



California Estuarine Wetland Monitoring Manual (Level 3)

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Estuarine Wetland Monitoring Manual V.2

Application and Assessment of USEPA Three-Tiered Monitoring Strategy to California Coastal Wetlands

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Wetland Monitoring Program Abstract:

Monitoring and assessment strategies developed by the State of California and USEPA universally call for coordinated and consistent approaches. Unfortunately, the ability to meet this goal is often limited. Although progress has been made over the last decade in developing standardized rapid assessment methods (i.e., Level 2) for California, there have been fewer resources and attention dedicated to standardized site-intensive (i.e., Level 3) assessment methods. Compared to Level 2 methods, intensive assessment methods provide information on ecological function and process, are more diagnostic of restoration performance and regulatory compliance, and are important as validation measures for rapid assessment methods. The lack of consistent approaches to intensive assessment has limited our ability to share information between projects, precluded use of Level 3 data in ambient monitoring and regional health assessments, and fostered inefficiencies as each project developed independent protocols. Major coastal wetland restoration projects are planned throughout California, and the development and testing of standardized Level 3 assessment procedures is imperative.

This program prioritized coastal estuarine systems to facilitate baseline information transfer to several imminent large-scale restoration projects and developed standardized protocols for several indicators and parameters for the implementation of site-intensive assessments. This program took the first regional steps by compiling and analyzing existing site-intensive California estuarine wetland assessment

procedures, developing proposed standardized approaches with scientific review, exploring the covariance between these Level 3 protocols and existing Level 2 (i.e., CRAM) assessment tools, and developing protocol documents and implementation recommendations to assist the development of comparative monitoring programs and facilitate information transfer to other projects. Version 2 updates to this manual include refining approaches and standardized protocols (SOPs), two new SOPs, additional methods within individual SOPs, revised recommendations, updates throughout the manual, metadata recommendations, and the addition of data consolidation approaches, considerations, and challenges.

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Executive Summary

This manual updates a previous framework to guide the development of coastal, perennial estuarine wetland monitoring programs throughout California by providing Standard Operating Procedures (SOPs) and recommended protocols. As a living document that continues to be developed, this version has new and modified protocols, and it is anticipated that further changes to these will be made over time. It is important to have standardized, scientifically valid, and repeatable protocols and metrics to evaluate the health of a system. Protocols should be able to be replicated temporally and spatially, with a thorough evaluation of their applicability to different habitat types within wetland systems. The manual provides a framework to guide the development of site-intensive wetland monitoring and demonstrates how programs may begin to structure their protocol and method choices to reflect a more standardized approach.

The primary goal of this manual is to begin to develop standardized protocols for several indicators and parameters for the implementation of site-intensive assessments of coastal wetlands in California and to provide monitoring recommendations. At this site-intensive scale, a rigorous assessment is possible that provides high resolution information on the condition and function of wetlands within an evaluation area. Version 2 updates to this manual include refining approaches and standardized protocols (SOPs), two new SOPs, additional methods within individual SOPs, revised recommendations, updates throughout the manual, and the addition of data consolidation approaches, considerations, and challenges. This manual may be a support tool for wetland scientists and practitioners to guide the development of new programs or use existing monitoring data for larger analyses.

Final Suite of Recommended Protocols

The protocols and methods described in this manual were chosen for inclusion because they have broad applicability and proven efficacy. They focus on a subset of broad parameters (e.g., vegetation, birds) measured by most monitoring programs that were evaluated as part of the program development. Through the evaluation of multiple protocols for each of the main parameter categories, this manual provides the beginnings of basic monitoring “toolkit” recommendations which should be supplemented by additional protocols and/or additional parameters on a site- or project-specific basis, based on monitoring program goals or questions. Recommendations were primarily based on scientific evaluations of data quality, cost and effort, expertise requirements, and disturbance. Individual monitoring programs may build on this suggested framework to meet project objectives.

While site-specific goals should be the principal consideration to inform protocol selection and sampling design, this manual provides a suite of protocol recommendations based on analyses weighing multiple factors influencing implementation, including resource requirements, quality and importance of data outputs, and site disturbance. If implemented for new monitoring programs, data should have improved transferability. Table ES-1 presents two groupings of Level 3 estuarine wetland protocols, including a column for recommended protocols for each parameter, and a second minimum protocol recommendation for programs with fewer resources or funding. The recommended protocols include

higher resolution or better quality data to inform wetland functions and processes. Recommended protocols also include the low resource column.

In addition to the recommended Level 3 protocols listed in Table ES-1, Level 2 California Rapid Assessment Method assessments are also recommended for implementation for all monitoring programs to provide a broad, site-wide rapid condition assessment and provide supporting and transitional information between the Level 1 and Level 3 implementation assessments. Additional discussions of each parameter follow Table ES-1.

Table ES-1. Final suite of Level 3 recommended protocols and low-resource protocols, for programs with very limited funding. Note: monitoring programs or research that are targeted towards specific questions or needs would require additional sampling methods.

Parameter	Recommendation	Low-Resource Recommendation
Water Quality	Data sonde (SOP 1.1)	Spot sampling associated with beach seine (SOP 4.1)
Soil Characteristics	Grain size and organics (SOP 2.2), hydrometer method; soil texture and salinity (SOP 2.1)	Soil texture (SOP 2.1)
Vegetation	Vegetation mapping (SOP 3.5); cover class quadrat (SOP 3.2)	Cover class quadrat (SOP 3.2)
Fish	More replicates and higher frequency of beach seine (SOP 4.1); fish cameras (SOP 4.4)	Beach seine (SOP 4.1)
Birds	More replicates and higher frequency of point count (SOP 5.1) or site-wide surveys (SOP 5.1)	Point count (SOP 5.1)
Mammals	None recommended	None recommended
Invertebrates	Benthic invertebrates (SOP 6.1)	Presence associated with beach seine (SOP 4.1)

Water Quality

Ambient water quality plays an integral role in influencing habitat and species distributions and is often a good indicator of the efficacy of management interventions. Given dramatic short- and long-term temporal variability in water quality parameters (e.g., salinity, temperature, pH, and dissolved oxygen), water quality is best tracked with long-term deployment of data sondes. Due to the high costs associated with purchasing and maintaining monitoring equipment, programs with limited resources should use office-based GIS aerial image analyses and rapid assessments to evaluate the surrounding landscapes, freshwater inputs, and impairments to dominant hydrology to broadly infer water quality characteristics in combination with spot sampling when possible.

Soil Characteristics

Soil grain size, salinity, and organic content are defining characteristics of soft-sediment intertidal habitats. Protocols examining these characteristics can be labor-intensive and require specialized

equipment; however, general soil characteristic parameters can influence hydrology and the wetland community may respond directly (i.e., vegetation). Therefore, basic soil characteristics are recommended such as soil texture, grain size, and organic content. These require some specialized equipment but can be done cost-effectively. Soil characterization options, including methodologies not explored in this manual (e.g., chemical constituents, toxicity), will need to be evaluated on a project-specific basis. For low resource programs, soil texture can be done for almost no cost (except for labor) in any habitat and is an easy alternative, though it provides less valuable data (categorical rather than quantitative).

Vegetation

Some form of vegetation monitoring is recommended as a key component for every monitoring program, regardless of site-specific needs. High-resolution vegetation data can allow logical inferences to be made about multiple parameters including hydrology, soil characteristics, disturbances, and the distribution of associated wildlife such as mammals, birds, invertebrates, and herpetofauna. As a result, resources required to assess some additional parameters may be reduced if broad assumptions are sufficient to meet project goals (i.e., resulting from the vegetation data). Of the transect-based vegetation cover protocols evaluated, the cover class quadrat method is recommended as it is the most rapid and flexible survey across all habitat types and conditions while maintaining high precision and comparable accuracy to the laser quadrat. For programs with more dedicated resources, the creation of a site-wide vegetation map can provide an extremely useful foundational data layer and large-scale supplementary data to support site-wide analyses and restoration planning efforts or assessments.

Fish

The fish community is a common indicator evaluated by estuarine wetland monitoring programs (approximately one-third of evaluated program documents) and can serve as a proxy for the function of intertidal channels and habitats. As such it is recommended for surveying as part of this manual using a combination of beach seines and blocking nets to survey intertidal channels. While beach seine surveys can be fairly time- and labor-intensive and can only provide a snapshot of data in time due to their highly mobile nature, fish community and diversity are still common indicators of water quality and restoration activities. Additionally, as estuaries and wetlands provide essential nursery habitat for juvenile commercially important species, they are often tied to wetland ecosystem functions and services. If more resources are available, an increase in sample replicates or higher sample frequency (e.g., more seasons) are recommended. Additionally, fish cameras are a relatively newer technology survey that should be utilized if resources allow. The one-time costs associated with setting up several systems for deployment will provide supporting data to the beach seines and can be used more frequently and with less disturbance.

Birds

Of the Level 3 protocols evaluated for bird monitoring, the point count method is recommended based on ease of implementation, lower relative levels of disturbance, lower time/effort commitments, and comparable resulting data. While similar or equal to the low time requirements for box count surveys, higher visibility is associated with the point count method. Traversing through the entire sampling box was found to increase site disturbance while high tides made the visual delineation of box edges nearly impossible. The greater ease and lower habitat impacts implementing the point count surveys did not

yield any noticeable loss in data quality and bird populations were equally characterized by both methods. However, if more resources are available, an increase in sample replicates or higher sample frequency (e.g., more seasons) are recommended. Additionally, if a baseline- or species-level assessment (or geospatial assessment) is desired, a site-wide survey is recommended to provide the largest inventory of bird species and a more complete representation of site use by birds.

Mammals

Mammal survey protocols are not recommended for implementation by this manual. In addition to only infrequently being included in the documents evaluated by the monitoring report literature review, mammal presence in intertidal wetland habitats is intermittent and requires time-intensive protocol implementation and specialized permits. If adequate resources are available, and medium to large sized mammals are a target parameter of the developing monitoring program, then wildlife cameras are a feasible alternative to cover a variety of habitat types but may need to be deployed in large arrays to determine abundances or larger wildlife movement patterns. For smaller mammals, catch-and-release survey methods can be used with various sized traps to target specific wildlife or in combinations to assess the broader mammal community. Specialized methods with specific protocols would need to be implemented for some groups of species such as shrews.

Invertebrates

Benthic infauna are important in sediment turnover and bioturbation, activities that mix and transport particles, water, and solutes within the sediment and across the sediment water interface (Rhoads, 1974; Coen and Luckenbach, 2000; Nogaro et al., 2009; Belley et al., 2010). As an integral part of the consumer food chain, benthic infauna provide important trophic support to species of commercial and intrinsic importance like crab, fish, and birds (Sacco et al., 1994; Levin et al., 1996; Moseman et al., 2004). Infauna usually construct tubes or burrows and are commonly found in intertidal and subtidal waters. They reflect local environmental conditions and are used as bio-indicators for pollutant studies (Smith et al., 2001).

Methods focused on benthic invertebrates provide valuable information about the lower trophic levels within a given wetland area, but there are typically high labor, time, and resource costs associated with their implementation. This manual recommends using cores to sample the benthic invertebrate community and sorting to recognizable taxonomic units. Additionally, as a lower resource option, invertebrates may be captured in association with beach seining, providing information on larger, typically nektonic organisms (such as shrimp and crabs). Broader diversity or productivity patterns may be assessed using terrestrial invertebrate sampling protocols; however, there has been relatively little work on this on relationships to other, more traditionally sampled parameters remain unclear.

Data Consolidation

Data consolidation is essential to comparing different datasets and ecosystem, and while data consolidation can be complicated, using standardized protocols (SOPs) during the creation of new monitoring programs as well as collecting and sharing detailed metadata can facilitate comparison among existing program datasets or among locations. Clearly defining the goals of analysis has to be central to any data consolidation effort because the ability to effectively combine data will depend on

what is trying to be extracted from the data. Comparison of similarly collected data is the simplest case; yet data collected with substantially different methodologies can be synthesized to provide meaningful insight. This can be accomplished via the use of species lists, transformation to presence/absence data, indices, conversion factors, and development of multi-metric indicators.

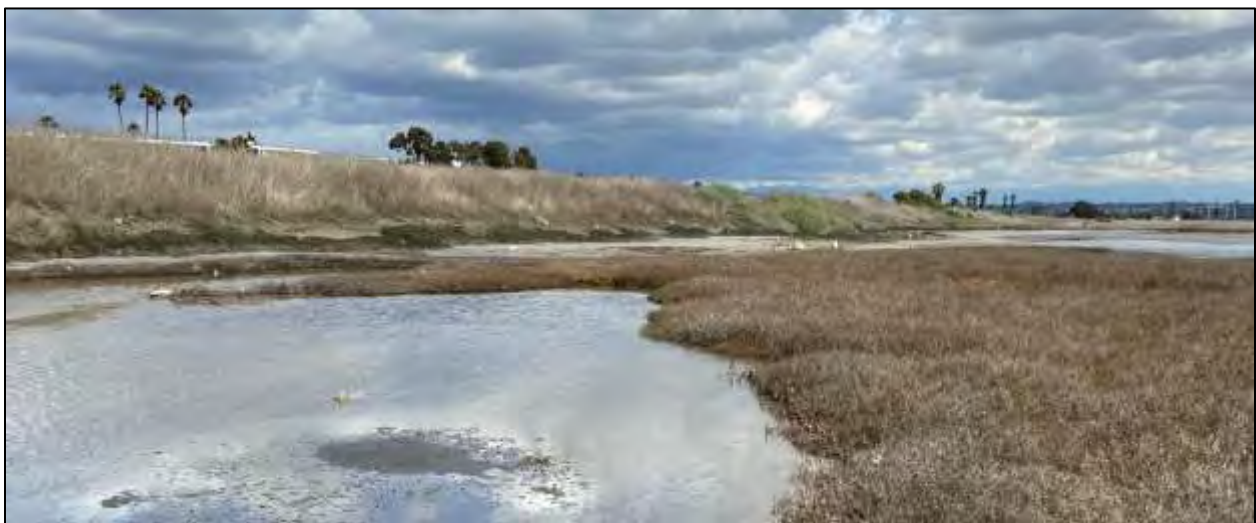
Metadata

Well-documented metadata are extremely important for data preservation, sharing, and analyses. Existing databases from regional monitoring programs, independent research programs, and peer-reviewed literature supported this assessment. Metadata as it pertains to this wetland monitoring manual can be categorized into three types including program, protocol, and field / laboratory metadata. This document provides recommendations for all three levels.

Conclusions and Applications

The principal purpose of this manual is to serve as a tool for resource managers, scientists, researchers, agency representatives, students, or anyone with the goal of developing an estuarine wetland monitoring program. Thus, this manual provides a framework to guide the development of Level 3 (site-intensive) wetland monitoring and shows how programs may begin to structure their protocol and method choices to reflect a more standardized approach.

The protocols and methods described in this manual were chosen for inclusion because they have broad applicability and proven efficacy. They focus on a subset of broad parameters (e.g., vegetation, birds) measured by most monitoring programs that were evaluated as part of the program development. Most of this manual relied heavily on previous or existing wetland monitoring programs for its development. Through the evaluation of multiple protocols for each of the main parameter categories, this manual provides the beginnings of basic monitoring “toolkit” recommendations which should be supplemented by additional protocols and/or additional parameters on a site- or project-specific basis. Recommendations were primarily based on scientific evaluations of data quality, cost and effort, expertise requirements, and disturbance.



Introduction

About the Manual

This manual was written to provide a framework to guide the development of coastal, perennial estuarine wetland monitoring programs throughout California by providing Standard Operating Procedures (SOPs) and recommended protocols. It is meant to be a living document that continues to be developed, with supplemental additions of new or modified protocols over time (this is Version 2.0). Although site- and project-specific considerations are always necessary, it is important to have standardized, scientifically valid, and repeatable protocols and metrics to evaluate the health of a system. Protocols should be able to be replicated temporally and spatially, with a thorough evaluation of their applicability to different habitat types within wetland systems.

The primary goal of this manual is to begin to develop standardized protocols for several indicators and parameters for the implementation of site-intensive assessments of coastal wetlands in California. At this site-intensive scale, a rigorous assessment is possible that provides high resolution information on the condition and function of wetlands within an evaluation area. This intensive scale often employs bioassessment procedures or intensive biological, chemical, edaphic, and human use analyses. The robust measures used in these intensive assessments produce information that can be used to (a) refine or validate rapid assessment methods based on a characterization of reference condition, (b) assess causes and consequences of wetland change, (c) evaluate the efficacy of management interventions, (d) develop designs and performance standards for wetland restorations, including compensatory wetland mitigation and restoration trajectories, and (e) support the development of water quality standards that are protective of wetlands.

One of the key purposes of the development of the manual is to increase the understanding of regional or statewide wetland conditions. If standardized protocols are followed, interested scientists and researchers can begin to improve monitoring coordination on a large scale, including a potential future application of assisting in the development of restoration trajectories, success criteria, and adaptive management thresholds.

Purpose and Use of this Document

The principal purpose of this manual is to serve as a tool for resource managers, scientists, researchers, agency representatives, students, or anyone with the goal of developing an estuarine wetland monitoring program. The manual provides a framework to guide the development of Level 3 (site-intensive) monitoring and shows how programs may begin to structure their protocol and method choices to reflect a more standardized approach. Additionally, suggestions are made on data consolidation across programs, when possible.

The manual is not intended to mandate new methods or override those currently being used by monitoring groups or for mitigation purposes. Instead, it presents methods that have been adapted from those used successfully by existing wetland monitoring programs throughout California, including modifications to provide more standardized approaches to data collection. The conclusions of the

manual provide a recommended suite of monitoring protocols. The manual and specifically, the individual Standard Operating Procedures (SOPs) for each protocol, describe methodologies and techniques for 30 protocols covering a broad range of monitoring parameters including: ambient water quality, soil characteristics, vegetation, fish, birds, mammals, and invertebrates. These protocols are combined into 18 SOP documents as appendices to this manual and posted individually online as stand-alone documents.

The manual is not intended to be used for regulatory purposes at this time, though there is potential for regulatory adoption of specific protocols in the future. Rather, it is intended to be used by local, state, and federal agencies, resource managers, scientists, and landowners to support monitoring objectives.

Literature Review Summary

The protocols and methods described in this manual were chosen for inclusion because they have broad applicability and proven efficacy. They focus on a subset of broad parameters (e.g., vegetation, birds) measured by most monitoring programs that were evaluated as part of the program development. Most of this manual relied heavily on previous or existing wetland monitoring programs for its development. These sources are listed within each of the Standard Operating Procedures and in the literature cited at the end of this report. Appendix A contains a breakdown of each protocol utilized by each of the different monitoring programs that were part of the literature review (N = 71 program documents). Table 1 summarizes the total count of monitoring program documents that surveyed for each parameter included in the statewide literature review. Protocols for the vegetation cover parameter were identified in over two-thirds of the evaluated monitoring program documents. Mammals, terrestrial invertebrates, marine sediment, and herpetofauna survey protocols were each included in less than one-quarter of the evaluated program documents. There are many protocols and methods available to wetland monitoring practitioners for each parameter. The protocols presented within this manual establish a recommended basic monitoring “toolkit,” which may be supplemented by additional protocols and/or additional parameters on a site- or goal-specific basis (e.g., targeted special status species surveys).

Table 1. Count of monitoring program documents that survey each parameter (N = 71 program documents).

Parameter	Number of Monitoring Program Documents / Reports	Percentage of Total Documents Evaluated
Vegetation	48	68 %
Water Quality	34	48 %
Benthic Invertebrates	29	41 %
Avifauna	24	34 %
Ichthyofauna	23	32 %
Terrestrial Soil	18	25 %
Physical Characteristics	18	25 %
Mammals	12	17 %
Terrestrial Invertebrates	11	15 %
Marine Sediment	11	15 %
Herpetofauna	9	13 %

Establishing a Monitoring Program

It is important to ensure that a monitoring program is effectively and efficiently designed with established goals, objectives, timelines, and detailed protocols. This manual is meant to address several components of wetland monitoring program development, namely specific protocol implementation strategies as well as suggested frequency and timing. While monitoring needs often differ between projects and sites (e.g., restoration, mitigation, reference sites), or are based on a range of goals (e.g., assess water quality changes over time, track vegetation development or condition trajectories, education), implementing standardized approaches can begin to make data transferrable across and within regions.

Given the broad range of considerations when developing a monitoring program, this manual does not provide a rigid step-by-step development instruction process, but rather, provides a detailed synthesis of method-specific data outputs and resource requirements to inform the decision-making process once monitoring goals and restrictions have been identified. This manual is a first step in the development of a site-intensive framework for standardizing protocols to be implemented with different frequencies or as part of various sampling designs.

Sampling efforts should be designed to collect information capable of answering management questions by means of robust statistical analysis. In addition, site selection, characterization of reference sites or systems, and identification of appropriate index periods (e.g., peak growing period for vegetation) are all of particular concern when selecting an appropriate sampling design (USEPA 2002a). Additionally, there are likely many site-specific monitoring practitioner considerations to consider that are not directly addressed by this manual such as permitting or site access requirements.

Connection to WRAMP and EPA

The State of California and the California Wetlands Monitoring Workgroup (CWMW) call for consistency in wetland monitoring. This manual attempts to address several challenges and gaps identified in the California Wetland Monitoring Workgroup's Tenets of a State Wetland and Riparian Monitoring Program (WRAMP), namely the standardization of protocols, inclusion of comparable metadata, and the recommendation of quality assurance and quality control methods.

The WRAMP consists of coordinated, comparable regional and statewide efforts that use standardized methods to monitor the effects of natural processes, climate change, and government policies, programs, and projects on the distribution, abundance, and condition of wetlands and riparian areas. The direct application of standardized intensive protocols fits within the WRAMP framework structure to address identified challenges in the following ways (Figure 1):

- Condition assessment protocols;
- Data transfer protocols and data quality control procedures; and
- Analytical and reporting methods.

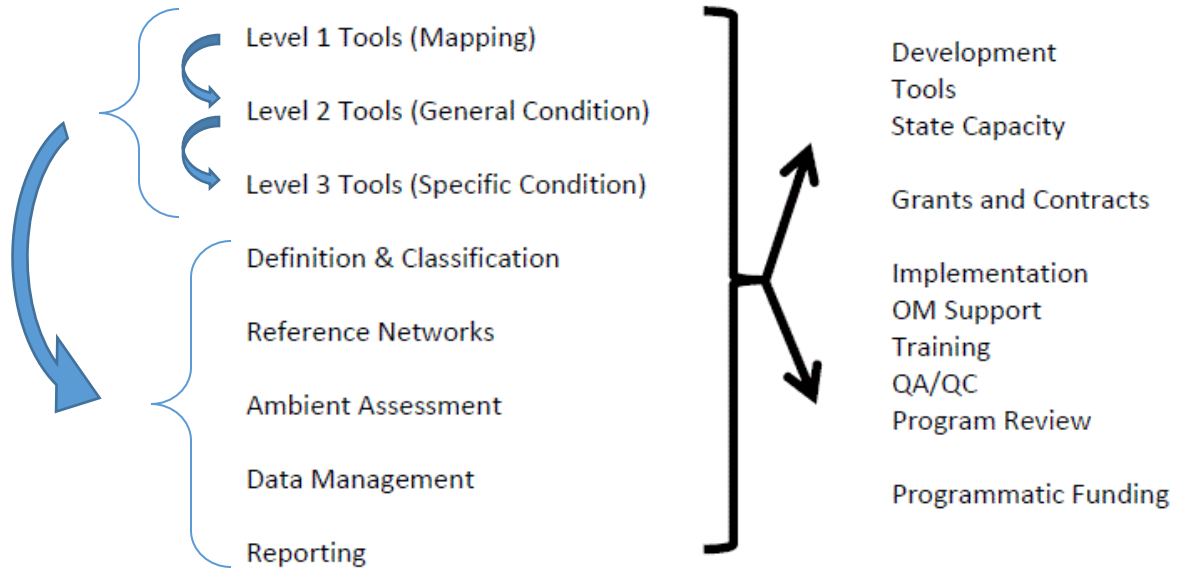


Figure 1. WRAMP strategic elements and framework (modified from WRAMP 2010).

Introduction to EPA Three-Tiered Monitoring Structure

In 2002, a consortium of scientists and managers from around the state began developing a monitoring and assessment program modeled after USEPA’s Level 1-2-3 framework for monitoring and assessment of wetland resources. The fundamental elements of this framework are as follows (modified from WRAMP 2010 and USEPA website, accessed June 2015; Figure 2):

Level 1: A broad landscape-level characterization consisting of wetland and riparian inventories (e.g., National Wetland Inventory) or to answer questions about wetland extent and distribution. Assessment results can also provide a coarse gauge of geology and hydrology of a watershed, broad impacts, or wetland type.

Level 2: Rapid assessment methods, which use cost-effective field-based diagnostic tools to assess the condition of wetland and riparian areas. Level 2 assessments answer questions about general wetland health along a gradient through qualitative assessments and “stressor checklists”.

Level 3: Intensive site assessments to provide data to validate rapid methods, provide more thorough or rigorous datasets, characterize reference conditions, and diagnose causes of wetland condition observed in Levels 1 and 2. Level 3 assessments can be used to test hypotheses and provide insight into functions and processes.

All three Levels of the USEPA’s three-tiered structure should be implemented to some extent; however, the strength of site-intensive assessments to provide data on function, specific species, or detailed restoration trajectories is a vital component of any monitoring program. Level 1 and 2 provide needed preliminary information on wetland area and condition which is needed to develop and implement a site-intensive (Level 3) monitoring program.

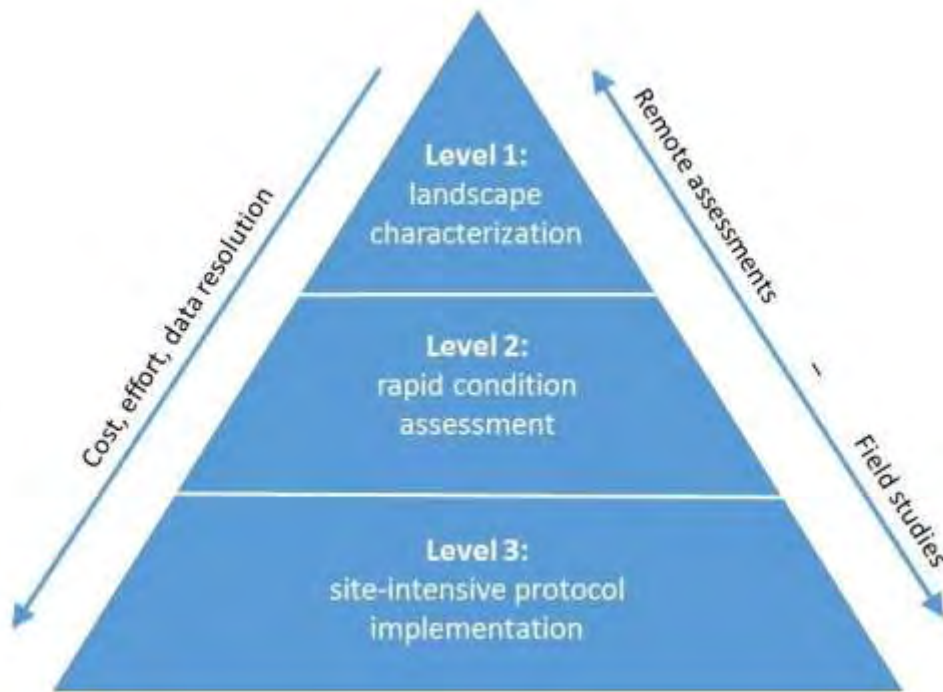


Figure 2. Conceptual model of USEPA three-tiered wetland monitoring structure.

Need for Level 3 Assessment

General practice has been for each individual restoration project to independently develop monitoring approaches and protocols. Not only is this inefficient, compilation and comparison of data across projects is then difficult at best or infeasible in many situations. This in turn limits regional synthesis and broader-scale condition assessments.

Although progress has been made over the last several years in developing standardized rapid (i.e., Level 2) assessment methods, there has been much less attention paid to standardized intensive (i.e., Level 3) assessment methods. Intensive assessment methods provide information on ecological function and process, are more diagnostic of restoration performance and regulatory compliance, and are important as a validation measure for rapid assessment methods. The lack of consistent approaches to intensive assessment limits the ability to share information between projects, precludes the use of Level 3 data in ambient monitoring, and fosters redundancy as each project develops its own protocols. While not comprehensive, this manual begins to standardize the Level 3 implementation process.

Organization of the Manual

This manual is organized into several chapters focused on the three tiers of the USEPA monitoring program (Figure 2, above), with substantial emphasis placed on the rigorous, site-intensive chapter (Level 3). The Level 1 chapter should be regarded as containing introductory information only, including several links to various databases and literature for more details. The Level 2 chapter includes summaries of two rapid assessment strategies, the California Rapid Assessment Method (CRAM) and Photo Point. CRAM is a standardized condition assessment score and is broadly discussed, with detailed

methods in the referenced literature and manuals specific to CRAM. The Photo Point Level 2 survey protocol is included primarily as a method of qualitative visual observation over time which serves to provide supplementary or ancillary information to Level 3 assessment methods.

Within the Level 3, or site-intensive monitoring protocol chapter, there are sections pertaining to protocol development for each of the focus parameters, including ambient water quality, soil characteristics, vegetation, fish, birds, mammals, and invertebrates. Each parameter section contains detailed evaluations and comparisons (if applicable) of one or multiple protocols [or Standard Operating Procedures (SOPs)] based on ecological indicators such as the biological diversity of a community (Figure 3). The protocols were chosen based on frequency of use in other monitoring programs throughout the State of California (Appendix A) and are therefore not a comprehensive list of protocols that may be necessary to implement a full monitoring program. Individual monitoring programs may need to supplement the recommended list, depending on individual project goals. Each parameter section contains summary information related to implementation time/effort and data quality for protocols outlined in each SOP, with full detailed objectives, implementation details, and comprehensive categorical protocol comparison matrices (individually for each SOP) included as Appendix B (Table 2).

In addition to the Level 3 SOPs, two Level 2 protocols (CRAM and Photo Point) each also have implementation details in the Level 2 chapter below, as well as individual SOPs in Appendix B.

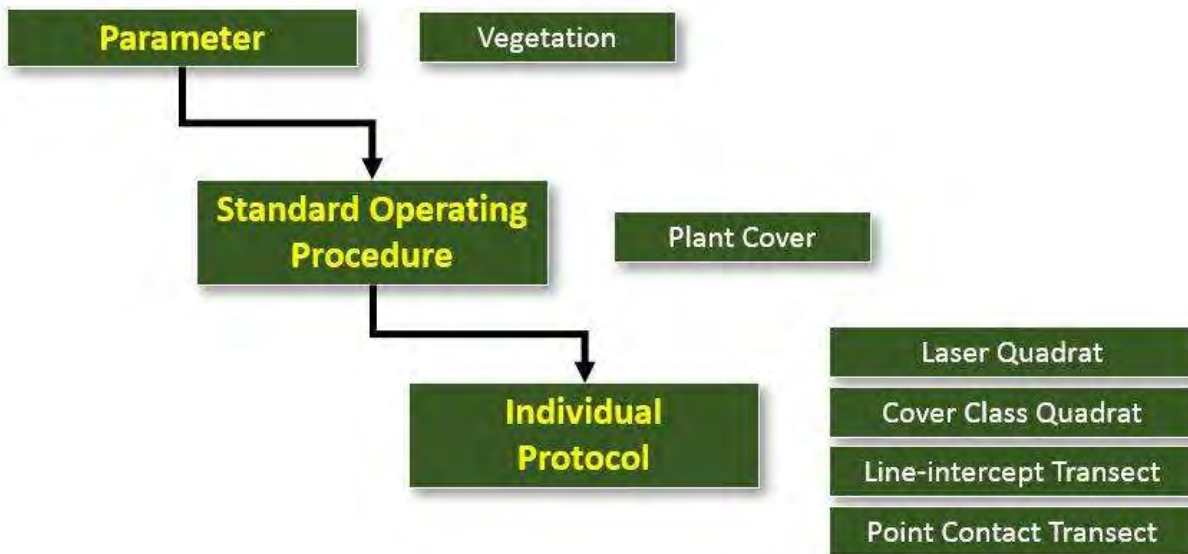


Figure 3. Graphic flow chart of parameter section outline.

After the Level 3 chapter is a new set of chapters for this version of the manual (Version 2.0) specific to data consolidation and the ability to combine disparate data sets from different monitoring programs or projects. Additionally, there is another new chapter (Version 2.0) for the importance of metadata and recommendations for standardization of its use.

