Final Report to Massachusetts Bays Program Research and Planning Grants

CREATING THE BASIS FOR A SUCCESSFUL RESTORATION: TEST-TRANSPLANTING MULTIPLE EELGRASS DONOR POPULATIONS IN PLUM ISLAND SOUND, MASSACHUSETTS

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Project Summary

Eelgrass meadows are both ecologically and economically valuable and have become the focus of resource management initiatives in Massachusetts. Plum Island Sound, located on the Upper North Shore, once contained acres of lush, thriving eelgrass beds that disappeared by the mid-1900s. With funding from the Massachusetts Bays Program (MBP) in 2012, our research team developed a model for Plum Island Sound that identified areas with good potential for the re-establishment and growth of eelgrass. In 2013 we began test-transplanting eelgrass at some of the most suitable sites using multiple donor sources. Shoots that were transplanted between June and August did not survive while shoots transplanted after September are still alive at sites in the central and southern portion of the Sound. The low success rates of summer transplants are attributed to multiple stressors including warm waters, poor water clarity, as well as bioturbation from a hyper-abundant European green crab population. Based on our results we recommend that the suitability of sites in this system be further evaluated before considering a large-scale restoration. We suggest that test-transplanting efforts continue and be directed towards the southern part of the system where the waters are well-flushed and we are having success with recent transplants. In addition, we suggest managing transplant sites for green crabs through trapping and fencing, as well as acquiring further information on the population structure of this invasive species to advise future eelgrass restoration and management initiatives in this system.

Introduction

Eelgrass (*Zostera marina* L.) is a temperate marine angiosperm that grows in the intertidal zone to approximately 10 m below mean low water in Massachusetts. Eelgrass meadows are highly productive communities and contribute to the coastal environment by stabilizing and enriching sediments, trapping and cycling nutrients, maintaining water quality and clarity, and providing habitat for microbes, invertebrates, and vertebrates (Heck et al., 1995; Short and Coles, 2001). In recent decades, eutrophication caused by increased nutrient loading associated with development of coastal watersheds has resulted in significant declines in eelgrass populations in Massachusetts (Valiela et al., 1992; Short and Burdick, 1996). Because eelgrass meadows are both an ecologically and economically valuable resource they have become the focus of resource management initiatives in the state, with the Massachusetts Department of Environmental Protection (MassDEP) mapping the distribution of eelgrass on a three-year cycle and several government and academic groups participating in monitoring programs (e.g., SeagrassNet, see Short et al., 2006) to assess eelgrass habitat quality for management.

Plum Island Sound is located on the Upper North Shore in northeastern Massachusetts and is part of the Great Marsh Area of Critical Environmental Concern (ACEC; Figure 1). The Sound once contained acres of lush, thriving eelgrass beds that were destroyed by multiple stressors during the mid-late 1900s (Addy and Aylward, 1944; MassGIS, 2001; Costello and Kenworthy, 2011). Although the environmental conditions are now suitable for growth, the waters are still devoid of eelgrass and the Massachusetts Division of Marine Fisheries (DMF), the Environmental Protection Agency (EPA) - Region 1, and the U.S. Fish and Wildlife Service (USFWS) at the Parker River National Wildlife Refuge on Plum Island have jointly recommended that eelgrass be restored to the Sound.

In 2012, as part of the Massachusetts Bays Program (MBP) Research and Planning Grants our research team developed a quantitative model in geographic information systems (GIS) using the formulation of Short et al. (2002) to identify and prioritize sites within Plum Island Sound for future transplanting efforts. The model assessed multiple parameters (i.e., bathymetry, sediment type, water quality and clarity, wave exposure, and the location of tidal flats and mooring fields) and weighted them according to their degree of influence on eelgrass establishment and growth. The model identified a number of sites in the Sound with good suitability for eelgrass, having scores of 8 and 16 (total area of 314 ha (776 ac) and 246 ha (608 ac), respectively; Figure 2; Novak and Short, 2012).

