island and Great South Bay had the highest shoot density (105.0 and 101.1 shoots sub-plot^{-1} , respectively) (Tukey's test $p < 0.05$). In HOM, Nannies, Prudence and Great South Bay had the highest shoot density (79.0, 71.0 and 67.1 shoots sub-plot^{-1} , respectively) (Tukey's test $p < 0.05$). Leaf lengths were shorter in HOM ($p < 0.0001$), longer in reduced light ($p = 0.0004$), varied among environmental treatments (sediment x light $p = 0.0060$) and populations ($p < 0.0001$) and among populations and between sediment treatments (sediment x population $p = 0.0167$). Leaves were wider in LOM and full light ($p < 0.0001$, $p = 0.0071$,

respectively) and varied among populations ($p < 0.0001$) and among populations between light treatments. Mean leaf area ($\text{cm}^2 \text{shoot}^{-1}$) was greater in LOM ($p = 0.0005$) and varied among populations ($p < 0.0001$). Overall, Duck Island plants had the highest leaf area ($53.12 \text{ cm}^2 \text{shoot}^{-1}$), but were not significantly different from Prudence, Ninigret Pond, Ram Island, or Nannies Island ($46.2, 46.1, 44.1$ and $40.0 \text{ cm}^2 \text{shoot}^{-1}$, respectively). Great South Bay plant had the lowest leaf area ($23.4 \text{ cm}^2 \text{shoot}^{-1}$), but was not significantly different from Southway, Shelter Island, West Island or West Falmouth Harbor ($34.7, 32.3, 31.7,$ and $27.3 \text{ cm}^2 \text{shoot}^{-1}$, respectively) (Tukey's test $p < 0.05$).

Belowground morphology varied significantly among treatments and populations; in particular, rhizome length, number of internodes and mean internode length were greater in LOM ($p < 0.0001, = 0.0018,$ and $< 0.0001,$ respectively), full light ($p < 0.0001$) and varied among populations. Rhizome and mean internode length varied among population between sediment treatments ($p < 0.0001$ and $p = 0.0019$). A three-way interaction effect was detected for number of internodes per ramet ($p = 0.0382$). Overall, Nannies Island had the longest rhizomes ($46.6 \text{ cm ramet}^{-1}$) followed by Prudence ($30.7 \text{ cm ramet}^{-1}$) (Tukey's test $p < 0.05$); both of these populations had the longest rhizome length in HOM (34.9 and $28.8 \text{ cm ramet}^{-1}$, respectively). Nannies had the longest rhizome length in the 50% light treatments ($40.4 \text{ cm ramet}^{-1}$) followed by Prudence, Great South Bay and Ninigret Pond ($28.8, 20.4$ and $17.7 \text{ cm ramet}^{-1}$) (Tukey' test $p < 0.05$).

Estimated total leaf area ($\text{m}^2 \text{plot}^{-1}$), rhizome length (m plot^{-1}) and number of rhizome nodes per plot varied among populations and sediment and light treatments. Total leaf area was highest in LOM treatments ($p < 0.0001$), full light ($p = 0.0026$) and varied among populations ($p < 0.001$) and among populations between sediment and light treatments (sediment x population $p = 0.0001$; light x population $p = 0.0308$). Overall, Nannies and Prudence Island had the greatest leaf area in both sediment treatments and light treatments. Total rhizome length was highest in LOM and full light (p

<0.0001) and varied among populations ($p < 0.0001$) and among populations between sediment and light treatments (sediment x population $p < 0.0001$; light x population $p = 0.0422$). Nannies and Prudence Island had the highest total rhizome length overall and in the HOM treatment. Total number of rhizome nodes was higher in LOM ($p < 0.0001$), full light ($p = 0.0014$) and varied among populations ($p < 0.0001$) and among populations and between sediment and light treatments (sediment x population $p = 0.0029$; light x population $p = 0.0310$); again with Nannies and Prudence having the greatest number overall and within the HOM treatment.

Leaf absorbance and ETR

The fraction of incident PAR absorbed by leaves (AF) varied among populations and sediment treatments (Figure 28). AF was highest in HOM ($p = 0.0001$) and ranged from 0.70 (Nannies Island) to 0.81 (West Falmouth Harbor). Electron transport rates (ETR) varied among populations and environmental treatments (Figure 29). ETRs were significantly different in each environmental treatment (Tukey's test $p < 0.05$). ETR was highest in full light x LOM, lower in 50% light x LOW, even lower in full light x HOM and lowest in 50% light x HOM.

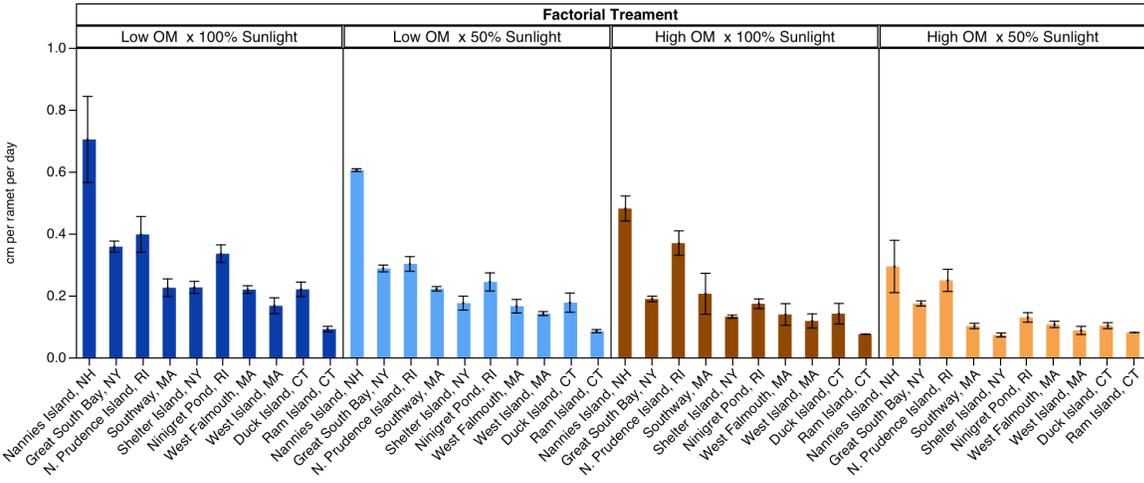
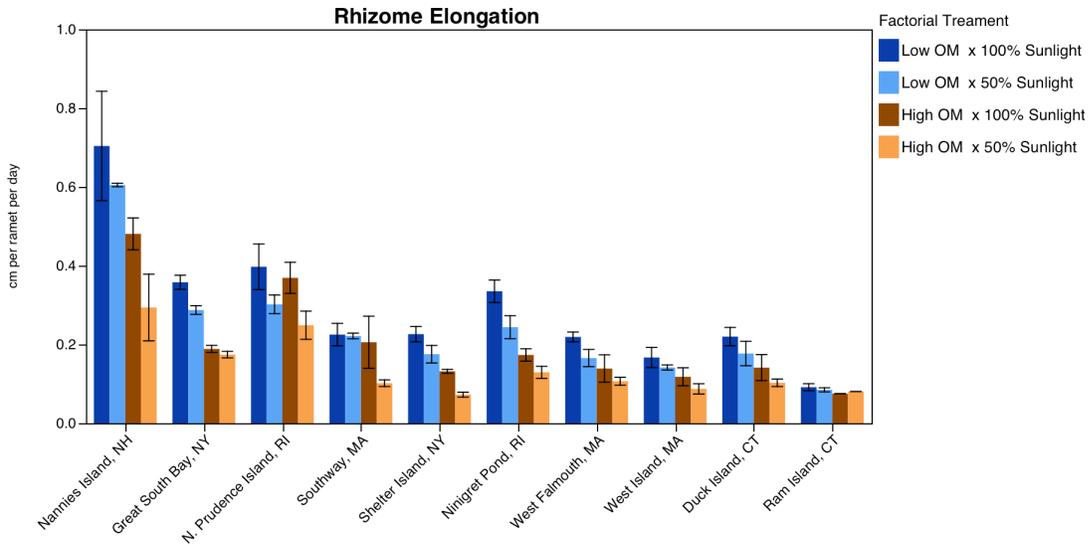


Figure 22: Rhizome elongation rate (cm day⁻¹) among *Z. marina* from ten genetically differentiated populations following a 13-week common-garden mesocosm experiment in which sediment organic matter content and sunlight were manipulated. Means plus or minus standard error for populations within an environmental treatment (n = 3). Plot size = 0.125 m². Results of eelgrass rhizome elongation rate for the light and organic matter treatment factorial are shown. The top panel shows treatments grouped by population; the lower panel shows the same data grouped by treatment.

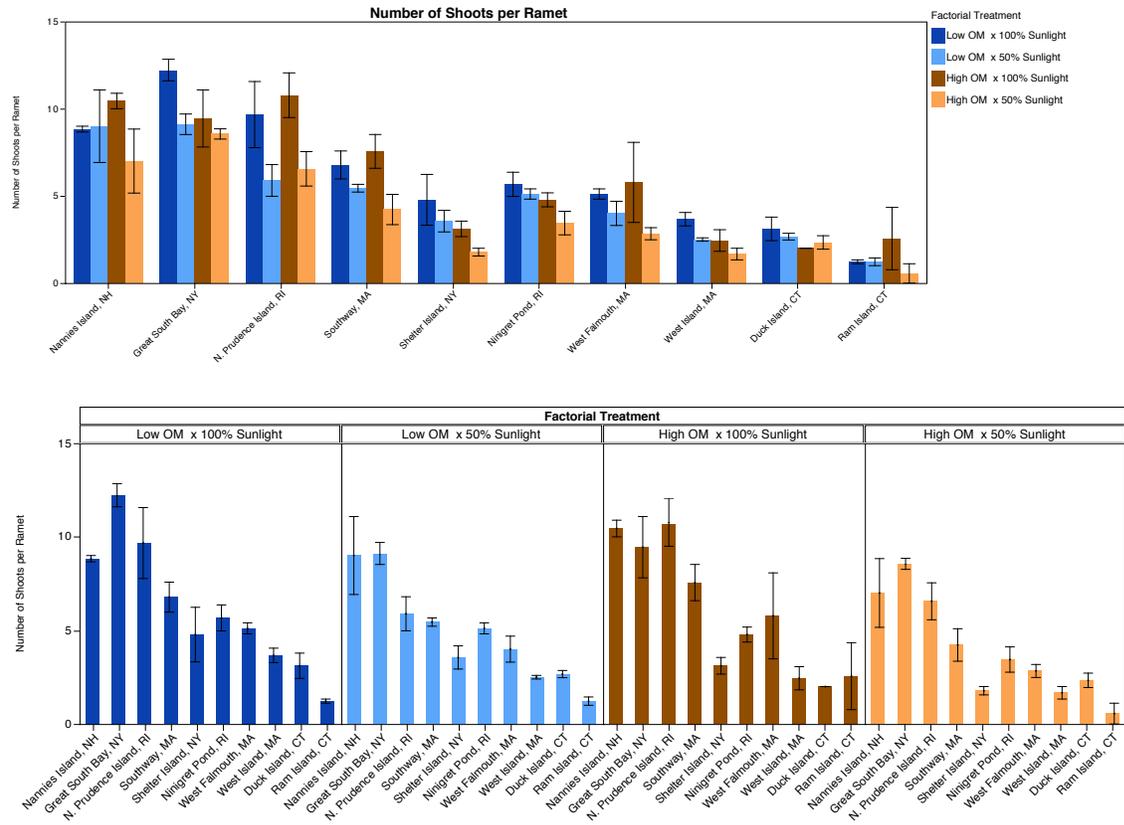


Figure 23: Total number of shoots (terminal and lateral) ramet⁻¹ among *Z. marina* from ten genetically differentiated populations following a 13-week common-garden mesocosm experiment in which sediment organic matter content and sunlight were manipulated. Means plus or minus standard error for populations within an environmental treatment (n = 3). Plot size = 0.125 m². Results of total number of shoots for the light and organic matter treatment factorial are shown. The top panel shows treatments grouped by population; the lower panel shows the same data grouped by treatment.

