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Successful Eelgrass (*Zostera marina*) Restoration in a Formerly Eutrophic Estuary (Boston Harbor) Supports the Use of a Multifaceted Watershed Approach to Mitigating Eelgrass Loss

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Abstract From a watershed perspective, Boston Harbor, MA, USA is an ideal site for eelgrass restoration due to major wastewater improvements. Therefore, by focusing on site selection and transplant methods, high survival and expansion rates were recorded at four large eelgrass-restoration sites planted in Boston Harbor as partial mitigation for a pipeline construction project. Transplanted sites met and exceeded reference and donor bed habitat function after 2 years. Hand planting and seeding in checkerboard-patterned transplant plots were efficient and effective methods for jump-starting eelgrass growth over large areas. Although restoration through planting can be successful, it is highly site specific. Even using a published site-selection model, intensive fieldwork was required to identify sites at fine enough scale to ensure successful planting. Given the effort required to identify scarce potential sites, we recommend that future focus includes alternative mitigation strategies that can more adequately prevent eelgrass loss and address water quality degradation which is the leading cause of dieback, site unsuitability for planting, and lack of natural re-colonization.

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Introduction

Eelgrass (*Zostera marina*) is the dominant marine plant in coastal waters of New England. Its ecosystem value is well-documented. Eelgrass acts to stabilize sediment, buffer wave energy, and provide habitat for juvenile fish and shellfish (Stauffer 1937; Orth et al. 1984; Heck et al. 1989; Hughes et al. 2002; Lazarri and Tupper 2002). Eelgrass throughout the North Atlantic was devastated by wasting disease in the 1930s (Tutin 1942) and again in some regions in the 1980s (Short et al. 1986). It enjoyed somewhat of a resurgence once the disease abated, but despite its capabilities for re-populating vegetatively or from existing seed stock (Harwell and Orth 2002; Frederiksen et al. 2004), the continued decline of this important marine plant has been tracked throughout its range (Jacobs 1979; Short et al. 1986; Valiela et al. 1992; Short and Burdick 1996, C. Costello MA DEP, unpub. data, 2009, Paling et al. 2009). It is thought that eelgrass growth has been thwarted by degraded water quality from coastal development and by physical disturbance (Valiela et al. 1992; Valiela 1995; Koch 2001).

In Massachusetts, 90% of embayments have experienced declines in eelgrass cover since 1995 (C. Costello MA DEP, unpublished data, 2009), coincident with increases in nutrient concentrations from septic systems, fertilizer, and atmospheric deposition (Valiela et al. 1997; Cloern 2001; Hauxwell et al. 2001, 2003). This correlation suggests that loss of eelgrass in embayments may be a result of eutrophication. The relationship between eutrophication and eelgrass loss is driven primarily by light attenuation from enhanced algal growth (Kemp et al. 1983; Valiela et al. 1992; Short and Wyllie-Echeverria 1996; Hauxwell et al. 2001, 2003; Cardoso et al. 2004; Fox et al. 2008). Because of increased coastal development and its deleterious effect on water quality and eelgrass, the number of sites coast-wide where eelgrass can grow, but does not already, is limited (Fredette et al. 1985, Paling et al. 2009). In fact, two intensive searches of the Massachusetts coast were recently conducted by The Nature Conservancy and a private consultant. Despite hundreds of thousands of dollars spent on site selection and test transplants, only a few potential areas were identified as even possible to support eelgrass. None have yet been planted at a large scale. Most areas are not likely to be suitable candidates for restoration until eutrophication is remediated (Paling et al. 2009).

Eelgrass can return to an area if conditions improve, but this process can take decades (Frederiksen et al. 2004; Paling et al. 2009 and references therein). In addition, physical and biological changes in the water column and seafloor caused by dredging, construction, storms, or disease-related eelgrass dieback may not revert back to original conditions once pressures are removed, and may inhibit natural re-vegetation (Rasmussen 1977; Duarte 1995; Short et al. 2002a; Munkes 2005; Krause-Jensen et al. 2008; Duarte et al. 2009; Paling et al. 2009). Sediment composition, for example, can be altered by these processes and become unsuitable for eelgrass (Koch 2001). Also, depending on wind and current patterns, otherwise suitable areas may be isolated from potential seedbeds as eelgrass grows scarce (Frederiksen et al. 2004).

In order to jump-start eelgrass growth along the east coast of the USA, and to mitigate for direct impacts caused by construction, eelgrass restoration is fast becoming a commonly employed management tool, as it can potentially decrease the time needed for eelgrass to be re-established. Instead of simply restoring where eelgrass was once known to grow, careful site-selection is recognized as an essential precursor to any restoration project (Fonseca et al. 1998; Short et al. 2002a; Kopp and Short 2003; Palmer 2009). In Boston Harbor, Massachusetts, significant water quality improvements were made as a result of the installation of the Deer Island secondary wastewater treatment facility and outfall pipe from 1991 to 2000. Also, it was determined that natural repopulation of eelgrass in the harbor was unlikely since tide and wind-driven current patterns would prevent reproductive shoots from reaching many areas of the estuary from existing beds (Signell and Butman 1992; Leschen et al. 2009). Therefore, the harbor was targeted for active eelgrass restoration.

The Massachusetts Division of Marine Fisheries (MDMF) conducted an eelgrass restoration project from spring 2004 to fall 2007 with financial support from a mitigation requirement for seafloor impacts associated with the construction of the HubLine natural gas pipeline in Massachusetts Bay. The project purpose included the examination of issues associated with site selection and transplant methodology and, finally, the restoration of eelgrass at suitable locations in Boston Harbor. This effort resulted in a better understanding of the limits to eelgrass restoration and the importance of a watershed approach that incorporates other strategies that may in the long run be more effective at mitigating eelgrass loss. In this paper, we describe our restoration project in Boston Harbor and then propose management recommendations for future restoration and mitigation efforts for impacts to eelgrass.