Organization of Standard Operating Procedures (SOPs)

Each SOP within Appendix B is structured as a stand-alone document and is referred to as individual sections labelled by parameter and protocol number (e.g., Vegetation mapping = Appendix B, SOP 3.6 or Appendix B, Parameter 3, SOP 6). The detailed structural outline of each SOP includes an introduction regarding the sampling objective(s), a description of equipment and supplies needed to implement the SOP, a summary of field preparations and methods, any applicable laboratory methods, data entry and quality assurance / quality control procedures, a summary of potential data analysis methods, suggested datasheets, and any required health and safety precautions that should be considered before implementation. Each SOP also contains a detailed list of references and applicable literature related to the development of the SOP.

Each of the 18 SOPs (i.e., Appendix B, SOPs 1.1 – 8.1) includes an evaluation matrix describing the applicability of each protocol within a set of principal habitat types for coastal, perennially open estuaries (e.g., flooded marsh plain to degraded fill sediments) that characterize the ambient condition of estuarine wetland resources. For example, some protocols are not easily applied in intertidal habitat types and some are not applicable within areas of modified or restricted tidal regimes (e.g., degraded fill areas). Additionally, each SOP contains a detailed evaluation matrix assessing the time and effort levels required to implement, personnel requirements, the survey (or data) quality (e.g., accuracy, precision, and type of output), and the potential limitations of that SOP. These evaluations are largely categorical, but the analyses and/or logical reasoning used to inform the recommended SOPs/protocols can be found in the conclusions section of this manual.

Table 2 contains a list of the SOPs (N = 18) and protocols (N = 30) described in Appendix B of this manual within the broader parameter-based categories of ambient water quality, soil characteristics, vegetation, fish, birds, mammals, and invertebrates. Additional Level 2 parameters (i.e., CRAM and photo point) were included to allow for cross-level correlations and evaluations and to further validate the CRAM scoring metrics. Most parameters included a variety of evaluated protocols (e.g., nine protocols to assess vegetation), with several exceptions. As water quality can be evaluated for a multitude of biological (e.g., bacteria), chemical (e.g., constituents of concern), or physical (e.g., hydrological connectivity, flow) elements, and due to the large number of potential protocols and site-specific concerns, fully evaluating water quality was outside the scope of this manual/program. And as mammals are not commonly part of regional monitoring programs, assessing the variety of trapping techniques was also outside the scope of this manual/program. Instead, the focus was on the most commonly surveyed parameters based on a statewide monitoring program literature review (see also “Indicator and Protocol Development” section, below).

Table 2. List of Standard Operating Procedures described in Appendix B of this manual.

Parameter	SOP	Protocol	Appendix
Water Quality	Continuous Data Sonde	General WQ Parameters	B – 1.1
Soil Characteristics	Soil Salinity and Characteristics	Soil Salinity	B – 2.1
		Pore Water Salinity	B – 2.1
		Soil Texture	B – 2.1
	Soil Grain Size Analysis	Soil Particle Grain Size	B – 2.2
Vegetation	Submerged Aquatic Vegetation and Algae	Submerged Aquatic Vegetation and Algae	B – 3.1
	Cover	Point Contact	B – 3.2
		Line-Intercept	B – 3.2
		Cover Class Quadrat	B – 3.2
		Laser Quadrat	B – 3.2
	Biomass	Biomass	B – 3.3
	Seedbank	Seedbank	B – 3.4
	Vegetation Mapping	Alliance and Association Mapping	B – 3.5
Seed Collection and Germination	Seed Collection and Germination	B – 3.6	
Fish	Beach Seine	Beach Seine	B – 4.1
	Minnow Trap and Enclosure Trap	Minnow Trap	B – 4.2
		Enclosure Trap	B – 4.2
	Fish Cameras	Baited Remote Underwater Videos (BRUVs)	B – 4.3
Birds	Abundance and Activity	Site-Wide	B – 5.1
		Box Count	B – 5.1
		Point Count	B – 5.1
Mammals	Motion Wildlife Cameras	Motion Wildlife Cameras	B – 8.1
Invertebrates	Benthic Invertebrates	Large Cores	B – 6.1
		Small Cores	B – 6.1
	Terrestrial Invertebrates	Aerial Arthropod Traps	B – 6.2
		Pitfall Traps (non-tidal)	B – 6.2
		Pitfall Traps (tidal)	B – 6.2
Level 2 - Rapid Assessment Methods	California Rapid Assessment Method	CRAM	B – 7.1
	Photo Point	Photo Point	B – 7.2

The suite of recommended protocols for Level 2 and Level 3 implementation are described in the conclusions section of this manual. Each SOP is contained in the appendices and is also available for download individually as a stand-alone document for implementation of that specific protocol. This Manual and associated SOPs will be available for free download at multiple websites, including [The Bay Foundation](#) and the [California Wetland Monitoring Workgroup portal](#). By focusing on an electronic release, a broader audience of potential users may be attained. When periodic updates are made, they will be included on each of the websites.



(photo included for illustrative purposes; photo: The Bay Foundation)

Level 1: Spatial and Landscape Assessment

Introduction

Wetland functions are not solely dependent on biological communities and chemical interactions but also physical position within the larger landscape features. Level 1 is the broadest and most financially efficient level of assessment across a large scale which relies primarily on office-based GIS tools and aerial images to assess wetland condition based on landscape level analyses (USEPA 2006). Level 1 assessments can provide a sample framework for on-the-ground higher intensity Level 2 and Level 3 monitoring assessments.

Level 1 assessments can include wetland acreage trends and assessments characterizing adjacent landscapes including road density, land use, and presence of disturbances (e.g., drainage ditches, roads, levees) (USEPA 2006). Within the framework of this manual, Level 1 assessments are used to establish a geographic foundation for identifying locations to implement more intensive monitoring strategies (e.g., Figure 4). Existing databases or data layers may be useful for implementing a Level 1 analysis such as the [National Wetland Inventory](#), [California Aquatic Resource Inventory \(CARI\)](#), [EcoAtlas](#), [CA Wetland Status and Trends Program](#), [Surface Water Ambient Monitoring Program \(SWAMP\)](#), and many more.

While limited in its capacity to collect high-resolution condition information, Level 1 assessments can be useful for collecting quality broad-scale landscape data including:

- 1) Summary of wetland distribution, type, and abundance;
- 2) Identification of specific wetland area locations, size, and type to be monitored;
- 3) Characterization of land uses of wetland areas and adjacent landscapes including the identification of wetland buffer resulting in a coarse gauge of wetland condition;
- 4) Identification of ownership within selected wetland parcels to facilitate appropriate communication to obtain site access; and
- 5) Identification of broader status and trends of wetland types across a large geographic region (e.g., probabilistic approaches) (Stein et al. 2015).

Level 1 assessments are a necessary first step to developing a sound monitoring plan by creating the geographic foundation to help prioritize appropriate areas to implement more intensive Level 2 and Level 3 protocols. These assessments assist in the identification of potential reference sites for the monitoring program and areas which may require more robust survey methods to accurately characterize and assess wetland condition. While in-depth Level 1 assessments (e.g., landscape disturbance indices) may not be required to achieve most monitoring goals, at a minimum Level 1 methods should be used to preliminarily identify monitoring areas or appropriate criteria for in-depth evaluations. Prior to allocating monitoring stations, the properties of selected areas should be validated through *in-situ* field verification or Level 2 assessments to ensure their applicability for inclusion within the context of the monitoring goals (e.g., verify areas are the correct habitat and wetland type).

Detailed descriptions of potential Level 1 assessments are beyond the scope of this manual, but additional information may be found on the EPA's website (<http://www.epa.gov/>).

Limitations and Challenges of Level 1

Level 1 assessments are limited to coarse scale analyses appropriate for gauging wetland condition over large or inaccessible areas where more intensive methods will not be implemented and/or identifying appropriate areas for Level 2 and 3 assessments. Level 1 is the most cost-effective, yet least detailed tier of assessments within the Level 1-2-3 structure. It provides useful information at large geographic scales and allows basic wetland extent and distribution questions to be answered.

GIS-based assessments of wetland areas within highly urbanized landscapes may be challenging given the increased complexity of identifying disturbances caused by anthropogenic structures and landforms (e.g., roads, levees, culverts). Identifying the intensity of degradation may be difficult to evaluate without *in situ* field verification. Therefore, it is recommended that all areas identified as appropriate for more intensive monitoring through Level 1 assessments should be ground-truthed prior to finalizing a monitoring plan.

One example demonstration of a basic application of Level 1 data can be found in Figure 4, which contains data layers identifying wetland type from the National Wetlands Inventory overlaid on an aerial image of Steamshovel Slough at the Los Cerritos Wetlands Complex in southern California. This Level 1 example identifies specific areas within the larger complex which may be appropriate for implementing protocols described in this manual. However, in this example and throughout southern California estuarine wetlands, historic impacts and modifications to the landscape may have made identifying habitat types, continuity of tidal regimes, and degrees of disturbance difficult to determine. Thus, *in situ* ground-truthing is recommended using Level 2 or Level 3 protocols to verify appropriate habitat types and to conduct detailed condition evaluations. In one example, two on-the-ground Level 2 Photo Point landscape photographs (Figure 5) depict areas of varying hydrology and condition within the original Level 1 wetland habitat classification which may not be appropriate for grouping when designing a sampling plan.



Figure 4. Example of wetland habitat data layers at the Los Cerritos Wetland Complex downloaded from the National Wetlands Inventory database (accessed: June 2013).



Figure 5. Panorama photographs of Steamshovel Slough at Los Cerritos Wetlands showing tidally influenced reference wetlands (A) and degraded, non-tidal wetlands (B).

Level 2: Rapid Condition Assessments

Introduction to CRAM

California has adopted Level 2 rapid wetland assessment methods to conduct standardized monitoring and condition assessments (CWMW 2010, USEPA 2006) and to facilitate information transfer between projects, while allowing for a condition-level comparison to reference or more 'natural' wetland sites (Sutula et al. 2006). In California, the California Rapid Assessment Method (CRAM) was developed by the California Wetland Monitoring Workgroup (CWMW) as a field-based diagnostic tool that can be used to cost-effectively monitor the condition of streams and wetlands throughout California (CWMW 2013). CRAM supports the State's Wetland and Riparian Area Monitoring Plan (WRAMP) as developed by the CWMW. All CRAM testing, validation, and implementation are coordinated on an ongoing basis by an oversight committee of the CWMW that focuses on the development and implementation of RAMs in California. According to the CRAM User Manual (CWMW 2013): "The overall goal of CRAM is to provide rapid, scientifically defensible, standardized, cost-effective assessments of the status and trends in the condition of wetlands and the performance of related policies, programs and projects throughout California..."

CRAM can be used as a measure of general aquatic resource health and produces condition scores that are comparable and repeatable for all wetlands and regions in California yet accommodates special characteristics of different types of wetlands. For the purposes of CRAM, *condition* is defined as the state of a wetland assessment area's buffer and landscape context, hydrology, physical and biological structure relative to the best achievable states for the same type of wetland. Condition is evaluated based on observations made at the time of the assessment, the results of which can be used to infer the ability to provide various functions, services, values, and beneficial uses to which a wetland is most suited (CWMW 2013), although these are not measured directly by CRAM. CRAM also identifies key anthropogenic stressors that may be affecting wetland condition with a checklist. Additional guidance for the use of CRAM for project assessment as an element of regulatory, grant, and other management programs is available in a technical bulletin update (CWMW 2019). Thus far, the California State Coastal Conservancy is the first agency to adopt the protocol by requiring it for new projects.

According to Solek et al. 2012, "the integration of rapid assessment methods with probability-based regional survey designs provides a cost-effective means for making unbiased assessments of wetland condition over a relatively large area within a short period of time." While limited in its capacity to collect site-intensive or species-specific quantitative information, Level 2 assessments are useful for collecting information related to the four primary attributes within a given Assessment Area (AA): (1) landscape and buffer context, (2) hydrology, (3) physical structure, and (4) biotic structure.

The attributes are all averaged to quantify a final assessment score between 25 (lowest condition) and 100 (highest condition) for each wetland module and AA analyzed which is related to functional capacity of health of a wetland area (Table 3). Additionally, these data provide context for the application of a

more structured Level 3 monitoring program. For example, the plant layer sub-metric may help inform an appropriate vegetation cover assessment method by identifying broad-scale height and biodiversity estimates. Each SOP references a connection to CRAM metrics, when appropriate.

Limitations and Challenges of CRAM, Level 2

Careful consideration should be undertaken in evaluating the specific requirements for a project-level assessment and AA placements. In some cases, an appropriate evaluation may involve the universe of potential AA locations and random allocations within an error margin. As CRAM is a categorical qualitative assessment of wetland condition and impacts and is not quantitative or specifically diagnostic of loss of function or causes of degradation, care should be taken on the interpretation of the scores. The overall condition of a wetland depends more on the diversity and levels of all its functions than the level of any one function.

Due to the slightly subjective nature of some CRAM metric assessments, effort should be made to maximize the accuracy of each assessment in accordance with the CRAM methodology. This effort should include several strategies: (1) CRAM practitioners attend a training course prior to field implementation; (2) field teams consist of multiple trained individuals to avoid observer bias; and (3) quality control checks performed by the Quality Assurance Officer.

Implementation of CRAM

CRAM implementation requires application of the most appropriate wetland type-specific module (e.g., depression versus bar-built estuary). Different modules exist for different types of wetlands and a flow chart to determine which CRAM module to use is available in the CRAM resources. Additional guidelines to choose CRAM modules in problematic AAs and determining CRAM AAs are available in the 2019 technical bulletin (CWMW 2019). There are both field and office components (below and Table 3); one AA takes approximately 2-4 hours to complete. Additionally, accurate CRAM assessments require multiple certified scientists who have undergone calibration and training. CRAM scores for each attribute and for the final score range between 25 (poorest possible condition score) and 100 (maximum possible points or the best possible statewide reference condition). For additional implementation details, see the CRAM SOP (Appendix B, SOP 7.1), and the CRAM User Manual (CWMW 2013).

Steps of a CRAM Assessment (replicated from CRAM Training documents online, accessed May 2015):

1. Assemble the background information;
2. Classify the wetland;
3. Verify the appropriate season;
4. Sketch the CRAM Assessment Area (AA) (e.g., Figure 6);
5. Conduct the office assessment portion of the AA;
6. Conduct the field assessment portion of the AA (including completing the stressor checklist);
7. Complete the quality control check of the data; and
8. Submit results online.

Table 3. Summary table of CRAM attributes; descriptions modified from the CRAM User Manual (CWMW 2013).

Attribute	Metric	Sub-metric	Description	Assessment Location
Landscape and Buffer Context	Aquatic Area Abundance	---	Spatial association to adjacent areas with aquatic resources	Office
	Buffer	Percent of AA with Buffer	Relationship between the extent of buffer and the functions it provides	Office
		Average Buffer Width	Extent of buffer width assesses area of adjacent functions provided	Office
		Buffer Condition	Assessment of extent and quality of vegetation, soil condition, and human disturbance of adjacent areas	Field
Hydrology	Water Source	---	Water source directly affects the extent, duration, and frequency of hydrological dynamics	Office / Field
	Hydroperiod	---	Characteristic frequency and duration of inundation or saturation	Office / Field
	Hydrologic Connectivity	---	Ability of water to flow into or out of a wetland, or accommodate flood waters	Office / Field
Physical Structure	Structural Patch Richness	---	Number of different obvious physical surfaces or features that may provide habitat for species	Field
	Topographic Complexity	---	Micro- and macro-topographic relief and variety of elevations	Field
Biotic Structure	Plant Community Composition	Number of Plant Layers	Number of vegetation stratum indicated by a discreet canopy at a specific height	Field
		Number of Co-dominant Species	For each plant layer, the number of species represented by living vegetation	Field
		Percent Invasion	Number of invasive co-dominant species based on Cal-IPC status	Field
	Horizontal Interspersion	---	Variety and interspersion of different plant “zones”: monoculture or multi-species associations arranged along gradients	Field
	Vertical Biotic Structure	---	Interspersion and complexity of plant canopy layers and the space beneath	Field



Figure 6. Map of CRAM Assessment Areas at Steamshovel Slough in the Los Cerritos Wetlands created using GIS.

The CRAM web site (www.cramwetlands.org) provides access to an electronic version of the user and field manuals, training materials, eCRAM and the CRAM database. It is important that the user checks for updates to manuals and technical bulletins before implementing CRAM to ensure the most recent methods are being used. CRAM results can be uploaded to the database, viewed, and retrieved via the CRAM web site using eCRAM.

Level 2: Photo Point Survey

The primary purpose of this sampling method is to visually capture broad changes in the landscape and vegetation communities over seasons or years. This method collects georeferenced photos for use in site management (e.g., invasive species tracking) and long-term data collection. Each year (or seasonally), a set of landscape or panorama photographs is taken at permanent locations and bearings to ensure comparable photos. If annually, the targeted time is during mid- to late summer or during the peak growing season depending on the habitat's specific vegetation community.

Photo Point surveys do not yield quantitative data but are informative for visual landscape-scale changes and can be useful as visual baselines for areas that have undergone significant changes (e.g., post-restoration changes over time). Additionally, Photo Point can be used to visually assess variability

in hydrology, such as low and high tides (Figure 7). Detailed methods can be found in Appendix B (SOP 7.2, Photo Point). Photo point surveys are appropriate for all habitat types.

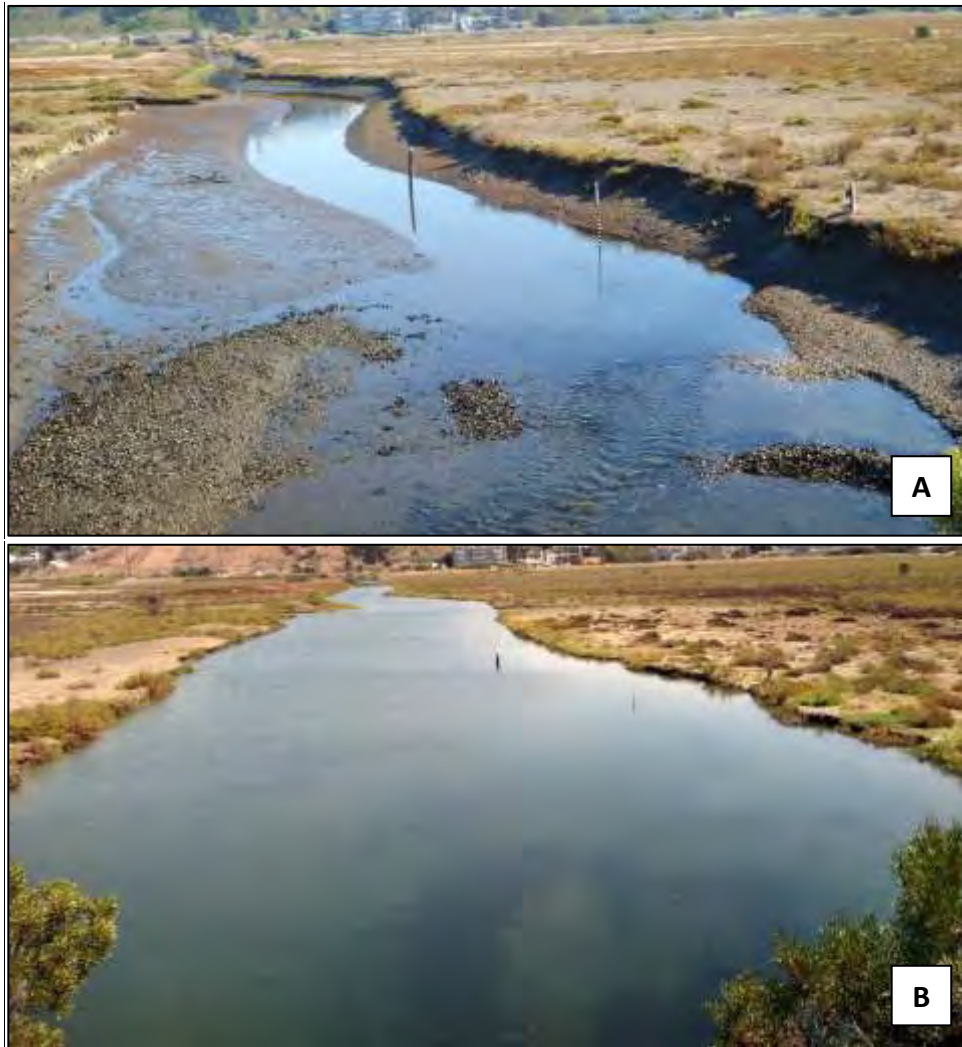


Figure 7. Example photographs at Photo Point Station 3 at the Ballona Wetlands Ecological Reserve, bearing 140° on (A) 27 October 2011 (low tide), and (B) 15 August 2013 (high tide).

Additional Level 2 Rapid Assessments

Additional standardized rapid assessments may also be used when the characteristics of a particular area are ambiguous, or finer scale detail and higher quality is desired or required for a particular parameter. These other types of rapid assessments frequently apply to vegetation communities. For example, the vegetation-specific Relevé assessments (CNPS 2007) are described in Appendix B, SOP 3.5 – Vegetation Mapping) and can be used to provide supplementary information to a larger vegetation survey map or for broader cover estimates over a larger area than a Level 3 transect. Additionally, the USEPA prepared the “Review of Rapid Assessment Methods for Assessing Wetland Condition” (USEPA 2004). While it does not contain information specifically about CRAM, it compares a variety of assessment methods throughout the United States.

Level 3: Site-Intensive Protocol Implementation and Evaluation

Introduction

Level 3 assessment methods are a collection of more rigorous monitoring methods that provide high resolution information on the condition of wetlands within an assessment area, often employing wetland bioassessment procedures or intensive plant, faunal, soil, or water quality analysis. The focus of this manual is to evaluate a variety of protocols assessing several key parameters (i.e., water and sediment quality, vegetation, birds, and invertebrates) within a variety of estuarine wetland habitats.

The robust measures used in Level 3 assessments produce information that can be used to:

- Refine or validate rapid assessment methods based on a characterization of reference condition and specific functions;
- Assess causes and consequences of wetland change;
- Judge the efficacy of management interventions;
- Evaluate specific wetland functions;
- Develop design and performance standards for wetland restoration, including compensatory wetland mitigation; and
- Support the development of water quality standards that are protective of wetlands.

Level 3 intensive assessment methods also provide information on ecological function and process at a higher resolution and provide more specific information than Level 1 and 2 assessments. The lack of consistent approaches to intensive assessments has previously limited our ability to share information between projects, precluded use of Level 3 data in ambient monitoring, and fostered inefficiencies as each project developed its own protocols.

Level 3 tools allow for the collection of quantitative data within a variety of wetland habitat types to assess site specific issues or to provide information on specific species, including presence data and abundances or cover assessments. Often Level 3 tools facilitate the collection and evaluation of more accurate or more precise data, with less susceptibility to subjectivity. Additionally, analyses of Level 3 data often allow for the determination of higher level connectivity or function.

Broad Guidelines for Setting up a Level 3 Program

It is important to ensure that a monitoring program is effectively and efficiently designed with established goals, objectives, timelines, and detailed protocols. This manual is meant to address several pieces in wetland monitoring program development, namely specific protocol implementation strategies as well as suggested frequency and timing. However, the sampling design of monitoring programs will depend on site-specific management needs. Monitoring needs often differ between projects and sites, (e.g., restoration, mitigation, reference sites), or with variable goals (e.g., assess water quality changes over time, track vegetation development or condition trajectories, education). However, as a general rule, large-scale or highly diverse wetlands may require increased frequency of implementing monitoring protocols.

The protocols may be applied in multiple types of sampling designs set up to assess wetland condition with statistical rigor, while maximizing available management resources (USEPA 2002a). For example, restoration projects may require a before/after, control/impact (or BACI) design, while others may focus solely on a stratified random sampling design by habitat type. Both types of designs allow for the specific application of the protocols with a flexible approach.

Identifying the appropriate protocols to implement for a monitoring program will be assisted by the implementation of Level 1 and 2 assessments. For example, Level 1 assessments will allow for the determination of wetland area, distribution, and type, and Level 2 CRAM assessments will allow for the identification of an area-specific hydrology regime within each wetland and the preliminary evaluation of several habitat types to identify which protocols are the most feasible and appropriate for that particular site (see habitat applicability tables in Level 3 section, below).

Limitations and Challenges of Level 3

While individual protocols provide high resolution datasets for discrete parameters, broader scale questions regarding overall wetland health or ecological function will require the implementation of multiple SOPs to provide supplementary information covering a range of ecological indicators. However, given adequate resources and an appropriate monitoring plan, the range of possible site-intensive data sets should provide sufficient information to address most research or monitoring goals.

Although monitoring plans based on standardized Level 3 protocols allow for detailed data collections, the application of Level 3 sampling designs and protocol-level assessments may be constrained by restrictions or limitations of a larger institutional framework (e.g., regulatory or permitting agencies). For example, the California Coastal Commission applies an extensive assessment process to individual restoration project protocols and monitoring to best evaluate success criteria. In another example, some long-term or national monitoring programs have limited ability to alter their existing sampling design or monitoring program. Crosswalks may need to be applied to achieve comparable data sets, or potentially the application of different protocols implemented concurrently. Based on research informing this manual, slight variations in protocols (e.g., vegetation cover) may have the consequence of making the data non-transferrable. These topics are discussed in the 'Data Evaluation and Consolidation' chapter and individual SOPs in Appendix B. The local implementation of any recommended protocol should allow for the opportunity to be tailored to site-specific constraints.

Site-specific requirements may also prohibit the implementation of standardized Level 3 monitoring methods. On-the-ground application of Level 3 protocols can have significant challenges in categories that range from permit limitations, varying degrees of impacts, to the higher costs and effort needed to implement a range of intensive protocols. There are often site-specific limitations such as accessibility and required permits. In California, there are also often challenges associated with a high degree of urbanization surrounding the site. For example, several of the larger coastal, estuarine wetlands in southern California have semi-permanent encampments or large amounts of trash or debris in some of the transitional or upland areas adjacent to the wetland habitats resulting in potential safety issues (Figure 8). There may also be major roadways or freeways bisecting the site that significantly alter the habitat types and/or may require modifications to the sampling design.



Figure 8. Photographs of human impacts at the Ballona Wetlands Ecological Reserve (top), and a higher condition estuarine habitat area (bottom).

There are also individual protocol limitations based on their individual suitability to a range of habitat types. For details on each protocol, see the “Tested Protocol” evaluation section, below, or refer to the individual evaluation matrices in each SOP (Appendix B).

Indicator and Protocol Development

Ecological indicators for the primary parameters evaluated for the Level 3 assessments are included in Table 4. Three categories of protocols (or methods) were evaluated for each of the parameters and indicators, including “simple visual estimates or categorizations,” “structured methods,” and “tool-based methods.” For some indicators, not all three categories of protocols had associated methods (e.g., no tool-based method for SAV cover was evaluated). The simple visual estimate category can be defined as closer to a Level 2 protocol. These are rapid, field-based methods, and often provide qualitative or categorical data results. The structured methods are site-intensive, Level 3 protocols that involve quantitative measurements in the field and provide an immediate *in situ* assessment. The tool-based methods are site-intensive, Level 3 protocols that involve specific quantitative measurements, but require some additional degree of processing, analysis, or specialized equipment (e.g., laboratory post-collection processing or equipment) to occur subsequent to the field data collection phase.

While not all the over 75 protocols described below were evaluated as part of this program, a representative subset from each parameter were chosen (total N = 30). Each individual monitoring program should go through its own decision support matrix for the selection of Level 3 indicators based on specific monitoring objectives and restoration goals.

Table 4. Ecological indicators for each of the primary parameters evaluated for the Level 3 assessments and the associated protocol options.

Parameter	Indicator or Metric	Method or Protocol			
		SIMPLE VISUAL ESTIMATE / CATEGORIZATION	STRUCTURED METHOD	TOOL-BASED METHOD	
VEGETATION	SAV / algae	gross cover	cover classification and/or categorical	line-intercept; point-contact; cover classes within a quadrat	----
		compositional diversity	cover classification and/or categorical	line-intercept; point-contact; cover classes within a quadrat	----
		biomass	gross visual estimate or category	harvested tissue weights	additional laboratory assessment / processing (constituents; carbon)
	Adult	gross cover	cover classification and/or categorical (nativity); broad estimate	line-intercept; point-contact; cover classes within a quadrat	laser quadrat; photo quadrat (post processing)
		compositional diversity	cover classification and/or categorical; broad estimate	line-intercept; point-contact; cover classes within a quadrat	laser quadrat; photo quadrat (post processing)
		biomass	gross visual estimate or category	harvested tissue weights (above and/or below ground)	additional laboratory assessment / processing (constituents; carbon)
		structure	categorical topographic complexity and/or canopy height (rugosity)	measured canopy heights; quantified physical assessment; individual-level physiology (branching)	pin-drop board for small-scale rugosity
		reproductive health / recruits	presence of flowers or indicators; evidence of recruitment (germinated seedlings)	number of flowers; stage classes	----
	distribution	presence / absence; categorical cover	presence within area / habitat; grouping	mapping extent of species or alliances; tracking invasives spread	

Parameter		Indicator or Metric	Method or Protocol		
			SIMPLE VISUAL ESTIMATE / CATEGORIZATION	STRUCTURED METHOD	TOOL-BASED METHOD
		vigor / health	presence / absence; categorical cover of live/dead; color	line-intercept; point-contact; color or live/dead frequency	color cards / pigment-based; laser quadrat for live/dead
	Seed Bank	density	categorical presence of recruits	quantitative field counts	soil core germination (greenhouse)
		compositional diversity	categorical presence of recruits	quantitative field counts	soil core germination (greenhouse)
AVIFAUNA	Adult	compositional diversity	presence / species lists	bird counts (all); point counts; call back surveys	georeferenced points (post-processing using GIS)
		density	visual ID, presence; categorical	bird counts (all); point counts; call back surveys	georeferenced points (post-processing using GIS)
		distribution	habitat-based categorical	territory maps; probable range; special status species	georeferenced points (post-processing using GIS); habitat-based analyses
		behavior	actions; sounds	movement (migration); counts of actions or sounds	banding; tracking
	Reproductive Capacity	health (functional assessment)	nest presence; fledgling presence; apparent condition	counts; clutch size	weights; gut content
TERRESTRIAL INVERTEBRATES	Aerial	productivity	----	sticky traps (size class & biomass)	----
		density	visual ID, presence; categorical	sticky traps (number per trap?)	----
		compositional diversity	visual ID, presence; categorical; presence of indicator species (e.g., mosquitoes)	sticky traps (species or group-level ID)	----

Parameter	Indicator or Metric	Method or Protocol			
		SIMPLE VISUAL ESTIMATE / CATEGORIZATION	STRUCTURED METHOD	TOOL-BASED METHOD	
	distribution	presence of indicator species (e.g., mosquitoes)	habitat or area-based assessments using sticky traps	-----	
	Epigeal	productivity	-----	pitfall traps (size class & biomass)	-----
		density	visual ID, presence; categorical	pitfall traps (number per trap?)	-----
		compositional diversity	visual ID, presence; categorical; presence of indicator species (e.g., tiger beetle)	pitfall traps (species or group-level ID)	-----
		distribution	presence of indicator species (e.g., tiger beetle)	habitat or area-based assessments using pitfall traps	-----
BENTHIC INVERTEBRATES	Infauna	density	visual ID, presence; categorical	counts in cores	-----
		compositional diversity	visual ID, presence; categorical; presence; cores w/presence	species- or group/taxa-level ID in cores	-----
	Epifauna	density	visual ID, presence; categorical	surface counts	-----
		compositional diversity	visual ID, presence; categorical; presence; cores w/presence	species- or group/taxa-level ID on surface	-----
	Invert-based functional assessment	trophic complexity (parasites)	presence; categorical	-----	speciate trematodes (post-processing in laboratory)
		pollinators	presence	collecting pollinators on transects	species-level analyses in laboratory or through taxonomist

Habitat Types Evaluated using Level 3 Assessments

As part of this manual development, protocol testing was conducted within six habitat types at five coastal, perennial estuarine wetlands in southern California. At some wetlands, all habitat types were evaluated, but not all wetland locations had each habitat type. Habitat types evaluated for each SOP (within all or a subset of the wetland locations) included: tidal channel, mud/sand flat, emergent salt marsh, non-tidal salt marsh, salt pan, and “degraded” or fill habitat. Figure 9 displays representative photographs of each habitat type at the Ballona Wetlands Ecological Reserve in Los Angeles, CA. The “degraded” habitats were identified *a priori* based on known impacts, stressors, and Level 1 analyses and then validated using CRAM scores. Details for the applicability or feasibility of each protocol in each habitat can be found in each SOP (Appendix B) and are summarized in the parameter sections, below.