In 2013, we used the results of our model and began test-transplanting in Plum Island Sound at sites with the highest scores (8 and 16) as survival of test-transplants is highly indicative of how well a large-scale transplanting effort will succeed at a given site (Figure 3; Short et al., 2002, 2005). Multiple donor sources were used to identify the best donor sources for this system and to build a genetically diverse population with potentially enhanced resilience to multiple stressors, including climate change. The results of our 2013 test-transplanting efforts are discussed in this report and should be used to inform future eelgrass restoration initiatives in Plum Island Sound.

Methods

Study Area

Plum Island Sound Estuary is located in the Upper North Shore Region of the Massachusetts Bays Program and is part of the Great Marsh ACEC. The estuary is the largest wetland-dominated estuary in New England, supporting a diversity of flora and fauna, and is formed by Plum Island Sound, the Parker, Plum Island, Rowley, Eagle Hill, and Ipswich Rivers, as well as a vast network of tidal creeks (Figure 1). Plum Island Sound is relatively shallow with an average depth from 3 m (MHW) to 1.6 m (MLW) and a tidal amplitude ranging from 2.6 m to 3.6 m during the neap-spring cycle. The large amount of tidal flushing between tides makes Plum Island Sound less sensitive to nitrogen than other estuaries in Massachusetts where eutrophication has been well documented (Buchsbaum et al., 2000).

Donor Populations

Eelgrass used for test-transplanting was collected from five different locations in Massachusetts and New Hampshire. The donor source beds include:

1) Southway, Cape Cod, MA: population with relatively moderate genetic diversity (Table 1) that showed high resilience to multiple stressors in mesocosm studies (Bayley, 2012; Short et al., 2012) and high survival rates during pilot studies in East Harbor, MA (Bayley, 2012);

2) West Island, Fairhaven, MA: population with relatively moderate genetic diversity (Table 1) that showed moderate resilience to multiple stressors in mesocosm studies (Bayley, 2012; Short et al., 2012) and was the primary source for the New Bedford eelgrass restoration (Short et al., 2005).

3) Dorothy Cove, Nahant, MA: population frequently used as a donor source for restorations in MA (N. Tay Evans, pers. comm.);

4) Essex Bay, MA: closest naturally occurring population with environmental conditions similar to Plum Island Sound. The population was recently identified (Phil Calarusso pers. comm.); and,

5) Nannie's Island, Great Bay, NH: population with relatively high genetic diversity (Table 1) that showed high resilience to multiple stressors in mesocosm studies (Bayley; 2012; Short et al., 2012). The population is within the same metapopulation as other eelgrass beds in MA (Short et al., 2012).

Genetic differentiation of populations and information on genetic diversity was obtained for the eelgrass populations from Southway, West Island, and Nannie's Island prior to test-transplanting (Table 1; Bayley, 2012). Genetic analyses were not performed on shoots from Nahant and Essex Bay although samples were collected and are being stored for future analyses.

Harvesting

Approximately 650 shoots were haphazardly collected along a 50 meter transect from each of four donor sites (i.e., Southway, West Island, Nahant, and Nannie's Island) between May and August and transplanted among seven test-transplant sites in the Sound (sites 1-7; Figure 3). An additional 250 shoots were haphazardly collected from two donor sites (i.e., Nahant and Essex Bay) in September and October and transplanted among three test-transplant sites (sites 6-8; Figure 3). Collection of shoots at donor sites was spatially dispersed in the middle of the eelgrass beds to minimize impacts (~2m MWL). Shoots were removed by uprooting 3-5 cm of the rhizome and snapping the rhizome at the base of plants. Harvested

eelgrass was cleaned of epiphytes and immediately stored in a cooler with aerated seawater for less than 24 hours before being transplanted at test-sites (Davis and Short, 1997).