Study Site

Boston Harbor is a relatively shallow (4.9 m average depth) estuary located on the western edge of Massachusetts Bay within the Gulf of Maine (Fig. 1). The 125 km^2 surface area is broken up by numerous small islands. It is a mesotidal system with an average tidal range of 2.7 m (Signell and Butman 1992). Geometry of the estuary mouth, combined with regional bathymetry, results in an ebb tide-dominated system, with net flushing of water from within the harbor on each outgoing tide (Signell and Butman 1992). The harbor is a mixed-energy system, with variable tide- and wind-driven current patterns throughout the estuary (Signell and Butman 1992; Knebel 1993). Sediment within the

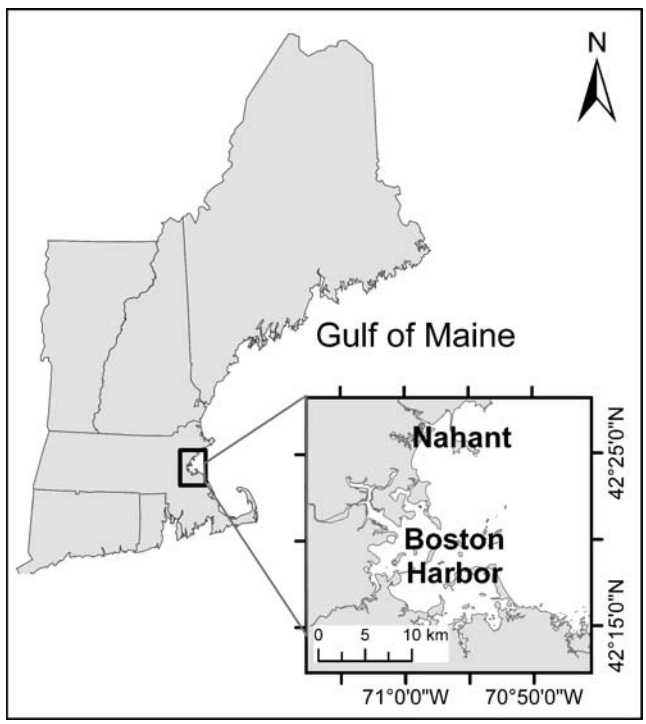

Fig. 1 Study site in Boston Harbor, and donor bed location in Nahant, MA, USA

harbor is dominated by fine-grained sediment (silts, clayey silts, and sandy silts) in a patchy distribution. Due to underlying glacial deposits, gravel is also found within the Harbor (Knebel et al. 1991; Knebel 1993; Knebel and Circé 1995; Diaz et al. 2008).

An urban estuary, Boston Harbor is surrounded by a population of 2 million people. Prior to 1991, Boston Harbor received 11,400 t C annually (Taylor 2006; Diaz et al. 2008). As part of a massive wastewater collection and treatment project for the Boston area, sludge discharge ceased in 1991. Over the next 9 years, effluent treatment was upgraded to secondary; in 2000, treated wastewater discharge was diverted from within Boston Harbor to an area 15 km offshore in Massachusetts Bay. There was a resulting 90% decrease in loading to 1,200 t C year^{-1}. Intensive studies conducted by the Massachusetts Water Resources Authority, United States Geological Survey (USGS), and others have led to a good understanding of the impacts of the wastewater system upgrades on water and sediment quality in the harbor. As of 2006, almost all water quality parameters improved within the harbor (Table 1; Taylor 2006), in most cases meeting current thresholds for eelgrass requirements where these have been established (Batuik et al. 2000). Sediment grain size increased and oxygen demand and flux of nutrients from the sediment decreased (Tucker et al. 2006; Diaz et al. 2008). These changes are indicators of reduced organic loading and improved health of the ecosystem and were more rapid in better flushed areas (Tucker et al. 2006; Diaz et al. 2008). Boston Harbor presented an excellent opportunity to assess eelgrass restoration potential in an area that was subjected to significant eutrophication, but where recent wastewater management upgrades achieved major sediment and water quality improvements.

The MDMF undertook an eelgrass restoration project in the Harbor with the goal of jump-starting the re-colonization of eelgrass to this recovering embayment. Eelgrass surveys conducted by the Massachusetts Department of Environmental Protection (DEP) Wetlands Conservancy Program in 1996 and 2001 showed four beds in the harbor. By 2006, acreage in the harbor had declined by 42% from 1996 levels (Charles Costello, MA DEP, unpublished data). Because of the slow and patchy nature of recovery (Tucker et al. 2006) due to the physical and ecological changes that had occurred in the harbor from years of degradation and the fact that natural beds were still declining despite overall water quality improvements, it was recognized that rigorous attention to site selection would be essential to ensure restoration success.

Boston Harbor is dominated by fine-grained sediment. Existing eelgrass beds often contain very fine sediment (Wanless 1981; Smith et al. 1988), largely due to the trapping and settling of suspended particles by leaves extending into the water column. The accumulation of organic matter and inability of oxygen to diffuse very far into fine sediments often creates anoxic sediment below 1-2 cm (Klug 1980; Thayer et al. 1984; Huettel and Gust 1992). Nevertheless, very fine-grained sediment in unvegetated areas may be problematic for eelgrass transplants. Fine-grained sediment is easily re-suspended, and can increase light attenuation, and the reducing environment can stress transplants (Kuhn 1992; Koch 2001). Existing sediment guidelines for eelgrass transplanting range from a silt/clay fraction of <20% (Koch 2001) to <70% (Short et al. 2002a). Tucker et al. (2006) and Diaz et al. (2008) found variation in sediment grain-size composition and redox chemistry between sampling stations in the harbor, though far less so than right after outfall diversion. These findings,

Table 1 Summary of differences in selected water quality parameters in Boston Harbor at baseline and 5 years after outfall went online

Variable	Percent change at 5 years	2005 value	Recommended requirements for eelgrass
Total nitrogen (μmol l^{-1})	−35	20.2±2.9	NA
Dissolved inorganic nitrogen (mg l^{-1})	−55	0.074±.049	<0.15 (mg l^{-1})
Total phosphorus	−28	1.48±0.31	NA
Dissolved inorganic phospohorus	−38	0.02±.0084	<0.02 (mg l^{-1})
Total chlorophyll-*a* (μg l^{-1})	−26	4.8±2.4	<15
Total suspended solids (mg l^{-1})	**5**	3.8±1.1	<15
Percent organic carbon as percent TSS	−33	12±3	NA
Secchi depth (m)	4	2.7±0.70	NA
Dissolved oxygen (mg l^{-1})	5	8.9±1.3	NA
k (m^{-2})	**1**	0.53±0.12	NA

Adapted from Table 1 in Taylor (2006). All differences are considered “improvements” for eelgrass except those in bold. Recommended requirements for eelgrass (Batuik et al. 2000) are provided for comparison where available and the wide range of recommended grain-size in the literature, underscore the need to conduct sediment analysis as a component of site selection. Such data would better characterize sediment on a localized scale and define the suitable range for eelgrass restoration within Boston Harbor.