Figure 9. Representative photos of the six habitat types at the Ballona Wetlands Ecological Reserve: (A) Tidal channel, (B) mudflat, (C) emergent salt marsh, (D) non-tidal salt marsh, (E) salt pan, and (F) degraded.

SOP Evaluation Matrix

A detailed, categorical evaluation of each Standard Operating Procedure (SOP) and protocol was conducted for this manual. Table 5 is a summary of all of the metrics used in the evaluation matrix and the type of output that is included in the summary tables for each SOP and protocol. The “Tested Protocol” section, below, contains summary time/effort and data quality information, with full evaluations included in the first appendix of each SOP.

Table 5. Full Standard Operating Procedure evaluation matrix and type of output (Note: L2 = Level 2; L3 = Level 3).

Category	Evaluation Metric	Type of Output
L2	Correlation to L2 CRAM	List of Attributes
L3: Time / Effort	Office Preparation Time	Categorical
	Equipment Construction Time (one time)	Categorical
	Field Time	Categorical
	Laboratory Time	Categorical
	Post-Survey Processing / QAQC Time	Categorical
	Minimum Repetition (site-dependent)	Categorical
	Relative Cost (equipment and supplies)	Categorical
L3: Survey / Data Quality	Accuracy (at a survey area level)	Categorical
	Precision (at a survey area level)	Categorical
	Type of Output	Categorical
	Qualitative-Quantitative Score	Categorical
	Subjectivity-Objectivity Score	Categorical
	Active or Passive Monitoring Style	Categorical
	Specialty Computer Software Required	Categorical
	Availability of Online / External Resources	Categorical
L3: Personnel Requirements	Specialty Equipment or Clothing Required	Categorical
	Ease of Transport (amount or weight of supplies)	Categorical
	Ease of Implementation	Categorical
	Expertise / Skill Level	Categorical
	Number of Personnel	Categorical
	Training Requirements	Notes
	Seasonality of Survey Time	Time Range
	Suggested Frequency	Categorical
L3: Potential Limitations	Wetland Type Applicability	Notes
	Images or Multi-Media Required	Categorical
	Degree of Impact / Disturbance	Categorical
	Vegetation Height Limitation	Categorical
	Appropriate for Tidal / Wet Habitats	Categorical
	Tide Height	Categorical
	Regional or Broad Implementation	Categorical
	Potential for Hazards / Risk	Categorical
	Restrictions	Notes

Evaluated or Tested Protocols

This manual includes a discussion of evaluated protocols only, not an exhaustive list of possible survey methods and parameters. Each of the SOPs contains the full categorical evaluation matrix described above, but summary information related to time/effort and data quality evaluations are summarized in each parameter section, below. Additionally, each SOP (Appendix B, SOPs 1.1 – 8.1) contains detailed information on how to implement each protocol along with suggested datasheets and analysis methods.

Each parameter section, below, contains the overall monitoring objectives for those SOPs (e.g., to collect ambient data, to assess the vegetation community, etc.), a summary habitat suitability table, an abbreviated comparative evaluation matrix, and a comparative discussion of the protocol evaluation. The monitoring objectives at the wetlands evaluated for this manual varied from collecting pre-restoration baseline data at several degraded wetlands (i.e., Ormond Beach, Ballona Wetlands Ecological Reserve, Los Cerritos Wetlands) to identifying potential reference sites (i.e., Carpinteria Salt Marsh, Mugu Lagoon, Tijuana Estuary). A subset of each of the protocols was evaluated at each wetland based on the goals of the individual monitoring program (e.g., there were no baseline vegetation data to support the restoration project planning at Los Cerritos, so vegetation data collection was a priority at that site). Version 2.0 of the manual did not collect new data, but instead evaluated existing monitoring data for the potential for data consolidation and assessment.

Water and Soil Quality

The assessment of water and sediment quality can provide supporting information about the physical forces affecting habitat distribution. Prevailing vegetation communities are directly linked to dominant hydrologic regimes, soil salinity, and composition (James-Pirri et al. 2002). Although it is typical (and suggested) to collect water quality information with hand-held instruments to spot-check conditions, such as during sampling associated with other parameters (e.g., seining), here we focus on water quality as collected with deployed data sondes. Water quality probes on multi-parameter sondes are used to measure water parameters in continuous monitoring mode by collecting data at user-defined intervals and storing those data until download at discrete intervals. Water quality multi-probes can be deployed continuously at monitoring stations to characterize parameters over multiple tidal cycles, through freshwater-input events, or over longer periods of time. Water quality sampling objectives may include quantifying specific water parameter (e.g., pH, temperature, salinity, chlorophyll depth) variations over time. Additionally, long-term deployment may begin to capture data on climate change impacts such as pH decreasing (acidification), temperature warming, or average depth change (coastal flooding).

Protocols assessing soil composition are aimed at characterizing soil properties such as salinity and texture. Salt composition and distribution within the soil profile affects many biological and chemical parameters including plant response, ion effects, and nutritional imbalances (NSSC 2009). Soil texture and individual phenotypic characteristics of each plant species are also widely understood to influence vegetation growth under various saline soil conditions.

It should be noted that this manual demonstrates sampling protocols designed to provide general water and sediment quality information and should not be used specifically to ensure regulatory compliance, but rather to provide supplementary information to support site-level analyses. While there are many other biological (e.g., bacteria) and chemical (e.g., constituents, heavy metals) parameters that can be

monitored, this manual focuses on several that are straightforward to implement, appropriate for long-term monitoring programs, and correlated to the vegetation community and habitat distribution.

This manual includes methods for one water quality and five soil quality monitoring protocols. Table 6 is a habitat suitability index containing appropriate estuarine wetland habitat types (of those evaluated) for each protocol. Habitat types that do not contain an “X” are either not compatible with the specific protocol or are not feasible (e.g., any habitats that are not fully submerged are not appropriate for a permanent data sonde assessing water quality parameters; habitats that are not saturated are not appropriate for pore water salinity).

Table 6. Appropriate habitat types for water or sediment quality monitoring protocols.

Survey Protocol	Habitat Types					
	Tidal Channel	Mud / sand flat	Emergent salt marsh	Non-tidal salt marsh	Salt pan	'Degraded' / fill
General WQ Parameters	X	X	X			
Soil Salinity		X	X	X	X	X
Pore Water Salinity	X	X	X		X	
Soil Texture	X	X	X	X	X	X
Soil Particle Size – LISST	X	X	X	X	X	X
Soil Particle Size – Hydrometer	X	X	X	X	X	X

A comparative assessment of cost, effort, and data quality are shown in Table 7. A matrix of additional detailed categorical evaluations to compare evaluated water and soil quality sampling protocols can be found in Appendix B, SOP 1.1A, 2.1A, and 2.2A. Evaluated protocols included several expensive, tool-based protocols (i.e., data sonde and soil particle analysis – LISST) that required the purchase, maintenance, and use of specialized equipment; and several protocols (i.e., soil salinity, pore water, and soil texture) that were easy to implement and replicate and had a very low associated cost. However, the specialized, tool-based protocols that collected quantitative data will have a higher level of accuracy and precision.

Table 7. Categorical assessment of cost/effort and data quality for water or sediment quality monitoring protocols.

Evaluation Metric	General WQ Parameters (sonde)	Soil Salinity	Pore Water Salinity	Soil Texture	Grain Size – LISST	Grain Size - Hydrometer	
	Time / Effort	Office Preparation Time	0-10 minutes	0-10 minutes	0-10 minutes	0-10 minutes	0-10 minutes
Equipment Construction Time (one time)		> 60 minutes	0-10 minutes	0-10 minutes	0-10 minutes	10-20 minutes	
Field Time (per deployment)		30-60 minutes	10-30 minutes	10-30 minutes	10-30 minutes	30-60 minutes	
Laboratory Time (per deployment)		> 60 minutes	> 60 minutes	0 minutes	0-10 minutes	> 280 minutes	Multiple days
Post-Survey Processing / QAQC Time		> 60 minutes	10-30 minutes	0-10 minutes	0-10 minutes	> 30 minutes	> 30 minutes

	Evaluation Metric	General WQ Parameters (sonde)	Soil Salinity	Pore Water Salinity	Soil Texture	Grain Size – LISST	Grain Size - Hydrometer
	Minimum Repetition (site-dependent)	Few Repetitions	Many Repetitions	Many Repetitions	Many Repetitions	Once	Once
	Relative Cost (equipment and supplies)	Very High (> \$10,000)	> \$50	\$15 - \$50	\$0	> \$15,000	> \$25
Survey / Data Quality	Accuracy (at a survey area level)	High	High	High	Medium	High	Medium
	Precision (at a survey area level)	High	High	High	Medium	High	Medium
	Qualitative-Quantitative Score	Quantitative	Quantitative	Quantitative	Qualitative	Quantitative	Quantitative
	Subjectivity-Objectivity Score	Objective	Objective	Objective	Subjective	Objective	Objective

Vegetation

Long-term monitoring of vegetation is one of the most common methods of evaluating the health and functioning of a wetland system (Zedler 2001). Changes in the composition of native and non-native plant species may affect the distributions of associated wildlife species and can be used as a proxy to infer physical characteristics and the effects of human impacts or restoration actions.

Vegetation cover sampling methods will likely vary depending on program objectives. As a result, this manual compares four vegetation cover sampling methods including: point-contact transect, line-intercept transect, cover class quadrat, and laser quadrat (SOP 3.2). Additional protocols evaluated include vegetation biomass (SOP 3.3), algae/submerged aquatic vegetation (SOP 3.2), seed bank (SOP 3.4), and mapping (SOP 3.5). Though not a “survey” per se, the seed collection and germination protocol is also included in this evaluation (SOP 3.6). Table 8 is a habitat suitability index containing appropriate estuarine wetland habitat types (of those evaluated) for each vegetation cover protocol. Habitat types that do not contain an “X” are either not compatible with the specific protocol or are not feasible. For example, the laser quadrat survey method is not appropriate in degraded habitat types with individual plant heights greater than approximately 1 m (quadrat placement infeasible) or those with grasses or thin-stemmed vegetation (reduced accuracy in wind, high variability). Similarly, the tidal, unvegetated habitats are most appropriately surveyed using the SAV/Algae protocol, and biomass and seed collection are also only appropriate in vegetated habitats.

In any study, the number of plots to sample is an important consideration. The appropriate number can be determined by plotting species numbers (or the cover of a given species) as a function of the number of quadrats sampled and then identifying where species richness “levels off” (USEPA 2002b). Another recommendation is that a total of 1% of the total wetland area be sampled (Krebs 1999). Transect-level surveys can be supplemented by Level 2 rapid assessments such as Relevé surveys (see [Level 2](#), above).

Table 8. Appropriate habitat types to implement vegetation survey protocols.

Survey Protocol	Habitat Types					
	Tidal Channel	Mud/sand flat	Emergent salt marsh	Non-tidal salt marsh	Salt pan	'Degraded' / fill
Algae	X	X	X	X	X	X
SAV (subtidal)	X	X				
Point-contact		X	X	X	X	X
Line-intercept		X	X	X	X	X
Cover Class		X	X	X	X	X
Laser Quadrat		X	X		X	
Biomass			X	X	X	X
Seed Bank			X	X	X	X
Mapping	X	X	X	X	X	X
Seed Collection			X	X	X	X

A comparative assessment of cost, effort, and data quality are shown in Table 9. A matrix of additional detailed categorical evaluations to compare evaluated vegetation assessment protocols can be found in Appendix B, SOPs 3.1A through 3.6A. Four transect-based quantitative cover protocols were compared (i.e., point contact, line-intercept, cover class quadrat, and laser quadrat). Vegetation mapping provides broad cover estimates, is closer to a Level 2 protocol on smaller scales and may need to be supplemented by one of the four transect-based methods or other vegetation rapid assessment methods. However, depending on desired resolution, vegetation mapping is the most time-consuming yet yields a site-wide picture of categorical vegetative cover while providing a foundation for a more exacting geographic sampling plan.

Of the cover estimates, laser quadrat was identified as the most time intensive and costly, but also the most accurate and objective. Both quadrat-based methods (i.e., laser quadrat and cover class) were more precise than either the point contact or line-intercept methods. Biomass and seed bank methods provide very different information than the cover estimates, and are thus, project specific. Seed collection protocols are likely only applicable associated with restoration activities.



Table 9. Categorical assessment of cost/effort and data quality by vegetation survey protocol.

	Evaluation Metric	Algae	SAV (subtidal)	Point Contact	Line-Intercept	Cover Class Quadrat	Laser Quadrat	Biomass	Seed Bank	Vegetation Mapping	Seed Collection	
Time / Effort	Office Preparation Time	10-30 minutes	10-30 minutes	10-30 minutes	10-30 minutes	10-30 minutes	10-30 minutes	10-30 minutes	10-30 minutes	> 60 minutes	10-30 minutes	
	Equipment Construction Time (one time)	30-60 minutes	30-60 minutes	10-30 minutes	10-30 minutes	10-30 minutes	> 60 minutes	0-10 minutes	10-30 minutes	Not Applicable	10-30 minutes	
	Field Time (per transect)	10-30 minutes	3-4 hours	10-30 minutes	10-30 minutes	10-30 minutes	30-60 minutes	10-30 minutes	30-60 minutes	Multiple days	> 60 minutes	
	Laboratory Time	30-60 minutes	0 minutes	0 minutes	0 minutes	0 minutes	0 minutes	30-60 minutes	> 60 minutes	Not Applicable	> 60 minutes	
	Post-Survey Processing / QAQC Time	10-30 minutes	10-30 minutes	10-30 minutes	10-30 minutes	10-30 minutes	10-30 minutes	10-30 minutes	30-60 minutes	> 60 minutes	10-30 minutes	
	Minimum Repetition (site-dependent)	Few Repetitions	Few Repetitions	Many Repetitions	Many Repetitions	Many Repetitions	Many Repetitions	Many Repetitions	Many Repetitions	Many Repetitions	Many Repetitions	Many Repetitions
	Relative Cost (equipment and supplies)	< \$15	< \$1,000	< \$15	< \$15	< \$15	\$15 – 50	< \$15	< \$15	> \$100	< \$15	
Survey / Data Quality	Accuracy (at a survey area level)	Medium	Medium	Low	Medium	Medium	High	High	High	Low to High	Not Applicable	
	Precision (at a survey area level)	High	High	Low	Medium	Medium	High	Medium	Medium	Medium	Not Applicable	
	Qualitative-Quantitative Score	Quantitative	Quantitative	Quantitative	Quantitative	Quantitative	Quantitative	Quantitative	Quantitative	Qualitative and Quantitative	Not Applicable	
	Subjectivity-Objectivity Score	Objective	Objective	Objective	Objective	Subjective	Objective	Objective	Objective	Subjective	Not Applicable	

Fish

Defining the full fish assemblage of a wetland can be difficult, due to the highly mobile nature and differing sizes of the fauna. Fish are often among the first organisms to rapidly colonize restored habitats (Zedler 2001, Johnston et al. 2011). Wetlands act as nursery habitat for commercially important species such as halibut (Beck et al. 2001) and are an easily assessed component of food web complexity, vertebrate diversity, overarching water quality conditions, and/or anthropogenic stressors (WRP 2006).

The primary purpose of this sampling method is to quantitatively assess the distribution, relative abundances, species richness, and diversity of fish in intertidal wetland habitats. While each type of fish sampling equipment (i.e., seines, trawls, minnow traps) exhibit some degree of preferential capture or limitations to specific fauna, beach seines, minnow traps, and enclosure traps are generally appropriate for shallow, slow-moving water in tide channels or the equivalent habitat, while trawls are appropriate for subtidal, deep water habitats that can tolerate a high degree of disturbance (e.g., deep water, high order channels with no sensitive benthos habitats or species) (Table 10). Protocols evaluated for this manual included beach seines, minnow traps, enclosure traps, and fish cameras.

Table 10. Appropriate habitat types for fish survey protocols.

Survey Protocol	Habitat Types					
	Tidal Channel	Mud/sand flat	Emergent salt marsh	Non-tidal salt marsh	Salt pan	'Degraded' / fill
Fish Beach Seine	X	X				
Minnow Trap	X	X				
Enclosure Trap	X	X				
Fish Camera	X	X				

A comparative assessment of cost, effort, and data quality are shown in Table 11. A matrix of additional detailed categorical evaluations to compare evaluated fish survey protocols can be found in Appendix B, SOP 4.1A. Minnow traps offer several advantages and disadvantages. They are relatively easy to deploy and their use can be designed to have relatively minimal impact. They thus can be used to characterize fish during times when more intensive sampling might not be possible (e.g., the bird nesting season), and they are appropriate for species-specific targeted surveys (e.g., if the monitoring goals include collecting adult killifish). However, abundances are not tied directly to a specific area size and the data are therefore only comparable to other minnow trap surveys or to a broader abundance to time-deployed ratio. The minnow trap survey also does not work well for juvenile fish, small gobies, or other fish that are sensitive to reduced oxygen levels (e.g., topsmelt). While the beach seine is time-intensive, it is also more likely to more accurately sample the fish population. Baited cameras are highly effective at attracting scavengers and subsequent predators (Wilson and Smith 1984, Henriques et al. 2002, Davis et al. 2019). Additionally, they are non-extractive, cost-effective, and particularly useful when other forms of sampling are challenging due to uneven substrate (e.g., shell hash or oyster beds), fish handling restrictions, or other reasons (Dorman et al. 2012).

Table 11. Categorical assessment of cost/effort and data quality for fish beach seine survey protocols.

	Evaluation Metric	Fish Beach Seine	Minnow Trap	Enclosure Trap	Fish Camera (BRUV)
Time / Effort	Office Preparation Time	30-60 minutes	30-60 minutes	30-60 minutes	30-60 minutes
	Equipment Construction Time (one time)	> 60 minutes	10-30 minutes	> 60 minutes	10 minutes
	Field Time (per station)	> 60 minutes	10-30 minutes	> 60 minutes	2 + hours
	Laboratory Time (per station)	0 minutes	0 minutes	0 minutes	0 minutes
	Post-Survey Processing / QAQC Time	10-30 minutes	10-30 minutes	10-30 minutes	Variable
	Minimum Repetition (site-dependent)	Few Repetitions	Many Repetitions	Many Repetitions	Few Repetitions
	Relative Cost (equipment and supplies)	> \$1,000	> \$100	> \$100	> \$500
Survey / Data Quality	Accuracy (at a survey area level)	Medium	Low	Medium-Low	Medium
	Precision (at a survey area level)	Medium	Medium	Medium	Medium
	Qualitative-Quantitative Score	Quantitative	Quantitative	Quantitative	Quantitative
	Subjectivity-Objectivity Score	Objective	Objective	Objective	Objective

Birds

The presence and distribution of avifauna within an ecosystem is often used as an index of habitat quality due to their diet and vulnerability to environmental conditions (Conway 2008, Johnston et al. 2011, 2012). Bird communities are in constant flux. Turnover, especially at isolated sites, can be high with new species colonizing and rare species becoming extirpated (Cooper 2006). Regular, repeated surveys help maintain a clear picture of bird communities on a site. Additionally, sites with high habitat variability may employ multiple survey types to represent avifauna populations more accurately.

The primary purpose of these observational sampling methods is to develop maps of species presence, assess bird community distributions and activities, and collect information on species-specific site use. Additionally, bird survey methods may provide information on rare species and supplement historical or volunteer data. Recording the activity of each species will allow for an assessment of higher ecological function of the area or wetland. Bird surveys are conducted as an integral part of most monitoring programs, though each program has variations on the specific details of the surveys. Table 12 is a habitat suitability index containing appropriate estuarine wetland habitat types (of those evaluated) for each bird abundance and activity protocol.

Table 12. Appropriate habitat types for bird abundance and activity protocols.

Survey Protocol	Habitat Types					
	Tidal Channel	Mud/sand flat	Emergent salt marsh	Non-tidal salt marsh	Salt pan	'Degraded' / fill
Site-wide Survey	X	X	X	X	X	X
Box Count	X	X	X	X	X	X
Point Count	X	X	X	X	X	X

A comparative assessment of cost, effort, and data quality are shown in Table 13. A matrix of additional detailed categorical evaluations to compare evaluated bird survey protocols can be found in Appendix B, SOP 5.1A. The site-wide survey method was by far the most time-intensive assessment, but also was more accurately representative of the specific bird community in a given area and may be most appropriate for baseline assessments. Site-wide surveys were also more likely to capture cryptic or lower abundance species. However, both the box count and point count methods were effective at capturing most bird species present, involved considerably less disturbance, and could be implemented easily in significantly less time. Similarly, the point count method was the least disruptive to the birds and least intrusive into the wetland habitats. There was an edge effect for the boxes as their boundaries were difficult to discern at high tide and fairly inaccessible, whereas the point count method included most of the area within line-of-sight of each point.

Table 13. Categorical assessment of cost/effort and data quality for bird abundance and activity protocols.

	Evaluation Metric	Site-wide	Box Count	Point Count
Time / Effort	Office Preparation Time	10-30 minutes	10-30 minutes	10-30 minutes
	Equipment Construction Time (one time)	Not Applicable	Not Applicable	Not Applicable
	Field Time (per unit)	> 120 minutes	5 minutes per station	5 minutes per station
	Laboratory Time	Not Applicable	Not Applicable	Not Applicable
	Post-Survey Processing / QAQC Time	> 120 minutes	10-30 minutes	10-30 minutes
	Minimum Repetition (site-dependent)	Few Repetitions	Many Repetitions	Many Repetitions
	Relative Cost (equipment and supplies)	< \$150	< \$150	< \$150
Survey / Data Quality	Accuracy (at a survey area level)	High	Medium	Medium
	Precision (at a survey area level)	Medium	Medium	Medium
	Qualitative-Quantitative Score	Quantitative	Quantitative	Quantitative
	Subjectivity-Objectivity Score	Objective	Objective	Objective

Mammals

Mammalian species and other medium and large fauna fill a wide range of ecological roles and are a central component to maintaining balance within an ecosystem (IUCN 2014). From seed dispersal to the regulation of invertebrate and smaller mammal populations, the presence and abundance of large mammals may act as indicators of general ecosystem health (Jones and Safi 2011). Documenting the presence and relative abundances of larger wildlife can be difficult due to their high mobility, acute senses, nocturnal behavior, or general aversion to human interaction; however, the use of motion activated cameras provides a non-invasive, cost-effective method to capture medium and large wildlife presence (Moruzzi et al. 2002). The primary purpose of this sampling method is to visually confirm the presence of medium or large wildlife species residing within an area (Table 14). Additionally, this method can be used to assess movement of different species within or between specific geographical locations.

While there are many methods to survey mammals in upland habitat types (e.g., Sherman live traps, scent station monitoring, track station monitoring, etc.), only one of them other than anecdotal or observational evidence was appropriate for intertidal wetland habitats (i.e., wildlife motion cameras). Additionally, as mammals are not generally a focal point of wetland monitoring programs (Appendix A) and several other indicators may be used as a proxy for mammal use of the site (e.g., burrow holes or indirect evidence), only one protocol was evaluated for this parameter. The motion-activated camera traps were effective at capturing the presence of medium to large fauna in a specific area and somewhat useful for determining species-based ranges; however, in small arrays (e.g., 1-4 cameras), they do not provide quantitative data to assess abundance. Additionally, the motion cameras imprecisely capture the presence of smaller animals due to limitations in the camera activation sensitivities.

Table 14. Appropriate habitat types to implement the motion wildlife camera survey protocol.

Survey Protocol	Habitat Types					
	Tidal Channel	Mud/sand flat	Emergent salt marsh	Non-tidal salt marsh	Salt pan	'Degraded' / fill
Wildlife Camera	X	X	X	X	X	X

A summary assessment of cost, effort, and data quality are shown in Table 15. A matrix of additional detailed categorical evaluations for wildlife camera protocols can be found in Appendix B, SOP 8.1A.

Table 15. Categorical assessment of cost/effort and data quality for the motion wildlife camera survey protocol.

	Evaluation Metric	Wildlife Camera
Time / Effort	Office Preparation Time	10-30 minutes
	Equipment Construction Time (one time)	> 30 minutes
	Field Time (per station)	30-60 minutes
	Laboratory Time (per transect)	0 minutes
	Post-Survey Processing / QAQC Time	30-60 minutes
	Minimum Repetition (site-dependent)	Many Repetitions
	Relative Cost (equipment and supplies)	> \$50
Survey / Data Quality	Accuracy (at a survey area level)	High
	Precision (at a survey area level)	Medium
	Qualitative-Quantitative Score	Qualitative
	Subjectivity-Objectivity Score	Objective

Invertebrates

Aquatic benthic invertebrate taxa are useful ecological indicators because they provide a reflection of the state of the environment, especially at the transition from water to land and can indicate local biodiversity (Hilty and Merenlender 2000). The presence or absence of certain infauna (i.e., burrows into and lives in bottom sediments) or epifauna (i.e., lives on the surface of bottom sediments) within tidal channels can serve as indicators of water quality, anthropogenic stressors to the estuary, and the potential to support other trophic levels (WRP 2006); these benthic communities provide essential ecosystem services and support (Schreiber 1981). Additionally, terrestrial invertebrates are a vital component of wetland food webs and are indicators of the overall health of a system (Zedler 2001).

Invertebrate-related ecosystem function has traditionally been measured by enumerating and identifying invertebrates or insects to the species level to calculate compositional biodiversity. In practice, such approaches are costly, require extensive periods of sample interrogation, and therefore have resulting processing times on the order of months to years for monitoring efforts with robust / frequent sampling plans. Logistically, simpler and more rapid measures that more directly describe functions or rates of arthropod productivity may be better indicators of ecosystem health (Anderson 2009). The high diversity of coastal arthropods, a lack of existing, complete baseline inventories, and the growing dearth of qualified invertebrate taxonomists also make traditional high-resolution taxonomically focused terrestrial invertebrate assessments in this habitat expensive and difficult. Taxa should be assessed for both benthic and terrestrial invertebrate methods by sorting to the lowest taxonomic level possible including recognizable taxonomic units (RTU).

The primary purpose of the aquatic benthic invertebrate sampling method is to assess the benthic invertebrate community by collecting data on the density and distribution of infauna within wetland tidal channels. The primary purpose of the terrestrial invertebrate sampling methods is to document aerial and epigeal (above soil surface) arthropod productivity (as biomass per unit area, or productivity as biomass per day) for each habitat or area by extrapolation from enumerated arthropods via length-fresh weight regressions.

Table 16. Appropriate habitat types for invertebrate survey protocols.

Survey Protocol	Habitat Types					
	Tidal Channel	Mud/sand flat	Emergent salt marsh	Non-tidal salt marsh	Salt pan	'Degraded' / fill
Benthic Cores	X	X	X	X	X	X
Aerial Traps			X	X	X	X
Pitfall Traps (non-tidal)				X	X	X
Pitfall Traps (tidal)		X	X			

A comparative assessment of cost, effort, and data quality are shown in Table 17. A matrix of additional detailed categorical evaluations to compare evaluated invertebrate survey protocols can be found in Appendix B, SOP 6.1A and 6.2A.

As each protocol assesses an independent component of the greater invertebrate community or is specialized for a particular tidal regime, a cross-protocol data comparison is not possible. However, it is possible to independently evaluate each protocol based on its resource expenditures and quality of data output. While invertebrate survey protocols are generally more labor and resource intensive than other survey methods, their implementation may be crucial to project goals or needed to provide supplementary information. For recent restoration projects, the assessment of benthic invertebrate communities can be highly informative of restoration trajectories as they comprise the lower trophic levels and provide key biological support for water quality and intertidal habitat data.

Similarly, terrestrial aerial and epigeal invertebrate communities can provide vital information about lower trophic levels to better provide a better understanding of ecological function and process within a wetland area, but they may not be worth the required resources for some projects. While comparing data quality for pitfall traps within varied tidal regimes is not possible, as an implementation comparison it should be noted that deploying pitfall traps within tidal areas requires up to four times additional labor and travel resources as the traps must be covered or collected between each high tide as opposed to every four days.

Table 17. Categorical assessment of cost/effort and data quality for invertebrate survey protocols.

	Evaluation Metric	Benthic Invertebrates	Aerial traps	Pitfall (non-tidal)	Pitfall (tidal)
Time / Effort	Office Preparation Time	10-30 minutes	10-30 minutes	10-30 minutes	10-30 minutes
	Equipment Construction Time (one time)	30-60 minutes	0-10 minutes	10-30 minutes	10-30 minutes
	Field Time (per station)	> 60 minutes	0-10 minutes	10-30 minutes	10-30 minutes
	Laboratory Time (per station)	> 60 minutes	> 60 minutes	> 60 minutes	> 60 minutes
	Post-Survey Processing / QAQC Time	> 30 minutes	10-30 minutes	30-60 minutes	30-60 minutes
	Minimum Repetition (site-dependent)	Few Repetitions	Many Repetitions	Many Repetitions	Many Repetitions
	Relative Cost (equipment and supplies)	\$ 15-50	> \$15	> \$15	> \$15
Survey / Data Quality	Accuracy (at a survey area level)	Medium	Medium	Medium	Medium
	Precision (at a survey area level)	Low	Low	Medium	Medium
	Qualitative-Quantitative Score	Quantitative	Quantitative	Quantitative	Quantitative
	Subjectivity-Objectivity Score	Objective	Objective	Objective	Objective

Community-Based Science

Community-based science, also commonly called citizen science, has greatly increased in popularity in recent years. The iNaturalist (www.iNaturalist.org) and eBird platforms (<http://www.ebird.org/>) are two sources of community-based science data that could support monitoring efforts of California estuarine wetlands. Both serve as a way for users to identify and keep track of the organisms they encounter. Users can receive help with species identifications through a computer vision analysis of their photographs, as well as a community-based identification process. Researchers can export the records of what users have observed, which include location, date and time, taxon name, photographs or sounds of the organism, and any additional notes recorded by the original observer. Many other community science programs and platforms exist (e.g., Grunion Greeters through Pepperdine University, Audubon Society Christmas Bird Counts, and others); however, other programs were not explored in depth for this manual. Monitoring programs should familiarize themselves with opportunities to incorporate community science in their regions.

iNaturalist and eBird observations provide a potentially rich source of biodiversity information, particularly in urbanized areas or those with high human visitation rates (Jacobs and Zipf 2017). Considering the high population density in southern California coastal areas, iNaturalist or eBird records may provide a reasonable approximation of the species present in areas accessible to the general public. It also provides an easy way for researchers to request help with identification of unknown species.

It is important to keep the limitations of community science platforms in mind when considering the potential uses for wetland monitoring programs. The lack of a species on the platforms does not mean it is not present in a given location, just that no user was present or found it interesting enough to record. Species vary in suitability for observation by the general public. Conspicuous species are often very well sampled where they occur along trails, but less accessible or obscure species are likely to be under sampled. Due to this effect, and the non-systematic way in which observations are collected, community science observations cannot be used as an indication of abundance, except perhaps at very coarse levels with the limitations of the sampling clearly explained.

While more consistent datasets might be obtained by formally training volunteers and organizing data collection, such as in the Reef Check California program (Gillett et al. 2012), the advantage of online community science platforms is that data can be contributed by users at any time without a need for an organizational structure beyond the websites. Community-based platforms are oriented around the experience of the user, with potential utility for scientific research considered only as a secondary priority.

Despite these limitations, community science observations have been important sources of data for numerous studies. Uses applicable to coastal environments include species distribution modeling (Tanner 2018), range expansion (Lonhart et al. 2019), non-native species detection (Taylor et al. 2018), and vegetation mapping (Uyeda et al. 2020). Managers and researchers in coastal wetlands could benefit by considering iNaturalist, eBird, or other platforms as a source of monitoring data.



(photo included for illustrative purposes; photo: Neysa Frechette, Friends of Ballona Wetlands)

Data Consolidation and Metadata

Data consolidation is the organization, analysis, and storing of varied data and datasets in a single location. It lets users manipulate different types of data, compare data from one project or location to other locations and project datasets, and convert raw data into shared data that can help resource managers with decision-making. While data consolidation can be complicated, there are several ways to streamline the process of data consolidation: (1) gather the data via standardized protocols (SOPs) that ensure data are very similar or (2) provide detailed metadata that allow users to determine post-collection if data can be compared among existing programs or among locations. The differences between these two approaches have specific implications for the ability to standardize, compare, or cross-walk datasets. In general, programs that develop monitoring in concert are more likely to be translatable over time and space. Analyzing and identifying feasible cross-walking, comparability, and analyses between monitoring protocols allows increased understanding to help customize new monitoring programs and understand the limitations of existing monitoring programs.