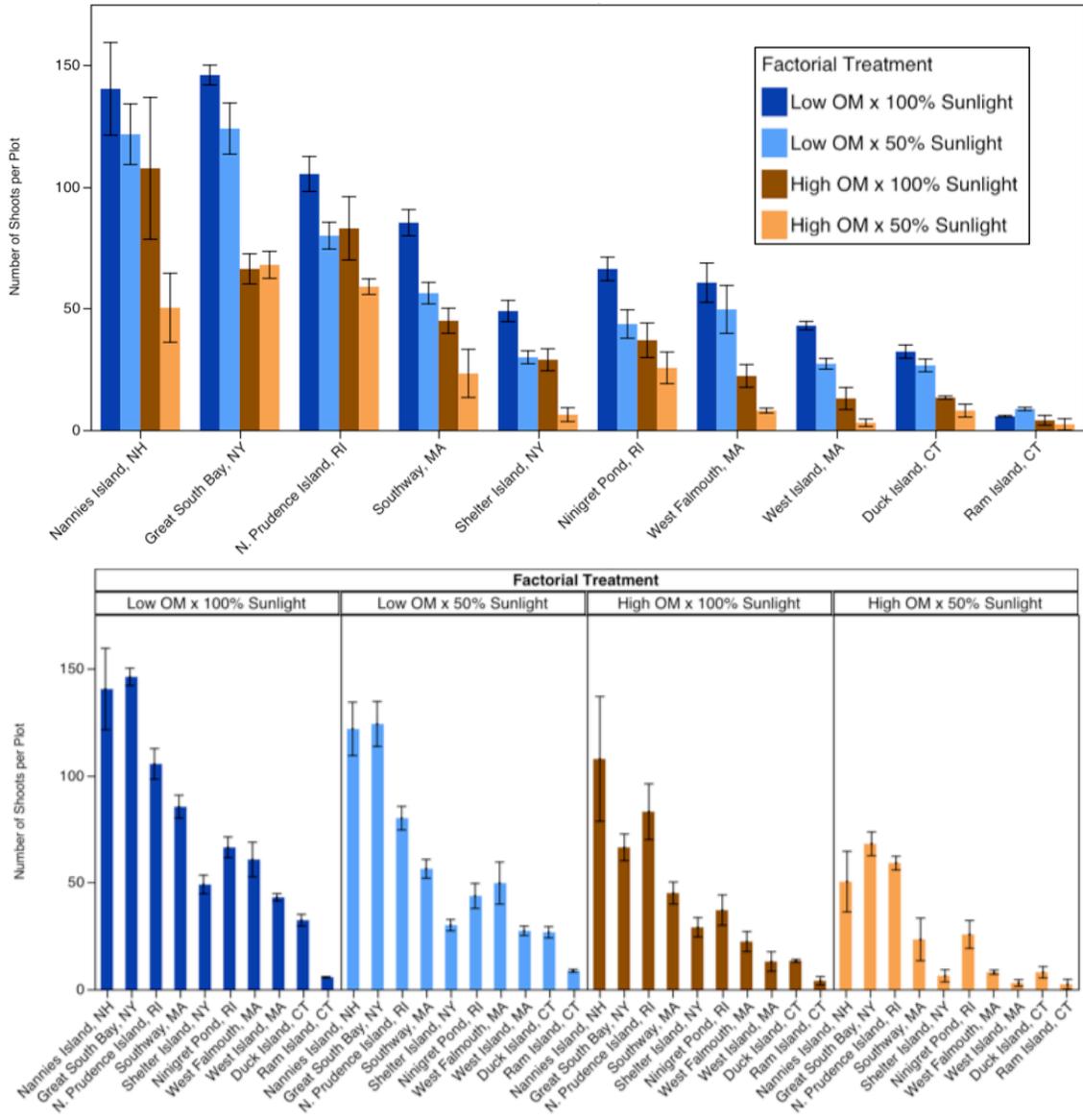


Figure 24: Shoot density (number of eelgrass shoots plot⁻¹) among *Z. marina* from ten genetically differentiated populations following a 13-week common-garden mesocosm experiment in which sediment organic matter content and sunlight were manipulated. Means plus or minus standard error for populations within an environmental treatment (n = 3). Plot size = 0.125 m². Results of eelgrass density for the light and organic matter treatment factorial are shown. The top panel shows treatments grouped by population; the lower panel shows the same data grouped by treatment.

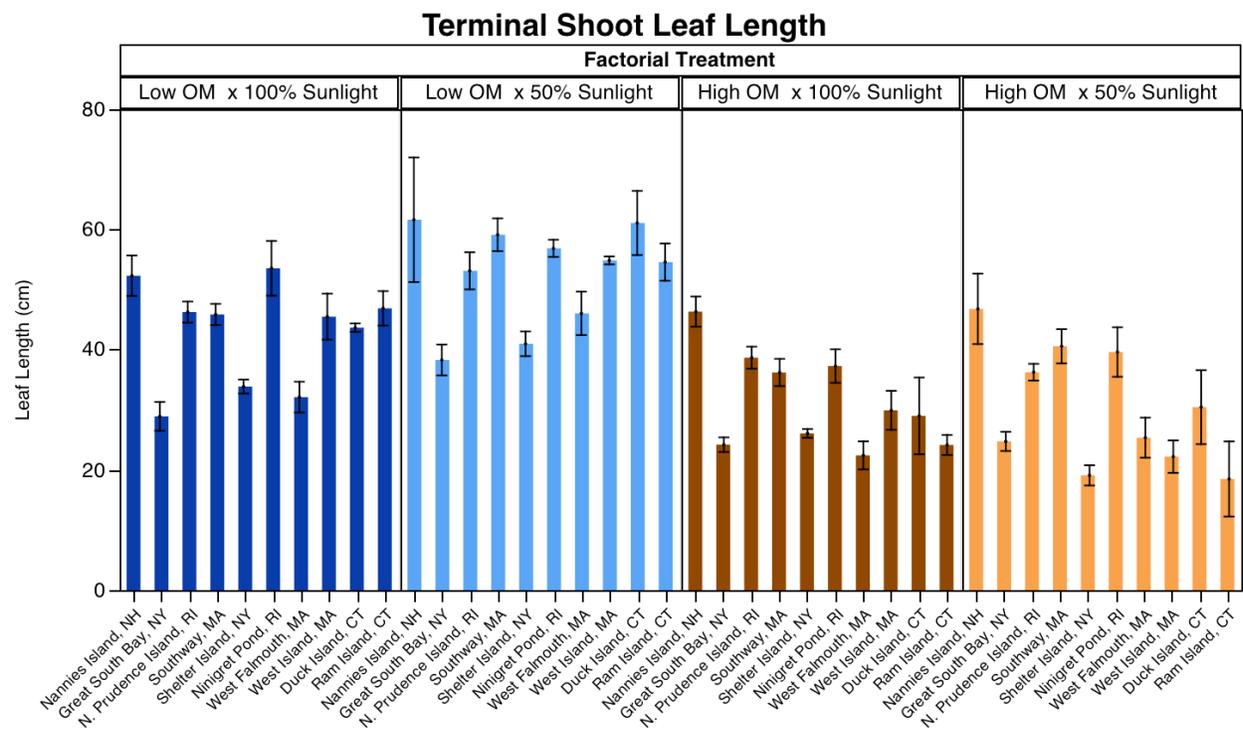
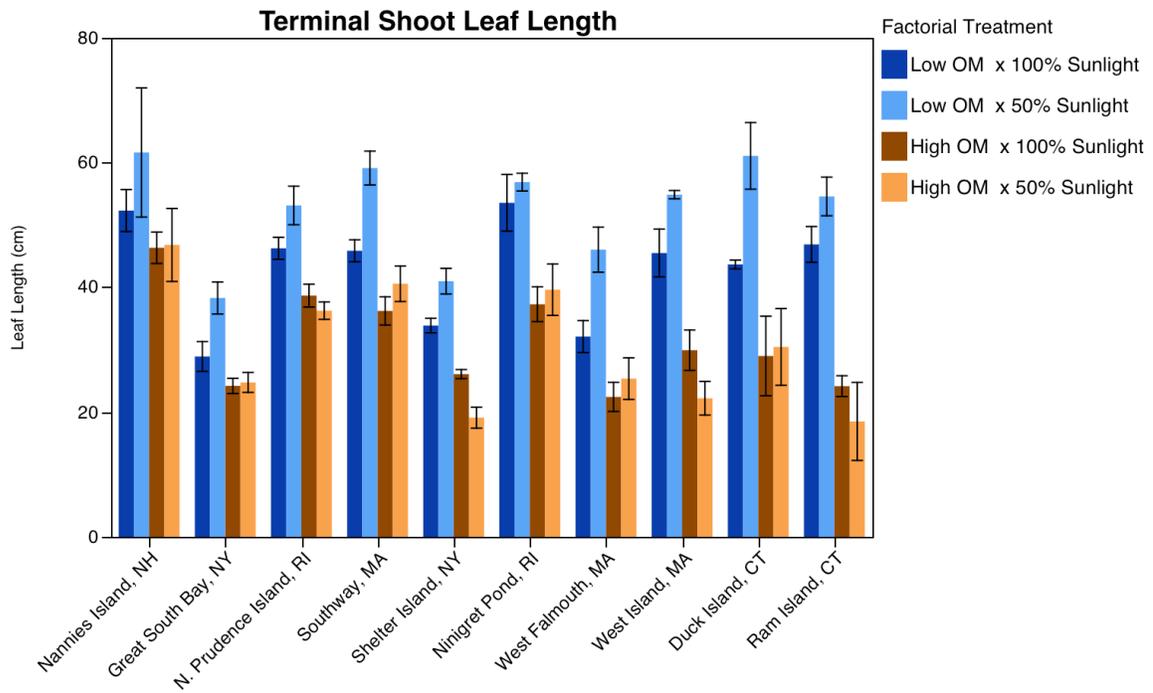


Figure 25. Leaf length (cm) of terminal shoots among *Z. marina* from ten genetically differentiated populations following a 13-week common-garden mesocosm experiment in which sediment organic matter content and sunlight were manipulated. Means plus or minus standard error for populations within an environmental treatment (n = 3). Plot size = 0.125 m². Results of leaf length for the light and organic matter treatment factorial are shown. The top panel shows treatments grouped by population; the lower panel shows the same data grouped by treatment.

