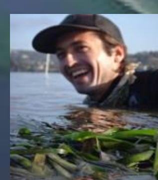
2014-2022 MONITORING REPORT

Cosco Busan Trustee Council
Eelgrass Restoration and Monitoring Services
Cosco Busan DARP
San Francisco Bay, California



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INTRODUCTION

PROJECT BACKGROUND

On November 7, 2007, the freighter Cosco Busan struck a bridge pier on the San Francisco-Oakland Bay Bridge (SFOBB) rupturing the vessel's hull and discharging approximately 53,000 gallons of bunker fuel oil into San Francisco Bay. Inside the Bay, the oil primarily impacted waters and shoreline within the central portion of the Bay, from Tiburon to San Francisco on the west side and from Richmond to Alameda on the east side. The spill was of a scale warranting a Natural Resource Damage Assessment (NRDA) process. Under the NRDA the trustee agencies were organized under the Cosco Busan Trustee Council (CBTC) agencies, including the National Oceanic and Atmospheric Administration (NOAA), United State Fish and Wildlife Service (USFWS), National Park Service (NPS), California Department of Fish and Wildlife (CDFW), and the California State Lands Commission (CSLC).

Oil from the spill was documented within eelgrass beds throughout central San Francisco Bay. Shortly after the spill, Pacific herring (*Clupea pallasii*) spawn was observed to occur within the oiled eelgrass and on non-eelgrass spawning habitat that was also oiled. Due to the physical properties of the Cosco Busan oil, the CBTC concluded that exposure of aquatic organisms to the oil; and accordingly, injury was most likely to occur in nearshore areas where oil stranded along shorelines. Since nearshore areas are also the primary spawning location for herring, the Trustees conducted an in-depth assessment of the potential for injuries to the spawning fish (Cosco Busan Oil Spill Trustees 2012). Because of their spawning behavior and high sensitivity to oil toxicity, the CBTC considered herring to be a reasonable proxy for nearshore spawning species of fish in San Francisco Bay at risk for exposure from the spill. As a result of the investigations and ultimate assessment, injuries to herring populations and to eelgrass within central San Francisco Bay were a component of the natural resource damages settlement approved by a federal court on January 27, 2012 (United States of America v. M/V Cosco Busan, et al. (Case No. C 07-6045 (SC)). A portion of the settlement funds were directed towards restoration and monitoring of eelgrass within San Francisco Bay to off-set both direct and indirect impacts to the ecosystem supported by eelgrass. The overall intent of the restoration and monitoring efforts is to expand the current densities and spatial coverage of eelgrass in the bay to provide suitable spawning and nursery grounds for multiple forage fish, in particular herring. The program has a 9-year goal of expanding eelgrass in San Francisco Bay by 70 acres, with 36 acres resulting from planting and the remainder generated by bed expansion.

For eelgrass restoration work, the National Fish and Wildlife Foundation (NFWF) serves to administer the eelgrass restoration and monitoring funds under the Cosco Busan Oil Spill natural resource damages settlement on behalf of the NOAA and the other CBTC agencies. NFWF issued a competitive request for proposal (RFP) for the first three years of the nine-year restoration program to complete eelgrass restoration and monitoring to evaluate the success of eelgrass habitat establishment and spawning use by herring as well as other species of concern. Merkel & Associates (M&A) and its partner San Francisco State University, represented by the Estuary & Ocean Science Center, Romberg Tiburon Campus (EOSC-RTC), were selected to undertake the restoration and monitoring efforts. Following the completion of the first three years, the contract was extended to cover Years 4–6, which were originally intended to be 2017–2019. However, because eelgrass in San Francisco Bay, including the eelgrass restoration and donor sites, suffered tremendous damage from the high winter discharges of early 2017 and heavy snowpack was anticipated to prolong the low salinities into the spring months, a decision was made not to plant in 2017 pending the return of more suitable planting conditions and an assessment of restoration site recovery from low salinity impacts associated with the 2017 wet winter discharge period. As a result, Year 4 planting was ultimately delayed until 2019. The Year 5 planting, which was scheduled to be conducted in 2020, was also subsequently delayed to 2021 due to state mandated work restrictions associated with the COVID-19 pandemic response in 2020. As a result, only 6 years of the 9-year program have been completed to date.

The present report documents the 2014 through 2022 transplanting and associated 2014–2022 monitoring of the transplants conducted under the eelgrass restoration program effort. The report is organized in an accumulating structure such that it provides a means to track conditions through time at the various planting areas. The Cosco Busan eelgrass restoration work has been closely coordinated with a similar restoration program established by permit special conditions of the San Francisco Bay Conservation and Development Commission (BCDC) under which the California Department of Transportation (Caltrans) established a restoration fund for habitat restoration projects to mitigate impacts to eelgrass (*Zostera marina*) resulting from the SFOBB East Span Seismic Safety Project. While these projects are separate, they have been conducted in parallel to capitalize on synergy of both implementation resources as well as information developed from each project. As a result, this report occasionally discusses elements of the SFOBB eelgrass program to provide context to the Cosco Busan DARP restoration work.

METHODS

Restoration Site Selection

Restoration sites were selected based on an assessment of herring spawning history, the predicted potential for successful eelgrass restoration, and land-owner willingness to allow the restoration efforts. To identify potential restoration areas, two spatial data layers were overlain. The first was the San Francisco Bay eelgrass habitat suitability model developed within the Ecological Limits Viability and Sustainability (ELVS) modeling structure. This model integrates several physical environmental parameters and defining ranges or tolerances for eelgrass. This model was used as a tool in the development of eelgrass restoration objectives under the San Francisco Bay Subtidal Habitat Goals (California State Coastal Conservancy et al. 2010). The ELVS model was initially prepared as a tool for screening areas of San Francisco Bay to eliminate areas needing to be surveyed for eelgrass and as such, the model is more inclusive of habitat potential than exclusive because, for the purpose intended, exclusion of potential areas was considered to be of greater concern than including too much area (M&A 2005). Conversely, it is important to note that factors such as salinity levels and sediment loading are transitory and very much climate dependent. As such, drought and flood conditions may have tremendous influence over the distribution of eelgrass and the suitability of areas to support eelgrass at any given time. This was clearly seen in 2017 and was also clear during wet periods of 2005 and 1997 where monitored eelgrass beds declined in association with prolonged brackish conditions in the North Bay (K. Merkel, pers. obs.). The existing San Francisco Bay Eelgrass ELVS model was based on long-term average conditions rather than extreme conditions. As a result, eelgrass occurrence has been known to extend beyond the predicted suitable habitat extents during drier years and extended periods of low delta outflows. In wet years and following flood periods, eelgrass may be reduced in geographic distribution in both the north and south bay extents, both as a result of prolonged depressed salinity as well as subsequent increased suspended sediment loading that may linger following initial inputs. Clear examples of extreme variations from average conditions have occurred during the period of the present restoration efforts. In 2014, prolonged drought had allowed eelgrass to extend well beyond predicted ranges up into Suisun Bay as far inland as Pacheco Creek, approximately 1.5 miles inland from the Benicia-Martinez Bridge. This area typically supports brackish water submerged aquatic vegetation dominated by *Stuckenia* spp. (M&A 2015). In 2015, water temperatures in the bay reached highs that were as much as 10–15 °F (6–8 °C) above normal. After a brief return to normal temperatures and salinity conditions in 2016, early 2017 saw tremendous and prolonged flood discharges from the Sacramento-San Joaquin River Delta and depressed salinities that lowered much of the North Bay area salinities to osmotically lethal levels for eelgrass for an extended period of time. As such, three of the first four years of the restoration program suffered from anomalous and differing climatic conditions that deviated significantly from average conditions on which the ELVS model was generated. Post-2017, more normal water temperatures and salinity conditions have prevailed from 2018 through 2019 and increasing atmospheric and shallow water temperature again occurring from 2020 through 2021.

The second spatial data layer used in the selection of potential restoration sites for the Cosco Busan eelgrass restoration program was developed from existing data on the spawning distribution of Pacific herring. The CDFW maintains data on annual herring spawning locations within San Francisco Bay, and estimated tonnage of spawn dating back to 1973–1974 (CDFW 2014a). Within the Bay, Pacific herring are known to spawn on marine vegetation including eelgrass or rocky intertidal areas; however, man-made structures such as pier pilings and riprap are also frequently used spawning substrates (CDFW 2014b). For the first year, site selection, general spawning data were used that defined the extent of herring spawning as geographic reaches along the shoreline. These data indicated that herring spawning extended from just north of the San Mateo bridge to near Point San Pedro on the west side of the Bay and from Point Pinole to Bay Farm Island on the east side of the Bay.

For the 2015 transplant, the existing data on herring spawning (CDFW 2014a) along with additional information provided regarding herring spawning in 2014 (R. Bartling, pers. comm.) were used in the site selection analyses. In 2014, herring spawned further north in Marin County than had been recorded in the prior 40 years (R. Bartling, pers. comm.). This was likely related to the drought conditions and salinity gradient present at the time. As an element of the screening process for the Coastal Conservancy's creosote pile removal project to support Pacific herring, the historic records of herring spawning were used to develop a spatial data layer for herring spawning potential; the vertical spatial range of herring spawning was developed based on discussions with DFW staff (R. Bartling). This layer allowed the presentation of the historic herring spawning within the Bay as a frequency of spawning occurrence over time (M&A 2014).

By overlaying the ELVS eelgrass model with the historic herring spawning frequency it was possible to identify coincident occurrences of restoration potential with areas that would be expected to benefit the development and expansion of spawning habitat for Pacific herring (Figure 1). Following the identification of potential eelgrass habitat restoration opportunities to support herring on a regional scale, it was unnecessary to examine potential site availability based on ownership and willingness of property owners to allow restoration activities. In addition, setbacks from publicly maintained navigation channels and the operations of the Ports of San Francisco, Oakland, and Richmond berths and basins were requested by BCDC and the United States Army Corps of Engineers (USACE) during the 2014 and 2015 restoration site screening process. On August 18, 2015, the USACE issued Regional General Permit (RGP 2013-00408N) to National Marine Fisheries Service (NMFS) to conduct eelgrass restoration work in San Francisco Bay, formally incorporating a 350-meter setback from the Ports and publicly maintained navigation channels as a condition under the permit.

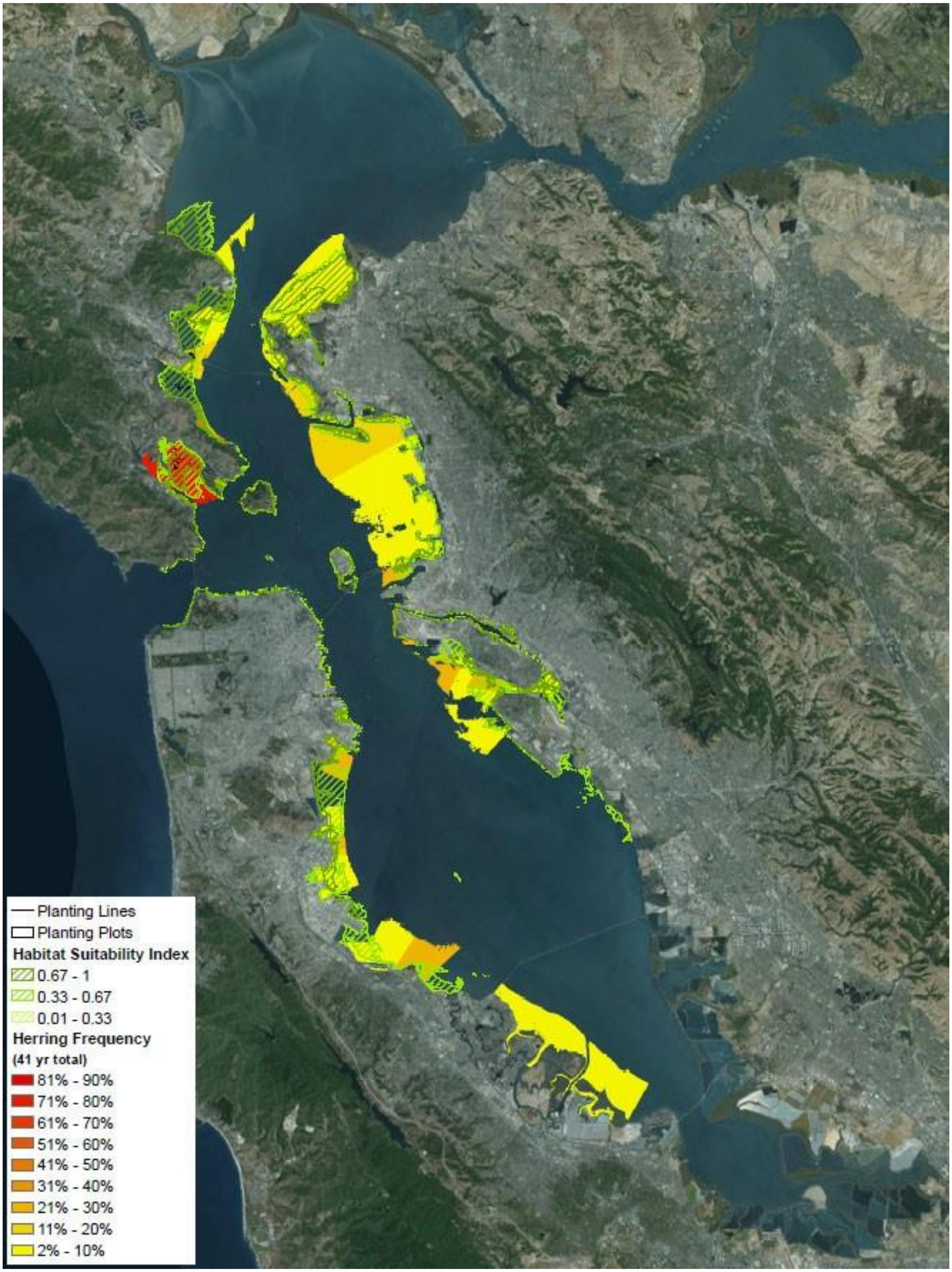


Figure 1. ELVS Eelgrass Habitat Suitability Index Overlay on Pacific Herring Spawning Frequency

Another factor used to select restoration sites was the absence of naturally occurring eelgrass. Based on initial screening of sites that both fell within the region of prior historic herring spawning and areas predicted to be suitable to support eelgrass, sites were reviewed to verify bathymetric conditions, absence of eelgrass, and ownership that was anticipated to be supportive of the objectives of habitat restoration. The application of these parameters to restoration site selection resulted in a general favoring of the Marin County shoreline and East Bay shoreline between Bay Farm Island and Point Pinole. South of Port of San Francisco the most suitable area to meet the project goals is located near Coyote Point. In 2014, a baywide eelgrass survey was completed by NOAA NMFS to update information for eelgrass management purposes in the Bay (M&A 2015). This survey further aided in restoration site identification for the 2015 planting season, as it identified precipitous declines in eelgrass in northern Richardson Bay since the prior 2009 comprehensive eelgrass inventory had been completed (M&A 2009).

Sites were vetted based on a combination of past information available from prior eelgrass restoration studies on the sites or the immediate area, land-owner support, pre-site surveys to determine bathymetric conditions and eelgrass presence or absence, and any other potential promoters or detractors from site use. Sites evaluated during the first years of the restoration program are identified in Figure 2. Since this period, additional sites have been evaluated and added for future planting activities. In addition, areas where success had been achieved with prior restoration were augmented with expanded planting following the stepwise progression from site suitability testing by installation of test plots to larger scale eelgrass restoration developed within the San Francisco Bay Subtidal Habitat Goals Project - Eelgrass Conservation and Restoration in San Francisco Bay: Opportunities and Constraints (Boyer and Wyllie-Echeverria 2010).

Over the prior several years, individual property owners within the identified target restoration regions have been supportive of eelgrass study and restoration efforts undertaken by Dr. Boyer. These property owners were specifically sought out for the first round of restoration due to both their prior cooperation and the information available on the sites. Property owner partners, such as The Nature Conservancy (TNC), Marin County Parks (MCP), and Marin Rod & Gun Club (MRGC), have been organizations that have regularly shown stewardship interest in the Bay's resources by allowing access for ecological study and restoration and were the ultimate property owners for the sites selected for the 2014 transplant efforts. In 2015, Richardson County Audubon Sanctuary (RCAS) was added, and efforts were initiated to add sites within China Camp State Park, East Bay Regional Park District (EBRPD) properties, and CSLC parcels. Additional sites have been identified for restoration planting in South San Francisco, northern San Rafael, and in the central East Bay, but have been delayed due to potential conflicts with on-going projects, requirements for additional environmental review, or landowner approvals. In 2021 the Richardson Bay Regional Authority (RBRA) provided access to conduct plantings within vacated mooring areas where scars from mooring tackle and vessel dragging had removed eelgrass and scoured depressions in the bottom of the bay. In 2021 five mooring scars were planted. These plantings were the beginning phases of restoration of broader damage to eelgrass from moorings and were to serve as pilots to understand what is required to restore the mooring damage. Planting of the mooring scars continued in 2022 with an additional twelve scars being planted under the Cosco Busan DARP funding. These efforts have subsequently resulted in anchoring a much larger effort to restore broad-scale damage to these central Richardson Bay eelgrass beds with the goal of restoring an estimated 73 acres of eelgrass damaged by long-standing moorings that are being removed from the bay over the next few years.

Figure 2. Restoration Sites Considered and Those Selected for Planting from 2014 through 2022 Planting Seasons

San Pablo Bay

China Camp State Park – Point San Pedro

Based on herring spawning off McNears Beach County Park during the 2013-2014 season, investigations were initiated into eelgrass restoration at the adjacent China Camp State Park. The beach at China Camp offers potentially suitable restoration opportunities for eelgrass as do the small coves to the north of the beach. Favorable contacts were made with State Parks staff and the San Francisco Bay National Estuarine Research Reserve (NERR) that has a reserve site at China Camp State Park. While this area has promise for potential eelgrass restoration and expansion of the bay range of eelgrass, the initial logistics of obtaining approval for site use were determined to exceed potential value for the present program. Streamlined approval for pilot plot restoration was sought; however, concerns were subsequently raised about potential for eelgrass offshore of the small marshes to result in a net loss of sediment from the marshes either by fostering marsh erosion, or enhancement of nearshore sediment trapping at the expense of marsh capture of sediment. We do not believe eelgrass would result in a reduction of sediment capture in the marsh, but would more likely enhance sediment capture due to energy dissipation in the adjacent waters and thus a lower energy environment on the shore fringe, or would have a neutral influence due to eelgrass being at a substantially different elevation than the marsh plain and thus principally influencing the wave and circulation environment at elevations below marsh vegetation rather than at the same elevation range. However, demonstration of the lack of negative effect on the adjacent marsh is beyond the scope of the restoration so we have dropped this site from consideration, pending a separate determination from State Parks and the NERR that eelgrass would not adversely affect the shoreline accretion conditions at China Camp. Should a determination be made that eelgrass restoration is compatible and desirable at this location, we would propose reconsidering the site.

McNears Beach County Park – Point San Pedro

McNears Beach County Park received herring spawning use in 2013-2014. This use by Pacific herring was the first known spawning occurrence in 40 years. Prior to this spawning, the site was outside of identified herring spawning range in the Bay and therefore did not meet the objectives of the restoration program. The beach area at McNears Beach is generally very rocky at the south end of the site transitioning to sandy beach at the north end. This area is suitable for investigation with pilot plantings but has not been advanced for restoration efforts.

San Pablo Cove – Point San Pablo

San Pablo Cove is located adjacent to the Point San Pablo Yacht Harbor. The cove is located on a shoreline with limited patches of eelgrass, principally found to the north along Point San Pablo. The cove provides a quiescent environment across a shallow channel from the largest eelgrass bed in San Francisco Bay. This region of the Bay has uncommonly been used by spawning herring and it is not clear why the area does not presently support eelgrass given the high abundance of potential seed source material in the area. As such, pilot plantings only should be pursued at this site. This area has not been investigated in the present restoration efforts. However, restoration of eelgrass in this area has been conducted by the California State Coastal Conservancy (CSCC) in association with the Red Rock Warehouse Creosote Pile Removal Project and thus restoration efforts for the benefit of herring are being conducted at this location. As of May 2020, the Red Rock Warehouse eelgrass restoration has yielded gains of 110 percent above pre-restoration eelgrass coverage levels (M&A 2021).

San Rafael Bay

San Rafael Bay (Point San Quentin to Point San Pedro) supports both predicted restoration potential and historic herring spawning activities (Figure 1). It also has a history of eelgrass restoration activities. While herring have not been observed to spawn in eelgrass on this shoreline, herring have been observed to spawn along the riprap shoreline on many years (B. Abbott, pers. comm., CDFW data). This shoreline has been considered a good location to focus larger-scale eelgrass restoration due to the prior success of several smaller eelgrass restoration projects that were conducted on this shoreline. Some of these restoration efforts have survived for a period of over 5 years and have been source beds for natural expansion of eelgrass along the nearby shoreline area. The creation of additional spawning habitat in the form of eelgrass at low intertidal and shallow subtidal waters would be expected to enhance herring reproductive success. Sites in this area considered for restoration efforts are discussed below.

Marin Islands National Wildlife Refuge

The Marin Islands site offshore of San Rafael generally has a rocky shoreline. However, the configuration of the islands funnels high velocity tidal waters between the islands in a manner that creates sand and gravel bars that have resulted in the development of semi-protected coves on the north sides of the islands. These coves appear to be well suited to support eelgrass restoration and, if successful, would enhance the suitability of the islands to support herring spawning. At present, there is no history of eelgrass on the islands, so pilot transplants are proposed. No transplanting has been performed to date, but planting of pilot plots remains a goal.

The Nature Conservancy (TNC) Norman Tidelands

This is an approximately 10-acre parcel that lies along the San Rafael shoreline between the MRGC and the TNC Edwards Parcel. The Edwards Parcel is a 20-acre parcel on which small eelgrass test plots were established successfully in 2007 (Boyer 2008). These persisted over many years, and in 2012 a portion of this site was used to establish the CSCC-managed Living Shorelines Project that includes eelgrass restoration that persisted and expanded from 2012 through 2015. Because of the success of the bracketing sites at the Edwards Parcel and MRGC, the Norman Tidelands were identified as a potentially suitable restoration site. However, in subsequent investigations, it was determined that this site is extremely shallow at approximately 0 ft MLLW and above. As such, the site was only explored with pilot plots given the expectation that the area is at the upper margin of potentially suitable elevations for eelgrass in this area. Pilot plantings were installed at this site in 2014. These plots failed and no subsequent plots have been installed. The area is still considered potentially suitable at lower elevations outside of the approved parcels.

Marin Rod & Gun Club

Eelgrass was established in a half-acre plot on this property in 2006–07 and persisted and spread over subsequent years (Boyer et al. 2008; Boyer and Wyllie-Echeverria 2010). In addition, a bamboo stake transplant method developed by Boyer Lab graduate student Stephanie Kiriakopolos was used to incorporate small plots of eelgrass among oyster shell mounds in another half-acre plot in 2009. These plantings also persisted and expanded (Abbott et al. 2010; Boyer, unpublished data). Out-migrating salmonid juveniles have been found to linger in the area of these previous projects according to telemetry stations signaled by tagged fish (Abbott et al. 2010), and additional restoration could further increase habitat value for a number of fish species of interest, including the primary target species, Pacific herring. Multiple additional acres of potential restoration habitat were identified as available in between these two former restoration areas and north and south of the

MRGC pier. M&A has also completed multiple sidescan sonar surveys of the site since 2006 and tracked expansion of eelgrass both within the initial planting plots and in areas distant enough from the plots to suggest that intrinsic reseeding from these transplants is occurring on this site and serving to colonize additional areas. For this reason, this area was selected as an initial restoration site that was planted in multiple half-acre and test plots in 2014. Success during early test plot installation has resulted in the area being used for expanded transplants in subsequent years with planting occurring in this area in 2014, 2015, and 2016. Further planting in this area has not been done as the beds have become resilient and self-propagating to near the extent believed to be site capacity.

Corte Madera Bay

Corte Madera Bay is located at the north end of the Tiburon Peninsula at the mouth of Corte Madera Creek. This large embayment is of particular interest with respect to the potential for establishment of eelgrass in that the bay supports over 800 acres of subtidal bottom that occurs within ELVS eelgrass suitability model predictions. As such, establishment of eelgrass within this Bay would be particularly advantageous relative to potential for garnering significant benefits from natural eelgrass habitat expansion.

Marin County Parks Open Space District Corte Madera Bay Parcels

Marin County Parks Open Space District has granted access for the restoration of eelgrass within their multiple subtidal parcels in Corte Madera Bay. Because there have not been prior eelgrass transplant efforts or subtidal environmental investigations within Corte Madera Bay, restoration efforts spanned the broadest elevation range within the eelgrass predicted suitability range and which also falls within properties for which access has been granted. This area extends from elevations of approximately 0 ft MLLW to below -4 ft MLLW.

The location of this parcel between two historically successful eelgrass restoration areas (MRGC and sites on the Tiburon Peninsula) makes this a very desirable location for the current project. This parcel is currently bare substrate and thus the entire area has potential for active and passive eelgrass restoration over time. This site has tremendous potential for using multiple smaller restoration plots to enhance the rate of infill from natural expansion from vegetative growth and seedling recruitment from initial plantings, if initial parent stock is effectively established. The site was used during the 2014 and 2015 planting seasons. Additional test plots were attempted in 2016 under the SFOBB restoration program, but not the Cosco Busan program.

Corte Madera Marin Country Day School

This site is owned by Marin County and managed by their Department of Parks and Open Space in the center of Corte Madera Bay. This was the site of a previous half-acre seed-buoy restoration plot established in 2006–07 (Boyer et al. 2008) that persisted for 5 years but then disappeared. Small test plots are desirable in this location to help determine suitability of this site for future persistent restoration. Successes would be used to guide expanded restoration. Test plots were installed in 2014 and 2015 but were unsuccessful.

East Tiburon Peninsula

Eelgrass has been documented along this shoreline since the early 1920s (Setchell 1929) but has been shown to be sparse in surveys over the last decade (M&A 2004a, 2009, and 2015). Prevailing winds to the east may limit the arrival of drifting flowering shoots that help to maintain both the supply of seeds and the genetic diversity of established plants that may enhance resiliency to disturbance (Hughes and Stachowicz 2004; Ruesch et al. 2005). Thus, recruitment is likely to limit eelgrass beds along this shoreline, and active restoration could dramatically increase acreages. Herring spawn along this shoreline in most years (Boyer, pers. obs; CDFW 2014a) and are likely to benefit by additional eelgrass coverage. Individual parcels for the planting have not been selected; however, based on early involvement, it is believed that several of these parcel owners may grant access for eelgrass restoration purposes.

The Nature Conservancy Day-Ring/Robbins

This approximately 4-acre parcel just south of Paradise Cay along the east side of the Tiburon Peninsula has suitable depths and substrate for eelgrass. As with the other Nature Conservancy parcels, a willing landowner makes this a desirable site for eelgrass restoration. This site was not advanced in the present planting efforts due to a saturation of opportunities available and the relatively small size and narrow nature of this parcel.

Lori Grace/Sunrise Center Parcel

This parcel is owned by an environmental activist wishing to establish a marine protected area along this shoreline in part to enhance herring reproductive success. The owner is interested in eelgrass restoration on her property and also leads a local neighbor group with similar affinities. Strong public support and broader outreach opportunities may be garnered through use of this site. This site has not been used in the present transplants as there is existing eelgrass along much of this shoreline and natural expansion is expected to fill available suitable areas.

Paradise Park

This 20+ acre property owned by Marin County Parks has sparse eelgrass present, which could potentially be extended by active restoration. The landowner representative contacted Dr. Boyer with interest in subtidal restoration or living shoreline type projects. Education and outreach potential with a nearby pier and actively used picnic and park facilities are a plus and provide potential for enhanced stewardship. This area has not been advanced in the first wave of restoration as the opportunities for new eelgrass beyond what could be accomplished by natural processes is not clear and the focus has thus far been to establish beds in areas that generally lack eelgrass and which could benefit from introduction of new nuclear beds.

<u>Richardson Bay</u>

Richardson Bay has historically received the highest frequency of herring utilization for spawning of any San Francisco Bay region (Figure 1). The 2014 baywide eelgrass survey documented losses of eelgrass totaling over 340 acres between 2009 and 2014 (M&A 2015). Most of this loss in eelgrass occurred within the northern and eastern portion of the Bay. Eelgrass depression within the eastern and northern portions of Richardson Bay continued through 2017 although a notable commencement of recovery in eelgrass had commenced along the western margins of Richardson Bay beginning in 2016. The recovery within the western portions of the bay occurred along the Sausalito shoreline and within the northern portion of the Richardson Bay mooring field between clearings generated by the moorings. Some recovery was noted to extend into portions of the eastern areas of Richardson Bay by 2017 although beds remained considerably reduced from the conditions observed in 2009.

Richardson Bay Audubon Sanctuary

Based on concerns over precipitous losses of eelgrass within Richardson Bay, the Richardson Bay Audubon Sanctuary approached the Cosco Busan DARP restoration effort to offer the Sanctuary as an area for eelgrass restoration that has the benefit of being preserved and located within an area of historically high herring spawning activities. As a result, this area was included in the 2015, 2016, 2019, and 2021 restoration efforts.

Richardson Bay Regional Agency Vessel Moorings

As of 2019, it was estimated that approximately 73.58 acres of eelgrass restoration potential exists as a result of mooring scar damage resulting from anchor out moorings in eelgrass beds (M&A 2019). In 2019, the Richardson Bay Regional Agency (RBRA) commissioned a study to determine potential means of deconflicting moorings with eelgrass habitat and other ecological resources in the bay with the intent of developing an ecologically sensitive mooring strategy (M&A 2019). This effort has led to the identification of potential mooring opportunities as well as identifying areas where mooring removals would lead to potential for significant eelgrass restoration. Since this time, the RBRA has embarked on a program driven by settlement agreement with the Bay Conservation and Development Commission (BCDC) to remove anchor-out vessels from the bay over time. In 2021, the RBRA adopted an Eelgrass Protection and Management Plan (Lesberg 2021). The RBRA subsequently authorized planting of abandoned vessel mooring scars and a total of five scars were planted in 2021 under Cosco Busan DARP funding. In 2022 an additional twelve vessel mooring scars were planted. These planted scars as well as vacant scars are being investigated under a separate but related effort to evaluate potential means of accelerating recovery in areas where moorings have been removed (Boyer, in progress). Vessel mooring damage will continue to be a focus of restoration in Richardson Bay as the removal of moorings provides the greatest single opportunity for major eelgrass gain within herring spawning habitat in San Francisco Bay.

City of Sausalito Vessel Moorings

As with the Richardson's Bay Regional Authority moorings, similar moorings in Sausalito must also be removed and several vacant scars exist in the eelgrass beds within Sausalito waters. These scars are being evaluated for future planting under the present program.

East Bay

Brooks Island Shoal

A shallow shoal exists between Brooks Island and the Richmond Small Boat Harbor. This shoal supported approximately four acres of eelgrass in 1996. The eelgrass was lost from this shoal area prior to 2000 and has not returned since, although on rare occasion a few plants have occurred for short durations since the original loss. The shoal is bounded on the west by the deepwater navigation channel of Richmond Harbor and is bounded on the north by the small boat harbor navigation channel. It is anticipated that this shoal likely receives donor seed from the eelgrass beds located both outside and inside the Richmond Harbor training wall. However, given the winds and currents in the area around the island and channels, it is not certain that this is the case. If authorization may be obtained, a pilot transplanting effort back to this shoal would be conducted in future years of this program.

The potential use of the primary shoal environment is restricted by the 350-meter setback from the ports and publicly maintained navigational channels. This would restrict planting to more marginal environments to the southeast of the region of the shoal that historically held eelgrass. The area remains under consideration but has not been pursued for the present planting efforts.

Eastshore State Park Multiple Sites

There are several areas along Eastshore State Park that are managed by EBRPD that have been identified as potentially suitable to support eelgrass via modeling and site surveys. Plots under consideration but not yet incorporated into the planting effort include Albany Point adjacent to the recently reconstructed shoreline, Berkeley North Basin, expansion of the initial restoration at Brickyard Cove, the Emeryville Shoreline, and Emeryville Crescent areas within EBRPD.

An eelgrass restoration project conducted within the Berkeley North Basin was planted with eelgrass in 2005, and spotty occurrences of eelgrass were intermittently present on the engineered planting site through the term of the monitoring period in April 2008. Eelgrass was never observed to be present on the site in winter and early spring but was often found as small stands in summer and fall months (M&A 2008). Similarly, Dr. Boyer has planted pilot plots along the eastern shore within the Emeryville Crescent with poor long-term establishment success. Conversely, M&A successfully restored 0.25 acre of eelgrass at Berkeley's Brickyard Cove through bareroot planting in 2002 (M&A 2004b). This restoration area has persisted since the original planting as verified in the 2014 baywide eelgrass survey (M&A 2015). The intermittent presence of eelgrass along the east bay shoreline in areas that are separated into multiple segments by intervening headlands may suggest limited opportunities for natural recruitment by seed stalk drift. However, the lack of identification of specific factors that would preclude eelgrass persistence once established remains problematic. To investigate the potential for restoration in these areas, future restoration plans would include a "prospecting" pilot planting approach with small scale plots within several segments of the shoreline that presently do not support eelgrass but appear well suited. Where plots are persistent over multiple years, more extensive restoration would be undertaken, using the plots as a core, and building off the physical environmental conditions observed in the successful plots as a guide for more extensive efforts.

In 2016, consideration was also given to restoration planting within the Albany Point area where shoreline restoration and enhancement of the Albany Neck region had just been completed. However, concurrence on eelgrass restoration at this site was postponed pending evaluation of how the restoration actions that were taken naturally influenced the nearshore area of the Albany Neck relative to colonization by eelgrass and pending final design of the adjacent Albany Beach enhancement elements of the park improvements. As a result, no new planting regions were added to the program in 2016. Pilot planting in this area is planned for future restoration years.

South San Francisco Bay

There are a few potential restoration areas in South San Francisco that are under consideration for eelgrass expansion under the Cosco Busan restoration program. However, these have not yet been advanced due to potential conflicts with other restoration planning efforts. One such location is offshore of Heron’s Head Marsh. This area is presently being advanced with a living shoreline restoration project and eelgrass in offshore areas may conflict with equipment access. As a result, eelgrass restoration is being deferred until after living shoreline restoration is completed. A second potential area is located at Pier 94 in the Port of San Francisco. This area was planned to be planted in 2020, prior to COVID-19 restrictions that curtailed the larger restoration effort. However, a small effort was undertaken by the Boyer Lab to install a few test plots in July 2020. The site was determined to not be highly suited to support eelgrass but follow-up on the test plots in the future will be used to inform any further action at this location.

DONOR BED SITE SELECTION

In 2014, donor beds from which eelgrass was harvested to support the transplant efforts included the Point San Pablo-Point Pinole eelgrass bed, Point Molate, Keller Beach, and Richardson Bay (Figure 3). These donor sites distributed the source material harvest over a wide geographic area within beds that are known to be genetically separated (Talbot et al. 2004). As discussed later, the donor material

Figure 3. Eelgrass Transplant Regions and Donor Site Beds Used in Eelgrass Restoration Program

was kept separate to assist in tracking any performance differences between sources of transplanted material.

Between 2014 and 2015, eelgrass within the Keller Beach area declined precipitously across the entirety of the beds for reasons that are not known but are now believed to have been related to temperature or disease stressors. As a result, Keller Beach was dropped as a donor site and was not used in the subsequent 2015, 2016, 2019, 2021, or 2022 transplants. As a result, Bay Farm Island was substituted in to replace Richardson Bay for these years.

EELGRASS PLANTING CONDUCTED UNDER THE COSCO BUSAN DARP (2014-2021)

Transplanting of eelgrass was conducted in 2014, 2015, 2016, 2019, 2021, and 2022. Within each of the selected transplant sites, multiple plots were identified for planting as either small test plots, or larger half-acre plots. Planting was not completed in 2017, 2018, or 2020. In total, 20.6 acres of eelgrass have been planted over the six planting years (Table 1). An average of 3.43 acres has been planted during each year with plantings being distributed over 38 half-acre plots and 31 test plots.

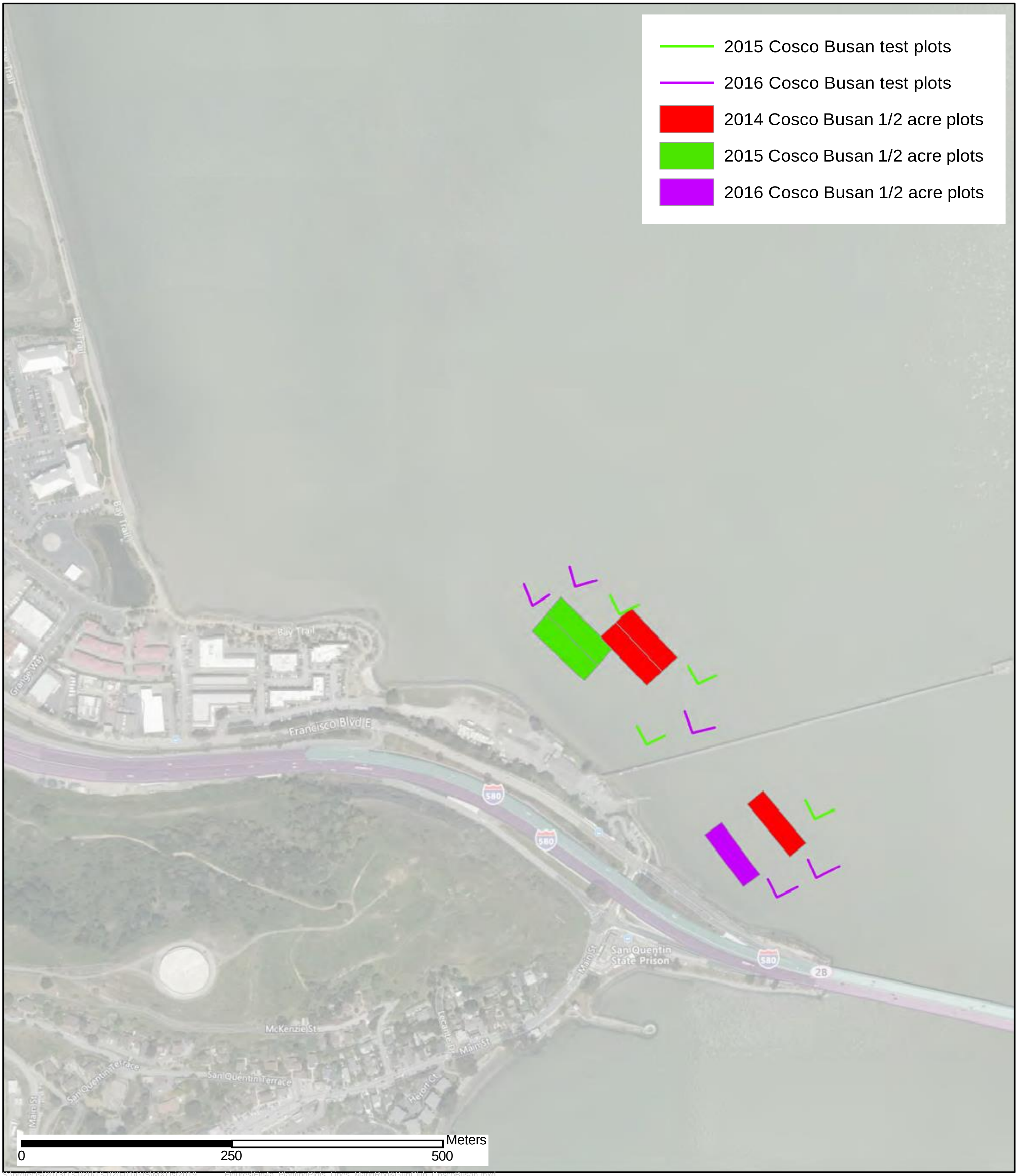
Table 1. Summary of Eelgrass Planting Conducted from 2014–2022 for the Cosco Busan DARP

COSCO BUSAN DARP EELGRASS TRANSPLANTS						
YEAR	**SAN RAFAEL BAY**		**CORTE MADERA BAY**		**RICHARDSON BAY**	
	1/2 Acre	**Test Plots**	**1/2 Acre**	**Test Plots**	**1/2 Acre**	**Test Plots**
2014	3	0	5	0	0	0
2015	2	4	1	4.5	3	7
2016	1	5	0	0	3	6.5
2019	0	0	0	0	5	4
2021	0	0	0	0	7	0
2022	0	0	0	0	8*	
Total Count	**6**	**9**	**6**	**4.5**	**26**	**17.5**
Total Acres	**3**	**0.5**	**3**	**0.2**	**13.5**	**0.9**

*Count includes 4 half-acre plots and 8 quarter-acre plots for an 8 half-acre plot equivalency.

Thus far, the planting program has been undertaken in three different areas including San Rafael Bay, mostly situated at the MRGC (Figure 4a), Corte Madera Bay (Figure 4b), and Richardson Bay (Figure 4c). Interspersed among the planted plots were several other planting plots installed for the SFOBB eelgrass restoration effort. The two programs provide synergy to each other by coordinated restoration activities and information transfer between the programs that allow strategic expansion of eelgrass beyond what either program could individually accomplish.