The other key element of any consolidation effort is articulating the goals of such an exercise because the ability to effectively combine data will depend on what is trying to be extracted from the data. In other words, the ability to conduct data consolidation and compare across ecosystems should always start with “What is your question?”. While the range of potential ‘questions’ is limitless, there are several common and overarching questions that can guide the development of monitoring programs. Many wetland monitoring programs and state efforts to understand the health and function of wetlands can be grouped into four types of inquires (described below). These types of questions can apply to a single wetland or multiple wetlands and typically involve identifying changes over time or under certain conditions.

- 1) **Extent-distribution** (e.g., how has the area of a wetland changed over time?)
- 2) **Typology** (e.g., has the proportion of wetland habitat shifted from salt flats to salt marsh?)
- 3) **Diversity** (e.g., has the composition of fish or invertebrate populations changed in response to restoration actions?)
- 4) **Function-based questions** (e.g., how did the composition of functional feeding groups in the trophic index vary as a function of wetland type?)

Cross-System Comparisons – Lessons Learned from Southern California Wetlands

Examining studies where datasets from different wetlands in southern California (and beyond) were combined reveals that a variety of approaches have been used to ask and answer these questions (Table 18). Studies evaluated below are divided into five categories: abiotic, vegetation, invertebrates, fish, and multi-metric. Table 18 identifies examples of the variety of parameters used, whether methods were consistent, methods, and abbreviated information on the study purpose. Studies that had consistent methods contained transferrable data; those that did not have consistent methods attempted to combine the data using some sort of conversion or scaled the data up to a usable comparison level (e.g., species list).

Table 18. Examples of cross-system comparisons of Southern California wetlands, including cases where consistent methodologies were used to collect data and cases where data collection methodologies varied among projects.

	Study Purpose	Parameters Compared	Methods	Consistent Methods?	Locations	Reference
Abiotic	El Niño effects on water level	Water depth / level	Dataloggers	No (estimated common datum)	Multiple Southern California estuaries	Harvey et al. 2020
	Ecosystem metabolism	Dissolved oxygen, chlorophyll, pH, temperature	Dataloggers	Yes	Tijuana Estuary & other NERR sites	Wenner et al. 2005; Apple et al. 2008; Baumann & Smith 2018
Vegetation	Probabilistic marsh sampling	Vegetation	Field plots; GIS	Yes	Southern California & San Francisco	Fetscher et al. 2010
	Marsh vegetation structure	Vegetation; Elevation	Field quadrats; surveying	No (but developed conversion)	San Diego & San Quintín Bays	Zedler et al. 1999
	Response to sea level rise	Vegetation; elevation; birds	RTK; LiDAR; bird surveys	No (but developed conversion)	Carpinteria; Mugu; Seal Beach; San Diego Bay; Tijuana Estuary	Rosencranz et al. 2018
	Marsh plant / invertebrate relationships	Vegetation; invertebrates	Quadrats; cores	Yes	Tijuana Estuary; San Diego, Mission, Newport, & Anaheim Bays; North Carolina	Levin et al. 1998; Talley & Levin 1999; Levin & Talley 2002
Invertebrates	Crab / marsh relationships	Crabs; vegetation	Pitfall traps, burrow counts, quadrats	Yes	Tijuana Estuary & other NERR sites	Wasson et al. 2019
	Community structure	Bivalves	Cores	Yes	Tijuana Estuary; Mugu	Peterson 1975, 1976, 1977

	Study Purpose	Parameters Compared	Methods	Consistent Methods?	Locations	Reference
	Spatial and temporal trends	Bivalves	Cores	No (used rarefaction)	Multiple Southern & Baja California	Novoa et al. 2016
	System comparisons	Invertebrates; fish	Cores; seines	Yes	Tijuana Estuary; Los Peñasquitos; San Diego Bay	Nordby & Zedler 1991; Desmond et al. 2000, 2002
	Warm-water species distributions	Algae; invertebrates; fish	Various	No (used species presence only)	Multiple California & Baja California	Lonhart et al. 2019
	Estuarine vs. coastal invaders	Invertebrates	Various	No (used species presence only)	Global	Preisler et al. 2009
	Parasites as indicators	Invertebrates; fish; trematodes	Cores; seines; haphazard	Yes	Morro Bay; Carpinteria; Mugu	Hechinger et al. 2007
Fish	Methods development for mitigation monitoring	Fish	Seines; enclosures	Yes	Multiple Southern California estuaries	Steele et al. 2006a, 2006b
	Species / area relationships	Fish species richness	Various	No (used species presence only)	Multiple Southern California estuaries	Horn & Allen 1976
	Nursery habitat	Fish (halibut)	Trawls; seines	No (developed conversion for catch per unit effort)	Multiple San Diego County estuaries	Fodrie & Mendoza 2006
Multi-metric	Eutrophication framework	Multi-metric (dissolved oxygen, algae, phytoplankton)	Quadrats; grab samples; data loggers	Yes	Multiple Southern California estuaries	McLaughlin et al. 2014
	Marsh resilience to sea level rise	Multi-metric (e.g., water level, marsh elevation)	sondes, RTK, accretion rates	Yes (but some standardization needed)	Tijuana Estuary & other NERR sites	Raposa et al. 2016

In some cases, and in answer to some questions, data collected with substantially different methods can be synthesized to provide meaningful insight. For species-level analyses (e.g., invertebrates, vegetation, fish, etc.), a species list is one of the most informative tools to describe an ecosystem (Slobodkin et al. 1980), and such lists are often compiled from many different sources, such as formal surveys, anecdotal observations, media accounts, or community science. Although there are challenges associated with compiling species-level data collected in different manners, such approaches have been used to describe patterns associated with invasions of non-native species inside versus outside estuaries (Preisler et al. 2009) (Figure 10), track range changes of warm-water species in relation to marine heatwaves (Lonhart et al. 2019) and develop species-area relationships for estuarine fish (Horn and Allen 1976). In addition to generating a species list, quantitative analyses using multivariate methods such as multivariate community analysis and ordination techniques can be conducted if abundance data can be reduced to presence/absence data. The literature suggests that these analytical techniques are more robust to variation in sorting mesh-sizes than univariate methods (e.g., Hammerstrom et al. 2012) (Figure 11).

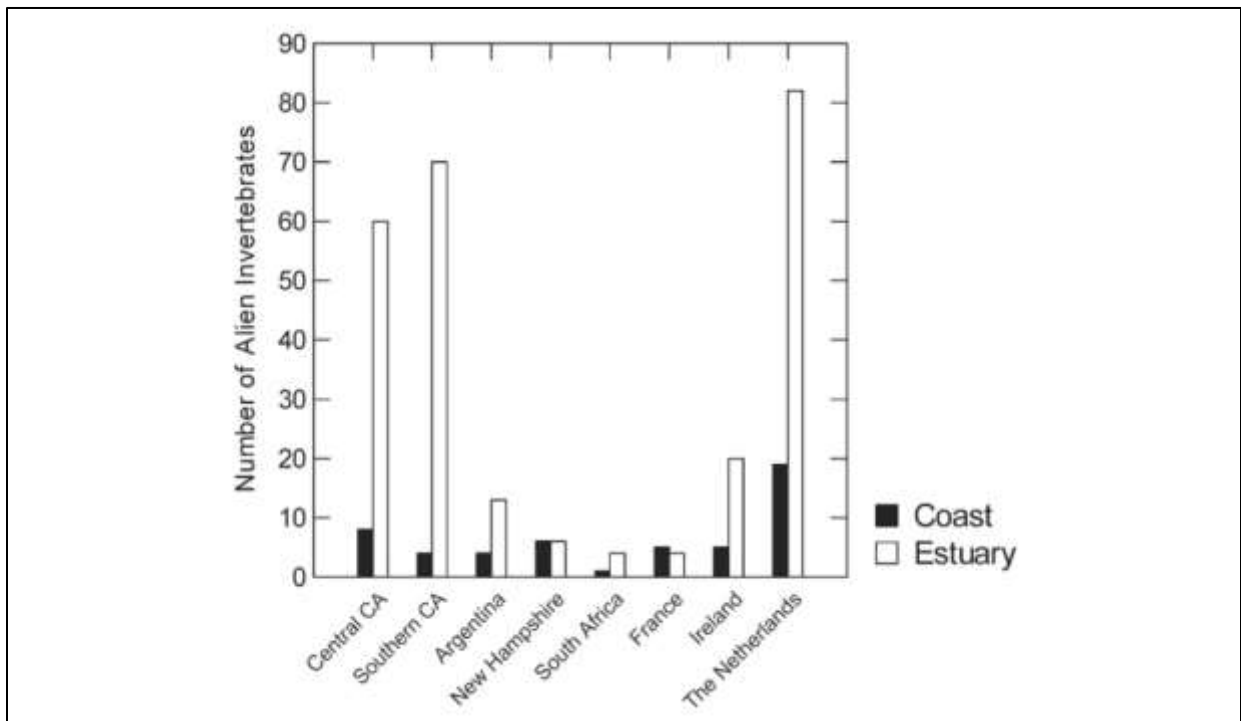


Figure 10. Number of non-native species in estuarine and coastal systems in Southern California and elsewhere in the world (Replicated from Preisler et al. 2019).

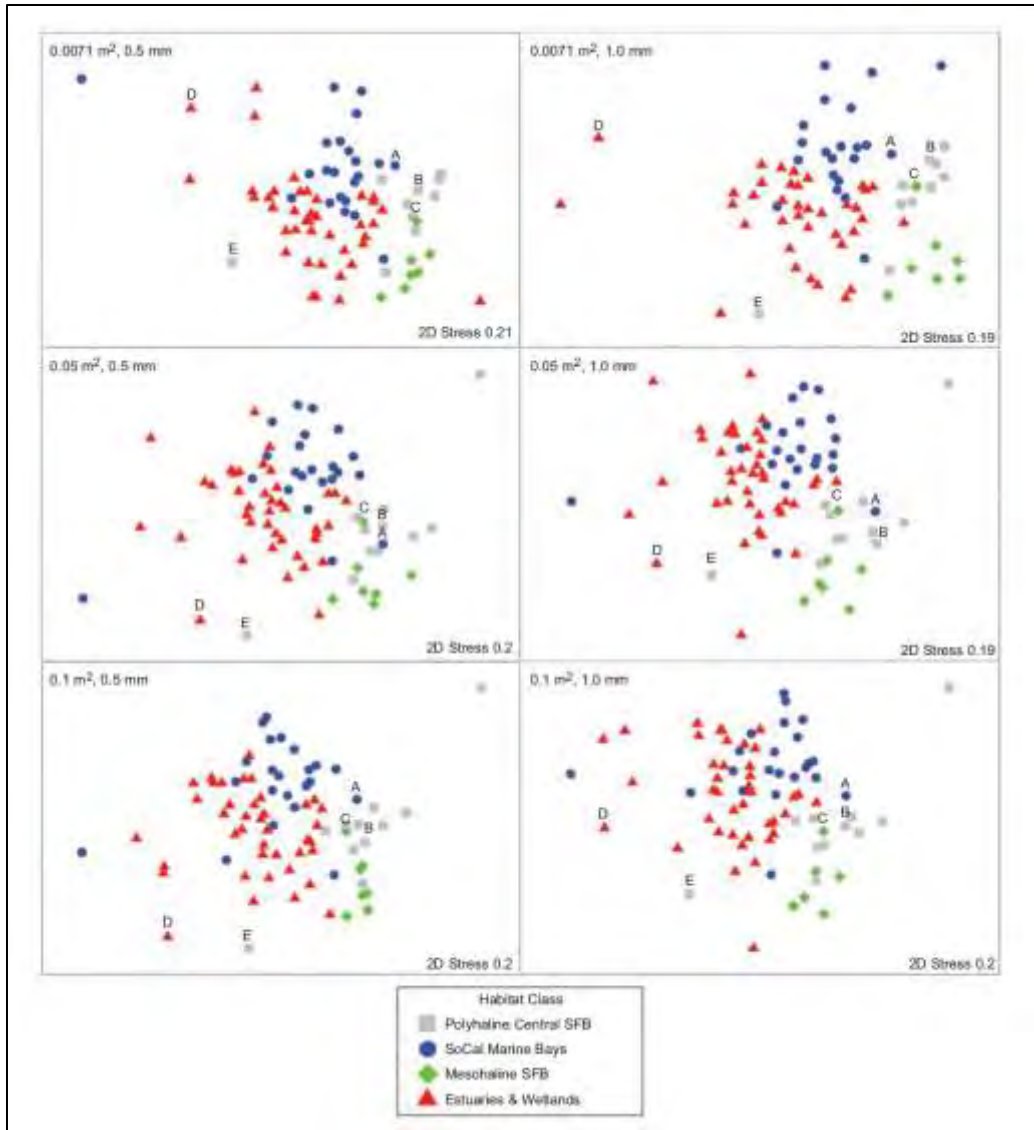


Figure 11. Multidimensional scaling (MDS) plots for each combination of gear and sieve size. Letters A through E indicate the position of five stations representing each of the four habitat types and illustrate station shifts among the MDS plots (Replicated from Hammerstrom et al. 2012).

Finally, biotic indices can be used to quantitatively assess ecosystem quality by grouping species based on functional traits, such as invasion status, feeding group or sensitivity to pollution or disturbance. While this relies on best professional judgment by experts, the resultant indices can be used to compare among sites in a similar manner to species list discussed above. For example, Resh and Jackson (1993) tested the ability of 20 benthic metrics used in 30 different assessment protocols to discriminate between impaired and minimally impaired sites in California. The most effective measures, from their study, were the richness measures, two community indices (Margalef and Hilsenhoff family biotic index), and a functional feeding group metric (e.g., percent scrapers).

Beyond compiling disparate data with little attempt at standardization, it is sometimes possible to develop “conversion factors” or other means of comparing data collected using different methods and/or with different levels of effort. For example, Fodrie and Mendoza (2006) developed a quantitative conversion to compare halibut caught in seines to halibut otter trawls (the different gear being necessary due to different geomorphology of the study systems), and Novoa et al. (2016) used rarefaction curves to compare bivalve assemblages across different systems and different time periods (Figure 12). To assess El Niño impacts on estuarine water levels and mouth dynamics, as a potential preview of climate change impacts, Harvey et al. (2019) used depth data, collected by a variety of methods. The elevation of most sondes in the study were surveyed and presented relative to NAVD88, but even relative water level from non-surveyed sondes proved valuable in determining regional effects of the El Niño in estuarine systems (Figure 13). For comparing salt marsh vegetation in Southern and Baja California, Zedler et al. (1999) converted different-sized monitoring plots to a standard size (a relatively common approach). It should be noted that in many cases attempting to standardize data or develop conversion factors can be unsatisfactory, based on fundamental differences in sampling designs. For example, attempts to reconcile two different long-term vegetation monitoring protocols (one local and one national) at the Tijuana Estuary proved unsuccessful, and both protocols are now employed essentially independently (J. Crooks, unpublished data).

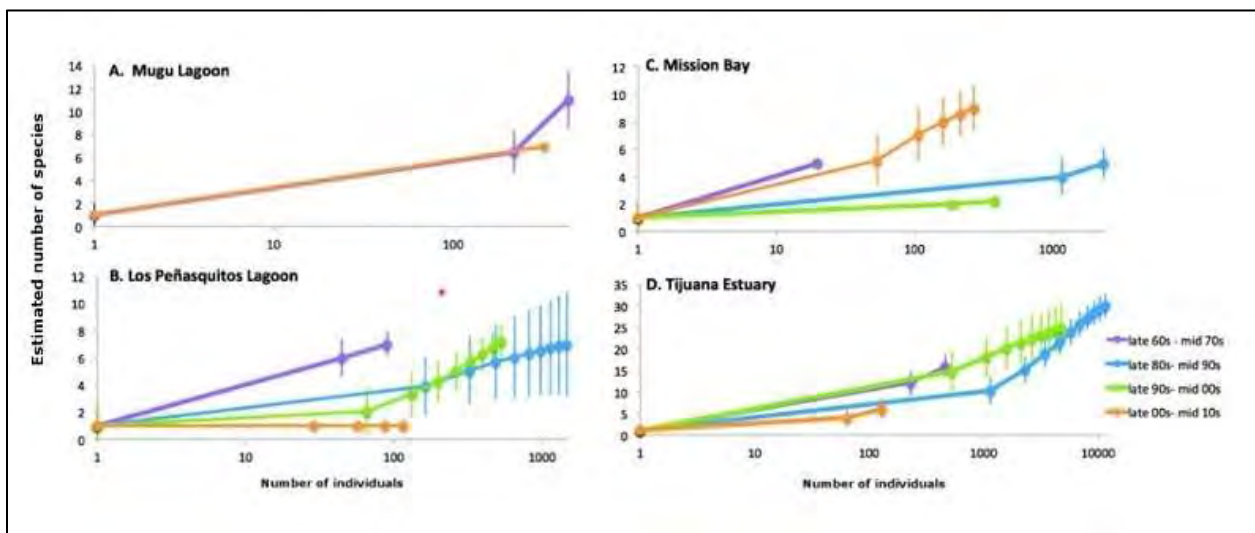


Figure 12. Rarefaction curves comparing number of individuals to estimated number of species for bivalves in Southern and Baja California (from Novoa et al. 2016).

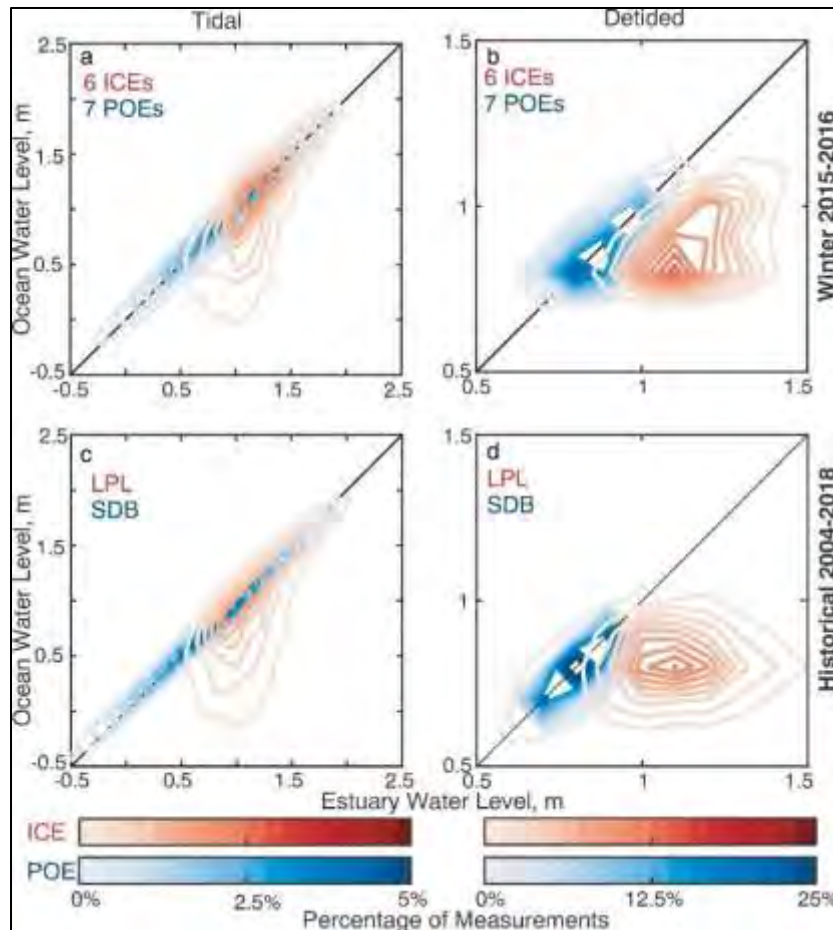


Figure 13. Comparison of water levels from Intermittently Closed Estuaries (ICEs) and Permanently Open Estuaries (POEs), including Los Peñasquitos Lagoon (LPL) and San Diego Bay (SDB). Figure replicated from Harvey et al. 2019.

The degree to which data are collected similarly will increase the ability to compare among datasets. Compared to other parameters of interest (e.g., biota), consolidation of water quality data across monitoring programs is relatively straightforward. While a variety of different instruments are available that can measure water quality parameters, SOPs, and Quality Assurance Quality Control (QAQC) measures are typically well-developed, and data that are collected and screened in accordance with these best practices are typically comparable (J. Crooks, unpublished data). There have been several studies using the standardized System Wide Monitoring Program (SWMP) of the National Estuarine Research Reserves (NERR), which involves programs across the country who use similar instruments and undergo training to implement monitoring programs. These broad scale comparisons of water quality have informed an understanding of basic estuarine processes, including ecosystem metabolism, and have also emphasized the potential utility of parameters such as pH (Wenner et al. 2005, Apple et al. 2008, Baumann & Smith 2018). Within Southern California, McLaughlin et al. (2014) examined the extent and magnitude of eutrophication by measuring dissolved oxygen and chlorophyll a from deployed dataloggers in 23 estuaries (Figure 14). This was coupled with assessments of macroalgae on tidal flats to explore multi-metric frameworks for assessing estuarine eutrophication.

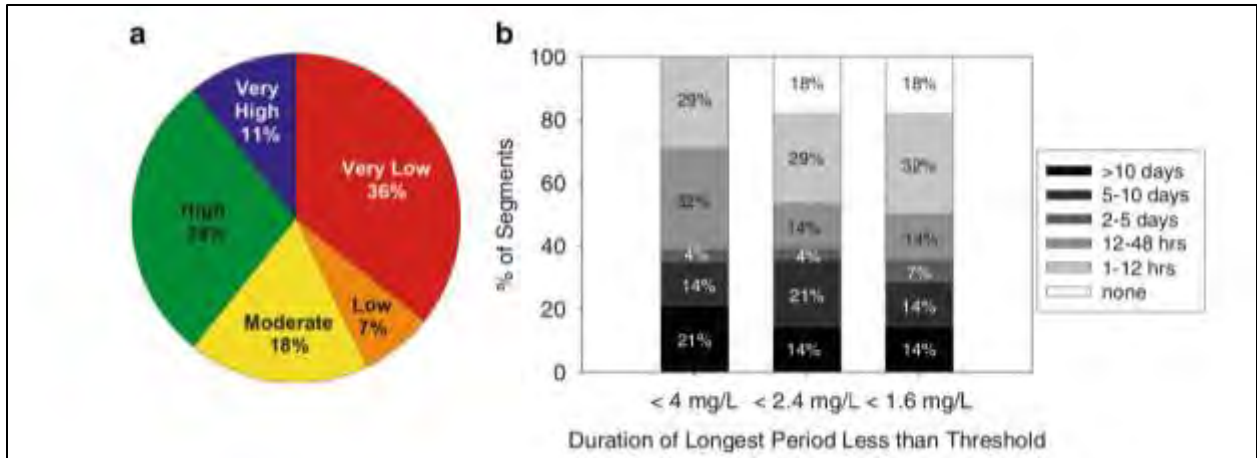


Figure 14. a) Percent of Southern California estuarine segments in different conditions, based on dissolved oxygen, b) duration of hypoxic events in estuarine segments. Figure replicated from McLaughlin et al. (2014).

In addition to indices, which are largely based on community composition, monitoring programs or studies can also incorporate multi-metric approaches, which cross parameter boundaries (e.g., water quality and biotic parameters). One multi-metric approach that used a variety of different indicators to ask questions of both local and national applicability was the development of the MARS (MARsh Resilience to Sea level rise) Index within the NERR system (Raposa et al. 2016). This included long-term measurements of tidal range, sea level surface change, turbidity (as a proxy for suspended sediments), marsh elevation distribution within the tidal frame, marsh elevation change, and accretion (Figure 15). Beyond consolidation of different data, this integration of indicators also can be viewed as standardization at the level of the question (i.e., how vulnerable are marshes to sea level rise?), and it utilizes a toolbox of various approaches to address a key question. Multi-metric approaches, exemplified by the eutrophication framework and MARS Index, merit further attention with respect to the conservation and management of coastal California wetlands.

Metric thresholds	Percent of marsh below MHW	> 60%	> 60%	> 40%	> 20%	< 20%
	Percent of marsh in lowest third of plant distribution	> 60%	> 60%	> 40%	> 20%	< 20%
	Skewness	> 1.5	> 0.5	0.5 to -0.5	< -0.5	< -1.5
	Elevation change rate (mm yr ⁻¹)	> 2	> 3	> 3	> 4	> 5
	Short-term accretion rate (mm yr ⁻¹)	> 2	> 2	> 3	> 4	> 5
	Long-term accretion rate (mm yr ⁻¹)	> 2	> 2	> 3	> 4	> 5
	Turbidity (NTU) / Total suspended solids (mg l ⁻¹)	> 10	> 10	> 20	> 30	> 40
	Tidal range (m)	> 0.6	> 0.6	> 1.2	> 1.8	> 2.4
	Long-term rate of SLR (mm yr ⁻¹)	> 0.5	> 0.6	> 1.8	> 1	> 1
	Short-term inter-annual variability in water levels (mm)	> 25	> 15	> 5	5 to -5	< -5
Scoring	All metrics	1	2	3	4	5
	All categories	1-5	< 1	< 4	< 5	5
	MARS - risk	5	2	3	4	5
	MARS - average	1	> 1	> 2	> 3	> 4
	MARS - ratio	> 0.5	> -0.5	> 1.5	> 2.5	> 3.5

Figure 15. Numeric thresholds and color codes for individual metrics and all categories and indices in the MARS index study (replicated from Raposa et al. 2016). For metric scoring, red = 1, brown = 2, yellow = 3, light green = 4, and dark green = 5.

An examination of data consolidation efforts makes it clear that the more protocols can be standardized, the easier consolidation will be. In the southern California examples (Table 18), highly consistent datasets typically arise from a single research group using consistent methods across multiple systems (e.g., Nordby and Zedler 1991, Talley and Levin 1999, Hechinger et al. 2007), or teams that use consistent approaches (e.g., McLaughlin et al. 2014, Baumann and Smith 2018, Steele et al. 2006a, b). As methods become more widely adopted [e.g., those associated with the San Onofre Nuclear Generating Station (SONGS) marsh mitigation program (<https://marinemitigation.msi.ucsb.edu/>)], they can help form the foundation of consistent regional monitoring efforts. To that end, the methodologies developed over time in southern California (e.g., Callaway et al. 2001, Steele et al. 2006 a,b) have helped shape the SOPs and recommendations presented in this manual.

Although it is sometimes possible that widely disparate datasets with little cohesive underlying sampling can be combined to provide insight into broad questions, a standardization of protocols will facilitate data consolidation and its application to wetlands management. As such, this manual focuses on identifying protocols that can be adopted (or, if necessary, adapted) in different monitoring contexts. Another critical step in consolidation exercises, especially when combining data collected from different programs, is being able to determine the why, what, when, and how of sampling programs – captured in accurate and generous metadata.



(photo included for illustrative purposes; photo: The Bay Foundation)

Principles of Metadata

Well-documented metadata are extremely important for data preservation, sharing, and analyses. Existing databases from regional monitoring programs, independent research programs, and peer-reviewed literature supported this assessment. Metadata as it pertains to this wetland monitoring manual can be categorized into three types including program, protocol, and field / laboratory metadata (Figure 16).



Figure 16. Categories of metadata and examples.

Program metadata provides an overview of the monitoring program, while protocol and field/laboratory metadata provide details on metadata specific to parameters and field/laboratory sampling and analyses. While protocol and field/laboratory metadata may contain more specifics than program metadata, all are equally important to collect, or the data may not be useable. It is crucial to collect metadata at all three category levels as part of a monitoring program. Metadata can and should be general and extensive with rich descriptive information about the data being collected. Like the information needed to recreate the scientific process, detailed metadata accompanying a wetland monitoring program provides information to understand the who, what, where, when, and why. Some of the many reasons metadata collection are important include ensuring dataset continuity (i.e., change in field technicians, managers, or organization), data analyses and/or regional comparison, and maintaining an ability to check data integrity. Metadata helps maintain long-term datasets, share and transfer datasets, conduct site-specific and regional analyses, produce science-based monitoring reports and scientific publications, and maintain compliance for permitting regulators and enforcement.

Program Metadata

Program metadata is an overarching summary of a specific monitoring program, typically unique by wetland site, and should include at a minimum the site name, geographic location, purpose of monitoring program (i.e., restoration, baseline, or reference site), agency or organization collecting data, parameters collected, and survey dates or date range. Program metadata provides a snapshot of a

specific wetland monitoring program and can help facilitate regional data collection efforts and data sharing. An example of program level metadata is shown in Table 19. The program metadata should be updated when a major change occurs to the monitoring program or at the minimum updated annually.

Table 19. Example of program metadata documentation.

Program Metadata	Example
Site Name	<i>Malibu Lagoon</i>
Location (GPS coordinates)	34° 1'59.62"N, 118°41'3.73"W
Organization	<i>The Bay Foundation</i>
Point of Contact (POC)	<i>Karina Johnston</i>
POC Email	kjohnston@santamonicabay.org
Project Type	<i>Post-restoration</i>
Wetland Type	<i>Bar-built</i>
Protocol/Period/Frequency	<i>CRAM (bar-built)/2013-2018/annually, Vegetation Cover/2013-2018/semi-annually</i>
Monitoring Reports	Available at www.santamonicabay.org

Protocol Metadata

Protocol metadata includes metadata specific to individual monitoring protocols (SOPs). Protocol metadata should be collected whenever a new protocol is initiated or when a protocol changes. Protocol level metadata should reference to the program metadata by including the site name and/or monitoring program. Additionally, protocol metadata should be updated anytime a protocol changes and at the minimum reviewed annually. Protocol metadata may include information like location (latitude/longitude/elevation), equipment used (i.e., sieve size, core size, sonde model), or frequency (annually, quarterly, every 30 minutes). Protocol metadata are especially important for running site specific and regional analyses.

Field and Laboratory Metadata

Field and laboratory metadata are recorded when protocols are initiated in the field. These metadata include information like date, time, environmental conditions (i.e., wind speed and direction, temperature, tide), as well as observational notes that pertain to the data or conditions of the data being collected. Field and lab metadata can be dynamic inputs and therefore are required to be recorded every time protocols are implemented in the field or lab. Information like weather and hydrologic conditions should be included as well as logistical information such as field technician names and anyone participating in data collection (i.e., who recorded data versus who made observations). Sonde calibrations are also an example of field metadata. Lab metadata can include information such as who ran a grain size analysis or processed benthic invertebrate samples. Reference to the program metadata and protocol should also be included as part of field and lab metadata. Field and lab metadata are often recorded by the person(s) implementing a specific protocol on the original datasheet for quality control purposes. This information can be scanned and/or digitized following the field or lab event. There are circumstances where metadata can be digital and embedded in files like

photographs and/or geographic information software (GIS) files. Whenever possible, metadata should also be recorded on datasheets.

Data Sharing

For this manual, we are defining data sharing in two ways. First, data sharing is the practice of making data from scholarly research available to other researchers and the public (e.g., scholarly research and academic grants). Many funding agencies, institutions, and publication venues have policies requiring data sharing because transparency and openness are considered by many to be part of the scientific method. Second, data sharing is also a best practice for collaboration among field sites or within a certain geographic location (e.g., southern California), and should be incorporated into public databases.

Many funding agencies and journals require authors to make all data necessary to replicate their study's findings publicly available without restriction at the time of publication. This can be done via individual websites, DOI's for data, or on request. Another option is to use an existing centralized data platform, such as EcoAtlas, CEDEN, or iNaturalist. These efforts promote collaboration and coordination among multiple local, state, and federal agencies and organizations that are interested in estuarine protection, restoration, and conservation. Researchers can display information regarding the purpose, location, extent, status, and results of a variety of projects affecting aquatic habitats. As discussed on the platforms' webpages, this data sharing can improve coordination among monitoring efforts with the goal of protecting and restoring target ecosystems.

Challenges with integrating data across a diverse assemblage of estuaries include sample design for closing lagoons versus perennially open systems, or for systems of different sizes that support different habitat types and ecosystem services. In advance of a new regional monitoring program or a shared grant, researchers should try to design a suite of monitoring protocols to document the condition (and, if possible) the functioning of the estuarine ecosystem(s) in question. Choosing the exact SOPs should be done with all researchers involved as well as with consultation with a technical or scientific advisory board (again, if possible). In some examples, the process has been to first choose the key structure or functions that will be measured, and then select representative taxa, habitats, and abiotic conditions with which to document site-specific conditions and services.