Test-Transplanting

Test-transplanting in Plum Island Sound was performed at a total of eight sites between June and October (Figure 3). The selection of sites was based on the results of our model (i.e., suitable sites with scores of 8 or 16; Figures 2-3), the results of pilot studies conducted in April, and water quality conditions at the time of planting. All shoots were transplanted at the same depth as donor source sites (~2MWL). Two methods were used to transplant shoots:

TERF Method

The TERF (Transplanting Eelgrass Remotely with Frame) method consists of tying mature eelgrass shoots with biodegradable ties to the cross-hairs of a frame (Short et al., 2002). Each frame is 60 cm X 60 cm and contains 25 PUs (50 shoots). In June, our collaborators and over 20 volunteers participated in test-transplanting efforts using TERFs in Plum Island Sound. Four donor populations (Southway, West Island, Nahant, and Nannie's Island) were tied in monoculture or polyculture to thirty-two TERFs and deployed among four sites in Plum Island Sound (4 monocultures and 4 polycultures per site; sites 2-4, 7; Figures 2-4).

Horizontal Rhizome Method

The horizontal rhizome method consists of anchoring two mature eelgrass shoots (PU) with a biodegradable staple. The rhizomes are aligned parallel, pointing in opposite directions, and are pressed horizontally into the top 2 cm of the sediment, and held in place with the staple (Davis and Short, 1997). The horizontal rhizome method was used to transplant shoots into four plots (1m x 1m) at five test-transplant sites (sites 1, 5-8; Figure 3) between July and October. Each plot contained 25 PUs (50 shoots) and contained shoots from different donor populations. The horizontal rhizome method was selected over the TERF method for all test-transplanting efforts after June because it required less field personnel.

Plant measurements

Test-transplant sites were monitored monthly between June and December. Shoot counts were conducted at sites when shoots were present and used to evaluate how each respective population was performing, to predict shoot survival, expansion of the transplant plot, and restoration suitability in Plum Island Sound.

Habitat quality measurements

Water samples were collected bi-monthly at three sites (sites 1,4,7) in the Sound between June and October and bi-monthly at two sites between August and November (sites 6, 8; Figure 4). Water samples were collected from the surface of the water column during a flood tide with a Niskin bottle and immediately prepared for laboratory analyses of total nitrogen, turbidity, nitrate-nitrite, and chlorophyll *a*. Detailed methods for sample collection and laboratory measurement of select water quality parameters are discussed in detail in the PCCS Quality Assurance Project Plan (QAPP; Novak, Short, and Costa 2013). Water quality data for each test-transplant site was compared to target values for eelgrass growth and survival.

Light intensity and temperature were measured underwater at two test-transplant sites (sites 4,7) between June and October, underwater at one test-transplant site between August and November (site 8), and on land at one site between June and November using HOBO pendant loggers (Onset; Figure 4).

Measurements were taken every 15 minutes and the underwater sensors were cleaned regularly to minimize interference from fouling. The percent of surface light reaching the canopy of test-transplants between (10:00–15:00) was quantified and averaged at each site and light and temperature data were compared to target values for eelgrass growth and survival.

Sediment grain size was determined at test-transplant sites following the protocol described by Folk (1974). Sediment samples were collected using a 3cm diameter core sampler. Samples were placed in 50ml plastic tubes and transported to the laboratory in ice coolers. The sediment samples were placed in 400ml beakers and cleaned from shells and debris. Samples were dehydrated in a 60°C oven until constant weight was obtained. Organic matter was oxidized using 35% (v/v) H_2O_2 for approximately 1 week until no oxidation was evident and then samples were dehydrated in a 60°C oven until constant weight was obtained. Organic matter content in the sediment samples was obtained by difference in weight after oxidation with 35% (v/v) H2O2 and dehydrating the samples to constant weight in a 60°C oven as described previously. Sediment grain size and organic content was compared to target values for eelgrass growth and survival.