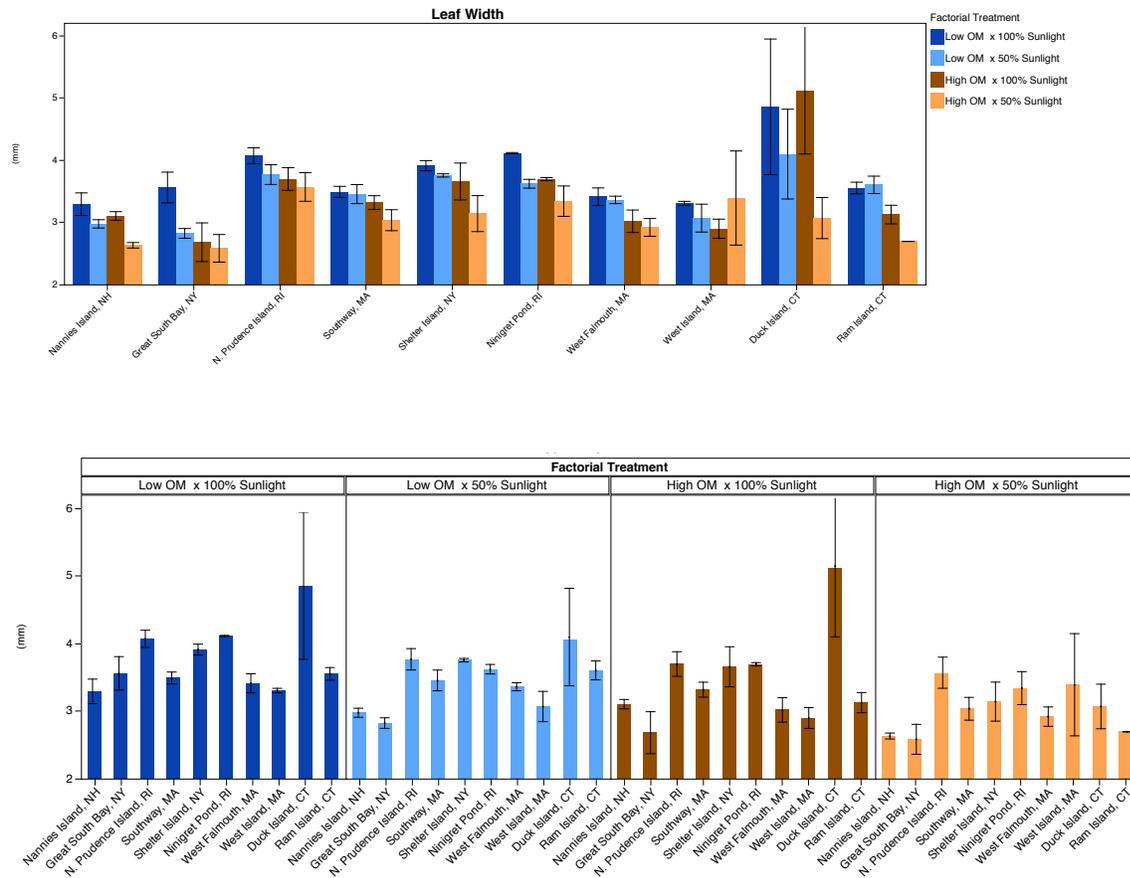


Figure 26. Leaf width (mm) variation among *Z. marina* from ten genetically differentiated populations following a 13-week common-garden mesocosm experiment in which sediment organic matter content and sunlight were manipulated. Means plus or minus standard error for populations within an environmental treatment (n = 3). Plot size = 0.125 m². Results of leaf width for the light and organic matter treatment factorial are shown. The top panel shows treatments grouped by population; the lower panel shows the same data grouped by treatment.

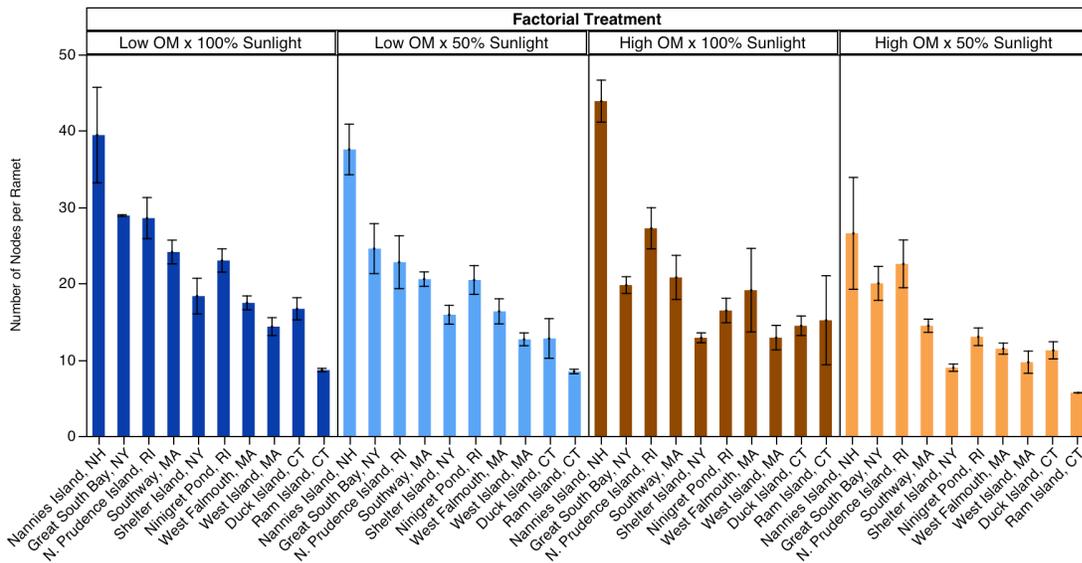
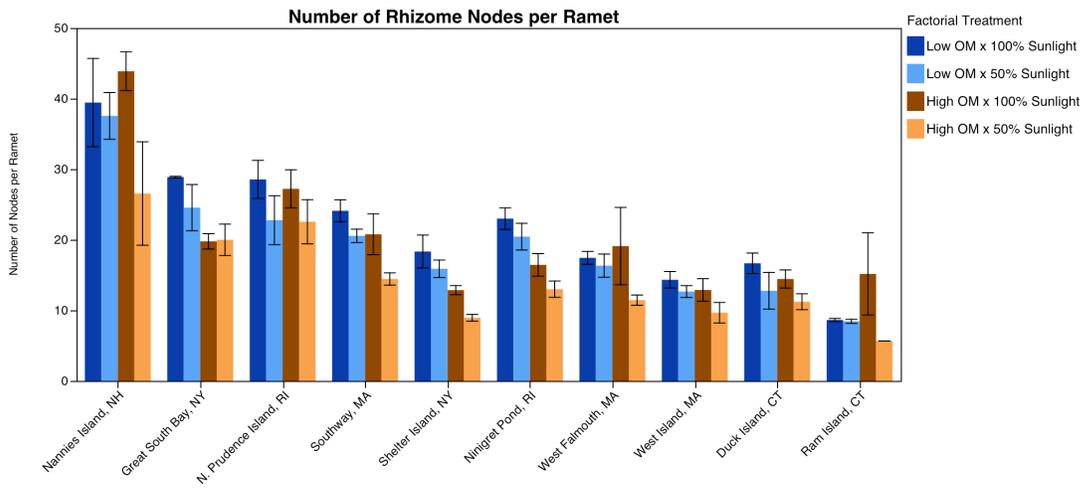


Figure 27. Number of rhizome nodes produced ramet⁻¹ among *Z. marina* from ten genetically differentiated populations following a 13-week common-garden mesocosm experiment in which sediment organic matter content and sunlight were manipulated. Means plus or minus standard error for populations within an environmental treatment (n = 3). Plot size = 0.125 m². Results of eelgrass rhizome nodes produced ramet⁻¹ for the light and organic matter treatment factorial are shown. The top panel shows treatments grouped by population; the lower panel shows the same data grouped by treatment.

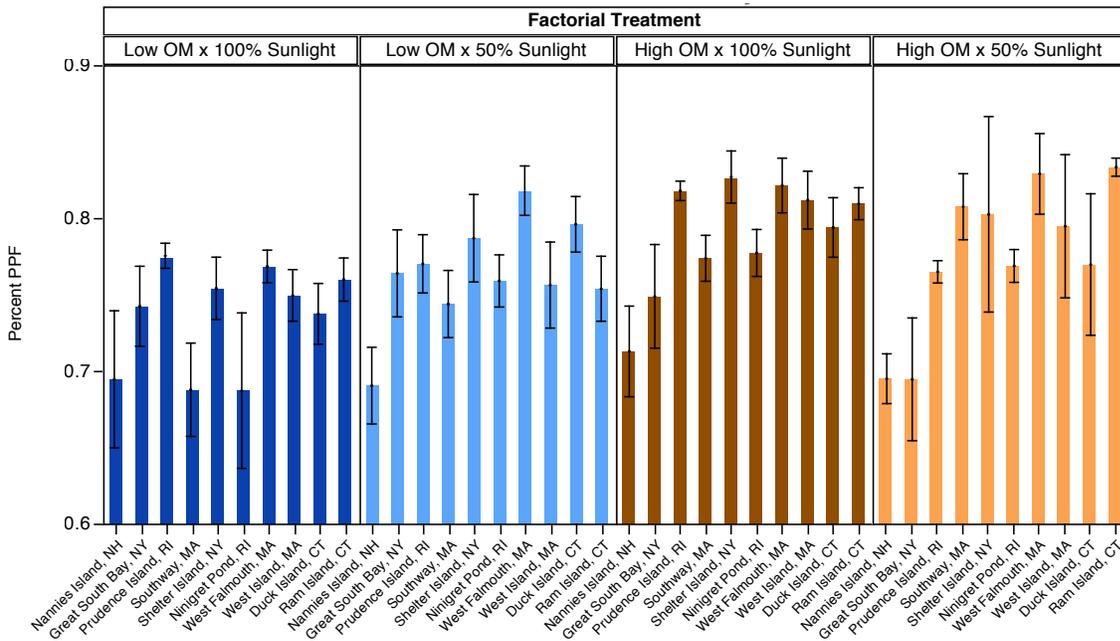
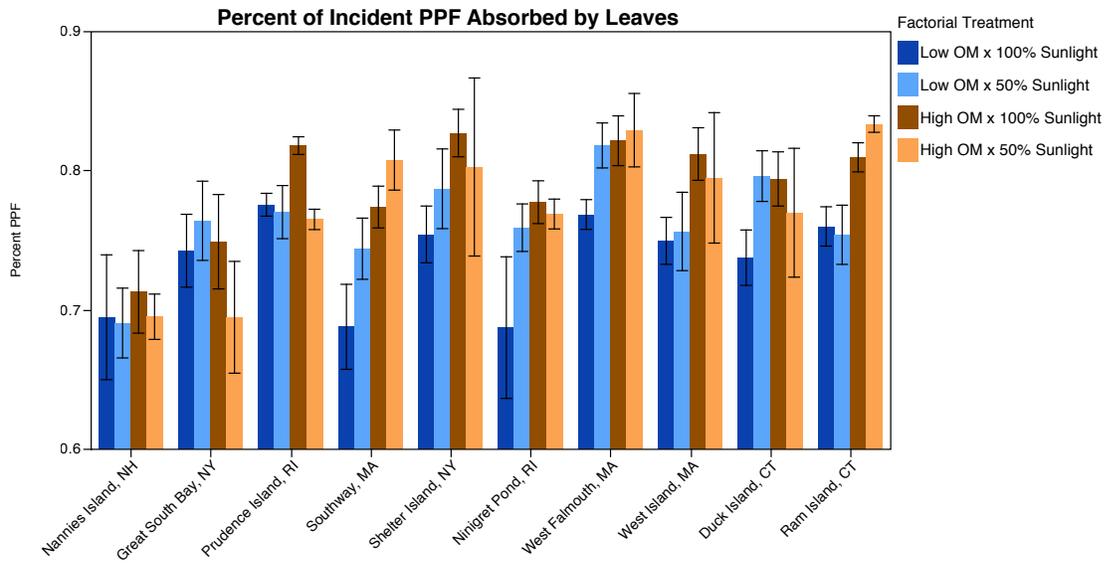


Figure 28. Leaf absorption (AF) (percent of incident PPF absorbed by leaves) among *Z. marina* from ten genetically differentiated populations following a 13-week common-garden mesocosm experiment in which sediment organic matter content and sunlight were manipulated. Means plus or minus standard error for populations within an environmental treatment (n = 6). Plot size = 0.125 m². Results of leaf absorption for the light and organic matter treatment factorial are shown. The top panel shows treatments grouped by population; the lower panel shows the same data grouped by treatment.

