The Bay Foundation
Santa Monica Bay Subtidal Eelgrass Restoration Project

Restoration and Monitoring Plan

Background:

Eelgrass (*Zostera spp.*) is a marine flowering plant and an economically and ecologically valuable marine habitat found in temperate regions throughout the world. It provides rearing habitat for juvenile fishes (Tanner et al. 2019; Obaza et al. 2015), filters nutrients (Burkholder et al. 2007) and reduces erosion (Hansen and Reidenbach 2012) among myriad other functions. As eelgrass beds are typically found in close proximity to the shoreline, they are vulnerable to anthropogenic influences and the impacts of climate change.

A direct product of deleterious anthropogenic impacts, seagrasses are exposed to local stressors such as increased sedimentation, eutrophication, invasive species, and fragmentation (Sherman and DeBruyckere, 2018; Unsworth et al 2015), as well as global stressors including increasing dissolved CO_2 levels, increasing water temperatures, and sea level rise (Orth et al. 2006; Waycott et al 2009). More locally, California is also afflicted by critical eelgrass (*Zostera* spp.) habitat loss, which has been identified as one of the rarest state habitats (NOAA, 2014). In the Southern California Bight (SCB), human impacts associated with shoreline development, dredging and filling for harbor development, shading, and watershed inputs of sediment and nutrients have resulted in *Zostera* spp. habitat loss (Bernstein et al. 2011; NOAA 2014; Tanner et al. 2019; Obaza et al. 2015). Anthropogenic influence, compounded by global stressors have caused historic seagrass habitat loss globally (Waycott et al. 2009) and locally in the SCB (NOAA 2014). Yet, multi-billion dollar investments to address poor water quality in SMB have precipitated substantial ecosystem health enhancements, providing the opportunity to reestablish eelgrass beds in SMB. As the nearshore benthos of SMB is predominately soft bottom habitat, which is the requisite substrate for *Zostera* spp*.,* the expansion of eelgrass along the coast could be of regional significance. The multidisciplinary approach to this project, which integrates *Zostera* spp. genetics with biophysical oceanic research, will address coastal threats by establishing eelgrass habitat using various transplant methods and site characterizations, ultimately providing innumerable ecosystem services and benefits (NOAA 2014; Sherman and DeBruyckere 2018; Theyer et al. 1984).

Eelgrass beds are highly productive systems (NOAA 2014; Sherman and DeBruyckere, 2018), and the complex structure of seagrasses compared to unvegetated sediments greatly enhance biodiversity (Duffy 2006). The proposed eelgrass transplantation and restoration work associated with this project has the potential to exponentially increase fish biomass (Pondella et al. 2003), improve sediment and water quality (Huesemann et al. 2009; Theyer et al 1984), mitigate impacts of ocean acidification (Kapsenberg and Hofmann 2016; Fourqurean et al. 2012), ameliorate climate change through carbon sequestration (Duarte and Chiscano 1999; Duarte and Krause-Jensen 2017), and buffer against coastal erosion through sediment accretion and dissipation of near bottom wave energy (Hansen and

Reidenbach 2012; Duarte et al. 2013). These impacts will simultaneously provide valuable information to academics, resource managers, and the public stakeholders.

Past and ongoing restoration efforts for *Zostera* spp. that have employed various strategies to help mitigate such negative impacts on eelgrass habitat have been on-going in the Southern California Bight (SCB) for more than 30 years. Most of these efforts have focused on restoring *Z. marina* (common eelgrass) in bays and estuaries as mitigation following impacts from coastal development projects (i.e., Coastal Resources Management. Inc. 2007 and 2016; Merkel and Associates, Inc 2014; Obaza et al. 2015). Far less is known about open coast eelgrass along the mainland and the Channel Islands, with few noteworthy studies (Engle and Miller 2005; Santa Barbara Channelkeeper 2010; Obaza et al. 2019). The only known attempted open coast eelgrass transplant in California used *Z. pacifica* (Pacific eelgrass) on Anacapa Island and was proven successful (Altstatt 2003; Altstatt et al. 2014).

Presently, there remain only a handful of *Z. pacifica* beds within SMB, located exclusively on the western edge in Malibu. Yet, as the majority of the nearshore benthos of the SMB is soft bottom habitat, the expansion of eelgrass along the coast could be of regional significance, generating greater sustainability through habitat creation for nearshore fishes and related socioeconomic factors. The proposed work of this project to actively restore *Z. pacifica* is coupled with components that will further our ability to expand the knowledge of offshore eelgrass in the Southern California Bight (SCB), including monitoring of restored, donor and reference beds in the SCB, and development of methodological approaches most effective for offshore eelgrass transplants.

To this end, The Bay Foundation (TBF) and project partners have developed a strong methodological approach to enhance currently unvegetated subtidal habitat in SMB through *Z. pacifica* transplants. Although open-coast eelgrass restoration methods have not been comprehensively tested in the SCB, we have leveraged the extensive experience of both project partners and preeminent Submerged Aquatic Vegetation (SAV) researchers, who serve on our Technical Advisor Committee (TAC) for this project. The project will utilize successful methodological approaches of prior SCB eelgrass transplants. These methods were used for both *Z. pacifica* and *Z. marina* (primarily), and include Single Shoot, Bundle Shoot, and SOD methods (see Altstatt 2005; Zhou et al. 2014; Paulo et al. 2019 for methodologies). This proposed work aims to apply these approaches to pilot-project-scale *Z. pacifica* transplants, further informing the efficacy of future scalable eelgrass restoration in SMB and the SCB.

Project Location:

The Santa Monica Bay Subtidal Eelgrass Restoration Project intends to transplant *Z. pacifica* from donor beds on Catalina Island to three geographically distinct sites in SMB (Fig. 1).

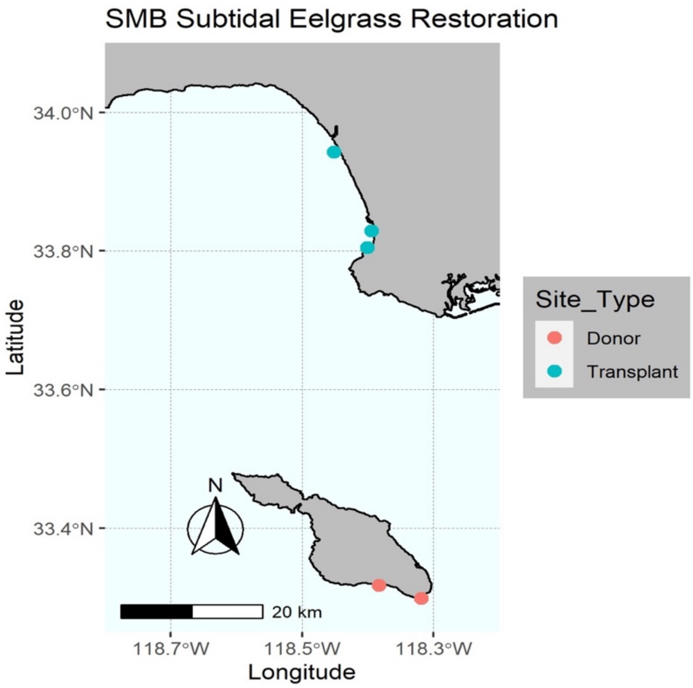

Figure 1. Project map displays locations of two donor sites in red (East End and Palisades) on Catalina Island and three transplant sites in blue (Dockweiler, Redondo Canyon, and Malaga Cove) in Santa Monica Bay.