Results

Plant Measurements

Approximately 3,600 shoots were harvested from four donor sources between June and October and planted at eight sites in Plum Island Sound (Figure 4). Shoots transplanted between May and August did not survive more than six weeks and 60% of the shoots (200 shoots) transplanted in September and October were still alive during December monitoring. The shoots that survived the fall transplanting were located at sites 7 and 8 and were from Nahant and Essex Bay. No additional measurements were made as restoration guidelines recommend monitoring shoot survival for one year prior to fully evaluating transplant success (Fonseca et al., 1998).

Habitat Quality Measurements

The results of measurements for select water quality parameters (total nitrogen, turbidity, nitrate-nitrite, and chlorophyll *a*), light and temperature, as well as sediment grain size and organic content can be found in Table 2. All measurements except for summer temperature and light values were within the target values recommended for eelgrass growth and survival (Batiuk et al., 2000; Lee et al., 2007; Goshorn, 2006; Wazniak et al., 2008; Yarish 2006).

Discussion

In 2013, our research team test-transplanted eelgrass at eight sites in Plum Island Sound using donor sources from New Hampshire and Massachusetts (Figure 4). Shoots that were transplanted between June and August did not survive more than six weeks while shoots transplanted after September are still alive at sites in the central and southern portion of the Sound, near Roger's Island and Sandy Point (sites 7 and 8; Figure 4). The shoots that survived at both sites were from Nahant and Essex Bay in Massachusetts. Based on our preliminary results, two of our eight sites may be good candidates for future restoration efforts in Plum Island Sound.

The time of planting appears to have played a critical role in determining eelgrass survival. Restoration guidelines suggest that eelgrass transplanting activities occur during the spring (April–June), in accordance with populations located in more northerly habitats (Fonseca, et al., 1998). We found that spring and summer transplants did not survive while fall transplants are doing well. The difference in

survival may be attributed to the presence of additional stressors during the spring and summer compared to fall months including, warmer water, poorer water clarity from a series of rain events, and more wave action from boating activity. While water quality levels were on the whole within target values for eelgrass, temperature and light levels were out of the range required for eelgrass growth and survival throughout the summer months (Table 2).

Bioturbation by the invasive European green crabs may have also had a significant impact on transplant survival. Green crab activity was observed at all of our sites and we consistently found green crabs burrowing under TERFs, shoots that had been cut by crabs, and uprooted staples at sites 2-6 (Figure 3; Figure 5). While European green crabs have been present along the Northeast Atlantic coast since the early 1800s (Say, 1817), a recent explosion of the green crab population in Nova Scotia and Maine due to warming waters and/or the introduction of a more cold-tolerant clade has caused significant declines in eelgrass and shellfish populations (Roman, 2006; Neckles et al. 2009; C. McCarthy and H. Neckles, pers. comm.) Local fishermen in Ipswich River and nearby Essex Bay both reported catch per unit efforts (CPUE) greater than 40 crabs per trap in a 24 hour period, suggesting that crabs may be hyper-abundant in this system as well.

Based on our results, we recommend continuing test-transplanting efforts in Plum Island Sound to evaluate the system for a large-scale eelgrass restoration. We suggest planting more frequently in the fall compared to spring and summer time and directing efforts towards the southern part of the system where it is well-flushed and we are having success with recent transplants. Finally, we suggest managing transplant sites for green crabs through trapping and fencing, as well as acquiring further information on the population structure of this invasive species to advise future eelgrass restoration and management initiatives.

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Table 1. Genetic and genotypic diversity information for three eelgrass donor populations used for test-transplanting in Plum Island Sound (Bayley, 2012). Genotype data was acquired from Short et al. (2012) and used to calculate genetic diversity parameters[1]. Samples for from Nahant and Essex Bay have been collected for genetic analyses but have not been analyzed.