While the development of a new electronic tool for data sharing was outside the scope of this program, further work with the Level 3 subcommittee of the California Wetlands Monitoring Workgroup will help develop a specific online toolkit strategy for the consolidation and easy transferability of online Level 3 data. Several tools already exist [e.g., California Environmental Data Exchange Network (CEDEN), EcoAtlas, etc.], and should be further explored or used.

Conclusions and Applications

The principal purpose of this manual is to serve as a tool for resource managers, scientists, researchers, agency representatives, students, or anyone with the goal of developing an estuarine wetland monitoring program. Data consolidation for existing programs is challenging if not conducted from the outset using standardized protocols. Thus, this manual provides a framework to guide the development of Level 3 (site-intensive) wetland monitoring and shows how programs may begin to structure their protocol and method choices to reflect a more standardized approach.

The protocols and methods described in this manual were chosen for inclusion because they have broad applicability and proven efficacy. They focus on a subset of broad parameters (e.g., vegetation, birds) measured by most monitoring programs that were evaluated as part of the program development. Most of this manual relied heavily on previous or existing wetland monitoring programs for its development. Through the evaluation of multiple protocols for each of the main parameter categories, this manual provides the beginnings of basic monitoring “toolkit” recommendations which should be supplemented by additional protocols and/or additional parameters on a site- or project-specific basis. Recommendations were primarily based on scientific evaluations of data quality, cost and effort, expertise requirements, and disturbance.

Final Suite of Recommended Protocols

The protocols and methods described in this manual were chosen for inclusion because they have broad applicability and proven efficacy. They focus on a subset of broad parameters (e.g., vegetation, birds) measured by most monitoring programs that were evaluated as part of the program development. Through the evaluation of multiple protocols for each of the main parameter categories, this manual provides the beginnings of basic monitoring “toolkit” recommendations which should be supplemented by additional protocols and/or additional parameters on a site- or project-specific basis, based on monitoring program goals or questions. Recommendations were primarily based on scientific evaluations of data quality, cost and effort, expertise requirements, and disturbance. Individual monitoring programs may build on this suggested framework to meet project objectives.

While site-specific goals should be the principal consideration to inform protocol selection and sampling design, this manual provides a suite of protocol recommendations based on analyses weighing multiple factors influencing implementation, including resource requirements, quality and importance of data outputs, and site disturbance. If implemented for new monitoring programs, data should have improved transferability. Table ES-1 presents two groupings of Level 3 estuarine wetland protocols, including a column for recommended protocols for each parameter, and a second minimum protocol recommendation for programs with fewer resources or funding. The recommended protocols include higher resolution or better quality data to inform wetland functions and processes. Recommended protocols also include the low resource column.

In addition to the recommended Level 3 protocols listed in Table ES-1, Level 2 California Rapid Assessment Method assessments are also recommended for implementation for all monitoring programs to provide a broad, site-wide rapid condition assessment and provide supporting and

transitional information between the Level 1 and Level 3 implementation assessments. Additional discussions of each parameter follow Table ES-1.

Table ES-1. Final suite of Level 3 recommended protocols and low-resource protocols, for programs with very limited funding. Note: monitoring programs or research that are targeted towards specific questions or needs would require additional sampling methods.

Parameter	Recommendation	Low-Resource Recommendation
Water Quality	Data sonde (SOP 1.1)	Spot sampling associated with beach seine (SOP 4.1)
Soil Characteristics	Grain size and organics (SOP 2.2), hydrometer method; soil texture and salinity (SOP 2.1)	Soil texture (SOP 2.1)
Vegetation	Vegetation mapping (SOP 3.5); cover class quadrat (SOP 3.2)	Cover class quadrat (SOP 3.2)
Fish	More replicates and higher frequency of beach seine (SOP 4.1); fish cameras (SOP 4.4)	Beach seine (SOP 4.1)
Birds	More replicates and higher frequency of point count (SOP 5.1) or site-wide surveys (SOP 5.1)	Point count (SOP 5.1)
Mammals	None recommended	None recommended
Invertebrates	Benthic invertebrates (SOP 6.1)	Presence associated with beach seine (SOP 4.1)

Water Quality

Ambient water quality plays an integral role in influencing habitat and species distributions and is often a good indicator of the efficacy of management interventions. Given dramatic short- and long-term temporal variability in water quality parameters (e.g., salinity, temperature, pH, and dissolved oxygen), water quality is best tracked with long-term deployment of data sondes. Due to the high costs associated with purchasing and maintaining monitoring equipment, programs with limited resources should use office-based GIS aerial image analyses and rapid assessments to evaluate the surrounding landscapes, freshwater inputs, and impairments to dominant hydrology to broadly infer water quality characteristics in combination with spot sampling when possible.

Soil Characteristics

Soil grain size, salinity, and organic content are defining characteristics of soft-sediment intertidal habitats. Protocols examining these characteristics can be labor-intensive and require specialized equipment; however, general soil characteristic parameters can influence hydrology and the wetland community may respond directly (i.e., vegetation). Therefore, basic soil characteristics are recommended such as soil texture, grain size, and organic content. These require some specialized equipment but can be done cost-effectively. Soil characterization options, including methodologies not explored in this manual (e.g., chemical constituents, toxicity), will need to be evaluated on a project-specific basis. For low resource programs, soil texture can be done for almost no cost (except for labor)

in any habitat and is an easy alternative, though it provides less valuable data (categorical rather than quantitative).

Vegetation

Some form of vegetation monitoring is recommended as a key component for every monitoring program, regardless of site-specific needs. High-resolution vegetation data can allow logical inferences to be made about multiple parameters including hydrology, soil characteristics, disturbances, and the distribution of associated wildlife such as mammals, birds, invertebrates, and herpetofauna. As a result, resources required to assess some additional parameters may be reduced if broad assumptions are sufficient to meet project goals (i.e., resulting from the vegetation data). Of the transect-based vegetation cover protocols evaluated, the cover class quadrat method is recommended as it is the most rapid and flexible survey across all habitat types and conditions while maintaining high precision and comparable accuracy to the laser quadrat. For programs with more dedicated resources, the creation of a site-wide vegetation map can provide an extremely useful foundational data layer and large-scale supplementary data to support site-wide analyses and restoration planning efforts or assessments.

Fish

The fish community is a common indicator evaluated by estuarine wetland monitoring programs (approximately one-third of evaluated program documents) and can serve as a proxy for the function of intertidal channels and habitats. As such it is recommended for surveying as part of this manual using a combination of beach seines and blocking nets to survey intertidal channels. While beach seine surveys can be fairly time- and labor-intensive and can only provide a snapshot of data in time due to their highly mobile nature, fish community and diversity are still common indicators of water quality and restoration activities. Additionally, as estuaries and wetlands provide essential nursery habitat for juvenile commercially important species, they are often tied to wetland ecosystem functions and services. If more resources are available, an increase in sample replicates or higher sample frequency (e.g., more seasons) are recommended. Additionally, fish cameras are a relatively newer technology survey that should be utilized if resources allow. The one-time costs associated with setting up several systems for deployment will provide supporting data to the beach seines and can be used more frequently and with less disturbance.

Birds

Of the Level 3 protocols evaluated for bird monitoring, the point count method is recommended based on ease of implementation, lower relative levels of disturbance, lower time/effort commitments, and comparable resulting data. While similar or equal to the low time requirements for box count surveys, higher visibility is associated with the point count method. Traversing through the entire sampling box was found to increase site disturbance while high tides made the visual delineation of box edges nearly impossible. The greater ease and lower habitat impacts implementing the point count surveys did not yield any noticeable loss in data quality and bird populations were equally characterized by both methods. However, if more resources are available, an increase in sample replicates or higher sample frequency (e.g., more seasons) are recommended. Additionally, if a baseline- or species-level assessment (or geospatial assessment) is desired, a site-wide survey is recommended to provide the largest inventory of bird species and a more complete representation of site use by birds.

Mammals

Mammal survey protocols are not recommended for implementation by this manual. In addition to only infrequently being included in the documents evaluated by the monitoring report literature review, mammal presence in intertidal wetland habitats is intermittent and requires time-intensive protocol implementation and specialized permits. If adequate resources are available, and medium to large sized mammals are a target parameter of the developing monitoring program, then wildlife cameras are a feasible alternative to cover a variety of habitat types but may need to be deployed in large arrays to determine abundances or larger wildlife movement patterns. For smaller mammals, catch-and-release survey methods can be used with various sized traps to target specific wildlife or in combinations to assess the broader mammal community. Specialized methods with specific protocols would need to be implemented for some groups of species such as shrews.

Invertebrates

Benthic infauna are important in sediment turnover and bioturbation, activities that mix and transport particles, water, and solutes within the sediment and across the sediment water interface (Rhoads, 1974; Coen and Luckenbach, 2000; Nogaro et al., 2009; Belley et al., 2010). As an integral part of the consumer food chain, benthic infauna provide important trophic support to species of commercial and intrinsic importance like crab, fish, and birds (Sacco et al., 1994; Levin et al., 1996; Moseman et al., 2004). Infauna usually construct tubes or burrows and are commonly found in intertidal and subtidal waters. They reflect local environmental conditions and are used as bio-indicators for pollutant studies (Smith et al., 2001).

Methods focused on benthic invertebrates provide valuable information about the lower trophic levels within a given wetland area, but there are typically high labor, time, and resource costs associated with their implementation. This manual recommends using cores to sample the benthic invertebrate community and sorting to recognizable taxonomic units. Additionally, as a lower resource option, invertebrates may be captured in association with beach seining, providing information on larger, typically nektonic organisms (such as shrimp and crabs). Broader diversity or productivity patterns may be assessed using terrestrial invertebrate sampling protocols; however, there has been relatively little work on this on relationships to other, more traditionally sampled parameters remain unclear.

Other

A suite of other protocols are available for use by wetland monitoring practitioners to assess the site-specific conditions of a wetland site, e.g., detailed water and soil chemical analyses, heavy metals, nutrients, bacteria, etc. Additionally, high resolution information on physical parameters such as elevation and detailed hydrological data can inform the distribution of vegetation assemblages and associated wildlife of estuarine wetlands. These additional protocols and parameters may be further explored in future versions of this manual or through supplemental program development.

Data Sharing

While the development of a new electronic tool for data sharing was outside the scope of this program, further work with the Level 3 subcommittee of the California Wetlands Monitoring Workgroup will help develop a specific online toolkit strategy for the consolidation and easy transferability of online Level 3 data. Several tools already exist [e.g., California Environmental Data Exchange Network ([CEDEN](#)), [EcoAtlas](#)] and should be further explored.



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** Additional literature cited for the development of each Standard Operating Procedure can be found in the individual SOP appendices. **

Appendix A

Summary of Wetlands Program Monitoring Literature Review by Protocol

Reference Number	Wetland or Organization	County	Water Quality	Marine Sediment	Terrestrial Soil	Vegetation	Fish	Herpetofauna	Mammals	Birds	Benthic Inverts	Terrestrial Inverts	Physical Characteristics	Aerial Veg
1	Ballona Wetlands	Los Angeles	X			X	X			X	X	X		
2	Ballona Wetlands	Los Angeles	X			X	X			X	X	X		
3	Ballona Wetlands	Los Angeles												
4	Batiquitos Lagoon	San Diego	X			X	X			X	X		X	X
5	Batiquitos Lagoon	San Diego				X								X
6	Bolsa Chica	Orange	X		X	X	X			X	X		X	X
7	Bolsa Chica	Orange	X	X	X	X				X	X		X	X
8	Bolsa Chica	Orange	X		X	X	X			X	X		X	
9	Bolsa Chica	Orange				X				X				
10	Bolsa Chica	Orange								X				
11	Bolsa Chica	Orange				X								
12	Calleguas Creek	Ventura	X	X			X			X	X		X	
13	Carpinteria	Santa Barbara			X	X							X	
14	Carpinteria	Santa Barbara			X	X								
15	Devereux Slough	Santa Barbara				X								
16	Devereux Slough	Santa Barbara				X								
17	Devereux Slough	Santa Barbara				X								
18	Devereux Slough	Santa Barbara				X								
19	Elkhorn Slough	Monterey County				X								X
20	Elkhorn Slough	Monterey County					X							
21	Elkhorn Slough	Monterey County									X			
22	Elkhorn Slough	Monterey County									X			
23	Elkhorn Slough	Monterey County	X			X								
24	Elkhorn Slough	Monterey County	X											
25	Elkhorn Slough	Monterey County	X											
26	Elkhorn Slough	Monterey County				X								
---	EPA Wetland monitoring modules	Regional	X			X		X	X	X	X	X		
27	Integrated Wetland Regional Assessment Protocol (IWRAP)	Regional					X			X	X			
28	Los Cerritos Wetlands	Los Angeles/Orange											X	
29	Los Pensaquitos	San Diego	X		X	X	X	X	X	X	X			

Reference Number	Wetland or Organization	County	Water Quality	Marine Sediment	Terrestrial Soil	Vegetation	Fish	Herpetofauna	Mammals	Birds	Benthic Inverts	Terrestrial Inverts	Physical Characteristics	Aerial Veg
30	Los Pensaquitos	San Diego	X											
31	Los Pensaquitos	San Diego	X			X								
32	Los Pensaquitos	San Diego	X		X	X	X	X	X	X	X			
33	Los Pensaquitos	San Diego	X		X	X	X	X	X	X	X			
34	Los Pensaquitos	San Diego	X		X	X	X	X	X	X	X			
35	Los Pensaquitos	San Diego	X			X	X	X	X	X	X			
36	Los Pensaquitos	San Diego	X		X	X	X				X			
37	Malibu	Los Angeles	X	X	X	X	X	X	X	X	X	X	X	X
38	Malibu	Los Angeles	X	X										
39	Malibu	Los Angeles							X					
40	Malibu	Los Angeles	X	X	X	X	X			X	X		X	
41	Morro Bay	San Louis Obispo	X											
42	Morro Bay	San Louis Obispo				X								
43	Mugu Lagoon	Ventura		X	X	X	X		X	X	X	X	X	
44	Mugu Lagoon	Ventura				X								
45	Mugu Lagoon	Ventura	X		X	X	X					X		
46	Mugu Lagoon	Ventura			X	X	X						X	
47	Mugu Lagoon	Ventura	X				X							
48	Mugu Lagoon	Ventura										X		
49	Newport Bay	Orange	X			X								
50	Ormond	Ventura				X					X	X		
51	Ormond	Ventura				X						X		
52	San Dieguito	San Diego				X							X	
---	San Dieguito	San Diego	X	X							X		X	
53	San Dieguito	San Diego	X			X	X			X	X		X	X
54	San Elijo	San Diego								X				
55	San Elijo	San Diego		X										
56	San Elijo	San Diego				X								
57	SF Bay	Bair Island				X							X	X
58	Surface Water Ambient Monitoring Program (SWAMP)	Regional	X	X										
59	Tijuana	San Diego					X	X						

Reference Number	Wetland or Organization	County	Water Quality	Marine Sediment	Terrestrial Soil	Vegetation	Fish	Herpetofauna	Mammals	Birds	Benthic Inverts	Terrestrial Inverts	Physical Characteristics	Aerial Veg
60	Tijuana	San Diego				X								
61	Tijuana	San Diego				X					X			
62	Tijuana	San Diego	X								X			
63	Tijuana	San Diego	X										X	
64	Tijuana	San Diego			X	X							X	
65	Tijuana	San Diego			X	X								
66	USGS	San Francisco Bay Area	X	X		X			X	X	X	X	X	
67	USGS	EPA Region 5	X	X	X	X	X			X	X			X
68	Wetland Monitoring Series (Wisconsin)	Wisconsin	X			X		X	X	X	X	X		
69	WRAMP	San Francisco Bay Area				X			X		X			X

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Appendix B

Complete List of Standard Operating Protocols



Standard Operating Procedures (1.1): Continuous Water Quality Monitoring – Data Sonde

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Prepared for the United States Environmental
Protection Agency



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Standard Operating Procedures: Continuous Water Quality Monitoring – Data Sonde

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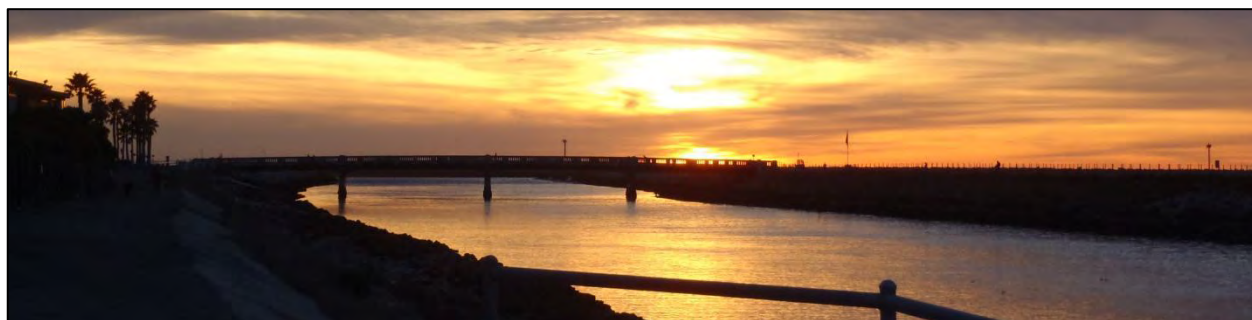
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Protocol Suitability Evaluation

A habitat suitability table containing appropriate estuarine wetland habitat types (of those evaluated) to implement continuous water quality monitoring protocols is displayed in Table 1. A comparative assessment of cost, effort, and data quality are shown in Table 2. A matrix of additional detailed categorical evaluations of continuous water quality monitoring can be found in Appendix 1.1A. For emergent salt marsh habitats, data sonde protocols are only applicable in areas (e.g., tidal channels) that allow for the sonde sensors to be continuously or frequently submerged.

Table 1. Appropriate habitat types for continuous water quality monitoring protocols.

Survey Protocol	Habitat Types					
	Tidal Channel	Mud/sand flat	Emergent salt marsh	Non-tidal salt marsh	Salt pan	'Degraded' / fill
General WQ Parameters	X	X	X			

Table 2. Categorical assessment of cost/effort and data quality for continuous water quality monitoring protocols.

	Evaluation Metric	General WQ Parameters	Notes
Time / Effort	Office Preparation Time	0-10 minutes	May involve printing maps and identifying site locations
	Equipment Construction Time (one time)	> 60 minutes	Involves constructing permanent sonde housing, if applicable, and the first calibration
	Field Time (per deployment)	30-60 minutes	Highly variable, depending on field location and access to site (hiking / boating); housing may require cleaning due to biofouling
	Laboratory Time (per deployment)	> 60 minutes	Monthly calibration and cleaning required (minimum)
	Post-Survey Processing / QAQC Time	> 60 minutes	Requires checking data against calibration standards and equipment specifications
	Minimum Repetition (site-dependent)	Few Repetitions	Usually, 1 permanent sonde in one or multiple locations
	Relative Cost (equipment and supplies)	Very High (> \$10,000)	One-time fee plus recurring maintenance, new probes, and calibration standards
Survey / Data Quality	Accuracy (at a survey area level)	High	----
	Precision (at a survey area level)	High	----
	Qualitative-Quantitative Score	Quantitative	----
	Subjectivity-Objectivity Score	Objective	----

Resulting Data Types

The application of continuous water quality data sonde monitoring survey protocols will yield quantitative data for all measured parameters including pH, salinity, temperature, water depth, dissolved oxygen, turbidity, and chlorophyll (optional). These variables are displayed as discreet readings for each monitoring interval (e.g., every 15, 30, or 60 minutes). Resulting data are useful in the identification of trends over varying time scales (e.g., daily, seasonally, annually) and can be helpful in identifying the times, durations, and individual parameter variability during anomalous events such as

freshwater inputs or storm events, nutrient discharges, or algal blooms. If a site is telemetered (i.e., automatic transmission to receiving equipment), results can be viewed in near real-time.

Objective

Water quality measurements may be used as indicators of both human health concerns and the overall chemical, physical, and biological conditions of a site (Johnston et al. 2012). Variations in water quality affect the biota and physical properties of wetlands, including vegetation, ichthyofauna, benthic and pelagic invertebrates, salinity profiles, and anoxic conditions.

Water quality probes are used to measure water parameters in continuous monitoring mode by collecting data at user-defined intervals and storing those data until downloaded at discrete intervals. Water quality multi-probes can be deployed continuously at monitoring stations to characterize parameters over multiple tidal cycles, through freshwater-input events, or over longer periods of time. The number and spatial distribution of monitoring stations depends upon restoration and monitoring objectives and site-specific considerations. The primary objective of this SOP describes a single multi-probe deployed within a primary channel in an estuary or smaller tidal channel to identify the overall general water quality condition of that area and identify gross problematic events (e.g., periods of low dissolved oxygen).

A variety of options are now available for long-term monitoring with dataloggers. Due to the different maintenance requirements of various water quality multi-probe sondes, general guidelines are presented below. General principles should apply reasonably well to most multi-probe sondes, but details will vary with sonde make and model. A focus on the YSI EXO multi-probe is provided (Figure 1), as this model has received extensive consideration as part of the NOAA National Estuarine Research Reserve (NERR) System-Wide Monitoring Program (nerrsdata.org). Other sondes are available (e.g., OTT Hydrolab DS5X). Additionally, multi-probe sondes may be upgraded or replaced over time, especially for long-term monitoring programs. Documentation of dataloggers including model and specifications should be recorded and included in metadata.



Figure 1. YSI EXO sonde (credit: YSI).

Water Quality Parameters

Water quality parameters identified in this SOP include:

- Temperature, salinity, conductivity, dissolved oxygen, depth, pH, turbidity, and chlorophyll (additional optional parameters include oxidation-reduction potential, blue-green algae, ammonium, nitrate, and chloride)

Installation

Methods for sonde deployment will vary by site. Considerations include (modified from USGS 2012, see also nerrsdata.org):

1. Sampling intent (e.g., capturing hypoxic events)
2. Sonde location (e.g., river, tidal channel, or subtidal habitat)
3. Method of access (e.g., boat on high tide vs. wading on low tide)
4. Substrate type (soft substrates may require additional reinforcements)
5. Potential threats to instrument (e.g., biofouling, logs, *Ulva sp.*, boats, vandalism)

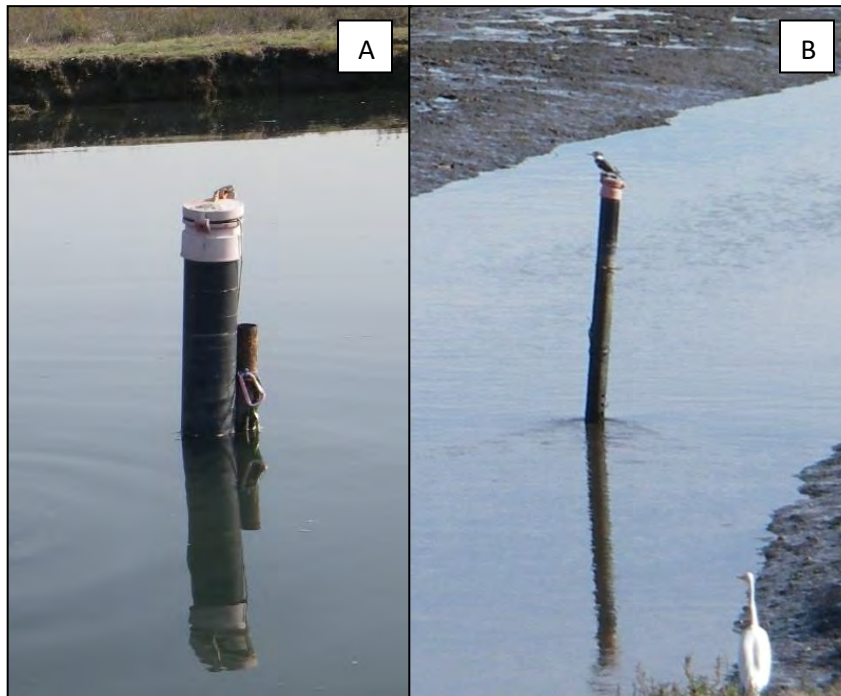


Figure 2. Installed YSI 6600 V2 multi-probe with PVC housing at differing tide heights: (A) high tide, (B) low tide.

For most purposes, installation includes driving a post (stake or PVC) into the substrate to which the sonde housing (PVC tube) is attached. Where available, sondes also can be attached to existing infrastructure such as pier pilings. Sondes should be placed inside a PVC (or ABS) tube, which should have larger holes cut into the sides and bottom of the tube to allow natural water movement on and around the probes. This tube acts as protection for the sonde and as a stilling well.

The elevation of the depth sensor on the sonde body in the field should be measured using surveying equipment or RTK GPS so that water depth data can be converted from relative depth to elevation (relative to a geodetic datum such as NAVD88). It is critical that the probe be returned to the same location after each sonde calibration to help ensure the accuracy of the depth / elevation conversion, and sonde elevation should be re-surveyed periodically (e.g., annually or following a major event).

Helpful hint: Cutting small holes in the bottom of the housing may reduce sedimentation in the housing. Additionally, if subject to tampering, a locking mechanism is recommended for the housing.

Methods

These SOPs are based on those developed by the NERR system and are available at the Centralized Data Management Office website (nerrsdata.org). They will be briefly summarized

below. While the following SOPs are specific to EXO sondes, other model sondes will have similar operating procedures. Always refer to the sonde manufacturer instructions and tutorials when operating sondes and for detailed calibration and download information (e.g., for YSI EXO: <https://www.ysi.com/file%20library/documents/manuals/exo-user-manual-web.pdf>).

Programming and Calibration

Communication with the EXO sonde is conducted with KORS software, a copy of which is provided with each EXO on a USB thumb drive. This software is used for EXO calibration, post-deployment readings, data file download, sonde/probe firmware updates, and any other necessary interaction with the sonde via a PC. Communication with the sonde and a laptop (running Windows) can occur either through a cabled connection or Bluetooth.

The probe should be calibrated before the initial deployment and post-checked after retrieval, or approximately every two to four weeks (although longer deployments are possible with the EXO sonde, depending on site characteristics such as degree of fouling). Detailed calibration instructions for each parameter are provided at nerrsdata.org, but some general considerations and recommendations include the following:

1. Good laboratory practices should always be followed when handling calibration standards. Please refer to MSDS sheets for any standard when necessary.
2. A **Calibration Log** and **Field Log** should be completed for each instrument's calibration, deployment and retrieval, and post-calibration (see Appendix 1.1B).
3. The sonde should be visually inspected for any abnormalities, such as a broken probe or damaged bulkhead.
4. Remove the wiper brush from the sonde, as the brush can trap residual standard and affect the calibration accuracy.
5. Calibrations are best performed using a guard and calibration cup that are dedicated to calibration only and never taken in the field. This ensures a high level of cleanliness and non-contamination during the calibration process.
6. During the calibration of the probes **NEVER** accept any calibrations that display an error message. Troubleshoot the cause of the problem, correct it, and recalibrate or replace the probe before deploying the instrument.
7. Standards must be active (check expiration date) and fresh for all calibrations. Previously used, clean standards may be used to rinse probes but must not be used to calibrate probes. Discard and replace all expired standards.
8. All diagnostic parameters (pH millivolts, DO gain, and conductivity cell constant) for EXO sondes are presented after calibration of the respective parameter on the KORS generated calibration sheet and should be recorded on the data sheet once calibration of those probes is complete.
9. Recommended probe calibration order: Temperature (not a true calibration, but a check against NIST source), Specific Conductivity, pH, Depth, Turbidity, Dissolved Oxygen.

10. Prior to calibration it is a good idea to record serial number for the sonde and probe on in the Calibration Log's "Datasonde & Probe Identification Numbers" section.
11. Batch calibrations are possible, where multiple versions of the same probe type are installed into an EXO sonde and calibrated one after the other. Once calibrated in this fashion, which can reduce costs associated with calibration solutions, the probes are then installed into their respective EXO sondes.

Deployment

1. For sonde transportation, it is best to use a cooler where the sondes can rest horizontally. A tap-water-soaked white towel must be wrapped around each sonde. This is to be done during both deployment and upon retrieval. The wet towels reduce shock and vibration damage and ensure a saturated environment for the oxygen probe during transport. Alternately, keep the calibration cup with a small amount of water fixed on the sonde during transfer. Towels or other soft materials will keep the sonde from vibration damage. Probes should not be submerged during short- or long-term storage for any substantial length of time.
2. Independent, paired field data readings are suggested at all sonde retrievals / deployments. Ideally, use a hand-held meter, Winkler titration, or other properly calibrated instrument to collect this data alongside the deployed sonde for its last reading and the newly deployed sonde for its first reading. At a minimum, take an independent paired reading with the freshly calibrated sonde against the deployed sonde before replacing. Record the data from the independent instrument in the Field Log with one log following each sonde through its deployment.

Retrieval

1. Retrieve the sonde from the water and visually examine it and the probes for fouling and/or damage. Note any fouling type and amount in the "Fouling Presence" section of the Field Log; however, **DO NOT** remove fouling, so that true post-deployment readings may be obtained.
2. Record field data on the data sheets.
3. Wrap the EXO sonde in a tap-water saturated white towel, and place in a secure container (e.g., a cooler) to prevent severe vibrations to the EXO sonde during transportation. Alternately, replace the sensor guard with a calibration cup with a small amount of DI water. Secure in a similar manner as above with towels or soft materials.

Data Download and Post-Deployment Readings

1. Post Deployment Calibration Checks – These checks note any changes or drift of the probe during deployment combined with effects of biofouling. This process is critical not only for data QA/QC, but also for data users to know if the data were affected by biofouling, wear and tear, or other issues. Ideally these checks will take place within 24 hours of sonde retrieval. If not, it is critical to make a visual inspection of the conductivity cells and note, either photographically or via notes, any visible fouling to document related drift. Bubbles and saturated water bath currents may dislodge material and significantly impact drift. Batch calibration is NOT suggested

during post-deployment readings, since it is important to take these readings with the sonde remaining in a condition similar to what it was while deployed in the field.

2. **Data Download** – Place the sonde into a bucket of clean water that has been aerated for at least 60 minutes to create a 100% air saturated water bath. Allow the sonde ample time to reach temperature equilibration prior to beginning the download and post-deployment readings procedures.

Probe Care and Storage

1. Most of the probes, except Conductivity, have a limited shelf life, so do not purchase replacements too far in advance. The procedure for storage of probes is different for short-term (1 month or less) and long-term (greater than 1 month).
2. For short term storage, it is important to keep the probes moist but not immersed in water. Keep probes attached to the EXO sonde and place the sonde in approximately 0.5 in of tap water (not distilled) in the sealed EXO calibration cup.
3. **Long-term Storage** – Clean conductivity sensors and store them either dry or wet. If they are in contact with solution, it should not be corrosive. The pH probe should be removed from the EXO sonde if storage will exceed 30 days and stored in the pH storage cup (the one it was shipped in) containing 1 molar KCL or pH 4 buffer. Dissolved Oxygen probes should be stored in a water-saturated air environment (attached to the sonde with a small amount of water in the calibration cup to maintain humidity) to avoid the need for a 12-hour membrane rehydration at a later date (the probe can be stored dry, but if done so it must be re-hydrated in saturated water for a 12 hour period). No special precautions are necessary for the Depth sensor.
4. Remove the brush from the wiper probe and store dry (make sure it is clean and dries in original shape – no gaps or forks in the bristles). The wiper itself can be stored in a humid environment or dry environment.
5. Remove copper tape applied directly to the sensors prior to long-term storage to prevent the glue from hardening and becoming difficult to remove. Copper tape can remain in place if a protective barrier is applied underneath like packing tape or YSI clear anti-fouling sleeve.
6. Remove batteries prior to storage greater than 30 days. The battery compartment and compartment cap should be cleaned thoroughly and re-greased prior to storage.
7. Clean and re-grease the two sonde connectors (located at the top of the sonde) and place connector caps on both.
8. Plug ports of any missing sensors.

