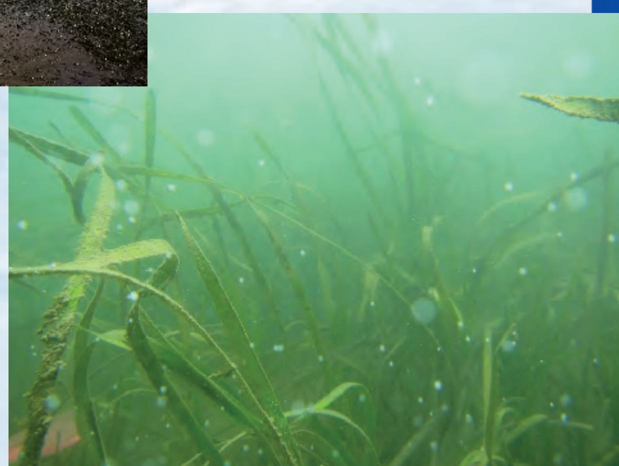
Methods and Guidance on Assessing the Ecological Functioning of Submerged Aquatic Vegetation in Southern California Estuaries and Embayments



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PREFACE

Submerged aquatic vegetation (SAV) is a conspicuous and ecologically important feature of the coastal zone of California. However, it is also a habitat for which we lack a standardized bioassessment framework. This document briefly describes a three-tiered framework we have developed for assessing the extent, health, and functioning of SAV beds. More importantly, the bulk of this report describes the methodology we have developed to conduct a survey of SAV beds to assess their ecological functioning (the 3rd tier of our framework). The goal of this document is to provide resource managers with conceptual material to help understand the development of our the recommended monitoring metrics and the types of data that will be produced, as well as methodology that can be applied in a variety of monitoring programs concerned with the ecological functions provided by SAV beds. The results of a pilot study are used to develop these methods are also presented to illustrate the types of outputs that can be produced.

ACKNOWLEDGEMENTS

This work represents an ongoing effort to establish a statewide standardized bioassessment protocol for an oft overlooked habitat within the State of California. As such, it has been the product of many contributors, who have helped gather information, provide time in the field collecting data, provide laboratory space to analyze samples, and improve the readability of this document. We thank student volunteers that contributed to field surveys as kayakers or divers from California State University Long Beach – Nick Da Silva, Chloe Van Grootheest, James Chhor, Juliana Vitagliano, Aaron Sugimoto, David Boehmer, and from California State University Fullerton – Mason Emery. We are indebted to the California State University Long Beach biology department staff and Dr. Christine Whitcraft for providing access to financial and laboratory resources for processing samples, equipment transportation, and general project advising. We would also like to thank Rick Ware for providing equipment and advisement used for environmental data collections.

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Executive Summary

Submerged aquatic vegetation (SAV) is an ecologically, economically, and societally important component of estuarine and coastal systems across southern California. These systems are host to a variety of SAV species, but eelgrass (*Zostera marina*) is the dominant species, forming expansive beds in shallow, soft-bottom sediments. Eelgrass comprises an important functional component of the mosaic of shallow subtidal and intertidal habitats, interspersed among emergent wetlands, biotic reefs, and expanses of mud and sand. Like many other “habitat engineering” flora and fauna, eelgrass has a dual nature, both as semi-permanent biological resources, whose condition can be indicative of ecosystem health and integrity, as well as a unique habitat that facilitates unique ecological functions that are absent from adjacent habitats in shallow coastal waters.

Despite its ecologically important and spatially prominent role in the coastal ecosystem, there is no standardized bioassessment framework for eelgrass, or any other species of SAV. Consequently, the health and functioning of a large part of the coastal ecosystem is unknown. There are ongoing efforts to monitor eelgrass in the region, but these efforts are only focused on mapping the extent of the seagrass, not evaluating its condition in sense of traditional bioassessment. Developing a monitoring and assessment approach that addresses both the resource and the habitat aspects of eelgrass poses a unique challenge that will differ from traditional bioassessment efforts. The goal of the present work was to begin the process of creating a unique bioassessment framework to evaluate the multiple roles of eelgrass in the coastal ecosystem.

This report covers three pieces of the process for developing bioassessment tools that evaluate the ecological functions of *Zostera marina* and is divided into three chapters. Chapter 1 provides a brief description of a three-tiered framework for assessment of all types of SAV, including eelgrass. Chapter 2 details the development of eelgrass-specific indicators selected to approximate priority ecological functions of eelgrass. Chapter 3 details our work in applying and refining methods for collecting the eelgrass indicators, including testing different methods for those indicators where there were multiple options.

A three-tier bioassessment framework was developed for eelgrass and the different other species of SAV. It was organized to provide an evaluation of 1) Presence and extent; 2) Waterbody condition, and 3) Ecological function. This assessment framework lays the groundwork for moving beyond the basic mapping and inventory of eelgrass that monitoring programs across the region are presently focused on. Critically, the different tiers of the framework speak to the dual nature of habitat engineering seagrasses, with extent and ecological function tiers addressing eelgrass as habitat for other organisms, while the ecological condition and ecological function address eelgrass as a biological resource.

To populate the third tier of the assessment framework (Ecological Function), nineteen different indicators of eelgrass function were identified and associated with the seven priority ecological functions. Standardized methods for data collection were identified for ten of the nineteen indicators, including measures of seagrass bed dimensions, robustness of the bed, and characteristics of the individual plants. For three of those ten indicators – shoot height, shoot density, and percent cover – there were multiple potential methods of collection, so each method was tested in a field study.

In order to evaluate the utility of the eelgrass indicators and to determine the best method for those indicators that had multiple options, eight eelgrass beds were surveyed in Newport Bay, California and San Diego Bay, California across the Summer, Fall, and Winter seasons. Eelgrass beds were selected to represent a nominal gradient in stressor exposure, represent a variety of bed sizes/typologies, and geographic locations. All indicators had large within-bed variability, but shoot density, percent cover, above ground biomass, and below ground biomass were the most responsive indicators to the nominal stressor gradient among the sample sites. Bed-to-bed patterns in the indicators were similar across seasons, with magnitude of the indicators being greatest in winter. A field-based method is recommended for estimating shoot density and percent cover, while a laboratory-based method is recommended for estimates of shoot height.

Recommendations

- Opportunities should be identified to begin applying the methods detailed in this report to eelgrass beds throughout southern California and other regions of the State in order to generate a larger, temporally and spatially diverse dataset of eelgrass indicator measures. Sampling efforts should be targeted to include eelgrass beds of different typologies from waterbodies in different geographies and with different levels of anthropogenic stressor exposure.
- Of the indicators with multiple potential methods, a field-based method is recommended for estimating shoot density and percent cover, while a laboratory-based method is recommended for estimates of shoot height
- Methods for measuring the indicators not tested in this study, especially the infauna and epifauna-related indicators, should be evaluated in the field.
- As more eelgrass indicator data are collected, they should be evaluated against direct measures of the different eelgrass ecological functions. Direct measures of nekton utilization would be most valuable to the management community, while measures of primary/secondary productivity would be most informative towards filling data gaps
- The tier 1 (habitat prediction and extent assessments) and tier 2 (water body condition) elements of the bioassessment framework should be developed to allow for a more fully realized, multi-faceted evaluation of these ecosystems.

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BACKGROUND

Submerged aquatic vegetation (SAV) is an ecologically, economically, and societally important component of estuarine and coastal systems across southern California, as well as the world (Nordlund et al. 2016; Dewsbury et al. 2016; Ruiz-Frau et al. 2017; Cullen-Unsworth et al. 2014). SAV plays an important role in the ecology of coastal systems, as it provides unique structure and enhancement of biogeochemical processes. The physical structure of SAV can function as temporary refuge from environmental threats, substratum as a permanent point of attachment, and a direct or indirect mechanism for food acquisition (Boström et al. 2006; Hemminga and Duarte 2000; Orth et al. 1984). Within many southern California estuarine environments, SAV forms expansive beds in shallow, soft-bottom sediments, comprising an important functional component of the mosaic of shallow subtidal and intertidal habitats, interspersed among emergent wetlands, biotic reefs, mudflats, and other intertidal habitats (e.g., Heck et al. 2008; Polis et al. 1997). Seagrasses, like many other "habitat engineering" flora and fauna (e.g., Wright and Jones 2006; Jones et al. 1994), have a dual nature, both as semi-permanent biological resources, whose condition can be indicative of ecosystem health and integrity, as well as a unique habitat that facilitates or enhances novel food webs and biogeochemical cycling that are absent from adjacent habitats in shallow coastal waters.

Constructing a monitoring framework that addresses both the resource and the habitat aspects of SAV, poses a unique challenge, which differs from traditional bioassessment efforts. Most traditional bioassessment tools focus on the organisms that reside in a system (e.g., benthic fauna, microalgae, nekton) as resources. The health of individuals or the composition of multi-taxa assemblages represent the condition of a waterbody and provide insights into its functioning. Seagrasses can be viewed from this perspective, where the size/biomass of the plants and the robustness of a given bed can be used to indicate waterbody condition (e.g.,García-Marín et al. 2013; Neto et al. 2013; Montefalcone 2009) and some ecosystem functions. However, only focusing on the health and condition of the SAV ignores the benefits it provides to other types of organisms (i.e., as critical habitat). Assessment of biological habitats tend to focus on measures of presence, extent, and complexity (e.g., MBC Applied Environmental Sciences 2017), which have inherent economic, societal, and ecological value. To date, the majority of SAV-based assessments in southern California have focused on it as habitat (Merkel and Associates 2011; Merkel and Associates 2014; Coastal Resource Management, Inc 2017).

Southern California's coastal embayments are host to a variety of SAV species, including *Ruppia maritima* (widgeon grass), *Zostera pacifica* (wide-leaved eelgrass), and *Zostera marina* (narrow-leaved eelgrass), but *Z. marina* is the dominant species present in these habitats (Green and Short 2003; Olsen et al. 2014)[1]. Given its dominance in the region's enclosed bays and estuaries, and high ecological value (Moore and Short 2006), most efforts at monitoring, restoration, and mitigation of SAV habitat in southern California coastal waters have focused on *Z. marina*, with more than 50 different eelgrass mitigation projects conducted in southern California over the last 30 years (NOAA National Marine Fisheries Service, 2014).

[1] Note that *Phyllospadix* (surfgrass) is also a SAV species found extensively in California but is largely absent from enclosed bays and estuaries.

Bernstein et al. (2011) assembled a panel of regulated, regulatory, environmental, and research organizations to develop a roadmap for parties interested in eelgrass to develop a regional-scale monitoring program. This expert panel was given the charge to identify what the goals of a monitoring program for eelgrass should be, summarize the status of monitoring efforts to date, and describe what further advances needed to be made in order to develop a regional monitoring program. This report identified a series of key management questions for an eelgrass monitoring program focused on mapping and extent measurements and produced a number of recommendations for realizing their goal of a monitoring program (Box 1). This report was important in kickstarting new rounds of eelgrass monitoring, but the focus of these efforts failed to consider the dual nature of all types of SAV, though the infrastructure they propose could be applied to all types of SAV monitoring.

Box 1. Key management questions and recommendation from Bernstein et al. 2011 "Recommendations for a Southern California Regional Eelgrass Monitoring Program" report

- Key Questions:
  - **Question 1**: What is the extent of eelgrass habitat and how is it changing over time?
  - **Question 2**: Where does potential eelgrass habitat exist and where is eelgrass vegetation currently not persistent?
  - **Question 3**: What is the condition of eelgrass habitat?
  - **Question 4**: What are the effects of [coastal modification] projects on regional eelgrass habitat?
  - **Question 5**: What are the significant stressors on eelgrass habitat and what are their effects?
- Recommendations:
  - **Survey methods**
    - Supplement aerial data in Morro Bay with sidescan sonar surveys in deeper water areas that are not well represented by current multispectral mapping methods
    - Standardize eelgrass bottom coverage categories across all programs
    - Adjust timing of individual surveys to concentrate on the late summer – early fall time period
    - Develop protocols for integrating survey methodologies for maximized efficiency (e.g., blending aerial photography with sidescan sonar surveys)
  - **Data management**
    - Create eelgrass webpage as part of the Wetlands data portal on the California Water Quality Monitoring Council's "My Water Quality" website
    - Load maps of current eelgrass extent into the eelgrass webpage
    - Complete revisions to the project tracking form to capture data appropriate to the five management questions
    - Develop data upload protocols for loading project tracking and routine survey data into the eelgrass webpage
  - **Filling key data gaps**
    - Make provisions for surveys in eelgrass habitat that has not been surveyed
    - Make provisions for collecting bathymetric data as a part of routine surveys
    - Collect and organize currently available bathymetric data
  - **Program management**
    - Empanel a more permanent regional workgroup to manage program implementation and regional assessments
    - Investigate the costs and benefits of including regional eelgrass surveys as a part of the Southern California Bight Program
    - Make necessary changes to regional environmental stewardship programs and regulatory structures to facilitate funding and implementation of the regional program

Most *Z. marina* monitoring programs in southern California focus on the seagrass as a habitat; monitoring the location and extent of the eelgrass beds across the region (e.g., Coastal Resources Management, Inc 2017; Merkel and Associates 2014; Merkel and Associates 2011). Under this type of assessment framework, the primary concern is where and how much of the resource there is across the region, as well as the how those values are changing through time. Underpinning this approach is the implicit assumption that the presence of the beds implies that they are providing the ecological functions they should. There has been some testing of this assumption in small-scale studies (e.g. Potouroglou et al. 2017; Boström et al. 2014; Hansen and Reidenbach 2012; McGlathery et al. 2012; Hovel 2003; Attrill et al. 2000) but there has been little work scaling patterns up to a regional or statewide monitoring program.

Despite the variety of ecological roles it serves in the coastal ocean and the high value it has to those who use the ecosystem, there is no robust framework for monitoring and assessing the resource and habitat function (or condition) aspects of SAV in southern California. To that end, we developed a framework for assessing SAV structure and function in the region (Gillett et al. 2018a). We have proposed a three-tiered assessment approach that focuses on SAV Extent, SAV Condition/Health, and SAV Ecological Function to better capture the multiple aspects of SAV meadows (i.e., a living natural resource and a biologically-based habitat for other flora and fauna). The three elements or tiers of the framework can be seen to operate in a sequence of ecological completeness (*sensu* Haines-Young and Potschin 2009; Vlachopoulou et al. 2013) for the habitat:

1. If the landscape is ecologically suitable, is SAV present?;
2. If present, what is the condition of the SAV bed (health, structural integrity, etc.) and the waterbody where it is located?; and
3. Given the condition of the bed, how well is it functioning in the habitat mosaic of the coastal zone?

We have developed our assessment framework to be general enough that it could be applied to any type of SAV. However, the component pieces of framework (i.e., the actual measurements collected in the field) and the benchmarks used to interpret the data (i.e., assign high, moderate, and low values) will vary among species of SAV. Furthermore, it is likely that assessment components and benchmarks will also vary for a single species growing in different environments (e.g., intertidal vs. estuary vs. open coast) and different geographic regions (e.g., northern California vs. southern California). Despite the potential for variation, we believe that concepts of the framework – assessing extent, health, and then function – are flexible enough to be applied across the different species or habitats. Given this flexibility, even if assessment tools are populated with different species-specific or habitat-specific metrics and thresholds, it will allow for consistent interpretation of SAV systems within a given region or across the state.