The Santa Monica Bay Subtidal Eelgrass Restoration project intends to harvest *Z. pacifica* material for transplant from two donor beds on the backside of Catalina Island. Palisades and East End are the two beds that we intend to harvest material from due to the extant bed stability, size, high turion density, and selected depth range. Due to the sheer size of the donor beds, this project also proposes to utilize a separately distinct area of the two beds to serve as reference beds, which will be established in 2021 prior to transplant activities. Project partners have characterized Southern California Bight open coast eelgrass beds from 2018-2020, including the proposed donor beds. These extant *Z. pacifica* beds have remained persistent and stable from 2018-2020, with minimal changes observed since surveys conducted by Engle and Miller (2005). The overall size of both the Palisades (97 acres) and East End (21 acres) beds are much larger than what was mapped in 2020 (Obaza et al. 2019). Excluding the overall size and focusing on just the 2020 area (Fig. 2), Palisades has an estimated 753,566 turions and East End has an estimated 932,047 turions. This project aims to only harvest a total of 1500 turions (750 from each bed), a minimally invasive and negligibly impactful number (less than 0.01%) when considering the sheer size and density of the donor beds. The Scientific Collecting Permit issued by California Department of Fish and Wildlife that is required for this work is approved and in hand.

Figure 2. Depicts the location of both East End (Top) and Palisades (Bottom) donor beds in relation to Catalina Island. The green polygon represents a tiny subset of the total area of both beds. East End has a total area of 21 acres and Palisades has a total area of 97 acres from 2021 mapping effort. The area above was mapped by a single dive pair in one dive, swimming a polygon over an entirely continuous *Z. pacifica* patch in 2020.

Collection of *Z. pacifica* will occur at two sites, both on the south easterly side of Catalina Island. Transplants will occur at three sites within Santa Monica Bay. Specifics in harvesting and transplant methodology is detailed in a following section. Area of donor beds from mapping conducted by Vantuna Research Group in April 2021.

General Geographic Area	Site ID/ Site Name	Targeted Species	Site Type	# Turions	GPS Coordinates	Approx.. Area of Bed (m^2)
Santa Catalina Island, CA	SC-1 / Palisades	*Z. pacifica*	Donor	750	33.3177 -118.3835	> 392,000
Santa Catalina Island, CA	SC-2 / East End	*Z. pacifica*	Donor	750	33.2990 -118.3188	> 84,000
Los Angeles County, CA	LA -1 / Dockweiler	*Z. pacifica*	Transplant	500	33.9426 -118.4515	0
Los Angeles County, CA	LA -2 / Redondo Canyon	*Z. pacifica*	Transplant	500	33.83184 -118.39446	0
Los Angeles County, CA	LA -3 / Malaga Cove	*Z. pacifica*	Transplant	500	33.80980 -118.39783	0

No vessel mooring will be required for either harvesting at donor sites nor during activities at transplant sites. Temporary staging areas for eelgrass bundling stations will be set up at TBF lab space located at the Southern California Marine Institute (SCMI). These temporary bundling stations will be established to provide shading, aeration, and flowing seawater to eelgrass as it is bundled. SCMI facilities are located at 820 S Seaside Ave, San Pedro, CA 90731.

__Project Purpose and Performance Criteria:__

Eelgrass habitat restoration and conservation has been identified as a high priority by national, regional, and local agency planning documents, including the 2014 NOAA California Eelgrass Mitigation Policy and Implementing Guidelines, the 1976 Magnuson- Stevens Fishery Conservation and Management Act (MSA), the 2016 California Senate Bill 1363, the Ocean Protection Council's Ocean Acidification Management Tool (Nielsen et al. 2018), the 2018 SMBNEP's Comprehensive Conservation and Management Plan (CCMP) and the California Coastal Act section 30230, 30231 These documents consistently recognize the value of eelgrass as a direct contributor to improved ecosystem structure and function. Yet, the management of seagrasses, especially in remote and isolated areas (in which *Z. pacifica* is often found) has proven particularly challenging. Subtidal *Zostera* spp. beds exist throughout the SCB in shallow coastal wetlands, protected embayments, rocky coastlines and open coasts. Offshore expanses of open coast (deeper water) *Z. pacifica* beds are among the least understood subtidal habitats in the SCB highlighting difficulties in necessary management (Obaza et al. 2019; Engle and Miller 2005).

The dearth of *Z. pacifica* restoration activities and research in the literature is understandable, given its relative inaccessibility and less direct impacts from coastal development, as compared with *Z. marina*. However, the ecosystem services provided by seagrass coupled with ongoing losses (Duarte 2002), leave TBF biologists and project partners seeking to fill knowledge gaps. This proposed work encompasses an integrated, multi-habitat climate resiliency beach project currently funded by the California Coastal Commission (TBF 2020), which envisions a living shoreline from offshore eelgrass, to intertidal, foredune, and back dune restoration. As part of this project, educational signage will be posted along the coastal strand walkway and bike paths to display important components of the various habitats. Furthermore, TBF and project partners will conduct comprehensive project outreach to community groups, universities, schools, and public stakeholders through speaking engagements, newsletters, and social media platforms. These educational efforts are noteworthy as eelgrass habitats are among the least understood subtidal ecosystems in the SCB (Engle and Miller 2005) and have been identified as one of the rarest California State habitats (NOAA 2014). This lack of awareness and public interest must be addressed considering the highly productive and immense ecosystem services *Zostera* spp provide (Sherman and DeBruyckere 2018; Bernstein et al. 2011).

TBF biologists and project partners intend to answer questions regarding the optimization of pilot-project-scale *Z. pacifica* transplants in SMB in order to contribute to filling data gaps on open coast eelgrass by mapping and conducting biophysical characterizations of extant *Z. pacifica* beds. The innumerable benefits associated with successful eelgrass restoration will directly contribute to restoring water quality, reversing habitat degradation, and enhancing coastal resilience. The purpose of the Santa Monica Bay Eelgrass Subtidal Restoration Project is to coalesce the various methodological approaches of successful *Zostera* spp. transplants within the SCB and apply them in a scientifically defensible manner to conduct pilot restoration and monitoring of *Z. pacifica* in Santa Monica Bay.

Direct objectives from this work include: (1) Transplanting 306 m^2 (102 m^2 per site) of *Z. pacifica* to three geographically distinct unvegetated sites in SMB, while simultaneously advancing restoration methodology in order to address threats to coastal areas; (2) Characterizing biophysical oceanic conditions (wave characteristics) within extant *Z. pacifica* beds and restored beds to facilitate deeper understanding of potential drivers of restoration success; (3) Reviewing possible causative factors in potential success of rare *Z. pacifica* transplant; and (4) Strengthening partnerships that contribute to the ecological improvement and climate resiliency of SMB. TBF and project partners aim to act as a catalyst for beneficial ecological change, through implementation of these transplants, the outcome of the project will be a potentially significant enhancement in the structure and function of coastal habitat, informing the efficacy of scalable eelgrass restoration in SMB and the SCB.