QAQC Procedures

Quality Assurance and Quality Control (QAQC) procedures should be conducted on all data. Detailed data management procedures are available at nerrsdata.org. Also, the NERRS Centralized Data Management Office (CDMO) has a free upload service that allows users to upload raw water quality data files (collected using NERR System-Wide Monitoring Program or similar protocols) for automated QC and formatting. The returned files may be used with the NERR QAQC macro for further automated coding and error checking, editing using the CDMO's flagging / coding system, automated graphing, and

summary statistics. This is available at: <http://cdmo.baruch.sc.edu/nonswmp/>. Some considerations related to QAQC are:

1. Always archive the data. Backup and archive on a regular basis to ensure there will be no data loss. Third party software can be purchased to accomplish this. Archive the raw .DAT and raw .BIN data files from the data sonde as they are retrieved.
2. Record in local Standard Time **NOT** Daylight Savings Time. Set the clocks on your instruments and the computers that interface with them to Standard Time and DO NOT adjust them to Daylight Savings. Try to get in the habit of recording the time off your watch in Standard Time as well.
3. If the temperature sensor fails on a YSI EXO sonde, all data except turbidity will need to be rejected. In the event of such severe failure of the EXO CT probe that the sonde powers it down, DO%, pH, and ChlFluor data may not need to be rejected if they pass other quality control checks.
4. If the conductivity sensor malfunctions or is poorly calibrated then salinity, specific conductivity, DO mg/L (DO concentration) and depth will need to be rejected.
5. Poor calibrations or sensor malfunctions are evident when plotting appended files for multiple deployments, i.e., monthly, seasonal and yearly files.
6. Negative temperature, depth, and turbidity data: The **ONLY** potentially acceptable negative data are from the temperature, depth or turbidity probes. All other negative data must be rejected.
7. Do not remove data values from the dataset under any circumstances, except for the removal of pre- and post-deployment records.

Data Analyses

After corrections have been made, data may be used in multiple analyses. Examples include graphing the data over time, grouping the data by hour or day to look at broader trends over time, correlating variables, pinpointing events such as the frequency of freshwater inputs, and analyzing percent time against a threshold (e.g., percent time of dissolved oxygen below 1 mg/L).

Health and Safety Precautions

Sharp mollusks are often present within the substrate surrounding the sonde housing and on sondes that have been deployed for an extended period. Appropriate foot protection (e.g., neoprene dive / surf booties with a thick sole, if not collecting by boat) and hand protection (e.g., neoprene gloves) should be worn when wading and handling the sonde. Occasionally, an animal such as a crab or octopus may be inside the housing or sonde.

References and Applicable Literature

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Contact Information

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APPENDIX 1.1A

	Evaluation Metric	General WQ Parameters	Notes
	Correlation to L2 CRAM	Attribute 1 & 2	----
Personnel Requirements	Specialty Equipment or Clothing Required	Many Specialty Items	Data sonde plus handheld reading device or laptop; calibration standards
	Ease of Transport (amount or weight of supplies)	Few Items / Easy	Can be heavy
	Ease of Implementation	Moderate	Sonde often needs troubleshooting, depending on the model
	Expertise / Skill Level	Some Technical Knowledge	Familiarity with the manual is needed
	Number of Personnel	1	----
	Training Requirements	Yes	Familiarity with the manual is needed; "How To" YouTube videos available
	Seasonality of Survey Time	Continuous	----
	Suggested Frequency	Continuous	15-30 minute intervals are recommended
Survey / Data Quality	Type of Output	Numerical	----
	Active or Passive Monitoring Style	Passive	----
	Specialty Computer Software Required	Yes	----
	Availability of Online / External Resources	Many	Extensive manuals, videos, and suggested use documents
Potential Limitations	Wetland Type Applicability	All	Must be submerged in water for all or part time
	Images or Multi-Media Required	None required	----
	Degree of Impact / Disturbance	Low Disturbance	Sonde housings must be anchored in ground
	Vegetation Height Limitation	Not Applicable	----
	Appropriate for Tidal / Wet Habitats	Yes	Yes
	Tide Height	All tides	All tides
	Regional or Broad Implementation *	Almost always used	----
	Potential for Hazards / Risk	Low to No Risk	----
Restrictions	None	----	

* based on monitoring literature review

APPENDIX 1.1.

CALIBRATION WORK SHEET

Date of Calibration: _____
Technician: _____

Sonde ID: _____

RP DO membrane changed? Y N *Note: Wait 3 to 6 hours before calibrating for unattended*
RP DO membrane o-ring changed? Y N *deployments; run in Discrete mode for 10 minutes to accelerate*
burn in. (Rapid Pulse DO Only)
Turbidity wiper changed? Y N Chlorophyll wiper changed? Y N
ROX DO wiper changed? Y N BGA-PE wiper changed? Y N
BGA-PC wiper changed? Y N Rhodamine wiper changed? Y N

Note: If parking problems occur with optical probes having a serial number 07L (Dec 07) or older, be sure the firmware is 3.06 or later. Parking issues with optical probes having a serial number prior to 07L may be related to a dirty wiper body or pad.

Record sonde battery voltage: _____ (if applicable)

Record Calibration Values
Standard Pre Cal / Post Cal

Record the following diagnostic numbers after calibration.

6560 Conductivity cell constant _____ Range 5.0 ± .45

Integrated conductivity cell constant _____ Range 5.0 ± .70

pH mv Buffer 7 _____ Range 0 ± 50 mv

pH mv Buffer 4 _____ Range +180 ± 50 mv*

pH mv Buffer 10 _____ Range -180 ± 50 mv *

*Note: Millivolt span between pH 4 and 7 should be ≈ 165 to 180 mv

Millivolt span between pH 7 and 10 should be ≈ 165 to 180 mv

DO charge (RP only) _____ Range 25 to 75

DO gain _____ Range 0.7 to 1.4

ODO gain _____ Range 0.85 to 1.15

Turbidity standard used in calibration _____

Manufacturer and part number _____

Barometric Pressure: _____ mmHg

DO % Calculated – (BARO mmHg divided by 7.6) = % saturation

Example: $760 \div 7.6 = 100.0\%$

Depth Calibration - If zero was entered, record barometric pressure at time of calibration _____ mmHg

Depth Calibration - If offset depth was entered, record value _____ meters/feet and pressure _____ mmHg

Depth Calibration (Vented) – Acceptable calibration constant: 0.0 psig ± 0.15 _____

Temperature _____ Sonde

Conductivity _____ / _____

pH 7 _____ / _____

pH 4 _____ / _____

pH 10 _____ / _____

ORP _____ / _____

Turbidity _____ / _____

Turbidity _____ / _____

Turbidity 0.5 _____ / _____

Chlorophyll _____ / _____

Chlorophyll _____ / _____

DO RP _____ / _____

DO ROX _____ / _____

BGA PE/PC _____ / _____

BGA PE/PC _____ / _____

Rhodamine _____ / _____

Notes: _____

Appendix 1.1C

Symptoms	Probable Cause	Action
Data set collected is incomplete (stopped logging prior to pick-up)	Batteries died during unattended sample	<ol style="list-style-type: none"> 1. Check battery voltage of sonde during calibration. The voltage should roughly equate to the voltage of each individual battery x the total number of batteries) 2. Make sure the 650 Handheld is not powering the sonde (System Menu: "Power Sonde" checked-off) during the setup for deployment
	Auto sleep functions were not set to off	<ol style="list-style-type: none"> 1. If the auto sleep functions are set to "Off" they will draw battery power between samples, using the batteries up quicker – Check battery volt output records to detect usage rates
No data recorded during an unattended sample	Batteries were dead before deployment	<ol style="list-style-type: none"> 1. Check battery voltage of sonde during calibration. The voltage should mirror the voltage of each individual battery times the total number of batteries) 2. Make sure the 650 Handheld is not powering the sonde (System Menu: "Power Sonde" checked-off)
	Sonde and handheld were connected during the first reading during the unattended sample	<ol style="list-style-type: none"> 1. It is common practice to wait and observe the first reading before unattended deployment. However the 650 Handheld needs to be disconnected from the sonde prior to the first sample. This will allow the data to be stored in the bulkhead versus the 650 handheld
650 Handheld will not connect with Sonde	There may be water, or other debris, disturbing the connection	<ol style="list-style-type: none"> 1. Use a Q-tip and blot dry the Sonde Connector as well as the connector on the Field Cable. You may also use compressed air to rid the connection of water and other debris
	Data Sonde batteries are dead	<ol style="list-style-type: none"> 1. If this is the case, you want to change your 650 Handheld settings, and check-on the "Power Sonde" option in the System Menu. This will power your sonde in order to upload the data. New batteries will be required for future deployment
New pH probe installed is not reading accurately (calibrating correctly)	"Shipping residue" has clouded the bulb	<ol style="list-style-type: none"> 1. Soak probe in pH 4 standard for 3 - 45 minutes
	Defective probe	<ol style="list-style-type: none"> 1. Return back to YSI (make sure they pay for the 1 day shipping to and from)

For additional troubleshooting tips see here: <http://www.ysi.com/media/pdfs/YSI-Calibration-Maintenance-Troubleshooting-Tips-6-Series-Sondes-2-8-10.pdf>

Data set is missing parameters (probes not recording)	The specific probe has been disabled	1. Access handheld and enable probe
	The new probe has never been activated/enabled	1. Go in and enable/activate probe (the Sonde will not automatically enable a new probe, it needs to be told to activate said probe and what units to record in)
	The probe has not been set to record	2. Access handheld and set probe to record
Optical DO Probe not calibrating correctly	The DO Membrane has become bio-fouled	1. Replace DO Membrane (roughly \$150.00)
Bio-fouling on the bulb of the pH probe	Biologic reasons	1. Soak in 1 mol of Hydrochloric Acid for 30 minutes. Do not attempt to clean bulb with tools (Q-tip, sponge, pipe cleaner, etc.)
Error message "Date/Time" not set when setting probe to "Unattended Sample"	Date/Time needs to be reset	1. Access sonde "System" menu and set correct Date/ Time 2. If Date/ Time is correct, change Time by 1 second

For additional troubleshooting tips see here: <http://www.ysi.com/media/pdfs/YSI-Calibration-Maintenance-Troubleshooting-Tips-6-Series-Sondes-2-8-10.pdf>

APPENDIX 1.1D

Parameter	Range	Resolution	Accuracy
Optical Dissolved Oxygen	0 to 500%	0.1%	0 to 200%: $\pm 1\%$ of reading or 1% air saturation, whichever is greater; 200 to 500%: $\pm 15\%$ of reading
Conductivity 6560 Sensor*	0 to 100 mS/cm	0.001 to 0.1 mS/cm (range dependent)	$\pm 0.5\%$ of reading + 0.001 mS/cm
Salinity	0 to 70 ppt	0.01 ppt	$\pm 1\%$ of reading or 0.1 ppt, whichever is greater
pH 6561 Sensor*	to 14 units	0.01 unit	± 0.2 unit
Turbidity 6136 Sensor*	0 to 1,000 NTU	0.1 NTU	$\pm 2\%$ of reading or 0.3 NTU, whichever is greater**
Depth	Deep: to 656 ft, 200 m Medium: 0 to 200 ft, 61 m Shallow: 0 to 30 ft, 9.1 m Vented Level: 0 to 30 ft, 9.1 m	0.001 ft, 0.001 m 0.001 ft, 0.001 m 0.001 ft, 0.001 m 0.001 ft, 0.001 m	± 1 ft, ± 0.3 m ± 0.4 ft, ± 0.12 m ± 0.06 ft, ± 0.02 m ± 0.01 ft, 0.003 m



Standard Operating Procedures (2.1): Soil Salinity, Texture, and Pore Water

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Prepared for the United States Environmental
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Standard Operating Procedures: Soil Salinity, Texture, and Pore Water

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Protocol Suitability Evaluation

A habitat suitability table containing appropriate estuarine wetland habitat types (of those evaluated) to implement soil salinity and soil characteristic protocols is displayed in Table 1. A comparative assessment of cost, effort, and data quality are shown in Table 2. A matrix of additional detailed categorical evaluations of soil salinity and soil characteristic protocols can be found in Appendix 2.1A.

Table 1. Appropriate habitat types for soil salinity and texture survey protocols.

Survey Protocol	Habitat Types					
	Tidal Channel	Mud/sand flat	Emergent salt marsh	Non-tidal salt marsh	Salt pan	'Degraded' / fill
Soil Salinity		X	X	X	X	X
Pore Water Salinity	X	X	X		X	
Soil Texture	X	X	X	X	X	X

Table 2. Categorical assessment of cost/effort and data quality for soil salinity and texture survey protocols.

	Evaluation Metric	Soil Salinity	Pore Water Salinity	Soil Texture	Notes
Time / Effort	Office Preparation Time	0-10 minutes	0-10 minutes	0-10 minutes	Site selection and any GPS locations
	Equipment Construction Time (one time)	0-10 minutes	0-10 minutes	0-10 minutes	Collect field supplies
	Field Time (per transect)	10-30 minutes	10-30 minutes	10-30 minutes	Additional time is occasionally required for pore water to saturate within holes
	Laboratory Time (per transect)	> 60 minutes	0 minutes	0-10 minutes	Sample drying time, 48 hours to 1.5 weeks; sample processing, 30-60 minutes; post-processing, 24 to 48 hours
	Post-Survey Processing / QAQC Time	10-30 minutes	0-10 minutes	0-10 minutes	---
	Minimum Repetition (site-dependent)	Many Repetitions	Many Repetitions	Many Repetitions	---
	Relative Cost (equipment and supplies)	> \$50	\$15 - \$50	\$0	Cost will vary whether a refractometer or conductivity meter is used
Survey / Data Quality	Accuracy (at a survey area level)	High	High	Medium	---
	Precision (at a survey area level)	High	High	Medium	---
	Qualitative-Quantitative Score	Quantitative	Quantitative	Qualitative	---
	Subjectivity-Objectivity Score	Objective	Objective	Subjective	---

Resulting Data Types

The application of soil salinity and pore water protocols will yield quantitative salinity data displayed in parts per thousand. Data can be extrapolated up to the transect- or habitat-level. Salinity data can be correlated with vegetative cover or invertebrate biomass for assessing higher levels of wetland function.

Soil texture is a qualitative analysis meant to provide a general understanding of the broad categorization of different grain sizes in the soil (e.g., sandy clay).

Objective

Along with hydrology, soil salinity is one of the primary factors influencing vegetation communities and alliances in wetland habitats (James-Pirri et al. 2002). Salt composition and distribution within the soil profile affects many biological and chemical parameters including plant response, ion effects, and nutritional imbalances (NSSC 2009). Soil texture and individual phenotypic characteristics of each plant species are also widely understood to influence vegetation growth under various saline soil conditions.

The primary purpose of this sampling method is to understand the distribution of surface soil salinity and texture across estuarine wetland habitats. These data can be analyzed in conjunction with vegetation cover as well as seed bank data to better understand the responses of the vegetative community to general edaphic conditions. Soil salinity may also be assessed with other constituents to determine additional soil characterization at a particular location.

Equipment

The following supplies are recommended for full soil sampling (all three parameters); a subset may be appropriate if soil texture is the only target of the survey:

1. GPS and extra batteries
2. Refractometer (Figure 1) and eyedropper
3. Sealable plastic bags
4. Squirt bottles filled with distilled water
5. Data sheets (Appendix 2.1B) with clipboards and maps (optional)
6. "Determining Soil Texture By Feel Method" Flowchart card (Thien 1979; Appendix 2.1C)
7. Hand or gardening trawl (15cm long blade)
8. Glass jars (125 – 500 mL)
9. Conductivity meter
10. Clean plastic syringe
11. Graduated cylinder
12. Pens and markers
13. Duct or lab tape for labeling
14. Soil corer
15. Filter paper
16. Scoopula



Figure 1. Salinity hand refractometer (courtesy: Extech).

Field Preparation

Equipment described above should be collected prior to the field shift. Batteries for all electronic devices should be checked and replaced as needed, and relevant data sheets should be printed and attached to the clipboards. The refractometer and/or conductivity meter should be calibrated before the start of each salinity survey. If a survey event extends over many days, calibration should occur weekly at a minimum frequency.

Field Methods

Field methods described below are detailed for each of the three different soil survey parameters including: soil salinity, pore water, and soil texture. Frequency should be project- or goal-dependent, but annual or bi-annual surveying on a low tide during the dry season is recommended. Additional targeted surveying may be undertaken after rain events or during the wet season to assess pooled or ponded areas of inundated soil or to explore variability across seasons. Salinity assessed using the soil collection method is not directly comparable to pore water salinity.

Survey locations are also project dependent, but can be in depressions (e.g., salt pans), along vegetation transects, between transition habitats, or in areas where there are specific questions about vegetation growth or targeted restoration areas. Transects should begin from the point of lower elevation and continue with a sample approximately every 3-5 meters (unless more intensive salinity mapping is desired). Points can be further spaced out if less precision is needed. An additional option includes geospatially allocating grid points throughout a targeted survey area.

The depth of the soil collected is also an important consideration. In the surface option, detailed below, soil is collected at a depth of 1 cm. This depth is likely ideal for germination studies, but it will not provide complete information on the soil conditions at the rooting depth of perennials, and it might be too disruptive for long-term monitoring plots that are visited annually. In the rooting depth option, the top 10 cm of soil is collected with less disruption to the sampling area, which might be a more appropriate method for studies of perennial marsh plants. Depth should be considered based on the objectives of the monitoring program.

Soil Collection Methods – Dry Soils:

Surface option:

1. Clear desired patch of soil of any debris or above-ground vegetation, using the gardening trawl or knife.
2. Make a square on the soil surface with each side measuring approximately 15 cm in length (Figure 2a). *Helpful hint:* using the gardening trawl to measure the length of the soil square reduces equipment needed in the field.
3. Collect all soil within the square to a depth of 1 cm and place soil into a labeled and sealable plastic bag. Any noticeable vegetation (roots, runners, etc.) or subsurface debris should be removed to ease subsequent processing. This will provide approximately 200 mL of soil.



Figure 2a. 15 x 15 cm soil square being removed by a trawl.



Figure 2b. Soil corer.

Rooting depth option:

1. Use a soil corer (Figure 2b) to extract the top 10 cm of soil, discarding any plant material that might be present.
2. Place soil sample into a labeled and sealable plastic bag. *Helpful hint:* Some field samplers will prefer to use a gardening glove for this step, especially if repeated samples are required.

Pore Water (for use on saturated soils):

1. Using the hand trawl, push it into the target soil area and swing side to side/spin the handle back and forth to create an indentation in the soil (approximately 5 cm deep). Be careful not to allow surface water to flow into the indentation.
2. Mark the precise location of the indentation on the GPS and properly label the waypoint. *Helpful hint:* It is usually a good idea to make a few holes within the same area, to ensure one of them fills with soil pore water and to achieve repetition.

3. Wait several minutes to allow pore water to pool into the depression. *Note: Soils with high clay content or less infiltration will require additional time to accumulate pore water and may take upwards of 30-40 minutes.*
4. Use the eyedropper to collect pore water (Figure 3A), placing several drops on the refractometer (debris or sediment in the eyedropper will result in inaccurate readings), read the salinity (Figure 3B, for refractometer), and record the value on the datasheet.

Soil Texture (all soils):

1. Use approximately 100 mL of the collected soil sample (the rest will be returned to the lab for salinity processing).
2. Follow precise directions located on the “Determining Soil Texture by Feel Method” flowchart card (Appendix 2.1C).
3. Record the corresponding soil texture type on the datasheet (Appendix 2.1B).

Laboratory Methods (soil salinity only)

There are important differences in laboratory methods that will result in different readings and results. The saturated soil paste method is generally considered to produce results that are most representative of the conditions plant roots experience in the soil, but preparing a standard dilution is faster and therefore typically less expensive (Richards 1954). Results of the two methods are not directly comparable. While multiple studies have empirically calculated the relationship between the saturated soil paste method and standard dilutions, the resulting equations are variable and likely site specific (Zhang et al. 2005). If a reduced sample processing time is desired, it is recommended that a regression equation first be developed for the relationship between saturated soil paste and standard dilution for soil samples that represent the range of variability found at a particular site.

Initial steps:

1. Moist soil samples will need to be dried first. If necessary, dry the sample using a laboratory oven on a low heat setting (< 40° C) or by placing moist soil in direct sunlight. During this time, your plastic bag should be unsealed to allow the moisture to escape, taking care to not spill any of the sample. Depending on your method, the particular soil moisture content, and ambient humidity, drying may take anywhere from several days to 1.5 weeks.
2. Break up the dry sample so no large aggregates (clods of soil > 0.2 cm in diameter) remain using a coffee grinder, rolling pin, hammer, empty glass jar, etc.



Figure 3. Eyedropper removing pore water from a shallow depression (A) and reading the refractometer (B).

3. Remove as much foreign matter, plant material, and stones from the sample as possible.
4. Transfer sample from sealable plastic bag to a glass jar.

Saturated soil paste:

1. Add distilled water to soil, mixing as water is added, until the sample is “glistening” (Richards 1954). The sample should be wet enough that the soil slides off the scoopula, but not so wet that water is standing on the surface. The sample, if turned upside-down, should not slide out of the container or jar.
2. Let samples sit, undisturbed, for 24-48 hours to allow salts to fully dissolve
3. Place filter paper at the end of a 5 mL plastic syringe, add glistening soil sample, and express the resulting soil water onto refractometer. Record salinity on datasheet. Alternately, a low-pressure vacuum pump may be used.

Standard 1:1 Dilution:

1. Add one part soil by volume to one part distilled water by volume (Zedler 2001), e.g., 100 g of soil sample should be mixed with 100 mL of distilled water.
2. Shake the container vigorously by hand (do not use a shaker table) for at least three minutes to ensure all salts dissolve. In clay loam to clay soils, more shaking will bring more salts into the solution and increase the accuracy of the test (NSW 2000).
3. Let samples sit, undisturbed, for 24-48 hours to allow salts to fully dissolve and create a less turbid sample (Figure 4).
4. Heavier salt water will concentrate towards the bottom of the jar, forming a halocline. To eliminate this salt gradient and form a homogenous salt water sample, take the eyedropper and gently stir the water while using caution not to re-suspend the sediment.
5. Using the eyedropper, collect a sample of water from the middle of the water column, place on the refractometer, and take a reading. Rinse the refractometer and the eyedropper with distilled water. Repeat two more times and record all values onto the datasheet (Appendix 2.1B). If a water conductivity meter is available, this may also be used to take more precise readings by extracting sample water using a plastic syringe and transferring it to a graduated cylinder to take the readings (Figure 5). *Helpful hint:*



Figure 4. Eyedropper collecting water to test salinity.

A graduated cylinder with a slightly larger diameter than the conductivity probe will reduce the amount of sample water required (Figure 5).

6. When complete, discard the sample, wash the refractometer and sample jar with distilled water and air dry.

Data Entry and QAQC Procedures

Data should be entered in the field for pore water samples and in the lab for soil samples using the appropriate data sheet (Appendix 2.1B). All required fields should be completed in full, and the data recorder should assign their name at the top of the document(s). Data should be transferred to the appropriate electronic database within three days, and the hard copies filed in labeled binders.

Electronic copies of all data should be housed on an in-house dedicated server and backed up to a cloud-based or off-site server nightly. Hard copies should be saved for five years. Electronic copies should be saved indefinitely.

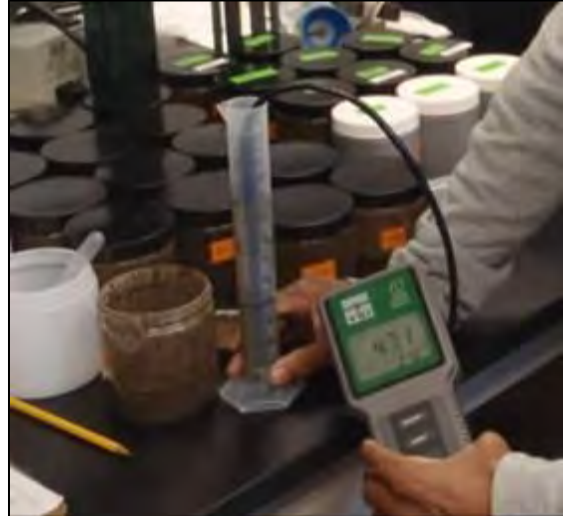


Figure 5. Reading the salinity in a graduated cylinder using a conductivity probe.

Quality Assurance and Quality Control (QAQC) procedures should be conducted on all data. QAQC procedures should be conducted by the QA Officer and include a thorough review of all entries, double checking of all formulas or macros, and a confirmation that all data sheets, Chain-of-Custody forms, and field notes are filed appropriately with electronic back-up copies available. QAQC should verify that the entered data match the hard copies of the field data sheets. Any discrepancies should be corrected, and the initial data entry technician notified.

Data Analyses

After data have been entered, corrections made and QAQC procedures completed, data can be used in multiple analyses. Examples include maps of soil salinity (or pore water) gradients and analyses of salinities with elevation contours and associations of vegetation alliances. Other examples include averaging soil salinity by micro-habitat or identifying a range in a particular habitat type. Soil texture can be broadly associated with other characteristics or used as a reference for other surveys.

Health and Safety Precautions

Not applicable.

References and Applicable Literature

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APPENDIX 2.1A

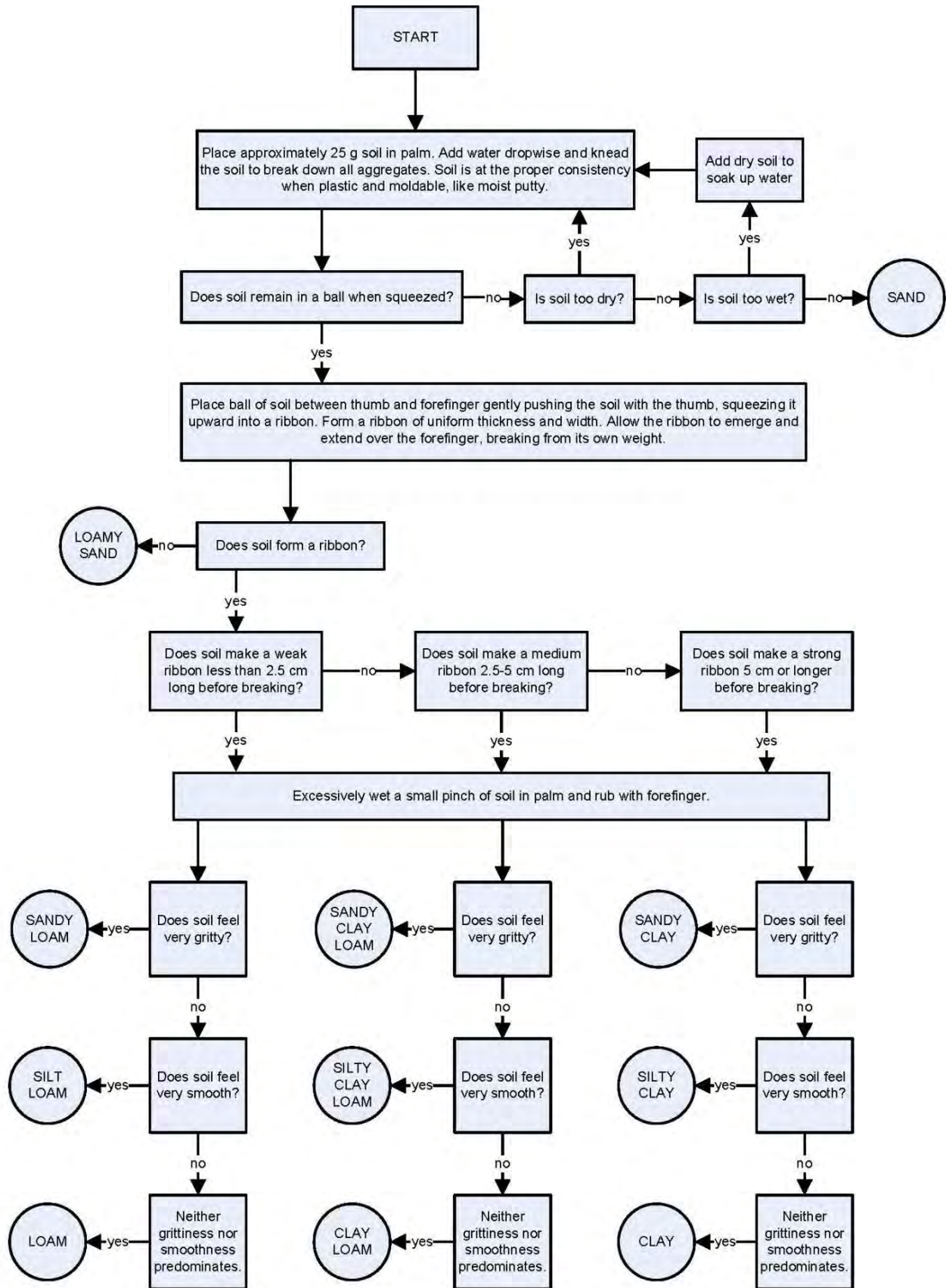
Evaluation Metric		Soil Salinity	Pore Water Salinity	Soil Texture	Notes
Correlation to L2 CRAM		Not Applicable	Not Applicable	Not Applicable	----
Personnel Requirements	Specialty Equipment or Clothing Required	Few Specialty Items	Few Specialty Items	None	Refractometer or conductivity meter, trowel
	Ease of Transport (amount or weight of supplies)	Few items / Easy	Few items / Easy	Few items / Easy	Depending on the number of soil samples collected
	Ease of Implementation	Moderate	Easy	Easy	---
	Expertise / Skill Level	Some Technical Knowledge	Some Technical Knowledge	None Required	Understand how to read refractometer or conductivity meter
	Number of Personnel	1	1	1	Multiple persons will expedite the soil processing for soil salinity
	Training Requirements	Some Technical Knowledge	Some Technical Knowledge	Some Technical Knowledge	Understand how to read and calibrate refractometer or conductivity meter
	Seasonality of Survey Time	Before wet season	Before wet season	Anytime	---
	Suggested Frequency	Annual	Annual	Annual or Biannual	---
Survey / Data Quality	Type of Output	Numerical	Numerical	Categorical	---
	Active or Passive Monitoring Style	Active	Active	Active	---
	Specialty Computer Software Required	No	No	No	---
	Availability of Online / External Resources	Some	Some	Some	YouTube and various online sources
Potential Limitations	Wetland Type Applicability	All	All	All	---
	Images or Multi-Media Required	None Required	None Required	None Required	---
	Degree of Impact / Disturbance	Low Disturbance	Low Disturbance	Low Disturbance	---
	Vegetation Height Limitation	No Limitations	No Limitations	No Limitations	---
	Appropriate for Tidal / Wet Habitats	No	Yes	Yes	---
	Tide Height	Low Tide Only	Low Tide Only	Low Tide Only	---
	Regional or Broad Implementation *	Infrequently Used	Infrequently Used	Infrequently Used	---
	Potential for Hazards / Risk	Low to No Risk	Low to No Risk	Low to No Risk	---
Restrictions	None	None	None	---	

* based on monitoring literature review

APPENDIX 2.1B

SOIL SALINITY DATASHEET					
Survey Area / Habitat (e.g. "A / seasonal wetland"):					
Date:		Staff (circle recorder initials):			
Survey Start Time: Stop:		Entered: _____	Date _____	QAQC: _____ Date _____	
Weather:			Days Since Rain (approx):		
General Soil Conditions:			Other Notes:		
High Tide: Height:		Time:	Page _____ of _____		
Salinity Measurements & Station Info Sampling (circle one): Soil OR Pore Water					
Station ID _____		GPS Coordinates:		General Notes:	
Time Collected _____ : _____		1			
Time Read _____ : _____		2			
Saturated (Y/N) _____		3			
Dilution (soil / H2O) _____ mL / _____ mL		Dilution- (soil/H2O) _____ mL / _____ mL		Vegetation (w/in 5m)	
Rep #1 (salinity) Refr: _____ YSI: _____		Rep #1 (salinity) Refr: _____ YSI: _____		1	
Rep #2 (salinity) Refr: _____ YSI: _____		Rep #2 (salinity) Refr: _____ YSI: _____		2	
Rep #3 (salinity) Refr: _____ YSI: _____		Rep #3 (salinity) Refr: _____ YSI: _____		3	
Salinity Measurements & Station Info Sampling (circle one): Soil OR Pore Water					
Station ID _____		GPS Coordinates:		General Notes:	
Time Collected _____ : _____		1			
Time Read _____ : _____		2			
Saturated (Y/N) _____		3			
Dilution (soil / H2O) _____ mL / _____ mL		Dilution (soil/H2O) _____ mL / _____ mL		Vegetation (w/in 5m)	
Rep #1 (salinity) Refr: _____ YSI: _____		Rep #1 (salinity) Refr: _____ YSI: _____		1	
Rep #2 (salinity) Refr: _____ YSI: _____		Rep #2 (salinity) Refr: _____ YSI: _____		2	
Rep #3 (salinity) Refr: _____ YSI: _____		Rep #3 (salinity) Refr: _____ YSI: _____		3	
Salinity Measurements & Station Info Sampling (circle one): Soil OR Pore Water					
Station ID _____		GPS Coordinates:		General Notes:	
Time Collected _____ : _____		1			
Time Read _____ : _____		2			
Saturated (Y/N) _____		3			
Dilution (soil / H2O) _____ mL / _____ mL		Dilution (soil/H2O) _____ mL / _____ mL		Vegetation (w/in 5m)	
Rep #1 (salinity) Refr: _____ YSI: _____		Rep #1 (salinity) Refr: _____ YSI: _____		1	
Rep #2 (salinity) Refr: _____ YSI: _____		Rep #2 (salinity) Refr: _____ YSI: _____		2	
Rep #3 (salinity) Refr: _____ YSI: _____		Rep #3 (salinity) Refr: _____ YSI: _____		3	
Salinity Measurements & Station Info Sampling (circle one): Soil OR Pore Water					
Station ID _____		GPS Coordinates:		General Notes:	
Time Collected _____ : _____		1			
Time Read _____ : _____		2			
Saturated (Y/N) _____		3			
Dilution (soil / H2O) _____ mL / _____ mL		Dilution (soil/H2O) _____ mL / _____ mL		Vegetation (w/in 5m)	
Rep #1 (salinity) Refr: _____ YSI: _____		Rep #1 (salinity) Refr: _____ YSI: _____		1	
Rep #2 (salinity) Refr: _____ YSI: _____		Rep #2 (salinity) Refr: _____ YSI: _____		2	
Rep #3 (salinity) Refr: _____ YSI: _____		Rep #3 (salinity) Refr: _____ YSI: _____		3	

APPENDIX 2.1C



Modified from S.J. Thien. 1979. *A flow diagram for teaching texture by feel analysis*. Journal of Agronomic Education. 8:54-55.