Structure of the Report

Given the breadth of material associated with these first forays into developing bioassessment tools for evaluating the ecological functions of *Zostera marina*, this report is divided into three chapters. Chapter 1 provides a brief description of the three-tiered framework for assessment of all types of SAV, including eelgrass. Chapter 2 details the development of eelgrass-specific indicators identified by our Technical Advisory Committee and selected to approximate priority ecological functions of this species of SAV. Chapter 3 details our work in applying and refining methods for collecting the eelgrass indicators and testing different methods for those indicators where there were multiple options. A detailed, step-by-step set of standard operating procedures for collecting the indicators detailed will be provided in a separate report (McCune et al. in prep).

CHAPTER 1. THREE-TIERED FRAMEWORK

As noted above, it is our vison that the assessment framework should be applicable across different species of SAV and different environments, but that the components of each tier are most likely species-specific in their construction and interpretation. The three tiers of the framework are meant to be implemented sequentially, building upon the information from the previous tier while simultaneously increasing the ecological meaning of the results and drawing closer to the beneficial uses they are meant to represent.

Between development and practical implementation of an assessment framework like this, there are a variety of incremental steps that need to be completed. Furthermore, within a multi-tier approach like we have put forth, these steps will need to be addressed for each tier, though hopefully there will be some degree of overlap given the related nature of the tiers. The first step is identifying what are biotic and abiotic measures that will serve as the underpinnings for the assessment. The second step is standardizing the methods for collecting the measurements, evaluating, among other things, reproducibility, cost, and degree of technical specialization needed to collect the measurements. The third step is developing an approach to interpreting the data; identifying the best ways to combine and scale different types of data, as well as understanding their response to disturbance. The last step is developing thresholds or benchmarks that allow the patterns in the data to be placed into a context that is relevant to the management and valuation of the ecosystem the framework applies to.

Tier 1 – Extent

The first tier of the proposed assessment framework is designed to address the questions of "where should SAV beds be present in coastal waters of southern California, based on physiological limitations in the absence of anthropogenic disturbance?", and "Is any SAV present in these suitable habitats?". This tier has three primary components: 1) Identifying the natural, abiotic characteristics that affect SAV distribution – its theoretical niche (e.g., Hutchinson 1959); 2) Mapping that niche space across southern California; and 3) Determining the presence or absence of SAV in those locations.

Tier 2 – Ecological Condition

The second tier of the proposed assessment framework is designed to address the questions of "How healthy is the SAV bed?" and "What is the ecological integrity of the waterbody in which the bed is found?" There are a variety of assessment tools available to evaluate the condition of unvegetated parts of southern California's embayments and coastal ocean (e.g., Pelletier et al. 2018; Ranasinghe et al. 2009; Smith et al. 2001), but there is no formal approach for the SAV beds in these waterbodies (Bay et al. 2014). As such, this tier of the framework will focus on evaluating the integrity of the bed as a whole and evaluate if the local environmental conditions are supportive of plant growth and persistence. There has been reasonable amount of research in this area, most frequently using the presence/extent of SAV bed growth as an assessment of eutrophication impacts in a waterbody (e.g., Corbett et al. 2005; Kraus-Jensen et al. 2005; Dennison et al. 1993). The pre-existing work in the literature will provide a good knowledge base for this part of our framework, however, there are only limited examples (mostly from Europe) where these patterns have been codified into a proper assessment tool (García-Marín et al. 2013; Neto et al. 2013; Montefalcone 2009).

Tier 3 – Ecological Function

The third tier of the proposed assessment framework is designed to address the question, "Are SAV beds providing the ecosystem functions they would be expected to?". This tier of the framework focuses on the extrinsic aspects of SAV beds; emphasizing how they are part of the mosaic of habitats in the coastal landscape and how they contribute to a healthy and fully functioning coastal ecosystem (e.g., Ruiz-Frau et al. 2017; Dewsbury et al. 2016; Nordlund et al. 2016; Cullen-Unsworth et al. 2014). Whereas tier 2 is focused around using structural aspects of SAV beds to infer the health and condition of their host waterbody, tier 3 is explicitly focused on evaluating if an SAV bed – natural or created – is providing the ecological functions it should.

The presence and rate of a habitat's functions (e.g., productivity, hydrological buffering, biogeochemical cycling) speaks to the most holistic and direct assessment of anthropogenic impacts to a system (Strong et al. 2015; Cortina et al. 2006). Most studies covering ecosystem functions of SAV beds provide direct estimates of a function(s) through relatively intensive, laboratory or local-scale measurements that provide insight into the magnitude of a function or how it may change under different abiotic or biotic scenarios (e.g., Lamb et al. 2017; Potouroglou et al. 2017; Thorhaug et al. 2017; Zarnoch et al. 2017). Much of this function-centric work is, however, not conducive to application in a large-scale monitoring program. As such, the present work has been focused on developing the pieces of this tier (identifying key functions and easily measurable proxies for the functions) and honing the best methods to collect these data in a fashion that would be more amenable to incorporation into regular monitoring programs.

This tier of the assessment framework consists of a series of assessment tools designed to evaluate the expression – and possibly magnitude/rate of flux – of different ecological functions in a given SAV bed. These tools are focused on a suite of ecological functions determined to be of primary importance to local management agencies and experts in SAV ecology (Table 1). Given the impracticality of directly measuring all of the ecosystem functions described in Table 1 at a regional scale, we have developed a series of SAV structural metrics (e.g., shoot density, above ground biomass, plant C:N ratio) that are representative of function, responsive to stressor exposure, and should be relatively easy to incorporate into a regional monitoring program.

Assessment Framework Conclusions

- We have developed a three-tiered bioassessment framework, the concepts of which can be applied to different species of SAV.
- This assessment framework lays the groundwork for moving beyond the basic mapping and inventory of SAV that monitoring programs across the region are presently focused on.
- The different tiers of the framework speak to the dual nature of habitat engineering organisms like SAV. The extent and ecological function (in part) tiers address SAV as habitat for other organisms, while the ecological condition and ecological function (in part) tiers address SAV as a biological resource with its own inherent value.

Table 1. Priority list of ecosystem functions that SAV beds are known to provide, as determined by SAV ecological experts and resource managers from across southern California. These functions are the focal point of Tier 3 assessment tools.

Function	Definition
Substrate Stabilization	Stabilization of soft bottomed sediments within and adjacent to SAV beds by sediment/organic matter retention and wave attenuation
Carbon Sequestration	Uptake and long-term retention of carbon
Water Quality Improvement	Enhancement of local water quality by a variety of mechanisms, including uptake of nutrients, settlement of sediment particles, production of oxygen, and increases in pH due to photosynthesis
Primary Production	Increased diversity and rates of primary production related to the above and below ground structural complexity of SAV beds
Secondary Production	Increased productivity of infauna and epifauna due to higher structural complexity and organic matter production in SAV beds
Fish Habitat Provision	Enhanced survival and greater food availability for all life stages of fish and other nekton within and adjacent to SAV beds
Waterfowl Habitat Provision	High productivity of SAV estuarine habitat make attractive feeding grounds for many species of waterfowl

CHAPTER 2. DEVELOPING AND MEASURING EELGRASS-SPECIFIC INDICATORS

In developing the SAV assessment framework, our goal was to create an approach to bioassessment whose concepts could be applied to nearly all marine and estuarine species of seagrass. Though the technical approach for each tier can be applied to different species of SAV, we recognize that the component pieces of those tiers may vary between species. Consequently, the transition from framework to working tools will most likely have occur independently from species to species. Eelgrass (*Zostera marina*) is the most common estuarine species of SAV in southern California (Green and Short 2003; Olsen et al. 2014) and therefore, it was the most logical species to begin the development of tools for. Though the indicators and methods of collection detailed in the rest of this document were conceptualized around eelgrass, the overall process could be easily applied to other species of SAV in the future.

Identifying indicators of ecological function

Based upon an extensive review of the primary and secondary literature (Gillett et al. 2018b), a variety of different eelgrass indicators were identified that could be used to estimate the presence and magnitude of the prioritized ecological functions. In conjunction with our Technical Advisory Committee (TAC) (Table 2), we evaluated those indicator-function relationships based upon practicality of collection (e.g., cost, effort), redundancy among indicators, sensitivity to anthropogenic stress, and orthogonality to natural environmental gradients. Based upon these evaluations, we settled upon a suite of measurements that all parties felt should be considered for inclusion in a regular, regional scale monitoring framework and can provide the information necessary to assess our targeted SAV functions (Table 3). This group of indicators included measures of SAV faunal communities (infauna, epifauna, nekton), SAV biomass, leaf area, shoot height, shoot density, and other whole meadow or patch scale measures (e.g., patch area to perimeter ratio).

Table 2. Members of our eelgrass Technical Advisory Committee and their respective affiliations

Name	Affiliation
Bryant Chesney	NOAA National Marine Fisheries Service
Betty Fetscher	San Diego Regional Water Board
Brian Hentschel	San Diego State University
Kevin A. Hovel	San Diego State University
Karina Johnston	The Bay Foundation
Chad Loflen	San Diego Regional Water Board
Rick Ware	Coastal Resources Management, Inc.
Christine Whitcraft	California State University, Long Beach

Table 3. A matrix illustrating the links of the SAV indicators (vertical axis) to prioritized ecological functions (horizontal axis) for an idealized SAV ecological function monitoring program. The color and the text at the intersections describe the strength of the linkage between indicator and the function as determined by the Technical Advisory Committee, with empty cells indicating no anticipated linkage. Green = a high strength relationship, yellow = medium strength, and red = low strength. An asterisk designates indicators for which were able to develop and apply methods in the field during this study.

	Substrate stabilization	Carbon Seqestration	Primary Production	Secondary Production	Improving Water Quality	Nekton Habitat	Waterfowl Habitat
* Above ground biomass		Medium	High	High	Medium		
Above ground Carbon and Nitrogen content			Medium	Medium			
* Below ground biomass	Medium	Medium	Medium	Medium			
Below ground Carbon and Nitrogen content			Low				
* Patch area	High	Medium					High
* Area to perimeter ratio						Medium	
* Percent cover					Low	High	
* Shoot density	High		High	High	High	High	
* Leaves per shoot			High	High			
Flowering shoot density			High				
* Shoot height	Medium			High	High	High	High
* Leaf area	Medium		High		High	High	
* Epiphyte biomass			High	High			
Redox potential discontinuity (RPD) depth		High					
Infauna diversity				Medium		Medium	
Infauna biomass				High			
Epifauna diversity				Medium		Medium	
Epifauna biomass				High			
Contaminant content of blades					Medium		

Within the scope of the present work, it was determined that we could not fully develop a methodology measuring each of the recommended indicators due to financial and technical constraints of the current study. As such, we worked with the TAC to prioritize a subset of indicators for which methods could be developed to maximize the available resources while still maximizing the number of ecological functions we could realistically address during our field work (* in Table 2). For the remaining indicators, we have provided descriptions of how one would theoretically measure them based upon our initial literature research and practical experience of other researchers we have interacted with. A brief description of each indicator follows:

Measuring Indicators

Among the variety of primary literature sources reviewed during this study, most sampling methods were based upon the review of Short and Coles (2001). For many indicators, Short and Coles (2001) detail more than one way to collect the appropriate data. To help narrow the focus and create a more standardized methodology, we interacted with the TAC to select the most reasonable methods that could be used within the framework of a large-scale, regularly occurring monitoring program. For some indicators, the TAC was unable to come to consensus on a single sampling method (i.e., some metrics could be assessed in quadrat field surveys and within core collections). For those multiple option indicators, we evaluated the different measurement options as part of our field program (Chapter 3).

The proposed sampling methodology is designed around SCUBA diver-based surveys that include visual observations, SAV tissue collections, sediment collections, and faunal collections. A sampling program to collect bioassessment data for SAV beds can be broken down in to four primary parts: 1) Site Selection and Reconnoitering; 2) Mapping Activities; 3) Field Measurements; and 4) Laboratory Measurements. Detailed step-by-step standard operating procedures, based upon our practical field experiences with each of these parts and collecting each indicator are available in McCune et al. (in prep).

Embayment/Site Selection - Reconnaissance

Site selection and reconnaissance is an important step in preparing for the sampling of previously unvisited sites. The goals of the recon should be to 1) Identifying access points to the bed; 2) Determining access permission, as needed; 3) Determining the presence of SAV; 4) Identifying the size and nature of the bed (see Distinguishing SAV Types and Respective Monitoring Methods for information about bed size and type details); and 5) Identifying potential hazards to divers or physical obstructions. These types of information should be used in modifying a survey's sample frame to classify locations as target sampleable (target species present and accessible), target non-sampleable (target species present, but not accessible), or non-target (target species not present).

Access points and permission – Most SAV beds can be accessed from the shore or from the water. Direct access from the shoreline to a bed may be limited by hardening structures (sea walls, docks, rip rap) or by an inability to traverse privately owned property. Conversely, access from the water via boat may be limited by water depth and similar navigational hazards. Public boat ramps, beaches or docks make convenient access points, so thorough scouting of a potential area in person and via satellite imagery is encouraged. For those areas not near a public access point, attempt to contact the owner of an adjacent shoreline to negotiate potential access to the SAV bed.

Presence and extent of SAV - Any proposed sampling should involve a preliminary literature (grey and white literature) search to obtain any information detailing the type and extent of SAV in the location. These sources can be used to highlight specific sites within an embayment where SAV would be expected to occur. Given the ephemeral nature of many SAV beds, more than one prior mapping surveys of SAV at a site can be informative in the initial search areas for commonly colonized SAV sediments. Much of the spatial information about SAV in southern California embayments will be found in management agency reports (see Sherman and

Debruyckere 2018 for a recent review). A large inventory of interactive SAV maps can also be found on https://www.ecoatlas.org/. If no prior data is available, verbal communication with a harbor or land manager might be sufficient to gather information about expected SAV presence.

Identification of hazards – Diver safety should always be paramount during sampling. As such, during the recon of a potential site(s) identification of potential hazards should be rigorously noted. Proximity of SAV beds to high traffic navigation channels, boat moorings, and fishing piers are important structural hazards to note. Diving or kayaking within boating channels may require special permitting as high traffic of overhead boats can be a hazard. Additionally, proximity to stormwater or other types of discharge pipes should be noted as they can be sources of contaminated water during rainfall events, which may in turn limit when divers should be in contact with the water. Communication with the appropriate harbor patrol and coast guard authorities is essential for safety during sampling events.

Mapping – Bed Perimeter and Area Measurements

After recon is completed, the initial underwater mapping of SAV beds by SCUBA divers and a kayaker can proceed. Two divers towing a floatable GPS unit or teamed with a kayaker with GPS unit on board are an efficient means of mapping shallow (< 5 m) SAV beds in low boat traffic areas. During mapping and perimeter measurement, divers should note locations of deepest and shallowest points along the bed for positioning of the sampling transects. Divers should carry extra surface markers to demarcate anomalous features of the bed – large holes in the center of the bed, small outlying patches, debris fields, algal blooms – can be noted in spatial data.