Methods:

The Bay Foundation and project partners intend to transplant *Z. pacifica* from donor beds on Catalina Island to three geographically distinct sites in SMB. Each site will consist of seven pilot project scale transplant plots (3 replicates of both single shoot and bundle shoot and 1 sod plot), where a total of 102 m^2 of *Z. pacifica* will be transplanted per site. The goal of this project is to enhance currently unvegetated subtidal habitat in SMB by establishing *Z. pacifica* beds through *Z. pacifica* transplants.

Site Selection and Setup

Characterizing site parameters that predict transplant efficacy is vital to project success considering the lack of historic eelgrass in the proposed transplant areas. Therefore, TBF biologists and project partners have conducted prior research at both extant *Z. pacifica* beds and potential transplant sites, in a conscious effort to select transplant sites based on similar biophysical characteristics found in extant *Z. pacifica* beds. Light regimes are the most significant determinant for *Zostera* spp. growth and survival (Zimmerman et al. 1995; Ward et al. 2003; Short et al. 2002). Thus, light and temperature sensors were deployed within two extant *Z. pacifica* beds in Malibu and the Dockweiler transplant site. Light intensity (lum/ft^2) and temperature (°F) were measured for a month at the three sites, concluding that while the transplant site light values were slightly lower, they were within bounds of the extant *Z. pacifica* beds. Further, sediment composition was characterized at five sites (four extant *Z. pacifica* beds in Malibu and the Dockweiler transplant site), resulting in a similar sediment configuration between extant beds and the transplant site. Further, wave exposure is an important site-selection parameter for seagrass restoration, largely because wave energy, particularly in these offshore eelgrass beds, can break leaves and uproot plants which can reduce transplant success (Short et al. 2002, Kopp 1999, Fonseca et al. 1998). While an upper wave threshold for *Z. marina* has been established in prior site selection studies (Short et al. 2002), none exist for *Z. pacifica.* To understand the physical conditions in which extant *Z. pacifica* beds persist, in October 2020, TBF and project partners began characterizing wave conditions within two eelgrass beds off Catalina Island that we propose to use as donor and/or reference beds through the deployment of bottom-mounted wave sensors. These sensors will allow us to quantify the capacity for *Z. pacifica* to attenuate waves.

TBF and project partners plan to incorporate additional biophysical characterizations throughout the transplanting process through deployment of light and wave sensors at both donor and transplant sites. TBF will continue to do final site-surveys in Spring 2021 to document transplant site conditions in order to ensure appropriate eelgrass conditions exist.

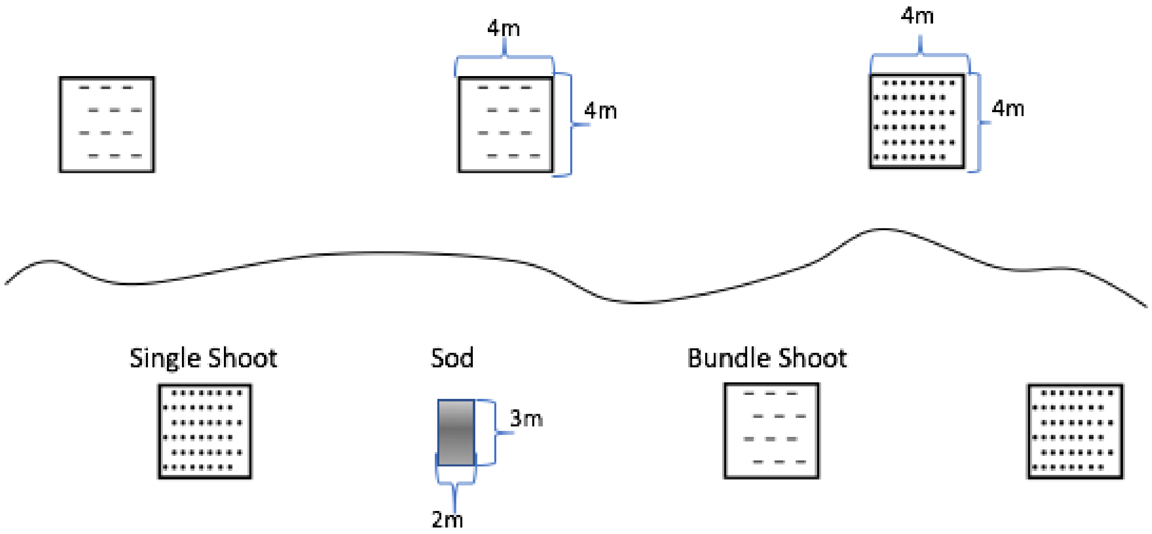

Figure 3. One eelgrass transplant site consisting of three replicates of two transplant methods (Single Shoot and Bundle Shoot) and one Sod plot, planted along a single depth strata of 35 fsw.

Once potential transplant areas have been identified and prior to collection and transplant activities, a team of divers will make a final assessment of the transplant area and establish the perimeters of each of three sites within the larger area to be planted. At a single transplant site, three unique methods may be used to transplant eelgrass in distinct plots, ensuring inter-site replication along a single depth strata for Single shoot and Bundle shoot methodologies (Fig. 3). Thus, the total project transplant area will consist of three distinct sites (Dockweiler, Redondo Canyon, Malaga Cove), each with seven plots (3 replicates of single shoot, 3 replicates of bundle shoot, and 1 sod). Transplant methods used will be determined during eelgrass collection based on the ease and success of the collection process (described in detail in next sections).

Each site will contain a baseline spanning the length of the planting site, in which planting plots are dispersed on +/- sides of the baseline. Planting plot locations will be determined through randomly generated meter marks and randomly generated assignment of methodology to those plots. To demarcate each plot, divers will deploy a buoy at one corner of each planting plot, using the same corner for each throughout the site for consistency. GPS coordinates will be taken at each temporarily deployed buoy so that the team can return to the location each day without needing to leave a buoy in place overnight. Divers will attach one end of a perimeter line (e.g., lead line or electrical cord, marked at 1-meter intervals) at the corner with the buoy. Divers will then run out the line alongshore to the length of the predetermined inshore edge of the plot for that transplant method (Fig. 3), following the depth contour, and recording a heading and distance once at the second corner. They will repeat the process to create the perimeter for each area, temporarily leaving perimeter line and markings during transplant activities. Removal will occur at culmination of the transplant, thus eliminating development. There will be a minimum of three meters buffer between each transplant plot. Once divers finish an area, they will deploy a meter tape and follow the depth contour to the next area to be established. Each buoy will be deployed on the same corner of each plot and a perimeter will be set up in the same direction (e.g., clockwise) so that plots are evenly spaced. This process will be repeated for each of the seven plots established at a site.

As dive teams set up each perimeter, they will carry a slate to record, (1) depth at each corner, (2) distance of each side, (3) heading from one corner to the next, and (4) any other relevant observations of the site (e.g., presence of eelgrass, hard substrate, other flora and fauna). Photos will be taken for pre-transplant visuals. Topside, TBF biologists and project partners will keep track of dive time and estimate how long it takes to set up each type of perimeter, so we can use these metrics in the future and for reporting purposes. Plots may or may not remain in place overnight depending on ocean conditions. It may be necessary to set up plots each day that transplanting will occur. Surface buoys will be removed when transplant activities are not underway.