Methods

Site Selection

To focus planting efforts on areas most likely to support eelgrass, environmental data specific to Boston Harbor were acquired (Leschen et al. 2009). The site-selection model produced by Short et al. (2002a), hereinafter the "Short model," was modified and adapted to a Geographic Information System (GIS) analysis (ESRI® ArcMap™ 9.2; Leschen et al. 2009). This analysis was based on 100-m grid cells covering the Boston Harbor area. Six parameters were input to each cell: depth, exposure to northeast winter storm winds, historical eelgrass distribution, current eelgrass distribution, water quality, and sediment type from USGS seafloor maps (USGS Open File 99-439). Parameters were assigned scores ranging from 0 to 2 (2 = most suitable for eelgrass growth) based on retroactive analysis of a restoration project, literature values, or from conditions at existing local reference eelgrass beds described in the Short model. All the parameter scores were then multiplied to get a preliminary transplant suitability index (PTSI) score (Short et al. 2002a) for each cell. Since the model employed a multiplicative index, a score of zero (0 = unsuitable) for any one parameter eliminated the site from further consideration, whereas high parameter scores made it more likely to support eelgrass. The PTSI results focused the search for suitable sites, thus reducing the number of areas requiring further investigation.

As required under the Short model, these potential transplant sites were groundtruthed in the field for actual depth, presence of human influence such as marinas and mooring fields, presence of bioturbators, and sediment type. An Atlantis underwater camera and a Ponar grab sampler were used to conduct a general characterization of sites identified by the model. Sites that were rocky, had high density of macroalgae or were gravelly were eliminated from further consideration. This allowed us to target our diver effort on sites where uncertainty existed. Cores taken by divers, stratified into the top 5 cm and below 5 cm, were analyzed for grain size using standard sieve methods described in Poppe et al. (2000). If observed sediment type was acceptable and high model scores were otherwise upheld after groundtruthing, the area was selected for test transplanting.

This high resolution groundtruthing resulted in twelve potential transplant sites, and all 12 received test transplants (Fig. 2).

Planting Methodology

Transplanting

Test transplanting began after potentially suitable sites had been identified. An existing bed in Lynn Harbor, Nahant, approximately 11 km north of Boston Harbor, was adopted as the primary donor site after investigation confirmed it was extensive and dense (263 ha, 436±24 shoots m^{-2}). Eelgrass was harvested by divers either in small clumps using a garden trowel (Susan Tuxbury, Save the Bay, RI, personal communication, 2004) or by snapping off one shoot at a time with 3-5 cm of rhizome attached (Davis and Short 1997).

We chose to plant with several methods, frames and hand planting, to test the success and efficiency of each. In addition, frame planting enabled the use of groups of on-shore volunteers, satisfying an outreach/education component of our project. We also used dive volunteers for hand planting, but the pool of candidates was more limited, therefore reducing outreach benefit. Twelve sites received preliminary small-scale test transplants in TERFs™ (Short et al. 2002b), weighted wire mesh frames to which eelgrass shoots were tied (four TERFs™, 50 shoots each, 200 total shoots, $site^{-1}$). Based on results at these sites, a subset of areas was chosen for medium-scale test transplants (1,000 shoots). Medium-scale planting occurred using a combination of TERFs™, a polyvinyl chloride (PVC)/jute frame we developed as an adaptation to TERFs™ (Leschen et al.

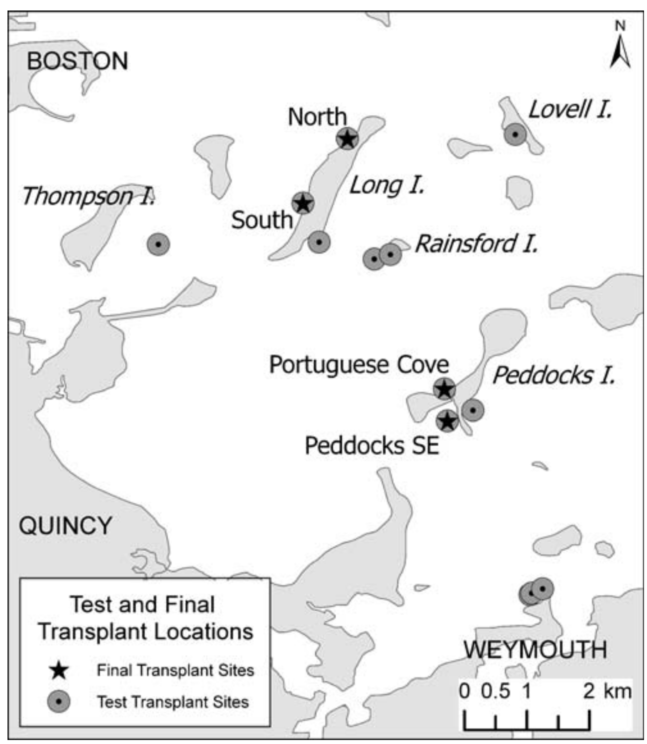

Fig. 2 Test and final transplant locations in Boston Harbor, MA, USA

2009), and hand planting. Hand planting was primarily done by anchoring clumps of shoots using bamboo staples (barbeque skewers bent in half; Davis and Short 1997).

Large-scale plantings (3,600-7,200 shoots) were done at transplant sites judged successful using hand-planted squares and improved PVC/jute frames. This final version of frames consisted of a 0.25 m^2 square of PVC pipe, with jute landscape mesh stretched over the frame and held in place with PVC and plastic cable ties (Fig. 3). Eelgrass shoots were tied to the jute by volunteers. Galvanized spikes (25.4 cm) were driven through holes drilled in two corners of the frame to anchor it, and bamboo staples helped hold the jute taut against the seafloor. After eelgrass had rooted, jute was cut away along the inside edges of the frame and left in place. PVC frames were retrieved for re-use. Both hand-planted and frame quadrats were arranged in a checkerboard grid pattern by alternating eighteen planted and unplanted 0.25 m^2 quadrats (total plot 3× 3 m). The planted squares contained approximately 50 shoots each (total plot 900 shoots). This pattern was adapted from a strategies used by Save the Bay in Rhode Island, University of New Hampshire (RK&K Engineers 2003), the Maryland Department of Natural Resources, and others; it is designed to cover more ground than continuous planting of shoots while providing voids for eelgrass to fill in. Initially, four to eight grid plots, spaced 30-50 m apart, were planted at each large-scale site (Fig. 4). More were added later.