	Nannies Is.	Southway	West Is.
Site			
N	22	30	22
No. genets determined	22	30	21
Genotypic diversity	1.00	1.00	0.95
Mean Allelic richness	4.61	3.95	3.20
Allelic richness per locus			
GA 12	3.77	2.35	1.00
GA 20	5.63	3.80	3.75
GA 17D	3.75	3.95	3.74
GA 19	2.50	2.00	1.00
GA 16	4.64	2.96	2.95
GA 2	5.75	5.35	6.93
GA 23	6.27	7.25	2.99
Mean H_O	0.55	0.36	0.27
Mean UH_E	0.59	0.45	0.37
H_O per locus			
GA 12	0.23	0.17	0.00
GA 20	0.68	0.27	0.43
GA 17D	0.36	0.40	0.38
GA 19	0.50	0.40	0.00
GA 16	0.59	0.33	0.24
GA 2	0.77	0.43	0.48
GA 23	0.68	0.53	0.33
Mean F_{is}	0.08	0.20	0.29
F_{is} per locus			
GA 12	0.23	0.37	NA
GA 20	0.00	0.19	0.14
GA 17D	0.45	0.00	0.19
GA 19	-0.02	0.16	NA
GA 16	-0.15	0.03	-0.07
GA 2	-0.07	0.17	0.40
GA 23	0.12	0.35	0.47

[1] Genetic and genotypic diversity information for Nannie's Island (NH), Southway (MA), and West Island (MA) was calculated using muli-locus genotype data (MLG) from Short et al. (2012) and analyzed by Bayley (2012). The data was derived from seven DNA microsatellites (GA 2, GA 12, GA 16, GA 17D, GA 19, GA 20, GA 23). Basic genetic diversity, including unbiased expected heterozygosity (UH_e), observed heterozygosity (H_o) and inbreeding (F_{is}), was calculated in FSTAT (Goudet, 1995). Allelic richness (*A*) was also estimated in FSTAT and was based on a minimum sample size of 11 individuals, using rarefaction because sample sizes were unequal (Leberg, 2002). Pairwise F_{st}, a measure of differentiation between populations, was calculated in GENPOP with significance determined at the 95% ($p < 0.05$) probability level after Bonferoni correction (Rousett, 2008). Genotypic diversity was determined by dividing the number of genets detected by the number of ramets sampled (Olsen et al. 2004).

Table 2. Comparison of recommended values for survival and growth of eelgrass to values measured in this study. Light and temperature data were averaged for two week intervals. The start of each interval is listed on the table.

		Turbidity (NTU)	Total Nitrogen (uM)	Total Phosophorus (uM)	Dissolved Inorganic Nitrogen (uM)	Dissolved Inorganic Phosphorus (uM)	Chl *a* (ug/L)	Water Column Light (%)	Temperature (°C)	Sediment Organic Content (%)	Sediment Grain Size (%)
		Target Values									
		<5.38 NTU (Goshorn, 2006)	<45.7 uM (Wazniak et al., 2008)	<1.19 uM (Wazniak et al., 2008)	< 2.2 uM (Yarish et al., 2006)	<0.64 uM (Yarish et al., 2006)	<5.5 ug/L (Yarish et al., 2006)	>22% (Batiuk et al., 2000)	< 23.3 °C (Lee et al., 2007)	< 5% (Yarish et al., 2006)	< 20% silt clay (Yarish et al., 2007)
Site	**Date**										
7	07/18/2013	1.80	24.16	0.81	0.88	0.34	5.06	**<10%**	**18-26**	0.3	0.3
4	07/18/2013	2.71	26.60	1.61	0.22	**0.78**	**5.65**	**20%**	**19-32**	0.4	0.2
7	07/29/2013	1.29	24.16	0.81	0.88	0.34	NA	**<10%**	20-23	NA	NA
4	07/29/2013	1.66	22.63	1.00	1.14	0.49	4.61	**21%**	**20-29**	NA	NA
7	08/15/2013	1.43	20.85	0.95	0.34	0.48	1.95	**<10%**	17-22	NA	NA
4	08/15/2013	1.88	29.79	**1.49**	0.65	0.60	3.56	**20%**	16-20	NA	NA
1	08/15/2013	2.25	38.76	**1.72**	0.43	0.59	3.46	NA	NA	0.5	0.8
7	08/25/2013	1.81	25.98	1.17	1.03	0.37	1.83	**<10%**	15-23	NA	NA
4	08/25/2013	2.04	27.24	1.01	0.85	0.52	2.54	**20%**	**19-25**	NA	NA
1	08/25/2013	2.14	29.91	1.27	0.84	**0.68**	NA	NA	NA	NA	NA
7	09/12/2013	2.57	21.54	1.08	0.37	0.48	2.78	30%	11-20	NA	NA
4	09/12/2013	1.12	25.74	1.02	0.33	0.57	NA	**<10%**	16-20	NA	NA
1	09/12/2013	**21.53**	37.83	**3.18**	1.36	**0.97**	NA	NA	NA	NA	NA
7	10/10/2013	1.70	18.04	1.07	0.18	0.23	3.56	28%	12-15	NA	NA
4	10/10/2013	2.38	24.55	0.86	0.17	0.62	3.06	30%	16-18	NA	NA
8	10/10/2013	1.59	22.00	0.86	0.23	0.29	2.38	31%	NA	0.5	0.8
7	10/30/2013	NA	20.95	0.66	0.48	0.26	NA	26%	7-11	NA	NA
4	10/31/2013	NA	NA	NA	NA	NA	NA	NA	12-16	NA	NA
8	10/30/2013	0.57	13.84	0.66	0.33	0.17	NA	30%	12-16	NA	NA
8	11/18/2013	NA	NA	NA	NA	NA	NA	30%	12-16	NA	NA