Donor and Reference Beds

The Santa Monica Bay Subtidal Eelgrass Restoration project intends to harvest *Z. pacifica* material for transplant from two donor beds on the backside of Catalina Island (Palisades and East End). These two beds were chosen due to bed stability, size, high turion density, and selected depth range. The magnitude of these donors beds will allow a separately distinct area of the two beds to serve as reference beds, which will be established in 2021 prior to transplant activities. Project partners have characterized Southern California Bight open coast eelgrass beds from 2018-2020, including the proposed donor beds. These extant *Z. pacifica* beds have remained persistent and stable from 2018-2020, further emphasized by minimal change since surveys conducted by Engle and Miller (2005). The overall size of both the Palisades (97 acres) and East End (21 acres) beds are much larger than what was mapped in 2020 (Obaza et al. 2019) and can be observed in the mapping conducted by Vantuna Research Group in April 2021. Excluding the overall size and focusing on just the 2020 area (Fig. 2), Palisades has an estimated 753,566 turions and East End has an estimated 932,047 turions. This project aims to only harvest a total of 1500 turions (750 from each bed), a minimally invasive and negligibly impactful number (less than 0.01%) when considering the sheer size and density of the donor beds. In totality from both beds, an estimated 51 million turions are available, this project will harvest 0.003%. TBF and project partners will conduct pre-collection monitoring of donor and reference sites in order to assess conditions of the extant *Z. pacifica* beds. Pre-collection monitoring will be conducted within 1-month prior to collection and will incorporate structural surveys (length, width, and turion density), counts and size of fish observed along timed roving diver surveys, and map eelgrass areal coverage in order to confirm size and health of the donor bed. Exact GPS location of harvested material will be taken during pre-collection and collection activities.

Eelgrass Collection and Preparation

We plan to use up to three different Technical Advisory Committee (TAC) supported transplant methods that have been successful in other restoration projects. These include the single shoot, bundle shoot, and sod methods outlined below.

Single Shoot Method

Placing single eelgrass shoots with associated rhizome into novel substrate using an anchor (e.g. bent bailing wire) has been used in the Channel Islands (Altstatt 2003), Chesapeake Bay (Orth et al. 1999), South Korea (Park and Lee 2007) and West Australia (Paling et al. 2007). Of note, this approach has found some success in higher energy environments (but see Paulo et al 2019) and reduces the harvest requirement by using only a single turion as opposed to a bundle of 8-12 turions. Note that Paling et al. 2007 only had success using this method when transplanting eelgrass turions with sediment from the collection site and this project will attempt to use methods described here as closely as possible.

Bundle Shoot Method

This method involves grouping eight to twelve eelgrass turions along with rhizome into a single bundle secured with biodegradable twine and an anchor such as a wooden tongue depressor or rock (Zhou et al. 2014). It is one of the most commonly used eelgrass transplant approaches in southern California. However, these transplants almost always occur in lower energy bays and estuaries. No evidence was found in the literature for this method achieving success in high-energy environments, though it is an admittedly small sample (Paulo et al 2019). Collection effort may be greater to accommodate additional turions in a bundle but risk of single turions failing would be lower.

Sod Method

In open coast environments, the most common transplant approach in the literature is that of sods, where small sections of an eelgrass bed are completely removed along with associated sediment and placed at a transplant site. This approach is mechanically difficult, and in West Australia an underwater excavator was designed for this specific purpose (Paling et al. 2001). Given the volume of sods, only so many can be excavated but the greatest success was evident in cases where large collections of sods (11 m^2) were grouped together, though sizes of 6 m^2 also worked (Paulo et al. 2019).

Transplant material will be collected by SCUBA divers by hand from donor beds, using a thinning approach where no greater than 10% of turions are collected (we intend to harvest less than 0.01% of donor bed material). Field operations will be conducted from The Bay Foundation’s Research Vessel, Xenarcha, currently docked at the Southern California Marina Institute, San Pedro, CA. The amount of eelgrass required per method to plant one site is outlined in Table 1. A total of 102 m^2 of eelgrass will be planted per site, which will require the collection of approximately 500 eelgrass turions. In total across the three transplant sites, a total of 306 m^2 of eelgrass will be transplanted utilizing 1,500 turions.

Table 1. Summary of the estimated amount of eelgrass material required to plant one site broken down by plot area (3 replicates for Single and Bundle shoot method; 1 Sod plot), the number of eelgrass transplant units and total number of turions required per plot, and total number of turions required per method per site.

Planting Method	Plot Area (m^2)	# Transplant Units/Plot*	# Turions/Plot	# Turions/Site
Single Shoot	16	33	33	100
Bundle Shoot	16	15	120	360
Sod	6	3.6	40	40
			Total # Turions	**500**

** Number of transplant units/plot for shoot methods calculated as the total planting area divided by the planting density and for sod method as the total number of 0.3 x 0.3m sods required assuming ~11 turions per sod.*

For the single shoot method, divers will select shoots and gently maneuver the plant (shoot and rhizome/root mass) out of the sediment such that approximately 4 inches, or three intermodal segments, of rhizome is attached to each shoot (Altstatt 2003). Rhizomes with a single shoot will be selectively used for this method and placed in dedicated mesh bags as in Altstatt (2003). Eelgrass for the bundle shoot method will be collected similarly, though rhizome with several shoots attached will also be collected along with a small amount of sediment, and placed in dedicated mesh game bags and brought to the surface. Small sod pieces (~0.3 x 0.3m squares) will be very carefully excavated from a bed by hand or by using small gardening shovels. Divers will dig deep enough to collect intact rhizome/root mass (generally 5cm deep), reducing as much breakage as possible within the small sod piece, as well as a thin layer of sediment to maintain the structure of the sod piece. The entire piece will be lifted into a tray, weighted on the seafloor during collection. A harness and small lift bag will be used to slowly lift the tray to the surface. Each tray will contain one sod piece (0.3 m^2) so that shoots are not buried. For all methods, plants with flowers will be collected along with other material opportunistically but will neither be targeted nor avoided. Eelgrass will be harvested from donor beds in a systematic fashion, using a thinning approach where no greater than 10% of turions are collected from within a donor bed, and in such a way that no substantial bare patches are created, thus minimizing inter-bed fragmentation. The Palisades donor bed has an area of 7,302 m^2 and a turion density/m^2 of 103.2, thus a rough estimate yields 753,566 turions in the Palisades donor bed. Similarly, the East End donor bed has an area of 7,646 m^2 and a turion density/m^2 of 121.9, thus a rough estimate yields 932,047 turions in the East End donor bed. The Santa Monica Bay Subtidal Eelgrass Restoration Project aims to only harvest 750 turions from both sites, a negligible impact on *Z. pacifica* donor beds of this size and density.