Seeds

Eelgrass reproduces sexually by producing seeds as well as spreading asexually by rhizome expansion. To determine if

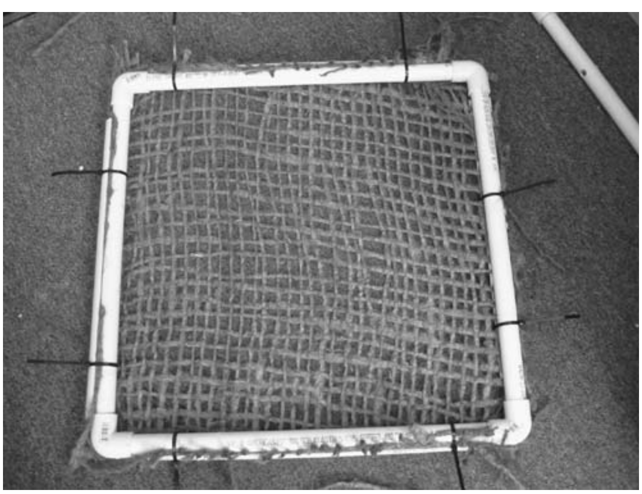

Fig. 3 Modified PVC/jute frame developed by MDMF for planting eelgrass. Eelgrass shoots can be tied to intersections of the jute by shore side volunteers. After eelgrass roots, frames are cut away from jute and removed for re-use, leaving jute behind

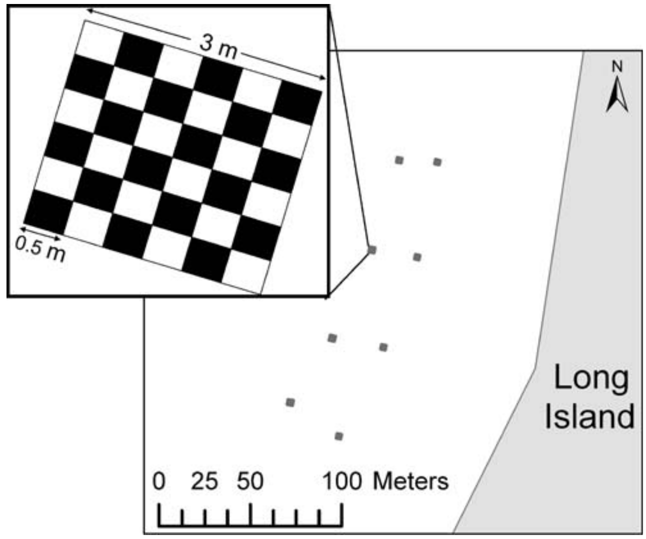

Fig. 4 Planting pattern showing checkerboard of alternating planted (*black*) and unplanted (*white*) 0.25 m^2 quadrats at a typical large-scale transplant site. Planted quadrats received 50 shoots each. Quadrats formed a 3 m×3 m plot. Plots were spaced 30-50 m apart

we could successfully grow eelgrass from seed, 12 fish totes of flowering shoots were harvested from Lynn Harbor in Nahant (Fig. 1) in July 2005 (Orth et al. 1994; Granger et al. 2002; Leschen et al. 2009). Shoots were maintained in flow-through seawater tanks at the Marine Biological Laboratory in Woods Hole for approximately 6 weeks until seeds ripened and dropped from the leaves. Thereafter, vegetation was discarded and seeds were sieved from detritus (Granger et al. 2002). Approximately 300,000 seeds were collected and distributed in late fall at three sites to complement the shoot planting. Divers brought pre-measured quantities of seeds to the bottom in small Ziploc™ bags and scratched seeds into the sediment using a small garden claw at two of the sites; at a third site, seeds were simply broadcast from the boat.

Restoration Monitoring

Major planting efforts occurred in 2005 and 2006. Complete monitoring occurred in 2006 and 2007; thus, we collected data from a minimum of 1 and 2-year-old beds. Several procedures were undertaken to determine success of eelgrass restoration efforts. Shoot density and size of randomly selected plots were measured at least once per year for the duration of the project to assess survival and expansion. Density was based on the mean shoot density count ± standard deviation from nine randomly selected 0.25 m^2 quadrats in each randomly selected sample of plots at each site. Mean areal cover was derived from measurements in perpendicular directions of individual plots using the outermost shoots as start- and end-points. This level of monitoring continues on an annual basis.

Biological monitoring was conducted for 2 years with the objective of comparing habitat function of our transplant sites to that of pre-existing natural beds and an unvegetated control site (Homziak et al. 1982; Heck et al. 1989; Fonseca et al. 1990; Heck and Wilson 1990). Faunal habitat use was measured as epibenthic/demersal and infaunal fish and invertebrate abundance (N), species richness (S), Pielou's evenness (J') and Shannon diversity (H'; Pielou 1966; Pearson and Rosenberg 1978; Krebs 1999). We selected several easily measured proxies to evaluate habitat function: provision of three-dimensional structure was measured as shoot density, two-sided leaf area index (LAI), canopy height, and above-ground peak eelgrass biomass (Short et al. 2000; Evans and Short 2005). Data were collected from a pre-existing Boston Harbor natural reference bed (Hull—which has since been declining), our donor bed in Nahant (considered an ideal healthy reference bed), our beds transplanted in 2005 and 2006, and an unvegetated control site near some of the planted sites. In 2007, we also conducted monitoring at one of the areas seeded in 2005.

To measure benthic and demersal species, ten 1 m^2 quadrats were distributed randomly within each site. Sampling of quadrats was delayed for a minimum of 0.5 h after placement to allow any disturbed fish and invertebrates to return to the area. Using two divers, a visual SCUBA survey was employed at all sites to assess the species present in each quadrat. Pratt and Fox (2001) found that underwater visual transects sampled more species than gillnets in medium and heavy macrophyte cover.

To measure infaunal abundance and diversity, 20 flow-through cores (4.9 cm diameter) were sampled to a depth of 15 cm. Samples were taken by divers from well-distributed, haphazard locations within each site. At vegetated sites, all cores were taken where eelgrass was growing.

Cores were sieved (0.5 mm mesh; Eleftheriou and Holme 1984; Tetra Tech, Inc. 1987; Mueller et al. 1992) and samples were fixed in ambient seawater with buffered formalin (4 oz borax per gallon 40% formaldehyde) and Rose Bengal stain solution (4 g/L; Holme and McIntyre 1984; Mudroch and MacKnight 1994; Raz-Guzman and Grizzle 2001). Samples were sorted and identified to species level where possible by ENSR, Inc., Woods Hole, MA, USA.

StatsDirect statistical software version 2.6.5 was used to test fauna and habitat structure with the Shapiro-Wilk *W* test for non-normality. The non-parametric Kruskal-Wallis test was employed for each parameter and for all pair-wise comparisons (between sites in each year, and between years for each site) to determine significance at α= 0.05. Shannon diversity index and evenness were calculated using Excel Statistical Add-In.