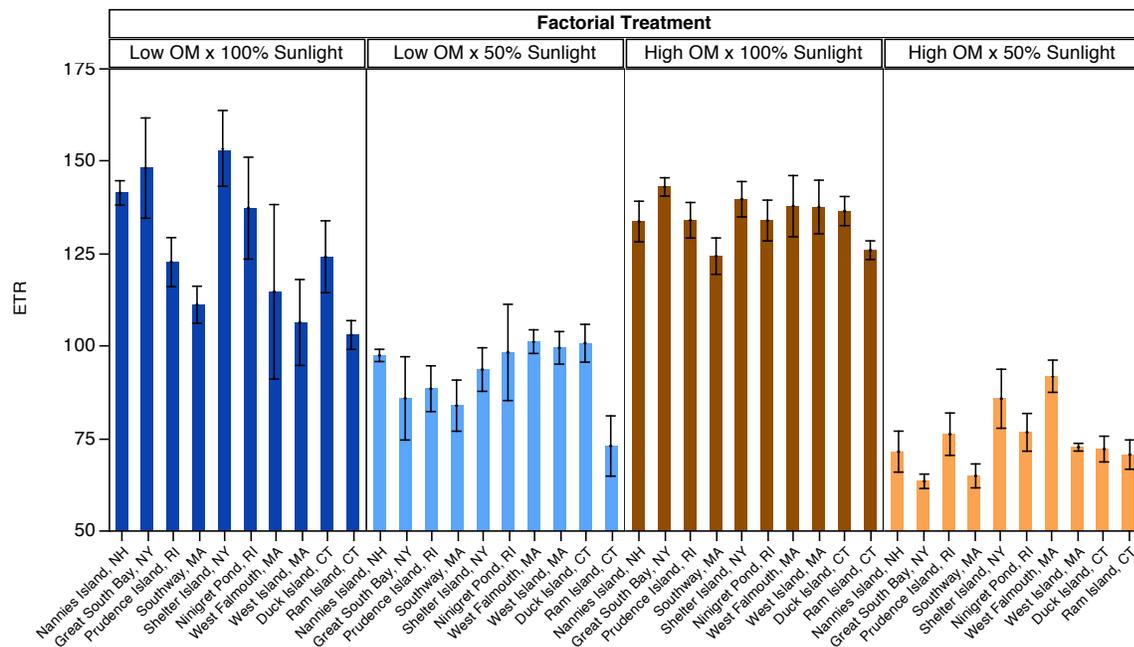
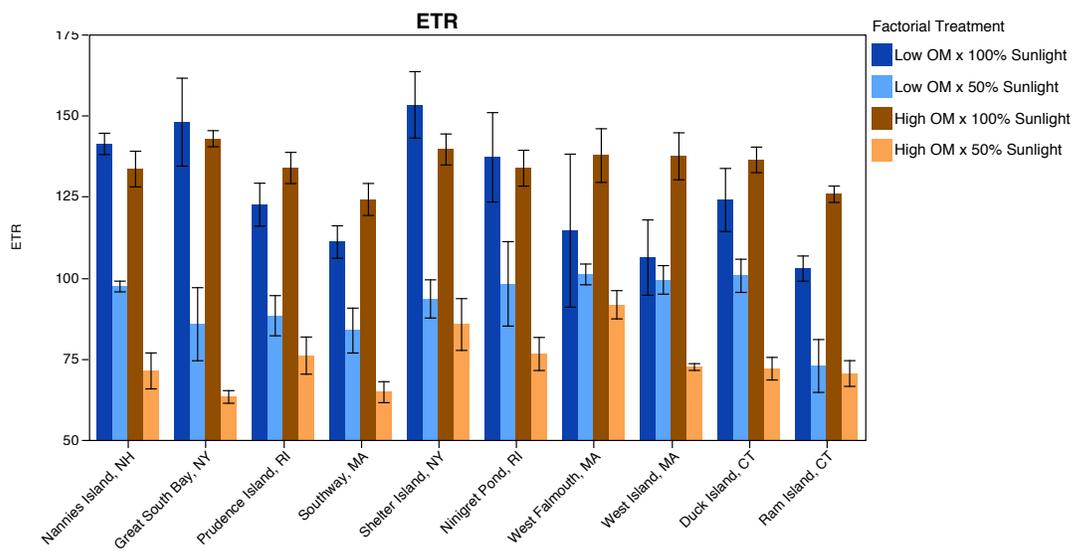


Figure 29: Electron transport rates (ETR) ($\mu\text{mol electrons m}^{-2} \text{s}^{-1}$) among *Z. marina* from ten genetically differentiated populations following a 13-week common-garden mesocosm experiment in which sediment organic matter content and sunlight were manipulated. Means plus or minus standard error for populations within an environmental treatment ($n = 6$). Plot size = 0.125 m^2 . Electron transport rates for the light and organic matter treatment factorial are shown. The top panel shows treatments grouped by population; the lower panel shows the same data grouped by treatment.

URI Eelgrass Mesocosm Evaluation of Temperature Stress

Comparable studies to the UNH mesocosm effort were conducted at the University of Rhode Island mesocosm system on the Narragansett Bay campus. These studies were looking in particular at temperature stress on the same selected eelgrass populations as analyzed at UNH; the base water temperatures at the UNH and URI mesocosm facilities were both in the range of 24 – 26° C. The experimental design at URI was to look at the stresses due to increased temperature (+2°C and +4° C) with the same sediment organic matter conditions as UNH. Light was ambient for all Rhode Island mesocosms and water was sourced from lower Narragansett Bay, making it lower nutrient and more oligotrophic than the mesocosms at UNH. The average nitrogen concentration in the water of the URI mesocosms was 2 µM while at UNH it was 20 µM. The studies were initiated in June 2011 but unfortunately, a shut-off of the water exchange system caused the experiment to fail. It was re-started in early August 2011, this time with 7 eelgrass populations re-collected from the sites.

The Great South Bay, NY (NY11) eelgrass population (producing 41 lateral shoots at ambient temperature and 24 lateral shoots at +4°C) outperformed all other eelgrass tested for resilience to high temperature stress (Table 6). Interestingly, the eelgrass did best at high sediment organic matter levels, because the low nitrogen of the Narragansett Bay source water meant that the plants were nitrogen limited. Nannies Island, NH (NH1) did not fare well in the Rhode Island experiments: the late start of the re-planted experiment was such that the majority of the New Hampshire plants were already in flowering mode and had begun producing flowering stalks rather than new ramets (essentially going reproductive) which meant they did not produce new lateral shoots, our measure of plant resilience. Plants in New Hampshire flower later than plants in southern New England and the experiment reflects that fact, rather than a temperature effect. North

Prudence Island, RI, Southway, MA, and Ninigret Pond, RI produced 19, 21, and 24 lateral shoots, respectively, at ambient temperatures and also were just below Great South Bay in performance at the higher temperatures (12, 17, 19 lateral shoots, respectively). The sites doing the worst in Rhode Island were West Falmouth Harbor (MA) and Shelter Island (NY) which produced 11 and 14 lateral shoots, respectively, at ambient temperature and 7 and 9 lateral shoots, respectively, at +4°C.

Table 6. Mesocosm results from URI experiments. Temperature increases show the replicates after the decimal, Shoot Count is original ramets planted, Shoot + lat is the ramet plus additional new shoots produced as laterals, and Canopy Height is a measure of the third longest leaf (cm). For each plant measure, data is presented for the high sediment organic (High OM) and low sediment organic (Low OM) matter treatments.

URI Mesocosm Results							
Degrees of Temperature Increase	Population	Shoot Count		Shoot+lat		Canopy Height	
		Low OM	High OM	Low OM	High OM	Low OM	High OM
0	Shelter Is, NY	15	14	23	34	28	20
2.1	Shelter Is, NY	11	10	21	21	32	38
2.2	Shelter Is, NY	12	11	19	21	31	28
4.1	Shelter Is, NY	13	12	24	19	32	28
4.2	Shelter Is, NY	14	12	19	23	28	31
0	Great South Bay, NY	15	11	66	42	28	22
2.1	Great South Bay, NY	14	13	44	45	24	38
2.2	Great South Bay, NY	8	12	26	48	26	21
4.1	Great South Bay, NY	8	12	25	36	29	21
4.2	Great South Bay, NY	12	7	12	7	22	30
0	Ninigret Pond, RI	15	15	36	41	37	37
2.1	Ninigret Pond, RI	15	12	35	34	32	38
2.2	Ninigret Pond, RI	15	12	21	21	34	30
4.1	Ninigret Pond, RI	12	12	28	26	32	37
4.2	Ninigret Pond, RI	10	8	31	34	37	37
0	North Prudence Is, RI	15	15	31	37	38	38
2.1	North Prudence Is, RI	15	12	30	24	42	38
2.2	North Prudence Is, RI	15	12	15	12	36	37
4.1	North Prudence Is, RI	13	11	28	23	38	36
4.2	North Prudence Is, RI	12	9	24	17	32	28
0	Southway, MA	14	13	36	33	38	44
2.1	Southway, MA	16	11	39	25	35	39
2.2	Southway, MA	13	15	36	15	34	37
4.1	Southway, MA	13	8	32	17	40	33
4.2	Southway, MA	14	10	33	32	34	38
0	Nannies Is, NH	7	3	7	3	34	43
2.1	Nannies Is, NH	11	9	24	15	34	40
2.2	Nannies Is, NH	14	0	31	0	31	0
4.1	Nannies Is, NH	6	8	8	16	29	38
4.2	Nannies Is, NH	9	0	14	0	31	0
0	West Falmouth, MA	11	10	20	23	38	39
2.1	West Falmouth, MA	0	0	12	10	0	0
2.2	West Falmouth, MA	10	12	15	21	38	43
4.1	West Falmouth, MA	10	10	12	13	47	40
4.2	West Falmouth, MA	13	14	20	28	41	37

Stressor Mesocosm Experiment: *Summary*

The responses of eelgrass populations to the stressors in the mesocosms of reduced light, elevated temperature and 2 levels of sediment organic content clearly distinguished some eelgrass populations as more resilient and better able to survive transplanting and to expand by producing lateral shoots, which was ultimately our measure of plant growth (and success). The ten eelgrass populations studied were chosen to be geographically representative of the region, but beyond that were chosen because in preliminary genetic screening, they demonstrated higher genetic diversity and allele richness, compared to eelgrass from other sites. However, among these ten sites, many differences of response were seen to the stressors to which they were subjected in the mesocosm experiments, showing us that different plant populations have abilities to withstand various stressor conditions and that these capacities are not simply demonstrated via the genetic markers that are available.

First, looking at the overall effect of the stressor treatments on eelgrass (Table 7a), rather than examining our results by individual population response, high organic content of the sediments was a greater stressor than the reduced light stress (50% surface light). This finding is new, and important – telling us that although eelgrass needs sufficient light, high sediment organic content can also create a major stress on eelgrass and impact its productivity. The high organic sediments are related to high rates of organic decomposition that create high concentrations of hydrogen sulfide (toxic to eelgrass roots) and high concentrations of ammonium (a valuable nutrient but too much is also toxic). Eelgrass can deal with high organic sediments by having enough light to pump oxygen into the sediments, eliminate the sulfide and ammonium toxicity. We saw this clearly in the mesocosm, where high light with high sediment organic matter (in the Rhode Island tanks) was the

most productive treatment, while the high organic sediments with low light (in New Hampshire) caused all plant populations to do poorly.

The best success for eelgrass was obtained with high light conditions and low organic sediment levels in the eutrophic waters of the mesocosms in New Hampshire (i.e., high levels of nitrogen in the waters of Great Bay). The next best eelgrass response was seen in the treatment of 50% light and low organic matter sediments. The two high organic matter treatments had low and very low eelgrass productivity under the 100% and 50% light treatments, respectively. Eelgrass was the most productive in ambient temperature conditions in both high and low sediment organic matter levels, while at +2° above ambient, eelgrass production was low for both organic matter levels and at +4°, it was very low.

In testing the resilience of specific eelgrass populations (Table 7b), the plants from Great South Bay (NY) and Nannies Island (NH) did the best in lateral shoot production under all stressor treatments, except the Nannies Island population could not be rated regarding the temperature stressor due to its flowering response. Independent of temperature and light, Great South Bay eelgrass did better than all other sites, and indeed rated “excellent” in the high sediment organic matter treatment when water nitrogen was low. With intermediate production of lateral shoots, eelgrass populations from North Prudence Island (RI), Southway (MA), and Ninigret Pond (RI) only did fair under both light and temperature stressors. With regard to organic matter, all plants did better in the high organic sediments when water nitrogen levels were low. The remaining 5 eelgrass populations, Shelter Island (NY), West Falmouth and West Island (MA), and Duck Island and Ram Island (CT), did poorly under all stressor treatments tested, indicating that these eelgrass populations have lower resilience to the kinds of stressors that have major adverse effects on eelgrass populations across the region.