Eelgrass will be stored on the boat deck for transport. Shoot material in game bags will be kept in 5-gallon buckets or similar holding tanks and replenished regularly with fresh, cool seawater. Sod will be kept in the original trays, carefully stacked, and replenished regularly with seawater. All eelgrass will be kept shaded during transport to SCMI. Time permitting, harvesting material and transplanting for single shoot and sod methods will be undertaken in the same day to minimize stress to plants. Unless immediately transplanted, mesh or burlap bags containing shoot material will be stored overnight in a flow-through seawater source (e.g., tied alongside the dock or in a nearby seawater tank in the SCMI/TBF aquaculture facility. Trays of sod will similarly be stored so that eelgrass is submerged, but in such a way that sediment loss is minimized. If sufficient time and personnel exist to start bundling eelgrass for the shoot bundle method upon arrival back at the dock, those tasks will be completed and resulting bundles placed in a mesh bag hung in a flow-through seawater system or over the side of a dock at SCMI. Timing of collection and transplant will be carried out such that no eelgrass is stored for longer than 24 hours.

On each day that eelgrass will be bundled using the bundle-shoot method, an eelgrass bundling station will be created at SCMI to provide shading, aeration, and flowing seawater to eelgrass as it is bundled. Mesh game bags or burlap bags with harvested eelgrass will be emptied into a flowing seawater system and eelgrass will be gently sifted out of the sediment and bundled. Each bundle will consist of approximately 8 turions (1 turion equates to a shoot with attached rhizome/root structure) held together by a pre-made biodegradable “anchor” composed of hemp string and a wooden tongue depressor. A loop in the hemp string will be gently tightened around the base of the group of shoots to hold them together for transplant. Finished bundles will be transferred to a jig intended to keep bundles from getting tangled during transport to the restoration site. Prepared bundles will be kept in flowing seawater and in the shade until ready to transport to the planting site.

Though we have developed collection and transplant protocols based on the use of three methods as outlined above, we may find that collection and transport of eelgrass, particularly using the sod method, is not feasible for this work. If so, we will collect enough eelgrass to use single and bundle shoot methods to meet our goal of planting 306 m^2 of eelgrass and 1500 turions.

Eelgrass Transplant

All planting activities will take place during June and July to provide adequate time for transplants to become established during the active growing season and prior to the storm season, beginning in the late fall. Increased storm activity may negatively impact newly transplanted eelgrass, as wave exposure can break leaves and uproot plants which can reduce transplant success (Short et al. 2002; Kopp 1999; Fonseca et al. 1998). Allowing ample time for transplanted eelgrass to establish will reduce negative impacts.

Material for the three transplant methods can be transported to the seafloor at the transplant sites in the reverse way they were brought up. Once on site, dive teams will work within one plot at a time to plant eelgrass, starting from one corner and working in a single direction towards the next corner/perimeter line and planting eelgrass in a grid pattern. Should visibility be reduced due to transplant activities and/or environmental conditions, divers will use a 1-meter PVC stick to maintain proper spacing of transplanted (particularly shoot method) eelgrass. Transplant strategies for each of the methods are described in detail in the following sections.

Single Shoot Method

Eelgrass will be planted using methods described in Altstatt, 2003. Divers will either use their hands or a gardening trowel to dig a small hole in the sediment. A single shoot will be taken from a game bag, the rhizome will be placed gently in the excavated hole, and a small “v” shaped piece of bailing wire will be pushed down over the rhizome to anchor it in the sediment. Excavated sediment will be pushed back over the rhizome/root mass so that the shoot is naturally situated above the sediment and the rhizomes are completely buried. Individual shoots will be planted at 0.5-meter intervals within each of three replicate, 16 m^2 (4 x 4m) plots at each site (Fig. 3). This method will use approximately 100 turions per site for a total of 300 turions total among all three sites.

Bundle Shoot Method

Two divers will descend with 1-2 jigs loaded with ~15 eelgrass bundles, each bundle consisting of ~8 turions. One diver in a buddy team will excavate a small hole, similar to that used in the single shoot method, while the second diver extracts a bundle from the jig and hands it to the first diver. The first diver will then place the entire root/rhizome mass into the excavated hole, take hold of the wooden tongue depressor, and gently wiggle it into the sediment so that it sits parallel to the seafloor, anchoring the bundle in the sediment. Approximately 15 bundles with 8 turions each will be planted at 1-meter spacing intervals within each of three replicate, 16 m^2 (4 x 4m) plots at each site (Fig. 3), similar to that of the single shoot method. Additional bundles remaining once the area has been planted will be used to fill in gaps or increase density within the plot. This method will use approximately 360 turions per site and 1,080 turions total among all three sites.

Sod Method

Each 0.3 x 0.3 m pieces will be placed at regular intervals within the 6 m^2 area, starting along the perimeter and working inwards to fill the center, within each of three transplant sites, and similar to Paulo et al (2019). However, planting the entire 6 m^2 space with sod as per Paulo et al (2019) would require a great deal of effort to harvest and thus, we do not intend to fill the entire plot but will place sods at regularly spaced intervals or closer together within that area to increase transplant density depending on site conditions. Trays containing sod pieces will be lowered to the plot by divers carefully using a lift bag. Sod pieces will be gently placed in similarly-sized holes that are slightly excavated to fit each piece so that the rhizomes are eventually buried in sediment and shoots float above the seafloor. Each 0.3 x 0.3 m sod piece will use an estimated 11 turions (~122 turions/m^2 were observed at the East End eelgrass bed at Catalina Island during 2020 monitoring of potential donor beds). Thus, each site will contain 3.6 sods and approximately 40 turions total, resulting in the potential transplant of up to ~120 turions among all three sites for the sod method.

Biophysical Characterization Methods

Biophysical characterizations of extant *Z. pacifica* and transplant sites will include light and wave sensors. Light sensors (HOBO loggers) will be attached to a sand anchor and deployed at depth inside the site to record light availability values. For wave characterizations, bottom-mounted wave sensors (Lyman et al. 2020) will be deployed within extant *Z. pacifica* beds for a period of two weeks per bed. Pressure record time series will be used to calculate significant wave height, dominant wave period, and wave energy within a given bed (see Lyman et al. 2020 for linear wave theory and methodology). Regional wave data from the nearest CDIP buoy (028, Santa Monica Bay) will be used to ensure the bottom-mounted wave sensor records capture a range of incident sea state conditions and to ensure records were not collected in an unusually calm or stormy time period. If the latter occurs, a two-week deployment will be repeated for that given bed. Characterization of local wave conditions within extant *Z. pacifica* beds will be used to inform site selection for this proposed restoration and could inform future *Z. pacifica* restoration efforts. To ensure wave conditions within potential transplant sites are comparable to those found within donor *Z. pacifica* beds, wave conditions will be characterized within each of the transplant sites prior to transplant activity. Following transplant efforts, waves will also be characterized within the restored beds. The experimental framework provides the opportunity to find quantitative change in wave conditions in novel deep subtidal vegetation.

Equipment

The proposed restoration project will have no impacts on public access to the shoreline, nor any negative impact to the subtidal area or to recreational opportunities.

TBF biologists and project partners will be assiduous in our effort to eliminate or considerably reduce the impact on non-target species and wildlife, and chosen methodology aimed to have negligible coastal development. To this end, our team possesses specialized training in harvesting, handling, and transplant techniques and procedures, distinctive *Zostera* spp. identification, and preeminent expertise in biological monitoring. To avoid incidental mortality to transplanted *Z. pacifica* associated with inclement oceanic conditions, all planting activities will take place during June and July to provide adequate time for transplants to become established during the active growing season and well before storm season, beginning in the late fall. Further, given the fine-scale, hand selective nature of this work, methodology utilizes no machinery for harvesting or transplanting eelgrass material, thus no bycatch of non-target species or intensive development is anticipated.