Results

Planting and Monitoring

In spring of the first year, 2005, small-scale test transplants in TERFs™ were placed at the 12 selected sites. Shoot survival after 6-8 weeks ranged from 5-90%. In four cases, reasons for failure were obvious and were due to the following factors: more macroalgae or gravel than had been detected in original surveys, stronger currents than anticipated, and heavy weekend boat traffic and anchoring not apparent during our weekday surveys. At another four sites, grass survival was extremely low and remaining grass looked very unhealthy. So we compared grain size composition among existing beds, successful transplant sites and the latter four that failed. Sites with 35% or less silt/clay were successful. Those with >57% silt/clay failed (Fig. 5). The remaining four sites showed good shoot survival and were selected for medium-scale test transplants that were conducted in late spring, 2005. Long Island South (LIS), Peddocks SE, Portuguese Cove, and Long Island North (LIN) were each planted with TERFs™, PVC/jute frames, and hand planting. At the end of the summer, we examined the health of the eelgrass and subjectively assessed the impact of the transplant methods themselves. For example, in several cases the TERFs™ attracted burrowing crabs which uprooted most transplants, but hand-planted eelgrass was healthy. Prototype PVC/jute frames had shifted on the ground due to inadequate anchoring, but remaining grass in them was healthy. Therefore, despite low overall shoot survival in some cases, we decided to keep these sites for large-scale transplants

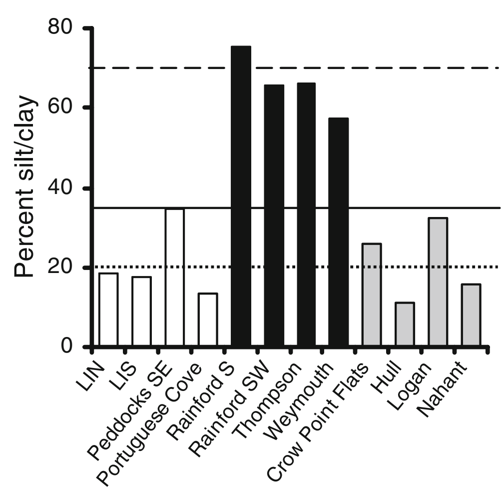

Fig. 5 Percent silt/clay at successful (*white bars*) and failed (*black bars*) transplant sites, and existing beds (*gray bars*). Top (*dashed*) line is recommended maximum per Short model. Middle (*solid*) line is maximum found at our successful sites. Bottom (*dotted*) line is maximum recommended by Koch (2001)

Table 2 Mean density and areal cover of our planted plots from 2005-2009

	2005	2006	2007	2008	2009[a]	Percent change
Density (.25 m^{-2})						
LIS	48	90	122	69[a]	56	17
Peddocks	57	84	73	73	60	4
LIN	NP	43	47	92	101	133
Port Cove	NP	34	83	99	83	143
Areal cover (m^2)						
LIS	9	16	25	27	41	351
Peddocks	9	17	21	34	39	329
LIN	NP	22	18	30	43	98
Port Cove	NP	14	20	30	44	206

[a] Density decreased because plots had spread and merged; thus measurements were taken over a larger area, some of which was still sparse

NP not planted yet—these two sites were first planted in 2006; *LIS* Long Island Southwest; *LIN* Long Island North; *Port Cove* Portuguese Cove off Peddocks Island

but eliminated TERF™ use. We also modified our PVC/jute frames and their anchoring system as previously described in the Methods section.

In fall of the first year, 2005, large-scale transplants using a combination of hand planting and PVC/jute frames were done at LIS and Peddocks SE. Due to time constraints that fall, LIN and Portuguese Cove were not planted until the spring of 2006. LIS also received further planting in 2006 and the two plantings are hereafter distinguished as LIS (05) and LIS (06). Eelgrass plots planted in 2005 were evaluated for shoot density and expansion in spring 2006 through 2009. By summer 2007, all plots looked healthy and most showed substantial shoot density increases and areal expansion (Table 2). When plots were measured in September 2007 at LIS, it was no longer possible to distinguish planted and test-seeded plots from one another. In 2009, beds at all sites continued to expand areally. Overall density was variable because sampling included newly colonized areas that were still sparse where the beds had merged and at the edges of the beds.

Overall, our planted sites were comparable to or exceeded the natural beds and control site after 2 years as measured by biomass and density (Tables 3 and 4), two structural proxies chosen to illustrate development of our planted beds (Short et al. 2000). This timeframe is slightly shorter than other studies that have shown 3-4 years to reach equivalency (Evans and Short 2005). Biomass at our beds increased over the 2-year monitoring period, whereas it declined at the existing beds. We believe the large reduction in density at Nahant between the years was due more to coincidental choice of an exceptionally dense area with tall plants for monitoring in 2006 (sampling areas were selected haphazardly within the large bed area), rather than an overall decline in the bed in 2007; that meadow continues to be expansive and healthy looking, but some areas are denser and taller than others. Hull, on the other hand, significantly declined bed-wide. Other parameters measured (LAI, canopy height) also indicated the restored sites were structurally robust (Leschen et al. 2009).

Overall, diversity indices for our planted sites were comparable to or exceeded those of natural beds at Nahant and Hull and the unvegetated control site after 2 years. From 2006 to 2007, H' index for epibenthic and demersal fish and invertebrates increased at all sites (Table 5); by 2007, our 2-year-old beds generated indices which exceeded those at Nahant and Hull (there is no comparative data for the seed bed because it was first assessed in 2007). Evenness increased at our planted sites and the control site; there was little change at Hull or Nahant. Data presented do not include *Mysis* spp. since they can number in the hundreds or thousands and greatly influence all indices.

Total number of species (S) showed less variation than diversity between years at our planted sites. It did not change at Peddocks, but increased slightly at all other sites. Total number of species at planted sites approached or

Table 3 Biomass and density, two measures of structure, in 2006 and 2007

Site	2006	2007	Percent change	2006	2007	Percent change
	Biomass (g 0.125 m^{-2})			Density (0.25 m^{-2})		
Nahant	27.0	21.4	−20.9	209.4	75.4	−64.0
Hull	13.2	2.3	−82.5	78.1	23.7	−69.7
Peddocks	17.7	30.4	71.6	89.5	121.9	36.2
LIS05	14.5	24.7	70.8	83.9	73.2	−12.8

LIS05 Long Island South planted in 2005. Hull is a remnant, but failing, Boston Harbor bed; Nahant is a healthy reference bed; Control (an unvegetated area near LIS05) is not included as it is by definition zero

Table 4 Habitat structure: significance of interactions between sites monitored in 2006 (*light gray*), in 2007 (*dark gray*), and at each site between 2006 and 2007 (*white*)

Aboveground biomass

	Nahant	Hull	Peddocks	LIS 05
Nahant	0.4406	0.0933	0.5819	0.2452
Hull	0.0065**	0.0004**	0.8239	0.9999
Peddocks	0.6566	0.0021**	0.0209*	0.7769
LIS 05	0.999	0.0112*	0.7506	0.0588

Shoot density

	Nahant	Hull	Peddocks	LIS 05
Nahant	0.0001**	0.0022**	0.0067**	0.0022**
Hull	0.0221*	0.0005**	0.9714	0.8624
Peddocks	0.0134*	0.003**	0.0263*	0.9999
LIS 05	0.9993	0.0499*	0.0447*	0.241

*$P<0.05$, significant difference

**$P<0.01$

exceeded reference levels at the natural beds in Nahant and Hull and the unvegetated control site by 2007. Nahant, Hull, and control site data exhibited slight increases in species number across years.