Table 7. a) Response of eelgrass to stressors of reduced light and elevated temperature in conjunction with high and low sediment organic matter treatments. The response of eelgrass to various combinations of stressors was determined by counting the number of sites having high levels of lateral shoot production. b) The resilience of eelgrass, measured as lateral shoot production, from various to locations to reduced light, high temperature, and conditions of high water nitrogen combined with high sediment organic matter and low water nitrogen combined with high sediment organic matter.

a)

<u>Stressor</u>	<u>Factorial</u>	<u>Results</u>
<u>Light</u>	<u>Treatment</u>	<u>Number of Sites</u>
High	100% Light L-OM	Very high
Low	50% Light L-OM	Moderate
High	100% Light H-OM	Low
Low	50% Light H-OM	Very low
<u>Temperature</u>	<u>Treatment</u>	<u>Number of Sites</u>
Ambient	T H-OM	High
Ambient	T L-OM	Moderate
High	T+2° H-OM	Low
High	T+2° L-OM	Low
Very High	T+4° H-OM	Very low
Very High	T+4° L-OM	Very low

b)

<u>Stress Resilience</u>	<u>Reduced Light</u>	<u>High Temperature</u>	<u>High water N, High Organic Sediment</u>	<u>Low water N, High Organic Sediment</u>
Great South Bay, NY	Good	Good	Low	Excellent
Nannies Is, NH	Good	-	Low	-
Prudence Is, RI	Fair	Fair	Low	Good
Southway, MA	Fair	Fair	Low	Good
Ninigret Pond, RI	Fair	Fair	Low	Good
Shelter Is, NY	Low	Low	Low	Fair
West Falmouth, MA	Low	Low	Low	Fair
West Is, MA	Low	-	Low	-
Duck Is, CT	Very low	-	Low	-
Ram Is, CT	Very low	-	Low	-

The genetic diversity of eelgrass populations we studied did not correlate with their ability to thrive under stress – based on number of private alleles, allele frequency and stressor responses seen in our mesocosm studies. We found that our most successful (resilient) eelgrass populations were part of the two most distinct metapopulations from the north (outer Cape Cod and New Hampshire, MA-NH) and the south (south shore of Long Island, NY) (Figure 30). In the New York (blue) metapopulation, one of our sites (NY11) did the best in responses to both light and temperature stressors and a second site (NY4) did moderately well (Figure 31). In the Massachusetts-New Hampshire (green) metapopulation, two populations did well and moderately well, respectively (NH1 and MA2). The North Prudence Island site, RI2, was also moderately resilient in its response to light stress, with fair temperature resilience.

When we group eelgrass metapopulations into potential restoration areas (Figure 31) we see that our study demonstrates that the resilient eelgrass populations are found primarily in the northern (MA-NH, green) and the southern (NY, blue) metapopulations. The resilient plant populations found within these metapopulations are likely not the only eelgrass having strong stressor resilience, but merely are the ones we tested. Since we do not have a genetic marker for resilience per se, more stressor tests need to be done on related eelgrass populations (within these two metapopulations) to see if other sites yield eelgrass populations that will be useful in restoration. In the meantime, we can recommend that the eelgrass populations we have determined to be resilient should be used sustainably for restoration within their metapopulation areas. In Long Island Sound, no resilient eelgrass populations were identified and it would probably be better to go outside the metapopulation area to find resilient donor eelgrass populations. For restoration efforts in this (red metapopulation) area, plants may do well, particularly in the face of global warming, when taken from the resilient populations within the New York (blue) metapopulation, which

showed better temperature tolerance in our stressor studies. The moderately resilient eelgrass found at North Prudence Island, RI is a long-standing eelgrass population that should be preserved but may not be robust enough to sustainably be used as a donor site. Planting could be done from this site in a limited way in order to preserve its genetic character in waters that are more conducive to growth, or possibly in culture facilities.

The new information gathered in this study indicates highly diverse eelgrass populations across the region which depend on sexual reproduction to a great degree, with some populations clearly more resilient than others. The study provides new knowledge about eelgrass genetics, genetic diversity, gene flow, clonality, and while it answers many questions, it also opens areas of future investigation. The preservation and restoration of eelgrass beds regionally will depend on wise and sustainable use of the resource as well as improvement of water clarity in the coastal zone throughout southern New England and New York. We must add our genetic and stressor insights to site selection for both donor eelgrass choices and locations where restoration efforts can be most effective.

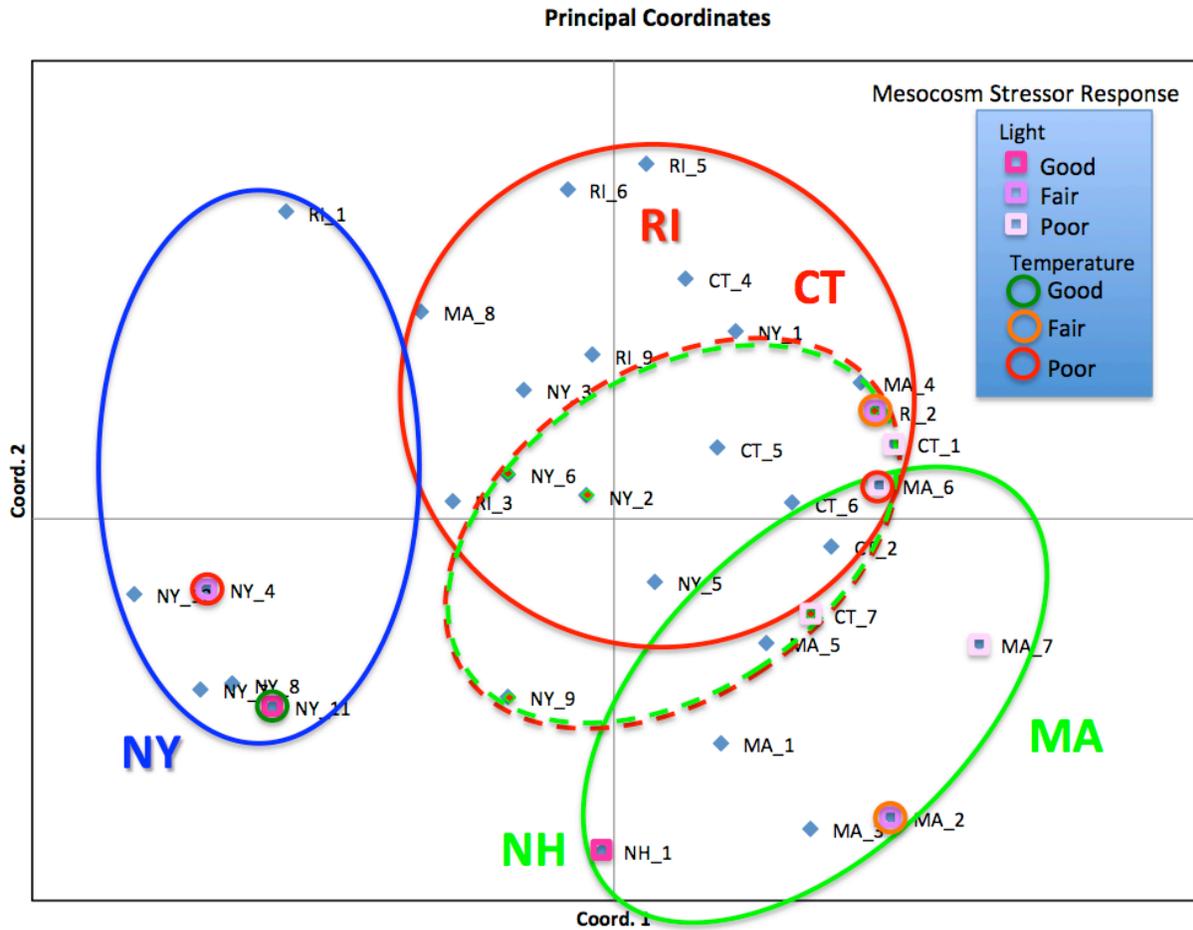


Figure 30. Overlay of the mesocosm light stress and temperature stress results on the genetic PCA (Figure G3). The best resilience to light stress is in the blue and green metapopulations, as is the trend in resilience to temperature stress.

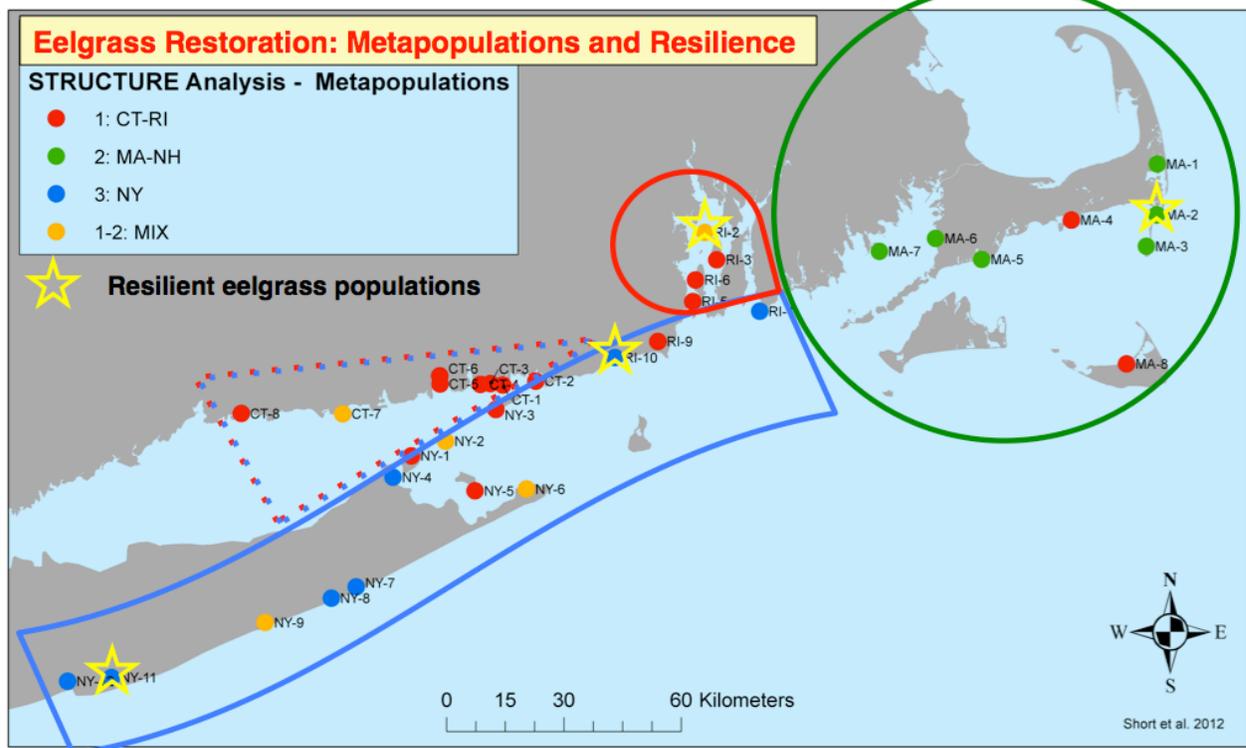


Figure 31. Groupings of eelgrass metapopulations into potential restoration areas; resilient eelgrass populations should be used for restoration within their metapopulation areas. In Long Island Sound, no resilient eelgrass populations were identified; for restoration efforts in this area, plants should be taken from the resilient populations within the New York (blue) metapopulation. The fifth site of resilient eelgrass (not shown) is Nannies Island, NH and is part of the MA-NH (green) metapopulation. The moderately resilient eelgrass found at North Prudence Island, RI is a long-standing eelgrass population that should be preserved but may not be robust enough to sustainably be used as a donor site.