Deeper water beds, those in high boat traffic areas, or very large meadows may be impractical for diver-based measures of bed perimeter and area. Boat surveys with side-scan sonar or aerial photography may provide a more effective means of mapping. In the present study, the focus was on diver surveys for mapping, but it is possible that recent mapping survey efforts at a location could be used to derive bed perimeter and area if need be. We suggest having mapping data within the same season and year as the field surveys to minimize potential fluctuations in SAV extent between mapping and bioassessment exercises

Patch Area:Perimeter

Patch area:perimeter ratio is the area of the SAV patch (or of the designated sample area in those cases where the entire patch cannot be surveyed for safety or practicality reasons) divided by its perimeter. Area:perimeter is used to inform the nekton habitat function. Divers mark a starting point with a surface buoy and follow the edge of the SAV bed while active GPS tracking marks points along the diver's swim paths. If a surface kayaker is used, the kayaker will follow the bubbles of the divers. Patch perimeter is estimated from the GPS spatial data loaded into a GIS using a function like Calculate Geometry in ArcMaps (v 10.6). The area:perimeter ratio is calculated by dividing the patch area estimate by the patch perimeter estimate

Patch Area

Patch area is the total area of SAV patch or designated sample area in those cases where the entire patch cannot be surveyed for safety or practicality reasons. Patch area is used to inform the substrate stabilization, carbon sequestration, and waterfowl habitat functions. Patch area is estimated from the spatial data recorded during perimeter measurement using a function like Calculate Geometry in ArcMaps (v 10.6). With some smaller beds, it may be difficult to get accurate spatial data with the GPS. In those instances, divers should use transect tape and tent stakes to measure perimeter and estimate area (length x width).

Transect and Quadrat placement

The recommended approach for sampling design utilizes a mixture of edge and interior, shallow and deep sampling within SAV beds, with most of the sampling occurring over a middle transect laid over the longest portion of the bed (parallel to shore). Observational data and quadrat samples are taken at five evenly spaced locations along the transect (zero point, left-middle, middle, right-middle, and transect end point). Core-based measurements are taken at three of these points (Figure 1). Additional quadrats and sample collections are taken at the shallowest and deepest portion of the bed – divers should consult mapping efforts to reference shallow and deep locations (Figure 1).

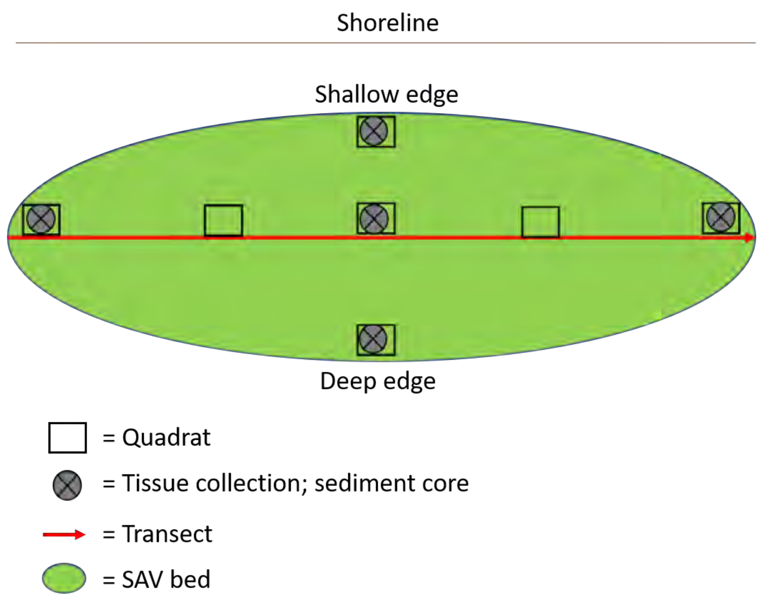


Figure 1. Recommended sampling design within SAV beds, using a transect tape (red arrow-line) across the middle section and selection of sampling points at deepest and shallowest locations within the bed. Quadrat sample locations indicated by black hollow boxes and tissue collections/core locations indicated by grey "X" filled circles.

Placement of transects, the number of transects, and number of sampling points (edge vs. interior locations; deep vs. shallow locations) can be adjusted to reflect a project's goals or the spatial peculiarities of a given SAV bed. A regular monitoring program focused on large-scale surveys of ecological function should entail at least five samples per site with sample locations spread evenly throughout edge, interior, deep, and shallow locations within a bed. More specialized monitoring programs centered on specific locations could deviate from this approach and use greater replication. SAV beds are heterogenous environments, so collecting replicate samples across the bed, capturing data from interiors and edges, is vital to draw meaningful conclusions about it. An edge measurement is defined here as any measurement where area directly adjacent to quadrat in any direction has zero percent cover of SAV. Edge portions of SAV beds can contain younger and less dense varieties of SAV in comparison to interior bed locations having older more dense vegetation (Moore and Hovel 2010; Tanner 2005). Sample and transect placement should also consider sample quantity and location based on prior recon – avoid sampling in areas of SAV beds impacted by changes in microtopography or small-scale impacts such as large holes or trash that do not affect the SAV bed as a whole.

Field Measurements

The observational field measurements and collection of SAV material represent the bulk of indicators needed to assess the ecological functions of SAV beds, all of which are most easily surveyed by divers using SCUBA. The observational measurements (e.g., shoot density, % cover, canopy height) should be conducted concurrently with the core/tissue collections (used for above/below ground biomass, infauna measures, etc.). The observational measurements are based upon samples collected along the transects with quadrats by two divers. Each Diver should collect independent measurements to control for measurement error and potentially difficult underwater conditions. If not noted during site recon and mapping, the presence and scale of anthropogenic disturbances (e.g., prop/anchor scarring, trash/debris, or stormwater pipes) should be noted while collecting field measurements.

During each sampling event, all participants in the field (divers and kayakers) should use a pre-dive check list including weather conditions prior to diving (> 72 hours after last rain), surf conditions, tidal height in compare to bed depth ranges (a minimum of +2 ft depth at shallow bed edge is needed). Snorkeling may be an option for some sites but is not recommended due to the need for close underwater inspection of quadrats during shoot density assessment in what are typically low visibility conditions. Our recommended field methodology has been constructed under the assumption of using high tide diver surveys while SAV beds are completely submerged.

Shoot Density

Shoot density is an abundance per quadrat measure of the number of vegetative eelgrass shoots growing in an area. Shoot density is used as an indicator for nekton habitat, primary production, secondary production and substrate stabilization. The number of vertical protrusions of SAV from the sediment-water interface is counted visually and by touch. In low visibility conditions or high-density areas, it can be easy to overestimate shoot density by divers counting blades rather than shoots. A slidable wire or string attached on both sides of the quadrat can help divers to keep track of counted vs. uncounted shoots by folding counted shoots under one side of the line. Alternatively, shoot density can also be measured from material brought back to the laboratory, as detailed below.

Flowering Shoot Density

Flowering shoot density is an abundance per quadrat measure of eelgrass shoots with reproductive structures. Flowering shoot density is used to inform the primary production function. Measurements are collected in a fashion similar to shoot density, where the number of reproductive protrusions from the sediment-water interface are counted visually and by touch. The presence of flowering shoots will vary throughout the year, in part dependent upon water temperature. In southern California, flowers would be expected in late Spring through the

Summer. However, certain genotypes only produce flowering shoot intermittently and do not produce flowers every year. *Flowering shoot density was not measured as part of our present study*.

Percent Cover

Percent cover is the percentage of SAV vegetative material relative to the total survey area. Percent cover is used to inform the improving water quality and nekton habitat functions. Percent cover is visually estimated in the field from a consistent angle – ideally from a top down view – at each quadrat. It is recommended that all divers should bench mark their cover estimates to standardized images depicting the gradient of cover (0-100%) for the species of interest (See McKenzie et al. 2003). It is important for surveyors to assess percent cover taking into account environmental conditions (surge, visibility), paying particular attention to the base of SAV shoots rather than tips of longer shoots than can fold down onto the base of a quadrat and create a perception of higher percent cover – i.e., vegetation from outside the transect can float into view and bias estimates of percent cover toward higher values. It is suggested that two independent replicates (one by each diver) are collected per quadrat.

Alternatively, photographs or digital images can be collected of each quadrat to assess percent cover. Images should be collected at a standardized distance calibrated in conjunction with the camera in the lab in order to insure as close a distance as possible while ensuring the full quadrat is in view. These images can then be analyzed by personnel in the less stressful setting of the laboratory or used in comparison to field estimates for quality control purposes.

Shoot Height

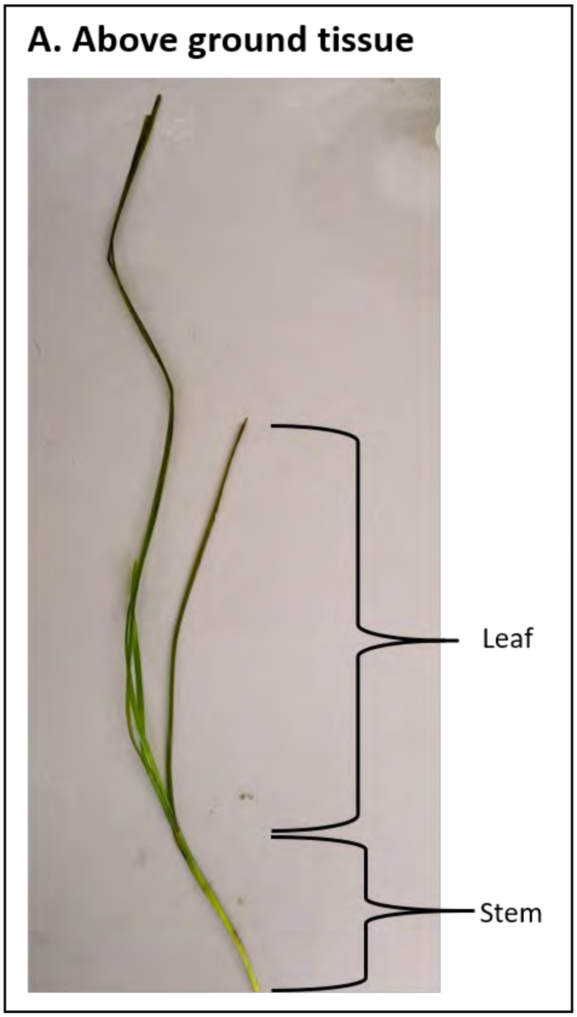
Shoot height is the average length from base to tip, of the longest leaf on a shoot for a subset of shoot per sampling quadrat. Shoot height is an indicator of the nekton habitat, secondary production, substrate stabilization, waterfowl habitat and water quality functions. Each diver should randomly select a handful of shoots within the quadrat area to measure. Shoot height is measured with a measuring tape extending from the sediment surface (at the base of the shoot being measured) and extending to the tip of the longest leaf of a given shoot. The tallest 20% of shoots should be excluded to avoid overestimating bed-scale height and thereby ensuring a more accurate estimate of overall canopy height for the entire bed. Alternatively, shoot height can also be measured from material brought back to the laboratory, as detailed below.

Laboratory Measurements

During field measurements, divers also collect material to measure indicators in the laboratory. Sealable sediment cores and sealable plastic bags are used to collect below ground and above ground material, respectively. Cores should be taken down to at least 10 cm in depth to effectively capture tissue and cores should have a sharpened end that will separate roots when force is applied on the core by samplers. Eelgrass material is to be collected at the five quadrat locations noted in Figure 1. To collect material:

- Place the quadrat along the transect and measuring all quadrat-based indicators.
- Identify the position within the quadrat where the core will be placed (e.g., upper right corner, center, lower left corner). The core can be used to indent the sediment surface to distinguish the sample area.

- Place the plastic bag over the eelgrass shoots within the corer circumference and then snapping or cutting shoots above the developing rhizome nodule at the base of the stem (easily felt by hand) (Figure 2).
- Quickly seal the bag to prevent loss of material.
- Insert the core 15 cm into the sediment and seal the top.
- Remove the core from the sediment and quickly seal the bottom to prevent loss of material.
- An additional core for infauna should be collected in a similar manner from a different location within or adjacent to the quadrat.
- All material should be placed in a dark, cold environment as soon as it has been collected.



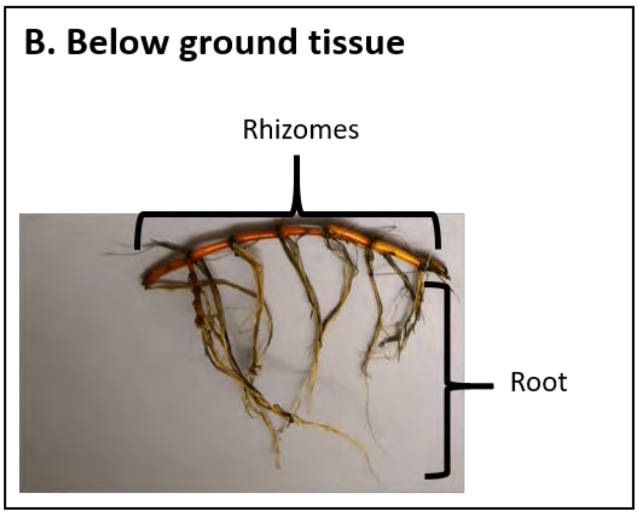


Figure 2. Image of a single eelgrass (Zostera marina) shoot and below ground tissue taken with recommended sampling protocols. A.) Above ground shoot structures of multiple leaves branching from multiple points above a rounded stem. B.) Below ground tissue is present as a section of root (severed by sediment core edge) with attached rhizomes.

Leaves Per Shoot

Leaves per shoot is an average number of individual leaves on each shoot collected with the above ground material. The number of leaves per shoot informs the primary production and secondary production functions. Leaves are counted on each shoot by separating the leaf tissue from the stem above the tip of the sheath and carefully examining the length of each leaf for smaller leaves – smaller, newer leaf growths can be hidden until separated from the stem.

Above Ground Biomass, Carbon, and Nitrogen Content

Above ground biomass is an area weighted measure of the dry weight of all the vegetative SAV material located above the sediment water interface (i.e., stem to leaf tip, see Figure 2). Above ground biomass informs the carbon sequestration, primary production, secondary production, and water quality functions. Carbon and nitrogen content of the above ground material is the area weighted estimate of organic carbon and total nitrogen in the vegetative SAV material above the sediment water interface. Above ground carbon and nitrogen content inform the primary production and secondary production functions. All indicators are measured from the collected above ground material, which is first scraped of epiphytes and epifauna and then dried. The mass of dried material is measured to produce estimates of total biomass. A small portion of the dried material is removed for analysis in a CHN elemental analyzer to produce measures of Carbon and Nitrogen content, which are then standardized to the biomass of all the above ground material. *Above ground Carbon and Nitrogen content were not measured as part of the present study.*

Above ground biomass and carbon content measures also be used to estimate bed-scale carbon stocks and therefore as direct estimates of the carbon sequestration function. Above ground tissue biomass and Carbon/Nitrogen content are typically reported on a per core-area basis (i.e., multiple shoots), but the values can also be measured per individual shoot and combined with shoot density to get a density standardized estimate of total biomass, carbon, and nitrogen values per bed.

Below Ground Biomass, Carbon, and Nitrogen content

Below ground biomass is an area weighted measure of the dry weight of all the vegetative SAV material (i.e., roots and rhizomes, see Figure 2) located below the sediment-water interface. Below ground biomass is an indicator of substrate stabilization, carbon sequestration, primary production and secondary production. Carbon and nitrogen content the below ground material is the area weighted estimate of organic carbon and total nitrogen in the vegetative SAV material below the sediment water interface. Below ground Carbon and Nitrogen inform the primary production function. All indicators are measured from the SAV material collected within the sediment cores. All sediments are washed from the vegetative material, which is then dried. The mass of the dried material is measured to produce estimates of total biomass. A small portion of the dried material is removed for analysis in a CHN elemental analyzer to produce measures of Carbon and Nitrogen content, which are then standardized to the biomass of all below ground material. *Below ground Carbon and Nitrogen content were not measured as part of the present study.*

Below ground biomass and carbon content measures can also be used to estimate bed-scale carbon stocks and therefore as direct estimates of the carbon sequestration function. Below ground tissue biomass and Carbon/Nitrogen content are typically on a per core-area basis (i.e., multiple shoots), but the values can also be measured per individual shoot and combined with shoot density to get a density standardized estimate of total biomass, carbon, and nitrogen values per bed.