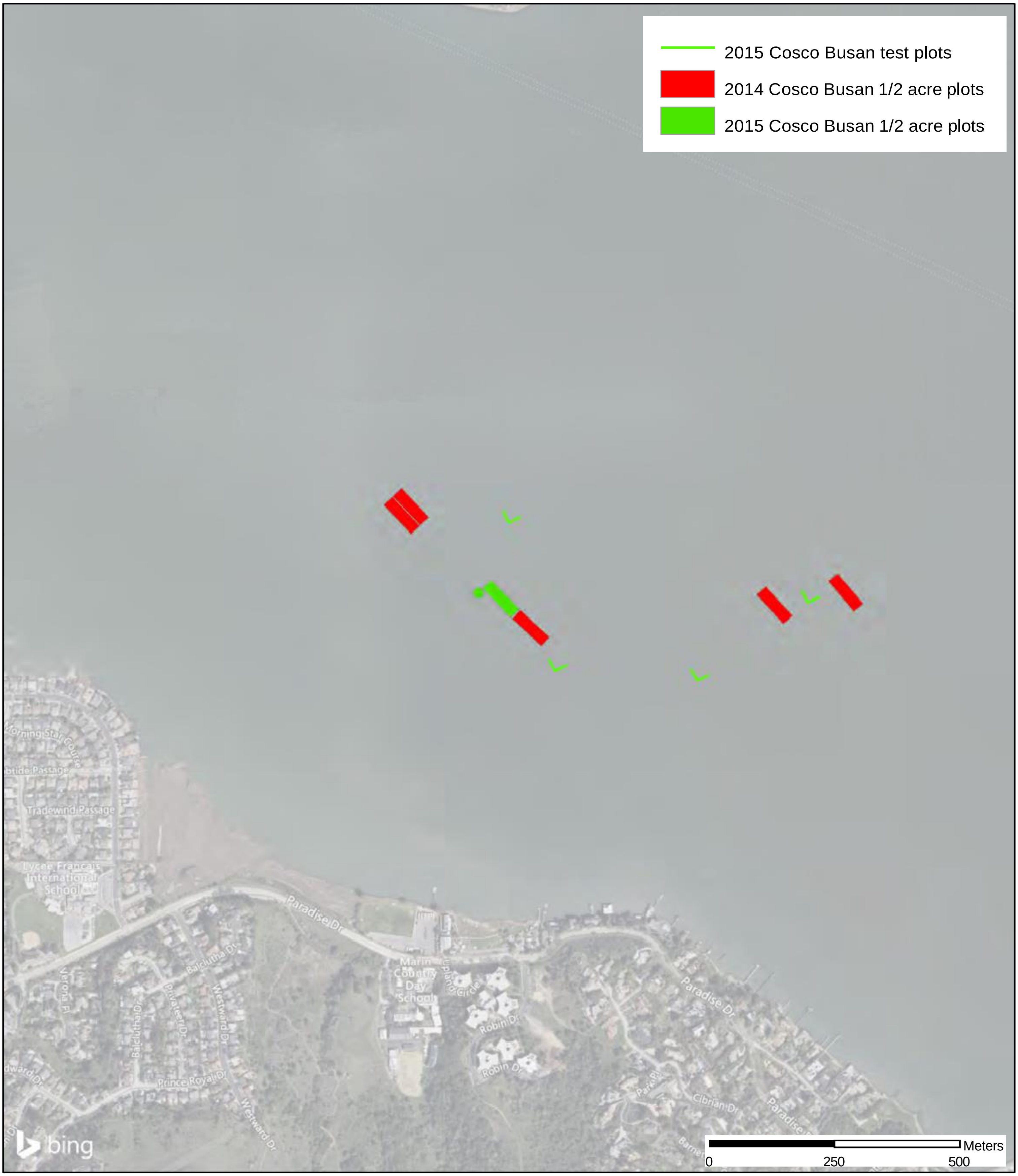
In 2014, Cosco Busan DARP plantings were performed at MRGC on the San Rafael Bay shoreline and within Corte Madera Bay. Plantings during this period focused on installation of half-acre restoration plots, while the SFOBB program implemented test plots in these same regions to test the potential for expanding shallower and deeper than eelgrass had been tested by research conducted by the Boyer Lab in prior years.



Cosco Busan San Rafael Bay Shoreline Eelgrass Planting Plots by Program Year
Cosco Busan Damage Assessment Restoration Plan

Figure 4a

Merkel & Associates, Inc.



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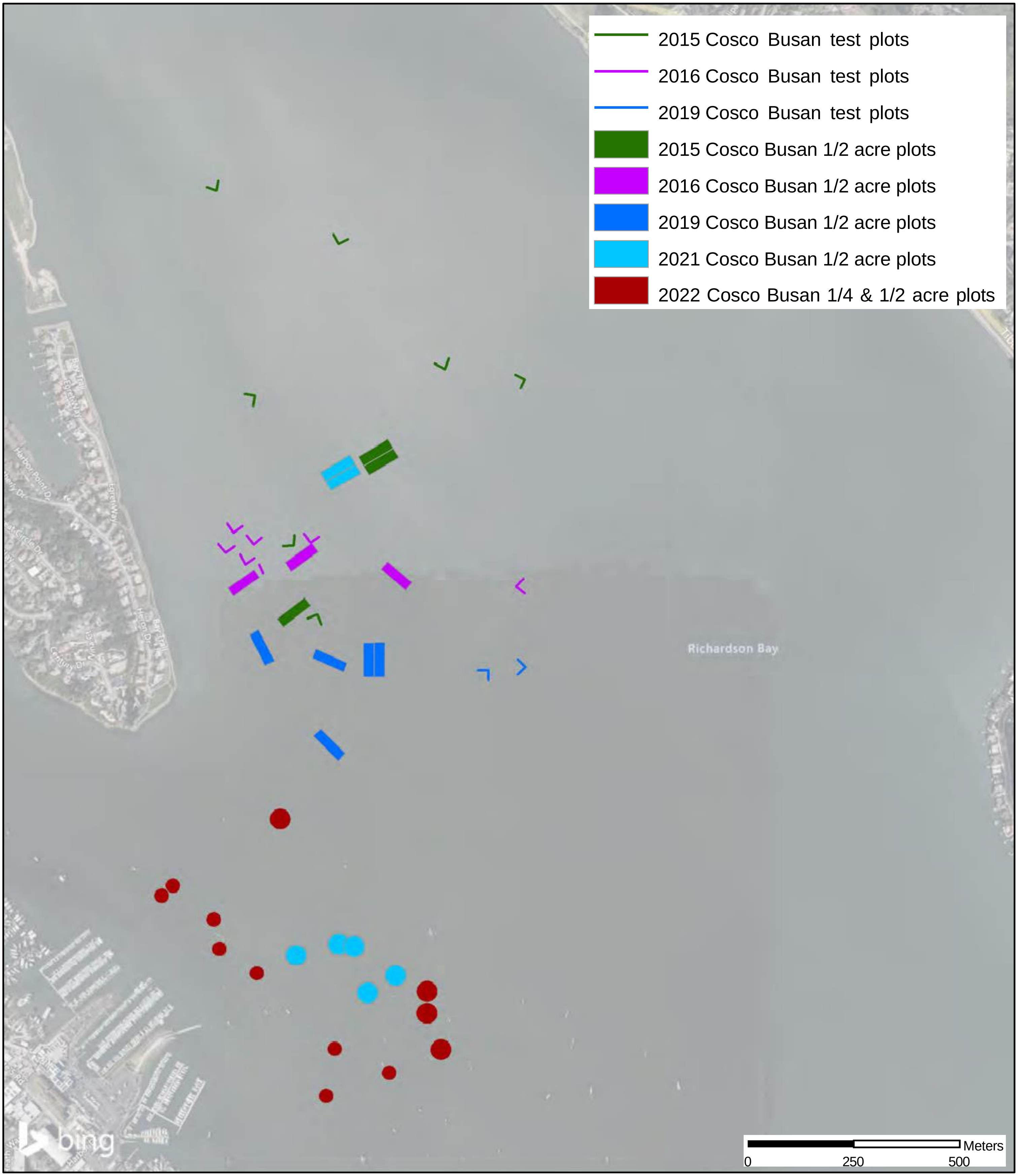
Cosco Busan Corte Madera Shoreline Eelgrass Planting Plots by Program Year

Cosco Busan Damage Assessment Restoration Plan

Figure 4b

Merkel & Associates, Inc.



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Cosco Busan Richardson Bay Eelgrass Planting Plots by Program Year
Cosco Busan Damage Assessment Restoration Plan

Figure 4c

Merkel & Associates, Inc.

In 2015, the DARP restoration program scaled back the larger plot restoration to six half-acre plots distributed over sites in San Rafael Bay, Corte Madera Bay, and Richardson Bay and focused considerable effort on expanding test plot implementation with 13.5 test plots being installed. The addition of plantings in Richardson Bay in 2015 followed low overall establishment from 2014 plantings and significant eelgrass declines being noted within Richardson Bay during the 2014 baywide eelgrass inventory (M&A 2015). Further, the Richardson Bay Audubon Sanctuary, noting the serious decline in eelgrass and reduction in herring spawning in the area, approached the Cosco Busan DARP program to offer the Sanctuary as an area for eelgrass restoration. As a result, this area was included in the 2015, 2016, 2019, and 2021 restoration efforts.

Site selection for the 2016 planting season made use of the results of the monitoring from the 2015 and early 2016 period to formalize plot selections. Low success of plantings in 2015 resulted in focusing restoration in 2016 around those areas that had previously been successful through the prior season. This meant planting only in San Rafael Bay and Richardson Bay and omission of planting within Corte Madera Bay. The SFOBB program planted four test plots within Corte Madera Bay in 2016 in order to continue exploration of potential to establish eelgrass within this area that has high potential for major gains in eelgrass if restoration of beds is ultimately successful.

In 2017, planting was not conducted due to the prolonged and severe freshwater inputs. Given the impact that these discharges had on donor beds and the transplant locations, it was determined that forgoing the planting season was appropriate to reserve funding and reduce risks of failure and excessive harvest within highly reduced donor sites. The 2018 planting season was also skipped due due to a delay in contract extension. The 2018 season was capitalized on for planting under the SFOBB eelgrass restoration program with an additional three acres of planting in Richardson Bay.

In 2019, planting was again performed under the Cosco Busan program within Richardson Bay to expand the extent of planted areas within the northeastern portion of the Bay. Differing from prior years, Richardson Bay was not authorized for use as a donor site for the 2019 transplants due to CDFW concerns over depressed Pacific herring spawning in the Bay in the prior season. As such, donor material from Bay Farm Island was substituted into the program.

Eelgrass restoration planned for spring-summer 2020 was cancelled due to state mandated COVID-19 restrictions that prevented SFSU facility operations and highly constrained staffing as a result of gubernatorial orders. As a result, the 2020 field season was limited to monitoring requiring smaller crews than is required for transplanting operations. This planting effort was postponed to 2021.

In 2021, planting was again performed under the Cosco Busan program within Richardson. As was the case in 2019, Richardson Bay was not authorized for use as a donor site for the 2021 transplants due to CDFW concerns over depressed Pacific herring spawning in the Bay in the prior season; although approval of Richardson Bay as a donor area was eventually granted, it was not timely enough to include the site as a donor as plots had already been replanned to substitute donor material from Bay Farm Island into the program.

In 2022, planting was conducted within Richardson Bay in twelve mooring scars. Four larger scars were planted with half-acre plots, while 8 smaller scars were planted with quarter-acre plots. Donor eelgrass for the planting was derived from Point San Pablo, Point Molate, and Bay Farm Island donor beds.

EELGRASS HARVEST AND TRANSPLANTATION

Restoration within San Francisco Bay must rely on an adaptive approach to be successful and not wasteful of resources. To accomplish the outlined project goals, it is essential to implement a process of trial, observation, and response in conducting the efforts. At the present time, while the science of eelgrass restoration in San Francisco Bay has expanded immensely over the past two decades, it must still be considered to be in its infancy when compared to restoration science elsewhere. For this reason, the program to be followed relies on the foundation of prior transplant programs but continues to strongly integrate a science-based restoration approach that makes use of multiple restoration techniques, mixed donor genomes, and multiple test plots to steer the larger scale restoration efforts.

The project team has followed this approach as a means that both maximizes the potential for success on an incremental basis, while developing the best practices for the future restoration phases as well as future restoration in the Bay under the Subtidal Habitat Goals Project. Because the restoration objective extends over many years, early investment in learning while implementing the program will provide direct payoffs in subsequent years of the eelgrass restoration program.

Elements of the Restoration Design

Increasing Resiliency of Restored Beds

Efforts to maximize genetic diversity, such as collecting transplants for restoration over large areas of a donor bed, are expected to increase the success of seagrass restoration efforts (*e.g.*, Williams 2001). Sufficient spacing of donor material collections to ensure inclusion of stock from different donor plants can help to promote genetic diversity of donor stock (Boyer and Wyllie-Echeverria 2010). Because seeds provide enhanced diversity via sexual recombination, they are inherently a greater source of variation in comparison to vegetative shoot transplants. As such, practitioners in other regions often add seed to supplement whole-shoot transplants to increase the genetic diversity in restored populations (C. Pickerell, pers. comm.). During the 2014 Cosco Busan planting efforts, seed buoys were used in an effort to establish eelgrass. However, no evidence was observed that seed buoys had been successful in 2014. As a result, seed buoys were omitted from the 2015 planting with the intent to establish eelgrass through transplant methods first and subsequently follow up with the augmentation of established planting with additional seed buoy efforts. Seed buoys were again deployed at the MRGC in 2016 after limited evidence of seedling recruitment was noted within the restoration plots in 2015. Extreme low success for restoration in Corte Madera Bay and high degree of benthic disturbance by infauna within Richardson Bay made it less likely that seedling establishment from seed buoys would occur in these areas and as such seed buoys were not deployed in these areas during 2016. Following a period of high seedling recruitment after 2017 freshwater flushes, eelgrass along the San Rafael shoreline were strongly impacted by the freshwater flows and recovery of these beds suggested a combination of both plant survival from previously established plantings and high seedling recruitment. For this reason, seed buoys were again deployed in 2018 plantings given the anticipation of good seedling recruitment from this planting methodology. Results of monitoring in 2019 showed prolific expansion of both planted beds and seedling establishment. However, it was not clear whether seedlings were derived from seed buoys or mature plants in the restoration area, or a combination of both.

Donor Success, Mixing and Matching

Increased genotypic diversity, as tested by inclusion of multiple genotypes in experimental plots, has been found to enhance resiliency to disturbances such as high temperatures and brant geese herbivory (Hughes and Stachowicz 2004; Reusch et al. 2005) and to increase ecosystem services

(Reynolds et al. 2012). Further, as extant populations in San Francisco Bay show significant genetic structure (Talbot et al. 2004; Ort et al. 2012), it is advisable to utilize multiple populations as donors in a single restoration site in order to improve the probability of including genotypes suitable for that site (Ort et al. 2012). One previous study comparing donors found differential success in seedling establishment and subsequent height, growth, and flowering that persisted over time, suggesting that genetic differences are maintained at restoration sites (Boyer and Wyllie-Echeverria 2010). As such, multiple genetically distinct donor sites have been used to test the success of different donors alone and in combination at the large-scale restoration sites in this project. This has been done without sacrificing elements of the restoration by tracking the source and placement of donor material from differing sites and blocking the monitoring effort by donor material source such that differences in early plant material performance could be tracked.

Consideration of Different Planting Methodologies

- Broadcast Seeding

While many techniques have been used for eelgrass restoration in other regions, past experience suggests that site-specific conditions within the Bay can and do influence the success of the various methodologies. Because the factors dictating success or failure of a particular restoration method are not always determinable, this makes wholesale acceptance of one technique over others, without performance testing, inappropriate and likely to generate lower levels of success than could otherwise be achieved. For example, broadcast seeding is commonly used for eelgrass restoration in Chesapeake Bay and the Virginia Coastal Bays (Orth et al. 2012) but was not successful in trials within San Francisco Bay at three different sites (Boyer and Wyllie-Echeverria 2010). Further, where seeding fails, it is often difficult to determine where and when in the restoration process the failure actually occurred (*e.g.*, low seed viability, seed loss from the site, low seedling establishment, unsuitable sediments, herbivory of young plants, inadequate growth prior to low growth periods).

- Planting Frames

Similarly, frame systems have been used with success on the East Coast for transplanting groups of shoots (Short et al. 2002). However, these have not been very successful when tried in San Francisco Bay (Boyer 2008; WRA 2009; reviewed in Boyer and Wyllie-Echeverria 2010). It is possible that wave and current environments, timing of frame placement, and other factors result in inadequate rooting of frame supported plants.

- Bareroot Planting Units

Merkel Paper Stick Planting Unit

In 1986, an eelgrass planting unit comprised of multiple rhizomes with several turions bundled with cotton string to a paper stick anchor was developed as an alternative to the wire staple anchor being used at the time. This anchor was developed to address concerns over public safety on popular wading beaches where eelgrass was being restored at the time (Merkel 1987). Since this introduction, well over a half million units have been planted along the U.S. Pacific coast with still more being used on the U.S. Atlantic coast and in Europe.

Past work using these units within San Francisco Bay has informed the recommendations for restoration in the current project and identified needs for additional evaluation. Merkel & Associates

(M&A 1998, 1999, 2002, 2004b, 2006, and 2008) conducted bareroot transplants using planting units at the Emeryville Flats, Bay Farm Island, and Berkeley North Basin. Survival varied among locations within the study area but averaged 35% after 48 weeks. Initial establishment was greater using larger bundles (8–20 shoots) compared to smaller bundles (2–6 shoots) and led to the recommendation that 6 or more shoots be included in a bundle to maximize success. In prior studies, trimming leaves did not increase rates of survivorship as had been hypothesized due to the damage sustained by eelgrass leaves during transplantation (M&A 2004b). This finding was contradictory to the results of similar studies conducted years earlier in southern California (Merkel 1987). Bundles collected from four donor locations (Bay Farm Island, Brooks Island, Emeryville Flats and Keil Cove) and transplanted into Emeryville Flats did not differ in percent survival or basal area (M&A 2006).

Multiple turion Merkel planting units tested

Kiriakopolos Bamboo Stake Planting Unit

In 2007, Boyer Lab graduate student, Stephanie Kiriakopolos, developed a new technique that has been used effectively in several small-scale restoration projects around the Bay (Boyer 2008; Boyer and Carr 2009; Kiriakopolos 2012). In this method, individual vegetative shoots are wrapped loosely at the base with a small piece of burlap and secured approximately 15 cm from the top of a bamboo stake with a paper-covered wire twist-tie. The bamboo stakes are pushed down into the sediment so that they emerge approximately 10 cm (with 35 cm beneath the sediment surface), thus permitting good root contact with the sediment while securely holding the plants in place until roots establish. Historically, the Kiriakopolos unit has been assembled with one or two turions rather than the higher counts in the Merkel units (Merkel 1990).


Bamboo stake (Kiriakopolos) transplant units

The long bamboo stake used in this method may offer advantages over other bareroot planting methods in anchoring plants within highly unconsolidated sediments as well as providing a positive indicator as to specific locations at which plants had been planted in the event plants are subsequently lost or spread and it is desirable to note the initial planting unit origin. However, in considering different bareroot methods, this technique had not previously been compared directly to others such as the Merkel bundled shoot transplant unit described above.

The efficacy of the two methods warranted early restoration program comparison for cost, effectiveness, and donor material demands, given the overall extensive nature of restoration under the program and future benefits to even broader restoration of eelgrass within the Bay under the Subtidal Habitat Goals Project. Both units were modified somewhat to more directly compare the planting unit designs rather than the variables in planting unit construction. The total turions used in the units were standardized to 2-3 turions per unit and the leaves were trimmed to a fairly long 0.5-meter length. Units were compared during the restoration to assess performance and efficiencies of use. In 2014, both units were utilized in equal ratios with paired treatments of bamboo stakes and paper sticks for all donor sites and planting plots. In 2015, 2016, 2019, and 2021, due to higher efficiencies with the paper stick planting units, planting was biased towards this planting unit type, while retaining the bamboo stakes equally in test plot applications and as end caps on larger plots where the stakes serve as plot markers in future monitoring.

- Seed Buoy

As described, seed-based restoration methods may help to alleviate the problem of genetic diversity decreases in previous restoration attempts using whole shoot transplants (Williams and Davis 1996; Williams 2001) and can be less time consuming than whole shoot transplant methods. The Boyer Lab has used buoy-deployed seeding (seed buoys or BuDS) with some successes and some failures in several locations in the Bay (Boyer and Wyllie-Echeverria 2010). This technique uses harvested flowering shoots suspended in mesh bags buoyed above the sediment of a targeted restoration area (Pickerell et al. 2005). This technique simulates long distance dispersal of detached reproductive shoots (Harwell and Orth 2002; Orth et al. 2012) and takes advantage of the natural slow release of seeds as they mature. This technique permits placing flowering shoots at the restoration site the same day as collection (or only temporary holding overnight), and thus does not require facilities for seed collection and storage as in broadcast seeding.

Seed buoy eelgrass transplants at Marin Rod & Gun Club, San Francisco Bay

Dr. Boyer has used seed buoy methods successfully and unsuccessfully at several sites in San Francisco Bay (Boyer et al. 2008) and the methods are ready to be “scaled-up” to larger restoration efforts. The seed buoy system was applied in 2014 transplants for the Cosco Busan DARP restoration and used a temporary anchor attached to a float that supported a 9mm mesh bag that held fertile flowering shoots harvested from donor populations. As the seeds within the flowering shoots matured, they were released and fell through the mesh to the bay floor beneath the bag.

The 2014 Cosco Busan seed buoys were ineffective at stimulating substantial seedling recruitment and were therefore not used in the subsequent 2015 SFOBB restoration efforts. Rather, seed buoys are now considered to be a secondary tool for reconsideration in expanding eelgrass once establishment of beds from bareroot transplants is achieved at a site. Subsequent discussions between K. Boyer and the method developer, Chris Pickerell, have indicated that similarly secondary application of the method have now been adopted elsewhere as well. In future planting efforts, seed buoys are expected to be added to the transplant methods once a site is determined to be suited to eelgrass restoration based on survival and growth of bareroot planting units.

Harvesting of Donor Eelgrass

Eelgrass Donor Sites

As indicated previously, eelgrass material for the Cosco Busan eelgrass restoration has been derived from five different donor sites: 1) Point San Pablo/Point Pinole, 2) Point Molate, 3) Keller Beach, 4) Richardson Bay, and 5) Bay Farm Island in order to prepare planting units. During the initial 2014 planting plots for the Cosco Busan DARP restoration efforts all sites except Bay Farm Island were used to prepare five different donor-source planting units (each separate site and mixed source units).

Donor sites were selected for multiple reasons. First, the donor areas all supported relatively extensive, well studied beds that could provide adequate donor material to support both the Cosco Busan restoration efforts and the parallel implementation of the SFOBB restoration program for all years of the two programs without taxing the donor sites. As such, any performance information garnered regarding the individual sites had long-term value to the restoration effort, allowing a concentration of donor harvesting to fewer beds, if information supported such a shift. Second, these donor sites occur under differing conditions with respect to substrate, salinity, water clarity, and environmental energy. For this reason, if there is any adaptive advantage to the material under differing environments, a broad spectrum of environmental conditions are represented by the donor stock. Finally, all of these beds have been investigated genetically and been found to be unique from each other (Ort et al. 2012; Talbot et al. 2004). This provides a high potential for maintaining and expanding genetic diversity within the restored beds, over the diversity of any single donor site.

Between 2014 and 2015, significant bed declines at Keller Beach rendered the site unsuited for use in 2015 and 2016 transplants. With the loss of this donor site, the material requirements were made up by the use of additional Point Molate donor material. As with the 2014 transplants, test plots installed in 2015 used the Point Molate eelgrass only to maintain consistency among the plots. In 2016 test plots were comprised of a combination of Point Molate and Richardson Bay eelgrass donor bed plant material due to limited availability of eelgrass at all sites following considerable declines in eelgrass observed baywide in 2014 and 2015. In subsequent years, Point Molate remained a mainstay of the test plots, but plots were also often mixed with plant materials from other donor sites to add diversity and to make full use of harvested eelgrass for the restoration.

In 2019 CDFW did not authorize the use of Richardson Bay eelgrass beds as donors due to low levels of spawning by Pacific herring in Richardson Bay eelgrass during the prior winters. While donor use was eventually provided for 2021, it was too late in the planning stages to be integrated. Instead, Bay Farm Island was substituted in to provide an additional source of donor plant material. For 2022, donor sites remained Point San Pablo, Point Molate, and Bay Farm Island.

Harvesting and Handling Donor Material

Eelgrass material was harvested by gleaning rhizomes with intact shoots from the sediment within the donor beds. The harvest targeted collection of rhizomes that included at least three intact rhizome nodes and an undamaged shoot. No more than 10 percent of the shoots were harvested per square meter of bed. The harvest was performed either by SCUBA diving within subtidal beds, or intertidally by walking or crawling through the beds and collecting donor materials. This harvesting methodology leaves a continuous bed with the low density harvesting of shoots generally being undetectable following the harvesting.

Harvesting of material from subtidal areas of Richardson Bay and intertidal areas at the Point San Pablo/Point Pinole donor sites by Merkel & Associates and SFSU project team members.

As plants were harvested, they were either laid in bins under wet burlap to avoid desiccation and thermal stress, or they were retained in mesh bags in the bay water until they were transported to the Estuary and Ocean Science Center within one to three hours of harvest where they were placed in flow-through large seawater tanks for storage prior to preparation of bareroot planting units. Tanks were set to circulate water and air stones were placed in each tank.

Flow-through seawater tanks used to store eelgrass from donor sites until processed into planting units. Tanks were then used to store processed planting units until they were planted at the transplant receiver sites.

In 2015, some concern was raised by CDFW over the effects of significant harvesting to support the two large eelgrass restoration projects underway in the Bay. To address this concern, two actions were undertaken. First, the large San Francisco Bay eelgrass restoration projects were folded into a single San Francisco Bay Eelgrass Restoration Program. This action was taken such that application for Scientific Collecting Permit (SCP) could be submitted with a full disclosure and consideration of cumulative harvest levels. Further, it allowed the harvest efforts to be fully coordinated such that there was no risk of double harvesting the same beds without knowledge. Second, the team agreed to conduct an opportunistic investigation on plots that were thinned by the Boyer Lab for other purposes associated with evaluation of the effects of bed density on predator-prey interactions as an element of the *Zostera* Experimental Network (ZEN) investigations for which the Boyer Lab is a collaborator. Plots were thinned in July 2015 from the original bed density by 0 percent, 50 percent, and 80 percent for the ZEN studies. These plots were subsequently tracked to determine the differences in eelgrass bed density over time. The study indicated that at a 50 percent harvest level there were no differences between harvested and unharvested plots within six months of the harvesting. However, notably at 80 percent harvest levels, depressed shoot densities were observed through at least April 2016, 10 months into the post-harvest monitoring (Boyer et al. 2016). The study demonstrates that at the standard harvesting rate of 10 percent, it is reasonable to expect a lack of substantial and lasting effects on the donor beds. Similar donor bed recovery studies have been completed in prior years in San Diego's Mission Bay (Merkel, 1986 unpublished data) and at Bay Farm Island in San Francisco Bay (M&A 1999) and documented similar rapid recovery of donor beds from high harvest levels.

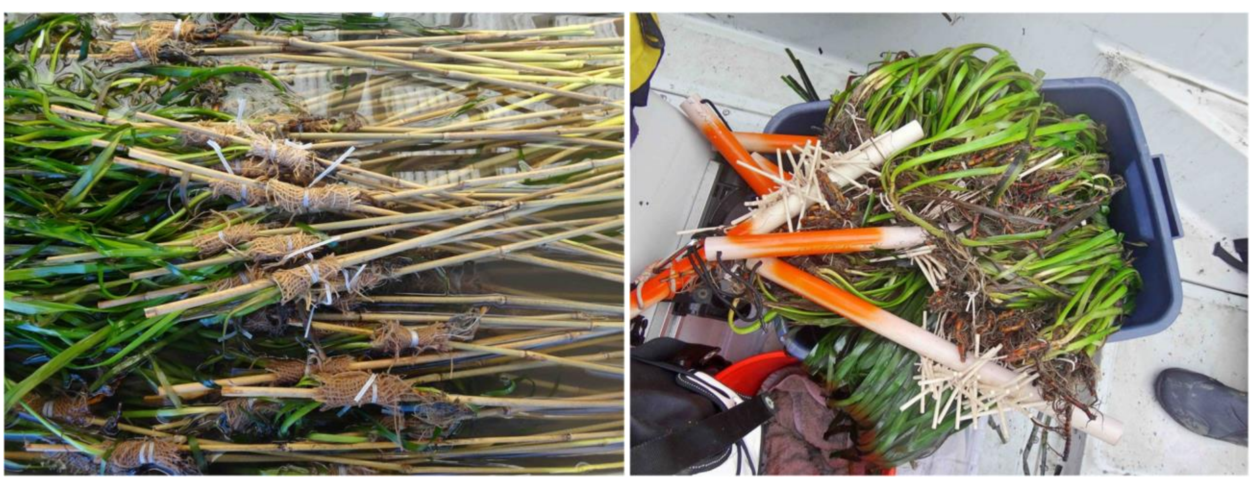
Preparation of Planting Units

Bareroot Planting Units

Eelgrass donor material was processed into uniform bareroot planting units. As needed, plants were moved from the storage tanks to water tables where they were prepared into bareroot planting units using either the Kiriakopolos bamboo stake or the Merkel paper stick anchors. Some of the donor eelgrass beds host the herbivorous invasive amphipod, *Ampithoe valida*, while others do not. As a result, eelgrass from infested donor sites was subject to multiple freshwater dips and rinses to remove amphipods prior to preparation of planting units. The collected amphipods were saved within a seawater tank for other experiments being conducted by the Boyer Lab.

Planting units were generally prepared within 24 hours of harvest of the eelgrass and were then stored once more in the flow-through seawater tanks to keep them cool and oxygenated. Planting units were identified by color coding of either the bamboo stakes or the carrying fids used for paper stick anchor units. Each donor site had a consistent unique color. When planting units were placed back into the seawater baths prior to planting, they were grouped in a manner that supported planting of each discrete planting area. This allowed plants to be picked up and loaded onto boats for each planting day in a manner that minimized handling and potential for planting errors.

Water-tables used to process donor eelgrass into bare root units. The eelgrass units being prepared in the photograph are Kiriakopolos bamboo stake units from the Keller Beach donor site as indicated by the painted yellow ends of the bamboo stakes.



Prepared planting units of Kiriakopolos bamboo stakes and Merkel paper stick anchors ready for transplant. Color coding denotes the donor source material in the planting units.

Seed Buoys

As indicated previously, seed buoys were used during the 2014, 2015, and 2018 transplant efforts but were not used consistently in plantings at Cosco Busan DARP transplanting locations. Seed buoys were prepared by harvesting mature flowering stalks from each of the donor beds and placing flowering stalks loosely within 9mm mesh bags. The bags were attached to a small line float and an anchor such that the bags could swing freely within a 3-meter radius. This radius avoided entanglement of buoys. The bags were separated by donor site by individual rows.

Eelgrass Planting Area Configuration

The broad scale planting efforts utilized the two whole plant bareroot transplant methods: the paper stick and the bamboo stake anchored bareroot units.

Eelgrass Test Plots

This type of planting is conducted under Phase I: Experimental Restoration as outlined in Appendix 8-1 of the San Francisco Bay Subtidal Habitat Goals Report and is used where information is lacking about a site or its capacity to support eelgrass. For the purposes of the present restoration, test plots have generally been used to also test the elevations within a site to establish the vertical extent of a site's suitability.

Test plots used in the present transplant consisted of an angled two-legged plot with each leg extending outward 25 meters at an approximately 90-degree angle from the central angle point. One leg of the plot generally extended normal to the prevailing wind patterns, while the second leg extended parallel to the prevailing wind (Figure 5). Each leg of the plot was planted at 1-meter centers, three plants wide such that each leg used 75 planting units. Test plots were comprised of plants from Point Molate and each leg of the test plot was planted with either bamboo stake or paper stick anchored units.

The ends of the test plot legs, the vertices of the angle, and the central gap between legs were retained for planting via seed buoys or other smaller scale experimental planting methods. Experimental planting methods applied have included high density planting plots using standard bareroot methods, broadcast seeding, weight anchored broadcast turion plantings, and free planting of unanchored turions. The association of highly experimental and limited scale planting methods with standardized test plot configurations has been used to assess experimental planting performance relative to standard planting methodologies. The experimental transplant methods were not implemented in an intensive or rigorous manner as the efforts are considered exploratory at the present time. Experimental planting methods, such as broadcast planting of eelgrass turions attached to non-galvanized washers were tested occasionally in an effort to find means of advancing planting methods suited to community-based restoration programs. In these instances, test plots were used mostly as a locator tool to facilitate monitoring of the experimental planting methods without undue additional efforts.

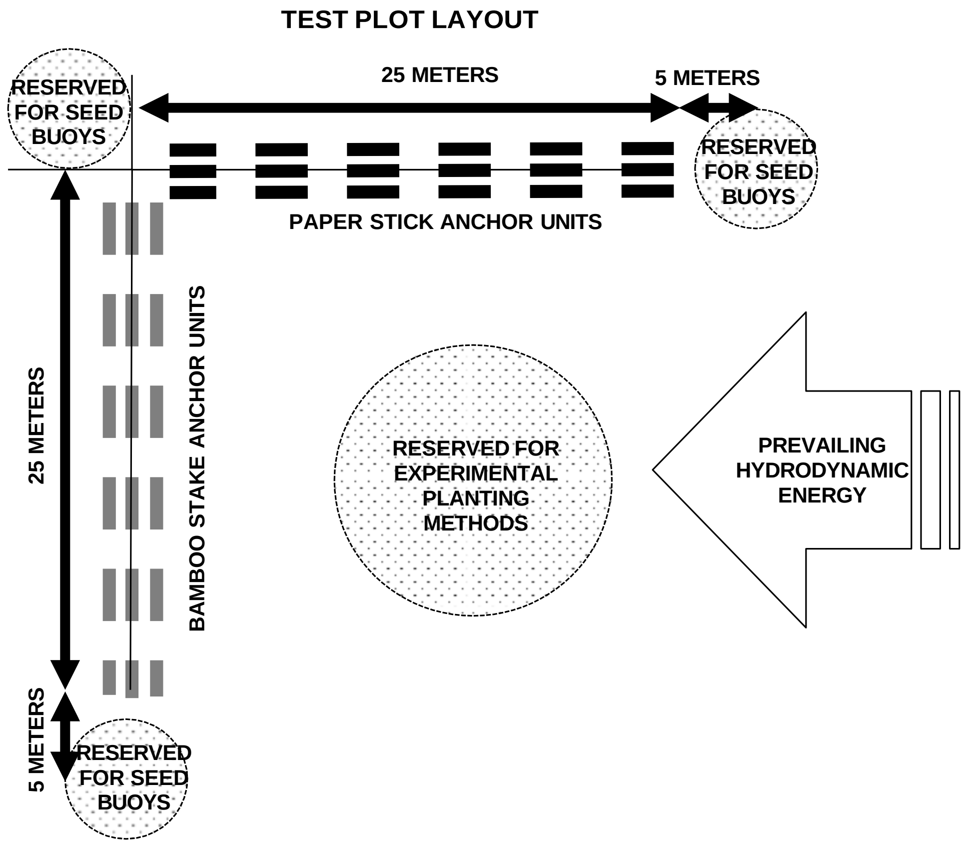


Figure 5. Typical Test Plot Planting Array Used for the Cosco Busan DARP Eelgrass Restoration (2014-2021 Planting Seasons)

Large-scale Planting Plots

The planting program used half-acre plots as well as the smaller test plots. These half acre plots were 81 meters long and 25 meters wide. Plots were planted in rows defined by planting unit type and donor site (Figure 6). Gaps were left between donor sites in order to accommodate plant performance tracking, seedling recruitment monitoring, and/or seed buoy deployment to facilitate infill of the planting areas. Plots used in 2014 and those used in 2015–2016 and 2019–2021 had the same general layout, but plantings varied between 2014 and the other planting years. In 2014, an equal ratio of bamboo stakes to paper stick units were planted in the plot with each of the four donor beds being independently planted in groups and a fifth treatment of mixed donor plant materials was also used. For field logistical reasons, the arrangement of all donors and treatments within the half-acre planting plots were maintained across all plots that were installed (Figure 6). This prevented potential for confusion in donor source within the plots during performance monitoring and analyses.

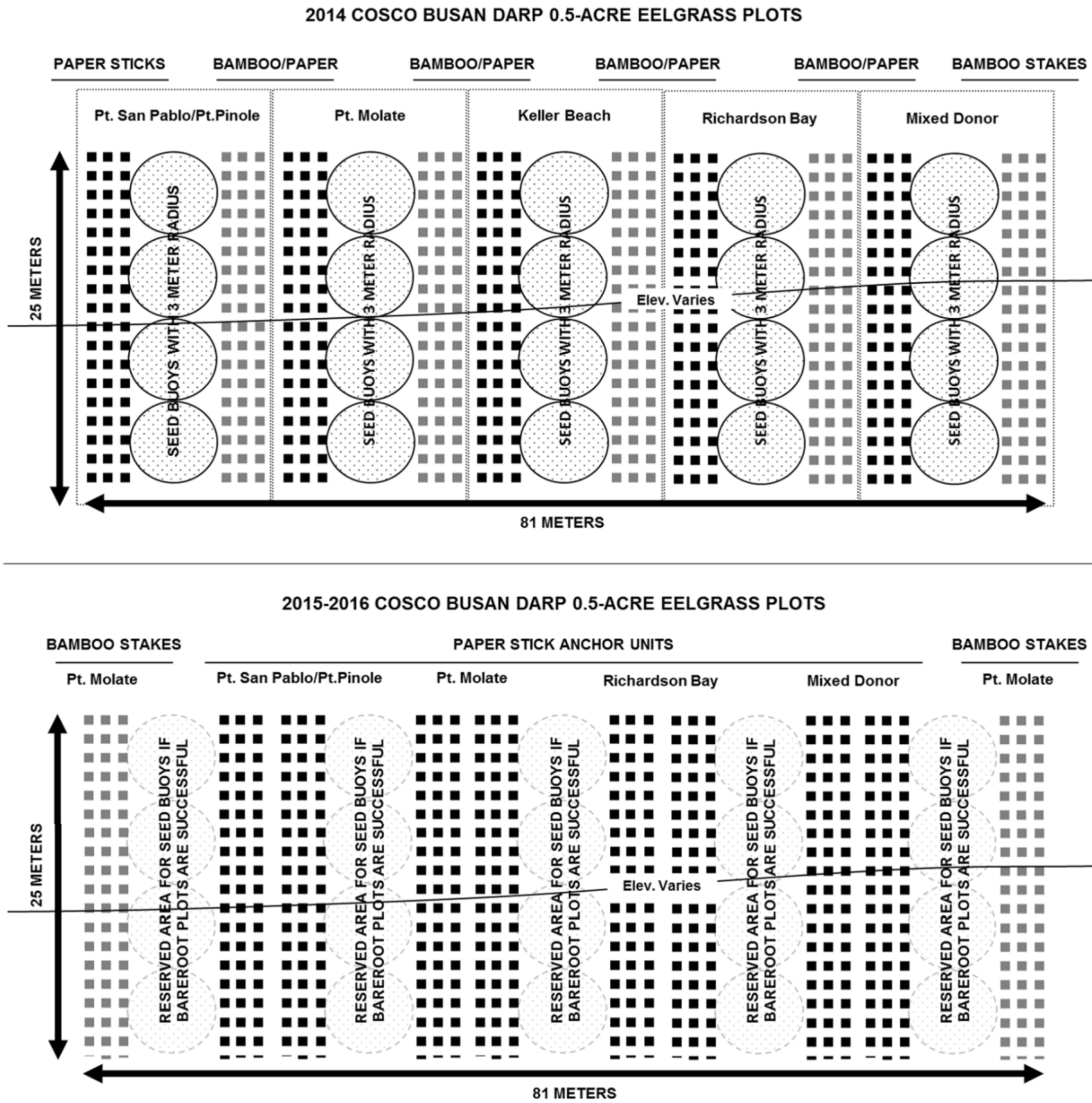


Figure 6. Typical Planting Array for the Cosco Busan DARP Eelgrass Restoration (2014, 2015-2016, 2019, and 2021 Planting Seasons)

Plots planted in 2022 utilized a cross pattern of planting units centered within each of the mooring scars. The legs of each of the four-legged crosses were planted with differing donor material with the north leg being Point San Pablo, east being Point Molate, south being Bay Farm, and west being mixed donor sites.

In 2015 and 2016, the same plot scale and planting row configuration as used in 2014 was used. However, the shortage of eelgrass from the Keller Beach donor site and the decision to postpone seed buoy use required a reconfiguration of plantings within the plots. In 2015, the planting layout for each half-acre plot followed a consistent pattern beginning with three 25-meter-long rows of bamboo stake units from Point Molate, followed by a gap and then six rows of paper stick anchored units from the San Pablo/Point Pinole donor, a gap, and repeated patterns with Point Molate, Richardson Bay, and mixed donor sites. At the end of the planting plot a second series of bamboo stake planting units from Point Molate was included (Figure 6). During 2019–2022, Richardson Bay donor material was substituted out and replaced with Bay Farm Island donor material. As plants are established from bareroot plantings, seed buoys may or may not be added to the gaps, depending upon the growth and expansion of initial plantings and any evidence of recruitment into the gaps from established plantings.