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VI. Supporting Materials

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Standard Operating Procedures

STANDARD OPERATING PROCEDURE
FOR DEPLOYMENT AND COLLECTION OF
LIGHT AND TEMPERATURE CONDITIONS
IN EELGRASS BEDS USING HOBO PENDANTS

SOP 50.1
Revision 1.0
July, 2010
Page 1 of 4

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I. OBJECTIVE

To characterize water temperatures (year-round) and quantify canopy light relative to surface light (for a two week period) for each eelgrass collection site.

Overview: The HOBO Pendant light and temperature logger has a waterproof housing and can record both temperature and light intensity. Each collection site receives 3 HOBO Pendants to collect light and temperature data. All loggers are pre-launched (at UNH), then placed in the field. For light, one logger is deployed in an eelgrass meadow and one above the water surface nearby; they are set up to record light levels for 2 weeks, then they are retrieved and downloaded (at UNH) to establish light levels at each site. For temperature, one underwater HOBO Pendant is attached to the eye anchor at the sediment surface at the time of light logger deployment, and is left to continue recording water temperatures for one year before it is retrieved.

II. MATERIALS AND EQUIPMENT

For each site:

- 3 HOBO Pendant loggers
- 1 PVC support rods
- 1 Eye anchor
- 1 Pipe as a handle to install anchor
- 10 Zip ties

III. METHODS

A. Deployment and Retrieval of the HOBO Pendant (Light/Temperature):

1. HOBO Pendants (3) to log light and temperature should be deployed at the time of eelgrass sampling. Two Hobo Pendants are deployed in the water (one for light and one for temperature) attached to a eye anchor in the area sampled for eelgrass. The third pendant is deployed on land (in the air), attached to a post or other un-shaded, permanent structure.
2. Send two cable (zip) ties through the HOBO Pendant bail, in opposite directions. Slide both ends of both cable ties through the PVC support rods.
3. In the field, using the cable ties, attach the HOBO Pendant assigned for light measurements to the PVC light pole and attach the pole to the eye anchor, orienting the HOBO Pendant toward the Equator (i.e., facing south in the Northern Hemisphere) and with the Pendant at the top of the seagrass canopy. If the canopy is greater than 50 cm above the bottom, the eye anchor and post will not be tall enough to evaluate the light without obstruction from the plants, so install the Pendants in an unvegetated 'hole' within the meadow. Sunlight reaching the HOBO Pendant should not be obstructed by shadows; the horizontal surface with sensors and flashing light should be level and face the sky. Cut off the unused ends of the cable ties. In a similar fashion, deploy the second HOBO Pendant for temperature collection on the eye anchor itself, just above the sediment surface (1 cm). Record the time and GPS location of deployment for each logger on data sheet (attached).
4. The final Pendant for light collection is deployed on land (in the air) in a convenient but protected location without shadows throughout the day. Attach it to the PVC light pole, then use cable ties to attach the light pole to a solid structure (fence post, dock piling, or similar). Record the time and GPS location of the logger on the data sheet.

5. After the HOBO Pendants have been deployed for 2 weeks, collect the Pendant and the PVC light pole from the eye anchor as well as the Pendant attached on land. Rinse in fresh water, dry with paper towel, and mail back to UNH immediately, within 24 hours of retrieval, for data download.
6. The full-year temperature sensors should be collected after one year. Rinse in fresh water, dry with paper towel, and return to UNH.
7. Mail all sensors to Nikki Sarrette, Jackson Estuarine Laboratory, 85 Adams Point Road, Durham, NH 03824, 603-862-5125.

IV. TROUBLE SHOOTING / HINTS

1. Write the location of the deployed pendants from the GPS unit on data sheet or book immediately upon collection.

V. STATISTICAL ANALYSIS AND DATA USAGE

No data reduction or statistical analyses are required at this stage. Pendants with data will be sent to Nikki Sarrette.

VI. REFERENCES

Short, F.T. and R.G. Coles (eds.). 2001. Global Seagrass Research Methods. Elsevier Science B.V., Amsterdam. 473 pp.

Short, F.T., L.J. McKenzie, R.G. Coles, K.P. Vidler, and J.L. Gaeckle. 2006. SeagrassNet Manual for Scientific Monitoring of Seagrass Habitat – Worldwide Edition. University of New Hampshire Publication, Durham, NH, USA. 76 pp.

STANDARD OPERATING PROCEDURE
 FOR DEPLOYMENT AND COLLECTION OF
 LIGHT AND TEMPERATURE CONDITIONS
 IN EELGRASS BEDS USING HOBO PENDANTS

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[Data sheet for recording Pendant sensor information]

Deployment Team Members: _____

Site Name and Site number: _____

LAND LIGHT PENDANT

Description: _____

Pendant serial number:		
Date and Time Deployed	GPS (N)	GPS (W)
Date and Time Retrieved		

UNDERWATER LIGHT PENDANT

Pendant serial number:		
Date and Time Deployed	GPS (N)	GPS (W)
Date and Time Retrieved		

UNDERWATER TEMPERATURE PENDANT

Pendant serial number:		
Date and Time Deployed	GPS (N)	GPS (W)
Date and Time Retrieved		



STANDARD OPERATING PROCEDURE
FOR SAMPLE COLLECTION OF EELGRASS
FOR MORPHOLOGICAL, PHYSIOLOGICAL
AND GENETIC ANALYSES

SOP 50.2
Revision 0.0
May, 2010
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POINT OF CONTACT:

NAME	Fred Short
ADDRESS	Jackson Estuarine Laboratory 85 Adams Point Road Durham, NH 03824
EMAIL	fred.short@unh.edu
PHONE	603-862-5134

I. OBJECTIVE

To assess the health and diversity of eelgrass meadows, a procedure is outlined to collect plants and limited physical data from a discrete meadow.

Overview: Air and water temperature data, water salinity and five sediment samples are collected from each sampling site along with 55+ plants. Preparation of the light and temperature pendants and the eelgrass plants for specific analyses are outlined in other SOPs.

II. MATERIALS AND EQUIPMENT

- Boat (appropriate for sampling needs and exposure) and safety equipment
- Navigation map
- Eelgrass meadow map and sampling map
- GPS unit
- Snorkel/SCUBA gear, dive flag, weight belt
- Sample Collection Data sheet, 2B pencils and clipboard using unique ID # System
- Pre-labeled collection bags for eelgrass (quart-size freezer bags)
- 2 gallons fresh water (for rinsing)
- Sediment piston core sampler (20 cc) and 5 sample bags
- Marker buoys and weights (2 each)
- Flotation basket and cooler
- Vials (60 ml) for salinity samples (5)
- Calibration solution for refractometer (to be used at lab)
- Optical refractometer (temperature corrected; to be used at lab)

III. METHODS

A. Field Collection

1. The eelgrass bed of the proposed sampling site is located and navigated to. Eelgrass

and other measures will be sampled at 50 stations within each collection site.

STANDARD OPERATING PROCEDURE
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2. The bed is visually inspected from the boat or in the water, depending on depth and size, to determine sampling boundaries. Select a 100-meter sampling path that traverses the middle of the depth range for the eelgrass meadow. Any deviations from expected meadow dimensions are recorded on the sampling map.
3. A sampling plan is chosen that will effectively sample eelgrass for genetic analyses, with stations about 2 meters apart (or more) and containing 50 sample stations (Olsen et al. 2004, Coyer et al. 2008). (If ‘holes’ in the bed are encountered, move on.) The sampling goal is to sample a portion of the bed about 100 m in length (Figure 1), unless the bed is small, and in that case the sampling path may curve around a portion of the bed. Safety, water depth and predicted tide currents and depths are considered in determining collection method (wading, snorkeling, SCUBA).
4. As soon as you arrive at the location, find the first station for sampling and drop a temporary marker buoy with weight to mark the site. At the weight, screw in an eye anchor to be used for attaching the HOBO Pendant temperature logger and the light logger on its PVC rod. Pendant loggers to measure light and temperature are deployed (see SOP 50.1) and the GPS position is written on the data sheet at the site before plant and sediment sampling. Be sure to note time of deployment on the data sheet.
5. A two-person team, one with pre-labeled collection bags and GPS, the other focused on sampling, get into the water with any other needed gear (e.g., dive flag, float to hold samples, flotation basket and cooler). GPS is marked at beginning and end points and waypoints are entered for each of the 50 collection stations. Be sure to record the GPS beginning and endpoint on the data sheet.

6. Sampling:

- 6.1 For the genetics only sample stations (50 stations per site), one terminal shoot is uprooted at the sediment surface with a small section of rhizome and placed in the sample bag. Refer to the sample form attached to the bags.

- 6.2 For the Wasting Index, NPI & N15 stations (every 4 stations: # 1, 5, 9, 13, 17, 21, 25, 29, 33, 37, 41, and 45), a longer section of rhizome is collected with the terminal shoot and one to two lateral shoots (all connected by rhizome). Three to four lateral shoots may be needed if the leaves are diminutive (less than 4 mm wide and 20 cm in height). All shoots for each station are placed in one bag.

- 6.3 At five stations (# 1, 13, 25, 37, 45), sediment and salinity samples are collected, as well as a second eelgrass shoot (with rhizome) for a herbarium voucher. A piston corer fashioned from a 20 ml plastic syringe is used to capture about 50 grams of sediment from the top 10 cm. Remove the piston and insert the tube into the sediment until the flange is reached. Insert the piston just to the top of the coring tube and withdraw the sample from the sediment. Above the water, extrude the sediment from the core into a labeled plastic bag. The sediment is kept cool until return to laboratory, where samples are air dried at room temperature (open bag and thumb tack it to a wall until all visible liquid has evaporated) and then shipped to UNH for analysis. Upon arrival it will be kept cool (4°C) until analyzed for organic matter using the Loss-on-Ignition method.

- 6.4 At the same five stations (# 1, 13, 25, 37, 45), a small pre-labeled 60 ml vial is used to collect water at mid depth for determination of salinity. The vials are returned to the lab and salinity measured with a temperature-corrected optical refractometer. The refractometer is calibrated with 15 ppt salinity immediately prior to measurements.
7. Once all plant samples are collected, the collection team boards the vessel, and retrieves the marker buoy with weight. Then, on the boat, plants are rinsed in fresh water and then placed in coolers to keep dark and cool until processing begins (SOP 50.3-50.5). To rinse, add ~50 ml of tap water to each plastic sample bag, slosh gently, and turn upside down holding the plant in the bag to drain. First and last station GPS positions are recorded on the data sheet. GPS coordinates for beginning and end points of the collection are also recorded in the GPS.
8. Once all samples are processed for a site (see SOP 50.3-50.5) - mail soil and plant samples to Nikki Sarrette, Jackson Estuarine Laboratory, 85 Adams Point Road, Durham, NH 03824 (603-862-5125). Record the Sent Date on the data sheet.

IV. TROUBLE SHOOTING / HINTS

1. The eelgrass meadow being sampled may not be continuous for a variety of reasons. If a station cannot be sampled, the team will move on to the next available sample station. No waypoint will be recorded for 'missing' stations.
2. If the team needs to leave the water for any reason, mark the endpoint with a small buoy (as well as the GPS) to aid in finding the location when sampling resumes.

V. STATISTICAL ANALYSIS AND DATA USAGE

No Data reduction procedures or statistical tests are planned for eelgrass sample and physical data collection.

VI. REFERENCES

- Coyer, J.A., K.A. Miller, J.M. Engle, J. Veldsink, A. Cabello-Pasini, W. T. Stam and J.L. Olsen. 2008. Eelgrass meadows in the California Channel Islands and adjacent coast reveal a mosaic of two species, evidence for introgression and variable clonality. *Annals of Botany* 101: 73–87.
- Olsen, J. L., W. T. Stam, J. A. Coyer, T. B. H. Reusch, M. Billingham *et al.* 2004. North Atlantic phylogeography and large-scale population differentiation of the seagrass *Zostera marina* L. *Molecular Ecology* 13: 1923-1941.