Leaf Area

Leaf area is the average surface area of all leaves within a random selected shoot from the collected above ground material. Leaf area is used as an indicator of the substrate stabilization, primary production, water quality and nekton habitat functions. Individual leaves are cut at the tip of the sheath from the selected shoot and epiphytes and epifauna are removed. The surface area of each leaf is measured with by scanning them with a Licor leaf area meter and multiplying by two to account for both sides of the leaf. Only whole leaves should be assessed – signs of breakage on the leaf tip and signs of grazing throughout the leaf should be noted or the leaf should be removed from analysis.

Alternatively, area can be estimated by manual measures of width and length, or other calibrated image scanning-based approaches. The width of a leaf can vary significantly from base to tip. As such, if manual measurements are used, multiple measurements should be made and integrated across the entire length.

Epiphyte Biomass

Epiphyte biomass is the dry weight estimate of epiphytic material growing on SAV above ground tissue. Epiphyte biomass is used to inform the primary and secondary production indicators. Epiphytes are measured from the above ground material used for estimates of biomass and leaf area. Prior to removal, epiphytes should be examined under a dissection microscope to determine the relative amount of sediment debris. Epiphytes are removed from both sides of leaves by carefully running the edge of glass slide across the surface of the leaf. Contents of the scraping should be dried at 60°C.

Epiphytes can be removed from SAV tissue in multiple other ways and users should consider the types of epiphytes present on SAV tissue when determining a method for removal and assessment. Dauby and Poulicek (1995) detail the efficiency of most common epiphyte removal methods. Similarly, if available, spectrophotometric analysis of the pigments in the epiphytes, which can be standardized to volume or surface area, is an option to measure biomass that quantitatively excludes sediments.

Shoot density

Shoot density is an abundance per core measure of the number of vegetative eelgrass shoots growing in an area. Shoot density is used as an indicator for nekton habitat, primary production, secondary production and substrate stabilization. The number of distinct shoots collected in the above ground vegetative material is counted. Alternatively, shoot density can also be measured in the field as noted above.

Shoot height

Shoot height is the average length from base to tip, of the longest leaf on a shoot among the shoots collected as above ground material. Shoot height is an indicator of the nekton habitat, secondary production, substrate stabilization, waterfowl habitat and water quality functions. Shoot height is measured with a measuring tape from the base of the shoot to the tip of the blade. Alternatively, shoot height can also be measured in the field, as detailed above.

Infauna Diversity and Biomass

Infauna diversity is an area weighted measure of the taxa richness and diversity of fauna living in the sediments of the SAV beds. Infauna diversity is an indicator of the secondary production and nekton habitat functions. Infauna biomass is an area weighted measure of the ash free dry mass of fauna living in the sediments of the SAV beds. Infauna biomass is an indicator of the secondary production function. Infauna are collected with a sediment core at the central location on the mid-transect of each SAV bed. The contents of the cores are sieved on a 500-mm screen and the material retained on the sieve is fixed in a buffered 20% formalin solution. Use of 95% ethanol-based preservation techniques are also a potential option, but ethanol will reduce biomass values, relative to formalin-based approaches. All of the individuals in the fixed material are then sorted from detritus, identified to the lowest possible taxonomic level, and enumerated. These data can then be used to calculate infauna biodiversity as both Shannon-Wiener diversity (H') and species richness (S). Each taxon identified and enumerated is then dried, combusted in a muffle furnace, and their mass is measured to produce infaunal biomass. *Infauna diversity and biomass were not measured as part of the present study.*

Epifauna Diversity and Biomass

Epifauna diversity is a volume weighted measure of the taxa richness and diversity of the motile and non-motile fauna living on the shoots of the SAV bed. Epifauna diversity is an indicator of the secondary production and nekton habitat functions. Epifauna biomass is a volume weighted measure of the ash free dry mass of the motile and non-motile fauna living on the shoots of the SAV bed. Epifauna biomass is an indicator of the secondary production function. Epifauna are collected along with the above ground material collection at the central location on the mid-transect of each SAV bed. The contents of the bags are returned to the laboratory, where the organisms can be sorted from the SAV itself and then fixed in buffered 10% formalin. Use of 95% ethanol-based preservation techniques are also a potential option, but ethanol will reduce biomass values, relative to formalin-based approaches. All of the individuals in the fixed material are then identified to the lowest possible taxonomic level and enumerated. These data can then be used to calculate epifaunal biodiversity as both Shannon-Wiener diversity (H') and species richness (S). Each taxon identified and enumerated is then dried, combusted, and their mass is measured to produce epifaunal biomass. *Epifauna diversity and biomass were not measured as part of the present study.*

Contaminant Content of Leaves

The content of toxic chemicals in SAV tissue is SAV biomass-weighted concentration of any variety of chemicals absorbed into the tissue of the plant. Contaminant content informs the improving water quality function. The specific chemicals may vary from study to study, but trace metals and PAHs are the most commonly observed compounds in SAV tissue. Tissue is collected in a similar fashion to above ground biomass, with vegetative material collected in plastic bags and returned to the laboratory. The leaves are cleaned of epiphytes and epifauna and then dried. Portions of the dried material are then measured for chemical content using standardized bioaccumulation methods for the compounds of interest. *Contaminant content of leaves was not measured as part of the present study*.

Stressor and Environmental Data Collections

In addition to the collection of biotic eelgrass indicators, abiotic measures should be collected as well to characterize the environmental setting of the beds and any anthropogenic stressors they may be exposed to. At minimum, a standard suite of water and sediment quality variables should be collected at each bed (Table 4). Additionally, measures of water currents, wave exposure, boat traffic, and other similar variables could be collected. These types of data provide a context for eelgrass indicators and help explain patterns observed in the data.

When possible, most water column variables (temperature, light, dissolved oxygen, turbidity, etc.) should be collected continuously over a 2-week spring/neap tidal cycle. Alternatively, these measures can be collected at the same time as the sampling of the eelgrass indicators. Probes measuring these variables should be deployed at the top of the canopy height of the SAV beds within the center of the bed. Additional probe measurements can also be taken outside of the SAV bed at the same depth in adjacent bare ground habitats. Similarly, secchi disk readings and water grabs should be done in a similar location to the probe deployments inside and outside of the bed.

Dive computers are a commonly used tool for divers, and they have the capability of recording depth within 1-foot intervals at a minimum, which can be used for recording SAV bed depth ranges and the depth of each sampling point during surveys. Sediment measurements should be collected from the interior of the seagrass bed.

Table 4. Recommended suite of stressor and environmental measures to be collected along with biotic SAV indicator variables that are helpful in contextualizing patterns in the indicator data.

Measurement	Instrument
Salinity	Multi-probe 2-week continuous logger deployment or single point measures
Dissolved Oxygen	Multi-probe 2-week continuous logger deployment or single point measures
pH	Multi-probe 2-week continuous logger deployment or single point measures
Water Temperature	Multi-probe 2-week continuous logger deployment or single point measures
Irradiance	2-week continuous sensor deployment or single point measures
Bed Depth	Dive computer
Turbidity	Multi-probe 2-week continuous logger deployment or single secchi disk measures
Water Nitrogen Content	Multi-probe 2-week continuous logger deployment or single water grab
Water Phosphorus Content	Multi-probe 2-week continuous logger deployment or single water grab
Sediment Grainsize	Single sediment core
Sediment Carbon Content	Single sediment core
Sediment Nitrogen Content	Single sediment core
Sediment Contaminants	Single sediment core

Eelgrass Survey Design

The goals of a monitoring program should inform the nature of the eelgrass survey. When designing a survey, it will be important to consider: 1) The extent of the sample frame – a single embayment, the Southern California Bight, the whole of California; 2) The spatial scale of the assessment unit – an embayment, a portion of an embayment, a discrete eelgrass bed, or a portion of an individual eelgrass bed or meadow (e.g., southern San Diego Bay); 3) The number of transects assigned per assessment unit; and 4) The frequency of sampling. Some examples of SAV surveys that could be expected to use the tier 3 assessment approach would include: 1) Probabilistic regional- or waterbody-scale assessments of function; 2) Site-specific monitoring to assess the impacts of a permitted discharge, construction project, or other activity; or 3) Monitoring the effectiveness and trajectory of restoration projects. Though all of these hypothetical monitoring programs are focused on evaluating eelgrass function, the disparate nature of their motivations will require considered design of their respective surveys.

Ideally, every eelgrass survey will include measures of the indicators we have detailed. This full suite of measurements would allow for a robust evaluation of all the key ecological functions of a meadow and therefore produce the best tier 3 assessment possible. However, if it is necessary to develop a monitoring program with a constrained number of indicators, we would suggest prioritizing ecological functions and measuring those associated indicators detailed in Table 2 in lieu of just picking indicators irrespective of the functions they may inform.

Indicator Development Conclusions

- With the help of the TAC, nineteen different indicators of eelgrass function were identified.
- Individual indicators were identified to approximate seven priority eelgrass functions.
- Based upon literature review and interaction with the TAC, standardized methods for data collection were developed for each indicator.
- For three of the indicators – shoot height, shoot density, and percent cover – there were multiple potential methods of collection, so each method was tested in the subsequent field study.

CHAPTER 3. COMPARISON OF METHODS AND EVALUATION OF INDICATORS

As detailed in Chapter 2, a suite of structure-based indicators was identified for inclusion in the development of a tier 3 bioassessment tool evaluating the ecological functioning of eelgrass (*Z. marina*) beds in the estuaries and embayments of southern California. Methods of data collection for each indicator were developed based upon review of the literature and interaction with the Technical Advisory Committee, taking advantage of their practical experience working within these habitats. In identifying methodologies, priority was placed upon those that would be practical to deploy in a regular monitoring program potentially covering multiple waterbodies sampled by multiple field crews on a repeated basis.

Eelgrass indicators could be classified into two classes: 1) Those for which there was clear consensus in the literature and among the TAC members on the best method to collect those data; or 2) Those for which a consensus on method of collection could not be reached (Table 5). For those indicators in the first group, our goal was to apply them to a series of eelgrass beds of varying degrees of health across the growing season. In doing so, we could evaluate their relative sensitivity to anthropogenic disturbance and quantify the within-bed variability. Furthermore, it would allow us to develop the practical knowledge of taking the measurements and develop guidance for conducting eelgrass surveys in the future.

As noted in Table 5, consensus on the best method data could not be reached for three indicators: shoot height, shoot density, and % cover. There was a split in opinion among experts on whether it was better to measure the indicator in the field (i.e., underwater) or in the laboratory. For these indicators, we had two goals: First, to apply both data collection approaches to a series of eelgrass beds of varying degrees of health across the growing season; Second, to determine the best method to use going forward on a basis of ease of measurement, time spend underwater vs. time spent in the laboratory, and the magnitude and variability of the data produced. As with the consensus methods, these experiences were also used to develop guidance on using the preferred method for each indicator in conducting future eelgrass surveys (McCune et al. in prep).

Table 5. Status of each eelgrass indicator identified to inform tier 3 assessment of ecological function. Consensus denotes whether the members of the TAC were in agreement on the best method to measure the indicator. An asterisk indicates those indicators for which we were able to collect data in the present study.

	Indicator	Consensus on Methodology?	Data Collection Approach
*	Above ground biomass	Yes	Lab-based
	Above ground Carbon and Nitrogen content	Yes	Lab-based
*	Below ground biomass	Yes	Lab-based
	Below ground Carbon and Nitrogen content	Yes	Lab-based
*	Patch area	Yes	Field-based
*	Perimeter to area ratio	Yes	Field-based
*	**Percent cover**	**No**	**Lab- or Field-based**
*	**Shoot density**	**No**	**Lab- or Field-based**
*	Leaves per shoot	Yes	Lab-based
	Flowering shoot density	Yes	Field-based
*	**Shoot height**	**No**	**Lab- or Field-based**
*	Leaf area	Yes	Lab-based
*	Epiphyte biomass	Yes	Lab-based
	Redox potential discontinuity (RPD) depth	Yes	Lab-based
	Infauna diversity	Yes	Lab-based
	Infauna biomass	Yes	Lab-based
	Epifauna diversity	Yes	Lab-based
	Epifauna biomass	Yes	Lab-based
	Contaminant content of blades	Yes	Lab-based

Methods

Sampling Locations

Measurements of all indicators were collected with two or three-person diver/kayak teams at a series of eelgrass beds in Newport Bay, California and San Diego Bay, California. Six eelgrass beds were sampled in Newport Bay, California (Figure 3) and two beds were sampled in San Diego Bay, California (Figure 4). In each bed transects were laid out along the long axis of the bed (typically parallel with the shoreline) and across the largest depth gradient of the bed. Samples for each indicator were collected along a single transect within each bed, with five quadrats and three sediment core/tissue collections along the length of the main transect and two additional quadrats and core/tissue collections at the shallow edge and deep edge of the bed as depicted in Figure 1.

The beds in Newport Bay were sampled in the summer of 2019 and the winter of 2019-2020. In order to investigate the response of the selected indicators to disturbance, the eelgrass beds in Newport Bay were selected to approximate different levels of stressor exposure. In the absence of existing SAV condition indices or pre-existing anthropogenic stressor data (e.g., bed-specific or bed adjacent measures of turbidity, water column nutrients, water column chl a), eelgrass beds were picked to try and capture the effects of shading stress due to suspended sediment and phytoplankton-related turbidity in the water column. This nominal stressor gradient presupposes the bulk of sediment and biostimulatory chemicals enter Newport Bay at its head via San Diego Creek and is diluted towards the ocean. As such the degree of stressor exposure should change longitudinally along the length of the bay. Following this hypothesis, the PCH site, the most landward eelgrass bed, was selected to be the nominal worst condition site and the BB1 site at the mouth of the bay was selected to be the nominal best condition site (Figure 3). Without actual measurements of turbidity, total suspended solids, chl a, etc., this spatial gradient provided our best, first principles-based organization of sites for data collection and data presentation. While the array of sites likely represents a gradient in stressor exposure, it likely also comprises a gradient in natural factors (e.g., currents, sediment composition, salinity) that directly or indirectly may affect the functioning of the eelgrass beds. As such, we acknowledge that any subsequent direct measures of stressor exposure will provide a more accurate representation of indicator-stressor response relationships and allow for the control of natural factors on indicator performance.

The two eelgrass beds in San Diego Bay were selected to capture the disparity of bed sizes seen there and in other large, shallow embayments across the state. There were few discrete, intermediate sized beds accessible in the bay like SB1 or NB1 in Newport Bay, so a smaller SAV patch and large continuous SAV bed were sampled from different San Diego Bay eelgrass zones or ecoregions (sensu Vantuna Research Group 2009). The smaller CP1 site was closer to the entrance of the bay in the north-central portion of the bay, while the large BP1 site was in the far south portion of the bay (Figure 4). The beds in San Diego Bay were sampled in the fall of 2019.