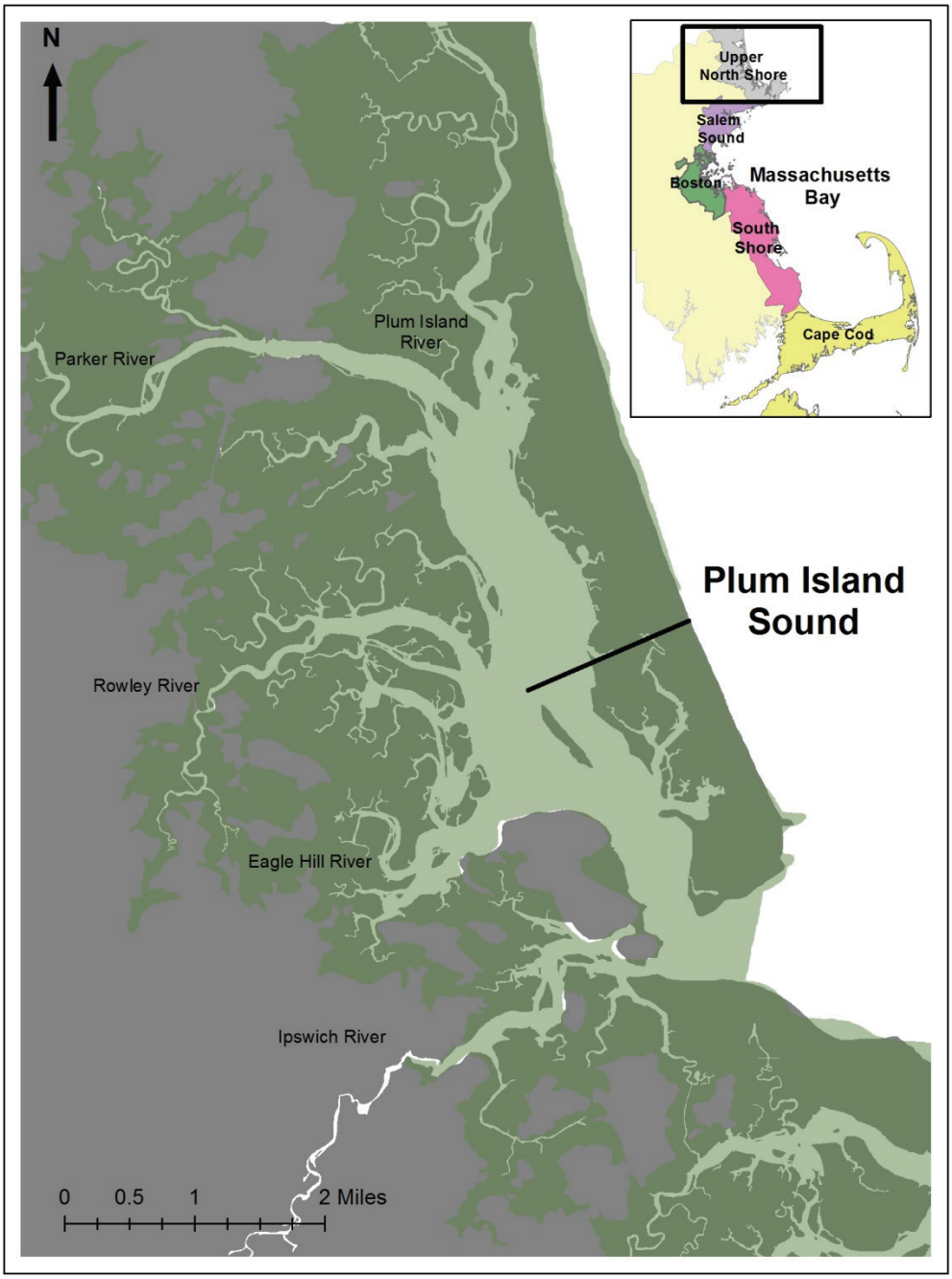

Figure 1: Plum Island Sound Estuary is located in northeastern Massachusetts in the Upper North Shore Region of the Massachusetts Bays Program (inset) and is part of the Great Marsh ACEC (boundary denoted in green). The estuary is formed by Plum Island Sound, the Parker, Plum Island, Rowley, Eagle Hill, and Ipswich Rivers, as well as tidal creeks. Eelgrass test-transplanting efforts focused on areas within the Sound.