Throughout the harvesting of donor material and preparation for transplant material, no coastal development will occur, and no equipment material will be left on site. During transplantation, minimally invasive methods will be employed. Sand anchors that will be used to demarcate the site (and attached biophysical sensors) will be McMaster-Carr steel ground anchors. Further demarcation of transplant sites will utilize lead line or ridged electrical cord, both of which would be secured to sand anchors to prevent creating marine debris. During the single shoot transplant method, a metal anchor made of a piece of biodegradable "v-shaped" bailing wire will be pushed over each rhizome to secure the plant a technique used by Altstatt, 2003. The bundle shoot method involves grouping eight to twelve eelgrass turions along with rhizome into a single bundle secured with biodegradable twine and a biodegradable wooden tongue depressor anchor as in Zhou et al. 2014. The sod method will utilize a plastic tub to transport the donor material to the transplant site, but all equipment will be removed after transferring material to the sediment. In summation, this project will be in accordance with the Coastal Act, will have negligible marine debris on account of using biodegradable materials and best management practices which avoid the discharge of marine debris.

All personnel transportation to and from the restoration site will occur in the TBF-owned research vessel and anchoring will occur in safe and appropriate manners. During harvesting material from donor beds, TBF biologists and project partners will anchor the research vessel offsite in the sandy boundary area, as to further minimize harm associated with anchoring fragmentation within the extant *Z. pacifica* beds. Storage of transplant material will occur in the certified flow through aquaculture facility operated by TBF located at SCMI.

Monitoring Plan:

A rigorous, accurate, and robust scientific monitoring plan is an essential component of any restoration project and will allow for the evaluation of restoration activities; especially pertinent in a pilot-project scale methodology optimization project such as this. While we aim to achieve a long-term restoration goal of full areal coverage of eelgrass within transplant plots and eventually sites with densities approaching those of reference sites, this project is in its infancy and it may not experience such success. However, the methods and results developed in this pilot project may be leveraged to inform ongoing restoration efforts and can only serve to improve the effectiveness of the process over time. To this end, TBF and project partners are not only uniquely qualified to conduct this subtidal *Z. pacifica* transplant, we also are adeptly qualitied to conduct the bio-physical oceanographic monitoring proposed for this project.

Pre-restoration baseline monitoring will occur prior to the implementation of the restoration project to allow a comparison of the pre- and post-project conditions of the area. Ongoing implementation monitoring will occur throughout the duration of the restoration activities. Post-restoration monitoring will occur after restoration activities are concluded and will allow a scientific evaluation of not only transplant methodology and differences across sites, but also allow us the opportunity to evaluate restoration effort, culminating to best inform future scalable *Z. pacifica* restoration. When possible, additional data will be collected and partnerships with universities and other entities will be undertaken to supplement research efforts. Examples include partnerships with LMU Coastal Research Institute Professor Dr. Demian Willette for population genetics, as well as UCLA researchers studying eDNA and sediment sampling of the nearshore benthos. Results will be disseminated in public annual reports, scientific presentations and conferences, potential future manuscripts, and to local communities via presentations and social media.

More specifically, baseline monitoring will occur at the three transplant sites within SMB, as well as the two donor sites on Catalina Island. Baseline monitoring of transplant sites will quantify the biological conditions of the site using fixed UPC transect to assess substrate and confirm absence of eelgrass as well as enumerate and size fish observed along timed roving diver surveys. To assess the interplot survival, divers will enumerate the planting units per plot (single shoot/bundle shoot) and during subsequent monitoring will track the survival within each plot. In addition, characterizations of the physical conditions of transplant sites will be measured using bottom-mounted wave sensors, PAR (Photosynthetically Active Radiation) sensors will measure light levels, deployment of temperature loggers will occur, and baseline sediment levels will be recorded to monitoring any accretion/erosional forces at work within the sites. Further, pre-collection monitoring at donor sites will be conducted within 1-month prior to collection (per permit requirements) and will incorporate biological structural surveys (length, width, and turion density), counts and size of fish observed along timed roving diver surveys, and map eelgrass areal coverage in order to confirm size and health of the donor bed. Exact GPS location of harvested material will be taken during pre-collection and collection activities.

To document progress and persistence of eelgrass habitat at transplant sites, post-transplant monitoring will be conducted annually for a period of five years (12, 24, 36, 48, and 60 months per Scientific Collection Permit requirements) following restoration at both restoration and reference sites. Monitoring will, at minimum, determine the area and density of eelgrass. All monitoring events will be conducted during the typical growing season for eelgrass in southern California, March through October. Immediate post-transplant monitoring will occur more frequently to assess fine-scale changes and monitoring whether the transplant was established within the desired site. To better inform transplant evaluation, post monitoring will occur on the day of transplanting, 1-week, 2-week, 1-month and then quarterly for two years.

Individual Protocol Details:

In addition to areal coverage and densities and, we plan to collect additional eelgrass structural data including length and width of eelgrass blades, and to enumerate and size fish observed along three timed roving surveys per annum at transplant and reference beds in accordance with McCune et al. 2020 and Obaza et al. 2019. These methods have been used annually from 2018 to 2020 to assess offshore eelgrass beds at Catalina Island and along the southern California mainland. It is our goal to continue this work in support of this and future restoration activities and long-term monitoring of this habitat within the SCB. Depiction of mapping monitoring and biological structural monitoring is visualized in figure 5.

Mapping:

To collect eelgrass areal coverage, we plan to use a combination of side scan sonar for mapping larger donor beds (e.g., Palisades and East End), and a Trimble R1 GNSS receiver linked with a smartphone attached to a bodyboard to map smaller areas, such as transplant sites. Small-scale mapping will be accomplished by a buddy dive team outlining the eelgrass bed perimeter underwater while a swimmer follows this path with the Trimble R1. This receiver, enabled with real-time SBAS correction, provides sub-meter accuracy during mapping. Data are exported to the Trimble Terraflex cloud system for review and are available as shapefiles. Percent vegetated cover will be determined by dividing the vegetated area by the total transplant area initially planted.

Biological Structural Data:

Important *Zostera* spp. structural data include length of blades, width of blades, and turion density. Density data will be collected at all eelgrass transplant and reference beds by counting turions within a PVC quadrat (0.07 m^2) and scaled up to attain a turions/m^2 measurement. Densities at donor sites will be collected immediately prior to and following collection activities. Per McCune et al. 2020 and Obaza et al. 2019, sampling design is as follows for donor sites. *Z. pacifica* density will be collected by quadrant sampling where divers randomly place a PVC quadrat (0.07 meter squared, which is scaled up to attain turions/meter squared) and enumerate turions within the quadrant, making sure to assess a representative area of the transplant site (edge, interior, shallow, and deep area within the transplant site). Divers will also collect length and width measurements from each quadrant. Shoot height is the length from base to tip, of the longest leaf on a shoot per sampling quadrat. Shoot width is the length across the eelgrass leaf. For transplant sites, sampling will be augmented from McCune et al. 2020 and Obaza et al. 2019, and density quadrat placement will be randomly collected within planting plots, as opposed to site-wide haphazard quadrant sampling.