STANDARD OPERATING PROCEDURE
 FOR SAMPLE COLLECTION OF EELGRASS
 FOR MORPHOLOGICAL, PHYSIOLOGICAL
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UNH - TNC Eelgrass Assessment: Field and COC Form			State:		
Date Collected:		GPS: Lat.	Long.	Site Name:	
Date Processed:		Start =		Site Number:	
		End =			
Station	Sampling	Processed	Station	Sampling	Processed
series start #					
#	1	2x (g + laterals) +			
	2	g	26	g	
	3	g	27	g	
	4	g	28	g	
	5	g + laterals	29	g + laterals	
	6	g	30	g	
	7	g	31	g	
	8	g	32	g	
	9	g + laterals	33	g + laterals	
	10	g	34	g	
	11	g	35	g	
	12	g	36	g	
	13	2x (g + laterals) +	37	2x (g + laterals) +	
	14	g	38	g	
	15	g	39	g	
	16	g	40	g	
	17	g + laterals	41	g + laterals	
	18	g	42	g	
	19	g	43	g	
	20	g	44	g	
	21	g + laterals	45	2x (g + laterals) +	
	22	g	46	g	
	23	g	47	g	
	24	g	48	g	
			49	g	
	25	2x (g + laterals) +	50	g	

g = sample for genetics
g + laterals = additional attached shoots if small shoots (all from the same rhizome)
2x (g + laterals) + = extra shoot(s) for herbarium vouchers + salinity and sediment

Send original with samples after making a PDF file to:
 Nikki Sarrette, JEL, 85 Adams Point Road, Durham, NH 03824

Email copies to: Fred.Short@unh.edu, Anita.Klein@unh.edu

STANDARD OPERATING PROCEDURE
FOR SAMPLE PREPARATION OF EELGRASS
FOR GENETIC ANALYSES

SOP 50.3
Revision 0.0
May, 2010
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POINT OF CONTACT:

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PHONE	603-862-5134

I. OBJECTIVE

To prepare eelgrass samples for genetic analysis.

Overview: Approximately 50 individual eelgrass shoots will have been collected from each site to determine the genetic diversity within, and at a larger scale, among sites in Southern New England. To prepare the tissue for genetic analysis, care must be taken to provide dry plant material.

II. MATERIALS AND EQUIPMENT

- Eelgrass samples with unique site/station labels from field collections
- Eelgrass Tissue Transfer Data Sheets
- Scissors
- Straight edge razor blades
- Forceps
- Kimwipes to remove epiphytes
- Soft, absorbent paper towels
- Microcentrifuge tubes, 2 ml, numbered, half-filled with fresh silica crystals
- Extra silica crystals to top off the tubes

III. METHODS

A. Field Collection has been covered previously in SOP 50.2.

B. Laboratory Processing

1. Enter the date, site and unique station numbers for each eelgrass sample on the Eelgrass Tissue Transfer Data Sheet.

2. For station numbers 1, 5, 9, 13, 17, 21, 25, 29, 33, 37, 41, and 45 (every fourth sample), the shoots must also be processed according to the Wasting Index/NPI & N¹⁵ processing instructions (SOP 50.4).
3. For all other stations, select the terminal vegetative shoot from the plastic bag, remove excess water with a paper towel, and place on bench top.
4. Using scissors, collect approximately a 10 – 20 cm long section of leaf from the youngest leaf. The material may come from within the sheath as well as from the recently emerged leaf.
5. Use a Kimwipe to wipe the plant tissue free of surface contamination and blotted dry. Cut the cleaned leaf section in 1 cm pieces with a razor blade. The aim is to collect 2 cm² of leaf tissue; this will amount to 4 pieces of leaf about 1 cm long if the leaf width is about 0.5 cm.
6. Open the labeled sample 2 ml tube containing dessicant and insert the sections of leaf tissue using the forceps. Cap the tube and shake. Be careful not to allow the leaf pieces to stick together – each piece should be surrounded by the silica gel crystals. Uncap the tube and add sufficient silica to nearly fill the tube. Then cap the tube a final time.
7. Once a site is completed, prepared plant samples for genetic analysis are sent to Nikki Sarrette, Jackson Estuarine Laboratory, 85 Adams Point Road, Durham, NH 03824, 603-862-5125.

IV. TROUBLE SHOOTING / HINTS

1. Silica crystals will absorb the water out of the section of eelgrass leaf very quickly. It is essential to blot the plant material as much as possible before putting it in the silica. The trick is to use enough silica crystals and to expose the maximum surface area of the leaf to the drying substance. "Top up" each vial completely with silica after putting in the leaf sample.
2. Plants should be processed as described above within 24 hours of collection. In the field, plants should be stored in the dark in coolers and then refrigerated immediately after return to the lab.
3. Have all bags and sample tubes marked ahead of time with the unique ID #s.
4. Always mail the sample(s) in their tubes with silica intact. If not, the samples will rehydrate and be ruined.
5. Do not air-dry samples for genetic analysis before putting in silica crystals.

V. STATISTICAL ANALYSIS AND DATA USAGE

No data reduction procedures or statistical tests are appropriate for this stage of analysis.

VI. REFERENCES

Burdick, D. M., and G. A. Kendrick. 2001 Standards for seagrass collection, identification and sample design. Pp. 79-100 *In*: Short, F.T. and R.G. Coles (eds.) *Global Methods in Seagrass Research*. Elsevier Science.

STANDARD OPERATING PROCEDURE
FOR SAMPLE PREPARATION OF EELGRASS
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Coyer, J.A., K.A. Miller, J.M. Engle, J. Veldsink, A. Cabello-Pasini, W. T. Stam and J.L. Olsen. 2008. Eelgrass meadows in the California Channel Islands and adjacent coast reveal a mosaic of two species, evidence for introgression and variable clonality. *Annals of Botany* 101: 73–87.

Olsen, J. L., W. T. Stam, J. A. Coyer, T. B. H. Reusch, M. Billingham *et al.* 2004. North Atlantic phylogeography and large-scale population differentiation of the seagrass *Zostera marina* L. *Molecular Ecology* 13: 1923-1941.

STANDARD OPERATING PROCEDURE
FOR SAMPLE PREPARATION OF EELGRASS
FOR THE WASTING INDEX, NUTRIENT POLLUTION
INDICATOR (NPI) AND STABLE ISOTOPES

SOP 50.4
Revision 0.0
May, 2010
Page 1 of 6

POINT OF CONTACT:

NAME	Fred Short
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PHONE	603-862-5134

I. OBJECTIVE

To measure amount of wasting disease on eelgrass plants, as well as processing eelgrass for nutrient pollution indicator (NPI) and stable isotope (N15) measurement.

Overview: Nutrient loads and wasting disease both can stress eelgrass. Wasting disease is responsible for destroying eelgrass populations in epidemic outbreaks (Short et al. 1987, Muelhstein et al. 1990), and can be an important stressor, limiting ecological success (Burdick et al. 1993). The procedure to assess the Wasting Index provides basic morphological data useful for characterizing the size and shape of individual plants in a specific eelgrass bed (Burdick et al. 1993). The eelgrass NPI is an indicator of nutrient enrichment to an estuary (Lee et al. 2004) and can be used to determine stress associated with excessive nutrient loading (Short et al. 1995) to an eelgrass meadow. Stable isotopes can reveal nutrient sources in an estuary (Tewfik et al. 2005). In combination, these three measures can provide important information regarding estuarine nutrient sources, nutrient and Wasting Disease stresses to eelgrass and eelgrass plant morphology.

II. MATERIALS AND EQUIPMENT

- Eelgrass plants in labeled bags
- Data Sheet: UNH – TNC Eelgrass Assessment Data Form
- Ruler to 1 meter (in mm)
- Calipers (to 0.1 mm)
- Straight edge razor blades
- Sample tubes (10 ml) filled with silica beads for N analyses

III. METHODS

A. Field Collection is covered in SOP 50.2.

B. Laboratory Processing

1. For station numbers 1, 5, 9, 13, 17, 21, 25, 29, 33, 37, 41, and 45 (every fourth sample) process as below for Wasting Index, NPI & Isotopes (N15) as well as for genetics. For all other samples, process only for genetics, according to the Genetics Protocol (SOP 50.3 Section III.B.).

2. Assessment of Wasting Index (WI)

2.1 Enter the date, site name and site number and the unique station number for the eelgrass sample on the data sheet. Select a terminal vegetative shoot and place on bench top.

2.2 Measure the height of the sheath length to the nearest mm: measure from the top of the youngest visible sheath (usually encloses the youngest 2 - 3 leaves) to the youngest root node in cm and record on the data sheet.

2.3 Visualize numbering the leaves for each shoot from youngest (1) to oldest.

2.4 In order of youngest to oldest leaf, measure and record the length of each leaf in cm to the nearest mm: measure from the leaf tip to the root node. If a tip is broken, record its length and indicate the broken tip with an asterisk.

2.5 Again starting with the youngest leaf, measure the Wasting Index for each leaf: using the Wasting Index Key (below) as a guide, enter the percentage of the wasting disease on the leaf under 'WI' on the data sheet. The percentage of disease on a leaf

is estimated by examining the portion of the leaf from the top of the sheath to the tip, then comparing the area of disease on the leaf to those shown on the Key (leaf areas 0, 1, 10, 20, 40 and 80% infection from disease are shown). Interpolate if the leaf appears to have a percentage of diseased area between those pictured on the Key.

3. Procedure for preparing eelgrass leaf tissue for the NPI and for Stable Isotope (N15) analysis (second half of the data form)

3.1 Use the Wasting Index shoot (above) or, if needed, remove an additional, lateral shoot from along the rhizome and cut the shoot(s) from the rhizome at the meristem above the rhizome node.

3.2 Remove the two oldest leaves from the shoot(s) with their sheaths and discard, keeping the youngest leaves for analysis.

3.3 Remove all surface water and material (slime, sediments) from the youngest leaves using a Kimwipe or paper towel.

3.4 Measure the shoot width at the top of the sheath to the nearest 0.1 mm with the calipers. Record the width under Shoot 1 on the data sheet.

3.5 To obtain the plant tissue samples for the NPI, cut the shoot exactly 20 cm above the top of the sheath, again at 10cm above the sheath and finally at the top of the sheath. Then, from the 0 – 10 cm part of these leaves (the part nearest the sheath), discard the youngest leaf (which will likely end at an intact leaf tip) and select the second-youngest leaf for genetic analysis (see SOP 50.3, Section III.B.).

STANDARD OPERATING PROCEDURE
FOR SAMPLE PREPARATION OF EELGRASS
FOR THE WASTING INDEX, NUTRIENT POLLUTION
INDICATOR (NPI) AND STABLE ISOTOPES

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Gather the third and fourth youngest leaf sections from the 0 – 10 cm part and the 3 to 4 youngest leaf sections from the 10 – 20 cm part for NPI and isotope analysis. Avoid using any floppy, immature leaf tissue, and avoid any grazed, damaged, or epiphytized leaf pieces. From these four to six leaf sections, choose a sufficient number of 10 cm sections to create leaf area to equal 20 cm² for analysis.

If there is insufficient material available from the original shoot (Shoot 1), select and section a second (and/or third) lateral shoot from the attached horizontal rhizome in order to obtain sufficient leaf area. Measure the shoot width at the top of the sheath to the nearest 0.1 mm with the calipers. Record the width under Shoot 2 or 3, as appropriate, on the data sheet.

Record on the data sheet the number of 10 cm leaf sections included in the NPI sample from each shoot. If need be, sections shorter than 10 cm can be used and recorded as partials with their lengths (i.e., 5.6 cm section).

3.6 When collecting leaf material, only mature plant material from within the sheath should be used. Do not include the leaf tip in the NPI/isotope samples. Do not use any plant material that is visibly epiphytized or diseased.