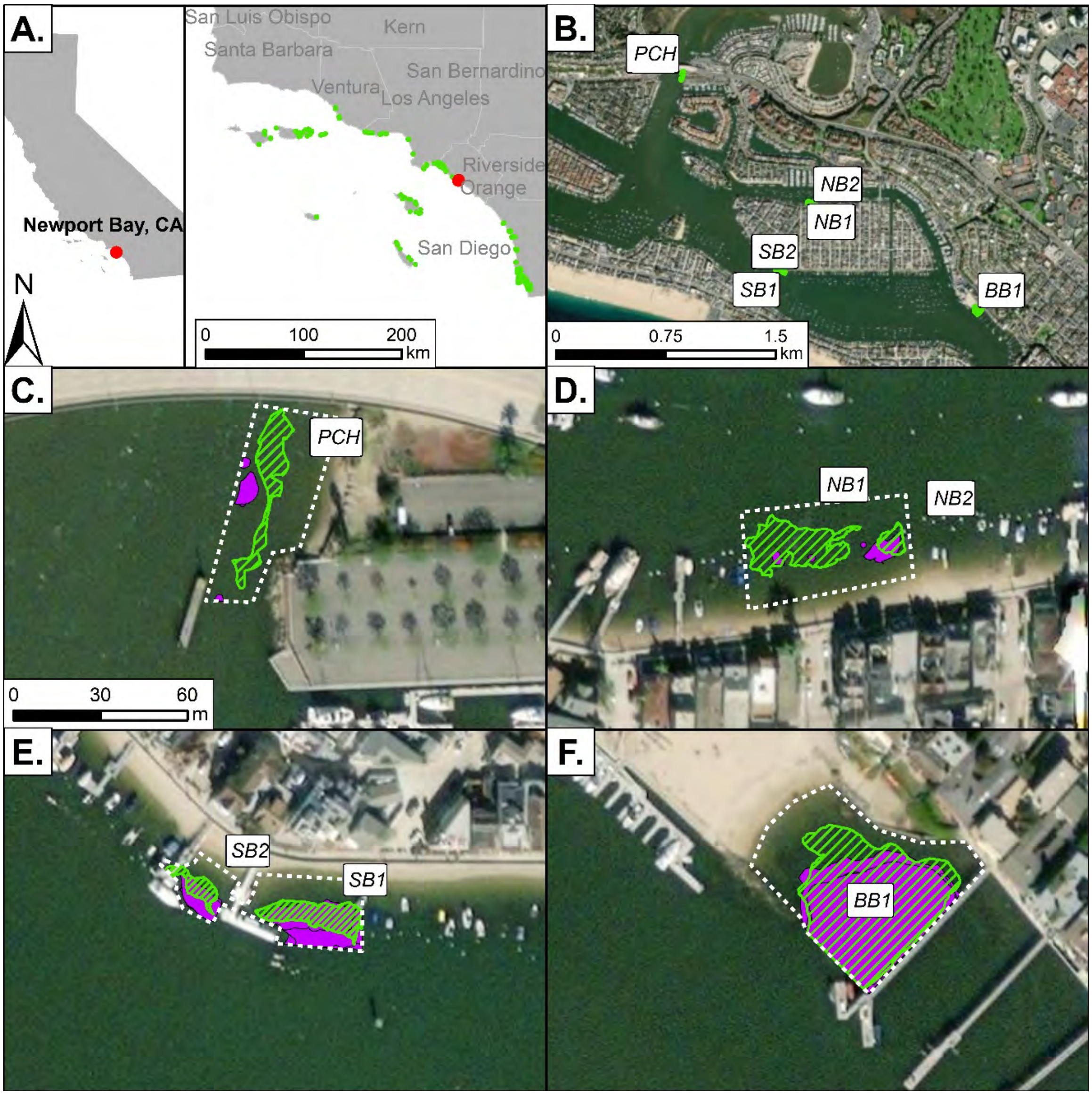


Figure 3. Areal images illustrating the location of eelgrass beds sampled in Newport Bay, California. Panel A indicates the location of the embayment along the southern coast of California and panel B shows the position of each bed within the embayment. Panels C-F show the 2003, 2007, or 2010 extent of each bed (purple polygon) (Coastal Resources Management 2003, 2007, 2010) and that measured in the summer of 2019 (green hashed polygon).

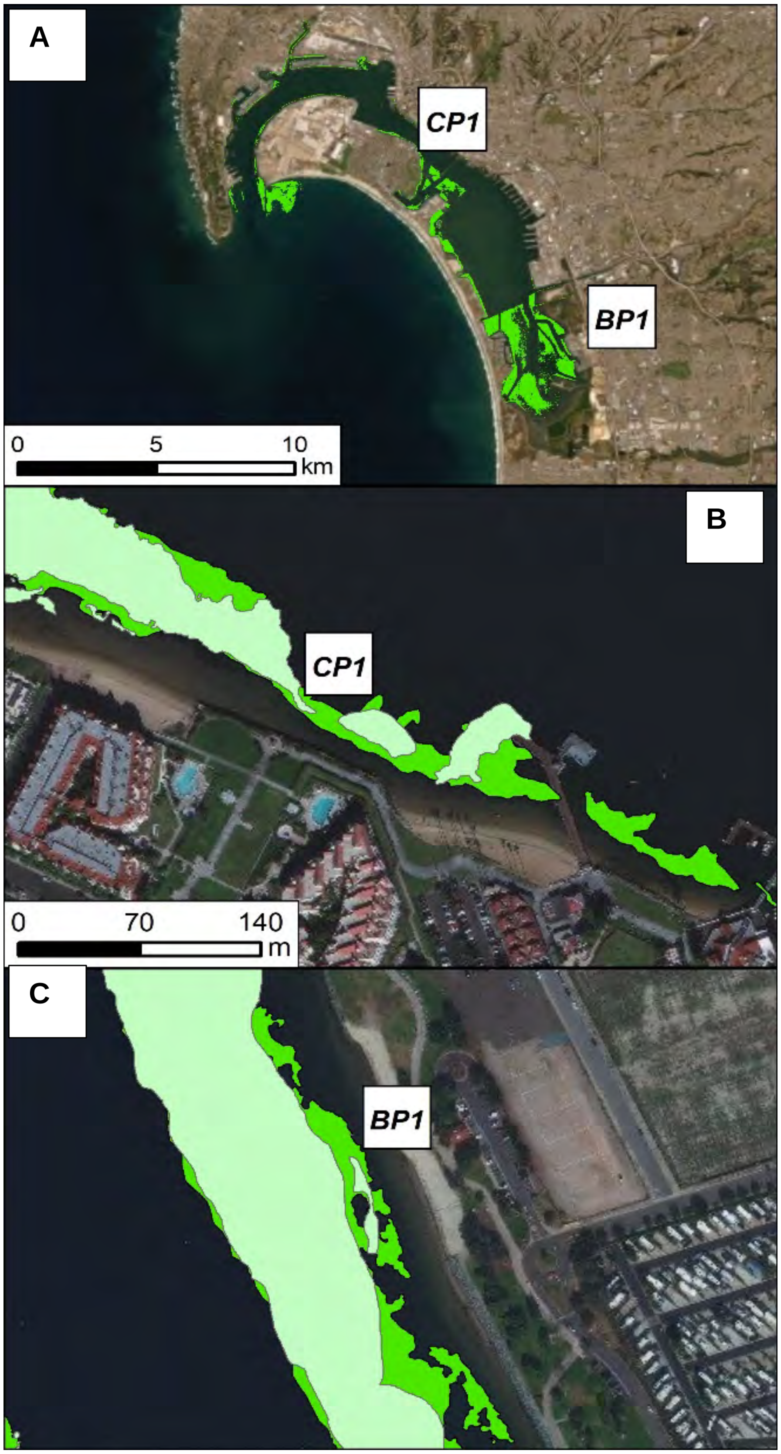


Figure 4. A map of San Diego Bay with mapped eelgrass beds depicted as green polygons CP1 and BP1 depict the samples sites where indicators were measured. The darker green indicates bed area in 2008 and the lighter green indicates bed area in 2011. Mapping data are from Merkel and Associates (2009 2011).

Results

Indicator Evaluation

Summer Sampling

SAV bed perimeter and area measurements done in summer 2019 show that sampled sites covered a wide range of areas and typologies. Bed sizes ranged from 1204 m^2 at BB1 to 35 m^2 at NB2, with most bed perimeters following a similar bed-to-bed pattern (Figure 5). The exception was the PCH site, which had a much larger perimeter relative to its area and consequently a long, narrow shape (Figure 3). Most beds in the more northern parts of the bay (PCH, NB1, NB2, SB1 and SB2) were easily identified as distinct patches with clearly defined edges. The BB1 site, however, appears to represent a different type of larger, continuous bed that extends throughout much of the harbor entrance, docks, and navigation channel. Within the smaller distinct beds, neither the area nor the area:perimeter indicators appear to track the nominal stressor gradient.

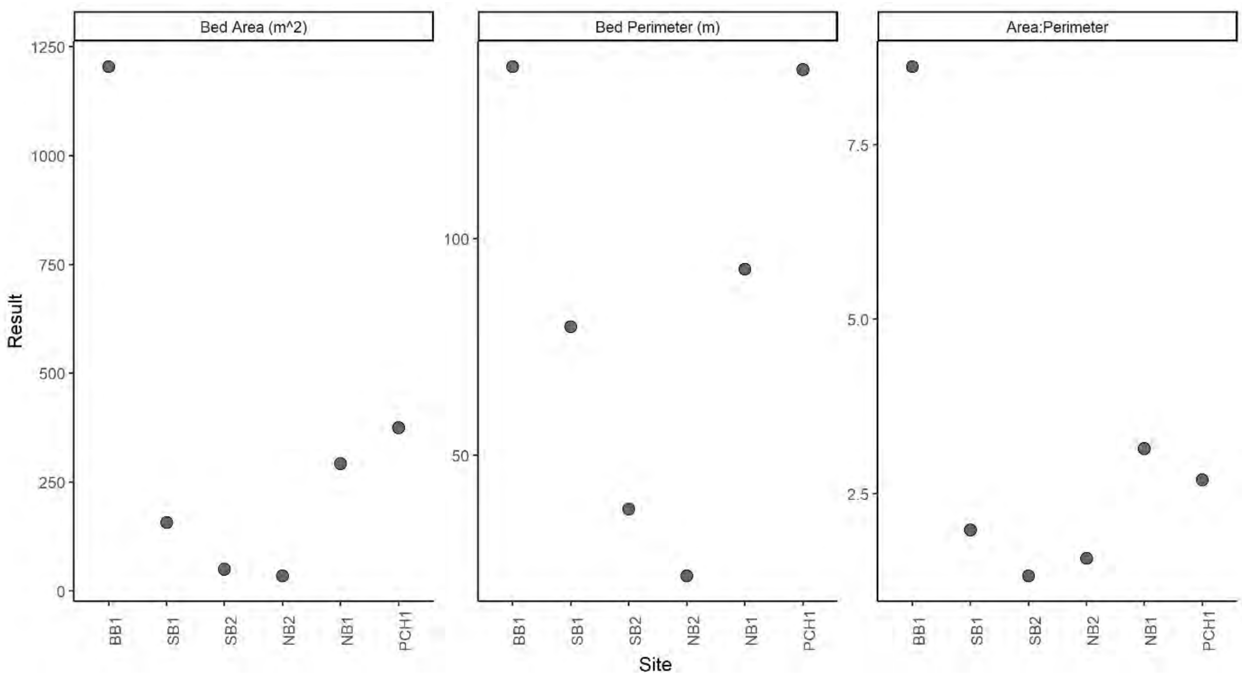


Figure 5. Dot plot of eelgrass bed dimension indicators collected in Newport Bay during the summer of 2019. Site abbreviations correspond to the beds depicted in Figure 3 and are arrayed along a nominal stressor exposure gradient from best on the left (BB1) to worst on the right (PCH).

All of the field-measured indicators (shoot density, % cover, and shoot height) were quite variable within a given bed. Despite the variance, there was a trend in the mean value of shoot density and % cover along the nominal stressor gradient (Figure 6). Field measurements of mean shoot density and percent cover steeply decrease when moving from sites BB1 and SB1 to the remaining sites (Figure 6). Conversely, shoot height field measurements were equivalent among all of the beds. Sites BB1 and PCH are at opposite ends of the range for field indicator that were observed, with BB1 having the highest mean shoot density and highest mean percent cover and PCH having the lowest mean percent cover and lowest mean shoot height.

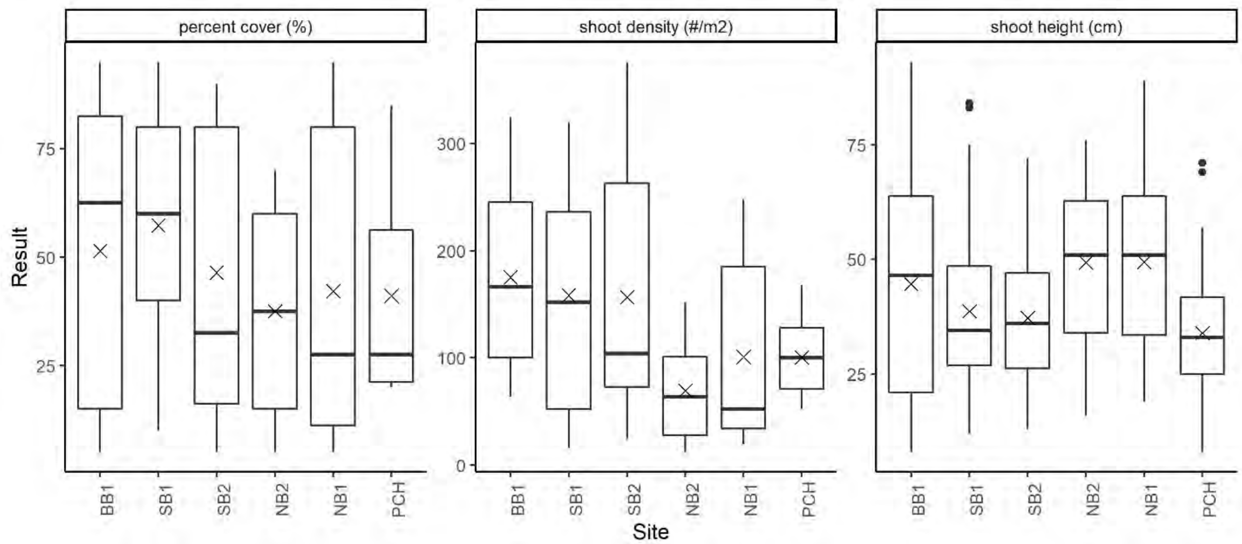


Figure 6. Schematic box and whisker plots for the field measured eelgrass indicators collected in Newport Bay during the summer of 2019. Each box represents the distribution of measurements within each bed. "X" shapes represent mean values. Site abbreviations correspond to the beds depicted in Figure 3 and are arrayed along a nominal stressor exposure gradient from best on the left (BB1) to worst on the right (PCH).

Eelgrass material was collected at all six sample sites during the summer sampling events, but due to laboratory access issues, the data for the different lab-based indicators were only available for the SB1 and PCH sites at the present moment. Though these beds are on relatively opposite ends of the nominal stressor gradient, the inferences that can be drawn from these data about indicator response to stress are limited. The average values and within bed variability were relatively similar for both eelgrass beds, with the exception of below ground biomass (Figure 7). Below ground biomass was consistent at the PCH site but was much more variable at the nominally less stressed SB2 site. No measurable amounts of epiphytes were present on blades from the SB1 and PCH samples.