Standard Operating Procedures (2.2): Soil Grain Size and Organic Content

March 2021

Prepared for the United States Environmental
Protection Agency



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Standard Operating Procedures: Soil Grain Size and Organic Content

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Protocol Suitability Evaluation

A habitat suitability table containing appropriate estuarine wetland habitat types (of those evaluated) to implement soil grain size protocols is displayed in Table 1. A comparative assessment of cost, effort, and data quality are shown in Table 2. A matrix of additional detailed categorical evaluations of soil grain size protocols can be found in Appendix 2.2A. The two protocols evaluated for soil particle grain size include a hydrometer and LISST particle analyzer.

Table 1. Appropriate habitat types to implement soil particle survey protocols.

Survey Protocol	Habitat Types					
	Tidal Channel	Mud/sand flat	Emergent salt marsh	Non-tidal salt marsh	Salt pan	'Degraded' / fill
LISST	X	X	X	X	X	X
Hydrometer	X	X	X	X	X	X

Table 2. Categorical assessment of cost/effort and data quality for soil particle grain size survey protocols.

	Evaluation Metric	Soil Particle Grain Size (LISST)	Soil Particle Grain Size (hydrometer)	Notes
Time / Effort	Office Preparation Time	0-10 minutes	0-10 minutes	Print data sheets
	Equipment Construction Time (one time)	10-20 minutes	10-20 minutes	Assemble equipment
	Field Time (per transect)	30-60 minutes	30-60 minutes	Collect soil samples, need larger samples for hydrometer than for LISST
	Laboratory Time (per sample)	> 280 minutes	Day 1 approx. 1 hour; Day 2 approx. 3 hours	Drying field samples (May take up to 10 days depending on soil moisture content); SOP processing time (approximately 280 min.)
	Post-Survey Processing / QAQC Time	> 30 minutes	> 30 minutes	Download LISST data; enter data sheet results (both protocols)
	Minimum Repetition (site-dependent)	Once	Once	----
	Relative Cost (equipment and supplies)	> \$15,000	\$25	LISST Particle Analyzer, drying oven, furnace, supplies
Survey / Data Quality	Accuracy (at a survey area level)	High	Medium	----
	Precision (at a survey area level)	High	Medium	----
	Qualitative-Quantitative Score	Quantitative	Quantitative	----
	Subjectivity-Objectivity Score	Objective	Objective	----

Eshel et al. (2004) state that there is no method for particle size distribution (PSD) determination of soil materials that can serve as a universal yardstick. All available methods whether classic (e.g., pipette) or newer (e.g., laser diffraction), suffer from some inherent flaws. The choice between methods depends, therefore, on the balance between the pros and the cons of each (Eshel et al. 2004). The advantages with the sieve/hydrometer method include limited cost investment and relative ease of the analysis. The disadvantages of the sieve/hydrometer method include the time involved per analysis, dependency

on lab technique and operator skill for accuracy, and the large amount of sediment needed for the analysis, approximately 10 g (Beuselinck et al. 1998). The sieve/hydrometer method is therefore not suitable for the rapid analysis of many small volume samples. However, given that this method has been the standard in grain size analysis, it can be used as a control against which the accuracy and precision of other methods can be statistically tested. The LISST particle analyzer is not recommended based on its cost and high variations in accuracy among samples.

Resulting Data Types

The application of soil particle size survey protocols will yield qualitative data displayed in percentage of sand and fines (silt and clay), which can also be turned into a mean phi. These data are useful to identify edaphic conditions which may be analyzed in conjunction with additional soil or biotic parameters (e.g., vegetation, inundation) to better inform physical processes influencing the distribution of habitat types.

Objective

Soil grain size and associated organic content of the soil are important parameters when measuring the health of an ecosystem, particularly for restored wetland habitats. Because soil grain size affects most other soil properties such as drainage rate, aerobic capacity, and contaminant transport, regular monitoring is necessary for restoration planning, long-term monitoring, and implementation of Best Management Practices (BMPs) (Alletson et al. 2005; Brown et al. 2013). For recommendations on a quick soil texture analysis, refer to SOP 2.1 (soil salinity and characteristics).

The objective of this Standard Operating Procedure (SOP) is to describe the equipment and protocols for analyzing soil grain size and the percentage of organic matter in the sample. With some modifications, organic content analyses can be modified to assess “blue carbon” stored in soils, which is critical for understanding the role of wetlands in the context of climate change (see Howard et al. 2014).

Field Collection and Lab Equipment

Equipment should be rinsed or washed for multiple soil samples. Equipment and supplies needed for this survey include:

1. LISST-Portable Laser Diffraction Particle Size Analyzer (Figure 1) (if doing in field LISST measurements)
2. Mortar and pestle
3. 1000 mL beakers
4. 500 mL beaker
5. 2 – 100 mL beakers
6. 25 mL beaker
7. 5-liter rectangular basin
8. 5-gallon bucket
9. 62-micron mesh screen (#230)



Figure 1. LISST-Portable Laser Diffraction Par (courtesy Sequoia Scientific).

10. DI water (~300 mL)
11. Soil sample (30 mL)
12. Scoopula, eye dropper, pen or marker, aluminum foil, turkey baster, DI squirt bottle
13. Drying oven
14. Duct or lab tape for labeling

Field Preparation

Equipment described above should be collected prior to the field shift. Batteries for all electronic devices should be checked and replaced as needed, and relevant data sheets should be printed and attached to the clipboards.

Field Methods

For details on transect or site selection, refer to SOP 3.2 (vegetation cover). These protocols are recommended to be conducted in conjunction with biological survey parameters. Field methods are the same for all protocols in this SOP.

Once the sample locations have been chosen, begin the collection process. Start by clearing the desired patch of soil of any debris or above-ground vegetation, using a gardening trowl, knife, or core. Make a square on the soil surface with each side measuring approximately 15 cm in length (Figure 2). *Helpful hint: using the gardening trowl to measure the length of your soil square reduces equipment needed in the field.* Collect all soil within the square to a depth of 1 cm and place soil into a labeled and sealable plastic bag. Any noticeable vegetation (roots, runners, etc.) or subsurface debris should be removed to ease subsequent processing. This should provide approximately 200 mL of soil (SOP 2.1 2015). Transfer soil sample information from bag to data sheet (Appendix 2.2C). Be sure to adhere to the Health and Safety Precautions as outlined by the US EPA.



Figure 2. Soil collection in the field.

Laboratory Methods

Steps 1-11 are for sample preparation for both organic matter and grain size (either LISST or hydrometer).

1. Start with a raw soil sample (Figure 3).
2. Place soil sample into 400 or 600 mL glass beaker and place into drying oven to a minimum of 60° Celsius for two or more days until sample is fully dry. With a piece of lab tape, label the



Figure 3. Raw soil sample with organic matter (pre-sorted).

beakers with the FID number and sample process date.

3. Once sample is completely dry, pour soil onto paper towel and pick out large pieces of organic matter / debris using tweezers or sieve through a 2 mm sieve. This debris can be discarded.
4. Measure approximately 60 mL of soil. Homogenize the sample by grinding the soil with a mortar and pestle. Continue until all clumps or large aggregates have been broken down (Figure 4).
5. Weigh empty crucible and record weight on data sheet (Appendix 2.2C). Note: if multiple crucibles are being used simultaneously, it helps to provide a unique identifier for each crucible (e.g., "#5" or "large crucible"). Tape cannot be applied to crucible because of intense heat, but pencil notation can be applied directly to the rough bottom of the mortar.
6. Measure 30 mL of homogenized sample and pour into crucible. Place excess sample in a labelled plastic bag if future testing is desired.
7. Weigh crucible with the sample and record weight on data sheet.
8. Subtract the crucible weight from the crucible plus raw bulk soil weight to determine the raw bulk soil weight.
9. With tongs and asbestos glove(s), place the crucible into muffle furnace at 500-550° Celsius for two or more hours to burn off any remaining organic matter (Figure 5). Sediments with more organic materials may need to be burned for up to 24 hours or until the sample is at a constant weight (American Society for Testing and Materials, ASTM, 2974-87).
10. With tongs and asbestos glove(s), carefully remove crucible and place on crucible stand and let cool until safe to touch, approximately 15-20 minutes (Figure 6). Crucibles can also be left overnight to cool and weigh in the morning.



Figure 4. Homogenized sediment sample with mortar and pestle.



Figure 5. Place crucible carefully in furnace.



Figure 6. Hot crucibles placed on crucible stand.

11. Weigh crucible with ash-soil and record weight on data sheet. Subtract this weight from crucible weight (see Step 5) to determine ash-soil weight.
12. Subtract the ash-soil weight from the raw bulk soil weight and then divide by the raw bulk soil weight to calculate the percent organic matter and enter on data sheet (for equation, see Data Analysis section below). This will conclude the organic matter portion of the analysis.

SOP – LISST Portable Diffraction Particle Size Analyzer

This SOP is based specifically on methods related to the LISST-Portable Diffraction Particle Size Analyzer, which is not recommended as the preferred grain size analysis. See Appendix 2.2B for a summary procedural flow chart. Steps 1 – 12 could be preparation for any grain size analyzer.

1. Label an empty beaker (400 mL is preferred) using a piece of tape with FID number and processing date. Weigh the empty beaker and record the weight on data sheet.
2. Transfer the soil from the crucible into the empty beaker using a wash bottle to wet the sample to minimize suspension into the air (because of their small mass, fines (silt and clay) are easily suspended into the air).
3. Fill beaker (minimum 150 mL) with water. Pour water from 1000 mL beaker into 400 mL sample beaker until approximately $\frac{1}{2}$ to $\frac{3}{4}$ full. Homogenize sample by stirring with metal scoopula to re-suspend settled soils. Let sit for 1-2 minutes.
4. Wet 62 micron mesh sieve with water.
5. Pour soil from beaker slowly through the 63 micron sieve into a large clean plastic tub. This allows suspended fine particles to be transferred from the beaker and through the sieve, but heavier sand particles remain in the bottom of the beaker and on the sieve screen. Tilt the sieve to keep contents in one side. Pour only until all water has passed through the sieve, leaving any sand that had settled in the bottom of the beaker. Use wash bottle to clean sieve and push all sand into one concentrated area. Do not use fingers to push soil through the sieve as this may cause sand particles to clog the screen or pass through the sieve. Soils remaining in beaker and on the sieve constitutes the sand sample (sand-size by definition is 62 microns and greater).
6. Use wash bottle to rinse off sides of beaker, add more water until beaker is about $\frac{1}{2}$ to $\frac{3}{4}$ full again, stir, and repeat process from Step 17. Continue to repeat the entire process until water is generally clear on top and sand is settled in the bottom of beaker. As the process is repeated multiple times, gradually allow more time for the sand to settle at the bottom of the beaker before pouring through the sieve (up to about 4-8 minutes). The number of required rinses (typically 6 – 10) increases for samples with higher proportions of fines.
7. Once water on top of beaker appears generally clear, pour through screen again and then use wash bottle to transfer sand that had collected on the sieve back into the beaker. At

this point, the clarity of the water does should be closer to a clear/grey color rather than brown, indicating most of the fines have passed through the sieve into the tub.

8. Once fines have been removed from the beaker sample, transfer any remaining sand left on the sieve back into the beaker using the wash bottle and scoopula. Be sure to remove all particles from the sieve (Figure 7). Squirt water around the sides of the beaker using the wash bottle to remove any sand from the sides and collect sample in the bottom of beaker.
9. Record time and date on the beaker's label and place into drying oven at 80° Celsius for two or more days. After sample is dry, weigh the beaker and record the weight as the beaker + sand weight. Subtract the beaker weight from this number to determine the sand weight. Divide the sand weight by the raw bulk soil weight to determine the sand percentage (for equation, see Data Analysis section below).
10. The soils remaining in the plastic tub are composed of fine-grained particles (silt and clay). Pour the contents of the entire tub into the 2-gallon plastic bucket (large enough to accommodate sample). Use wash bottle and scoopula to ensure all soil is transferred and none is left on bottom of tub.
11. Place round magnetic stirring bar (cross PTFE-coded magnetic stirring bar is recommended) into bottom of tall bucket and place entire bucket onto stirrer/hotplate. Turn on the hot plate stirrer to about the 7-9 level setting to stir the sample (only turn on stir setting, no heat). Allow sample to continue stirring for about 2-3 minutes.
12. With the stirrer still on, mix the sample with turkey baster by swirling in a circle a few times and repeatedly suctioning and then expelling the liquid. When sample is well mixed, use turkey baster to transfer a portion of the sample into a 100 mL beaker.



Figure 7. Rinsing sediment from sieve.

Steps 13-24 are specific to the LISST Particle Size Analyzer:

13. Set the 100 mL beaker aside. Plug in and turn on the LISST Laser Diffraction Particle Size Analyzer and attach yellow drainage hose. Make sure that the end of yellow hose is placed in a large bucket on the floor to allow water to drain. Remove the plain circular insert in the mixing chamber lid and replace with ultrasonic probe insert.
14. From the main menu on the LISST, tap the "Measure" button.
15. Remove the mixing chamber lid and make sure the drain lever is in the 'closed' position. Fill the chamber until water just spills over internal ring. Replace the lid back on tightly.
16. Set mixer speed to 50 to 75% using the on-screen slider and press "Sonics On" button. Let the water circulate for approximately 5 seconds. Press the "Sonics Off" button and set the mixer speed to 0%. Open the drain lever and make sure water drains completely. Repeat steps 28-29 two more times for a total of three rinses.

17. Before the LISST Particle Size Analyzer is used for sample analysis, fill the chamber with deionized water and press the "Next" button in the bottom right corner of the screen (do not run the mixer or sonics). Press the "Update" button on the next screen and make sure that the LISST Particle Size Analyzer reads "PASS." Note: the use of deionized water is only necessary during the calibration process, and regular water can be used for subsequent rinses. Press "Next" a total of 2 times to reach the "Step 3b: Prepare Sample" screen, skipping the "Step 3a: Add Sample" screen.
18. In the menu screen comment box, add FID number and necessary comments. This information will display in the data report.
19. Use an eyedropper to mix up the sample in the 100 mL beaker thoroughly until sample is uniform and all soils are suspended off the bottom. Place several drops of the sample into the chamber, thoroughly agitating the sample before each drop is taken. Continue adding sample until the concentration range percentage shows the sample is within the green section (75-95% transparency). Turn on the mixer and ultrasonic sliders to about 50% and make sure that the sample still reads from 75-95% transparent. If the sample is too concentrated, dilute until levels fall between 75-95%.
20. Press the "Next" button. The LISST Particle Size Analyzer will now analyze the sample for approximately 20 seconds and provide a read out of the results.
21. Press the "Return" button to start the process again two more times (steps 30-32). Between each sample analysis, rinse chamber three times with regular water. From the "Step 1: Rinse Chamber" screen, press "Next" a total of three times to reach the "Step 3b: Prepare Sample" screen, skipping the "Step 2: Get Background" and "Step 3a: Add Sample" screens.
22. Store the LISST Particle Size Analyzer with the drain open to allow ventilation of the chamber.
23. When rinsing all materials used throughout the process, use a wet paper towel to ensure all small particles are removed.
24. Download data to computer using LISST software or to an excel file.

SOP – Hydrometer method (grain size)

This method quantitatively determines the proportions of three sizes of soil particles as determined by their settling rates in an aqueous solution using a hydrometer. Proportions are represented by stated class sizes: sand ranging from 2000 – 63 μ m; silt ranging from 50 – 2.0 μ m and clay < 2.0 μ m, and those stated by the USDA Soil Survey and Canadian Soil Survey Committee.

Up to 20 samples can be done at once.

Estimated time: allow prep time for grinding and sieving samples; Day 1 - approximately 1 hour; Day 2 - approximately 3 hours.

Laboratory Supplies

Note: Hydrometers are used to determine the specific gravity of soil in the range of 0.995 to 1.038 or -5 to 60g/L (using a weight by volume scale)

Standard Operating Procedures: Soil Grain Size
The Bay Foundation

Laboratory supplies needed:

- 2 mm sieve
- 600 ml beakers (one for each sample)
- Sodium metaphosphate (5g per sample + 5g extra)
- 1 L graduated cylinder
- 1 L cylinders (1 per sample + 1 extra)
- Stir bar, timer
- DI water, squirt bottle
- Shaker table
- Thermometer
- Hydrometer (Figure 8)
- Parafilm



Figure 8. Fisherbrand™ Soil Analysis ASTM Hydrometers.

Prior to Day 1:

1. Follow steps as outlined earlier to dry and homogenize the soils.

Day 1:

1. Sieve dried, ground soil sample through 2 mm sieve.
2. Put 30-50 g (50 g preferable) of the sample into numbered beaker. Record beaker number next to sample number on data sheet. Be consistent with sample weights (i.e., 50 g for all).
3. Prepare sodium metaphosphate solution. Each sample gets 5g of dissolved sodium metaphosphate solution. To make solution for 10 samples; Slowly add 50 g of sodium metaphosphate to 1 L DI water while stirring vigorously until it has dissolved completely. This is best done on a stir plate. Decant 100 ml of this solution to each beaker.
4. Add DI water to bring the volume in each beaker to approximately 300 ml and use a spatula to briefly stir each beaker ensuring that the solution comes into contact with all of the sample.
5. Place the beaker on the shaker table for approx. 24 hours at 125 rotations/minute. Samples should be stirred until all clumps are broken up (less time with sandy samples).

Day 2:

1. After stirring, transfer contents of beaker to a 1 L cylinder with same number as beaker, using DI water to wash the remaining soil and residue into the cylinder. Add DI water to bring the volume of the cylinder to the 1 L mark.
2. Make a blank by adding 5 g of sodium metaphosphate to 1 L of DI water. Mix thoroughly on a stir plate.
3. Samples must be between 15.6 and 24.4 C.
4. Seal the top of the cylinder with parafilm and invert the cylinder several times until all the soil is suspended.
5. Immediately after completing mixing, start time and lower the hydrometer gently into the cylinder. Take the hydrometer reading and temperature after 40 seconds (Figure 9).

6. Rinse the hydrometer with DI water and continue to next sample. Do not forget to record the temperature and hydrometer reading for the blank also.
7. Record the temperature and hydrometer reading again two hours after the start time. (Do NOT shake the samples again!). Record the temperature and hydrometer reading for the blank also.



Figure 9. Hydrometer floating in a 1000 ml cylinder (left), diagram of how to read hydrometer (top right) from Amazon.com), and 1000 ml cylinders with sediment and sodium hydroxide (bottom right).

If you miss the 2-hour start time for the second reading or disturb the samples between the two readings, then you must start again from Step 4.

To wash cylinders:

1. Decant liquid into sink and dump remaining sediment and liquid into a bucket.
2. Wash all cylinders with soapy water and rinse into distilled water.
3. Empty bucket into dumpster on a regular basis.

Data sheets

GRAIN SIZE ANALYSIS											Temp Adjusted		% silt + clay	% sand	% clay	% silt				
Marsh	trans	elev	rep	beaker#	soil wt	40s	2hr	40s	2hr	T initial	T final	40s	2hours	corr. read *vol(L)/gms at 40 sec.	100-(%silt ar read *vol(L)/lit+clay)-%	at 2 hrs.				
					50	23	22	19	18	22.4		19.86328	10.806	39.7	60.3	21.6	18.1			

Figure 10. Example of data entry sheet for grain size analysis via hydrometer method. Headings explained below.

Formulas

Soil wt = dry soil weight (g or mg)

40s = 40 second reading of hydrometer

2 hr = 2 hour reading of the hydrometer

Subtract blank (blank-corrected readings) – These two readings are the corrected readings with the hydrometer reading from the blank subtracted from the 40s and 2 hour readings, respectively.

T initial, T final = temperature reading of the suspended sediment mixture at 40s and 2 hours, respectively

Temp-adjusted hydro readings (40s) = ((temp initial – 20)*0.3597) + blank-corrected reading

Temp-adjusted hydro readings (20hr) = ((temp 2 hr – 20)*0.3597) + blank-corrected reading

% silt and clay = (corrected reading 40s / soil weight)*100

% sand = 100 – (%silt and clay)

% clay = (corrected reading 2hr / soil weight)*100

% silt = (%silt and clay) = (% clay)

Data Entry and QAQC Procedures

Data output from the LISST is downloaded to proprietary software and/or as an excel spreadsheet. Quality Assurance and Quality Control (QAQC) procedures should be conducted on all data. QAQC procedures should be conducted by the QA Officer and include a thorough review of all entries, including the following:

1. Data should be removed for any operations that did not meet calibration standards.
2. Data should be removed if the processing was not conducted in specific accordance with manufacturer operating instructions.

Data Analyses

After the data have been entered, corrections made, and QAQC procedures completed, they can be used in multiple analyses. The LISST categorizes soil grain size in “bins” or size-classes from 0 to 500 µm. Clay is registered in bins 1-15 (~0.37 to 3.78 µm), silt in bins 16-32 (~4.46 to 63 µm), and residual sand in bins 33-44 (~74.5 to 500 µm). Sand percentage is determined through Steps 17 through 21 in Laboratory Methods above. LISST data output will automate bins summation, but equations are listed below to calculate percentages of organic matter, sand, fines, clay, and silt.

To determine organic matter (Step 12):

$$\% \text{ Organic Matter} = \frac{\text{Ash} - \text{soil}_{\text{weight}}}{\text{Raw} - \text{bulk soil}_{\text{weight}}}$$

Note: raw-bulk soil includes sand and fines.

To determine sand percentage (Step 21):

$$\% \text{ Sand} = \frac{\text{Sand}_{\text{weight}}}{\text{Total Sample}_{\text{weight}}}$$

To determine fines (silt + clay) percentage (Step 32):

$$\% \text{ Fines} = 1.0 - \% \text{ Sand}$$

To determine clay and silt percentage (Step 32):

$$\% \text{ Clay} = \frac{\% \text{ Clay}_{(\sum \text{bins } 1-15)}}{\% \text{ Total}_{(\text{silt}+\text{clay})}}$$

$$\% \text{ Silt} = \frac{\% \text{ Silt}_{(\sum \text{bins } 16-32)}}{\% \text{ Total}_{(\text{silt}+\text{clay})}}$$

Use the USGS soil texture classification chart (Figure 11) to identify the soil texture of the sample by identifying the intersection of all three grain size percentages. For example, a sample consisting of 45% clay, 45% silt, and 10% sand would be classified as 'silty clay'. These broad categorizations are the same as the soil texture analyses from SOP 2.1.

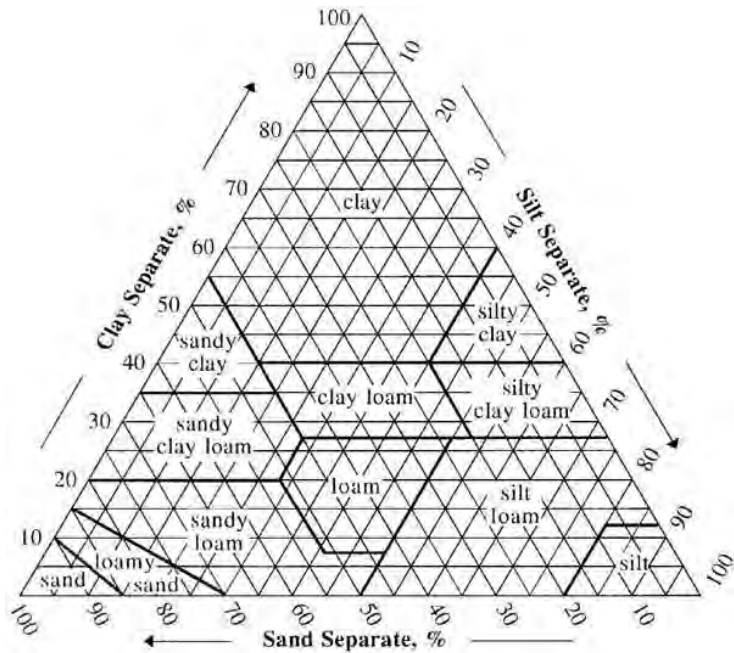


Figure 11. USGS Soil Texture Chart.

Health and Safety Precautions

The furnace used to dry soil samples must be set at high temperatures and can result in extreme burns. Asbestos gloves and tongs should always be used when placing into or removing from the furnace. Following removal from the furnace, crucibles will remain extremely hot for approximately 15 – 20 minutes and caution should be exercised whenever working in the immediate vicinity.

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- (ASTM) American Society for Testing and Materials. ASTM D 2974-87 Standard Test Methods for Moisture, Ash, and Organic Matter of Peat and Other Organic Soils 1916 Race St., Philadelphia, PA 19103. Reprinted from the Annual Book of ASTM Standards, Copyright ASTM.

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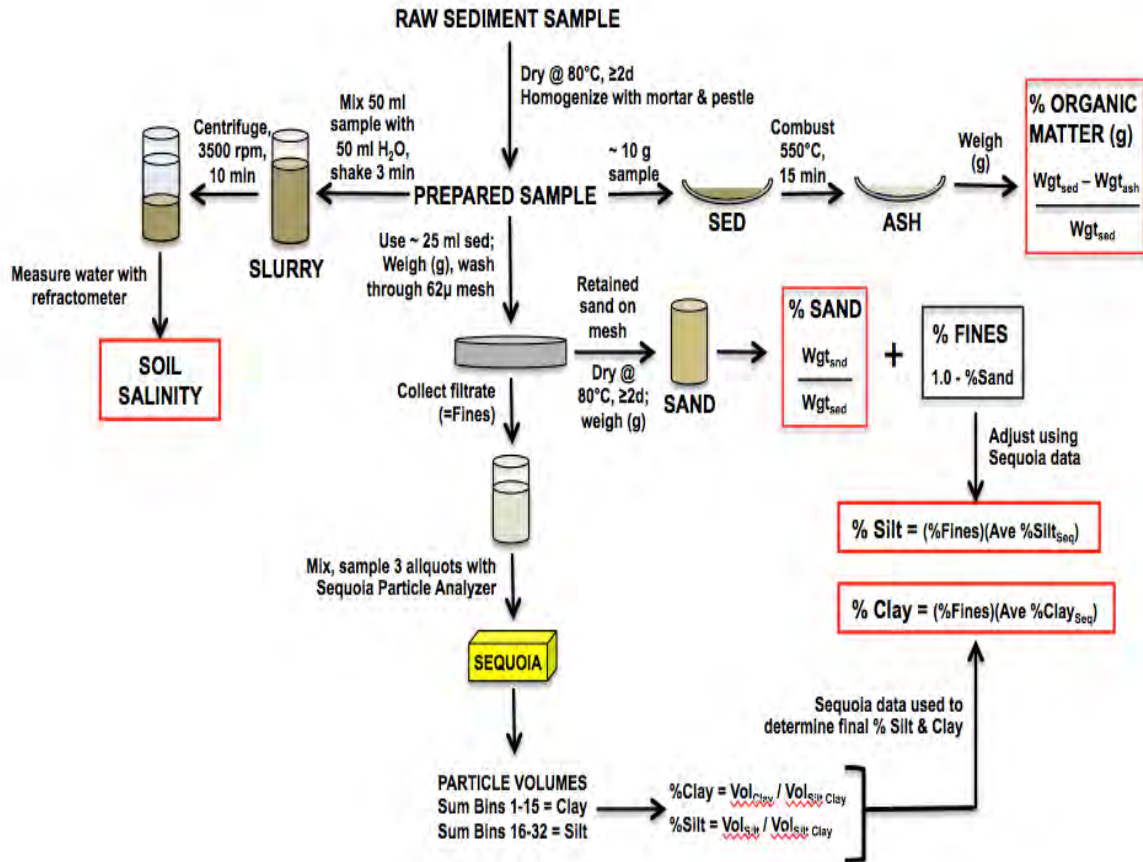
Appendix 2.2A

	Evaluation Metric	Soil Particle Grain Size	Notes
	Correlation to L2 CRAM	Not Applicable	----
Personnel Requirements	Specialty Equipment or Clothing Required	Few Specialty Items	LISST, drying oven, furnace, beakers, hotplate/stirrer
	Ease of Transport (amount or weight of supplies)	Some Items / Moderate	All items will be used in laboratory setting
	Ease of Implementation	Difficult	Time intensive; requires significant attention to detail
	Expertise / Skill Level	Some Technical Knowledge	Familiarity with LISST; adept with complex, multiple step process
	Number of Personnel	> 2	Teams of two personnel per sample
	Training Requirements	Required	Familiarity with LISST; complex, multiple step process
	Seasonality of Survey Time	Year round	
	Suggested Frequency	Annual	---
Survey / Data Quality	Type of Output	Numerical	Percentage of sand, silt, and clay
	Active or Passive Monitoring Style	Active	---
	Specialty Computer Software Required	Yes	Excel download of LISST data
	Availability of Online / External Resources	Yes	LISST manual online and with product
Potential Limitations	Wetland Type Applicability	All	---
	Images or Multi-Media Required	None required	---
	Degree of Impact / Disturbance	Low Disturbance	---
	Vegetation Height Limitation	No Limitations	---
	Appropriate for Tidal / Wet Habitats	Yes	---
	Tide Height	N/A	---
	Regional or Broad Implementation **	Infrequently Used	---
	Potential for Hazards / Risk	Low to No Risk	---
Restrictions	Special Status Species; Cultural	Soil disturbance	

* based on monitoring literature review

Appendix 2.2B

Procedural Flow Chart



Appendix 2.2C

Grain Size Data Sheet

Date Tested: _____

Tested By: _____

Sample #: _____

Site Location: _____

Visual Classification of Soil: _____

Weight of Beaker: _____ g
Weight Beaker + Dry Soil: _____ g
Weight of Dry Sample: _____ g

Particle Size Distribution:

Sand _____ %
Silt _____ %
Clay _____ %

Soil Texture Class:



Standard Operating Procedures (3.1): Algae and Submerged Aquatic Vegetation

March 2021

Prepared for the United States Environmental
Protection Agency



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Standard Operating Procedures: Algae and Submerged Aquatic Vegetation

SOP Identification: SOP 3.1 Algae and SAV

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Protocol Suitability Evaluation

A habitat suitability table containing appropriate estuarine wetland habitat types (of those evaluated) to implement macroalgae and submerged aquatic vegetation (SAV) protocols is displayed in Table 1. A comparative assessment of cost, effort, and data quality are shown in Table 2. A matrix of additional detailed categorical evaluations of implemented macroalgae and SAV protocols can be found in Appendix 3.1A.

Table 1. Appropriate habitat types for algae and submerged aquatic vegetation survey protocols.

Survey Protocol	Habitat Types					
	Tidal Channel	Mud / sand flat	Emergent salt marsh	Non-tidal salt marsh	Salt pan	'Degraded' / fill
Algae	X	X	X	X	X	X
SAV (subtidal)	X	X				

Table 2. Categorical assessment of cost/effort and data quality for algae and submerged aquatic vegetation survey protocols.