3.7 Cut the 10 cm sections in half and insert all the leaf sections into a 10 ml tube with silica beads. Top off the tube with silica beads after inserting the leaves. Tighten cap and check label, include unique ID #, and place it into a plastic bag.

4. Once a site is completed, prepared plant samples are sent to Nikki Sarrette, Jackson Estuarine Laboratory, 85 Adams Point Road, Durham, NH 03824, (603) 862-5125.

IV. TROUBLE SHOOTING / HINTS

1. The freshwater rinse performed in the field is critical for accurate assessment of wasting disease.
2. Plants should be processed within 24 hours of collection.

V. STATISTICAL ANALYSIS AND DATA USAGE

No data reduction procedures or statistical tests are appropriate for this stage of analysis.

VI. REFERENCES

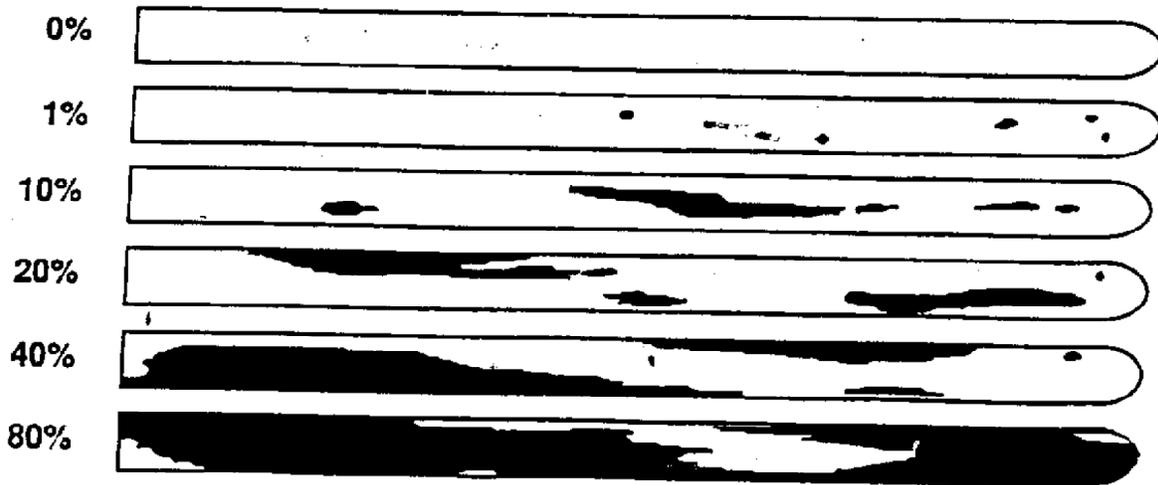
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- Short, F.T. and Burdick D.M. 2003. Eelgrass as an indicator of nutrient over-enrichment in estuaries: a final report submitted to the NOAA/UNH Cooperative Institute for Coastal and Estuarine Environmental Technology. University of New Hampshire, Durham, NH 60 pp. +CD-ROM. [web NPI - Nutrient Pollution Indicator Manual: <http://marine.unh.edu/jel/faculty/fred2/fredshort.htm>]
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STANDARD OPERATING PROCEDURE SOP 50.4
FOR SAMPLE PREPARATION OF EELGRASS
FOR THE WASTING INDEX, NUTRIENT POLLUTION
INDICATOR (NPI) AND STABLE ISOTOPES

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Short, F. T., D. M. Burdick, and J. E. Kaldy, III. 1995. Mesocosm experiments quantify the effects of coastal eutrophication on eelgrass, *Zostera marina* L. *Limnology and Oceanography* 40:740-749.

Tewfik, A., J.B. Rasmussen, K.S. McCann. 2005. Anthropogenic enrichment alters a marine benthic food web. *Ecology* 86: 2726–2736.



WASTING INDEX KEY

STANDARD OPERATING PROCEDURE
FOR PREPARATION OF ARCHIVAL
EELGRASS HERBARIUM SPECIMENS

SOP 50.5
Revision 0.0
May, 2010
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POINT OF CONTACT:

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I. OBJECTIVE

To prepare and press eelgrass as herbarium specimens that will be archived at UNH for later study and use.

Overview: The permanent record of eelgrass plants from specific sites and with known microsatellite distributions (genetic diversity) may be valuable for interpretation of results of the Eelgrass Genetic study and even more valuable for unknown studies conducted in the future (Burdick and Kendrick 2001).

II. MATERIALS AND EQUIPMENT

- Plant press
- Blotting paper / newspaper
- Herbarium labels
- Herbarium paper
- Floor fan

III. METHODS

A. Field Collection for herbarium samples is covered in SOP 50.2. For station numbers 1, 13, 25, 37, and 45, a shoot is collected for a herbarium archive.

B. Laboratory Processing (abstracted from Burdick and Kendrick 2001)

1. Five terminal shoots are collected from each sample site for archiving as a herbarium specimen. Eelgrass samples are pressed fresh, with a freshwater rinse, but without chemical treatment.

2. Place each plant separately on herbarium paper and arrange to clearly show the different tissues (rhizome, roots, shoot, reproductive portions, etc.). Fill out a herbarium label using collection data, including unique ID # and enclose with plant (see sample label below).
3. Separate each sample with blotter plus ventilation boards (corrugated cardboard) and place in a press. Dry in a well-ventilated room. A floor fan is ideal for improving ventilation.
4. Change the blotters daily until dry (2 to 3 days).
5. Once a site is completed, mail the press with the herbarium sheets to Nikki Sarrette, Jackson Estuarine Laboratory, 85 Adams Point Road, Durham, NH 03824, 603-862-5125.

V. STATISTICAL ANALYSIS AND DATA USAGE

No data reduction procedures or statistical tests are appropriate for this stage of analysis.

VI. REFERENCES

Burdick, D. M., and G. A. Kendrick. 2001 Standards for seagrass collection, identification and sample design. Pp. 79-100 *In*: Short, F.T. and R.G. Coles (eds.) *Global Methods in Seagrass Research*. Elsevier Science.

Dawes, C.J. 1981. *Marine Botany*. John Wiley, New York. 628 pp.

Herbarium Label:

Species Name: *Zostera marina* L. and Eelgrass

Collection Site: Use Site Name, Site Number for SNE Project
Lat/Long from GPS of the Site:

Collection Date: [dd MMM yyyy] 23 Jun 2010

Collector with Contact Information:

Unique ID# for Station:

STANDARD OPERATING PROCEDURE
FOR SEDIMENT ANALYSIS TO DETERMINE
ORGANIC CONTENT AND GRAIN SIZE

SOP 50.6
Revision 0.1
February, 2011
Page 1 of 4

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I. OBJECTIVE

To determine sediment organic content determined by loss on ignition and characterize distribution of sediment size classes (gravel, sand and silt/clay fractions). See Erftemeijer and Koch 2001.

II. MATERIALS AND EQUIPMENT

- Drying oven
- Muffle furnace
- Aluminum weighing dishes
- Temperature-corrected optical refractometer
- Dessicator
- 1L graduate cylinder
- Mortar and pestle
- 63 μ and 2 mm sieves with catch pan
- Deionized water
- Squeeze bottle with deionized water
- Small beakers (50 mL)
- Glass stirring rod, spatula, brush
- Plastic funnel

III. METHODS

1. Label the underside of the aluminum pan by etching the unique serial number for the sample you are processing. Any ink or pencil labels will be lost upon combustion.
2. Place sediment sample into pre-weighed aluminum pan; use glass stirring rod to disperse the sample evenly inside the pan. Add 10 ml of deionized water while picking out any large shell material using forceps and rinse; stir with glass rod; be careful not to lose any of the sediment. Set the sample aside to settle until water is clear, then measure the salinity using a few drops of the water and decant and discard the rest of the clear water fraction. Weigh sediment sample to the nearest 0.0001 g to determine the wet weight.
3. Dry in oven at 60° C for 24 hours or until completely dry. Place sample in dessicator until cooled to room temperature. Reweigh the sample to obtain the dry weight.
4. Heat sample in muffle furnace at 450° C for 4 hours to determine organic matter content. Place sample in dessicator until cooled to room temperature. Reweigh sample once it is sufficiently cooled to obtain the combusted weight.
5. Then make a calculation to remove the weight due to salinity from the dry weight and from the combusted weight: calculate the water weight in the sample (wet weight – dry weight). Multiply this weight by the salinity expressed in ppt; then multiply the water weight by 0.001 to determine the weight of salt. Calculate the corrected weight of the samples by subtracting the salt weight from the dry weight and the salt weight from the combusted weight, yielding the salt-free weights “dry wt” and “combusted wt”

6. Loss on Ignition is calculated as % weight loss after combustion using (Erfteimeijer and Koch 2001):

$$\text{LOI} = 100 * ((\text{dry wt} - \text{combusted wt}) / \text{dry wt})$$

7. Now that the organic content has been burned out of the sample, the sample must be gently ground by use of mortar and pestle to break up aggregates caused by combustion. Place sample into mortar and gently grind apart the aggregates using only the weight of the pestle.
8. After the sample has been gently disaggregated, dry sieve the sample through a stack of two sieves consisting of a 2mm and a 63 μ stainless steel sieve plus a catch pan. Be careful not to touch the sieve with your hand or anything that will damage the mesh. Put the disaggregated sample into the 2mm sieve on top, cover and shake with a circular motion and tap until the sand fraction has passed through the 2 mm sieve, leaving the shell and gravel material on top of the 2mm sieve. The sand fraction is left on the 63 μ sieve, and the silt-clay fraction (finest) is in the catch pan. Transfer the three grain size fractions into separate pre-weighed pans. Re-dry for an hour, then weigh when cool. The weight minus the weight of the pre-weighed pan yields the weight for each fraction.
9. The weights of the three grain size fractions: 1) gravel/shell, 2) sand, and 3) silt/clay are summed to provide the total weight and the percent of each fraction by weight is calculated.

IV. TROUBLE SHOOTING / HINTS

1. The salts do not affect estimates of grain size distribution.
2. Drying of sediment samples can be checked by placing them back in the drying oven and reweighing the following day.

3. Dry samples must be cooled in a dessicator before weighing. Dried and combusted samples will gain moisture from the atmosphere rapidly. Also, warm samples will give erroneous readings by creating convection currents around the pan of the balance.

V. STATISTICAL ANALYSIS AND DATA USAGE

1. Input pan weight, wet weight, salinity, dry weight, combusted weight and dried sediment fractions to spreadsheet. Calculate % LOI and size fractions using appropriate formulae (above).
2. Calculate mean and standard error of five sediment samples for each eelgrass collection site.

VI. REFERENCES

Erftemeijer, P.L.A., E.W. Koch. 2001. Sediment geology methods for seagrass habitat, p. 345-368, In Short, F.T. and R.G. Coles (eds.) Global Seagrass Research Methods. Elsevier Science B.V., Amsterdam. 473 pp.

UNH -TNC Eelgrass Assessment: Data Form													Date:	Site Name:			GPS:		Lat.				
													Site Number:					Long.					
Wasting Index													NPI & N15										
youngest													oldest							Shoot 1	Shoot 2	Shoot 3	Leaf Mass
no	Station	Sheath length cm	#1 Leaf length (cm)	WI %	#2 Leaf length (cm)	WI %	#3 Leaf length (cm)	WI %	#4 Leaf length (cm)	WI %	#5 Leaf length (cm)	WI %	#6 Leaf length (cm)	WI %	no. of 10 cm sections	Leaf width mm	no. of 10 cm sections	Leaf width mm	no. of 10 cm sections	Leaf width mm	Weight all sections combined mg		
1	1																						
2	5																						
3	9																						
4	13																						
5	17																						
6	21																						
7	25																						
8	29																						
9	33																						
10	37																						
11	41																						
12	45																						

* broken leaf tip box indicates collect extra eelgrass shoot for herbarium and samples for sediment and salinity