Fish Surveys:

Enumerating and sizing fish will be conducted by biologist using SCUBA based timed “roving diver” fish surveys during which they identify, count, and estimated total length in centimeters (cm) of fishes within a one meter cubed (m^3) box around the diver. Three roving diver surveys are completed in both middle

(1m of vegetation on either side of the diver) and edge (1m vegetated habitat one side of the diver, 1m vegetated habitat other side) in each bed, provided sufficient habitat is present. Visibility of 10ft or more are required to collect fish data.

Physical Oceanographic Characterizations:
To understand the physical conditions in which extant *Z. pacifica* beds persist, we will characterize wave conditions within two eelgrass beds off Catalina Island that we propose to use as donor and/or reference beds through deployment of bottom-mounted wave sensors. These deployments will continue to evaluate the wave conditions that exist within extant *Z. pacifica* beds.

Further, deployment of wave sensors in the presence and absence (pre-and post-transplant) of eelgrass provide a unique experimental framework to quantify the capacity of *Z. pacifica* to attenuate waves (via computing proportional change of wave energy flux over the length of the newly established beds; see Lyman et al. 2020 and Elsmore et al. *in Review* for wave theory and methodology). To this end, sensors will be deployed at transplant sites months prior to the commencement of transplant activities, to accurately gauge baseline conditions. Additionally, temperature and light sensors (HOBO loggers and PAR sensors) will record conditions within donor and transplant locations.

Pressure sensors (roughly the size of a can of tennis balls) will be affixed by a hose clamp to a single sand anchor (resembling a large screw that is turned into the substrate by hand by divers on SCUBA). TBF staff and project partners will additionally assess sediment accretion within transplant sites (utilizing markings on the exposed aspect of the sand anchor), allowing for an evaluation which will inform future restoration site selection.

Monitoring Design

Rigorous planning and incorporation of expert review from the project’s technical advisor committee, has yielded a scientifically defensible and robust monitoring scheme. Figure 4 displays the experimental design for each site. Site setup will contain a baseline which spans the length of the planting site, in which planting plots are dispersed on +/- sides of the baseline. Planting plot locations will be determined through randomly generated meter marks and randomly generated assignment of methodology. TBF and project partners will perform two fixed UPC transects, offset from the baseline in order to bisect the various replicates of planting plots located on the +/- side of the baseline. Within each plot biological structural data (turion density, shoot length and width) will be collected. As mentioned, fish will be enumerated and sized with site-wide roving diver fish counts. During site setup, a minimum distance of 3 meters will be allocated between planting plots, to assist with initial differentiation of planting plots.

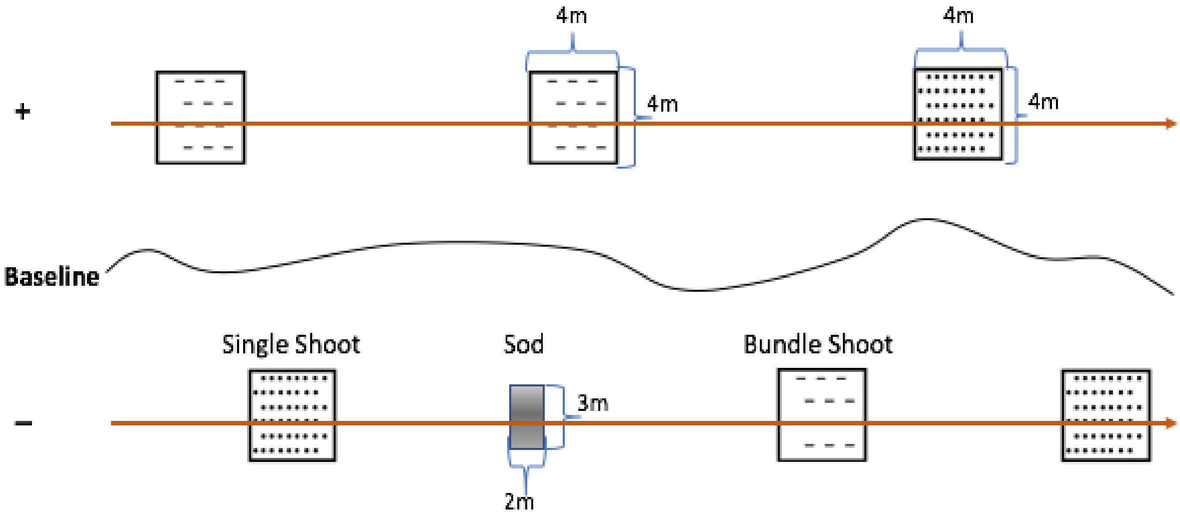

Figure 4. One eelgrass transplant site consisting of three replicates of two transplant methods (Single Shoot and Bundle Shoot) and one Sod plot, planted along a single depth strata. Two fixed UPC transects (one on the positive and negative side of the baseline) bisect planting plots.

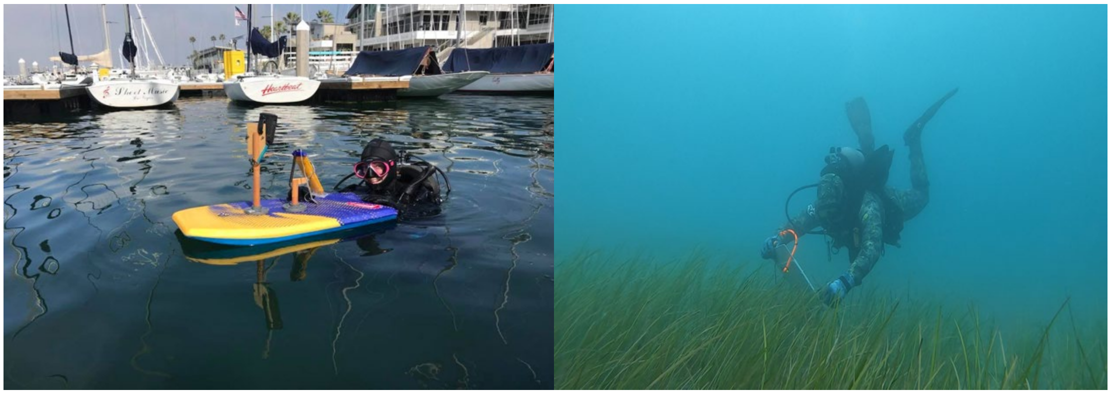

Figure 5. Photographs of monitoring protocols. Surface mapping utilizing Trimble R1 GNSS receiver linked with a smartphone attached to a bodyboard (Left). Diver taking biological structural data at extant *Zostera* bed (Right).

__Performance Thresholds and Contingency Plans:__

Past and ongoing restoration efforts for *Zostera* spp. have employed various strategies to help mitigate such negative impacts on eelgrass habitat in the Southern California Bight (SCB) for more than 30 years. Most of these efforts have focused on restoring *Z. marina* (common eelgrass) in bays and estuaries as mitigation following impacts from coastal development projects (i.e., Coastal Resources Management. Inc. 2007 and 2016; Merkel and Associates, Inc 2014; Obaza et al. 2015). Far less is known about open coast eelgrass along the mainland and the Channel Islands, with a few noteworthy studies (Engle and Miller 2005; Santa Barbara Channelkeeper 2010; Obaza et al. 2019). The only known attempted open coast eelgrass transplant in California used *Z. pacifica* (Pacific eelgrass) on Anacapa Island and was proven successful (Altstatt 2003; Altstatt et al. 2014).