The patterns of diversity for infaunal species are less clear than for epibenthic/demersal species. S, H', and J' remained highest at Nahant after 2 years (Table 6).

Transplanting

Within a year, it was impossible to tell the difference between plots planted with different methods (hand planting using either clump, horizontal rhizome method, or PVC/jute frames). However, other factors that may influence choice of methods are discussed below.

Clump harvest and hand planting were simple and worked well. With experience, two divers were able to transplant large numbers of plants in 1 or 2 days with no volunteer support. To conduct the frame planting, over 150 volunteers were used as shoreside helpers and divers. The final design of PVC/jute frames proved easy to work with, deploy, and retrieve. The spiked anchoring system effectively prevented frame shifting. The jute mesh silted over fairly rapidly at all but one site, allowing the eelgrass to

Table 5 Diversity indices of epibenthic and demersal samples at our planted sites, reference, and control sites

Index				
Site	Shannon (H′)	Pielou (J′)	Total no. spp. (S)	Abundance (N)
2006				
Peddocks	1.51	0.61	12	33
LIS 05	1.68	0.64	14	55
LIN06+LIS 06	1.41	0.49	11	51
Nahant	1.54	0.64	11	23
Hull	1.02	0.53	7	27
Control	0.82	0.37	9	89
2007				
Peddocks	1.72	0.69	12	26
LIS 05	1.97	0.73	15	49
LIS06+LIN 06	1.55	0.57	15	89
Nahant	1.61	0.63	13	23
Hull	1.31	0.55	11	67
Control	1.39	0.58	11	121
LIS seeds	1.13	0.54	8	68

We did not sample the LIS seeds site until 2007.

Data are with *Mysis* spp. removed from analyses. Shannon is the Shannon diversity index. Pielou is the Pielou's evenness value. Abundance is number of individuals per square meter

LIS 05 Long Island Southwest beds planted in 2005; *LIN+LIS 06* combine data from Long Island South- and Northwest planted in 2006

Table 6 Diversity indices of infaunal samples at our planted sites, reference and control sites

Index				
Site	Shannon (H′)	Pielou (J′)	Total no. spp. (S)	Abundance (N)
2006				
Peddocks	2.55	0.75	30	11
LIS 05	2.19	0.68	25	12
LIN+LIS 06	1.53	0.44	32	56
Nahant	1.78	0.47	44	53
Hull	2.59	0.77	29	7
Control	1.22	0.36	30	48
2007				
Peddocks	2.17	0.67	26	8
LIS 05	2.22	0.70	24	11
LIS+LIN 06	2.17	0.67	26	9
Nahant	2.70	0.73	40	20
Hull	1.92	0.56	31	20
Control	1.62	0.46	34	152
LIS seeds	1.22	0.37	27	50

We did not sample the LIS seeds site until 2007. LINLIS 06 combines data from Long Island South and North planted in 2006. Shannon is the Shannon diversity index. Pielou is Pielou's evenness value. Abundance is number of individuals per square meter

LIS 05 Long Island South beds planted in 2005

root. Eelgrass within the frames generally increased greatly in density. Expansion beyond the frame was limited; however, as the PVC frame itself apparently provided a significant, though not insurmountable, barrier to vegetative spreading. This confinement was primarily a problem for frames planted in spring; in the future, this issue could be resolved by removing the frames sooner after planting (in early spring for fall transplants or 4-6 weeks after spring planting). PVC frames had few excavation problems from crabs, in contrast to our experience with TERFs™ at these same sites. While restoration efforts in other areas have been plagued by damage from green crabs (*Carcinus maenas*; Davis and Short 1997; Davis et al. 1998; Garbary and Miller 2006) this species caused little or no destruction in our study area despite its presence in low densities. Excavation at our sites was caused by *Cancer* spp. crabs and juvenile lobsters (*Homarus americanus*) and only occurred with frames. Once the frames were removed from Portuguese Cove, excavation apparently ceased and eelgrass filled in.

Seeds

Initial monitoring of seed germination in late April 2006 appeared to indicate a low germination rate (<1%) at both Peddocks SE and LIS from seeds planted by scratching into the sediment in 2005. However, our site survey in July 2006 revealed a large, flourishing bed of eelgrass at the LIS seed-planting site. This bed continued to expand throughout the summer and by the end of August covered almost 180 m^2. Assessment in spring 2007 revealed an area of 3,100 m^2 harboring at least some tufts or bunches of eelgrass which spread from the original seed planting at LIS; by fall 2007, most of the area was fairly dense and the area continues to expand. Growth at Peddocks from the 2005 seed planting was less extensive and harder to measure due to poor visibility. Nevertheless, this site showed promising growth and expansion.

The LIN site, where seeds were simply broadcasted, covered approximately 100 m^2 by fall 2007. This cover was much less than at the sites where seeds had been scratched into the sediment.

Discussion

Site Selection

We successfully restored over 2 ha of eelgrass to well-chosen areas of a previously degraded estuary, Boston Harbor, by focusing intensively on site selection and refining planting methods. High survival and expansion rates were recorded at four large-scale sites. In just 2 years of monitoring, transplanted sites met and exceeded reference bed habitat function as measured by habitat structure and epibenthic/demersal species abundance and diversity. This project is the most successful eelgrass restoration in Massachusetts to date, in large part due to our attention to site selection. The location had undergone extensive water quality improvements, creating conditions favorable to eelgrass growth. Consistent with other studies, these two factors were crucial to the success of this project and should be integral to the consideration of any restoration attempt.

Use of a site selection model, such as the Short model, provided a way to initially narrow down potential planting areas, but had several limitations, and in fact was more effective as a low resolution site elimination tool than a site selection tool. Like any model, its output is defined by the data available for input. We found these data to be limited in two areas: (1) scale and (2) the variability of physical requirements for eelgrass restoration in the published literature.

As for scale, much GIS data for depth, sediment type, etc., is interpolated from a limited number of measurements which may not provide the resolution needed for eelgrass site selection, particularly in shallow water. These data also tend to be collected irregularly due to cost constraints, and probably cannot ever adequately deal with rapidly changing conditions of estuaries on temporal and spatial scales. Water quality data are often unavailable at high resolution or do not contain all the desirable parameters to assess suitability for eelgrass. These limitations of scale could be addressed with high resolution groundtruthing and test transplanting. However, this process was very labor intensive, taking the better part of a year with two full-time biologists, a seasonal technician, and many other advisors and occasional helpers. Of the large areas of coastline the Short model identified, we found only 12 sites were fit for consideration.