The large-scale planting plots are intended to result in the majority of the area of eelgrass planting under this restoration program. As such, the plots have been specifically targeted to areas where the greatest expectation of success exists with the smaller test plots being used to assist in defining the suitable planting area boundaries. Nominally, the plots are located between 0 m MLLW and -1.2 m MLLW with the majority of the plots being centered along the -0.6m ft MLLW contour. These ranges have been subsequently adjusted based on the natural depth range of eelgrass represented in planting plot proximity and performance of the test plots planted in both this effort and that of the SFOBB eelgrass restoration. The preliminary planting range selected for this effort is based on the fact that a full 94 percent of all eelgrass within San Francisco Bay was determined to occur within a 1.6 meter depth range between 0 and -1.6 m MLLW (M&A 2004a). More recent investigations have indicated that the depth distribution of eelgrass varies substantially within the Bay and that the eelgrass range within each of the targeted restoration areas is generally much narrower than that observed baywide (M&A 2015). However, the core of the planting range and the estimated percentage of eelgrass distribution remains similar to that previously reported.

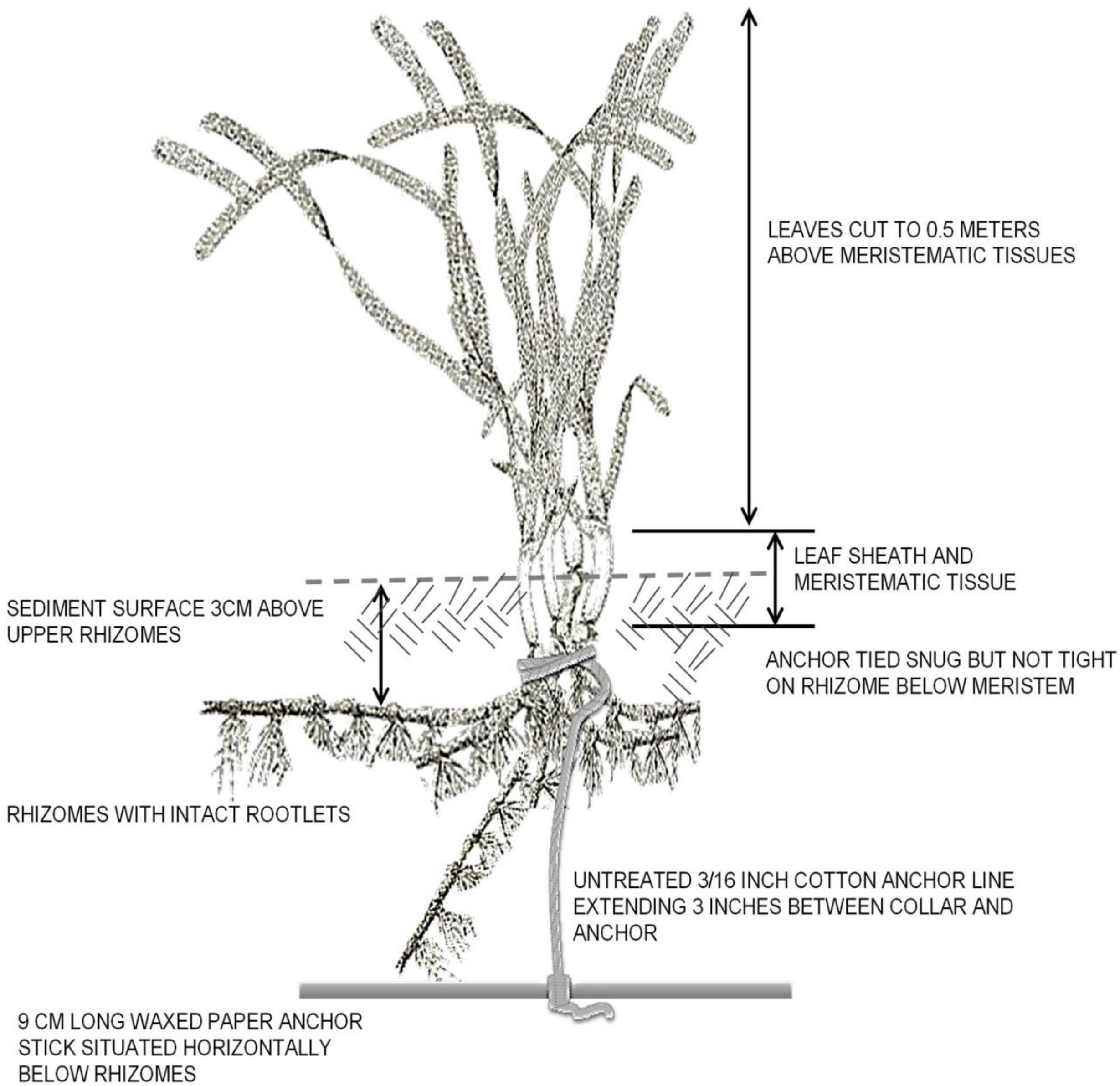
Planting Methods

To complete the plantings, temporary weighted lines were laid out across the bottom using corner points established by GPS using either sub-decimeter accurate real time kinematic (RTK) or sub-meter accurate WAAS-enabled differential GPS. Individual guidelines were subsequently stretched between corner points. A planting “T” with one-meter-long legs was used to guide the planting along the established planting lines. Planting depths ranged from approximately 0 m MLLW down to depths as low as -1.2 m MLLW. As such, planting methods similarly ranged from intertidal planting by field staff wading in the planting zone, to subtidal planting by SCUBA diving. Because the diving tended to be very shallow, often times divers were supported by a surface tender that handed planting units down and assisted with moving lines between planting zones.

Eelgrass planting at Corte Madera Bay, Marin Rod & Gun Club, and Richardson Bay Audubon Sanctuary. Planting varied from subtidal planting by divers to intertidal planting by wading. Temporary guidelines and corner stakes were set prior to planting and removed following the planting efforts.

- Planting Merkel Paper Stick Planting Units

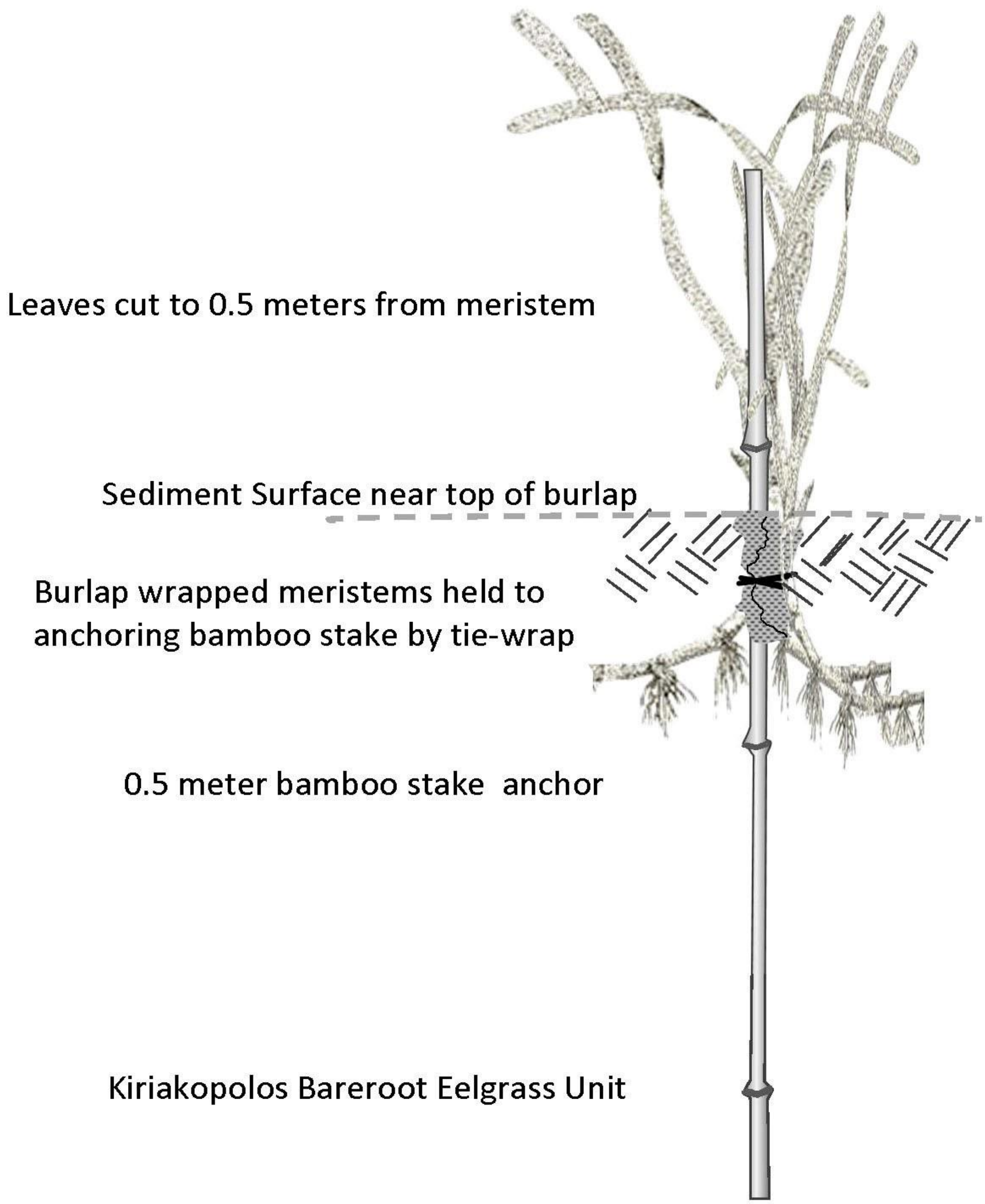
For the Merkel paper stick planting units, planting was conducted by burying the paper stick anchor units parallel to the sediment surface deep in the sediment such that the cotton string connecting the anchor to the rhizome bundle was tight with the rhizome bundle being installed approximately 3 cm below the sediment surface with the leaves of the unit extending vertically through the water column. When properly planted, the paper stick anchors are fully buried.



Typical bareroot planting for the Cosco Busan DARP restoration program using the Merkel paper stick anchor methods.

- Planting Kiriakopolos Bamboo Stake Planting Units

For the Kiriakopolos bamboo stake units, the stake is buried vertically with approximately three-fourths of its length in the sediment and the remainder of the stake extending vertically above the sediment surface. The burlap wrapped rhizomes of the planting unit are again buried approximately 3 cm below the sediment surface with the leaf bundles extending above the sediment surface adjacent to the bamboo stake.



Typical bareroot planting for the Cosco Busan DARP restoration program using the Kiriakopolos bamboo stake anchor methods.

Eelgrass Restoration Monitoring Methods

For the Cosco Busan DARP eelgrass restoration program the metrics to be monitored are metrics of the restored eelgrass and metrics of herring spawning use of the restored habitat areas. These monitoring elements are discussed separately.

Eelgrass Monitoring

For eelgrass restoration, the metrics include spatial metrics associated with the bed and plant metrics associated with individual plant properties within the restoration areas. Specific eelgrass metrics monitored for this restoration project include:

Eelgrass Bed Spatial Characteristics

- Area created and/or enhanced, expanded
  Areal extent
  Spatial distribution
- Percent vegetated bottom cover within bed
- Elevation range of established eelgrass

Eelgrass Plant Characteristics

- Turion (shoot) density
- Plant height
- Seedling recruitment and survival

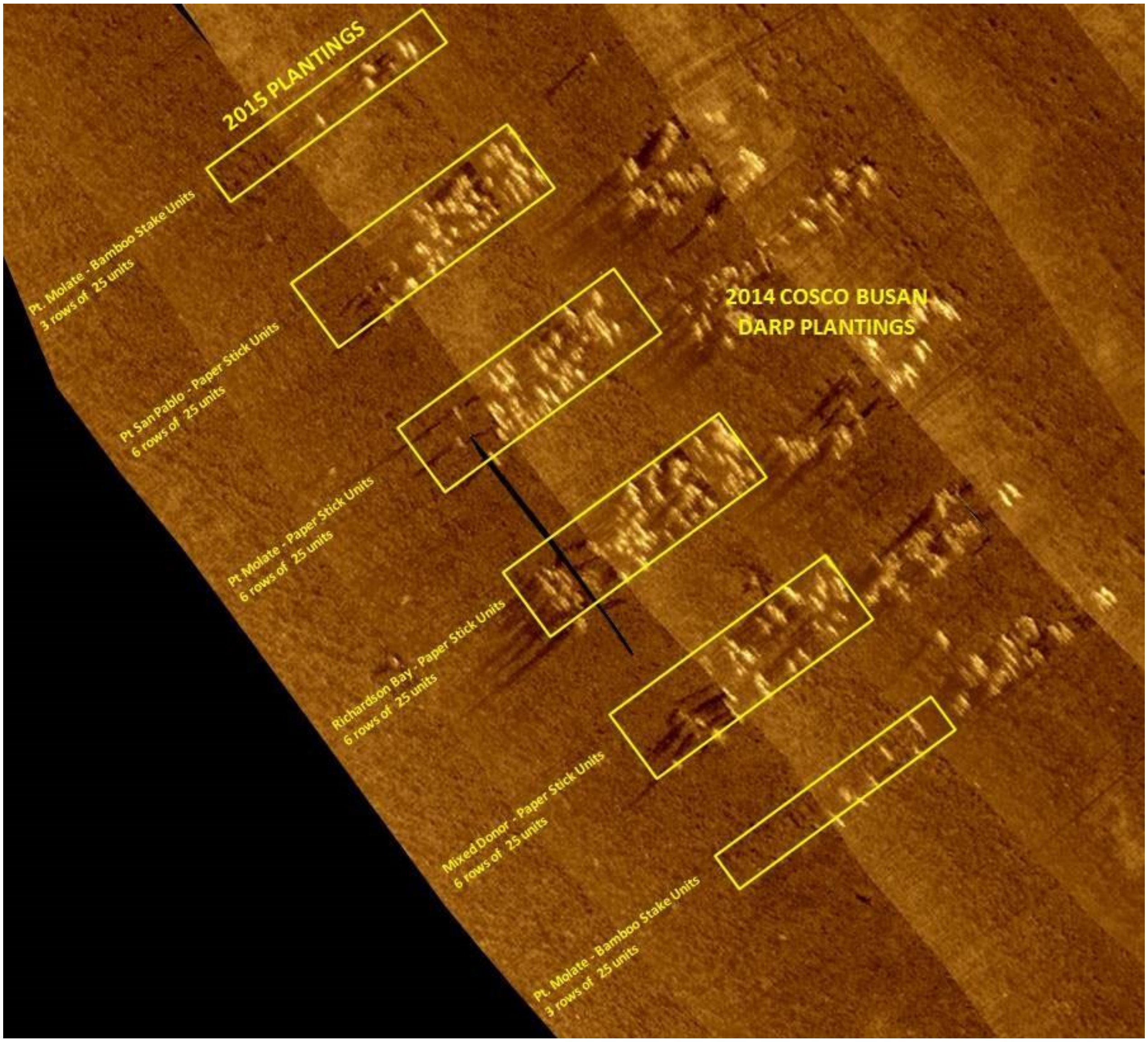
In addition, observations of other site conditions or plant characteristics that would assist in enhancing the success of eelgrass restoration were also made during the monitoring work. Monitoring for spatial characteristics and plant characteristics metrics uses two different methodologies. Eelgrass bed spatial extent and bottom coverage metrics are best assessed using wide swath acoustic mapping methodologies while plant metrics such as turion density, plant height, and recruitment of seedlings are assessed by direct plant observation. Seedling recruitment and survival are metrics that can be best assessed during early phases of seedling establishment through direct field observations, but which can be later assessed using acoustic mapping tools.

Interferometric Sidescan Sonar Mapping

The area of eelgrass beds and percent bottom cover within the beds within each transplanted plot were determined by interferometric sidescan sonar survey of the transplant areas conducted from 2014 through fall 2022. The surveys produced spatially accurate acoustic maps of the eelgrass habitat. Transplant plot by plot metrics of eelgrass cover area and percent bottom cover were produced for use in the interpretation of performance of the initial plantings. The interferometric sidescan sonar survey, mapping, and GIS spatial analyses were conducted by M&A, coincident with monitoring regional reference beds.

Sonographic surveys were undertaken using an interferometric sidescan sonar system. The interferometric sidescan system consisted of a dual channel hull mounted sonar operating at 468 kHz that integrates a vessel motion sensor to correct for vessel pitch, heave, and roll; a sound velocity sensor that corrects for speed of sound in water related to density differences resulting from changes in temperature and salinity; and a dual antenna RTK GPS that provides precision vessel positioning and correction for vessel yaw. Because the position of the interferometric sidescan sonar head is rigidly fixed to the vessel, the positional error is dramatically reduced from that associated with other mapping methodologies, including traditional towed sidescan sonar. Further, the system greatly reduces the complications of vegetation and other features that can foul towed sonar systems and limit survey coverage. With the survey system utilized in this effort, absolute positional error for eelgrass mapping is approximately ±1 meter. The relative positional error is estimated at ±0.25 meter as the positional error is substantially nullified across short distances represented within sonar mosaics. For the present mapping work, the interferometric sidescan was set to 31 meters on the port and starboard channels such that the full swath was 62 meters wide. At this swath width, the generated digital image is comprised of pixels that are 6 cm x 6 cm with the pixel intensity being generated by the average reflective conditions of the surfaces within the pixel. Because eelgrass is acoustically highly reflective due to air in the lacunae of the leaves, even a few leaves can generate an acoustic signature separating the eelgrass from the low reflectivity soft sediment.

Following completion of the field surveys, sonar traces were downloaded and processed into rectified mosaic images in a GeoTiff format using Chesapeake Technologies, Inc. Sonar Wiz. The planting plots were digitally overlain on the sidescan mosaic to assess the individual plot performance. Eelgrass was then mapped by manually digitizing from registered sidescan mosaics and aerial photographs using ESRI ArcGIS software. Mapping techniques and areal coverage determination have been made using of a mix of analytical techniques developed and employed in prior eelgrass surveys. For each area of the sidescan sonar mosaic that supported eelgrass, the spatial extent and bottom cover of eelgrass was determined. Patterns of restoration site performance were investigated by comparing eelgrass distribution and extent of bottom coverage across the multiple post-planting surveys. In addition, features such as bottom disturbance by biogenic agents (*e.g.*, bat rays, burrowing organisms) as well as anthropogenic agents (*e.g.*, propeller scarring) were noted from the image interpretation.



April 2016 interferometric sidescan mosaic illustrating an example of successful eelgrass transplant plot from 2014 Cosco Busan DARP and 2015 SFOBB plantings. The mosaic identifies the donor areas and types of planting units initially installed during the transplants. Because images are spatially rectified, they can be overlaid in ArcGIS to interpret patterns of change including areas of the bed that have experienced high growth or mortality. By evaluation of multiple images, the overall bed dynamics may be partially explored.

Direct Field Observations

From fall 2014 through July 2022, the Boyer Lab staff made multiple extreme low tide visits to each of the planting areas to evaluate the status of the transplants. These site visits included an assessment of: 1) the presence or absence of eelgrass within the planting plot; 2) the mean density of turions present within the surviving plants; 3) measurement of plant heights; 4) reproductive status of plants and the presence or absence of detectible seedlings in the transplant area. In addition, plants were assessed for overall vigor as well as any identifiable biotic or abiotic stressors.

To assess these parameters, the transplant plots were sampled individually by initial restoration treatments. Flowering frequency and inflorescence development were assessed following de Cock (1980), and overall non-destructive plant vigor assessment based on leaf color, turgor, epiphytic loading, macroalgal bed loading, tissue blemishes, herbivore grazing, as well as leaf tip erosion and new leaf development were evaluated.

For turion density, sampling was conducted using 0.5m x 0.5m (0.25 m^2) quadrats placed over randomly selected transplant units where multiple units existed and a full sampling of planting units where only a few planting units remained in a transplant. This sampling design is intended to provide spatial coverage across the half-acre plots while also permitting comparison of transplant methodologies and success of the donors at each site and depth.

In addition to assessing vegetative plant development, monitoring was intended to utilize spring quadrat sampling to determine seedling recruitment within each treatment of each plot. Seedling survival was to be assessed based on follow-up sampling in summer months to determine first year seedling survival ratios. Subsequent survival and growth tracking of seedling established plants were to be undertaken using the acoustic survey methods that allow tracking of individual plants until beds coalesce. While the seedling monitoring protocols were initially followed, the subtidal beds were not effectively investigated due to water turbidity and a limited extent of seedling recruitment in general, thus making the quadrat sampling an ineffective tool for seedling monitoring. Instead, comprehensive investigations were undertaken within the transplant areas to search for any seedling recruitment.

Biotic and abiotic stressors were evaluated through observations of the site conditions and plants during each field investigation. While not always obvious with point sampling, efforts were made during all sampling efforts to identify any plant conditions, substrate characteristics, site disturbance, water temperature, elevated turbidity, depressed salinity, or biological activities that may have adversely affected plot performance.

In order to monitor the vegetative shoot density for specific donors, the monitoring teams first located the bamboo end lines in each monitored plot. From the starting line, the plots were then followed in a north or south direction to complete monitoring by tracking each donor bed planting line. Changes in plot layout between 2014 and 2015 resulted in creating a larger gap between donor bed lines by repositioning of seed buoy gaps. This greater spacing between donor lines made it much easier to distinguish donor lines than was the case for monitoring 2014 planting plots. Once a donor line was identified, a 0.25 m^2 quadrat was placed over patches of eelgrass. Within the quadrats, the number of vegetative and flowering shoots and height of vegetative shoots (1 height per quadrat) were determined. Three 0.25 m^2 quadrats in each of the bordering bamboo lines, and five 0.25 m^2 quadrats in each of the other donor lines were sampled in 2015 to 2017, while in the 2014 plots 5 quadrats within each donor line were sampled where lines could be distinguished.

The monitoring of plots was often complicated by poor low tide windows that occurred at night or which were not low enough to reveal subtidal plantings. As a result, monitoring dependent upon visual observations of plants was often thwarted and monitoring was done by tactile based measurements and enumeration. This method of measurement, while possible, was less definitive than when measurements were aided by visual observation of plants.

Beginning in 2017 and continuing through 2022, direct field observation monitoring was aided by using an accurate Trimble GPS unit coupled with interpreted sidescan sonar data that allowed for strategic deployment of monitoring teams and graphic presentation of plot layouts to support the monitoring in subtidal environments where location of plants was often difficult due to turbid water or dark period monitoring windows.

Early morning low tide monitoring was conducted on August 29, 2015 in Richardson Bay. Plant survival, growth, and height were assessed for plants installed in June of the same year.

RESULTS

EELGRASS PLANTING SUMMARY

2014 Field Season

Planting for the 2014 season was conducted on multiple parcels owned by MRGC, Marin County Parks, and TNC. All of the 2014 plantings were conducted on the Marin County shoreline (Table 1, Figures 4a and 4b). Site selection was conducted in the spring of 2014, and focused site surveys to develop detailed bathymetry and document absence of eelgrass at the sites and in the vicinity were conducted in May 2014. Formal authorization to utilize sites was subsequently obtained from the various property owners and provided to BCDC to support CZMA Consistency Determinations for the work. From the period February through June, the M&A Team coordinated with the regulatory agencies and NOAA to support permitting, obtained CDFW harvest and transplant authorizations, and continued to coordinate with other property owners regarding future transplanting opportunities.

The 2014 eelgrass planting effort for the Cosco Busan DARP included planting of 1.5 acres in three half-acre plots at the MRGC and planting of an additional 2.5 acres in five half-acre plots within Corte Madera Bay. During the 2014 season, numerous additional plantings of test plots were undertaken within the separate SFOBB active eelgrass restoration and monitoring program. These test plots were reported on in a separate report (M&A and Boyer Lab, San Francisco State University 2016). Plantings were established in early summer 2014. Planting was implemented using the EOSC-RTC as a base of operation for plant material storage, unit preparation, and staging of work at each site. Plot performance was subsequently tracked and information on plot performance was used in the planning of 2015 plantings.

2015 Field Season

During the 2015 season restoration was conducted on multiple parcels owned by Richardson Bay Audubon Society, MRGC, and Marin County Parks. All of the 2015 plantings were conducted on the Marin County shoreline. Donor sites for eelgrass material included Point San Pablo, Point Molate, and Richardson Bay (Figure 1). Site selection was conducted in the spring of 2015 and focused on site surveys and documented absence of eelgrass at the sites during the baywide surveys conducted in October 2014. In addition, site selection made use of information following 2014 planting activities and monitoring results from the fall 2014 and spring 2015.

The 2015 eelgrass planting effort for the Cosco Busan DARP eelgrass restoration was conducted in late Spring 2015. Planting was again implemented using the EOSC-RTC as a base of operation for plant material storage, unit preparation, and staging of work from boats at each site. Bareroot plantings from multiple donor sites were prepared and planted within the planting plots. In total, 4 acres of eelgrass, including large half-acre and test plots were installed within three regions. These include planting of one acre within two half-acre plots at the MRGC along with 4 test plots to assess the vertical range of suitable conditions on the site (Table 1, Figure 4a). A single half-acre site and 4.5 test plots were planted within Corte Madera Bay (Table 1, Figure 4b). In addition, a small experimental plot was planted using washers as anchors for turions. This method used 5/8-inch non-galvanized washers attached to the rhizomes of three turions such that when dropped, the rhizome would settle on the bottom beneath the washer weight, holding the leaves upright. A total of 50 planting units were installed in this fashion as a small pilot seeking planting efficiencies for subtidal planting. Finally, 1.5 acres consisting of three half-acre plots and 7 test plots were installed within the Richardson Bay Audubon Sanctuary (Table 1, Figure 4c).

2016 Field Season

During 2016 planting was focused on areas that had success during prior planting years. One half-acre plot was planted at MRGC along with 5 test plots that were used to explore potential for expanded vertical range of planting (Table 1, Figure 4a). No additional plantings were installed at Corte Madera Bay in 2016. At Richardson Bay three half-acre plots were installed. An additional 6.5 test plots were widely distributed within the Audubon Sanctuary to determine suitability for both expanded planting based on horizontal and vertical constraints (Table 1, Figure 4c).

2017 Field Season

No planting was completed in 2017. Site selection was completed in support of the transplant effort, but due to extreme low salinities in the bay and loss of eelgrass early in the season, along with high snowpack levels in the Sierra-Nevada Range, it was anticipated that prolonged high discharge from the Sacramento Delta would continue into the late spring and eelgrass survival would be compromised. Further, it was determined that the substantial decline in donor bed condition reduced the suitability of harvest when coupled with anticipated low success of restoration plantings given the depressed salinity regime. For this reason, the project team and NMFS determined that planting in 2017 should be deferred until positive conditions were met.

2018 Field Season

No planting was conducted in 2018. The planting season occurred during a period when contracting was not in place for the program. As a result, the concurrent SFOBB planting was conducted and monitoring was undertaken for both programs, but no planting was completed for the Cosco Busan DARP.

2019 Field Season

During the 2019 season, restoration was conducted within Richardson Bay. Donor sites for eelgrass material included Point San Pablo, Point Molate, and Bay Farm Island. The 2019 eelgrass planting effort was conducted in mid-summer 2019. Planting was implemented using EOSC-RTC as a base of operation for plant material storage, unit preparation, and staging of work from boats at each site. Bareroot plantings from multiple donor sites were prepared and planted within the planting plots. In total, 2.7 acres of eelgrass were installed within 5 half-acre plots and 4 test plots (Table 1, Figure 4c).

2020 Field Season

Just prior to the commencement of the 2020 field season, COVID-19 pandemic restrictions took effect and continued through the planting season, thus terminating the restoration activities during 2020. However, monitoring that could be achieved with smaller crews and while implementing measures to minimize potential for disease spread was completed. This included acoustic surveys and limited crew size site *in situ* monitoring. Planned eelgrass restoration for 2020 was moved forward to the 2021 season.

2021 Field Season

Restoration was conducted within Richardson Bay during the 2021 season. Donor sites for eelgrass material included Point San Pablo, Point Molate, and Bay Farm Island. The 2021 eelgrass planting effort was conducted in mid-summer 2021. Planting was implemented using EOSC-RTC as a base of operation for plant material storage, unit preparation, and staging of work from boats at each site. Bareroot plantings from multiple donor sites were prepared and planted within the planting plots. In total, 3.5 acres of eelgrass were installed within 7 half-acre plots. Two of these plots were located within the Richardson Bay Audubon Sanctuary and five of these plots were vacated mooring scars within Marin County owned lands under the jurisdiction of the RBRA (Table 1, Figure 4c).

2022 Field Season

Restoration was conducted within Richardson Bay during the 2022 season. Donor sites for eelgrass material included Point San Pablo, Point Molate, and Bay Farm Island. The 2022 eelgrass planting effort was conducted in mid-summer 2022. Planting was implemented using EOSC-RTC as a base of operation for plant material storage, unit preparation, and staging of work from boats at each site. Bareroot plantings from multiple donor sites were prepared and planted within the planting plots. In total, 4.0 acres of eelgrass were installed within 4 half-acre plots and 8 quarter-acre plots. Plots were all located within mooring scars in the central portion of Richardson Bay (Table 1, Figure 4c). Moorings are being cleared out of eelgrass by RBRA and subsequently, cleared moorings are being restored to eelgrass.

ACOUSTIC SURVEY RESULTS

San Rafael Bay Shoreline (Marin Rod & Gun Club)

All plantings were investigated in July and October 2014; June and August 2015; April, July, and October 2016; April, July, September 2017; April and October 2018; June and October 2019; May and September 2020, April and October 2021, and April and October 2022. The acoustic monitoring has provided tremendous insight into the dynamics of transplanted eelgrass habitat in San Francisco Bay.

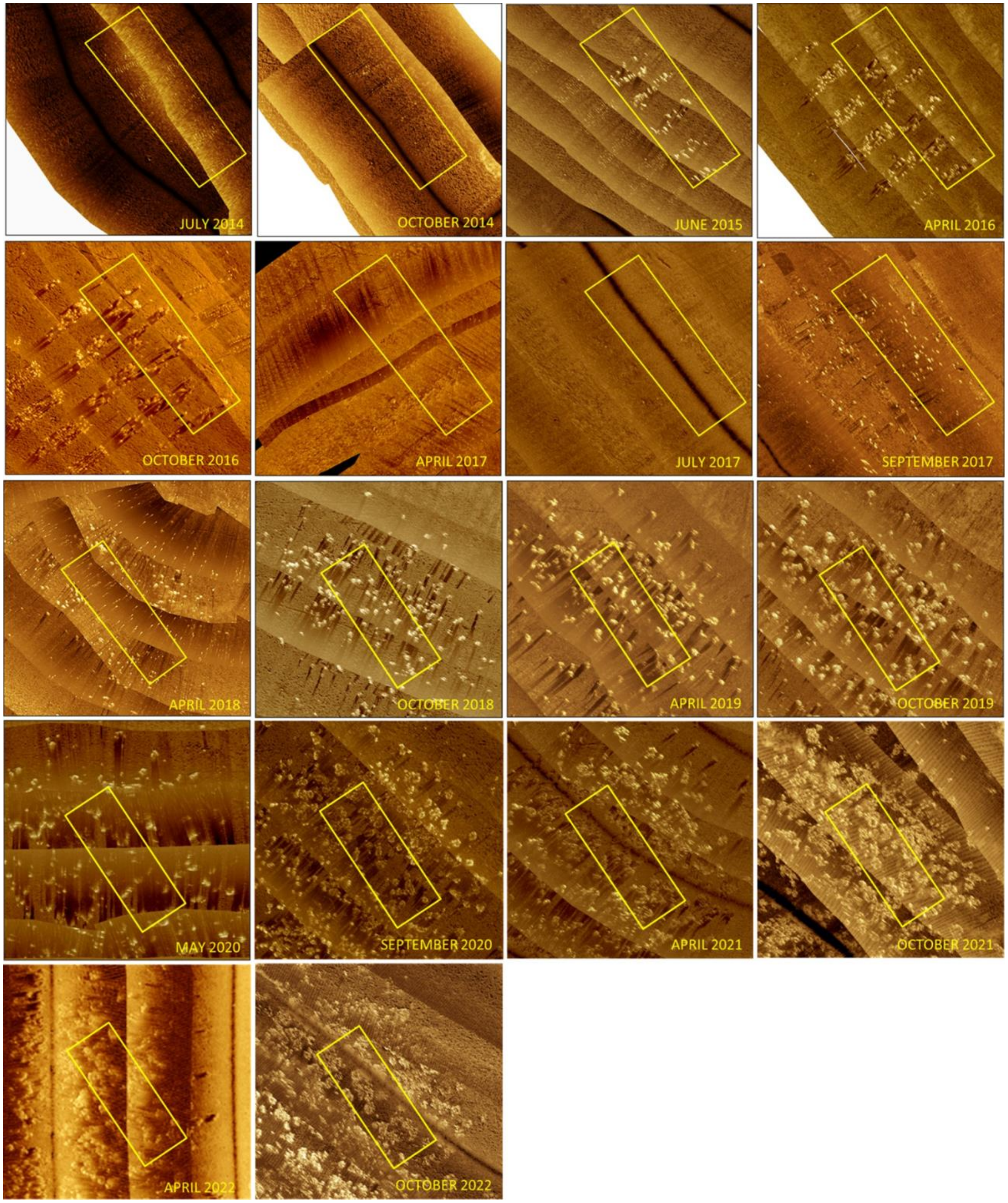
The spatial dynamics patterns of eelgrass bed development are well illustrated by a series of sidescan mosaic plates depicting the same portion of the transplanted eelgrass beds through time. Also of note is that the San Rafael shoreline has not supported naturally occurring eelgrass beds north of the Richmond-San Rafael Bridge during any surveys completed in the area from 1999 to present. However, eelgrass from the direct restoration and expansion of plants from these efforts has been reflected in many surveys completed through the area since restoration efforts commenced. This offered a unique opportunity to examine eelgrass persistence and spread through introductions of known origin and timeframe. It also allows examination of bed dynamics through time.

The Boyer Lab completed a number of test plantings at MRGC north of the pier for several years to examine eelgrass restoration in an experimental manner. In 2014, the first plantings were performed south of the MRGC pier using Cosco Busan DARP funding. After this effort, successful introductions were followed by additional Cosco Busan restoration in 2016 as well as SFOBB restoration in this area in 2015 and 2016. Eelgrass from this restoration has persisted as a highly dynamic mosaic bed now for six years. In addition, peripheral colonization of eelgrass in adjacent shoreline areas has also occurred, presumably due to the adjacent restoration beds providing seed. By tracking a small section of the transplant region through time, it is easy to see the extent of bed variability both seasonally and interannually. During 2018 and 2019 eelgrass expanded substantially northward along the shoreline from the more persistent cores around the transplant areas. However, in 2020 eelgrass retracted back towards the southern core of the original planting areas but remaining beds increased in the density of bottom coverage over that observed in prior years. In 2021 beds at MRGC continued to densify but did not expand outward along the borders of the beds established, suggesting that the site is nearing its natural carrying capacity under prevailing conditions.

During July 2014 eelgrass was noted to be persistent at most planting plots at the MRGC. By October 2014, most of the plants north of the MRGC pier were absent from the planting areas with only a handful of planting units being detectible within slightly deeper plots with all intertidal plants having been lost. Conversely, while planting units had exhibited considerable declines in both abundance and size from July to October 2014 in the half-acre plot installed south of the MRGC pier, the more southerly portions of the plot remained generally intact. This plot was slightly deeper (-0.9 m MLLW) than the other large plots at this site (-0.6 m to -0.8 m MLLW). Based on plot survival south of the pier, an additional plot was installed in May 2015 under the SFOBB active eelgrass restoration and monitoring program, and pilot plots were established north of the pier and in deeper water offshore of the remaining 2014 plot.

In June and August 2015, this site was again surveyed by interferometric sidescan sonar. These surveys revealed substantial regrowth of 2014 plants by June without much expansion of these plants by August of the same year. The new SFOBB plants continued to persist and expand slightly from the initial May planting period. The sites were again investigated in April 2016 and exhibited little change in the initial 2014 planting plots from the prior June but showed considerable expansion in plants installed in the adjacent plot from June 2015 to April 2016. In April 2016, the interferometric sidescan sonar revealed some limited evidence of seedling recruitment within the planting plots. Also, while not definitive, some bottom scarring by outboard motors in shallow waters near the south side of the MRGC Pier along with observations of vessel traffic to and from the MRGC launch ramp suggest a possible factor influencing distribution of eelgrass from the transplant expansion.

The rapid first year growth observed from the two half-acre plots followed by the apparent slowing of growth in the 2014 plot relative to the shallower 2015 plot is interesting, but not fully understood. It is possible that the differences are merely a reflection of the lower light levels and slower seasonal



Monitoring history of adjacent half-acre planting plots from July 2014 through October 2022 illustrating interval changes in eelgrass through time with a notable loss of eelgrass and restructuring of beds in response to 2017 freshwater flush. The oldest 2014 Cosco Busan plot (yellow) is bounded to the right and left by SFOBB planting plots.

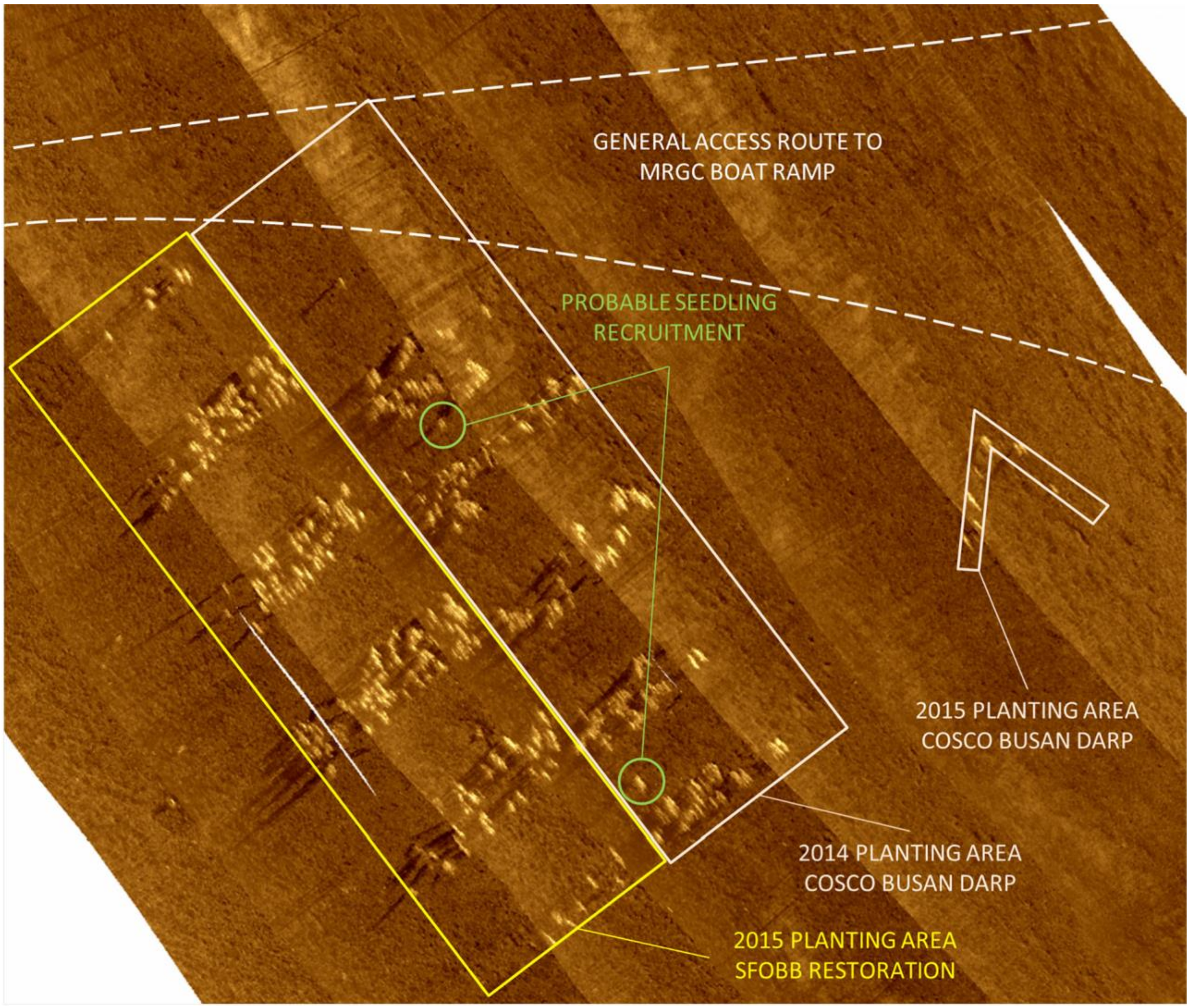


Diagram illustrating the MRGC southern planting plot status early in bed establishment as of April 25, 2016. Early losses of eelgrass at the northern end of the combined plot are believed to be related to boat traffic that may have resulted in early plant losses. Limited evidence exists that minor seedling recruitment may have occurred from the seed buoys placed in 2014, or as a result if subsequent plot seeding.

expansion in the deeper 2014 plot or it may be indicative of other controlling factors on eelgrass development. One potential controlling agent may be sediment chemistry, especially nutrient depletion, changing sediment chemistry with eelgrass colonization, or potentially microbial agents that affect eelgrass growth and development differently at differing stages of plant establishment. In any case, continued observation of these plots and investigations into controlling agents may assist in illumination of the reasons for plant expansion and survival differences.

Through 2016, eelgrass continued to expand surviving plots with a considerable increase in spatial extent of the beds and rapidly increasing areal extent of eelgrass occurring. However, in early 2017 prolonged flood conditions depressed salinity and increased turbidity resulting in devastating effects on eelgrass transplant beds on the San Rafael shoreline. During April 2017, surveys noted severe reductions in all spatial metrics, and corresponding direct plant observations made by the Boyer Lab during the *in situ* monitoring in January and February 2017 revealed precipitous declines in plant conditions occurring between the two field visits. Kathy Boyer noted that on February 26, 2017, raking her hand through the leaf canopy resulted in separation of leaves from the plants. The leaves were noted to lack any turgor and likely suffered from significant cell lysis due to osmotic stress from low salinity. By July 2017, the eelgrass at MRGC had declined so severely that the spatial distribution was reduced considerably from its October 2016 levels and the areal extent of the beds had been reduced nearly 100 percent from the prior October 2016 levels.