In the Anacapa Island restoration case, researchers were returning *Z. pacifica* to an area that a previously established bed existed and determined that it took a few years to see growth from the transplanted material (Altsatt et al. 2014). Yet, The Santa Monica Bay Subtidal Eelgrass Restoration Project exists under different circumstances, as no prior beds exist within the transplant sites. Thus, the primary objective of this project is to fill data gaps related to open coast *Zostera* spp., specifically transplanting *Z. pacifica.* Achieving this goal will produce 4 direct outputs: (1) Transplanting 102 m^2 of *Z. pacifica* to three geographically distinct unvegetated sites in SMB, while simultaneously advancing restoration methodology in order to address threats to coastal areas; (2) Characterizing biophysical oceanic conditions (wave, light, sediment characteristics) within extant *Z. pacifica* beds and restored beds to facilitate deeper understanding of potential drivers of restoration success; (3) Reviewing possible causative factors in potential success of rare *Z. pacifica* transplant; and (4) Strengthening partnerships that contribute to the ecological improvement and climate resiliency of SMB.

The pilot project scale evaluation of the methodological approaches outlined above (single shoot, bundle shoot, and sod) will determine the efficacy of large-scale open coast *Z. pacifica* restoration in SMB and the SCB. A successful transplant will establish and expand in area over time to mimic *Z. pacifica* reference beds' turion density and blade length. Further, the bed's function as fish habitat will emulate extant *Z. pacifica* beds. While the hope is that the transplants eventually mimic extant reference beds, at this stage in the pilot project scale examination of novel transplant methods within the SMB, the establishment and survival of transplanted *Z. pacifica* will be considered a success. A multi-tiered success spectrum was established, with guidance and support from our TAC, to help aid in the evaluation process. Although there are various desirable outcomes, every level of tiered success produces valuable results (Fig 6). The top of the pyramid correlates with the most highly desirable outcome being an expanding and thriving transplant site, and cascades in desirability towards persistent patches in distinct planting plots, to only temporary successful establishment of *Z. pacifica*, to potentially unsuccessful establishment yet gaining essential knowledge of metrics that can inform future restoration. Evaluation of

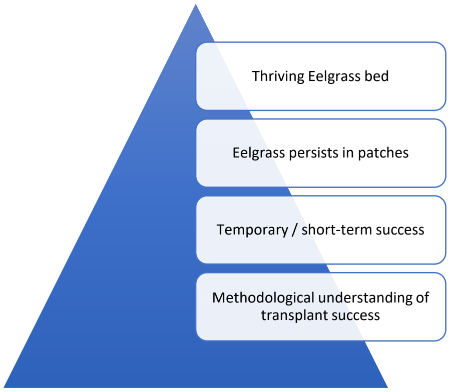

Figure 6. Success Spectrum. Highly desirable results from top to bottom, yet no invaluable outcomes.

methods, site selection, and biophysical data that will be collected throughout the project will culminate to advance the knowledge and opportunity to continue this work. Further, with limitations on collection from donor beds, an adaptive “second influx transplant” augmentation at a site experiencing loss is not planned, as it would additionally skew any evaluations of the three transplant methods.

In order to account for inclement weather windows or unforeseen setbacks, flexibility and contingency buffers have been built into the scheduling of harvest, preparation, and transplant activities, thus allowing TBF and project partners to eliminate delays in progress completion. Even with this contingency planning, it is of the utmost importance to transplant during the active growing window (June – July) for *Zostera* spp., which is supported by the preeminent seagrass researchers assembled for our TAC.

Biological Resource Information:

No sensitive biological resources are located within the transplant project area, nor is the project area adjacent to any marine reserves, critical habitat or protected areas. The project has no spatial conflicts with commercially or recreationally significant areas.

Relevant Qualification and Experience:

The Bay Foundation has extensive experience coordinating large scale projects involving diverse partners, implementing subtidal restoration/monitoring, and managing large federal, state and private grants to successfully achieve coastal and marine restoration goals (Grime et al. In Review; NMFS, 2015; TBF, 2020). Tom Ford will serve as Principal Investigator overseeing and advising all aspects of this project. Tom is the Director of the Santa Monica Bay National Estuary Program, Co-Executive Director of the Coastal Research Institute at Loyola Marymount University, and the CEO of The Bay Foundation. Tom has spent the past 20 years advancing science and community support to restore marine habitats off Los Angeles, CA. Tom, as well as the entirety of TBF biologists have extremely positive relationships with the project partners; having collaboratively worked on numerous projects including abalone restoration, offshore eelgrass surveys, and kelp forest wave attenuation studies.

Paua Marine Research Group (PMRG) is a woman-owned micro-business founded in 2016 to provide quality public and private sector marine and estuarine biological consulting services. PMRG excels in habitat mapping, subtidal biological surveys, species recovery, and community structure analysis. Examples of these projects include: a collaboration with University of Southern California to produce the first report on spatial distribution of eelgrass on Catalina Island (Obaza et al. 2019), co-authorship of a peer-reviewed study on non-native fouling species in Southern California’s bays and harbors (Obaza and Williams, 2018) and playing a central role in recovery of ESA-listed abalone (Neuman et al. 2018). In addition, PMRG has both assisted with and led several successful eelgrass mitigation and restoration projects in southern California bays and estuaries ranging in size from 3,000 ft^2 to 10,000 ft^2 (Obaza et al 2015). Though PMRG was created in 2016, both staff members worked with regulatory agencies, non-governmental organizations and academic institutions prior to forming the company, creating and maintaining many positive relationships. PMRG’s small size, scientific and field capability along with strong relationships with a variety of local institutions make them uniquely qualified for this open coast eelgrass restoration project.

Kristen Elsmore, PhD Candidate, University of California, Davis, will lead collection and analysis of wave data to characterize biophysical conditions within extant *Z. pacifica* beds and transplant sites. Kristen's research explores biophysical relationships across multiple axes, with emphasis on interactions among nearshore hydrodynamics and aquatic vegetation. She has experience leading hydrodynamic field studies in California's kelp forests, spanning Los Angeles, Monterey, and Mendocino counties. Additionally, she has forged collaborations across oceanographers, ecologists, restoration practitioners (including TBF), mechanical engineers, and electrical engineers to conduct her research and contribute to others'. Such work includes biophysical and biochemical interactions, with emphasis on waves (Lyman et al. 2020; Elsmore et al. In Review), currents (Elsmore et al. In Preparation), and water chemistry (Ricart et al. In Review; Jellison et al. In Review).

In addition to the collaborating partners above, to ensure and effectuate a scientifically defensible and compelling project, TBF has amalgamated preeminent California eelgrass specialists to establish a Technical Advisory Committee (TAC) to further inform and guide this project. This TAC has provided invaluable information informing site selection, experimental design and monitoring efforts, culminating in the support for the continuation of this work and the expansion of knowledge on *Z. pacifica* and open coast eelgrass in SMB and the SCB.

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