Out of the 12 sites selected after test transplanting and further assessment, only four resulted in significant eelgrass growth. We therefore concluded that the wide range and uncertainty in the literature of recommended physical conditions for eelgrass restoration would benefit from further study to improve the usefulness for site selection.

While some of this range is undoubtedly due to local variation found in any biological system, we speculate that the prevalent assumption that these conditions should mimic those in existing eelgrass beds (Kopp et al. 1995; Fonseca et al. 1998; Short et al. 2002a) may provide a good baseline, but is insufficient. Observations by our divers in eelgrass beds in other areas of Massachusetts and results of our study caused us to question the practice advocated in much eelgrass restoration literature of using conditions at existing eelgrass beds to set standards for transplant site suitability. It is known that established eelgrass beds can be found in fine grained, high organic, low oxygen sediment (Klug 1980; Thayer et al. 1984; Smith et al. 1988); in fact, it is likely that eelgrass creates these conditions by trapping fine particles from the water column and directing them to the seafloor. Established seagrasses can ameliorate reducing conditions and resultant sulfide toxicity by releasing oxygen from their rhizome and root systems into the sediment (Sand-Jensen et al. 1982; Smith et al. 1984; Goodman et al. 1995; Holmer and Nielsen 1997; Pedersen et al. 1998; Terrados et al. 1999; Lee and Dunton 2000). When light and photosynthetic biomass are plentiful, the oxygen released by the roots is able to keep reducing conditions at a minimum, thus neutralizing the effects of high organic content (Schlesinger 1991; Blackburn et al. 1994; Brüchert and Platt 1996; Lee and Dunton 2000; Koch 2001). In addition, if the sediment around the root zone is oxygenated, the plant does not have to continually send oxygen to the roots to maintain respiration in these structures. The supply of oxygen to the roots and surrounding sediment, where some diffuses, is therefore dependent on both the level of photosynthesis occurring in the leaves (Nienhus 1983; Smith et al. 1988; Kuhn 1992; Terrados et al. 1999) and the demand of the roots for oxygen; plentiful light available in good water quality may enable eelgrass to tolerate finer sediment by increasing the photosynthetic rates (Kuhn 1992; Goodman et al. 1995). However, our transplants did poorly in fine sediment conditions. We found all of our successful sites and all of the existing beds in Boston Harbor had <35% silt/clay, and that sites with >57% silt/clay failed (the Short model recommends <70% silt/clay). Therefore, some combination and interaction of the above conditions may have driven the failure of our transplants.

If individual shoots, or even small clumps, of eelgrass are transplanted into anoxic sediment, the net photosynthesizing biomass at the new site would be a fraction of that in the donor bed, thus making it more difficult for transplants to overcome an anoxic environment in very fine grained sediments; low light would exacerbate this difficulty. A study of *Phragmites australis*, an invasive marsh plant, found that severing rhizomes significantly lowered the photosynthetic rate of the plants, and that this effect was nearly double in anoxic versus oxygenated sediment (Amsberry et al. 2000). If this effect is also true for eelgrass, another clonal plant, severing the rhizomes during harvest would compound the already-diminished level of photosynthesis that occurs at a transplant site. The physiological effort involved in keeping roots oxygenated under these circumstances may stress the transplants to the point of death. Transplants, then, may need more oxygenated sediment, and/or better water quality than established beds until enough biomass is established to compensate for lower porewater oxygen in finer-grained sediments.

It may also be that TSS levels are still relatively high for eelgrass in Boston Harbor despite reduction in nutrient levels (Munkes 2005; Paling et al. 2009). All of our successful transplant areas were found adjacent to two inner harbor islands. These areas are flushed better than the shoreline of the inner harbor (Signell and Butman 1992; Diaz et al. 2008) and the recovery of their formerly impacted sediments to a “healthy” condition since the offshore outfall became operational has been more rapid than in lesser-flushed areas (Tucker et al. 2006). However, in order to encourage the greatest likelihood of success for future eelgrass restoration efforts, our data suggest a value between 35 and 57% silt/clay. Further study of newly created beds could better define a possible threshold and determine whether these thresholds apply elsewhere. Ideally, light measurements and/or Secchi depth should also be taken, to determine if or how light and sediment composition interact in determining transplant success.

Transplant Methodology

Clump harvest and hand planting were the most efficient methods of transplanting whole plants, but could only be accomplished by divers. Frame planting, while less efficient and still requiring divers, could involve many shore-side volunteers which may be a desirable goal of some restoration programs.

Baseline measurements of actual mean numbers of shoots 0.25 m^{-2}, as well as the areal cover of planted plots should be taken 1-10 ten days after planting. Since counting errors can occur while planting, it is important to base future survival and expansion data on the actual number of plants present and dimensions of plots. Evans and Leschen (2009) present more detailed recommendations regarding these different harvesting, planting, and monitoring methods.

If conditions are right for eelgrass growth, its self-spreading ability through a combination of vegetative shoot production and seed dispersal, enables plants to be transplanted at intervals, in a checkerboard pattern. Lessening these spacing intervals would decrease the time until full areal coverage, but would require more initial effort; these factors must be weighed based on the resources and needs of an individual project. We found that in suitable areas, planting a system of checkerboard plots spaced 30-50 m apart can eventually accomplish total area coverage while minimizing human effort. Based on our results and those of other projects in the region, we recommend 5 years of monitoring with the goal of parity with the reference bed and long-term persistence of the transplanted bed.

The success of our seed planting efforts corroborates that of other projects. (Pickerell et al. 2005; Orth et al. 2006; Maryland Department of Natural Resources). Seeding populated far more ground with eelgrass than our shoot transplant efforts, with a much smaller investment of time and resources. Large numbers of seed bearing shoots can be harvested in 1-2 days, and seeds planted in another 1-2 days. Additional time and expense are involved in storing the seeds in a flow-through seawater tank and sieving the contents, but overall effort per area colonized is much less than transplanting shoots. However, our success was uneven, as it has been with other programs, and the reasons are not well understood. Restoration efforts must still rely upon the site-selection process and test transplant stages to identify areas where seeds are likely to grow and spread, but seed planting may be a viable alternative to hand planting in some areas.

Management Recommendations

Despite protections for eelgrass under the Massachusetts Wetlands Protection Act (M.G. L. c. 131 § 40), the Federal Clean Water Act (33 U.S.C. § 1251 *et seq*. Section 401 and 404) and their associated regulations, projects that directly impact eelgrass are still occasionally permitted when there is an overriding public need or other demonstrated reason. In addition to direct impacts to eelgrass through dredge or fill projects, indirect impacts, such as shading from docks or degradation of water quality, occur regularly. Although some "best management practices" can minimize direct and indirect impacts to eelgrass beds, unavoidable impacts can and do occur and often require compensatory mitigation.