The losses both within the restoration beds and other natural beds in the north bay were severe and it was anticipated that the beds may have been lost completely. As a result, 2017 transplants were postponed. However, monitoring in September 2017 and subsequently in April 2018 revealed survival of some of the plants, albeit most plants never recovered within the transplant area. The recovery of plants was slow and plants that survived were widely scattered. This has resulted in a general loss of the initial planting grid patterns and some of the plants that have survived appear to be seedlings, while others are rootstock from prior planting. It is no longer possible to track plots due to the loss of plot pattern integrity.

Monitoring was conducted in October 2018 and revealed good survival of plantings within approximately half of the half-acre plot planted north of the pier at MRGC and moderate survival of plantings within only one of four test plots, specifically the plot located in waters of approximately -0.5 m MLLW. Concurrently, plants from prior plantings located south of the pier continued to expand and started to fill in creating an open, but coalescing bed architecture. During the subsequent monitoring in 2019, plants continued to generally expand and densify within the more established beds. However, a slight reduction in the average vegetated cover was noted, while the areal extent and spatial distribution of eelgrass continued to increase. This is the result of an increase in seedlings being detected between the June and October 2019 surveys while some core areas showed slight increase in patchiness.

During the May and September 2020 monitoring eelgrass showed a pattern of continued steady but slow increase in areal extent, while the vegetated cover and spatial distribution both rose from October 2019 through May 2020 but declined markedly from May 2020 through September 2020. This pattern reflects a densification of beds at the southern end of the shoreline, concurrent with a substantial decline in sparse small plants that had extended almost 400 meters north of planting plots in May 2019 but then retracted substantially by September.

From 2014 through early 2015 eelgrass spatial extent rose to approximately 1.5 acres from the DARP restoration (Figure 7). However, following the spring 2015 surveys, eelgrass declined by mid-summer 2015. This is believed to be related to exceptionally warm water that prevailed during the period.

Return of cooler waters in 2016 was marked by dramatic plant expansion with spatial distribution of eelgrass expanding by over 250 percent from April to October 2016. Following early flooding and prolonged depression of bay salinities in 2017, eelgrass declined significantly with scattered but prolific recovery of plants being observed in September 2017. In April 2018, plants persisted from the 2017 recovery, although they reflected an expected spring season depression in plant condition and distribution. The most notable expansion in eelgrass extent has occurred from 2017 to May 2020 when the spatial distribution reached a peak extent of 38,745 m^2 (9.57 acres) due principally to widely scattered individual plants. The seasonal infill of established beds in the restoration core, while simultaneous declines in scattered young plants lead to a decline in spatial distribution of eelgrass while the areal extent continued to rise (Figure 7).

From 2019 through 2021, eelgrass beds did not expand in overall distribution, but rather continued to densify in bottom cover within the occupied space. A slight reduction in spatial distribution of these beds was observed concurrent with continued increase in vegetated cover and vegetated areal extent of the beds (Figure 7). This is a typical pattern of bed coalescence coupled with a slight reduction in suitability of marginal areas surrounding the beds. The pattern of continued coalescence of the beds was also seen in April 2022 with a subsequent mid-year decline in outlying plants present in deeper waters as was revealed through the October 2022 survey. This resulted in a continued slight increase in vegetated cover as plants within the core of the beds fill out, coupled with more notable declines in spatial distribution associated with the loss of scattered outlier plants along the lower bed margin. In April 2022 spatial distribution of eelgrass was at 9.25 acres, falling to 7.15 acres (23 percent) by October 2022. This decline in deeper eelgrass is likely associated with the persistent red tide that occurred in San Francisco Bay in spring-summer 2022. Similar deeper eelgrass losses have previously been observed in other systems in response to shading from prolonged red tide events.

To exhibit how the Cosco Busan DARP restoration plots have changed through time, maps displaying the maximum areal extent and spatial distribution of eelgrass over each monitoring year have been prepared. For the MRGC planting areas maps were prepared for the period 2014 through 2022 (Figure 8a-i). Because each year may have one or more survey intervals, the cumulative distribution of eelgrass for the year has been presented in order to simplify the graphics.

From initial planting in July 2014, a decline in plantings occurred most notably on the northern side of the MRGC pier where two plots suffered much more substantial declines than occurred in the single plot on the south side of the pier (Figure 8a). In 2015, early season survival and infill of the 2014 planting plots was noted but plantings in the summer of 2015 did not fare well and high mortality of planting units occurred. As a result, the primary change in the eelgrass beds was associated with infill and expansion of the initial 2014 plantings (Figure 8b).

The pattern of DARP eelgrass observed in 2016 shows good survival and growth from multiple plantings made in 2016 as well as survival and growth of plantings from prior 2014 plots that had persisted through exceptionally warm periods of 2015. The 2014 plots installed to the north of the pier suffered substantial reduction in plant survival over 2015, while plants south of the pier did much better (Figure 8c). By the end of 2016, eelgrass transplants appeared to be well on the way to forming solid beds. Notable within the 2016 eelgrass distribution map, are the strong affinities to initial planting layouts.

In 2017 following a near complete demise of surface biomass as a result of depressed salinities, eelgrass returned through a combination of vegetative regrowth and seedling recruitment within the restoration areas. As a result, the pattern of plant distribution no longer reflects the initial planting layout (Figure 8d). The random pattern from DARP plantings that were observed in September 2017 and contributed most substantially to the cumulative maximum distribution observed in Figure 8d, continued to persist in April 2018 (Figure 8e). However, the observed slight reduction in distribution (Figure 8e) and spatial extent (Figure 7) between 2017 and 2018 is believed to be related to early seasonal timing of the 2018 survey and lack of later season surveys in 2018, rather than being an indication of bed decline.

In 2019, eelgrass along the San Rafael shoreline expanded substantially to the north of the initial planting areas through seedling recruitment while simultaneously filling in within the core transplants (Figure 8f). However, during the subsequent 2020 year, much of the seedling recruits to the north of MRGC had declined and the eelgrass within the main core planting areas had filled in substantially and established fairly coalesced eelgrass beds, particularly to the south of the pier (Figure 8g).

In 2021, eelgrass showed a slight retraction of the spatial distribution losing a few outlier patches; however, it continued to coalesce within the core of the beds (Figure 8h).

In 2022, eelgrass showed a retraction of the spatial distribution losing more outlier patches in deep water than in prior years, while the core of the bed continued to close to a continuous canopy (Figure 8i).

The most notable result from the San Rafael shoreline eelgrass restoration is that this is a successful establishment of self-sustaining eelgrass beds along an area of the shoreline where beds have not previously been documented to occur, but for which habitat suitability was modeled to occur (M&A 2005).

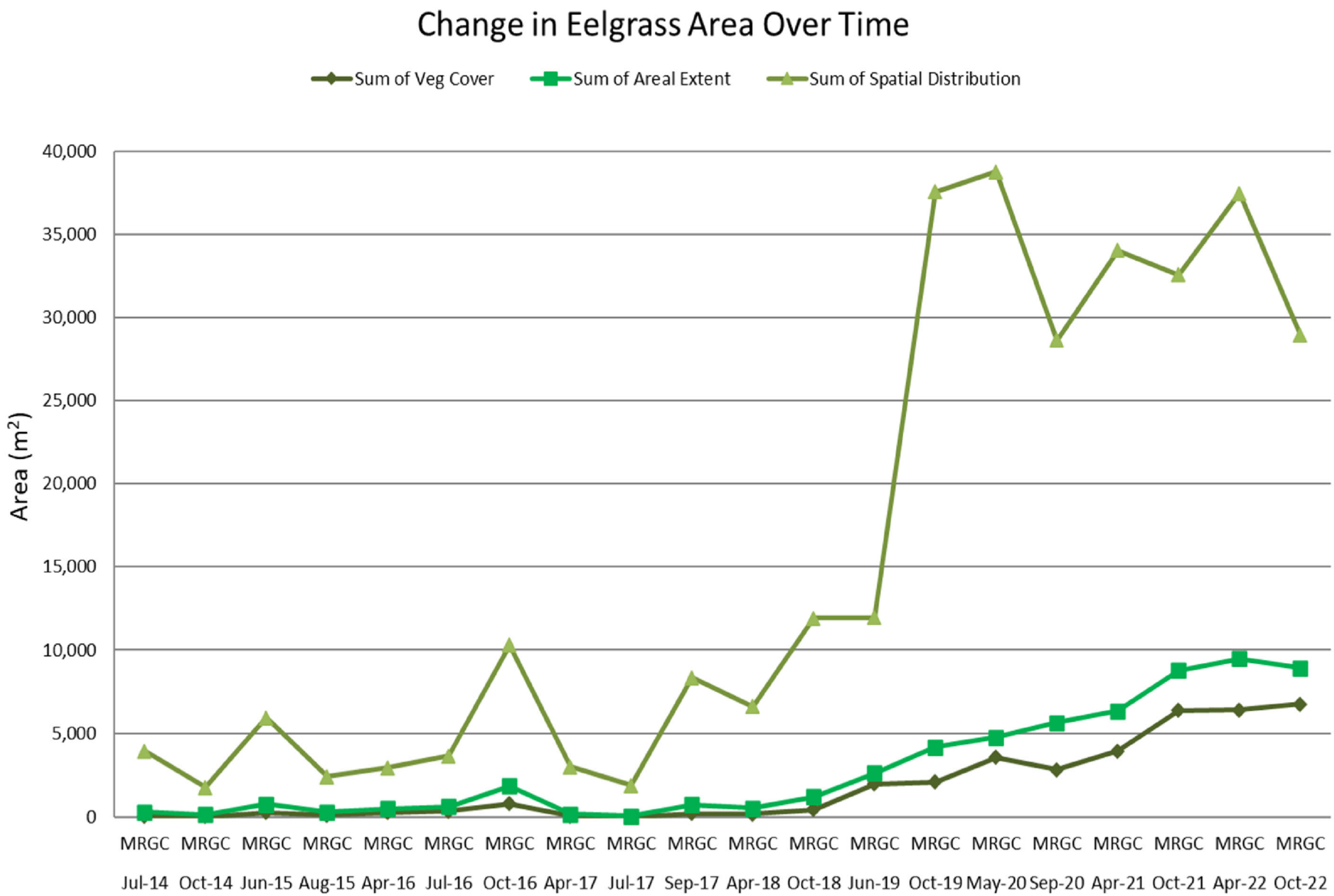
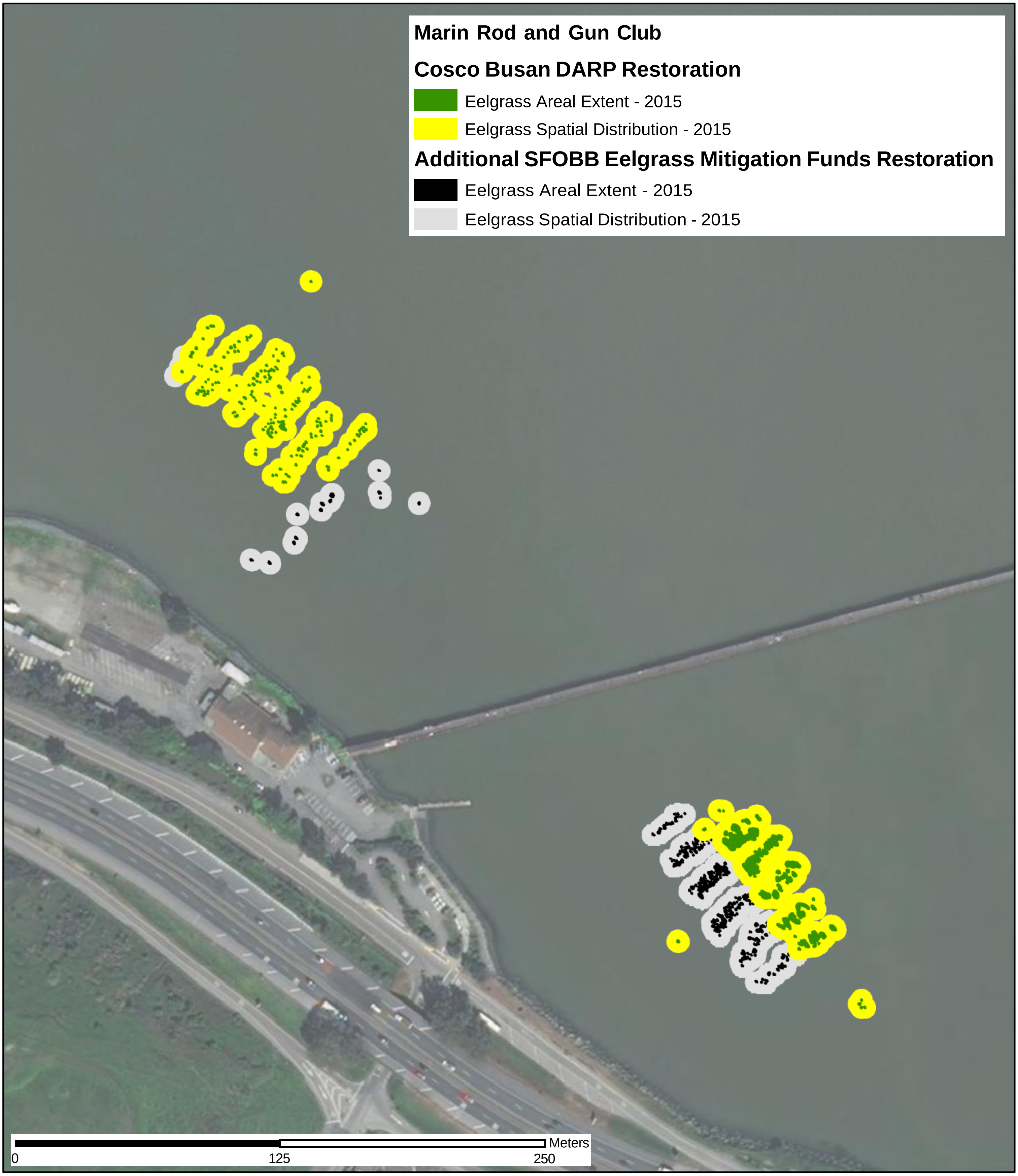


Figure 7. Change in Eelgrass Extent within Cosco Busan Transplants from 2014-2022 at Marin Rod & Gun Club

Cumulative Maximum Eelgrass - 2014
Marin Road and Gun Club
Cosco Busan Damage Assessment and Restoration Plan

Figure 8a

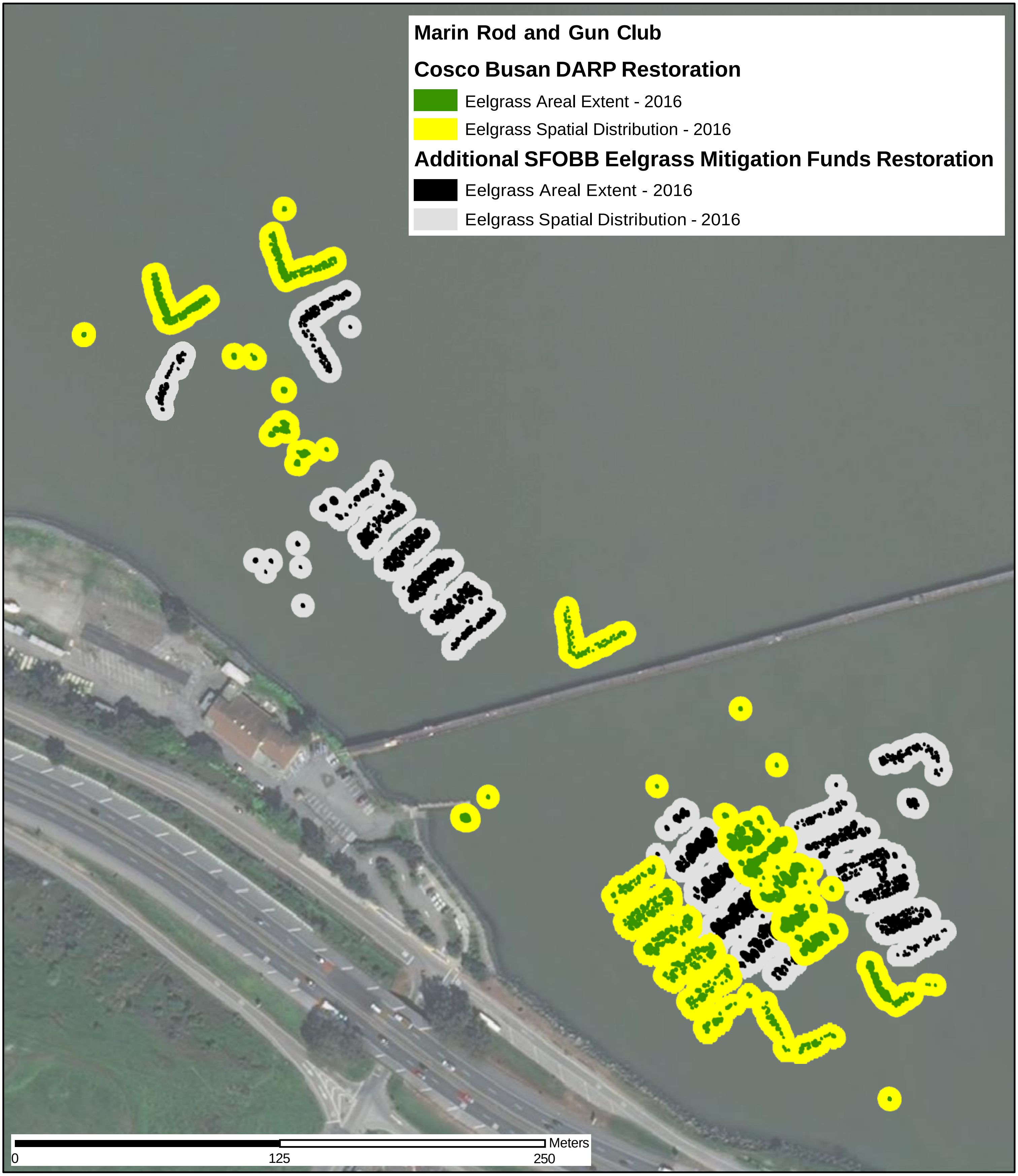
Merkel & Associates, Inc.



Cumulative Maximum Eelgrass - 2015
Marin Road and Gun Club
Cosco Busan Damage Assessment and Restoration Plan

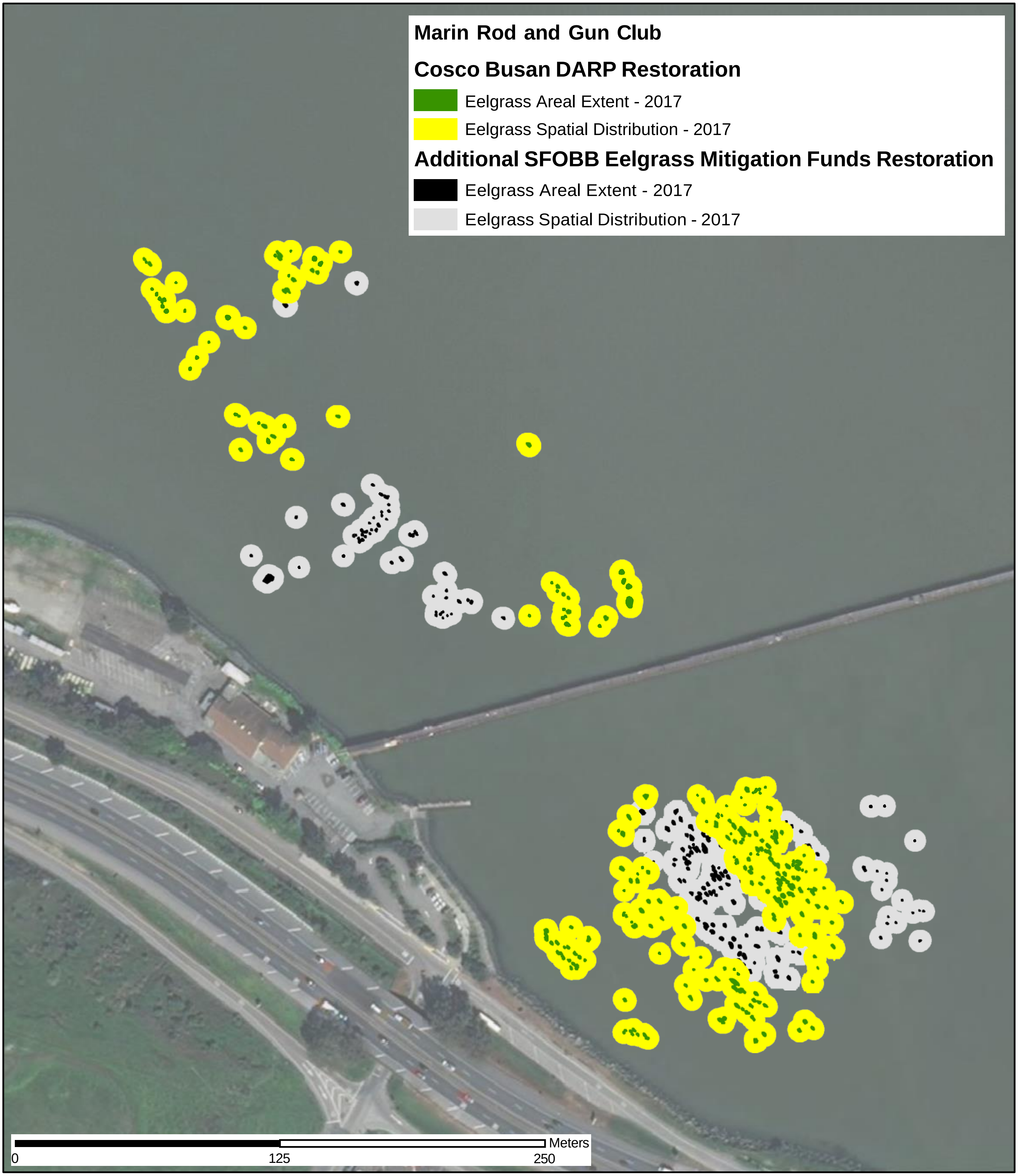
Figure 8b

Merkel & Associates, Inc.



Cumulative Maximum Eelgrass - 2016
Marin Road and Gun Club
Cosco Busan Damage Assessment and Restoration Plan

Figure 8c

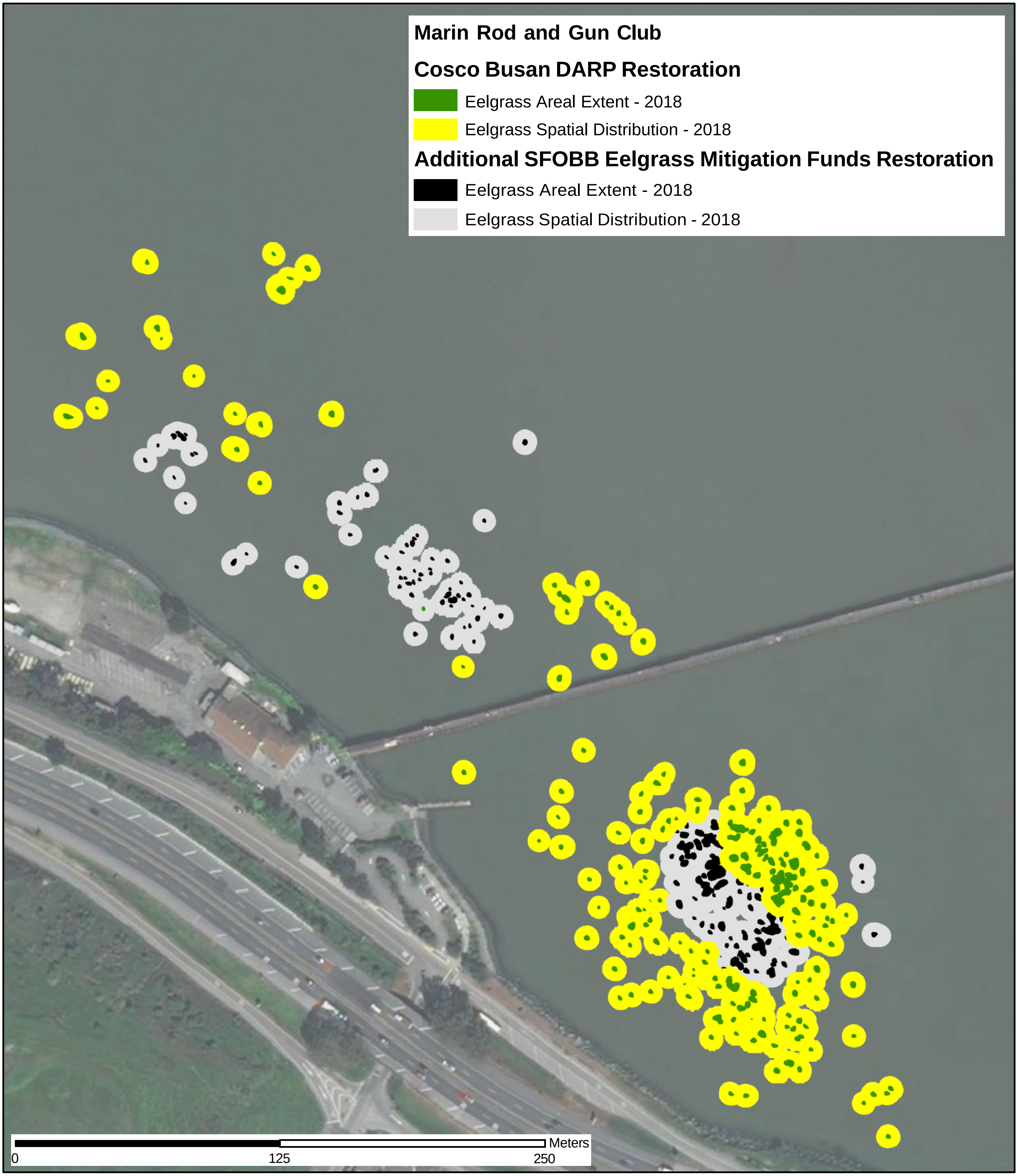


μ

Cumulative Maximum Eelgrass - 2017
Marin Road and Gun Club
Cosco Busan Damage Assessment and Restoration Plan

Figure 8d

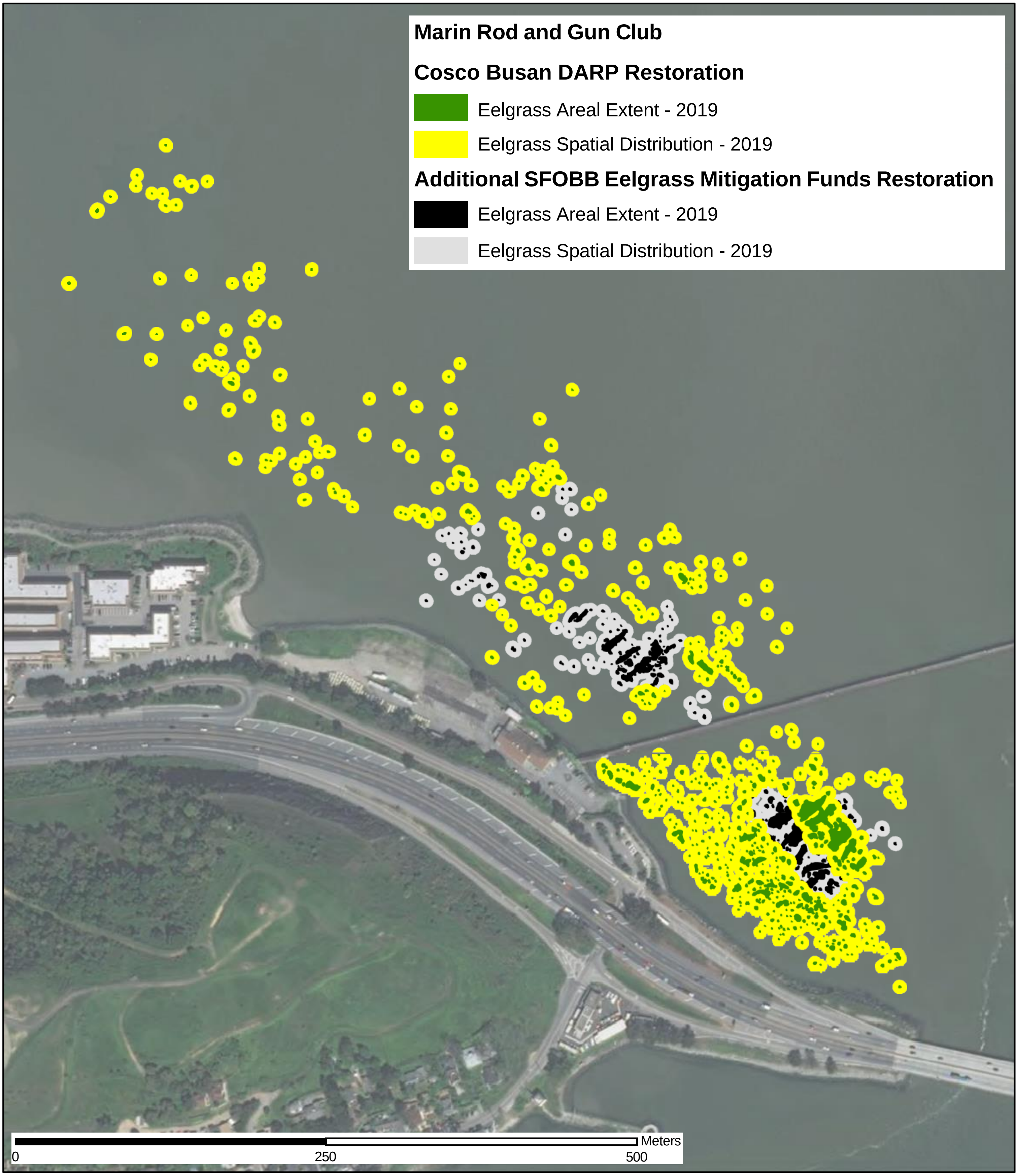
Merkel & Associates, Inc.



Cumulative Maximum Eelgrass - 2018
Marin Road and Gun Club
Cosco Busan Damage Assessment and Restoration Plan

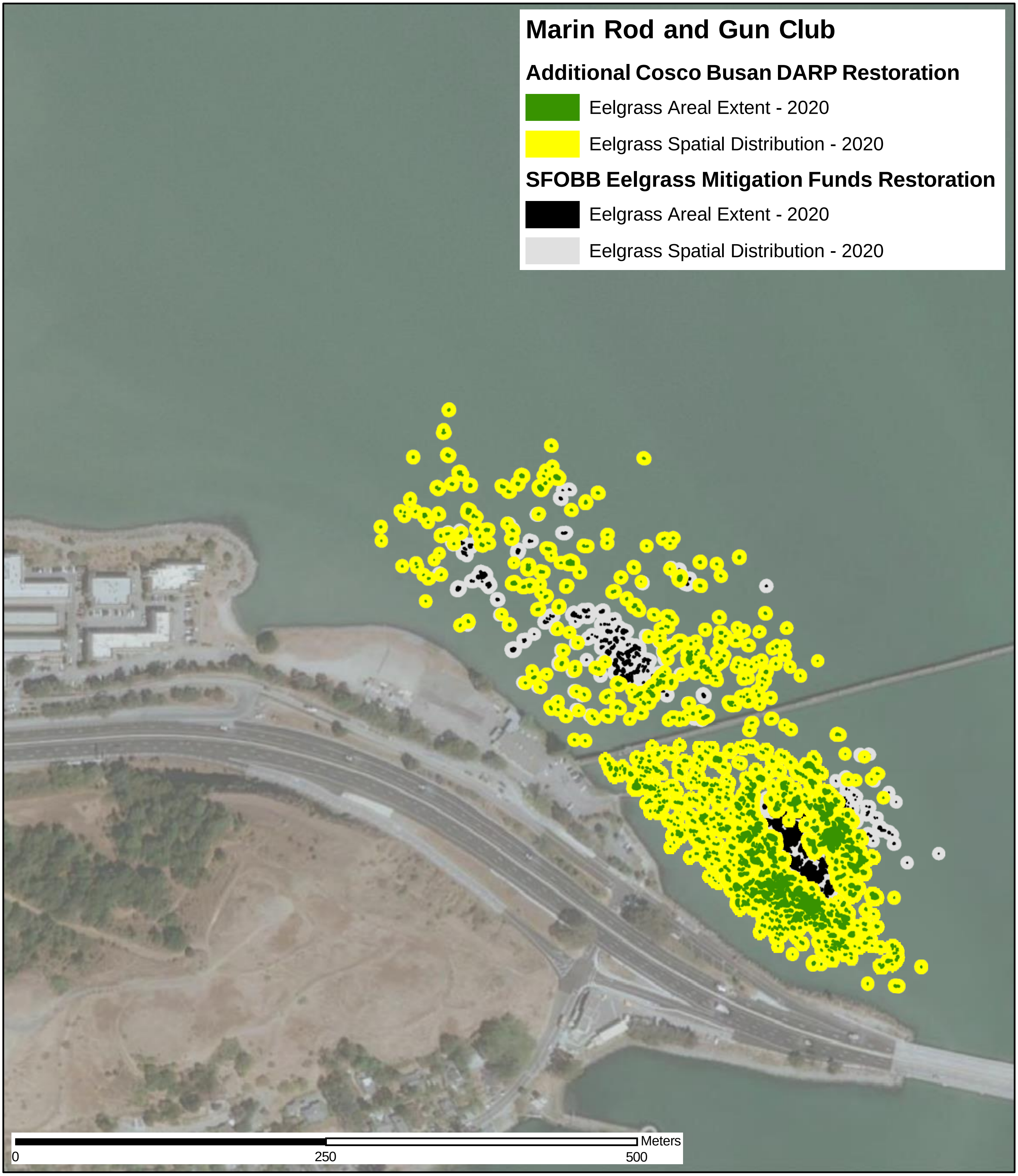
Figure 8e

Merkel & Associates, Inc.



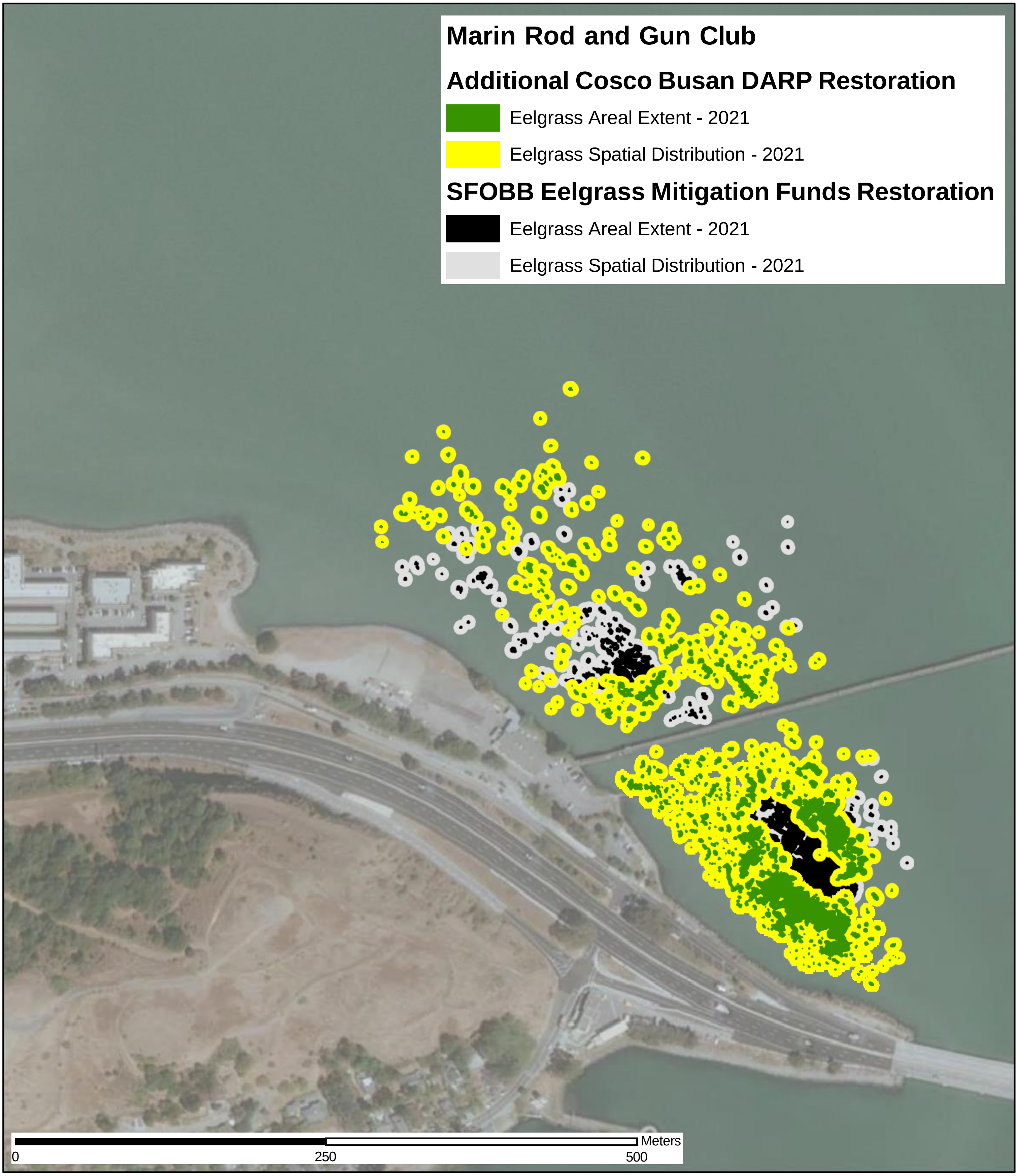
Cumulative Maximum Eelgrass - 2019
Marin Road and Gun Club
Cosco Busan Damage Assessment Restoration Plan

Figure 8f



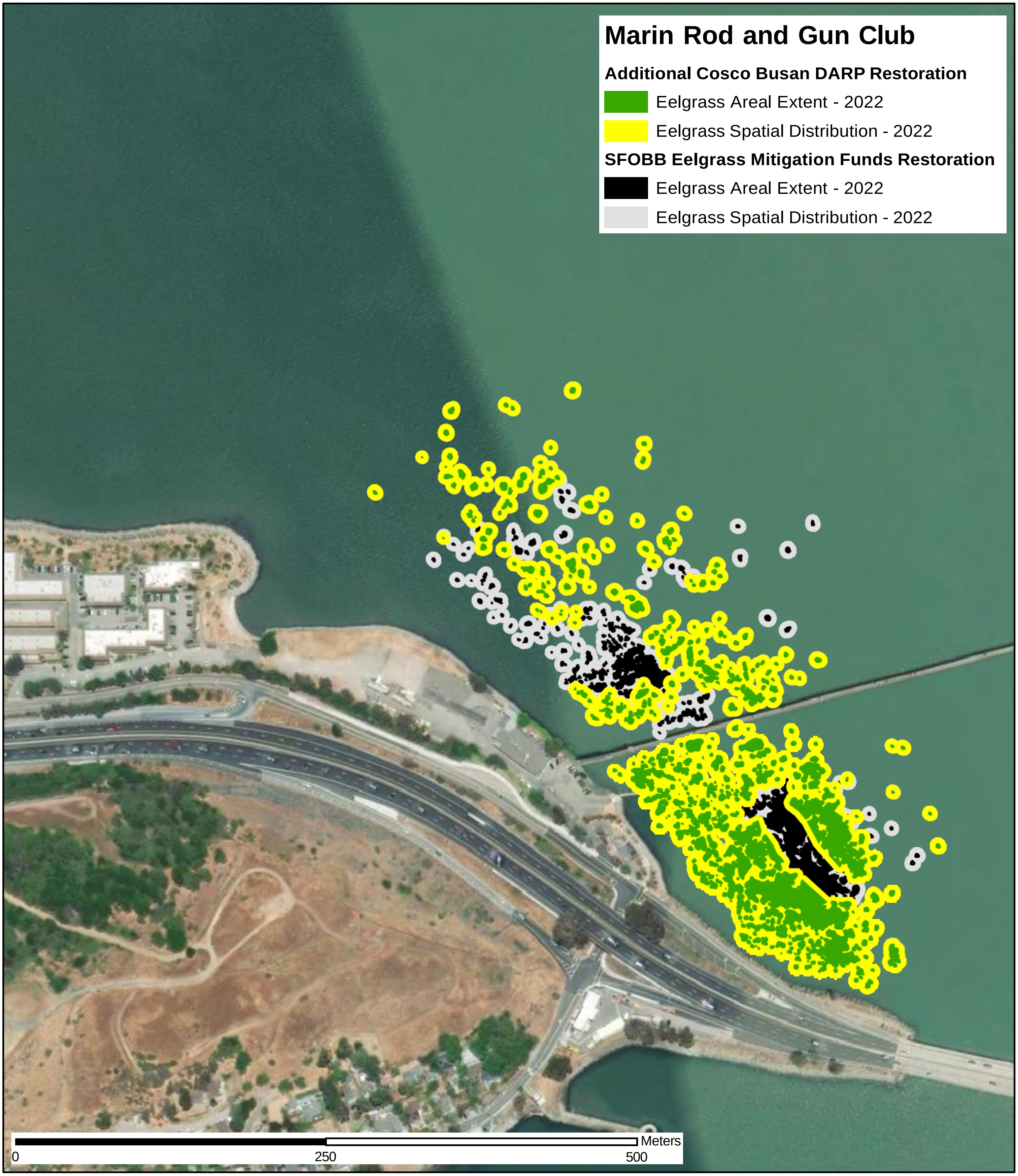
Cumulative Maximum Eelgrass - 2020
Marin Road and Gun Club
Cosco Busan Damage Assessment Restoration Plan

Figure 8g



Cumulative Maximum Eelgrass - 2021
Marin Road and Gun Club
Cosco Busan Damage Assessment Restoration Plan

Figure 8h



μ

Cumulative Maximum Eelgrass - 2022
Marin Road and Gun Club
Cosco Busan Damage Assessment Restoration Plan

Figure 8i

In monitoring the restoration plots in this region of the bay over the past multiple years, it is notable that beds have performed differently in response to various stressors depending upon where stressors operated, both horizontally and vertically. During benign years with cool temperatures, shallow water beds fared well and outgrew deeper beds (2016). However, during periods of warm water (2015) and depressed salinity (2017), deeper beds survived better than shallow beds. It is speculated that cooler bottom waters and more dense salt water tidal intrusion may have played a mitigating role allowing slightly greater plant survival at deeper depths than occurred within the shallower portions of the beds during these periods of differing stresses. It is likely that during 2017 flood periods, rhizomes within shallow water beds were fully bathed in freshwater as porewater in the sediment replaced salt water at low tides, while in deeper water more saline conditions in the porewater protected rhizomes even though leaves and above ground biomass was lost across nearly all elevations.