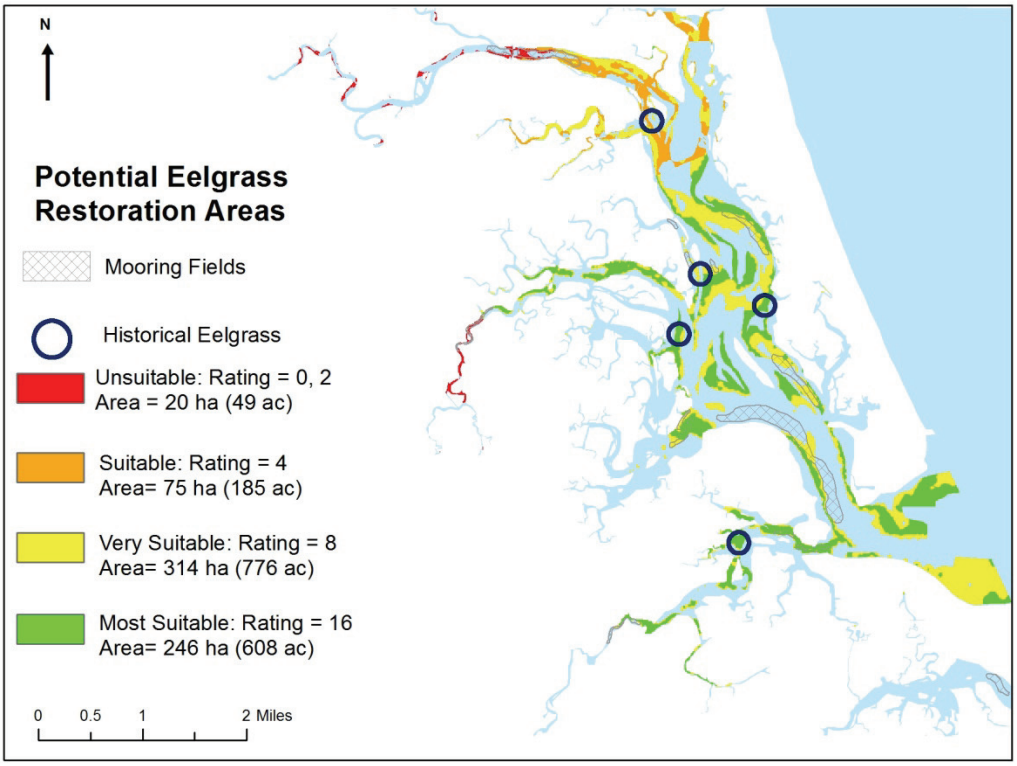

Figure 2. Map identifying the suitable areas for an eelgrass restoration in Plum Island Sound. The parameters of bathymetry, sediment type, water quality and clarity, wave exposure, and the location of tidal flats were used in a model to produce this map. Mooring fields and approximate locations of historical eelgrass beds are also shown.

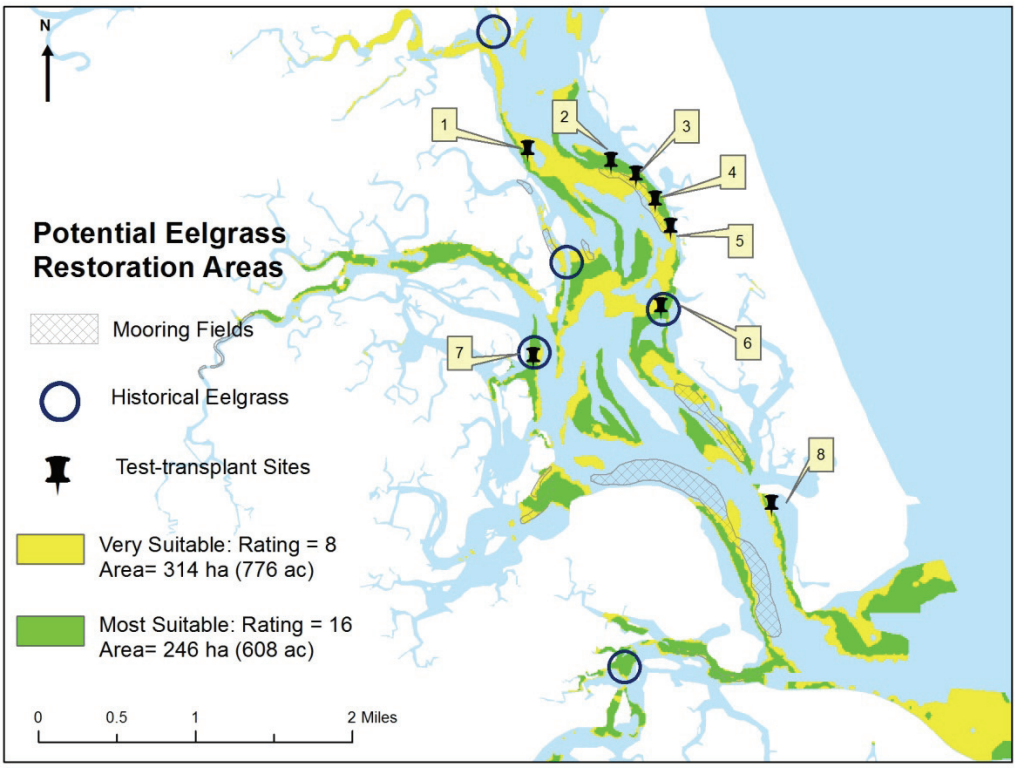

Figure 3. Map showing the location of eelgrass test-transplant sites (black push-pin). All test-transplant sites were in areas identified by the model as very suitable (yellow areas; rating= 8) or most suitable (green areas; rating=16). Mooring fields and approximate locations of historical eelgrass beds are also shown.

Figure 4. Collaborators and students tying eelgrass to TERFs in 2013.

Figure 5. Multiple green crabs in eelgrass transplant plots at site 5.