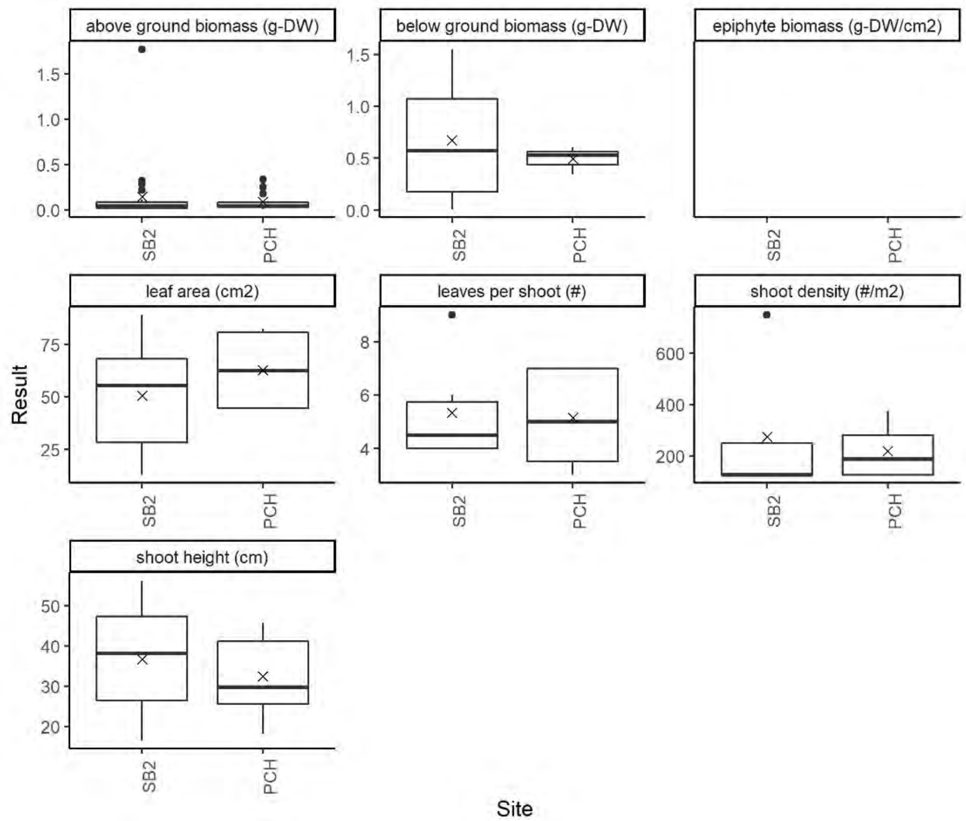


Figure 7. Schematic box and whisker plots for the lab measured eelgrass indicators collected from two sites in Newport Bay during the summer of 2019. Each box represents the distribution of measurements within each bed. "X" shapes represent mean values. Site abbreviations correspond to the beds depicted in Figure 3. Note that material was collected for the measurement of epiphyte load, but there were none present at either site.

Winter Sampling

Like the in the summer, the field-measured indicators were highly variable within beds, but generally decreased among beds along the nominal stressor gradient (Figure 8). The range in percent cover and field measured shoot density was greatest at the sites closest to the mouth of the bay (BB1, SB1, and SB2). Conversely, sites farther up the bay (NB2, NB1, and PCH) had less variability and lower average cover and shoot density than the beds lower on the stressor gradient (Figure 8). The pattern in field measured shoot height across all of the beds did not follow a trend along our nominal stressor gradient. However, the endmember sites, BB1 and PCH, did have the highest and lowest mean shoot heights, respectively.

The relative bed-to-bed pattern of field indicators measured in the winter followed a similar pattern to that seen in the summer data, though the differences along the nominal stressor gradient were more pronounced in the winter data. The absolute values of percent cover and shoot density were greater across all beds in the winter data. Patch area and patch area:perimeter were only measured in summer.

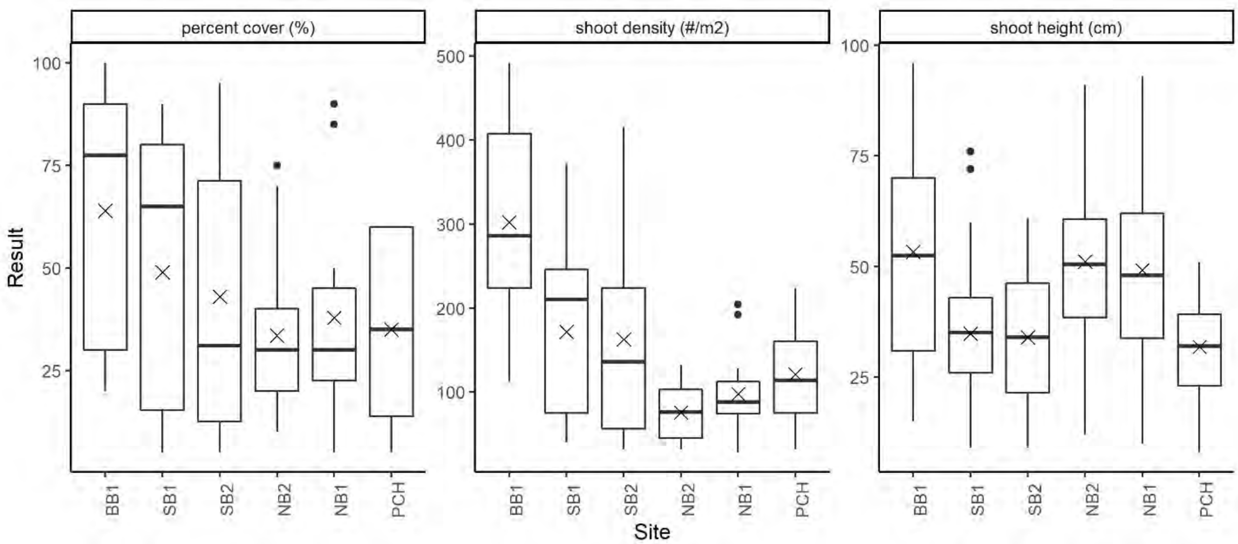


Figure 8. Schematic box and whisker plots for the field measured eelgrass indicators collected in Newport Bay during the winter of 2019-20. Each box represents the distribution of measurements within each bed. "X" shapes represent mean values. Site abbreviations correspond to the beds depicted in Figure 3 and are arrayed along a nominal stressor exposure gradient from best on the left (BB1) to worst on the right (PCH).

Among the lab measured indicators, above ground biomass, below ground biomass, and shoot density tracked along the nominal stressor gradient (Figure 9). The other indicators showed no trend along the stressor gradient. Epiphyte biomass was highly variable, while the leaf area, leaves per shoot, and shoot height measures were relatively similar among all of the beds. The lone exception being SB1 which had consistently broader leaves and more leaves per shoot. Across all of the beds, above ground biomass values had relatively low variability and similar means for all sites except the end member sites BB1 and PCH, which had the highest and lowest means respectively (Figure 9). Below ground biomass declined consistently along the nominal stressor gradient. Furthermore, in a pattern similar to the above ground biomass, the BB1 and PCH had highest and lowest means. Shoot density lab measurements were similar for most sites (median density of one shoot), except for BB1, which had much greater density. Epiphyte biomass was relatively low across all sites, with few samples containing any meaningful amounts of epiphytic material (usually three or fewer samples from a given site had measurable epiphytes). Leaf area data were relatively consistent across most sites, as there was high variability at all sites except PCH, which had consistently low leaf area.

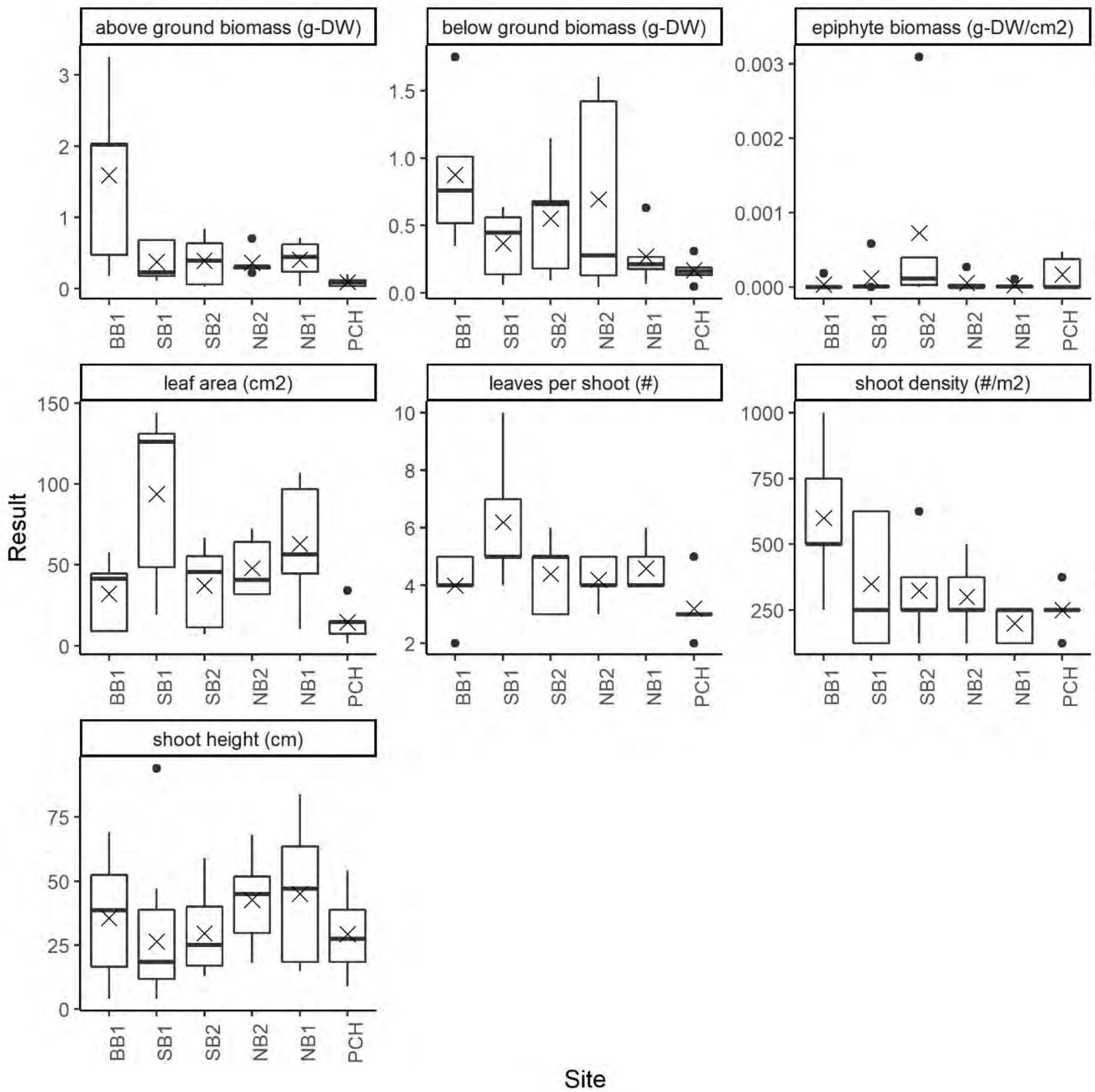


Figure 9. Schematic box and whisker plots for the lab measured eelgrass indicators collected in Newport Bay during the Winter of 2019-20. Each box represents the distribution of measurements within each bed. "X" shapes represent mean values. Site abbreviations correspond to the beds depicted in Figure 3 and are arrayed along a nominal stressor exposure gradient from best on the left (BB1) to worst on the right (PCH).

Fall Sampling

Two SAV beds were sampled in San Diego Bay in November 2019 (Figure 4). Bed area and bed perimeter measurements varied greatly between the two sampled sites in San Diego Bay (Figure 10). Site CP1 was a smaller sized, discrete bed similar to many that were sampled in Newport Bay and BP1 was a large continuous bed, very different from even the biggest bed (BB1) in Newport Bay. Given its size, the bed perimeter could not be measured by divers. Bed area and area:perimeter were estimated from aerial photographs and bed mapping data from Merkel and Associates (2011). Site CP1 was so narrow that it was not possible to collect the shallow and deep samples separate from the main transect, so only 5 samples were collected in that bed.

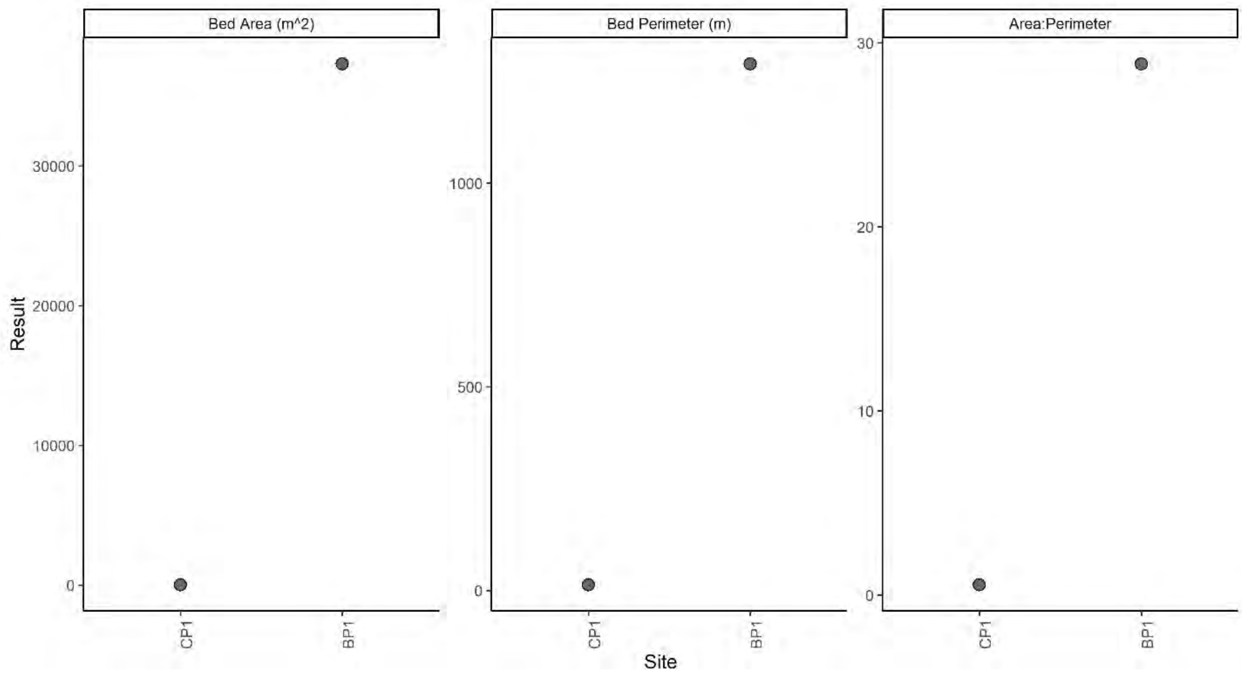


Figure 10. Dot plot of eelgrass bed dimension indicators collected in San Diego Bay during the fall of 2019. Site abbreviations correspond to the beds depicted in Figure 4.

Mean shoot density and percent cover were similar between the CP1 and BP1 beds (Figure 11), though the variability in both measures was much greater in the larger bed. Percent cover ranged from 10% to 100% in BP1 versus 35% to 80% for CP1. Conversely, the pattern for shoot height was reversed, where the mean shoot length was greater at the smaller bed but was also much more variable than at the larger bed.

The percent cover measures, were similar to those among the Newport Bay beds, but the shoot density, even at the smaller bed, was more than 2X that observed in even the most robust bed in Newport Bay (BB1). Interestingly, the shoot height in the summer and winter at all of the Newport Bay beds was the same or much higher than at the either CP1 or BP1.