Corte Madera Bay

Within Corte Madera Bay plantings were investigated in July and October 2014 following the initial June 2014 planting. In July, very few plants were observed to persist and by October 2014, no plantings were observed. The Corte Madera Bay planting plots were investigated subsequently in June, immediately after planting the 2015 plants. The planting plots were also investigated in April and July 2016 and again in April 2017. No evidence of plant survival was noted in the original 2014 Cosco Busan transplants during the June 2015 survey. The 2015 plantings were noted to be present in June 2015, but in August 2015, a partial survey (not included in this analysis), showed only a few plants remained from plantings conducted only three months earlier. Only a handful of widely distributed plants were noted in the Corte Madera planting plots during the April 2016 survey. In 2017 a clustering of plants extending along the primary planting area from 2014 and 2015 plantings was noted to persist. These plants were of a small stature and did not show substantial evidence of growth and may have been the result of seeding from these plants, or residual rhizome survival over a period of poor overall survival. While limited overall in total bottom cover, the widespread occurrence of plants resulted in considerable expansion of the spatial distribution of plants, prior to collapsing after the April 2017 survey. Plants never recovered after the 2017 losses (Figure 9).

The rapid unexplained losses of planting units in Corte Madera Bay following each transplant are extremely interesting and point to a wide range of potential causative agents. Given the generally greater depths of the majority of these plantings (-0.5 m to -1.2 m MLLW), avian herbivory is not likely to be a driving factor that would have resulted in the loss of all plants; however, wave energy, invertebrate herbivory, and/or inappropriate sediment chemistry may be potential controlling factors. In general, low light conditions alone do not result in eelgrass losses in such a rapid fashion during summer months with high sun angles and long days. While not definitive, the rate of plant loss and the completeness of the loss would suggest factors other than an unsuitable light environment.

When examining the changes in plant distribution on a spatial scale, the cumulative maximum eelgrass distribution was plotted for each year of survey. In 2014, the planting plots reflect the general residual survival of plants in July, one month after planting, since by October of the same year plants had declined to even lower levels (Figure 10a). In 2015, plants only occurred within the deeper of the transplant plots in June (Figure 10b). By August, plants were nearly non-existent as detectible above ground biomass. When surveyed in April 2016, a single individual plant was noted remaining in the prior tracked plots with additional plants occurring away from the initially identified April plant by July 2016 (Figure 10c). In April 2017, a string of plants existed along the initial planting zone, although plants could not be identified as belonging to any particular planting line (Figure 10d).

Corte Madera Bay continues to be an enigma with respect to eelgrass restoration suitability. In prior years, experimental planting in this area yielded multi-year plant survival (K. Boyer, pers. obs.). However, the current restoration program has not managed to establish persistent eelgrass, although inconsistent eelgrass has occurred in the planting areas. While the results within Corte Madera Bay have not been favorable overall, it remains a highly coveted restoration area due to the potentially huge gains in baywide eelgrass that could occur if eelgrass were to ever become well established here.

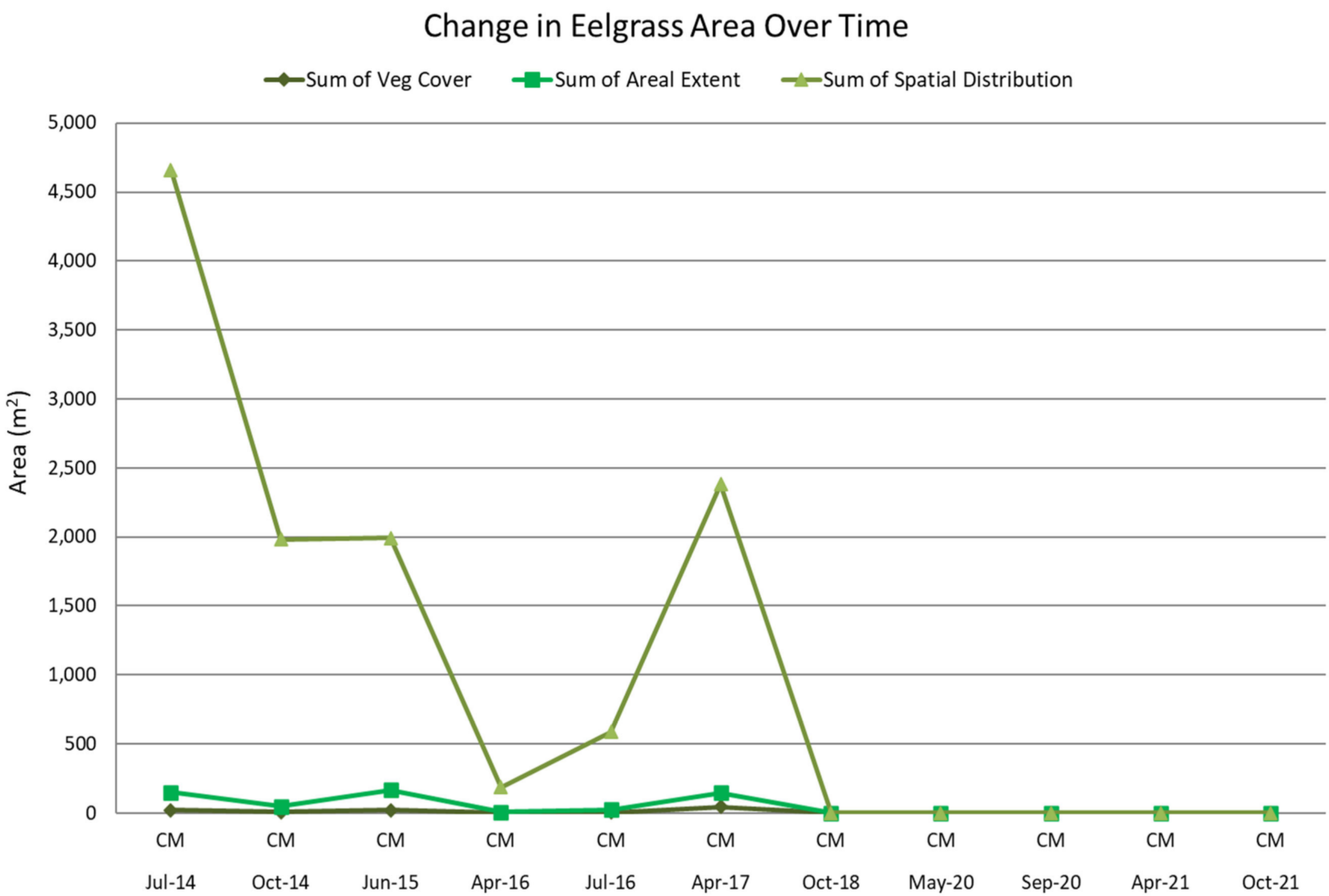
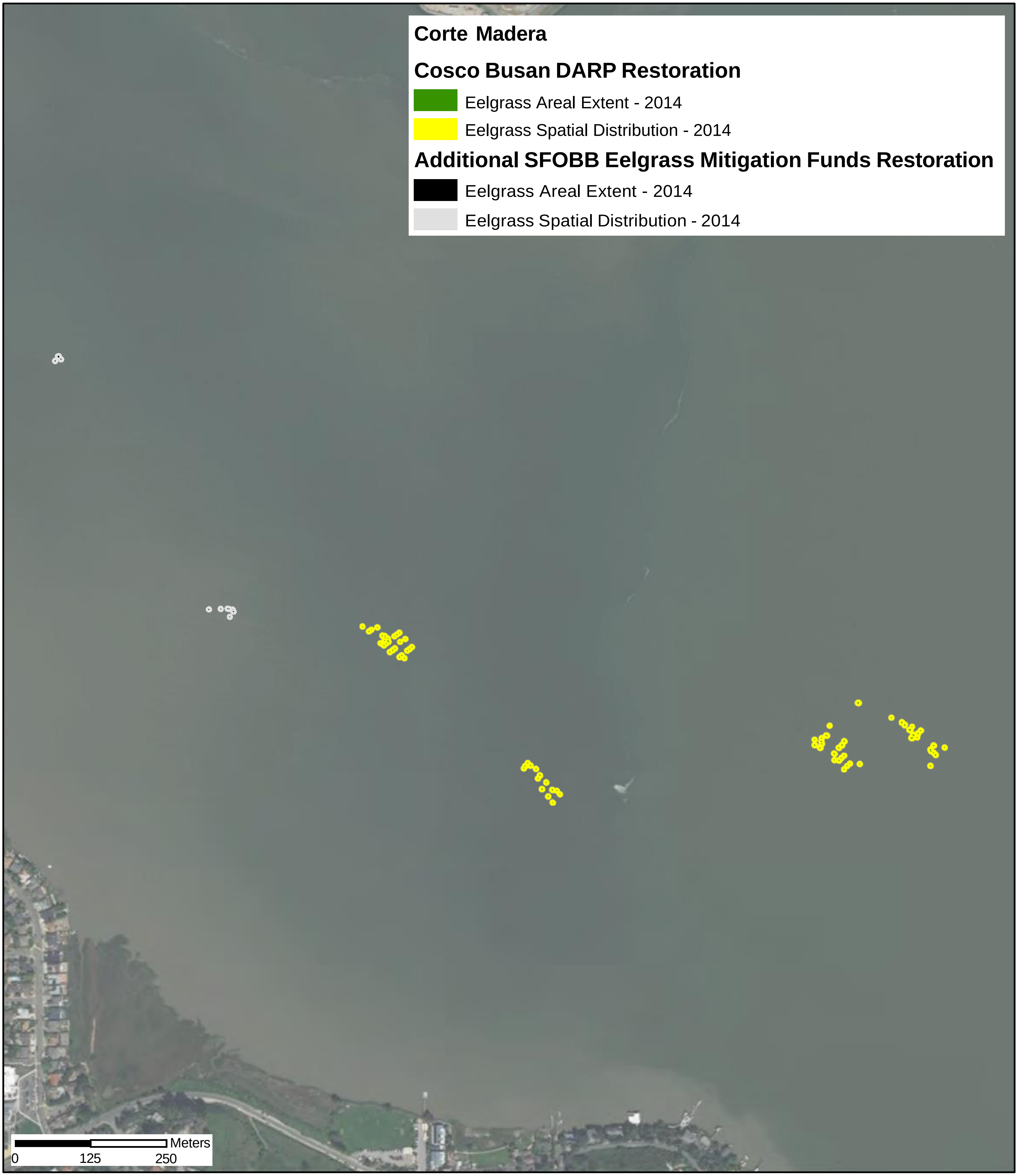
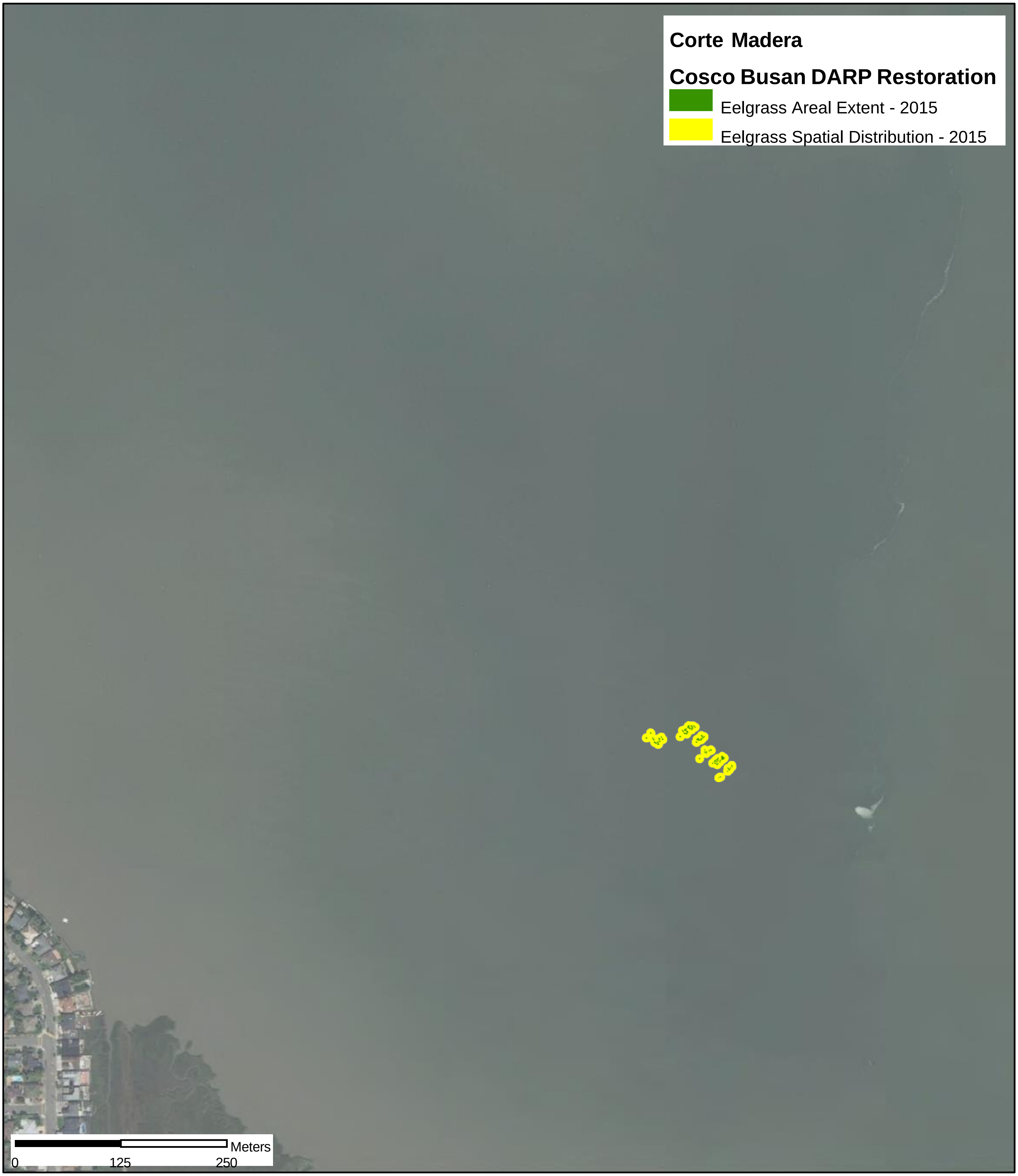


Figure 9. Change in Eelgrass Extent within Cosco Busan Transplants from 2014-2021 at Corte Madera Bay



Cumulative Maximum Eelgrass - 2014
Corte Madera
Cosco Busan Damage Assessment and Restoration Plan

Figure 10a

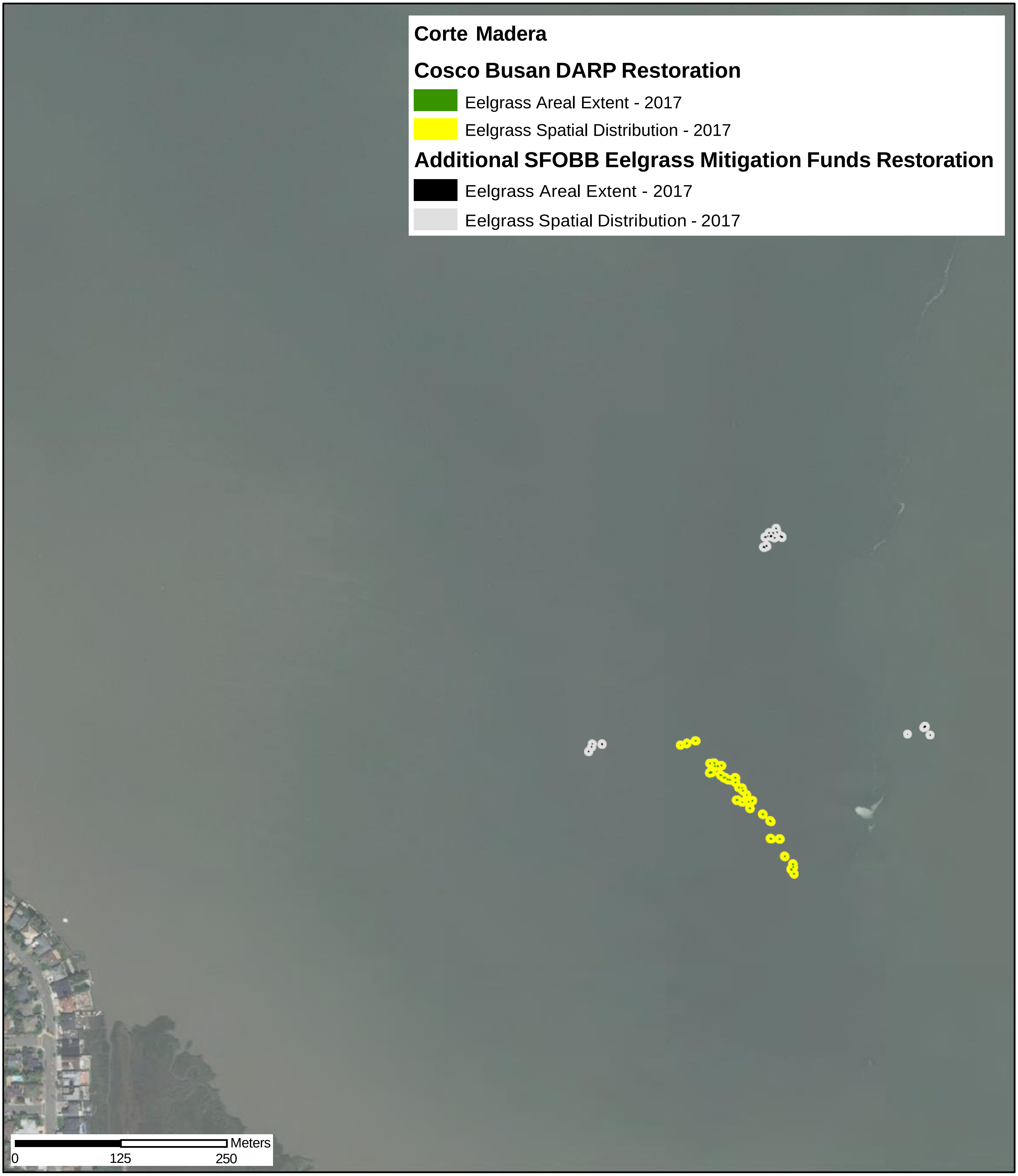


Cumulative Maximum Eelgrass - 2015
Corte Madera
Cosco Busan Damage Assessment and Restoration Plan

Figure 10b

Cumulative Maximum Eelgrass - 2016
Corte Madera
Cosco Busan Damage Assessment and Restoration Plan

Figure 10c



Cumulative Maximum Eelgrass - 2017
Corte Madera
Cosco Busan Damage Assessment and Restoration Plan

Figure 10d

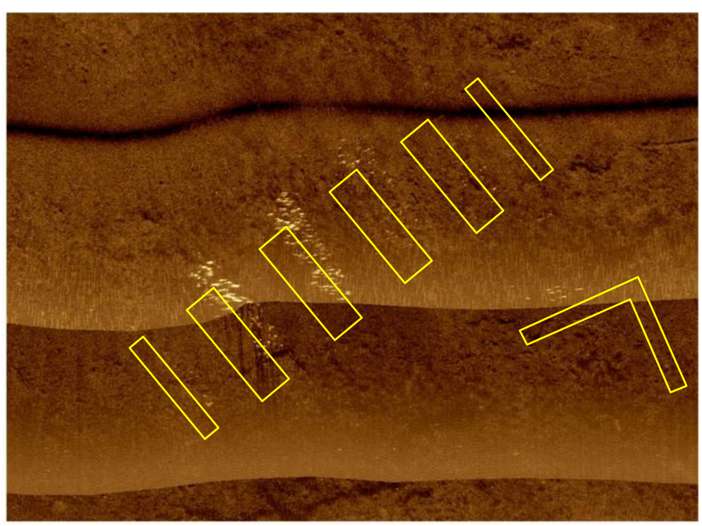
Richardson Bay

Plantings were performed within Richardson Bay beginning in spring 2015 with additional plantings being installed in summer 2016, 2019, 2021 and 2022. Plantings within the Richardson Bay Audubon Sanctuary spanned a broad area over which eelgrass had occurred as recently as 2009 with some areas holding eelgrass beds as late as 2012. During recent years, eelgrass declines had resulted in the loss of hundreds of acres in Richardson Bay with the majority of these losses being within the northern and eastern shallows of the Bay. Plantings in these areas spanned a wide depth range from 0 m MLLW to -1.2 m MLLW). Planting intentionally included restoration planting within the core of areas within which eelgrass had been previously documented to occur in 2009, as well as more broadly distributed test plots used to identify areas of potential for further expansion of eelgrass beds away from the historic core areas.

The plantings have been surveyed 18 times from June 2015 through October 2022. The overall extent of eelgrass was tracked across the multiple survey periods following CEMP metrics of vegetated cover, areal extent of the beds, and spatial distribution of eelgrass (Figure 11). Notably, the restoration generated eelgrass reached a maximum spatial distribution of 128,988 m^2 (31.87 acres) in October 2019, prior to starting a path of substantial declining to a September 2020 level of just 80,469 m^2 (19.88 acres). This decline in the beds within northern Richardson Bay has continued through October 2021 with the spatial distribution falling to just 40,612 m^2 (10.04 acres). This decline has been associated with a strong pull back in eelgrass distribution within the northernmost portions of the Audubon Sanctuary where the flats have suffered from high solar heating over recent years. While the decline has generally occurred within the shallowest plantings, areas lower in elevation and the recently planted mooring scar plots have done well and have steadily infilled with expanding eelgrass. In 2022, eelgrass restoration in Richardson Bay showed a substantial 144 percent rebound to 99,048 m^2 (24.47 acres).

Following the 2015 planting, regular plot monitoring was initiated. During the August 2015 surveys, eelgrass was noted to only persist in the southwesterly region of the plantings, specifically within two Cosco Busan DARP test plots, a Cosco Busan DARP half-acre plot, and a SFOBB test plot. These plots all exist at subtidal depths between -0.7 m and -1.0 m MLLW. None of the deeper plots at -1.2 m or shallower plots at -0.3 m MLLW, supported eelgrass just three months after planting.

The October 2015 survey revealed little change in the southwesterly most plots which continued to support eelgrass five months after planting. However, notably, by April 2016, the half-acre Cosco Busan DARP plot showed expansion with plants derived from Point San Pablo/Point Pinole and Point Molate generally outperforming either the Richardson Bay or mixed donor site plants, although plants from all donors did survive in the southwestern most half-acre plot. The low overall survival in a single multiple donor plot makes the data interesting but not significant.



Planting plot and test plot within the southwestern portion of the planting area shows differential survival of planting units by donor. The bottom throughout the planting region shows extreme biogenic disturbance from bat rays and benthos.

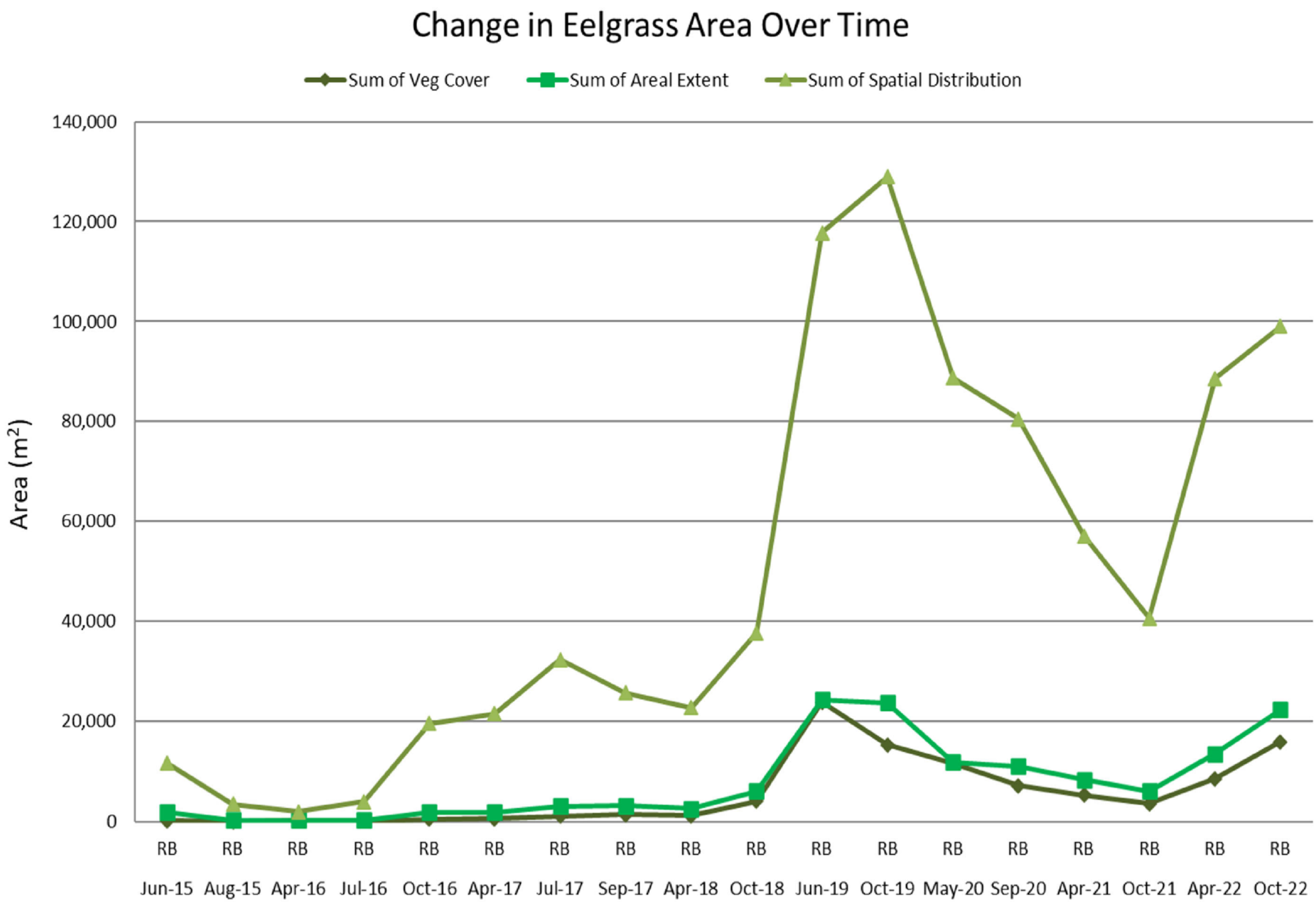
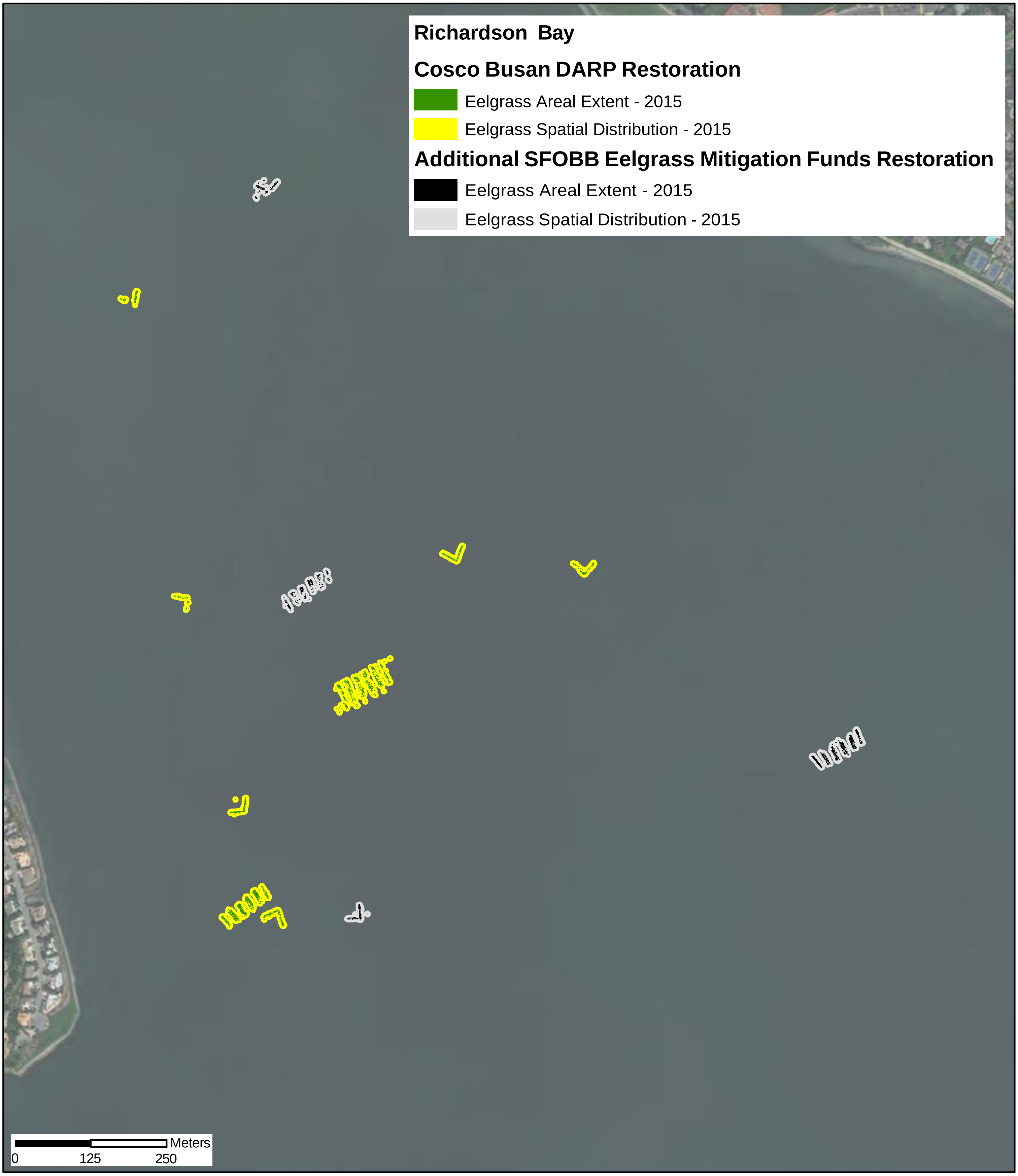


Figure 11. Change in Eelgrass Extent within Cosco Busan Transplants from 2014-2022 at Richardson Bay

During the initial planting of the Richardson Bay plots, it was noted that the bottom had experienced considerable disturbance by both burrowing organisms, likely bay ghost shrimp (*Neotrypaea californiensis*) as well as foraging bat rays (*Myliobatis californica*). Very little of the bottom was not pockmarked with burrows and massive foraging pits excavated by bat rays. These disturbances may be causative agents preventing effective colonization of some portions of the upper bay by eelgrass and may also have been contributory to eelgrass losses. However, it is also very likely that warm water conditions have been the most proximate stressor contributing to recent losses of eelgrass in the upper reaches of Richardson Bay. During restoration, it was noted that water temperatures within Richardson Bay were in excess of 24 °C (75 °F), much warmer than is typical during the spring in San Francisco Bay. While water temperatures had cooled considerably by the time of the June 2016 planting, biogenic activity patterns remained common in the area.

Eelgrass plantings in 2015 generally showed a distribution pattern indicative of the original planting (Figure 12a). Eelgrass declined through the year and into 2016. However, plants surviving to 2016 showed considerable recovery and growth through 2016 and 2017 (Figure 12b). Plantings conducted in 2016 also showed high survival and good expansion through the subsequent year. Notably, while eelgrass in northern San Francisco Bay in San Pablo Bay, along the Marin shoreline, and northern East Bay all showed catastrophic declines in eelgrass from depressed salinities in 2017, eelgrass in Richardson Bay fared very well over this period and showed seedling recruitment (Figure 12c). The 2018 eelgrass distribution is likely depressed from that observed in 2017 due to only including one spring survey (April 2018) in the cumulative extent (Figure 12d). From 2018 to 2019, eelgrass expanded substantially (Figure 12e). During 2019, eelgrass coverage naturally expanded in Richardson Bay to a record 837.3 acres, more than 20 percent higher than the prior high 2009 eelgrass extent (M&A 2019). Concurrent with this expansion, eelgrass expanded within the planting areas bringing the total extent of restored eelgrass derived from direct planting and spread to 41 acres. In 2020, eelgrass began a decline with once solid beds in the transplant areas beginning to open and more widely scattered plants disappearing (Figure 12f). The trend of declining condition and extent of beds within the northern portion of the Audubon Sanctuary continued through 2021 with the northernmost eelgrass distribution declining to scattered individual plants, while the more southerly transplant areas continue to sustain coalesced beds, although these beds, were also beginning to become patchy (Figure 12g). In 2022, the restored beds within the Richardson Bay Audubon Sanctuary continued to exhibit mixed response with increasing coalescence of core bed areas within shallow subtidal locations, but continuing patchy distribution of plants at intertidal elevations. The southwesterly most portion of the planting remained the most productive area in the Sanctuary (Figure 12h). Notable planting of mooring scars conducted in 2021 met with mixed results with one scar showing nearly complete infill while the remaining scars ranged from limited establishment from plantings to plant establishment wherein initial plantings survived but showed little spread. It is believed that the extent of survival and growth may be mediated in these scars by the effects of macroalgal mats present in the slightly incised scars that retain algal detritus.

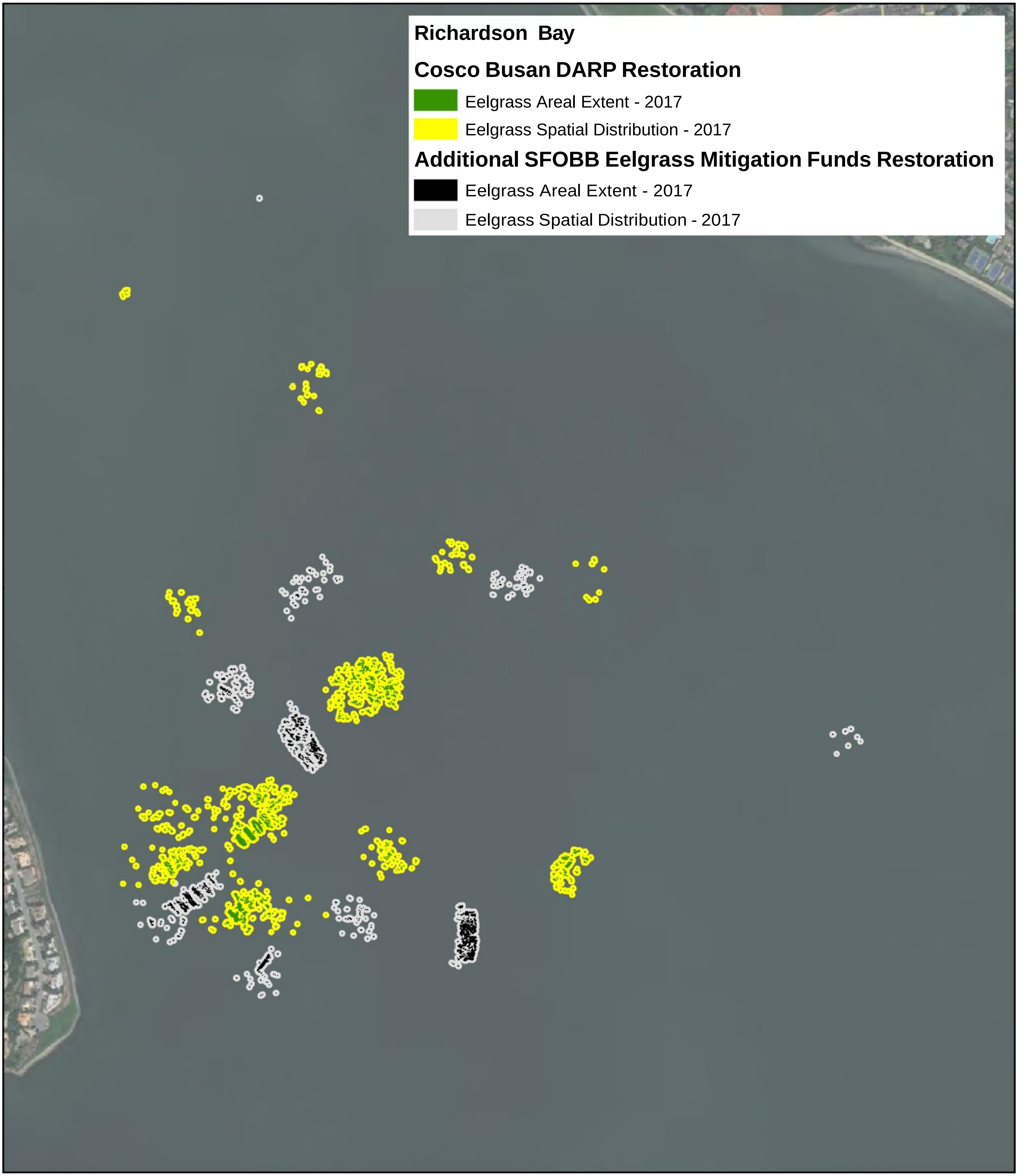


Cumulative Maximum Eelgrass - 2015
Richardson Bay
Cosco Busan Damage Assessment and Restoration Plan

Figure 12a

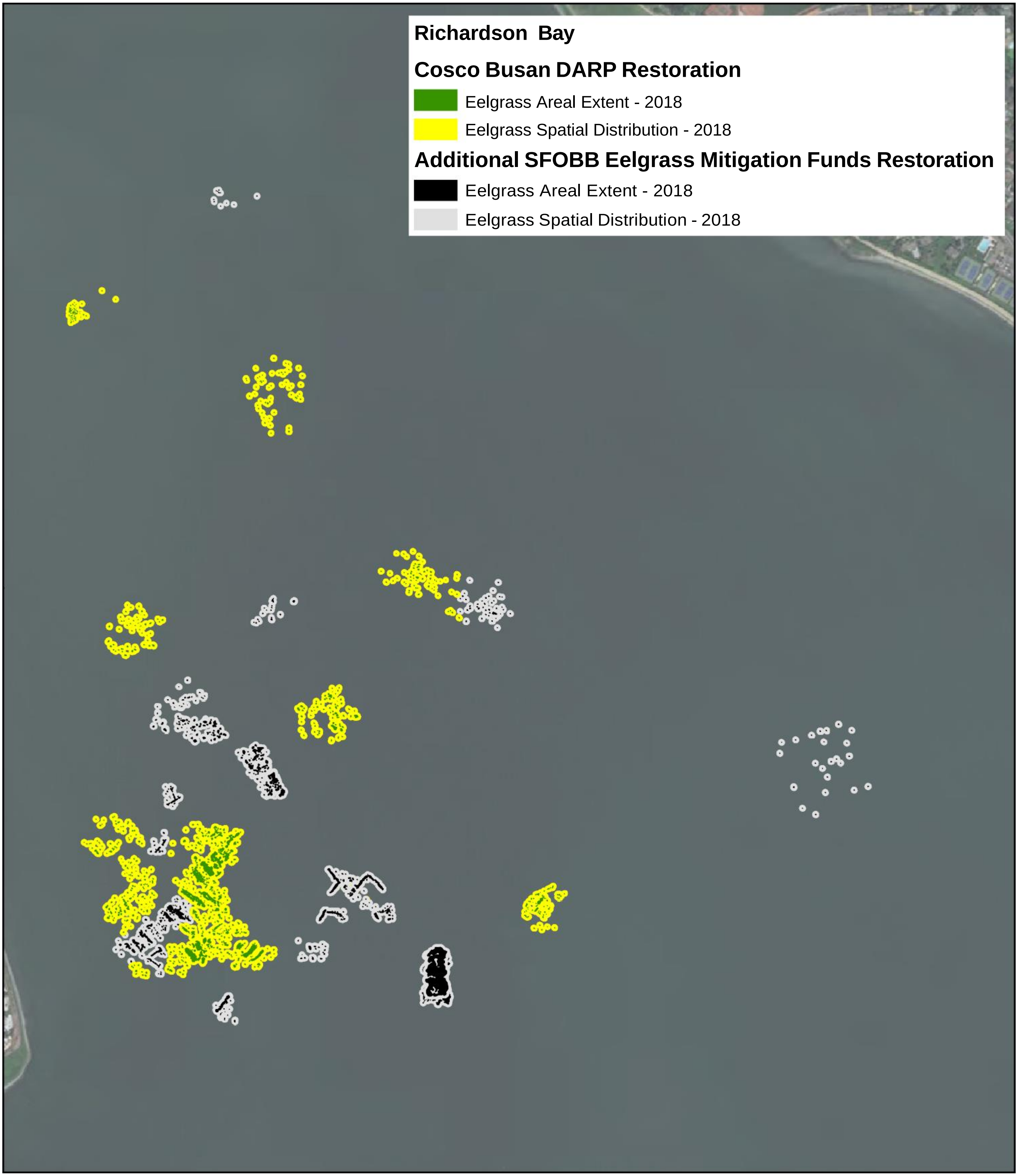
Cumulative Maximum Eelgrass - 2016
Richardson Bay
Cosco Busan Damage Assessment and Restoration Plan