The 1993 New Hampshire Port Authority (NHPA) Piscataqua River dredging project was the first in New England for which large-scale eelgrass restoration was used as mitigation for eelgrass loss. The NHPA restoration was successful; however, since that time, several small size restoration efforts (< 0.03 acres) were attempted with varying degrees of success, most of which ultimately failed. With increased awareness of the value and vulnerability of eelgrass and with improved restoration methods, it is now more common to see proposals that include eelgrass transplanting as mitigation for direct and indirect loss. However, successful, full-scale restoration and/or mitigation projects in MA are still relatively new and are limited to sites in Boston Harbor (this project), New Bedford (Kopp and Short 2003), and Gloucester (AECOM 2009), totaling approximately 4 ha successfully transplanted between 1998 and 2008; this pales in comparison to an estimate of 760 ha of eelgrass lost during that time (Charles Costello, MA DEP, unpublished data). Because of continued pressure on this resource and the gross imbalance between the amount of eelgrass lost and the amount that can successfully be restored, we need to consider other management tools that address the sources of eelgrass decline.

This project, together with the few other large eelgrass restoration efforts in New England, have shown that successful restoration requires careful site selection and groundtruthing, intensive planting effort, and several years of monitoring. Additionally, water quality in coastal embayments in Massachusetts is poor enough to eliminate many areas from consideration, including areas that previously supported beds. In areas where existing low flushing regimes compound long-term deposition of organic matter, it may take years for sediment to recover enough to support eelgrass, even after water quality has improved. The sediment remained unsuitable for eelgrass throughout much of Boston Harbor 5 years after the offshore outfall became operational. Therefore, areas that have compromised water or sediment quality may not be ready for eelgrass transplanting and a restoration project would not be the most desirable or efficient use of resources at that time.

In such cases, restoration alternatives that address the causes of eelgrass degradation from a watershed approach should be considered as part of a project mitigation plan. Some alternative mitigation strategies may have more far reaching effects on the broad-scale return of eelgrass to coastal waters than the construction of small transplant projects at a single location. In particular, projects such as upgrades to wastewater treatment plants and combined sewer overflows, sewer hook-up subsidies in critical areas, improved storm water management, and re-vegetation of previously armored stream channels, are important in improving water quality and creating the conditions necessary for natural repopulation of eelgrass over a larger area. In addition, minimizing boating impacts to eelgrass by reducing or eliminating mooring chain scour may also be a feasible mitigation strategy. The use of "conservation moorings", which may reduce impact to benthic communities including eelgrass beds by eliminating chain drag through use of a flexible rode and/or helical anchor, are currently being investigated. Better channel marking and management, and provision of public (conservation) moorings in popular anchoring locations, particularly in shallow areas, could also reduce direct boating impacts to eelgrass.

Two mechanisms to fund transplant projects and alternatives are mitigation banking and in lieu fee mitigation funds. A mitigation bank can be created by an entity, typically private, that restores wetlands or other aquatic habitats and sells credits to developers. The EPA has guidelines regarding what constitutes a mitigation bank (USEPA 1995). A mitigation bank is functioning prior to project impacts occurring; therefore the temporal impacts of loss of function of a habitat are minimized (US Dept. of the Army et al. 2000). Where mitigation banking is unavailable or inappropriate, an in lieu fee mitigation fund (ILF) can be utilized. An ILF is an agreement between a regulatory agency (federal, state, or local) and a sponsor (such as a resource agency or non-governmental organization). The sponsor collects mitigation fees after project impacts have occurred, causing a potential temporal lag in restored habitat function.

Mitigation banking and ILFs enable the pooling of compensatory mitigation fees to enable larger projects that can achieve efficiencies of scale and would be more successful than permittee-responsible mitigation. The fees charged for permitted impacts are designed to be commensurate with the value of restoration of the affected habitat.

While these types of funds have typically targeted wetlands mitigation, an ILF targeting impacts on coastal and marine resources (including salt marshes) was recently started in Massachusetts by the Army Corps of Engineers with the Division of Marine Fisheries as the sponsor. This program does not change the established requirement that an applicant demonstrate that they have avoided and minimized any resource impacts to the maximum extent practicable before mitigation is considered. If there are unavoidable impacts that require mitigation, resource and permitting agencies determine whether or not the applicant is eligible to pay into the in lieu fee fund. Payment into the fund is optional; the applicant still has the option to conduct their own mitigation activities if approved by the permitting agencies. A steering committee comprised of resource agents, permitting agents, and scientists oversees the distribution of funds to maximize the effectiveness of mitigation actions.

In summary, it is increasingly difficult to conduct successful on-site and in-kind restoration of eelgrass beds in Massachusetts as the sources of eelgrass decline remain largely unaddressed. Therefore, in circumstances where a recovering estuary such as Boston Harbor is not available, we recommend that mitigation plans take a watershed approach before resorting to transplanting in marginal locations. The Federal Rule for Compensatory Mitigation for Losses of Aquatic Resources (Federal Register 2008) also recommends this approach through the use of mitigation banks and ILF, rather than strict adherence to on-site, in-kind projects. Mitigation banks and ILF funds have not been fully studied in terms of effectiveness over other mitigation strategies (USGAO 2001), but they are a developing avenue for better implementation of a watershed approach to mitigation.

Summary

We successfully restored over 2 ha of eelgrass to a previously degraded estuary, Boston Harbor, MA, USA by focusing intensively on site selection and transplant methods. Our choice of planting locations was severely constrained by a vast area of unsuitable sediment, which persisted throughout much of Boston Harbor even 5 years after elevated wastewater treatment and improved water quality were realized. High survival and expansion rates were recorded at our four large-scale sites. In just 2 years of monitoring, transplanted sites met and exceeded reference and donor bed habitat function as measured by epibenthic/demersal species abundance and diversity, and habitat structure. Hand planting and seeding (with scratching) were the most efficient and effective methods for growing eelgrass. Employment of a checkerboard pattern of planting, with plots spaced tens of meters apart, added to the efficiency of creating larger beds, and were an efficient way to "jump start" eelgrass growth in an area that was suitable, but where eelgrass has failed to grow because of lack of local reproductive shoots. We found that a silt/clay content of <35% was a sediment characteristic at all of our successful sites, which is lower than some values found in the literature. Given the enormous effort required for successful eelgrass restoration and the lack of suitable transplant sites due to water and sediment quality issues, restoration efforts may be more effective by focusing on a watershed approach and identifying projects that improve water and sediment quality and minimize existing impacts to eelgrass. In lieu, fee mitigation programs offer a mechanism by which mitigation efforts for individual project impacts can be pooled to conduct more far reaching restoration efforts.

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