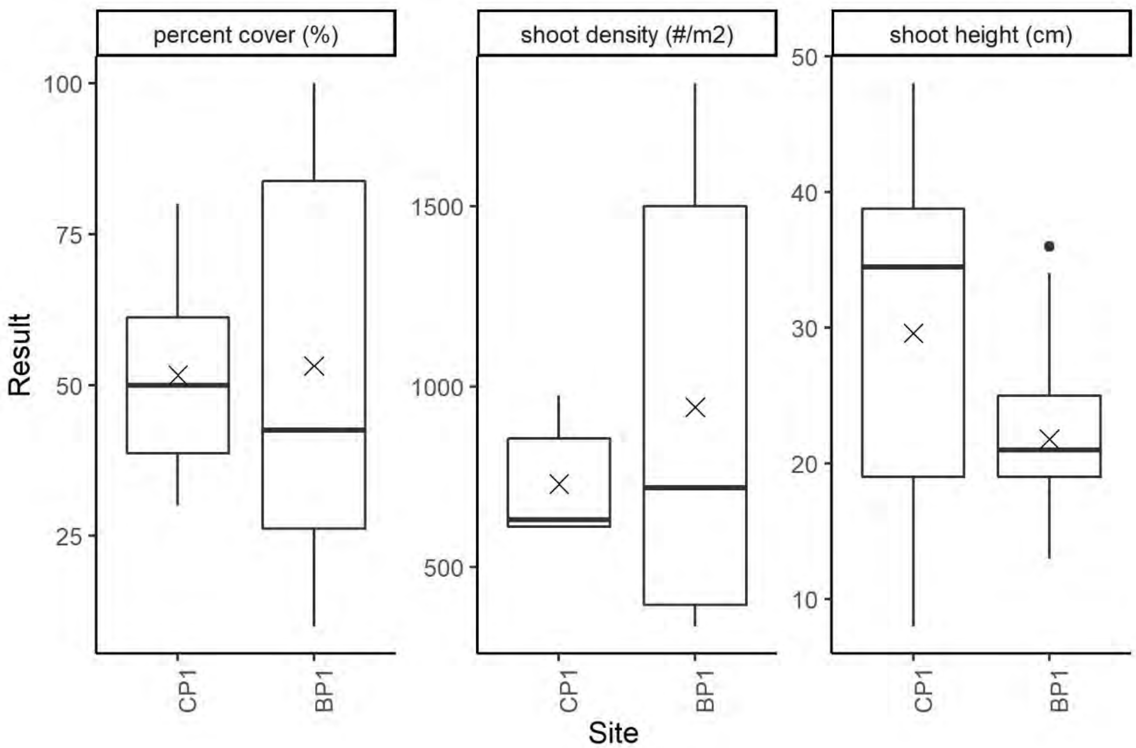


Figure 11. Schematic box and whisker plots for the field measured eelgrass indicators collected in San Diego Bay during the fall of 2019. Each box represents the distribution of measurements within each bed. "X" shapes represent mean values. Site abbreviations correspond to the beds depicted in Figure 4 and are arrayed along a nominal stressor gradient from left to right.

Of the lab measured indicators, above ground biomass, below ground biomass, epiphyte biomass, leaf area, and, to a lesser degree, shoot height were greater at the smaller CP1 site then the larger BP1 site (Figure 12). Leaves per shoot and shoot density was similar between the two beds, though there was greater variability in leaves per shoot at the BP1 beds. Like the field-based measures, nearly all of the lab-based indicator measurements from the beds in San Diego Bay were smaller than all of the beds in Newport Bay in winter. The lone exception was shoot density, which was similar between the beds from both embayments.

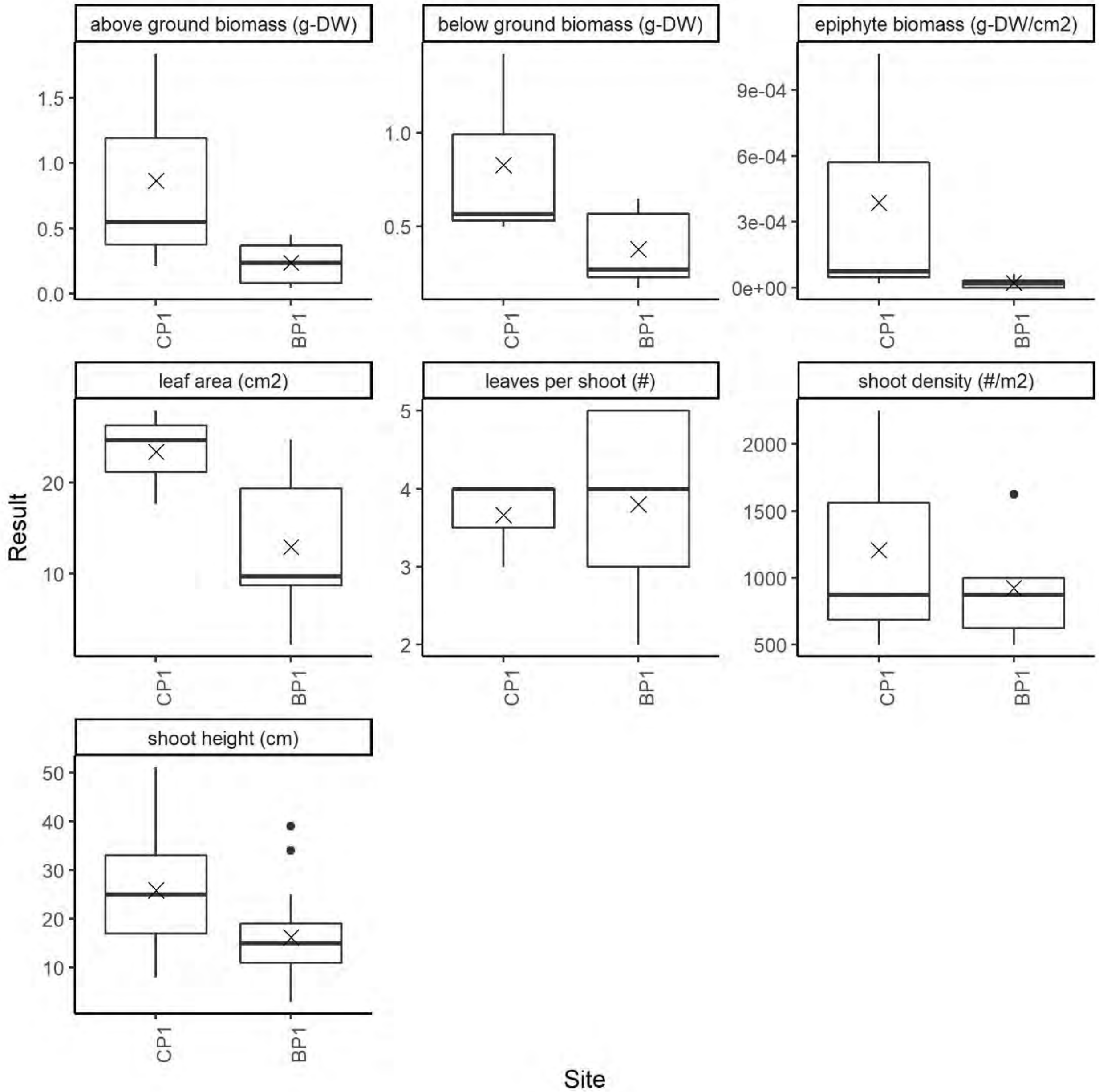


Figure 12. Schematic box and whisker plots for the lab measured eelgrass indicators collected in San Diego Bay during the Fall of 2019. Each box represents the distribution of measurements within each bed. “X” shapes represent mean values. Site abbreviations correspond to the beds depicted in Figure 4 and are arrayed along a nominal stressor gradient from left to right.

Methods Comparison

Shoot density was the first indicator that was measured with both a field-based method and a lab-based method. Field measures were made by divers feeling and counting the number of shoots with the sample quadrats while under water and the lab measures were made from sediment cores collected from the same location. As illustrated in Figure 13, there was consistent disagreement between the two methods, with the field measurements returning lower estimates in almost every instance. At the lower range of shoot densities that were observed (i.e., < 250 shoots m^{-2}), the lab measurements lost sensitivity; missing sample-to-sample variance captured by the field method (see the horizontal banding of points in the lower-left quadrant of Figure 13). The embayment from where the measurements were made did not appear to influence one method more than the other. However, there was greater discrepancy between the two methods at densities greater than 1,000 shoots m^{-2}, which only came from the San Diego Bay beds.

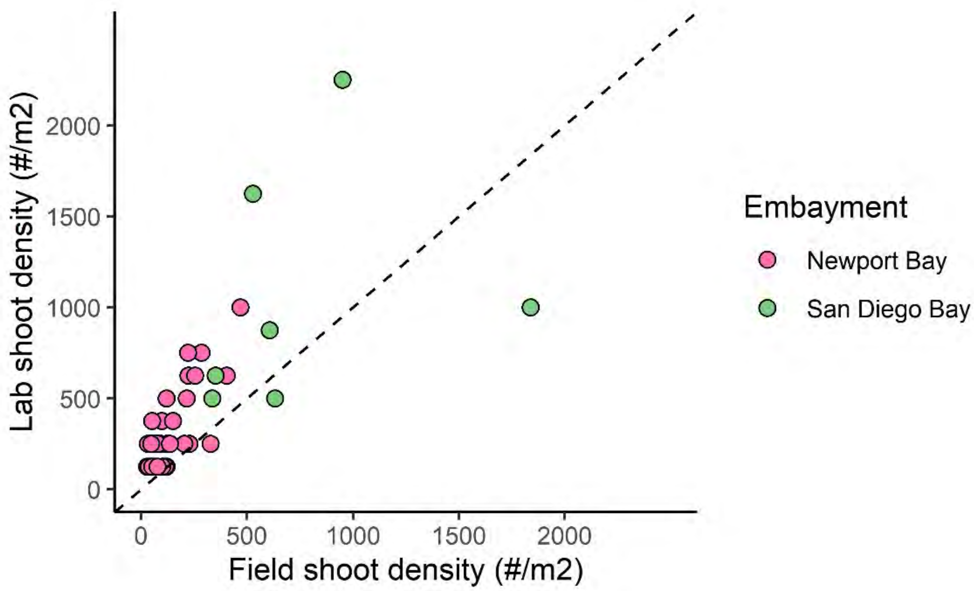


Figure 13. A scatter plot comparing estimates of eelgrass shoot density measured in the field (i.e., underwater) and from sediment cores subsequently brought back to the laboratory. The dashed line represents a 1:1 ratio between the two methods. The color of the dots indicates the embayment from where the data were measured

Blade length was the second indicator that was measured with both a field-based method and a lab-based method. Field measures were made by divers measuring a selection of shoots located within the sample quadrats while under water and the lab measures were made from sediment cores collected from the same locations. For blades less than ~60 cm, there was good agreement between the methods (Figure 14). However, for longer blades there was greater disagreement in the measurements produced by the two methods. No one method consistently over- or underestimated blade length and there was no apparent bias related to the embayment of origin, though nearly all of the longer blades (i.e., those with the largest disagreements) were from the Newport Bay beds.

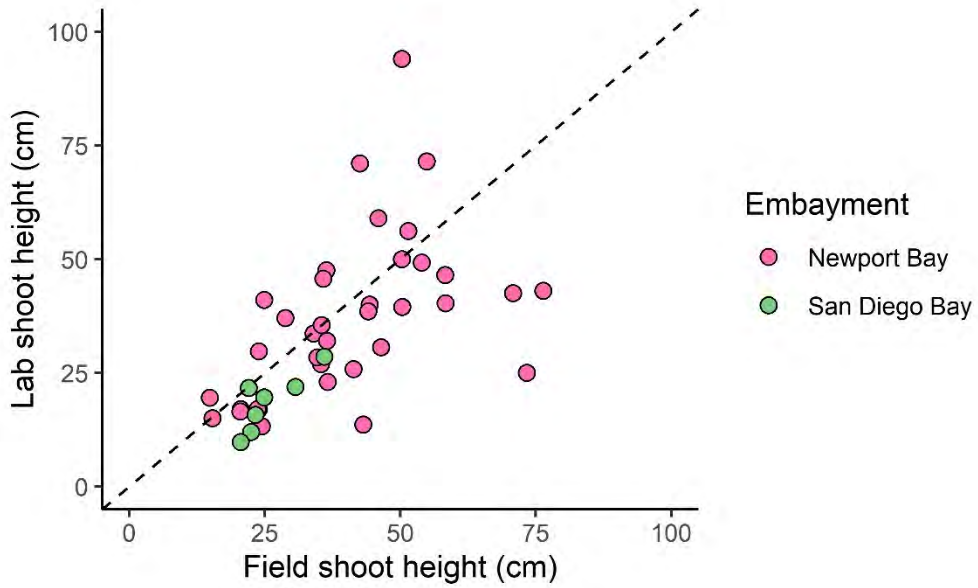


Figure 14. A scatter plot comparing estimates of eelgrass shoot height measured in the field (i.e., underwater) and from bagged vegetation subsequently brought back to the laboratory. The dashed line represents a 1:1 ratio between the two methods. The color of the dots indicates the embayment from where the data were measured.

Percent cover was the last indicator measured simultaneously with a field- and lab-based approach. Field measurements were underwater visual estimates made at each quadrat by the divers sampling the beds and the lab-based measurements were divers interpreting digital images captured at the same quadrats during sampling. For 23% of the samples, low visibility /low light conditions made it difficult to produce an estimate of percent cover from of the digital images. For those samples, no comparison of methods could be made. Among the samples were data could be produced from both methods, there was reasonable agreement between the two measurements. However, there was some bias in the denser beds (i.e., greater than 50% cover), where the estimates made from the digital images tended to be greater than those by the divers in the water (Figure 15). Conversely, there appeared to be no bias in the estimates related to the embayment in which the measurement was made.

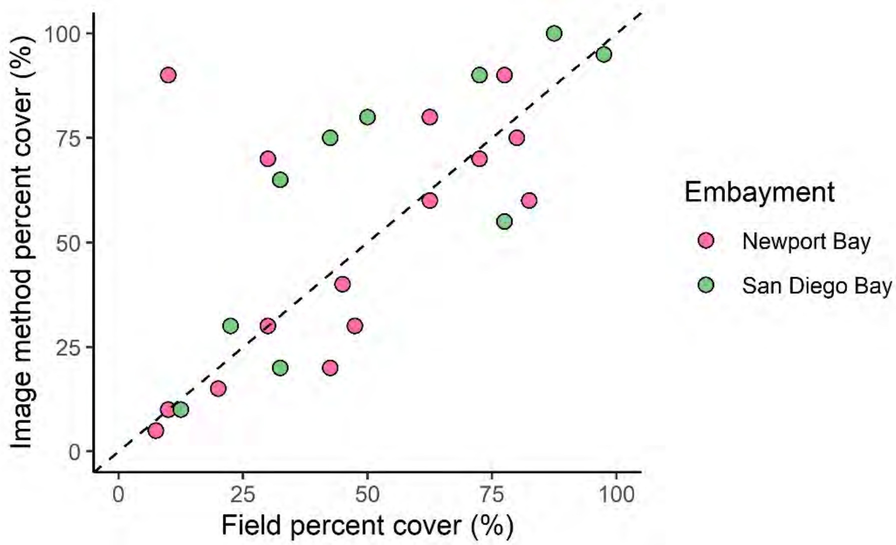


Figure 15. A scatter plot comparing estimates of eelgrass % cover by visual estimation of the divers and from digital images collected at the time of sampling. The dashed line represents a 1:1 ratio between the two methods. The color of the dots indicates the embayment from where the data were measured.