Figure 12b



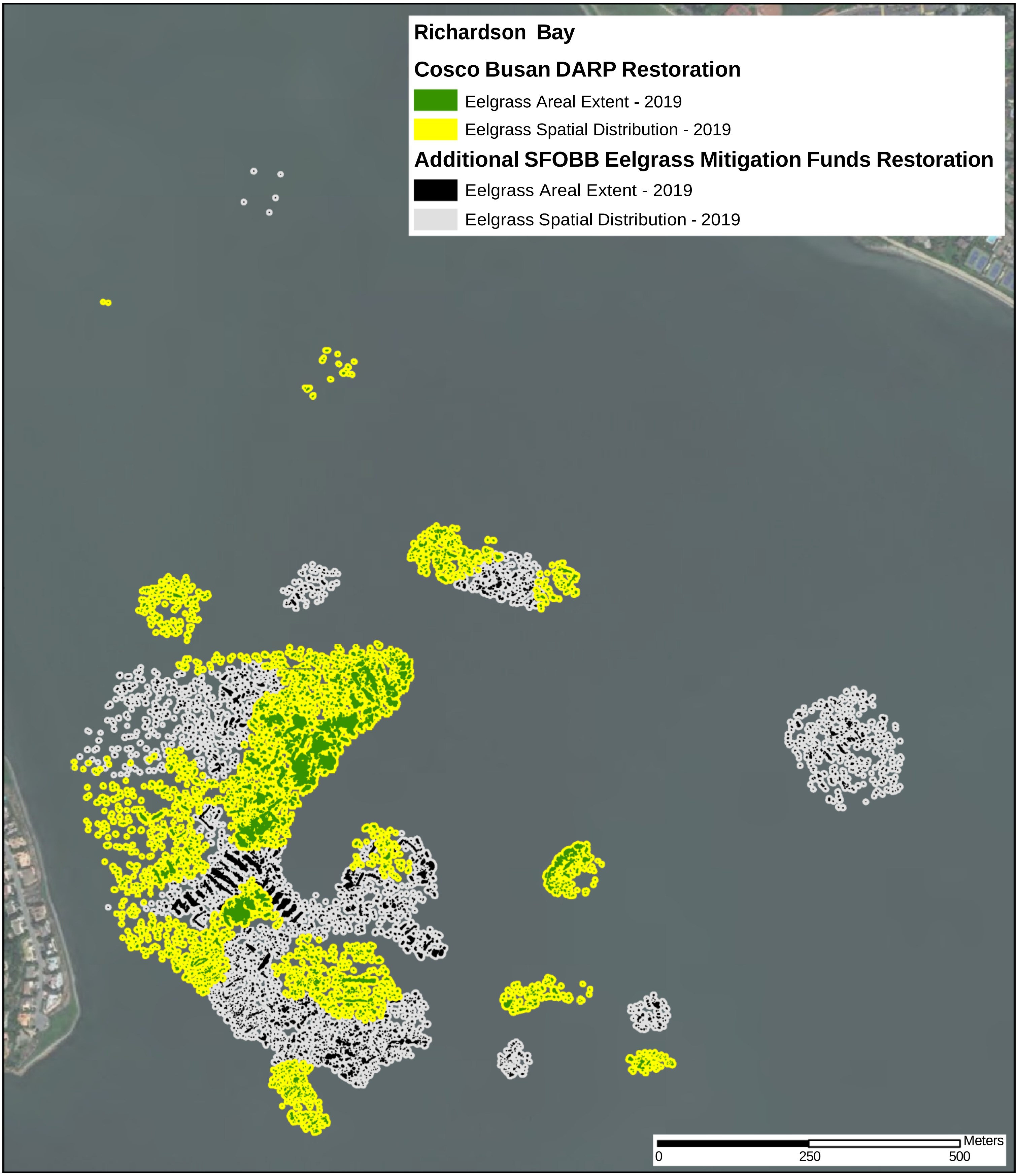
Cumulative Maximum Eelgrass - 2017
Richardson Bay
Cosco Busan Damage Assessment and Restoration Plan

Figure 12c



Cumulative Maximum Eelgrass - 2018
Richardson Bay
Cosco Busan Damage Assessment and Restoration Plan

Figure 12d



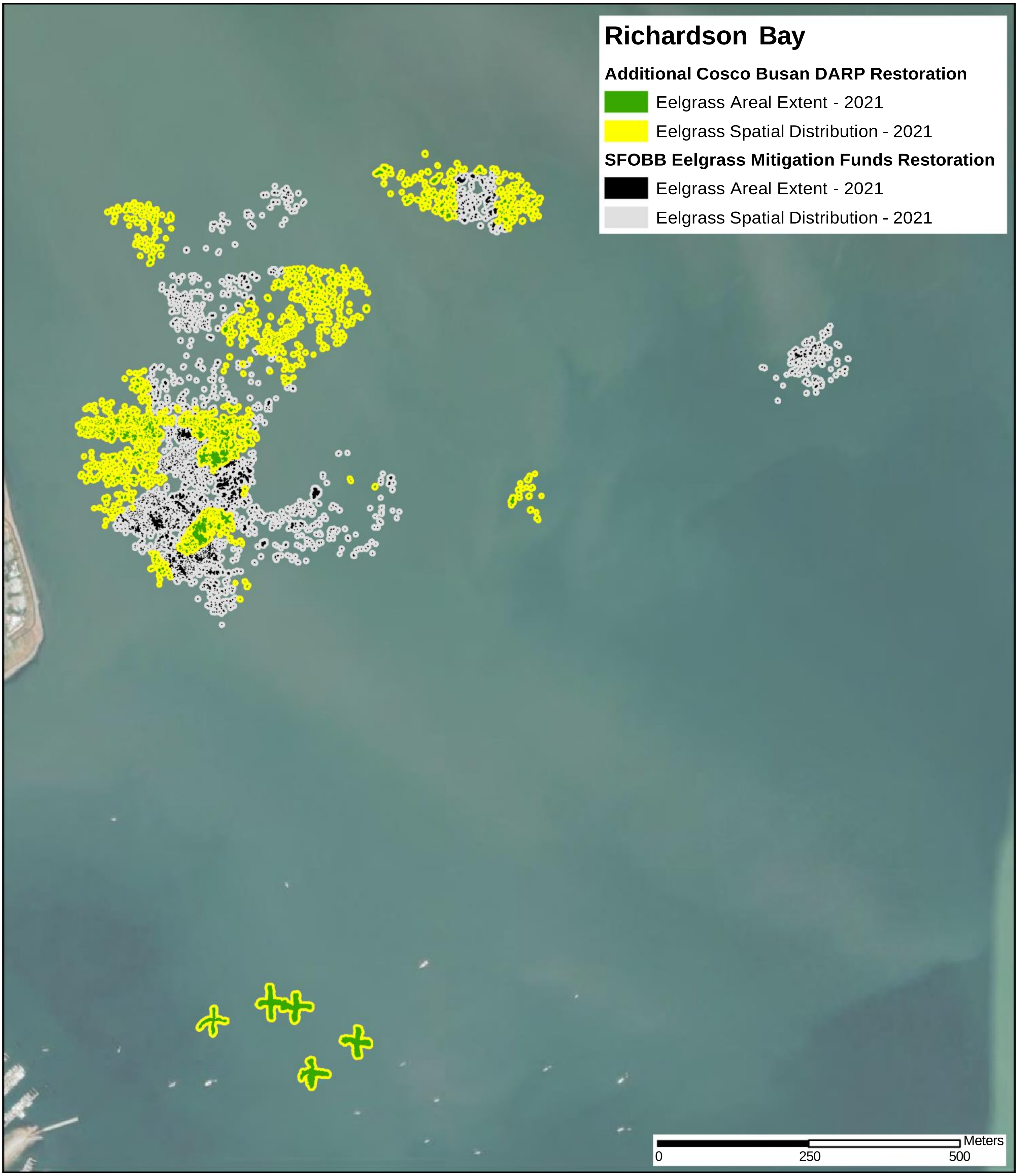
Cumulative Maximum Eelgrass - 2019
Richardson Bay
Cosco Busan Damage Assessment Restoration Plan

Figure 12e

Cumulative Maximum Eelgrass - 2020
Richardson Bay
Cosco Busan Damage Assessment Restoration Plan

Figure 12f

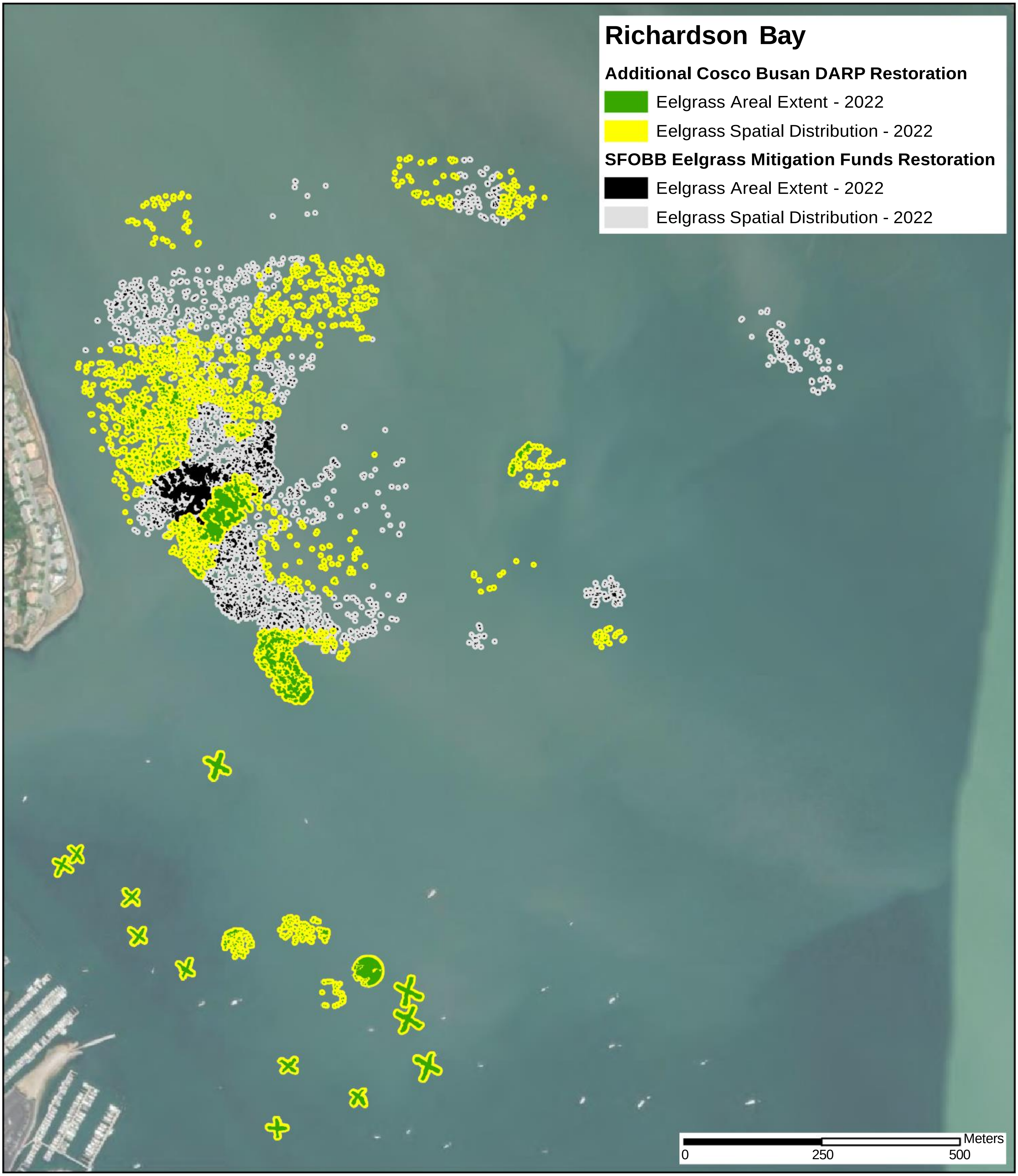
Merkel & Associates, Inc.



Cumulative Maximum Eelgrass - 2021
Richardson Bay
Cosco Busan Damage Assessment Restoration Plan

Figure 12g

Merkel & Associates, Inc.



μ

Cumulative Maximum Eelgrass - 2022
Richardson Bay
Cosco Busan Damage Assessment Restoration Plan

Figure 12h

DIRECT OBSERVATION SURVEY RESULTS

San Rafael Bay Shoreline (Marin Rod & Gun Club)

2014 Monitoring Results

In September 2014, monitoring of the survivorship of transplants at the MRGC was conducted using wading surveys within intertidal planting plots and extremely shallow subtidal plots, and SCUBA within subtidal plots. Low water visibility resulted in the need to investigate plots based on a combination of observation and tactile detection of plants and bamboo anchor stakes. In the Cosco Busan DARP one-acre plot north of the pier and oyster reefs, a total of six planting units were found to have persisted (Table 2). These varied in source of material (donor), with at least one planting unit persisting from each donor or the mix, with the exception of Keller Beach (no transplant units persisting). The bamboo stake technique tended to result in greater survivorship than the paper sticks; however, with this small sample size it was not possible to draw strong conclusions on the most effective bareroot planting method. The number of shoots per transplant unit was not recorded but was noted to be in the range of 1-6 shoots, suggesting some new shoot growth had occurred on some units since planting. No plants were noted within the test plots located north of the pier in 2014, although a few plants did persist in subtidal plots that were not captured during the direct plot inspection but were detected by interferometric sidescan surveys.

Table 2. Survivorship of Marin Rod & Gun Club 2014 Transplants, as of September 9, 2014

	One-acre plot (N of pier)		Half-acre plot (S of pier)	
	# Transplant units persisting		# Transplant units persisting	
Donor	**Bamboo**	**Paper Stick**	**Bamboo**	**Paper Stick**
Point San Pablo	0	1	9	0
Point Molate	2	0	22	40
Mix	1	0	21	7
Keller Beach	0	0	15	11
Richardson Bay	2	0	15	2*

* more transplants were present but strong currents and poor visibility precluded completion of counts

The half-acre Cosco Busan DARP plot south of the pier showed considerably greater survivorship than the plot to the north (Table 2). High survival and growth in the southern plot was the impetus for the subsequent addition of an adjacent half-acre plot under the SFOBB restoration effort in 2015. All donors had surviving transplant units, with a trend toward greater survivorship in the Point Molate transplants. There were no obvious trends by transplant method. The first band of plants from the north contained the Point San Pablo paper stick anchor units, of which none survived; the fact that this edge was nearest to the path that small boats take to the MRGC launch ramp leads to speculation that high disturbance may have confounded the survivorship results. Paper stick anchor units appeared to perform better with Point Molate plants than did bamboo stakes. With mixed donors, the bamboo stake anchor method tended to produce more surviving transplants. Transplant methods produced similar results with the Keller Beach donor material. For Richardson Bay transplants, it is not possible to draw conclusions as surveys could not be completed while monitoring the paper stick planting units due to strong currents that led to abortion of attempts to count surviving units using SCUBA gear as the tide rose. The number of Richardson Bay bamboo stake transplants was also an under-estimate as counts were rushed due to the strong currents present during the monitoring period. Overall, if the paper stick units for Point San Pablo and Richardson Bay are discounted (for the reasons described above), survivorship of transplant units was roughly 25 percent in this half-acre plot. A high prevalence of epiphytes (mostly filamentous algae) and bryozoans were noted on the older leaves on the plants in this plot.

2015 Monitoring Results

In May 2015 a follow up was made to assess planting persistence. In the one-acre plot to the north of the pier, only two transplant units were found after a thorough search of the entire area on a very low tide in which all transplants would have been detectable if present. These were both bamboo stake transplant units. Twenty vegetative and two flowering shoots were present in one plant, and twelve vegetative and one flowering shoot were counted in the other surviving unit. No seedlings were observed anywhere in the plot; with the good visibility in very shallow water, seedlings would have been detected had they been present. No plants were observed in the test plots north of the pier, though some plots remained subtidal and were difficult to effectively inspect.

In the half-acre plot located south of the pier, also monitored in May 2015, it was no longer possible to distinguish many of the transplant units due to spread and coalescence with other units. As a result, the number of patches per donor type and transplant unit type were counted but individual planting units could not be counted. About every fifth patch, the numbers of vegetative and flowering shoots within the patches were counted.

All donors continued to have surviving transplants present (Table 3). The Point Molate donor continued to have the most patches present and tended to have more shoots per patch. There continued to be little indication of differences by transplant method; however, the fact that many units appeared to have coalesced made direct comparison by donor problematic. Multiplying the number of patches by the mean number of shoots per patch, there was estimated to be over 1,500 vegetative shoots present. Flowering shoots were also present in most patches, suggesting that the transplants may contribute to further establishment by seedlings. Seedlings that might have been present through the previous summer's buoy-deployed seeding were not observed, although it is not possible to be certain of their absence due to the deeper setting of this restoration plot.

Table 3. Numbers of Patches and Mean Number of Vegetative (V) or Flowering (F) Shoots Per Patch in the Half-acre Plot South of the MRGC Pier (Planted in June 2014), on May 5, 2015

	# patches		mean # shoots per patch			
			Bamboo		Paper Stick	
Donor	**Bamboo**	**Paper Stick**	**V**	**F**	**V**	**F**
Point San Pablo	6	0	20	1		
Point Molate	18	22	26	5	20	3
Mixed Donor	6	4	19	2	9	2
Keller Beach	13	10	10	2	12	2
Richardson Bay	7	6	10	3	8	3

The invasive amphipod *Ampithoe valida* was observed grazing on the plants. As in other restoration projects (Boyer et al. 2014), this species was quick to establish even though plants were treated by fresh-water dips to remove clinging invertebrates before transplantation (per Carr et al. 2011). Although amphipods were present, they were not observed in high abundances that would be expected to negatively impact plants (Reynolds et al. 2012; Lewis and Boyer 2014).

The half-acre plot was again monitored in August 2015. At this point, many planting units had coalesced. Total counts of the shoots present were made, as patches were encountered moving through the plot from north to south. Based on their positions within the plot, it was possible to tentatively conclude that all the donors and the mixed donor plantings had plants persisting. However, it was not possible to quantify numbers of shoots per donor in several cases (Table 4). Both bamboo stake and paper stick methods were noted to have plants persisting. The rising tide prevented making precise counts in the southern portion of the plot where Richardson Bay shoots had been planted, but there appeared to be many shoots along the lines of bamboo stakes. In all, about 500 vegetative shoots (and only 1 flowering shoot) were counted, which represents a considerable underestimate, but total numbers were still likely lower than the 1,500 shoots estimated during the May 2015 monitoring. Heights of vegetative shoots were consistent with other measures within San Francisco Bay (Boyer and Wyllie-Echeverria 2010; Boyer et al. 2014).

Table 4. Numbers and Heights of Vegetative Shoots on August 29, 2015 in the Half-acre Plot South of the Marin Rod & Gun Club (Planted in July 2014)

Patch number (order monitored)	Donor (tentative)	Orientation	# vegetative shoots	Height of 1 vegetative shoot in patch (cm)
1	PSP	most NW patch	12	172
2	PSP or PM	to the east of 1st	10	154
3	PM	between 1 & 2, but S	27	129
4	PM	just west of 3	29	139
5	PM	just west of 4	60	169
6	PM?	just west of 5	46	156
7	PM?	just west of 6	12	N/A
8	PM?	just west of 7	13	N/A
9	Mix	to south	61	121
10	Mix	just south	40	127
11	KB		6	143
12	KB		32	155
13	KB?	just to south	10	139
14	KB?	just east	11	N/A
15	KB or RB		8	130
16	KB or RB		7	129
17	RB		27	168
18	RB	farthest south	60+	N/A

2016 Monitoring Results

At Marin Rod and Gun Club in May 2016 no plants were found in the 2015 half-acre north of the pier. On the south side of the pier, similar shoot densities and shoot lengths were found in both the 2015 and 2014 half-acre plots (Figure 13 and Figure 14, respectively).

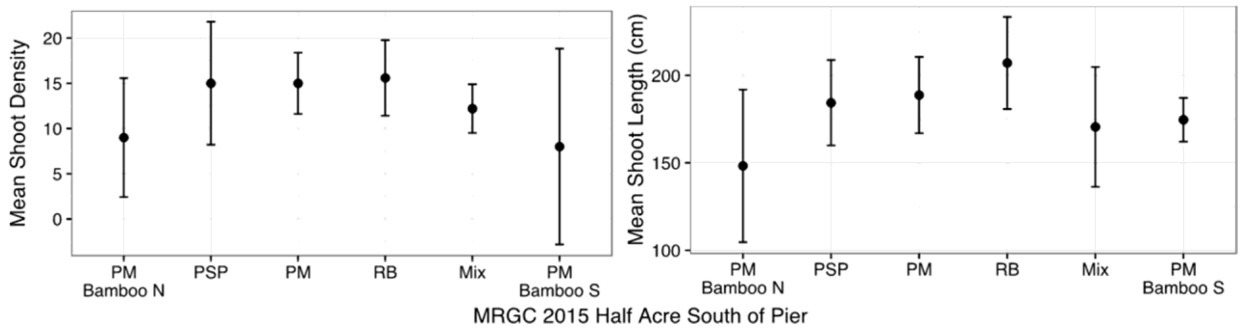


Figure 13. Mean Vegetative Shoot Density and Shoot Height (±95% CI) Per 0.25 m^2 Quadrat in the 2015 Half-acre Plot South of the Pier at the Marin Rod and Gun Club. Data was collected in May 2016. PSP = Point San Pablo, PM = Point Molate, Mix = Mixed donors, KB = Keller Beach, RB = Richardson Bay.

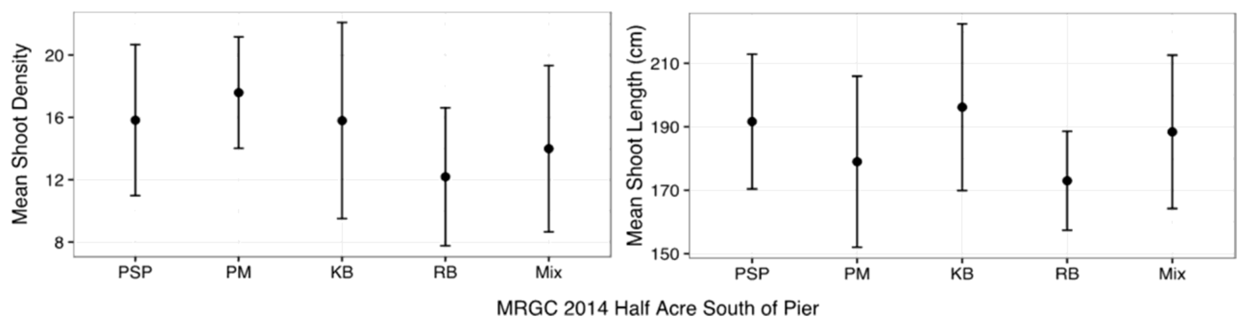


Figure 14. Mean Vegetative Shoot Density and Shoot Height (±95% CI) Per 0.25 m^2 Quadrat in the 2014 Half-acre Plot South of the Pier at the Marin Rod and Gun Club. Data was collected in May 2016. PSP = Point San Pablo, PM = Point Molate, Mix = Mixed donors, KB = Keller Beach, RB = Richardson Bay.

2017 Monitoring Results

In January 2017 the 2016 half-acre plot north of the MRGC pier and the shallow 2016 half-acre plot south of the pier were monitored. Both plots had similar densities across all donors (Figure 15 and Figure 16, respectively). Plants in the southern plot had some exposed rhizomes and some evidence of bird herbivory, while plants in the north plot did not show evidence of herbivory, and rhizomes were mostly fully buried in sediment.

In February 2017 a return visit was made to monitor plots at MRGC; however, the plants were significantly less abundant than in January. During this visit, leaves were noted to fall apart when handled, and many rhizomes were found in the sediment without attached shoots. It was impossible to measure or count the few remaining plants without damage, so the monitoring effort was abandoned. The leaves on plants during February were suffering from considerable cell lyses resulting from extreme salinity depression associated with the flood discharges to the bay at the time.

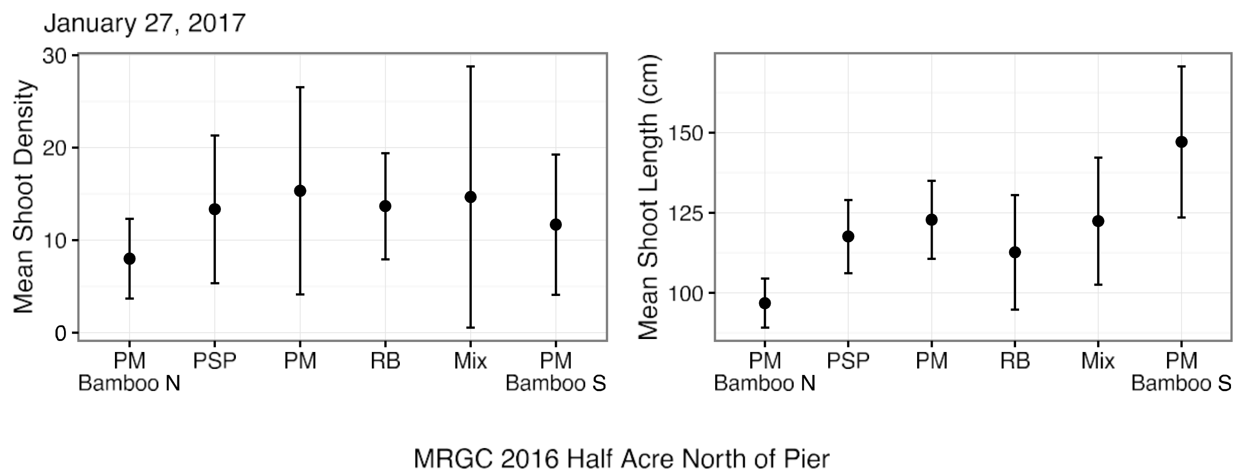


Figure 15. Mean Vegetative Shoot Density and Shoot Height (±95% CI) Per 1 m^2 Quadrat in the 2016 Half-acre Plot North of the Pier at the Marin Rod and Gun Club. Data was collected on January 27, 2017. PSP = Point San Pablo, PM = Point Molate, Mix = Mixed donors, KB = Keller Beach, RB = Richardson Bay.

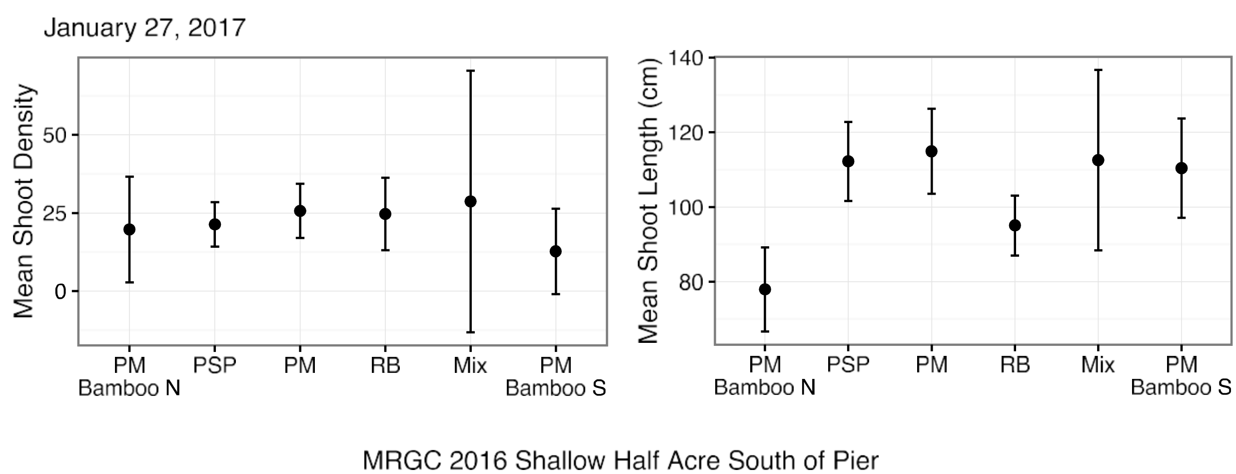


Figure 16. Mean Vegetative Shoot Density and Shoot Height (±95% CI) Per 1 m^2 Quadrat in the 2016 Shallow Half-acre Plot South of the Pier at the Marin Rod and Gun Club. Data was collected on January 27, 2017. PSP = Point San Pablo, PM = Point Molate, Mix = Mixed donors, KB = Keller Beach, RB = Richardson Bay.

A final 2017 monitoring was conducted in early December 2017. Interferometric sidescan sonar data showed few plants to the north of the pier and no plants within the full acre plots north of the pier. This agreed with previous field observations, so no monitoring was conducted in this plot. In the 2016 half-acre plot north of the pier, only 12 shoots were located. This included 6 in a patch in the mixed donor bed line, and 6 in a patch on the Point San Pablo donor line. This was a significant reduction from the conditions present in January prior to the impact of depressed salinities when plants from each donor line were present in this plot.

By December 2017 in the plots south of the pier, the plants had recovered significantly since February, although densities were still lower than in January and beds were no longer consolidated but rather marked by scattered plants within and around the original planting areas. In the 2016 shallow half-acre plot south of the pier, similar low turion densities were noted across all donors, except there were no plants present in the northern Point Molate bamboo line or the Richardson Bay lines (Figure 17). Note that some samples had only 2 replicate quadrats, leading to large confidence intervals. In the 2015 half-acre plot south of the pier, similar densities occurred across all donors. Richardson Bay plants were shorter than plants in the Point Molate and mixed donor lines (Figure 18). Plants in both plots had somewhat exposed rhizomes. The 2014 half-acre plot is becoming more difficult to distinguish between donor lines, with patches distributed somewhat evenly throughout the plot, except in the northern end where the Point San Pablo donor line was planted. Plants were similar in density and length across the rest of the donors (Figure 19), and rhizomes were not exposed.

Monitoring was difficult to complete during this sampling period, both because low tides fall after dusk, and because on the monitoring dates high wind and chop made it difficult to find plants. It is likely that monitoring slightly underestimated plant abundance and density in the monitored plots. Additionally, it was not possible to monitor the deeper 2016 half-acre plot. This plot is so deep that it generally requires SCUBA to conduct monitoring and as the original form of the planting area has been lost, this monitoring method has become ineffective at attributing encountered plants to particular donor source materials.

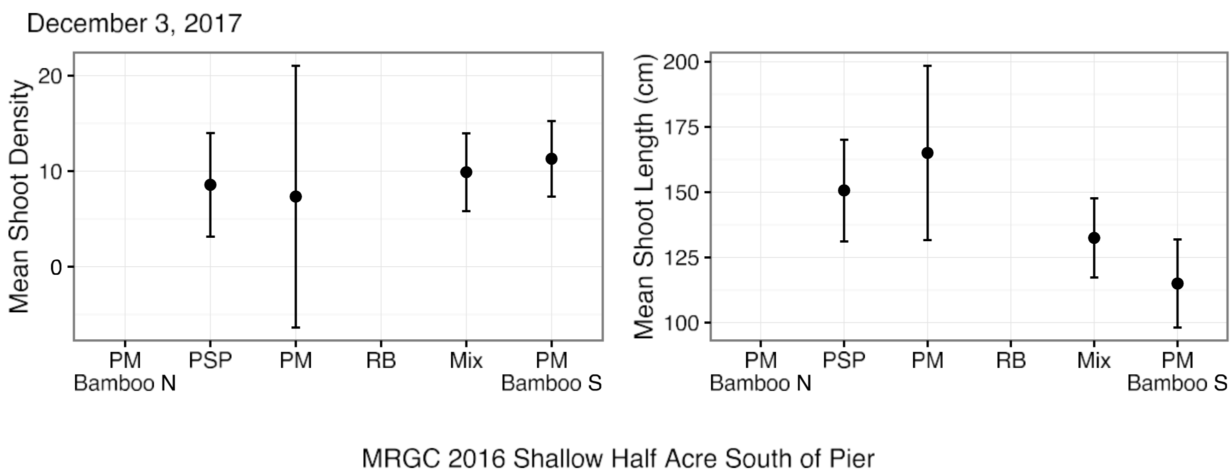


Figure 17. Mean Vegetative Shoot Density and Shoot Height (±95% CI) Per 0.25 m^2 Quadrat in the 2016 Shallow Half-acre Plot South of the pier at the Marin Rod and Gun Club. Data was collected on December 3, 2017. PSP = Point San Pablo, PM = Point Molate, Mix = Mixed donors, KB = Keller Beach, RB = Richardson Bay.

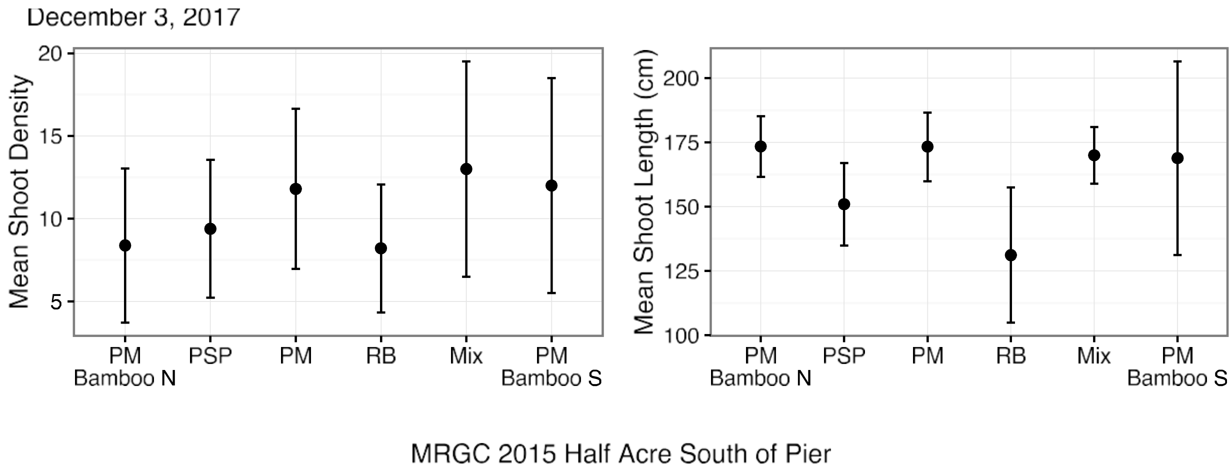


Figure 18. Mean Vegetative Shoot Density and Shoot Height (±95% CI) Per 0.25 m^2 Quadrat in the 2015 Shallow Half-acre Plot South of the Pier at the Marin Rod and Gun Club. Data was collected on December 3, 2017. PSP = Point San Pablo, PM = Point Molate, Mix = Mixed donors, KB = Keller Beach, RB = Richardson Bay.

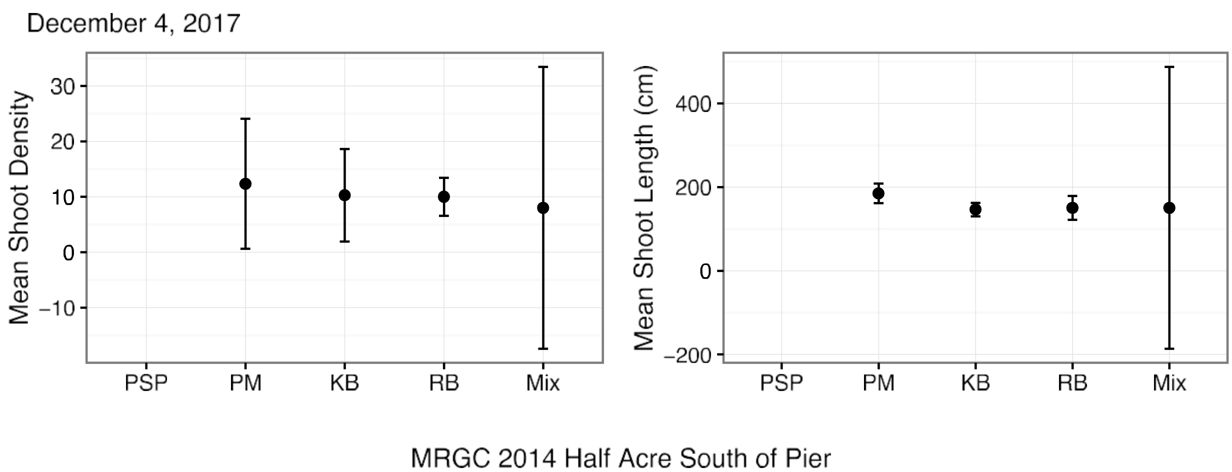


Figure 19. Mean Vegetative Shoot Density and shoot height (±95% CI) per 0.25 m^2 quadrat in the 2014 Shallow Half-acre Plot South of the Pier at the Marin Rod and Gun Club. Data was collected on December 4, 2017. PSP = Point San Pablo, PM = Point Molate, Mix = Mixed donors, KB = Keller Beach, RB = Richardson Bay.

On September 8, 2018, the 2018 half-acre plot was monitored, and seed bags were removed. The northern-most bamboo line (Point Molate donor) had very few plants present. The Point San Pablo donor line had only two plants present. The Point Molate donor line had some plants present, with some new leaf growth on shoots but still 1-2 shoots per planting. The Richardson Bay donor line had some plants present with 1-3 shoots per planting location. The mixed donor line had fewer plants present than the Richardson Bay donor line.

Overall plants in this plot showed low survivorship and little expansion. Plants did not seem healthy, showing little new growth. Rhizomes were occasionally exposed, with some feeling soft or rotted. Additionally, plant leaves were heavily fouled by a white bryozoan. Caprellid shrimp were abundant on shoots, and some amphipods were present but mostly small in size. Plants showed some damage from amphipod herbivory, as well as some "clipping" at the top of the blades from bird herbivory.

Bryozoan on an eelgrass blade

Seed bags were also heavily fouled with bryozoans, and there were numerous small nudibranchs associated with the bryozoan. The presence of the isopod *Synidotea laticauda*, and the crab *Cancer antennarius* on the seed bags were also noted at the site.

Bryozoan and nudibranch

Three shallower plots south of the pier were monitored in September 2018. The eelgrass in these plots was notably healthier than the northern plot. The plants exhibited less epiphyte fouling on the blades and coalesced plants forming small beds were common. Plants showed some evidence of avian clipping at the tops of the shoots, and some amphipod herbivory, but overall plants were robust and healthy. Some flowering shoots were also present.

Monitoring was again conducted on December 20, 2018. There were some plants present in all the donor lines in the 2018 plot north of the pier, except in the Point San Pablo line. Though survivorship was not high, the surviving plants showed signs of expanding from the planting density with 2-7 shoots per patch. Plant blades were relatively clean of epiphytes, with no signs of the bryozoans from September. Rhizomes were often exposed, and plants showed signs of bird clipping at the tips. There was some evidence of amphipod herbivory, but no amphipods were observed. Some caprellids were present. One shoot was flowering. Plants looked healthier in December than was the case in September 2018.

In the three shallow plots south of the pier, plants looked robust and healthy, as in September. There were few epiphytes on the blades, and less amphipod damage than in the recently planted 2018 plot. Avian herbivory was present, but less prevalent in deeper patches. Some flowering shoots were present throughout the beds.

Plots on the San Rafael Shoreline at Marin Rod and Gun Club were monitored in July 2019, and again in December 2019. In July monitoring was conducted in plots both north and south of the MRGC Pier. At plots south of the pier, monitoring was conducted in each of three shallow plots separately, but it was not possible to distinguish between donor rows within the plot due to the loss of initial planting patterns after the 2017 freshwater pulse impacts. However, at the more recently planted 2018 plot located to the north of the pier, plants were identified in three of the four donor rows. Plants from the mixed planting, Point Molate, and Richardson Bay donors were present, while Point San Pablo donor plants were not observed during the field inspections. The shoot densities varied across plots located south of the pier with the mean range generally falling between approximately 25 and 32 turions/0.25 m^2) while the younger transplanted plants within the 2018 plot to the north of the pier exhibited a generally lower mean density range across donor sites. The plants appeared healthy and robust throughout the site. On average, 20% of shoots were flowering at the site, with all flowering stages (De Cock 1980) being represented. There were signs of bird herbivory within the intertidal plants, with the tops of the plants being clipped by birds.

Notably, the extent of activities by the invasive amphipod *Ampithoe valida* was higher during July 2019 than has been noted in prior years. Both mature amphipods and nests were common on the eelgrass leaves in the transplanted plots. The nests of *A. valida* were the most common epiphytic loading on the leaves observed within this area during the monitoring period. In December 2019, the plants were again observed to be robust and healthy throughout the site, with low epiphyte loads being noted across all transplants. There were some exposed rhizomes above the sediment within the mid-elevation plots. Notably, there were a few flowering plants in early stages of development observed throughout the site. This is notable as flowers generally begin to develop in the early spring and not during the fall and winter. Caprellids were common on the plants. Nests of *A. valida* were observed on older leaves but were not common on younger leaves during the monitoring interval. Direct field monitoring was not conducted in spring 2020 due to state directives that precluded field activities by the SFSU team members. As a result, only the remote sensing monitoring activities were undertaken during this period of time. Quantitative sampling was conducted in July 2020. *Ampithoe* were present in moderate abundance with both nests and herbivory damage on leaves. Some caprellids were also present. Macroalgae was light with *Ulva, Mastocarpus*, and some filamentous red algae being present at the base of the beds. There was a moderate degree of epiphytic loading on the leaves. Some exposed rhizomes were noted on the sediment surface. Also noted was avian herbivory damage. Transplant plots to the north of the pier were denser and generally supported larger invertebrates and more herbivory damage than seen to the south of the pier. Shoot density was determined to be 74.3±23.8 turions/m^2 (n=42) with almost no difference between densities north and south of the MRGC pier. During the July 2021 sampling event it was determined that leaf lengths averaged 158.4±28.5 cm (n=41) while flowering shoots were only very slightly longer at 177.7±56.8 cm (n=41). Flowering was observed at an average rate of 4.0±5.5% (n=42). The high variance in flowering across the bed is indicative of patchy distribution of flowering stalks. In 2021, monitoring was conducted qualitatively in April and December with quantitative sampling occurring in July 2021. In July eelgrass shoot density was sampled along with leaf height, flowering percentage, and flower height. In addition, observations were made regarding the degree of damage from grazers on the plants. Shoot density was determined to be 59.0±13.3 turions/m^2 (n=40) with almost no difference between densities north and south of the MRGC pier. During the July 2021 sampling event it was determined that leaf lengths averaged 160.6±28.5 cm (n=40) while flowering shoots were only modestly longer at 195.9±42.4 cm (n=33). Minor damage by *Ampithoe* was noted; however, there were abundant nests on the leaves. No goose herbivory was noted. Also, there was no wasting disease noted in the restoration plots. Flowering shoots were present at all stages (De Cock 1980). Plants were healthy in appearance with low epiphytic loading that principally included filamentous algae, chain diatoms, and some bryozoans that were found on older flowering shoots.

In April 2022, in situ monitoring revealed healthy plants with moderate epiphytic loading and notable goose herbivory. There was a greater amount of epiphytic loading observed on plants in deeper water than shallow water. Caprellid amphipods were notably sparse and a few *Aphithoe* were present in the epiphytes.

Corte Madera Bay

2014 Monitoring Results

On September 8, 2014, the SFSU project staff monitored the Corte Madera Bay plots on SCUBA and on-foot. Test plots were visited concurrent with monitoring completed for the larger Cosco Busan DARP planting efforts completed in 2014. These plots were investigated by divers swimming along the v-shaped planting array looking and feeling for transplants within the easterly two test plots. For the shallower westerly test plots, staff walked the extent of the plots searching for any transplant units. No transplants were detected in any of the Corte Madera plots, although there were some limitations in the ability to confidently identify the presence of specific locations where paper stick planting units were used since these units lack surface manifestations without plants.

2015 Monitoring Results

The plots were again investigated on May 4, 2015 to determine if any seeding had occurred at the Cosco Busan plots where buoy-deployed seeding had been incorporated. At this time, neither seedlings nor mature plants were noted in the test plots, although examination was less extensive having documented plant absence during the prior fall. These investigations were consistent with the observations from the acoustic sampling performed where only a few individual plants were noted to persist.

No monitoring of plots was performed during 2016, 2017, 2018, 2019 or 2020 due to an extreme paucity of plants persisting in the area by 2016 and an absence of plants in later years.

Richardson Bay

2016 Monitoring Results

The first *in situ* monitoring conducted in Richardson Bay for the DARP program was conducted in May 2016 concurrent with monitoring of transplants completed for the SFOBB restoration effort. Plots were monitored together for the two programs to produce a more robust analysis by pooling the data sets. In May 2016, abundant macroalgae was observed on the sediment and in the water column. At times, algal mats were observed to be weighing down eelgrass leaves. In addition, during this period, a considerable covering of epiphytic worm tubes were also noted on the eelgrass leaves.

At Richardson Bay in May 2016, the mean number of shoots per 0.25 m^2 was similar between donors in the 2015 western half-acre plot (Figure 20). The donor with the greatest mean number of shoots was Richardson Bay (mean = 6.30 shoots/0.25 m^2) and those with the lowest were Point San Pablo and the mixed donor rows (both with a mean = 1.4 shoots/0.25 m^2). The mean height of vegetative shoots was similar across donors, although Point Molate plants from the bamboo lines were longer than Point Molate plants in the paper stick anchor line (Figure 20). Overall, plants at Richardson Bay were smaller and less dense than plants at Marin Rod and Gun Club.

2017 Monitoring Results

In February 2017 an attempt was made to monitor the 2016 western half-acre, and the 2016 eastern half-acre transplant sites. The plants showed signs of herbivory from birds and were clipped shorter than usual. This made it very difficult to find plants and to find donor lines, since the plants were below the water depth. In total, 15 plants were located in the plot, but it was not possible to confirm which donor line they were located on. The plant count obtained was likely an underestimate based on the extent of herbivory that had occurred prior to completion of the monitoring. In the eastern half-acre, only 7 plants were located for the same reasons and sampling accuracy was also considered very uncertain.

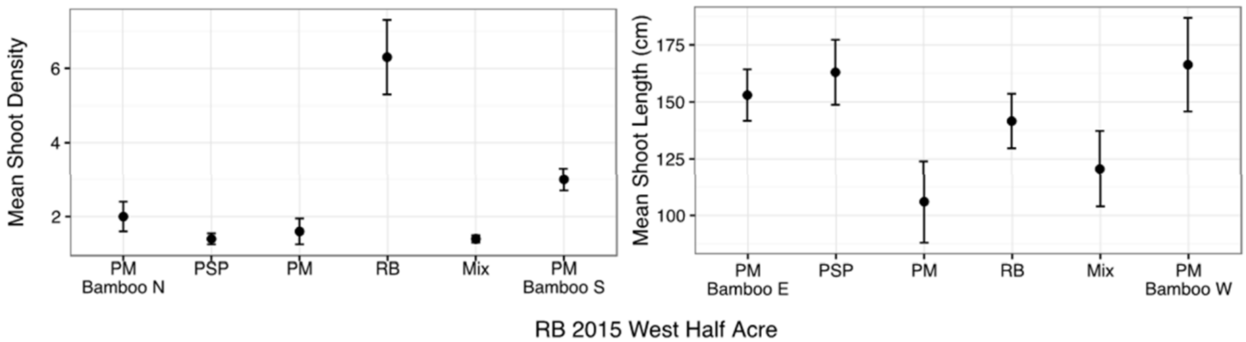


Figure 20. Mean Number of Vegetative Shoots, and Mean Shoot Length (±95% CI) Per 0.25 m^2 Quadrat in the 2015 Western Half-acre Plot in Richardson Bay (May 2016)

In April 2017 plants were more readily located and donor lines could be confirmed. In the 2016 western half-acre restoration site, plants were found along every donor line, and a complete count of plants was conducted for the entire plot. The Point San Pablo and Richardson Bay donor lines had the most shoots at 45 and 44 respectively (Figure 21). There were 151 plants identified in the entire plot. However, some plants were weighed down by macroalgae, making them difficult to locate, so these numbers are likely a slight underestimate. The 2016 eastern half-acre was much denser, with 626 total plants in the plot. There were plants in every donor line except the western Point Molate bamboo line (Figure 22). The Richardson Bay and mixed donor lines had the most shoots, with 273 and 269 total plants being identified (Figure 22). Plants in this plot were also weighed down by macroalgae and epiphytes, so again the turion counts reported for the plot are likely to be an underestimate of the actual abundance of turions present.

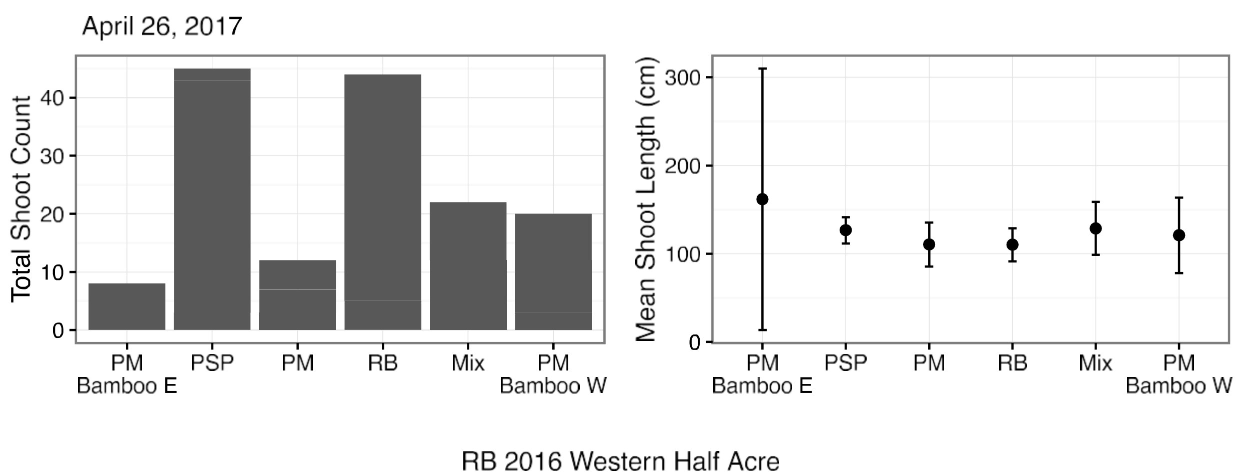


Figure 21. Total Shoot Count Per Donor Line, and Mean Shoot Length (±95% CI) in the 2016 Western Half-acre Plot at Richardson Bay (April 2017)

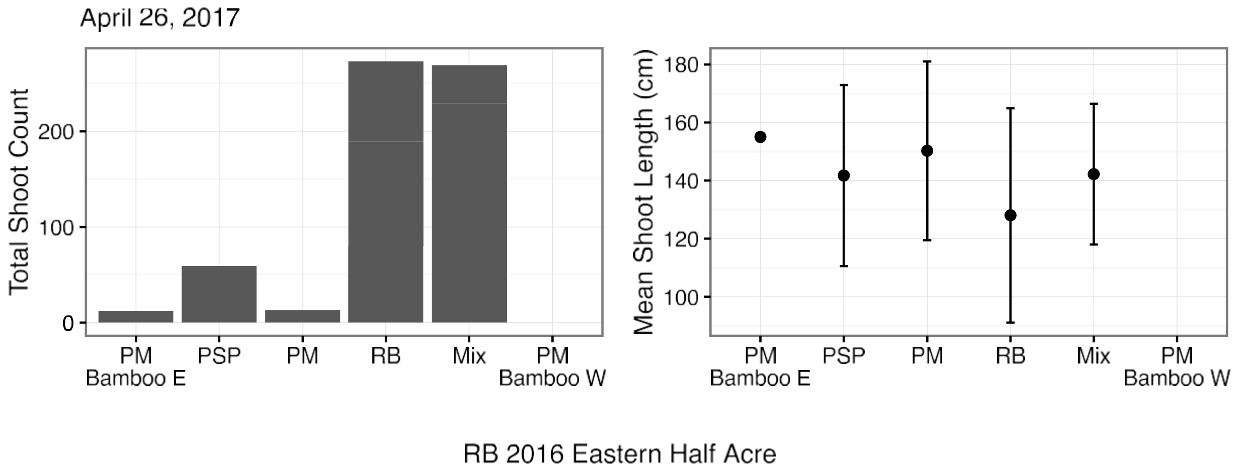


Figure 22. Total Shoot Count Per Donor Line, and Mean Shoot Length (±95% CI) in the 2016 Eastern Half-acre Plot at Richardson Bay (April 2017)

In August 2017 the 2015 western half-acre plot was monitored. Plants were found in all donor lines, although the plot end bamboo anchor unit lines supported only 6 and 3 plants (Figure 23). The mixed donor line had the most plants with 163, and there were an estimated 362 total plants in the plot. The previously high abundance of macroalgae noted in April 2017 was substantially reduced in August, although a number of the plant rhizomes in this plot were highly exposed above the sediment. It is believed the high rhizome exposure may be related to the avian herbivory.

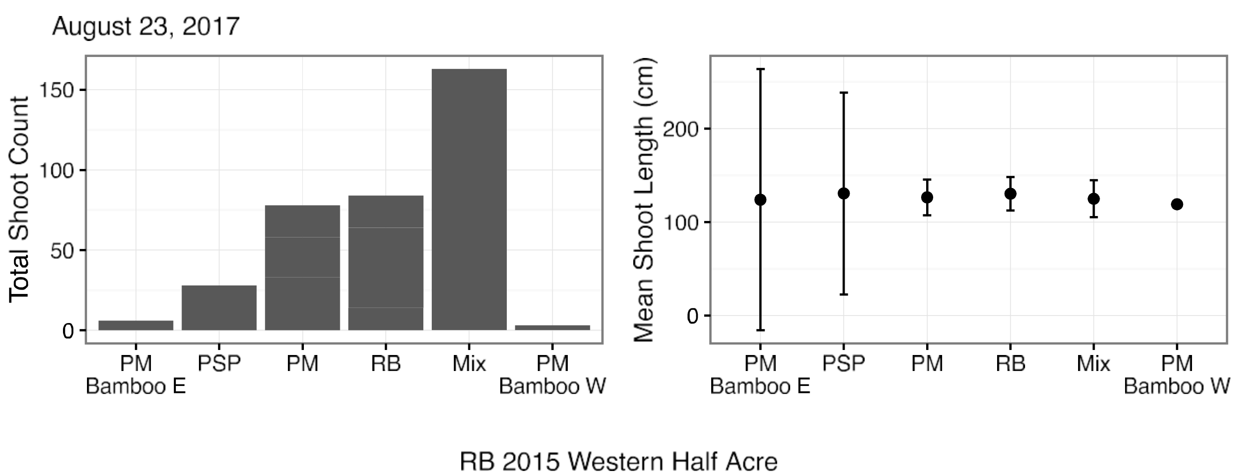


Figure 23. Total Shoot Count Per Donor Line, and Mean Shoot Length (±95% CI) in the 2015 Western half-acre Plot at Richardson Bay (August 2017)

In December 2017, the two 2016 plots from April were monitored. A part of an additional 2016 half-acre plot was also monitored. This plot showed a higher density of plants in the Richardson Bay line consistent with the sidescan sonar. The plot had a measured average density of 3.8 shoots per 0.25 m^2 on the evaluated transplant line (data not shown). There were other plants scattered throughout the plot, but these were not monitored further. In the 2016 eastern and western half-acres, similar densities and canopy heights were found across all donors and plants were found in each donor line (Figure 24 and Figure 25). In all three plots high amounts of a long feathery epiphytic algae was found attached to the leaves of the eelgrass. The presence of this epiphyte caused the plants to form large tangles, making it difficult to separate individual shoots to count and measure them. Plant rhizomes were somewhat exposed, though not as much as in August.

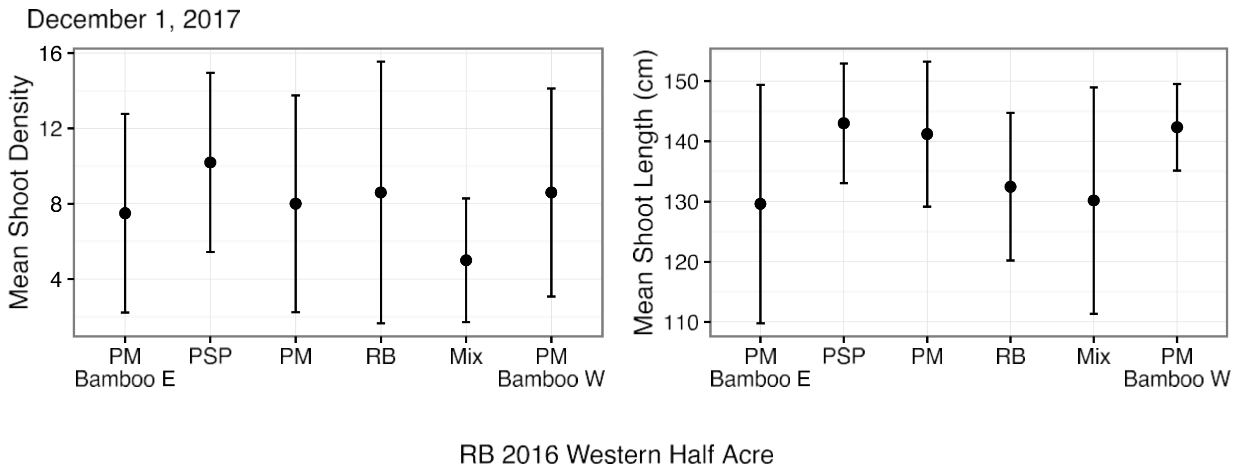


Figure 24. Mean Vegetative Shoot Density and Shoot Height (±95% CI) Per 0.25 m^2 Quadrat in the 2016 Western Half-acre Plot at Richardson Bay (December 2017)

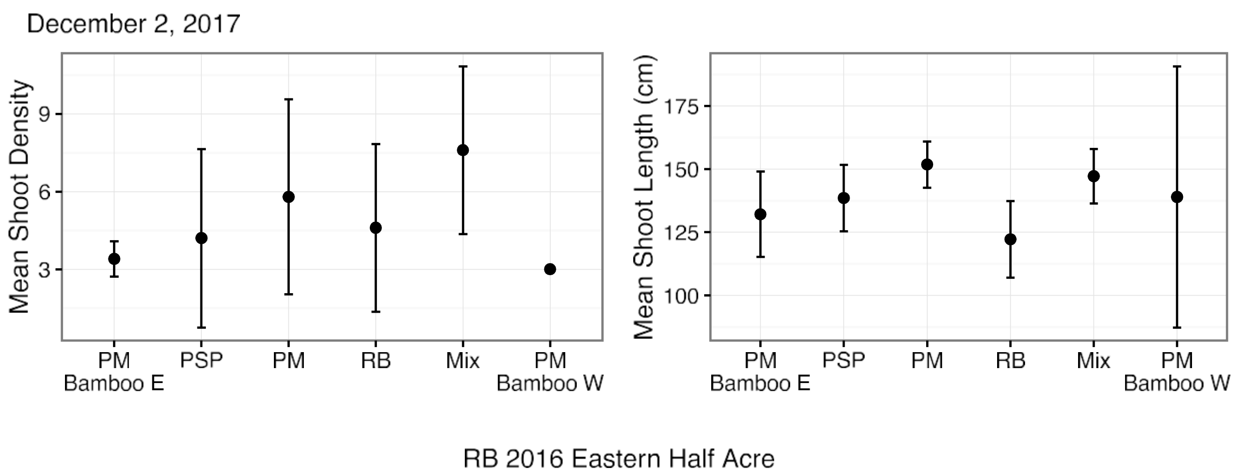


Figure 25. Mean Vegetative Shoot Density and Shoot Height (±95% CI) Per 0.25 m^2 Quadrat in the 2016 Eastern Half-acre Plot at Richardson Bay (December 2017)

2018 Monitoring Results

The full-acre plot and a half-acre plot planted in Richardson Bay in July 2018 were monitored on September 9, 2018. The plants looked much healthier than the ones planted in 2018 at MRGC. Plant blades had some epiphyte growth including a red filamentous alga but none of the bryozoans seen at MRGC. Plants were present at similar densities across all donor lines at both plots, with patches of 1-4 shoots. Some shoots were flowering. Seed bags were removed from these plots but were not as heavily fouled as at MRGC.

During monitoring on December 21, 2018, plants in both the 2018 full-acre and the 2018 half-acre plots were robust and healthy. Plants were in patches of 4-8 shoots. There were signs of bird herbivory, but little other damage to blades. Some caprellids were present, but fewer than at MRGC.

Epiphytes on eelgrass blades in Richardson Bay

Light Pacific herring egg deposition was noted on at least one plant in the half-acre that was monitored; however, eggs were not observed anywhere else throughout the area. There was a spawn reported on the same day in Richardson Bay at Schoonmaker Point by CDFW. This is the second year of observed herring spawning on the restored eelgrass plants. It is anticipated that spawning on eelgrass within this area of Richardson Bay will continue to occur on a regular to irregular basis as Richardson Bay is the heaviest used portion of the bay by spawning herring.

Example of "clipped" blades due to bird herbivory

In 2019, eelgrass was monitored on June 6, 2019 with monitoring plot identification and donor bed rows being identified using a handheld GPS loaded with the planting plot overlays over the October 2018 interferometric sidescan sonar data. At each donor row, excluding the bamboo rows on each end of the plot, the turion density of eelgrass was measured in 5 replicates by 0.25 m^2. Across all plots, plants were generally robust and healthy. Plants were typically heavily coated in epiphytes with diatom scum on the surface of the leaves, but not with significant amounts of larger epiphytic macroalgae that sometimes occurs at this site. The invasive *Ampithoe valida* was commonly observed in the transplant area as well as in nearby natural beds. Grazing scars and nests of this species were observed on plant leaves. On average, 33% of the shoots were flowering, with all flowering stages present. Also observed on the eelgrass leaves were eggs of the Japanese bubble snail *Haminoea japonica* and eggs of the sea slug *Elysia hedgpethi*.

Herring eggs on restored eelgrass at Richardson Bay (Dec. 2018)

In December 2019, monitoring of the same plots as were monitored in June was undertaken. The transplants were again noted to be generally healthy and robust, with lower amounts of diatom epiphytes than usual, but more extensive coverage by red filamentous macroalgae. Many caprellids, and some *A. valida* and their nests were observed on the leaves. A few flowering shoots persisted in the beds during the December monitoring. Also notable was the presence of exposed rhizomes on the sediment surface in some areas, although the cause of rhizome exposure could not be determined.

During the December 2019 monitoring, efforts were made to also monitor the plots planted in 2019. However, the 2019 planting plots are notably deeper than those planted in earlier years and thus they are more difficult to monitor by wading. This was especially true for the less-than-optimal tides occurring during daylight hours at this time of year. In slightly shallower plots the plants were noted as appearing healthy with some avian herbivory and considerable large caprellid amphipods. Some evidence of *Amphithoe* herbivory was noted, but it was not abundant and similar throughout the plots. In the deeper plots only a few plants were located, although this may have been due to the deep water and limited size of the recently installed planting units only a few months earlier. In spring

2020, direct monitoring was not conducted due to governmental directives in response to the COVID 19 pandemic. However, in July 2020, qualitative monitoring was conducted. During this period, filamentous green algae, possibly *Chaetomorpha*, was abundant across the surface of the sediment and was also overrunning eelgrass. The water was unusually clear allowing good visibility of the bottom, even in moderately deep water. This allowed observation to be made from the survey boat of many leopard sharks and bat rays, as well as bony fish within the eelgrass and open bottom in the project area. Eelgrass plants were generally chlorotic and lacked turgor pressure. Overall plants had low epiphytic loading. The contrasting synoptic conditions between the healthy eelgrass plots at MRGC and the declining conditions of Richardson Bay beds was striking. However, the declining conditions of beds within Richardson Bay appeared to be widespread across natural and restored beds with the declines extending southward from the Audubon Sanctuary. Eelgrass density within Richardson Bay in 2020 was notably low and highly variable across the transplants with an average of 16.8±9.0 turions/m^2 (n=120). Flowering was also low and highly variable with an average of 1.0±5.6% flowering stalks within the bed (n=120). In 2021 qualitative sampling was conducted within the Richardson Bay transplant plots during April and December, with quantitative sampling occurring in July 2021. In April, investigations revealed massive plant losses had occurred and plots were dominated by dense filamentous algal mats. Eelgrass rhizomes that were located lacked leaves and generally lacked turgor pressure and otherwise exhibited evidence of tissue rot. In some areas, floating mats of the algae were also prevalent. This appeared to be a continuation of the conditions observed in the prior year. Sampling in July 2021 revealed slightly improved conditions over those observed in April. Plants were generally healthy in appearance with little to no wasting disease and low epiphyte loading. Epiphytes were dominated by diatoms but also included bryozoans. There was little to know evidence of *Amphithoe* present on the plants, nests, or evidence of herbivory, although caprellids were abundant. Shoot density was determined to be low and variable at 14.3±10.0 turions/m^2 (n=48). During the July 2021 sampling event it was determined that leaf lengths averaged 137.7±40.1 cm (n=48) while flowering shoots were only slightly longer at 154.3.6±45.5 cm (n=48). At 4.1±12.0% (n=48) flowering was generally low but extremely variable across the transplant area in 2021. In April 2022, eelgrass was reviewed *in situ* with a finding of many smaller patches of eelgrass being present as disjunct occurrences with some larger plots. Patches had full width leaves suggesting recovery of vegetated material following the 2021 dieback. Epiphytic loading was moderate with egg masses being common. There was little evidence of *A. valida* damage on the plants; however, bryozoa were common epiphytes. Plants were generally healthy with mats of *Gracilaria* at the base of some of the plants.

DONOR BED INFLUENCE ON RESTORATION SUCCESS

One of the greatest questions associated with eelgrass restoration programs is the importance, or lack thereof, of using genetically diverse donor plant materials. The present restoration program has specifically been structured to assist in addressing questions regarding the benefits of multiple donor beds. While data continues to be collected and analyzed there are some patterns emerging that suggest that the source of donor eelgrass can influence eelgrass restoration success. The preliminary results of the present investigations were presented by Melissa Patten at the State of the Estuary meeting in October 2019 and at the Western Society of Naturalists meeting in Ensenada, Mexico, also in October 2019.

The importance of donor source is likely influenced by environmental variability and restoration site. At present information has been compiled on plantings performed in 2015, 2016, and 2018 within Richardson Bay, as well as plots planted in 2014, 2015, and 2016 at the MRGC. All of the half-acre planting plots through 2018 retained a consistent layout of donor sites with the two terminal ends of the plot being bounded by bamboo state plantings and four internal planting blocks being ordered north to south and west to east as Point San Pablo/Point Pinole, Point Molate, Richardson Bay, and Mixed Donor plots.

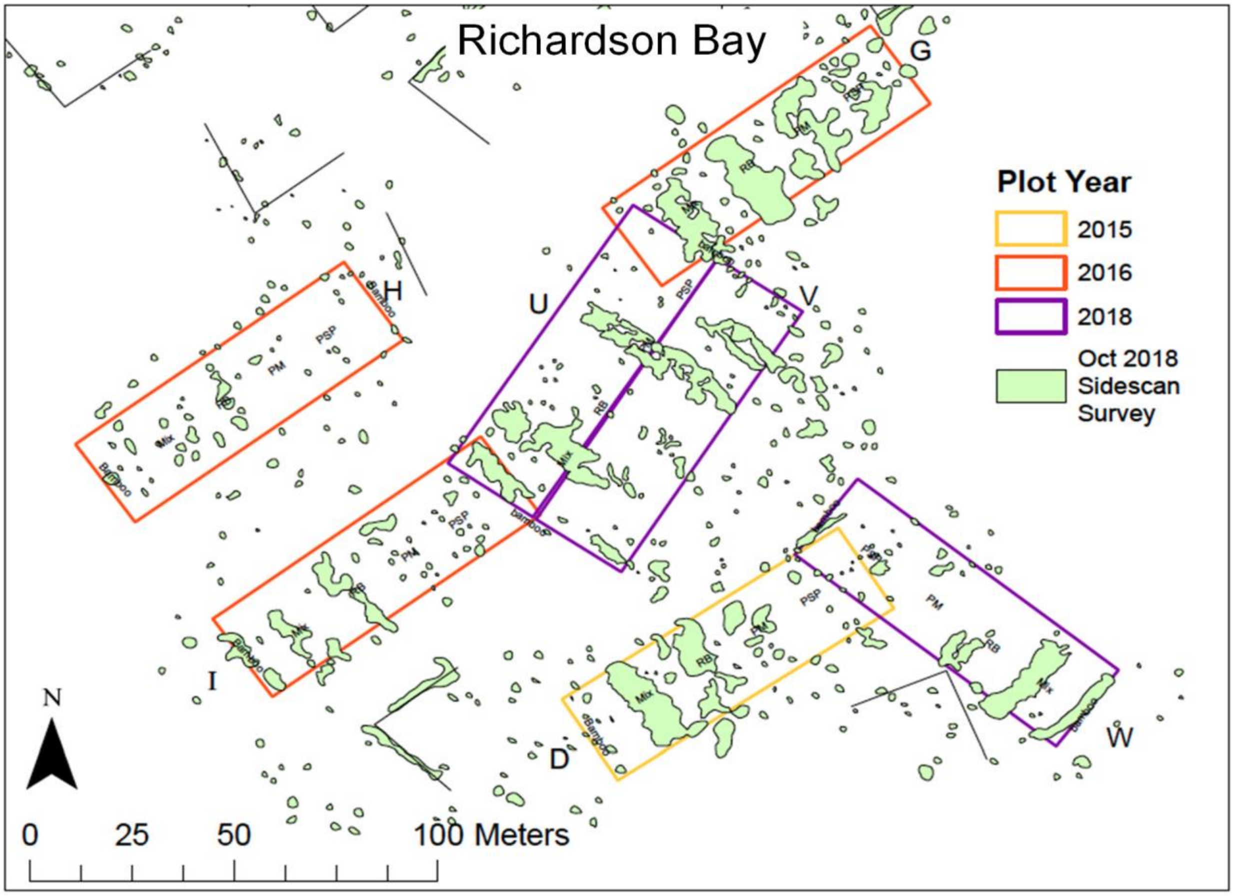
In 2019, the Richardson Bay donor was replaced by a Bay Farm Island donor site. Monitoring of the transplant plots using interferometric sidescan sonar has allowed the evaluation of how individual donor plantings have performed across transplant years and receiver sites by quantifying the eelgrass cover by donor site over planting years. By quantifying the eelgrass by donor source across the multiple years it is possible to evaluate how each donor has performed under the highly variable conditions experienced at the transplant sites over the course of the project.

In the Richardson Bay restoration plots, the Richardson Bay donor plants were consistently most successful in plots planted in 2015 and 2016. However, in plots planted in 2018, Point Molate and the Mixed Donor plants outperformed other donors.

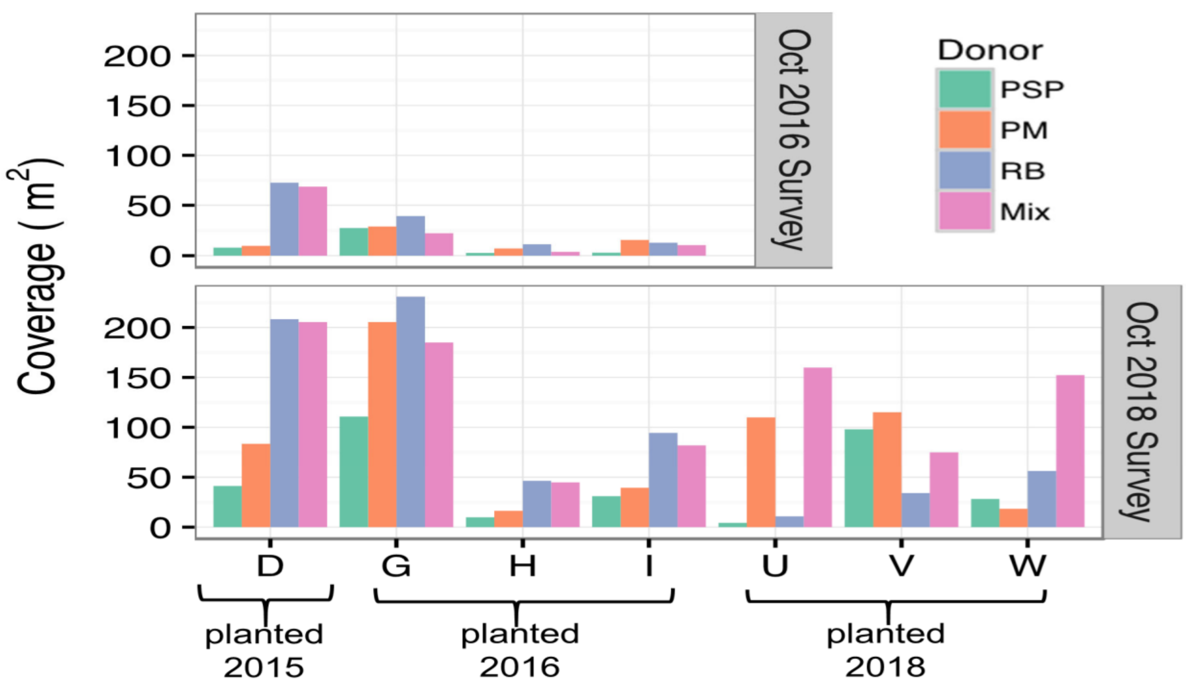
The patterns along the San Rafael shoreline at MRGC were not as clear as that observed in Richardson Bay. At MRGC plots planted in 2014, 2015, and 2016 all donor lines were equally successful during the 2016 surveys. However, in 2017, heavy rainfall and extended low salinity resulted in significant plant mortality. Some surviving plants recovered, and new plants germinated in 2018; however, donor lines could not easily be distinguished making it much more difficult to explore the role of donor beds on restoration success. While the depressed salinity had significant impacts across all planting plots and donor sources, there was a notable gradient of plant recovery favoring beds at moderate depth that likely suffered less salinity depression or shorter duration of exposure as well as less impact by elevated turbidity. This depth gradient coupled with considerable seedling recruitment following the freshwater pulse has resulted in obfuscating potential effect of donor source on restoration success.

In addition to spatial metrics, evaluations are underway into how donor source and planting year interact to influence other characteristics, including densities, flowering rates, and invertebrate use. In 2018, recovery was mostly concentrated in the mid-elevation plots, with less coverage in the shallowest plot, and almost none in the deepest plot where it has proven difficult to collect plant metric measurements due to extreme low water clarity. However, in 2019 eelgrass further expanded and occupied areas both deeper and shallower than the areas showing early recovery. Continuing through 2020 and 2021, while only spatial mapping was conducted in 2020, eelgrass beds continued to fill and densify from the mid-depth core outward. However, it appears the limits of suitability within the depth range available are beginning to be met. In 2022, deeper bed margins at San Rafael shoreline declined, while bed losses within core eelgrass areas were noted in Richardson Bay.

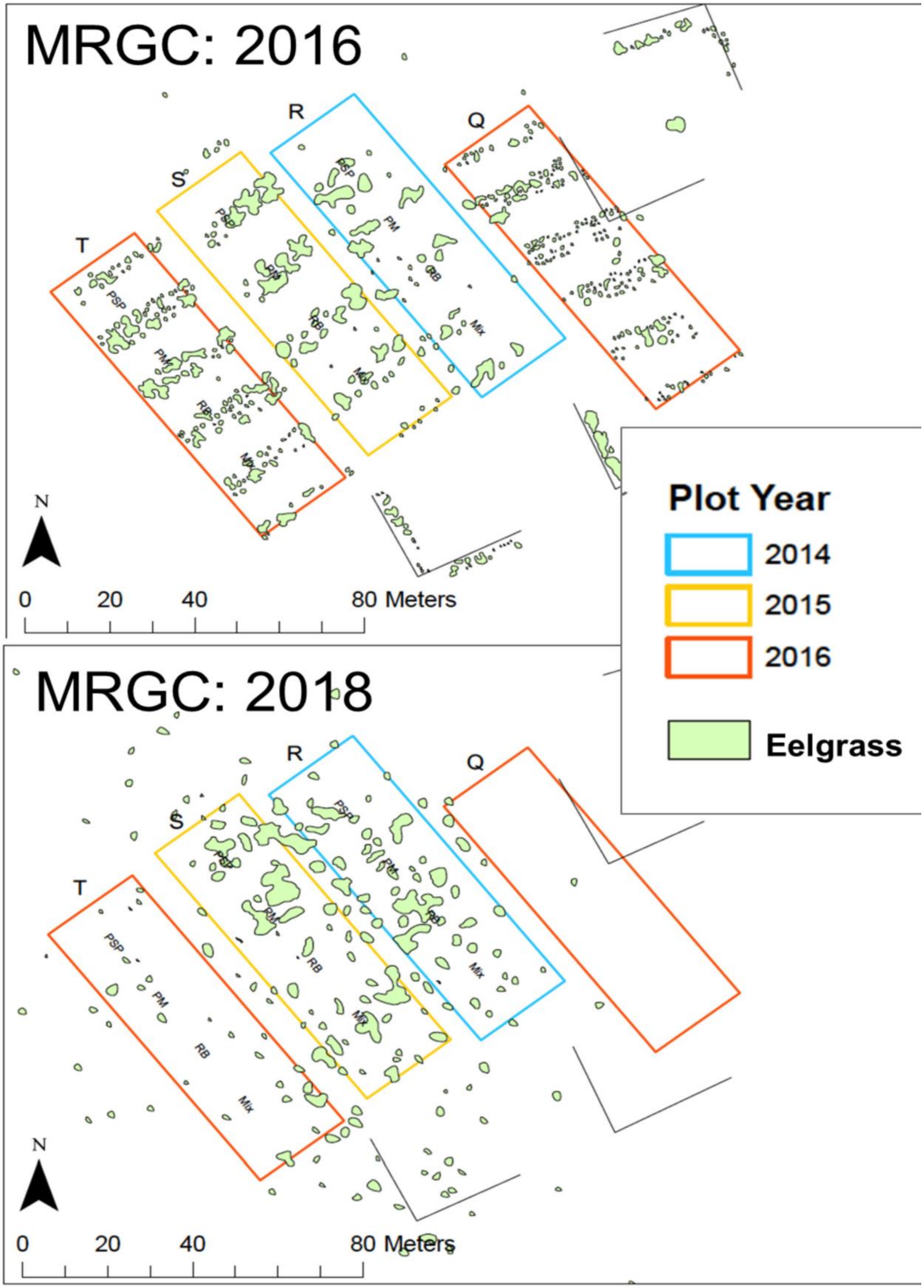
At some sites, such as Richardson Bay, monitoring results have suggested that matching donors to receiver areas may be important. However, this importance may only be manifested under certain environmental conditions. As a result, it may be important to hedge against fluctuating environmental conditions differentially influencing certain genotypes of transplanted eelgrass. This can be done by mixing donor sources. Continued plot monitoring and further multivariate analyses will continue to explore the potential effect of donor site on restoration in highly stressed environments.



Richardson Bay planting plots with 2018 eelgrass distribution reveals patterns of higher performance from some donors over others within different transplant years.



Eelgrass coverage by donor site and planting year within Richardson Bay restoration plots.



Marin Rod & Gun Club transplant site conditions before (2016) and after (2018) the larger freshwater pulse from flood events in 2017 that lead to significant losses of eelgrass in natural and transplanted eelgrass beds.

Pacific Herring Monitoring

Monitoring Approach

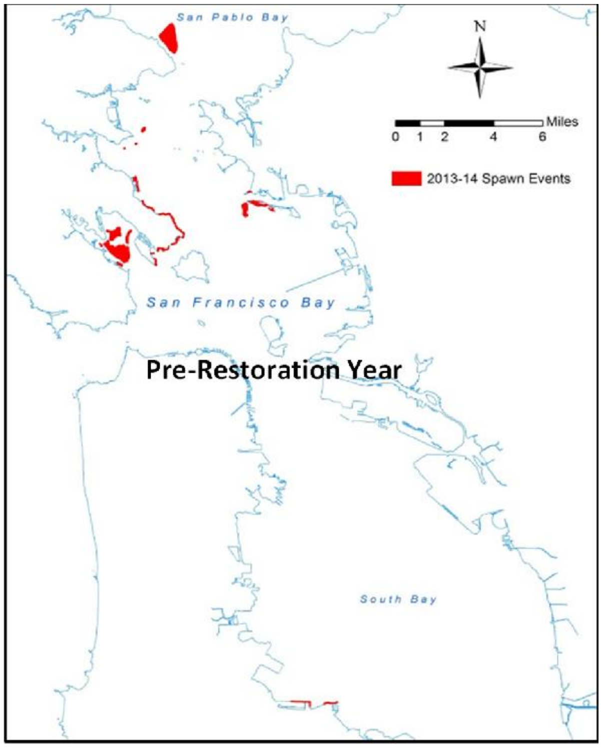
As an element of the restoration program, the spawning activities of Pacific herring were investigated to determine if restored habitat was being used by Pacific herring as spawning substrate. In addition, the tracking of herring spawning activities also facilitated identification of restoration opportunities that would overlap with herring use areas within the Bay. The timing and location of the herring runs were tracked by following the California Department of Fish and Wildlife (CDFW) blog posts by Ryan Bartling every week as well as monitoring bird activities offshore of EOSC-RTC. However, regular posting of herring spawning status ended during the COVID-19 pandemic. As such, herring spawning was less formally tracked during this period and formal reporting has been delayed.

The goal was to determine if herring spawning was occurring in the vicinity of eelgrass restoration areas. The CDFW monitors herring spawning activities and develops annual maps of spawning activities (Figure 26) and estimates of the biomass of herring as elements of the herring fisheries management program (Figure 27). The annual spawning maps (not preprepared for 2019–20 or 2020–21) are helpful in assessing potential restoration areas and likelihood of restored eelgrass use, while the annual biomass estimates are helpful in understanding the relative availability of spawning fish resources that may affect both distribution and abundance of spawning during any given year.