Discussion

Evaluation of Eelgrass Indicators

This work represents the first steps in developing a bioassessment framework for southern California's estuaries and embayments that focuses on eelgrass as the indicator. More importantly, it demonstrates a sampling approach, a set of protocols, and an organizing context to look not simply at condition of the habitat, but at the ecological functioning of these systems. There are a good set of protocols to measure the extent of eelgrass present across a region using SCUBA divers (e.g., Short et al. 2006, Short and Coles 2001), side scan sonar (e.g., Greene et al. 2018, Sanchez-Carnero et al. 2012, McCarthy and Sabol 2000) or aerial imagery (e.g., Lathrop et al. 2006, Pe'eri et al. 2016). However, as highlighted in Bernstein et al. (2011), these types of extent data do not provide any insight into condition and functioning.

The development of any bioassessment framework begins by identifying the aspects of the organisms to measure in order to best provide insight into their resident ecosystem (i.e., indicators). The next step is evaluating the practicality and efficacy of those indicators for yielding useful data and detecting disturbance. The present study encompasses these first two steps in developing a bioassessment approach for eelgrass. The results of the present study indicate that there were differential degrees of response to disturbance among the indicators. Within Newport Bay, where a gradient of potential disturbance could be established, measures of plant biomass and the robustness of the bed were the types of indicators that changed the most from bed-to-bed. Interestingly, this same pattern was also apparent between the two San Diego eelgrass beds that were sampled. This may imply that there is a similar gradient in stressor exposure moving away for the mouth of San Diego Bay and to the south, despite the seemingly healthy expanse of eelgrass in the southern part of the bay where the BP1 site was located. It is important to note that with only two data points firm conclusion should not be drawn, nor overly relied upon. Conversely, indicators that focused on the structural dimensions of the individual plants were the same along the nominal stressor gradient in Newport Bay and between the small and very large beds in San Diego Bay.

From the group of eelgrass beds that were sampled and the timing of the different sampling events, we were also able to begin to look at seasonal patterns in the eelgrass indicators. Fall and winter are the end of the vegetative growing season for eelgrass in southern California (Phillips 1972) and as such represent the maximum integrated experience of a given bed for that growing year. Where they could be compared, the general pattern of all the indicators along the stressor gradient were similar between summer and winter. However, within the more responsive indicators, the distinction between the nominal best and worst conditions sites was more apparent among the winter samples, which suggests that the end of the growing season may be the best time for sampling these habitats, with the Fall being more logistically feasible in southern California with regard to storms, water temperature, etc.

Though cursory in nature due to the imbalanced number of beds that were sampled, our sampling scheme did illustrate differences/similarities between beds from the southern and the central parts of the southern California Bight. For nearly all of the all of the indicators of bed composition (shoot density being the exception) and individual plant composition were greater in the Newport Bay beds (central bight) than the San Diego Bay beds (southern bight). Furthermore, anecdotal evidence suggests that actual shape and dimensions of the eelgrass leaves from San Diego Bay were different than that observed in Newport Bay, which may have contributed to the wholescale differences in the indicators between the two areas. Newport Bay eelgrass leaves in collected specimens were completely flat and possibly wider in shape, whereas San Diego Bay eelgrass leaves had horizontal curvature and formed more stiffened and thinner appearing leaves. Consequently, we would suggest that divers should record observations of blade morphology in their field notes.

A potential way to think about the individual indicators and how they speak to the dual organismal/habitat nature of SAV is grouping them into classes based on the aspects of the SAV bed they measured. First were the indicators of bed shape and size – area and area:perimeter ratio. Second were the indicators of bed composition – shoot density, % cover, above ground biomass, and below ground biomass. Third were the indicators of plant robustness – shoot height, leaves per shoot, leaf area. Lastly would be the indicators of SAV-associated organisms – epiphyte biomass. Of the indicators we recommend for collection, but were not measurable within the scope of this study, redox potential discontinuity depth would be in group one, above and below ground carbon/nitrogen content and flowering shoot density would be in group two, contaminant content would be group three, and infauna/epifauna diversity and biomass would be part of the fourth group of indicators.

Across our nominal stressor gradient, there were discernable patterns in the response of the bed shape/size and bed composition indicators to distance from the mouth of the embayment. In contrast, the indicators of plant robustness and SAV associated organisms were less responsive to our nominal stressor gradient. This pattern suggests that the stress levels associated with light availability and water circulation (approximated by our gradient) were not at a magnitude to impact the growth patterns of the individual plants (e.g., dimensions of the plants themselves) but were enough to alter the emergent properties of bed and all the component plants combined (size, density, biomass).

Ultimately, in the development of a tier 3 bioassessment framework that provides insight into the ecological functioning of eelgrass beds, the performance of individual eelgrass indicators along a disturbance gradient is not a stopping point. Though an important first step, next meaningful set of analyses will be to combine the different indicators associated with each priority ecological function (i.e., Chapter 2) and evaluate their performance in response to disturbance and as approximations of eelgrass function. There are number of potential ways indicators could be combined, but all should start with the conceptual relationships established by the TAC and detailed in Chapter 2. Mathematical approaches to combining indicators could include:

- Constrained linear combinations of indicators could be created from multiple linear regressions of the indicators with measures of ecological function at non-disturbed sites
- Unconstrained linear combinations could be extracted from axis loading values from PCA, PCO, DCA, or other ordinations of the indicators among disturbed and non-disturbed sites (e.g., Smith et al. 2001)
- Non-linear combinations could be derived from regression tree/forest models of the indicators and measures of ecological function at non-disturbed sites

The resultant combinations of indicators could then need to be arrayed along disturbance gradients to evaluate their responsiveness to disturbance in general and possibly to specific types of disturbance. Irrespective of the manner in which the indicator combinations are derived, a large dataset of indicator measures collected across a variety of different embayments and degrees of disturbance will be needed to account for natural environmental and temporal variability.

Furthermore, direct measures of eelgrass function will also have to be collected. These kinds of studies, some of which were conducted (e.g., sediment accumulation/stabilization, carbon sequestration) in parallel with the current work, are less practical to conduct over large spatial/temporal gradients. However, direct measures of ecological function collected across stressor gradients will also help in benchmarking functional rates and indicator values to reference/non-reference conditions and allow for the development of bioassessment tools to quantify and communicate levels of disturbance.

Methods Comparison

For three of the indicators we were testing, we could not come to a consensus method among the members of the technical advisory committee. A such, lab-based measurements and field-based measures for shoot density, % cover, and shoot height were compared to identify a method to recommend. Our recommendations were based in part on the precision and accuracy of the measurement techniques, but also from a practical aspect of trying to minimize diver time under water and minimize the amount of gear needed to take underwater.

Shoot density could be estimated from divers counts within the sample quadrats while under water or from sediment cores brought back to the laboratory. Both methods produced relatively similar estimates, though the values derived from material brought back to the laboratory were usually larger. However, within sparse beds that had low densities of shoots it was apparent that lab-based method was missing variability captured by the diver counts. The area of the cores was smaller than that of the quadrats, so during random placement of the core often only one or two shoots were collected. In contrast, random placement of the quadrat would yield a greater number of shoots given the larger area it covers. This larger area captured a more accurate estimate of shoot density. As such, we believe the field-based approach where the divers count by the shoots in quadrat by hand is the better method to use in estimating shoot density with eelgrass beds, despite an increase in time spent underwater. Additionally, the field-based counts are relatively non-destructive to the SAV and could therefore be applied more frequently and in situations where harvest of material is discouraged (e.g., restoration SAV beds). However, in very dense beds it may more practical to estimate shoot density both in the field and in the laboratory to ensure greater accuracy in the measurements.

Similar to shoot density, shoot height could be estimated directly in the field with divers measuring the length in situ or it could be measured from the shoots clipped at their base, bagged, and brought back to the laboratory. Both methods produced very similar measurements across most of the size ranges observed in the present study. There were some inconsistencies with larger leaves, but there was no clear bias in one method or the other. Given the similarity of the data produced, we recommend using the laboratory-based measures, as it reduces time spent under water. That being said, in monitoring programs where it has been decided to forgo the harvest of material, the field-based measurements are a reasonable alternative choice.

Percent cover of SAV within a bed can be estimated visually by divers underwater or from digital images collected under water and viewed later on a computer. When comparisons could be made, there was relatively good agreement between the two methods and no apparent bias in the visual estimates versus the digital images. However, nearly a quarter of the digital images could not be clearly interpreted due to low visibility (i.e., high turbidity and low light) conditions. Given the difficulty in consistently collecting images, we recommend the underwater visual estimate.

Bed Typology and Indicator Selection

During our sampling, we encountered three types of eelgrass beds: large, continuous beds, discrete small beds, and ephemeral patch beds. Furthermore, it became apparent that seagrass bed typology may need to be considered when conducting surveys of ecological function. SAV beds in southern California estuaries can be present in a variety of different sizes and, as they are living organisms, that size and even the presence of the bed can vary within a growing season and between years. This variability reinforces the need for adequate mapping and site reconnaissance before sampling but also suggests a need for some flexibility in sampling design.

Large, continuous beds are expansive, persistent spreads of eelgrass, often in excess of 500 m in length and width for which true whole-bed estimates are impractical to collect in a timely manner with small dive teams. Furthermore, considerations for this type of bed also extend to those beds that extend into unsafe diving conditions – heavy traffic boat channels, ports, fishing piers etc. These types of beds are common in lower Newport Bay, most of San Diego Bay, and Mission Bay. For these beds, other, non-diver-based means of mapping (e.g., side-scan sonar, aerial drones) are suggested to determine bed perimeter and area indicators. Subsequently, a sub-section of the bed can then be selected where the transect-based sampling can be (safely) conducted in a reasonable time frame. These transects would be conducted as normal and the measurements can be scaled to the discrete area measured or to the area of the whole bed if that can be measured.

Discrete beds are persistent, individual spreads of eelgrass with distinct barren patches of sediment between beds of seagrass that distinguish them from the large meadows. The breaks between beds may be caused by changes in depth and/or shoreline modification. These types of beds are of a size and shape that it is reasonable to sample the whole bed in a timely manner by a small dive team. These are the most common type of eelgrass beds that were encountered in our sampling surveys (especially Newport Bay), ranging from 20 to 150 m in length. Bed perimeter and area can be measured by divers and one set of transects will cover the entire bed. We expect that this type of bed would be the “standard” sampling example for most of southern California.

Ephemeral patch beds are small (< 10 m in length), typically sparse growths of eelgrass that may not persist across multiple years. The perimeter and area of beds of this typology are best measured with a measuring tape versus using a GPS. Also, depending on their dimensions and shape, a single transect along the long axis of the bed will be sufficient for sampling the indicators, forgoing the shallow-to-deep transect. For these small beds, reducing the number of replicates taken along the transect from 5 to 3 may be possible. Lastly, in particularly small or sparse beds, it may be prudent to limit the number of cores collected for above and below ground biomass to keep from damaging the bed and reducing the likelihood of it persisting through the next year. Alternatively, given these suggested sampling constraints, some monitoring programs may elect to exclude this bed typology from their sampling frame for a tier 3 assessment.

As more data are collected from additional eelgrass surveys across a broader geographic range, we will be able to better understand the importance of bed typology. Additional information will allow us to more robustly examine if there are true differences between different types of eelgrass beds and how bed morphology may limit structural measures and functional performance. Furthermore, it will allow for the examination of how the morphology of individual beds changes through time and whether they shift from one typology to another. Anecdotal evidence suggests that bed typology should not be considered a permanent attribute, but instead be treated as a factor to consider when scaling indicators from different beds against each other.

Methods Conclusions

- Shoot density, percent cover, above ground biomass, and below ground biomass were the most responsive indicators to the nominal stressor gradient among the sample sites.
- All indicators had large within-bed variability
- Bed-to-bed patterns in the indicators were similar across seasons, with magnitude of the indicators being greatest in winter
- Of the indicators with multiple potential methods, a field-based method is recommended for estimating shoot density and percent cover, while a laboratory-based method is recommended for estimates of shoot height

STUDY SUMMARY

With the completion of this study, the first steps have been taken towards the development of a fully realized, eelgrass-informed bioassessment program for the estuaries and embayments of California. There is now an assessment framework that encompasses the dual nature of eelgrass and other species of SAV and is organized around three key elements: 1) Where and how much of the habitat is there?; 2) What is the condition of the resource and its resident waterbody?; and 3) Is the resource/habitat functioning as it should? There is now a conceptual model linking structural elements of eelgrass to priority ecological functions that can be used to evaluate the third element of ecosystem functioning. Methods have been identified to collect the data that will populate the conceptual structure-function model. Lastly, there is now a small, but valuable eelgrass dataset that can be expanded and supplemented in the future.

Based upon the present work, we have an initial sense of how the indicators identified by our TAC perform in the field along gradients of potential stressor exposure, bed typology, and season. Indicators of eelgrass bed morphology and robustness were most responsive to the stressor gradient, while indicators related to individual plants were less responsive to the gradient. Nearly all of the indicators, except bed dimensions and shoot density, were similar between large meadows, discrete patches, and small, potentially ephemeral patches. The bed-to-bed patterns in indicators was relatively stable across seasons, though the magnitude of the indicator values increased as the growing season progressed.

It is important to note, however, that these patterns are only an initial impression, inferred from two water bodies in southern California, across an eight-month timeframe. A much larger pool of data, collected across multiple waterbodies and from different years, is needed to fully populate the conceptual model for assessing eelgrass ecological function. We do not have a complete dataset, but we have a very good starting point.

As mentioned in the beginning of this report, Bernstein et al. (2011) presented the infrastructural pieces they believed were needed to establish a persistent, regional-/state-wide program to monitor eelgrass. The present study and the associated SOP provide the technical underpinnings on what types of data should be collected to assess eelgrass ecological function and guidance on how those data should be collected within that hypothetical monitoring infrastructure. Though there is still more methods validation work to be done (especially on the collection of eelgrass associated fauna), the next step that needs to happen is the deployment of our technical recommendations into the infrastructure proposed by Bernstein et al. (2011) across southern California’s (or ideally all of California) estuaries and embayments through integration into existing monitoring programs.

STUDY RECOMMENDATIONS

- Opportunities should be identified to begin applying the methods detailed in this report to eelgrass beds throughout southern California and in other regions of the State. With a larger, temporally and spatially diverse dataset of indicator measures, an ecological function assessment scoring tool could be developed and subsequently benchmarked to management goals. Sampling efforts should be targeted to include eelgrass beds of different typologies from waterbodies in different geographies and with different levels of anthropogenic stressor exposure.
- As additional surveys are conducted across a broader geographic scale, multiple field crews will be collecting data. As such, a robust intercalibration and field auditing program should be developed to assure comparability of data through time and between locales.
- Methods for measuring the indicators not tested in this study, especially the infauna and epifauna-related indicators, should be evaluated in the field.
- As more eelgrass indicator data are collected, they should be evaluated against direct measures of the different eelgrass ecological functions. Direct measures of nekton utilization and primary/secondary productivity would be most valuable to a management audience and informative to SAV researchers, respectively.
- The tier 1 (habitat prediction and extent assessments) and tier 2 (water body condition) elements of the bioassessment framework should be developed to allow for a more fully realized, multi-faceted evaluation of these ecosystems.

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