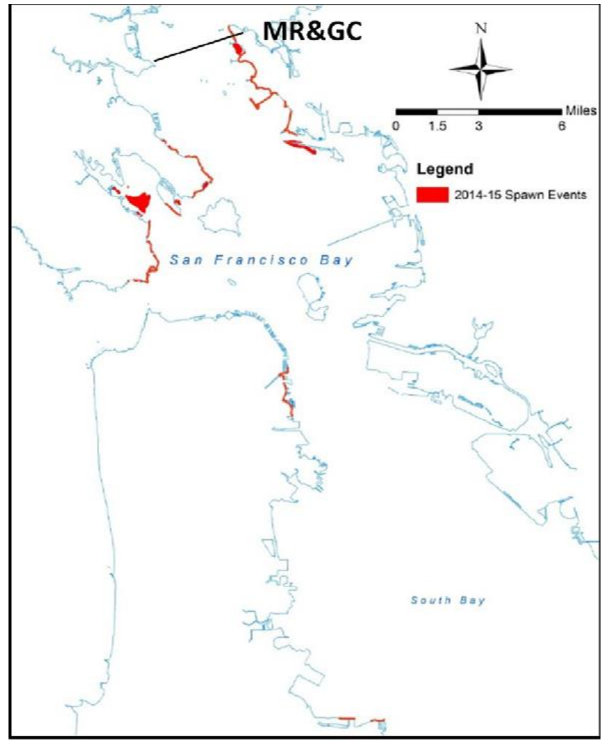
The long-term biomass data kept by the CDFW revealed a considerable reduction in biomass estimates over the past reporting years (2014–2015 through 2018–2019, with data from 2019–2022 not yet available) from the mean conditions. The biomass during the past recent years coincident with the DARP restoration program averaged only 30.8 percent of the long-term average and was only 24.2 percent of the preceding years (Figure 27). Also notable was a highly variable distribution from that historically seen. Herring did not spawn within the northeastern portions of Richardson Bay during the period 2013–2014 through 2015–2016. Even though, this has historically been an area of regular herring spawning activities (see Figure 1). During the DARP restoration and monitoring period, herring have only spawned in this area during 2016–2017 through 2020–2021. Similarly, herring never spawned in the vicinity of the MRGC during the monitoring period and also did not spawn in Corte Madera Bay during this same period.

Herring Spawning Habitat Utilization

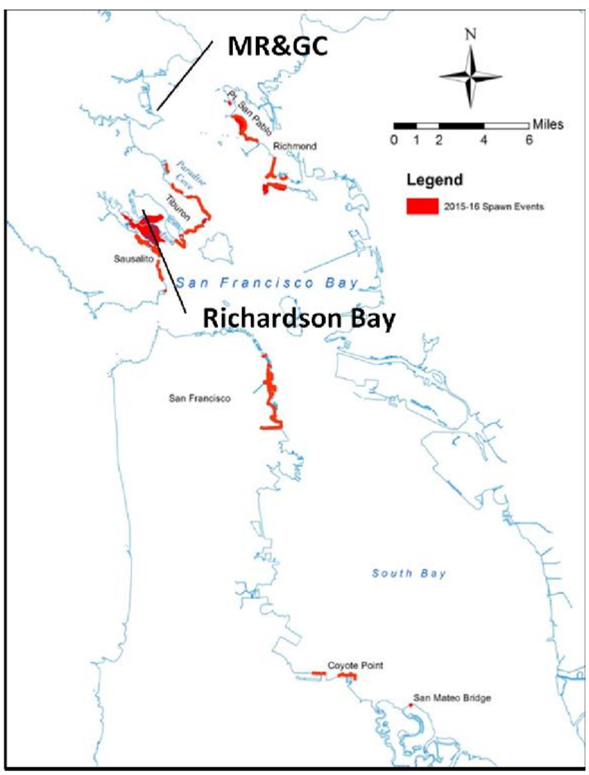
In January 2015, spawning was assessed along the shoreline in Tiburon at two sites to compare the use of different substrate types, and in January 2016 a natural eelgrass bed along the Richmond shoreline was investigated to assess herring egg deposits. Both these locations were near to known active spawning locations. On January 20, 2015, two beaches were visited along the Tiburon Peninsula shoreline, the beach at the EOSC-RTC and a private property approximately 1 mile to the north (Figure 28). At both sites, the percent cover of rock, cobble, algae and unconsolidated sediment was assessed along with the percent cover of herring eggs on those substrates. Samples of algae and eggs scraped from rock and cobble were collected to enable a biomass assessment from those substrates. Samples were taken back to the lab, weighed, dried at 55 °C for 24 hours and reweighed.



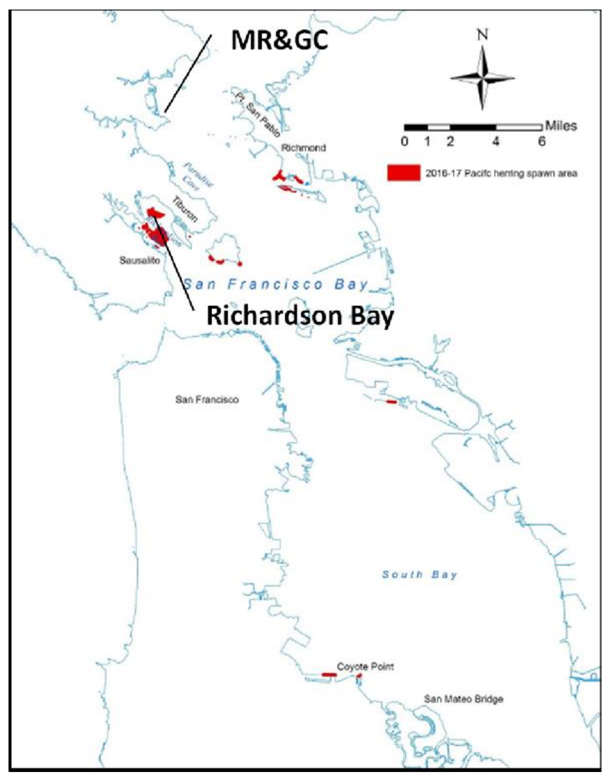
2013-2014 San Francisco Bay Herring Spawning (CDFW Pacific Herring Management News)



2014-2015 San Francisco Bay Herring Spawning (CDFW Pacific Herring Management News)

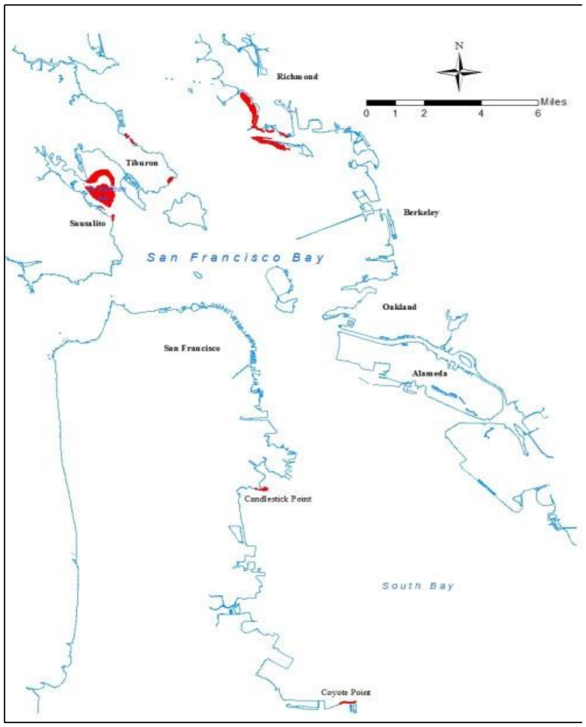


2015-2016 San Francisco Bay Herring Spawning (CDFW Pacific Herring Management News)



2016-2017 San Francisco Bay Herring Spawning (CDFW Pacific Herring Management News)

Figure 26a. Pacific Herring Spawning Areas Relative to Successful Eelgrass Restoration Sites



2018-2019 San Francisco Bay Herring Spawning (CDFW Pacific Herring Management News)

Figure 26b. Pacific Herring Spawning Areas Relative to Successful Eelgrass Restoration Sites

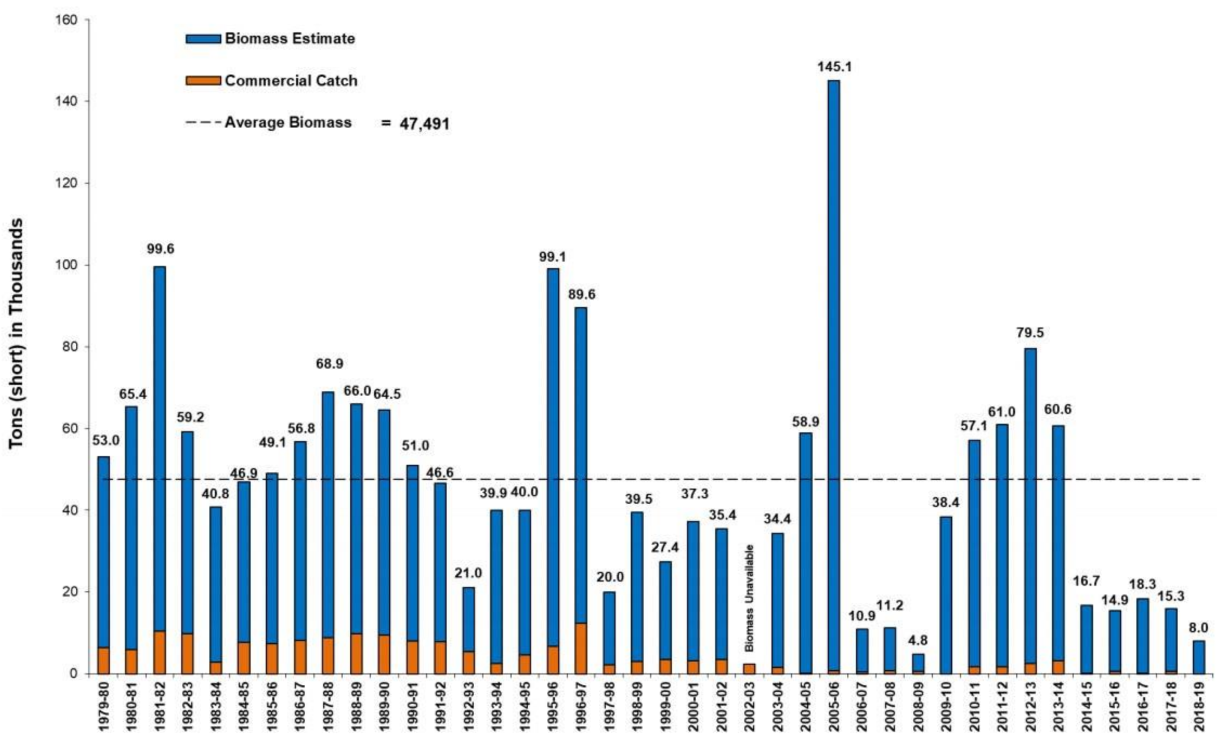


Figure 27. San Francisco Bay Pacific Herring Biomass estimates (1979-2019)
from CDFW https://nrm.dfg.ca.gov/FileHandler.ashx?DocumentID=177969&inline

Figure 28. Map Indicating the Locations of Investigated Herring Sites

EOSC-RTC beach and the private dock were visited in January 2015, and Keller Beach was visited in January 2016.

On January 23, 2016 at Keller Beach (Figure 28), spawning on eelgrass and the macroalgae *Gracilaria* sp. (likely *G. verrucosa*) was assessed at two depths along 25 meter long transects. At the shallower transect only *Gracilaria* sp. was present, and at the deeper transect both eelgrass and *Gracilaria* sp. were present. Quadrats were placed randomly over the substrate along the transects and an assessment of the available cover of *Gracilaria* sp., eelgrass, and sand was made.

The egg spawn assessment was made by counting the number of cells within the quadrat that contained eggs per each substrate type (rather than estimating the percent of the substrate within the quadrat that was covered by eggs). This method appeared to provide a more useful metric compared to percent cover. Samples of eelgrass and *Gracilaria* sp. or just *Gracilaria* sp. (depending on elevation) were taken from the center of each quadrat. The samples were taken back to the lab, where all eggs were removed and counted wet and dry weighed.

The goal of these investigations, absent herring spawning in or near the restoration areas was to work out monitoring methods and to develop an idea of proportionality of spawning substrate use when multiple habitat elements are present in a spawning area.

- *San Rafael Bay (Marin Rod & Gun Club)*

During the past seven years, it was hoped that it would be possible to determine if herring used the eelgrass successfully restored at Marin Rod & Gun Club. However, herring did not spawn in the vicinity of the Marin Rod & Gun Club plantings in the winters of 2014–2015 through 2020–2022. As a result, no monitoring of herring spawning was conducted at this location.

- *Corte Madera Bay*

No spawning was recorded within Corte Madera Bay during the monitoring period, although spawning did occur on the headlands north of Paradise Cay and south of Corte Madera Bay during 2013–2014 and during 2015–2016. This headland is located within a half mile of the Corte Madera Bay transplant locations. However, restoration eelgrass was not present during the 2013–2014 spawning event and was not present at adequate levels during the 2015–2016 herring season to support spawning or spawning monitoring this site. Spawning monitoring in Corte Madera Bay was ceased when the restoration areas in this area were deemed lost.

- *Richardson Bay*

While 2015–2016 herring spawning was recorded in Richardson Bay near successful 2015 eelgrass plantings, despite low tide survey attempts on January 23, February 5 and 19, and March 9, 2016, due to rough conditions during a stormy winter it was not possible to visit the restored site at Richardson Bay. Subsequent to the spawning in 2015–2016, Ryan Bartling's published maps of the spawning suggested that spawning may have been near but did not likely overlap with the restoration areas. Spawning did occur in the area of Richardson Bay transplants during the 2016–2017 and the 2017–2018 seasons and during these periods, restored eelgrass was used as spawning substrate during these periods. Monitoring results for these periods are discussed later. Spawning in the restored eelgrass also occurred in 2020 and again in 2021, but no efforts were made to visit the eelgrass during these spawning events. In 2022, herring again spawned within restored eelgrass in Richardson Bay, but not within the San Rafael shoreline sites.

Substrate Spawning Results

In 2015, eelgrass at the spawning sites visited did not support any herring eggs. However, eggs were found covering rock, cobble, algae and sand. Table 5 shows the percent cover of each substrate type within each quadrat, and the percent cover of eggs on that substrate. There was more spawning evident near the EOSC-RTC highest percent cover of eggs, especially on vertical faces. The dry weight biomass of egg samples showed a similar pattern as the percent of substrate cover with rock egg collections being more dense (and thus of higher mass; average = 0.17g) than those from cobble (average mass = 0.03g), and algae (average mass = <0.01g) (Table 6).

Table 5. Field Assessment of the % Cover of Eggs on Various Substrates at Two Tidal Elevations at Two Sites in Tiburon in 2015

Site	Habitat type	Quadrat #	Substrate	% cover	% cover eggs on substrate	Algae types
EOSC Beach	Upper tidal zone	1	rock	100	5-25	
			algae	50-75	5-25	90% *Fucus*, 10% *Mastocarpus*
		1a	rock	100	80	
		2	rock	90	15	
			sand	10	0	
			algae	10	10	
		2a	rock	100	50	
		3	rock	100	5	
			algae	50	10	
		3a	rock	100	80	

Site	Habitat type	Quadrat #	Substrate	% cover	% cover eggs on substrate	Algae types
		4	rock	95	5-25	
			sand	5	<5	
			algae	40	5-25	
		4a	rock	100	75-100	
		5	rock	100	5-25	
		5	algae	50	25-50	95% *Fucus*, 5% short red algae
		5a	rock	100	75-100	
	Lower tidal zone	1	cobble	90	<5	
			sand/ gravel	10	<5	
			algae	5	<5	
		2	cobble	95	5	
			sand/ gravel	5	0	
			algae	5	<5	
		3	cobble	95	10	
			sand/ gravel	5	0	
		4	rocks	95	<5	
			sand	5	0	
			algae	<5	<5	50% *Ulva*, 50% short red
		5	rock	95	5-25	
			sand	5	<5	
			algae	20	<5	50% *Ulva*, 50% short red
Private dock	Upper tidal zone	1	rock	100	0	
			algae	50-75	0	70% *Endocladia*, 25% *Ulva*, 5 *Sargassum*
		2	rock	100	<5	
			algae	60	<5	90% *Fucus*, 10% *Ulva*
		4	rock	100	<5	
			algae	75-100	5-25	90% *Fucus*, 5% *Mastocarpus*, 5% *Ulva*
		3	rock	100	0	
			algae	25-May	0	80% *Endocladia*, 20% *Ulva*
		5	rock	100	0	
			algae	75-100	5-25	70% *Fucus*, 20% *Endocladia*, 5% *Ulva*, 5% *Mastocarpus*
		1	cobble	85	0	
			sand	5	0	

Site	Habitat type	Quadrat #	Substrate	% cover	% cover eggs on substrate	Algae types
	Lower tidal zone	2	cobble	95	0	
			sand	5	0	
			algae	10	<5	75% *Fucus*, 25% *Mastocarpus*
		3	cobble	60	<5	
			sand	40	0	
			algae	10	<5	50% *Mastocarpus*, 50% *Ulva*
		4	cobble	20	<5	
			sand	80	0	
			algae	10	<5	90% *Mastocarpus*, 10% *Ulva*
		5	cobble	100	0	50% *Mastocarpus*, 25% *Ulva*, 25% *Gracilaria*
			algae	5	<1	
		6	sand	25	0	
			algae	10	<1	80% *Mastocarpus*, 20% *Sargassum*
			cobble	75	<1	

An 'a' following a quadrat number indicates the quadrat was placed on a vertical face of a rock.

Table 6. Percent Cover of Substrate and Eggs in Quadrats at EOS and Paradise Park (January 2015)

Site	Substrate	Rep #	% total cover	% egg cover	egg wet weight	wet substrate wt (g)	dry egg wt. (g)	dry substrate wt. (g)
RTC Beach	Rock	1	95	75	1.5434		0.239	
	Rock	5	100	50	0.646		0.125	
	Rock	4	100	5 to 25	0.2409		0.044	
	Cobble	1	100	5	0.0869		0.013	
	Cobble	2	100	<5	0.1404		0.026	
	Cobble	3	100	1	0.04		0.012	
	Cobble	4	100	20	0.2128		0.03	
	Cobble	5	100	15	0.2242		0.089	
	Rock	2	100	40	0.8945		0.201	
	Rock	3	100	50 to75	1.3568		0.26	
	Fucus	1	100	25 to 50	1.4873	25.2	0.26382	7.36
	Fucus	2	100	10	1.2117	29.4	0.18089	8.125
	Fucus	3	50	25 to 50	1.127	16.6312	0.14271	4.364
	Fucus	4	50	25 to 50	2.7118	21.976	0.20487	4.747
	Fucus	5	90	10	1.4686	19.7681	0.44879	5.273
Paradise Park	*Fucus*	3	100	5 to 25	0.5922	32.6301	0.33429	9.07

In 2016, more cells of the quadrat containing *Gracilaria* sp. had eggs attached than those containing eelgrass (Table 7). This pattern also held true when comparing the biomass of eggs found on *Gracilaria* sp. vs. eelgrass samples (Table 8). On average at the Keller Beach high transect the dry egg biomass on *Gracilaria* sp. samples was 9.9% of the dry substrate weight (average dry egg mass = 0.09g), compared to 0.33% when looking at *Gracilaria* sp. in the deeper transect (average dry egg mass = 0.03g) and 0.21% on eelgrass from the deeper transect (average dry egg mass = <0.01g).

Table 7. January 2016 Assessment of the % Cover of Eelgrass, *Gracilaria* and Bare Sediment and the Number of Quadrat Squares Containing Substrate with Herring Eggs Attached, Keller Beach

		% cover			# of quadrat squares containing eggs (/25)			
Transect	**Quadrat #**	***Gracilaria***	**Eelgrass**	**Bare**	**Total**	***Gracilaria***	**Eelgrass**	**Sediment**
Gracilaria	1	60	0	40	25	25	NA	0
Gracilaria	2	30	0	70	24	24	NA	0
Gracilaria	3	20	0	80	25	25	NA	0
Gracilaria	4	45	0	55	5	5	NA	0
Gracilaria	5	40	0	60	23	23	NA	0
Grac/EG	1	5	10	85	9	8	4	0
Grac/EG	2	5	5	90	12	9	3	0
Grac/EG	3	10	<5	85	8	7	1	0
Grac/EG	4	5	10	85	4	1	2	1
Grac/EG	5	10	<5	85	7	5	2	0

On January 23, 2017, restored sites at Richardson Bay were visited and a few eelgrass leaf samples from two restored plots were collected to quantify spawning density. However, the plots were difficult to find, and the tide was not low enough to access the plants easily, so it was not possible to collect many replicates or to determine if the collection of samples truly reflected the overall horizontal and vertical distribution of spawning in the area. Egg laden shoots of eelgrass and *Gracilaria* sp. were taken to the lab for processing.

On January 26, 2017 a natural eelgrass bed at Keller Beach on the Richmond shoreline was also visited and a survey of herring egg cover on eelgrass and *Gracilaria* sp. was completed. Haphazard sampling of ten 0.25 m^2 quadrats was used and visual estimates of the percent cover of each substrate type and of herring egg coverage within the quadrates was made. A sample of whichever substrate was rooted in the center square of the quadrat was collected for processing in the lab where the size of the eelgrass shoot or *Gracilaria* sp. segment was made and egg count and biomass was determined.

Herring eggs were found on shoots from two of the restoration plots in Richardson Bay during the January 2017 sampling. Although not many shoots were collected, it was clear that the restoration area was being used for spawning at a level comparable to native eelgrass in the area at the time. It was also determined that the number of eggs on each shoot from the Richardson Bay restoration plots was comparable to the number of eggs per shoot from eelgrass at Keller Beach site (Table 9).

Survey at Keller Beach found that both *Gracilaria* sp. and eelgrass were used as spawning substrate (Table 10). It is difficult to compare the use of *Gracilaria* sp. and eelgrass since they have very different structures. However, *Gracilaria* sp. may provide more surface area for egg deposition per unit of substrate mass (Table 9).

Table 8. The Wet and Dry Weights of *Gracilaria* sp. and Eelgrass Samples and Any Adhering Eggs Taken from Transects at Two Depths at Keller Beach in January 2016

Quadrat	Substrate type	Wet egg wt. (g)	Number of eggs in sample	Wet substrate wt. (g)	Dry substrate wt. (g)	Dry egg wt. (g)	Egg:Substrate biomass (%)
1	*Gracilaria* (deep)	0	0	0.3263	0.1096	0	0
2		0	0	0.9622	0.072	0	0
3		No sample					
4		0.0022	1	0.5022	0.0739	0.0003	0.41
5		0.0323	15	3.2002	0.439	0.0040	0.91
1	Eelgrass (deep)	0.0062	3	3.4526	0.286	0.0004	0.14
2		0	0	0.7627	0.1205	0	0
3		No sample					
4		0.0036	2	0.7904	0.1278	0.0009	0.70
5		0	0	1.5807	0.2695	0	0
1	*Gracilaria* (shallow)	1.6977	602	12.1412	1.5695	0.1557	9.9
2		0.5421	207	5.827	0.9477	0.0583	6.15
3		0.3614	165	3.0362	0.3841	0.0346	9.01
4		1.0580	354	12.3616	1.6796	0.1156	6.88
5		0.5612	225	7.1895	0.8085	0.0615	7.61

2016-2017 Herring Spawning within Richardson Bay Eelgrass Restoration Areas

Table 9. January 23, 2017 Herring Egg Monitoring at Richardson Bay and Keller Beach. Some samples collected from Keller Beach had no eggs on them, and so were not included.

Site	Substrate	n	Mean substrate length	Mean egg count	Mean # eggs/ mass of dry substrate (mg)
Richardson Bay, unrooted wrack	*Z. marina*	3	72.3	10	0.044
Richardson Bay 2016 W 1/2 acre	*Z. marina*	1	33.5	23	0.056
	Gracilaria sp.	2	49.6	52	0.155
Richardson Bay 2016 E 1/2 acre	*Z. marina*	5	56.1	38.2	0.096
	Gracilaria sp.	1	42	14	0.636
Keller Beach	*Z. marina*	2	47.8	5	0.012
	Gracilaria sp.	7	36.8	21	0.022

Table 10. Herring Egg Survey at Keller Beach on January 26, 2017. Percent cover of each substrate type, and overall percent cover of herring eggs, were estimated visually within a 0.25 m^2 quadrat.

Quadrat	Percent Cover			
	Eelgrass	Gracilaria sp.	Bare Ground	Herring Eggs
1	20	10	65	5
2	10	10	65	15
3	30	10	55	5
4	15	15	65	5
5	20	15	65	5
6	0	25	70	5
7	0	50	55	5
8	0	40	60	3
9	0	10	90	7
10	0	60	40	10

2017-2018 Herring Spawning within Richardson Bay Eelgrass Restoration Areas

On January 17, 2018, three restoration plots in Richardson Bay were visited. These included the 2016 east half-acre plot, the 2016 west half-acre plot, and the 2015 west half-acre plot. Since sampling was conducted in the dark and water levels were high, it was not possible to use the quadrat methods developed for the program to assess percent coverage of spawning on plants. However, herring eggs were identified as present on most shoots, and 20 shoots were collected from each plot for further quantification of spawning biomass in the lab. Unfortunately, the next day it was noted that most of the eggs were no longer on the shoots, possibly because they had burst or were rubbed off during handling. Based on our observations from the previous day, it was determined that the few remaining eggs did not accurately reflect the numbers of eggs present at the time of collection, and so no density or biomass determinations were undertaken. A further decision was made not to collect additional shoots from the beds to protect the beds from additional sampling impact.

On February 1, 2018, Keller Beach on the Richmond shoreline was visited; ten eelgrass shoots were haphazardly collected. Later in the lab, eggs were counted on each shoot and total wet egg mass per shoot was determined. The shoots and eggs were then dried at 50 °C for 24 hours and weighed to determine dry shoot and egg masses.

In 2018, eggs were found on eelgrass shoots at all three restoration plots visited in Richardson Bay (2016 east half-acre, 2016 west half-acre, and 2015 west half-acre). As stated above, these plots were not further sampled, so exact egg count data are not shown. However, it was estimated that there were 10-50 eggs per shoot, based on visual assessments at the time of initial collection. This count is similar to what was found in 2017 in Richardson Bay and at Keller Beach during 2017.

On February 1, 2018, at Keller Beach, much higher egg counts were found on plants. During this period up to 284 eggs per eelgrass shoot were noted (Table 11). It is important to note that after a spawning event, egg abundance will drop over time due to predation and other factors, so it is not possible to standardize density counts absent knowing when eggs were deposited or what the attrition history at the site included between deposition and sampling. Because it was not possible to pinpoint exact spawning times, and because sampling must sometimes wait for suitable low tide windows, this makes it difficult to directly compare egg abundance between sites and years. However, it is possible to confidently conclude that the restoration plots at Richardson Bay are being used as herring spawning habitat.

Table 11. February 1, 2018 Herring Egg Monitoring at Keller Beach

Sample #	Eelgrass shoot length (cm)	Eelgrass dry wt. (mg)	Herring Egg Count	Egg count/mg dry eelgrass mass
1	87	861.3	68	0.079
2	77	599.7	59	0.098
3	77	966	166	0.172
4	66	649.1	278	0.428
5	84	695.8	29	0.042
6	79	499.5	52	0.104
7	65	462.4	11	0.024
8	86	920.6	284	0.308
9	61	799.9	159	0.199
10	92	822.4	242	0.294

CONCLUSIONS AND RECOMMENDATIONS

Transplantation for expansion of eelgrass in San Francisco Bay commenced in 2014 and continued through 2022. Transplantation was not conducted in 2017 due to high flood discharges from the Sacramento-San Joaquin River Delta that depressed salinity levels and led to significant declines in native as well as restoration beds throughout most of the North Bay. Anticipation of continued salinity depression into the spring months and the impact to donor beds made it prudent to defer a season of planting in 2017, while continuing to conduct monitoring of restoration areas. Transplanting was also not conducted in 2018 due to a lapse in contracting. Restoration activities were reinitiated in 2019 with good success; however, transplantation was again not conducted in 2020 due to restrictions enacted in association with the COVID-19 pandemic. Restoration activities resumed in 2021 and 2022.

Substantial environmental deviations from normal conditions occurred for three of the eight years of the DARP restoration program. Eelgrass was noted to decline baywide between 2009 and 2014 losing 917 acres of eelgrass present in 2009. This represents a 25 percent loss of eelgrass between benchmark survey years (M&A 2015). Recent partial surveys in Richardson Bay would suggest that even greater declines occurred between 2014 and spring of 2016 (M&A, unpublished data). During this period, San Francisco Bay experienced exceptional water temperatures ranging as high as 24 °C

(75 °F) during periods when planting was underway in 2015. Even higher temperatures may have been met at an unprecedented level in recent years. During this same period of time, tremendous declines in eelgrass have been noted in many other California systems with the declines being attributable to multiple different factors including warm water, El Nino sea level rise, disease, and elevated eutrophication, depending upon the specific system. Finally, in 2017 considerable flooding depressed salinities to the point of resulting in additional eelgrass losses. Only the 2016 and 2018–2022 seasons fell within conditions considered to be normal for the bay.

During this period, eelgrass surviving from prior planting years of 2014 weathered 2015 better than plantings performed during 2015, and plantings conducted in 2016 did very well overall. However, subsequent early 2017 impacts to plantings significantly reduced the conditions of transplant beds in San Rafael Bay. Notwithstanding the considerable climatic variability during the restoration period and the setbacks individual planting areas have suffered during the program, survival of plots overall has been mixed and on the whole productive. As monitoring for the different restoration regions has demonstrated, there has been variable performance through time with each area performing differently than the other as a result of differing exposures to various stressors. While Richardson Bay suffered considerably during the 2015 warm water period, deeper plots in San Rafael Bay suffered a lesser degree of loss. Conversely, plots in San Rafael Bay suffered considerable impacts during the 2017 floods, while the more marine influenced beds in Richardson Bay did not show similar injury.

Following the episodic flooding conditions of 2017, eelgrass had been on a recovering and expanding trajectory baywide through 2019; however, following 2019 there has been a decline in eelgrass beds with the principal reduction being within the beds at the margins of established habitats. Concurrent with the declines in spatial distribution, many of the cores of the eelgrass beds have been densifying over the period of 2020–2022. In 2022, a rebound of spatial distribution was also noted with the transplanted beds.

Overall, the areal extent of eelgrass cover has risen through time due to the DARP restoration program with the cumulative of the program reaching a maximum of 27,832 m^2 (6.88 acres) in October 2019, while the wide distribution of plantings has allowed the restoration program to achieve a peak spatial distribution of 166,549 m^2 (41.15 acres), also in October 2019 (Figure 29). By September 2020 eelgrass was noted to decline to a spatial distribution of only 109,107 m^2 (26.96 acres), only 65.5 percent of the 2019 peak extent from the restoration program. This decline in spatial distribution continued such that by October 2021 only 73,158 m^2 (18.08 acres) of restored eelgrass persisted. However, spatial distribution rebounded in 2022 rising by 74.9 percent to 127,989 m^2 (31.63 acres) in October 2022 (Figure 29).

Perhaps as important as the establishment of eelgrass under the DARP, has been the finding that the restored beds are contributing to herring spawning substrate when herring are spawning in the area. For all years during which herring have spawned in the restoration region of Richardson Bay and monitoring was conducted, herring have made use of restoration eelgrass as a spawning substrate. As such, the restoration of eelgrass in this area has contributed to supporting herring spawning.

The restoration program has demonstrated fundamental benefits of establishing eelgrass in multiple locations to buffer against site specific differences with respect to exposure and response to variable environmental stressors. It is worth continuing to seek restoration in differing locations to further reduce risk of substantial eelgrass loss due to variability in climatic conditions. To this end benefits garnered by spreading restoration into the Richardson Bay mooring scars at this have provided some

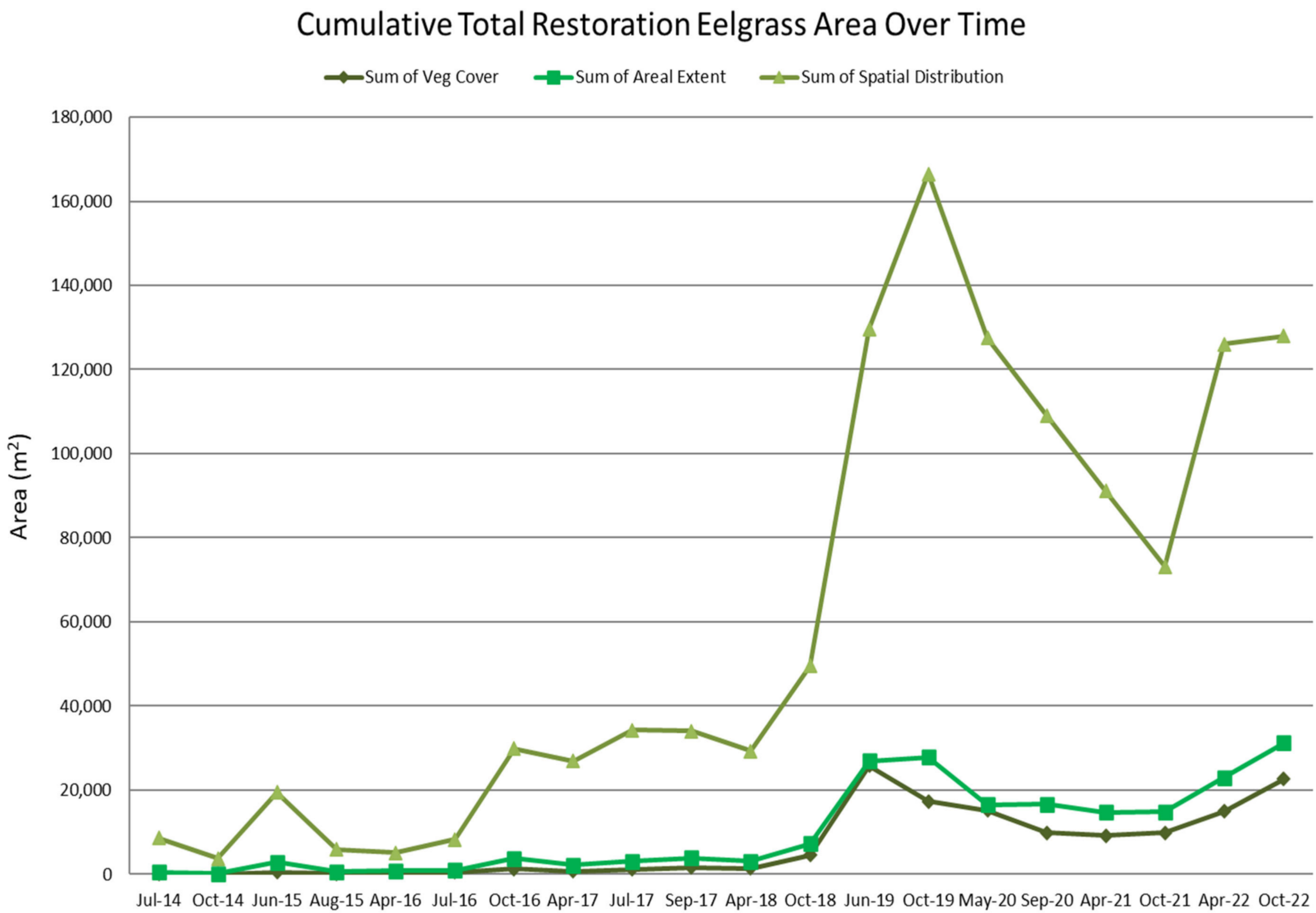


Figure 29. Cumulative Eelgrass Extent within Cosco Busan Transplants from 2014-2022

stabilizing benefits to the total eelgrass in Richardson Bay as the shallower margins have declined. This effort should be expanded while other planting areas in the bay should also be pursued.

It is worth noting the synergy of multiple eelgrass restoration projects underway in San Francisco Bay and how they may contribute cumulatively to the eelgrass within the system. In San Pablo Bay, eelgrass restoration has been conducted by the Coastal Conservancy at the San Francisco Living Shoreline site. At the Red Rock Warehouse Site, an area where creosote piling fields and debris were removed on the east side of the tip of the San Pablo Peninsula, was completed in 2019; and eelgrass restoration was also completed offshore of Giant Marsh in 2019 with additional plantings in 2021, work also managed by the Coastal Conservancy. Test plot plantings have been conducted at Dunphy Park in Sausalito within Richardson Bay as part of a living shoreline effort. Eelgrass restoration in the east bay has been initiated within the Middle Harbor Enhancement Area through funding provided by the Corps of Engineers and Port of Oakland. Test plots have also been installed at Pier 94 in South San Francisco by the Boyer Lab in 2020.

Finally, the SFOBB active eelgrass restoration effort that has been conducted in collaboration with the Cosco Busan DARP program has been funded by Caltrans and managed by NOAA Fisheries, West Coast Region. Collectively these various programs stand to add many acres of eelgrass to the Bay and would support the San Francisco Bay Subtidal Habitat Goals (California State Coastal Conservancy et al. 2010) effort to expand eelgrass resources in the bay. As important, these sites are distributed around the bay in a manner that further benefits overall eelgrass habitat stability.

When examining the Cosco Busan and SFOBB efforts together over the program period thus far, it is easy to see an overall contribution to eelgrass within the Bay. This includes establishment of eelgrass within new areas of shoreline along the San Rafael Bay shoreline. In total, the two programs have contributed considerably to the spatial distribution of eelgrass with a maximum of 304,826 m^2 (75.32 acres) being achieved in October 2019 with the total declining by October 2021 to a total of 131,918 m^2 (32.60 acres) with a rebound to 216,152 m^2 (53.41 acres) by October 2022 (Figure 30). As discussed previously, the later years' declines were generally the result of a pull back of sparse plants scattered widely beyond the core of the beds, while the bed cores have shown lesser decline.

While the combined results of the Cosco Busan DARP and SFOBB eelgrass restoration are extremely noteworthy, it is useful to note that either the Cosco Busan DARP or the SFOBB programs individually have yielded more restored eelgrass than all prior eelgrass restoration projects from central California to the California-Oregon border combined.

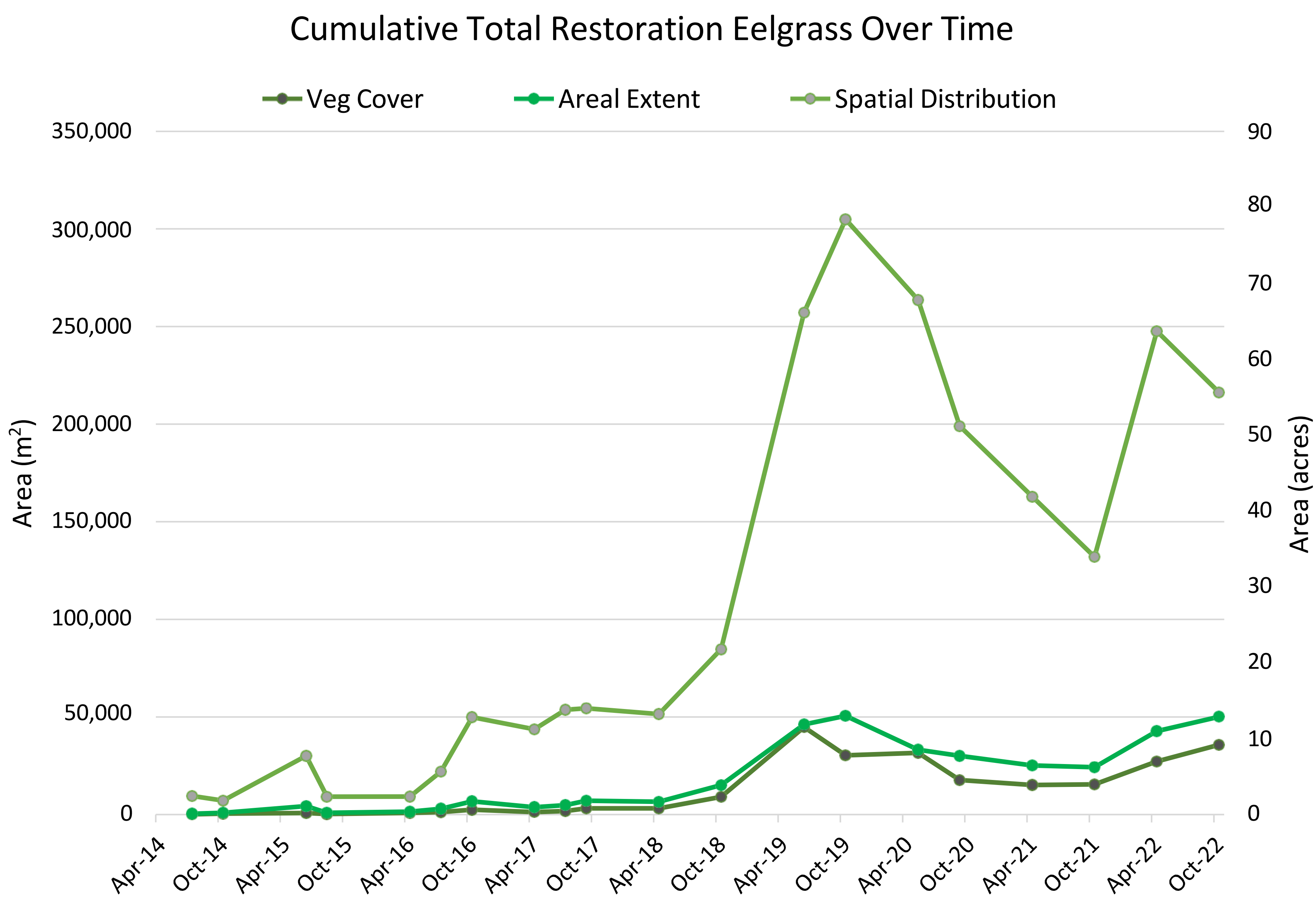


Figure 30. Cumulative Eelgrass Extent within Cosco Busan and SFOBB Program Transplants from 2014-Present

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