	Evaluation Metric	Algae	SAV (subtidal)	Notes
Time / Effort	Office Preparation Time	10-30 minutes	10-30 minutes	Site selection and any GPS locations
	Equipment Construction Time (one time)	30-60 minutes	30-60 minutes	Will need to construct quadrat with PVC and twine
	Field Time (per transect)	10-30 minutes	3-4 hours	----
	Laboratory Time (per transect)	30-60 minutes	0 minutes	Cleaning and weighing algae biomass
	Post-Survey Processing / QAQC Time	10-30 minutes	10-30 minutes	----
	Minimum Repetition (site-dependent)	Few Repetitions	Few Repetitions	Algal cover may vary across tidal channel areas; SAV may be patchy
	Relative Cost (equipment and supplies)	< \$15	< \$1,000	Significant costs associated with subtidal surveys (boat, SCUBA, etc.)
Survey / Data Quality	Accuracy (at a survey area level)	Medium	Medium	----
	Precision (at a survey area level)	High	High	----
	Qualitative-Quantitative Score	Quantitative	Quantitative	----
	Subjectivity-Objectivity Score	Objective	Objective	----

Resulting Data Types

The application of macroalgae and SAV survey protocols will yield quantitative data displayed in species-specific percent cover along individual transects or extrapolated to a habitat type or wetland. The application of subtidal SAV mapping survey protocols will yield quantitative data displayed in aerial cover along areas or as a Geospatial Information Systems (GIS) map. Additionally, biomass data result in grams per meter squared data that may be extrapolated up to a transect-level, habitat type, or wetland. These data are useful to identify algae and SAV cover trends over multiple time scales and may assist in identifying potential areas of eutrophication within estuaries or locations of subtidal SAV beds.

Objective

Macroalgae (or visible larger algae) and submerged aquatic vegetation surveys provide important information about primary productivity within a system, essential fish habitat, and given trophic structure. Algae abundance and growth can also be useful indicators of eutrophication and tidal flushing (Zedler 2001, Hughes et al. 2010). This document interchangeably refers to macroalgae or algae, but always means visible larger species (Figure 1).



Figure 1. Green algae and *Ruppia* sp. in a wetland tidal channel.

Repeated monitoring of macroalgal abundance provides information on when algal blooms occur and how long they endure as an indicator of primary productivity in each system. Macroalgal abundance is determined by measuring percent cover and algal biomass. The Southern California Bight 2008 Regional Monitoring Program (Bight '08) was part of an effort to provide an integrated assessment of environmental conditions through cooperative regional-scale monitoring and developed these protocols. One purpose of this sampling method is to continue to collect eutrophication data using the same regional collection methods from the Bight '08 program to assess long-term data trends over time. In southern California estuaries, some SAV will be intertidal especially if *Ruppia* (ditch grass) species are present. However, in some systems, much of the SAV is subtidal, requiring additional monitoring protocols including SCUBA (see SAV subtidal mapping protocols).

The macroalgae cover SOP is described based on standardized methods conducted by Johnston et al. (2011, 2012) and developed by the Bight '08 Program. The subtidal SAV cover SOP is described based on standardized methods conducted by Paua Marine Group (A. Obaza, A. Bird). However, more in-depth SAV measurements (e.g., turion density, invertebrates, etc.) are often required or important to understand. Those methods have been documented in other protocols (e.g., MarineGEO 2020, SCCWRP 2020, NOAA 2014) and are not included in SOP 3.1.

Equipment

Equipment and supplies needed for both survey types include:

1. GPS

Additional supplies needed for the macroalgae SOP:

1. Transect tape

2. 0.25 m² PVC quadrat (0.5 x 0.5 m) with 7x7 squares delineated using string to make 49 points of intersection (Figure 2)
3. Sealable bags
4. Small PVC cylinder (6 in diameter)
5. Data sheet (Appendix 3.1B)

Additional supplies needed for the subtidal SAV SOP:

1. Boogie board
2. SCUBA gear (Figure 3)
3. Data sheet (preliminary map or location)



Figure 2. Quadrat placement in a wetland tidal channel.

Field Preparation

Equipment described above should be collected prior to the field shift. Batteries for all electronic devices should be checked and replaced as needed, and relevant data sheets should be printed.



Figure 3. SCUBA gear is required for subtidal SAV surveys.

Field Methods

Macroalgae protocols:

Surveys should be conducted once quarterly in March, June, September, and December. Surveys should begin approximately one and a half hours before a low spring tide to obtain the maximum mudflat exposure and conclude after approximately three hours.

A minimum of three, 30 m transects should be laid out in the intertidal area parallel to the water's edge and along the same elevational contour (Figure 4; Bight 08, Johnston et al. 2011, 2012). Transects may be placed along the edge of the vegetation to reduce impact to the mudflat and channel bottom, but the quadrats should be

placed at approximately three quarters of the distance from the mean lowest low water line to the downslope end of vascular vegetation on the mid-to-upper mudflat (Figure 5). This area has been demonstrated to be representative of macroalgae accumulation in southern California estuaries (Kennison et al. 2003).



Figure 4. Transect deployment adjacent to a tide channel.

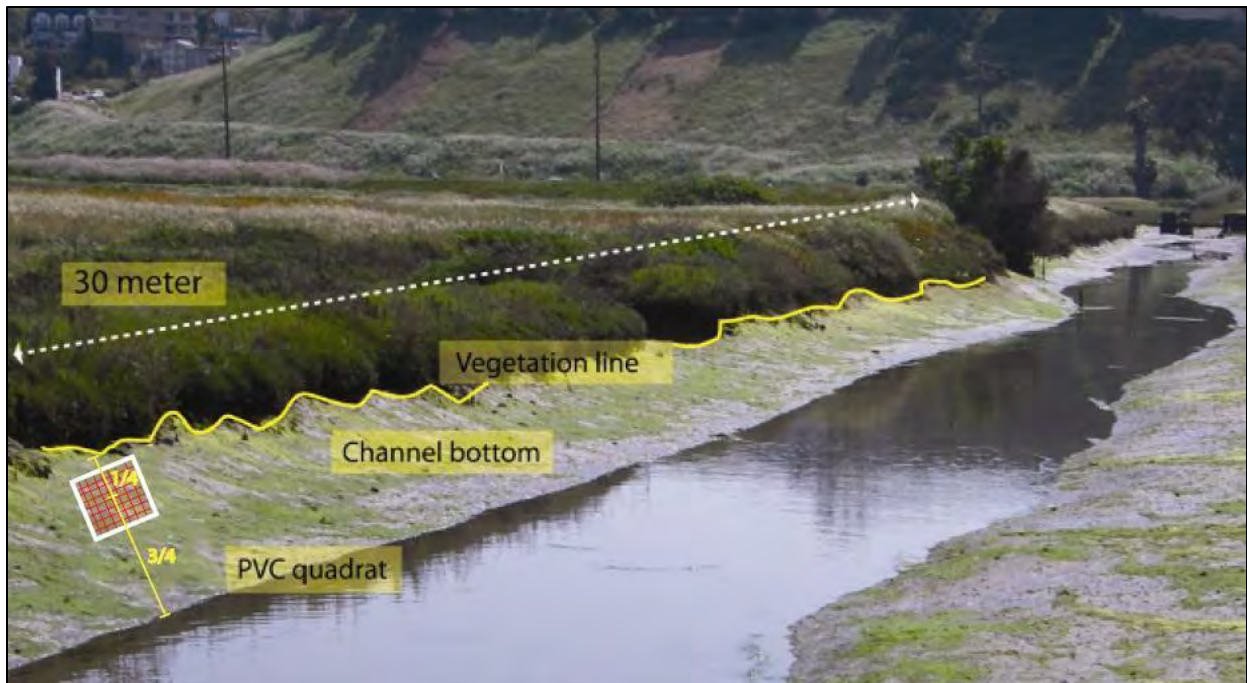


Figure 5. Diagram of algae/SAV transect showing placement of quadrat and vegetation line (replicated from Johnston et al. 2011). Note: diagram is not drawn to scale.

Percent cover should be measured at ten randomly chosen points along the transect using a random number generator. Place the 0.25 m² quadrat with 49 intercepts (Figure 2) on the benthos at each random transect point, and record the presence or absence of each macroalgae (e.g., *Ulva sp.*) or submerged vegetation (e.g., *Ruppia sp.*) species under each intercept point (see Appendix 3.1B for datasheet). Only one species per intercept point should be recorded. Intersecting points occurring over bare soil or mud should be recorded as 'bare'. The estimated maximum and minimum mat thicknesses

should also be noted in millimeters on the datasheet (Appendix 3.1B). Thickness can be measured by using the transect tape as a reference or a handheld ruler.

Biomass should be randomly collected at five of the quadrat locations using a 6-inch diameter PVC cylinder placed in the middle of the quadrat. Biomass samples should be collected from within the circumference of the PVC cylinder and placed into a labeled bag and sealed. Each biomass sample should be refrigerated until analysis and processed within 24-hours of collection (see laboratory methods).

Note: The additional “other” categories on the algae datasheet may be used for notating supplementary invertebrates (e.g., Cerithidea californica) and trash presence or absence.

SAV subtidal mapping protocols:

Surveys should be conducted twice yearly in June, September, (end of growing season) in line with regional monitoring efforts and NOAA California Eelgrass Mitigation Policy (CEMP) protocol. Aerial cover should be measured and mapped by divers on SCUBA using a Trimble R1 Global Navigation Satellite System receiver linked with a smartphone (or similar setup) (Figure 6). This mapping is done by having a single diver swim the outline of the eelgrass bed perimeter towing a Pelican float, while a second diver follows this path with the Trimble R1. This receiver, enabled with real-time Satellite-based Augmentation System correction, provides sub-meter accuracy during mapping. Data are then exported to the Trimble Terraflex cloud system for review and are available as shapefiles.

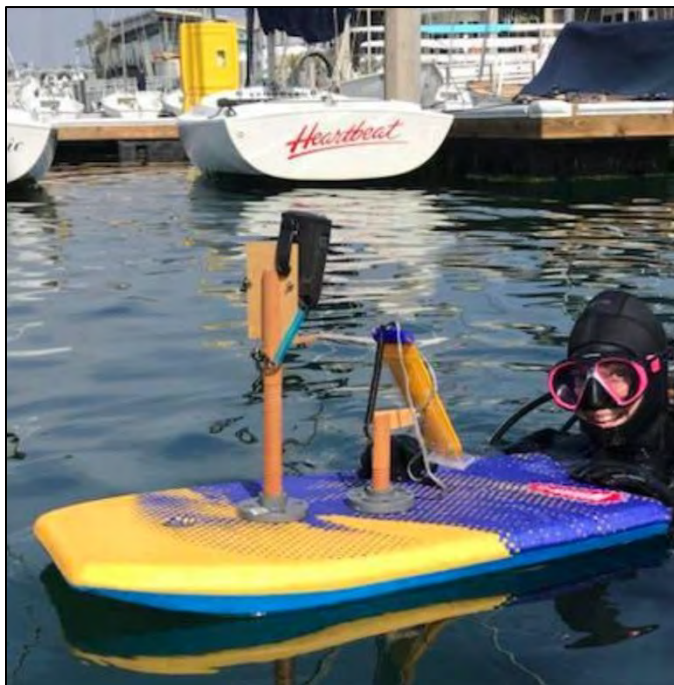


Figure 6. Biologist with Paua Marine Research Group outfitted with Trimble R1 GNSS Receiver used to map an eelgrass bed (photo: Paua Marine Research Group).

Laboratory Methods

In the laboratory, for biomass calculations, algal samples should be cleaned of macroscopic debris, mud, and animals, and sorted to genus level. Excess water should be shed from each sample, then weighed wet, and subsequently dried at 60°C to a constant weight, then weighed dry. During data analysis, all macroalgae genus weights should be summed for each quadrat to give a total macroalgae wet and dry weight by quadrat.

Data Entry and QAQC Procedures

Data should be entered in the field using the appropriate data sheet (Appendix 3.1B). All required fields should be completed in full, and the data recorder should assign their name at the top of the document(s). Data should be transferred to the appropriate

electronic database within three days, and the hard copies filed in labeled binders. Electronic copies of all data should be housed on an in-house dedicated server and backed up to a cloud-based or off-site server nightly. Hard copies should be saved for five years. Electronic copies should be saved indefinitely.

Quality Assurance and Quality Control (QAQC) procedures should be conducted on all data. QAQC procedures should be conducted by the QA Officer and include a thorough review of all entries, double checking of all formulas or macros, and a confirmation that all data sheets and field notes are filed appropriately with electronic back-up copies available. QAQC should verify that the entered data match the hard copies of the field data sheets. Any discrepancies should be corrected, and the initial data entry technician notified.

Data Analyses

Algae and intertidal SAV surveys can be analyzed by determining percent cover for each quadrat (i.e., number of points for a species / 49 x 100) by species or summed as one value for algae and one for submerged aquatic vegetation. Quadrats can be averaged by transect, and standard error used to determine variability. Graphs can be created using averages and standard errors by season, transect, or estuary.

Biomass data can be calculated for both wet and dry weight. Biomass data can also be evaluated at the transect level or up to habitat type or wetland. For biomass data, one, 6-inch PVC pipe equates to an area of 0.072963725 m². To extrapolate up to grams per meter squared, enter the resulting individual weight (g) of each biomass sample into the following equation:

$$\text{Weight of sample (g)} \times (1 / 0.072963725 \text{ m}^2) = \text{grams per m}^2$$

For subtidal SAV, data outputs are in the form of mapping GIS polygons (Figure 7), which can include specific attributes such as percent cover, number of turions, etc., as determined by the monitoring program.

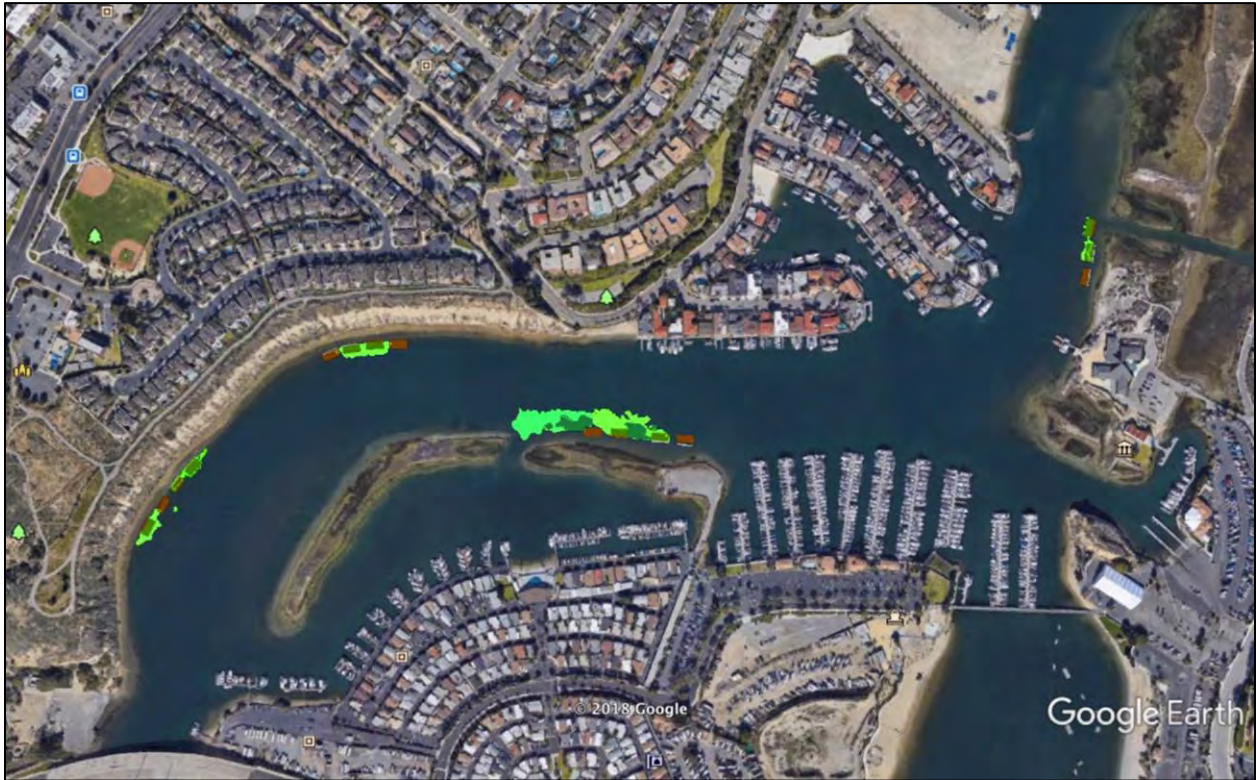


Figure 7. Upper Newport Bay subtidal SAV map (Zacherl et al. 2018, unpublished data).

Health and Safety Precautions

Not applicable.

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APPENDIX 3.1A

	Evaluation Metric	Macroalgae	SAV (subtidal)	Notes
	Correlation to L2 CRAM	Not Applicable (at the Attribute-level)	Not Applicable (at the Attribute-level)	Loosely tied to the patch size metric as one potential type of patch
Personnel Requirements	Specialty Equipment or Clothing Required	Few Specialty Items	Many Specialty Items	Hard-soled wetsuit booties work well in tidal channels; subtidal requires full SCUBA
	Ease of Transport (amount or weight of supplies)	Few Items / Easy	Few Items / Easy	----
	Ease of Implementation	Easy	Easy	----
	Expertise / Skill Level	Some Technical Knowledge	Some Technical Knowledge	Familiarity with species identification is recommended
	Number of Personnel	2	2	Includes one data recorder and one surveyor
	Training Requirements	None	None	----
	Seasonality of Survey Time	Every season	Late summer	Macroalgae: spring, summer, fall, winter
	Suggested Frequency	Quarterly	Annually	----
Survey / Data Quality	Type of Output	Numerical	Numerical	----
	Active or Passive Monitoring Style	Passive	Passive	----
	Specialty Computer Software Required	No	Yes	GIS required for mapping outputs (SAV subtidal)
	Availability of Online / External Resources	Some	Many	Other suggested use documents are available
Potential Limitations	Wetland Type Applicability	All	Subtidal only	----
	Images or Multi-Media Required	Images Required	GIS Required	----
	Degree of Impact / Disturbance	Moderate Disturbance	Low Disturbance	Walking through channels will disturb sediments
	Vegetation Height Limitation	Not Applicable	Not Applicable	----
	Appropriate for Tidal / Wet Habitats	Yes	Yes	----
	Tide Height	Low Tide Only	N/A	----
	Regional or Broad Implementation *	Almost Always Used	Frequently Used	----
	Potential for Hazards / Risk	Low to No Risk	Low to No Risk	----
Restrictions	None	None	----	

* based on monitoring literature review table

APPENDIX 3.1B (modified from Bight '08)

Submerged Aquatic Vegetation Sampling Data Sheet		
Date:	Transect #:	Page ____ of ____
Time (Start):	Time (End):	Notes:
Field Lead:	Entered: _____	Date: _____
Field Staff:	QAQC: _____	Date: _____

Site Observations

Days Since Last Rainfall (approx):	Tide Gate Position: Open / Closed
Weather: Clear / Partly Cloudy / Overcast / Rainy / Foggy	Time of Low Tide: _____ Height of Low Tide: _____
Photo Oceanward: Y / N	Time of High Tide: _____ Height of High Tide: _____
Photo Landward: Y / N	Direction of Tide: Ebb / Flood / Max / Min
Vertical Zonation of Macroalgae: Y / N	Describe: _____
Comments: _____	

Macroalgal Transect

Quadrat	1	2	3	4	5	6	7	8	9	10
Distance (M)										
Matt Thick (MM)										
Estimated?	Y / N	Y / N	Y / N	Y / N	Y / N	Y / N	Y / N	Y / N	Y / N	Y / N
Condition	Frsh / In / Des / Dd	Frsh / Int / Des / Dd	Frsh / Int / Des / Dd	Frsh / Int / Des / Dd	Frsh / Int / Des / Dd	Frsh / Int / Des / Dd	Frsh / Int / Des / Dd	Frsh / Int / Des / Dd	Frsh / Int / Des / Dd	Frsh / Int / Des / Dd
Bare										
Ulva intestinalis (string-like)										
Ulva lactuca (sheet-like)										
Ceramium										
Gracilaria										
Filamentous algae										
Ruppia (spp.)										
Macrocystis Wrack: Y / N										
Phyllospadix Wrack: Y / N										
Decayed and Unidentifiable										
Cerathidia										
Trash: Y / N										
Other 1:										
Total:										
Biomass: Y / N										
Field Lead Signature: _____										



Standard Operating Procedures (3.2): Vegetation Cover Surveys

March 2021

Prepared for the United States Environmental
Protection Agency



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Standard Operating Procedures: Vegetation Cover Surveys

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Protocol Suitability Evaluation

A habitat suitability table containing appropriate estuarine wetland habitat types (of those evaluated) to implement vegetation cover protocols is displayed in Table 1. For subtidal or heavily intertidal habitats, use Submerged Aquatic Vegetation cover protocol (SOP 3.1). A comparative assessment of cost, effort, and data quality are shown in Table 2. A matrix of additional detailed categorical evaluations of implement vegetation cover protocols can be found in Appendix 3.2A.

Table 1. Appropriate habitat types by vegetation cover survey protocol.

Survey Protocol	Habitat Types					
	Tidal Channel	Mud/sand flat	Emergent salt marsh	Non-tidal salt marsh	Salt pan	'Degraded' / fill
Point-contact		X	X	X	X	X
Line-intercept		X	X	X	X	X
Cover Class		X	X	X	X	X
Laser Quadrat		X	X		X	

Table 2. Categorical assessment of cost/effort and data quality by vegetation cover survey protocol.

Evaluation Metric		Point-contact	Line-intercept	Cover Class	Laser Quadrat	Notes
Time / Effort	Office Preparation Time	10-30 minutes	10-30 minutes	10-30 minutes	10-30 minutes	----
	Equipment Construction Time (one time)	10-30 minutes	10-30 minutes	10-30 minutes	> 60 minutes	----
	Field Time (per transect)	10-30 minutes	10-30 minutes	10-30 minutes	30-60 minutes	----
	Laboratory Time	0 minutes	0 minutes	0 minutes	0 minutes	----
	Post-Survey Processing / QAQC Time	10-30 minutes	10-30 minutes	10-30 minutes	10-30 minutes	Post QAQC for species identification may occur
	Minimum Repetition (site-dependent)	Many Repetitions	Many Repetitions	Many Repetitions	Many Repetitions	Fewer repetitions may be conducted in salt pan or lower diversity habitat areas
	Relative Cost (equipment and supplies)	< \$15	< \$15	< \$15	\$15 – 50	Laser quadrat is a specialized tool requiring construction
Survey / Data Quality	Accuracy (at a survey area level)	Low	Medium	Medium	High	----
	Precision (at a survey area level)	Low	Medium	Medium	High	May be decreased by wind, especially for laser
	Qualitative-Quantitative Score	Quantitative	Quantitative	Quantitative	Quantitative	----
	Subjectivity-Objectivity Score	Objective	Objective	Subjective	Objective	Subjectivity of cover class may be reduced by calibrations and trainings

Resulting Data Types

Each of the four cover protocols provide variable quantitative vegetation cover estimates at the quadrat- or transect-level that can be extrapolated up to a habitat- or site-level. Data can be presented by species or grouped for nativity or other considerations/needs. Data can be supplemented by biomass or other biological or physical parameters to evaluate a higher level of habitat function.

Objective

Long-term monitoring of vegetation is one of the most common methods of evaluating the health and functioning of a wetland system (Zedler 2001). Change in the relative presences of native and non-native plant species may affect the distributions of associated wildlife species. Many different approaches have been used to estimate plant species cover, especially for terrestrial vegetation (see review in Murray et al. 2006).

This Standard Operating Procedure (SOP) focuses on four types of cover surveys: point-contact transect, line-intercept transect, cover class quadrat, and laser quadrat. While all methods are based on transects allocated within habitats, they each provide a different degree of accuracy, difficulty, observer bias, and time commitment. All methods can be species-specific (or taxa-specific), and they all collect information on plant cover, live/dead/bare ground cover, plant canopy height, and general site conditions.

Cover surveys can be used to provide a wide range of information and data, including summarizing the prevalence of native and non-native plant cover in each habitat, determining species cover, relative species richness and diversity, and assessing canopy height.



Equipment

Equipment and supplies needed for these surveys varies depending on the specific type of vegetation survey to be conducted. Four types are discussed, including laser quadrat, cover class quadrat, line-intercept transect, and point-contact transect.

Several pieces of equipment are used in all vegetation cover surveys, including:

- 30m-transect tape (*Helpful hint: purchase the ones with both sides in metric units*)
- Meter stick or measuring tape (for vegetation canopy heights)
- Datasheets (Appendices 3.2B – E)
- Vegetation identification field guides (optional) or an electronic guide (e.g., phone app for plant identification)

Standard Operating Procedures: Vegetation Cover Surveys The Bay Foundation

- GPS equipped with compass (with additional power source if needed)
- GPS-enabled digital camera (preferred) or digital camera or smart phone camera application
- Two, 1m PVC pipes to permanently mark the beginning and ends of each transect (optional)

For the point-contact and line-intercept surveys, the above equipment is all that is needed. Individual surveys use some specialized equipment.

The cover class quadrat survey also requires:

- 1-meter square quadrat divided into 16 smaller squares using string, PVC pipes, and elbow joints (Figure 1)
Note: quadrat size can be adjusted based on the monitoring program objectives with somewhat comparable results, but it may affect species richness.
- Percent cover calibration guides (Appendix 3.2F)

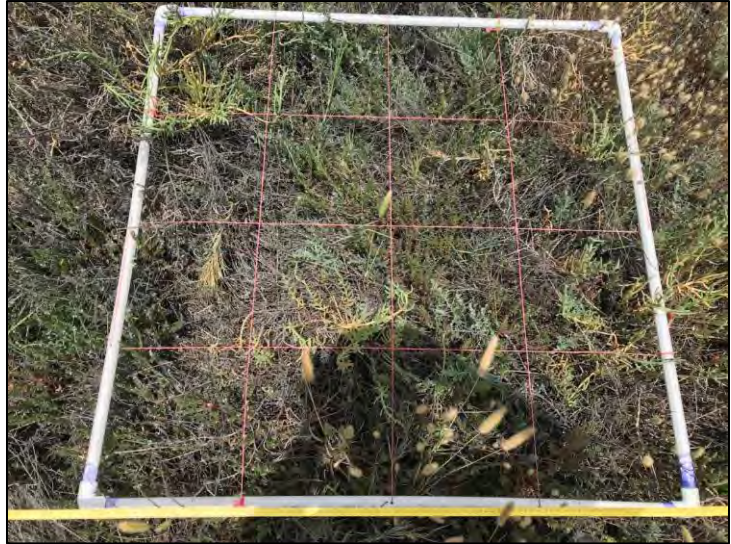


Figure 1. Cover class 1 m² quadrat with 16 sub-quadrants for increased accuracy.

The laser quadrat cover survey also requires:

- Laser quadrat (0.5 x 0.5 m square clear plexiglass board with 49 evenly spaced holes drilled through the board; Figure 2)
- Laser pointer; *Helpful hint: a weak laser will be very difficult to see on a sunny day; blocking the sun with the observer's body often increases the visibility of the laser in the field.*



Figure 2. Laser quadrat (0.5m x 0.5m) and laser pen with taped holes for increased accuracy.

Field Preparation

Equipment described above should be collected prior to the field shift. Batteries for all electronic devices should be checked and replaced as needed, and relevant data sheets should be printed and attached to the clipboards. Plant surveys should occur annually during the peak biomass season for each habitat type (e.g., estuarine wetland in late summer; Zedler 2001), or more frequently to capture seasonal variability.

The general sampling design consists of identifying distinct marsh zones or habitat types (e.g., low marsh, mid-marsh, upper marsh, etc.) within the site and randomly assigning transects within each zone using Geographic Information System (GIS) software and a stratified sampling design based on area of each habitat. The criteria for creating potential transects are as follows: Transects should be 25 m in length and spaced a minimum of 10 m apart; they should not be placed within 5 m of a zone (habitat) boundary or tidal creek (Ambrose and Diaz 2008) and should not cross the boundary. A random number generator may then be used to select a suggested minimum of three to five transects per habitat. More transects may be allocated to habitats that cover a larger area or that contain a diverse vegetation community to reduce precision error.

The beginning and end points of each transect should be mapped using a GPS (high resolution is recommended), and then permanently identified using thin, UV-resistant PVC piping and a waterproof tag (Figure 3; optional) to easily return to the same sampling location on subsequent trips.

Field Methods

Field methods were based on protocols used in the Ballona Wetlands Baseline Monitoring Program (Johnston et al. 2011, 2012, 2015). Laser quadrat protocols were developed by Dr. Sean Anderson at California State University, Channel Islands. Laser quadrat transects are not recommended in vegetation with an average height greater than approximately 1 meter.

For all transects, additional vegetation species occurring within 10 m of the transect should be noted on the back of the data sheet (presence, not quantified cover; see Appendices 3.2B – E). A minimum of three points for canopy height and the tallest vegetation height should also be recorded for each transect (see individual transect protocols below). Several additional places for site notes are also included in Appendices 3.2B - E.

For all transects, if the contact or quadrat cover is not plant tissue, the ground type should be recorded as bare ground, trash, wrack, or wood (as appropriate). Trash is defined as man-made debris, and wrack is defined as dead organic material. Algae on top of plants should be noted as present for each location, removed to reveal the plant tissue below, and not included in percent cover estimates (Ambrose and Diaz 2008); *Cuscuta salina* (salt marsh dodder) should be recorded similarly.

All transects begin by deploying the 25 m-transect tape between the permanent PVC pipes or GPS locations.



Figure 3. UV-resistant PVC pipe marking the beginning of a vegetation transect.

Point-contact Transects

Point-contact transects document every species observed below the transect tape at a set distance between points (e.g., every 20 cm). A minimum of 100 points per transect is recommended. The minimum length can be increased (i.e., fewer points) to reduce time commitment per transect, but this will also reduce the accuracy and precision of the average vegetation cover. Diverse plant habitats (e.g., transition zones) should record more points to increase accuracy.

The transect tape should be straightened between transect beginning and end points. If vegetation occurs below the top of the transect tape (i.e., if the tape is stretched taught and the vegetation is several inches below), then the first species (or ground type if no vegetation is present) that comes in contact with a hypothetical vertical line straight down from that point on the transect should be recorded (Ambrose and Diaz 2008). Canopy heights should also be recorded for plants every few meters. A minimum of four or five measurements are recommended per transect, as well as a maximum canopy height and species identification for each transect. A point-contact transect datasheet for transect with 20 cm point distances is shown in Appendix 3.2B.

Line-intercept Transects

Line-intercept transects are similar to point-contact. The transects document every species observed directly below the transect tape where the vegetation crosses a minimum of 0.01 m in length (the minimum length can be increased to reduce time commitment per transect, but this will also reduce precision). The start and end points are recorded on the datasheet (e.g., 1.05 – 1.22 m) as well as the species identification and whether the species is living or dead.

The transect tape should be straightened between transect beginning and end points. If vegetation occurs below the top of the transect tape (i.e., if the tape is stretched taught and the vegetation is several inches below), then the first species (or ground type if no vegetation is present) that comes in contact with a hypothetical vertical line straight down from that point on the transect should be recorded (Ambrose and Diaz 2008). Canopy heights should also be recorded for plants every few meters. A minimum of four or five measurements are recommended per transect, as well as a maximum canopy height and species identification for each measurement. A line-intercept transect datasheet is shown in Appendix 3.2C.

Cover Class Transects

The cover-class quadrat allows for surveys of taller vegetation and a more rapid assessment of the plant community in a given area. This survey method is based on the Daubenmire (1959) cover-class system using a 7-point scale (Table 3; Appendix 3.2F). Five to ten quadrats can be completed along each transect, depending on the degree of variability of the vegetation along the transect (higher variability = more quadrats). If quadrats are randomly assigned, they should be allocated by a random number generator (Excel is one option) prior to field deployment. If the quadrats are 'fixed', then the same meter marking can be used on every transect.

Surveys should be conducted using 1 m² PVC quadrats (Figure 1) subdivided into 16 sub-quadrats to increase the accuracy of cover estimates (Daubenmire 1959). Because canopies of different strata (e.g., grasses, shrubs) may overlap and the cover is broken down into classes, these cover estimates may total more than 100% (Ambrose and Diaz 2008), unlike the laser-based quadrat cover estimates. Each species (and whether it is alive or dead) should be recorded as one cover class. A cover class transect datasheet for a transect with seven quadrats is shown in Appendix 3.2D.

Table 3. Cover categories and associated cover class identification numbers used in the BAP surveys (modified from Daubenmire 1959).

Estimated cover category	Cover class
> 0 - 1 %	1
> 1 - 5 %	2
> 5 - 25 %	3
> 25 - 50 %	4
> 50 - 75 %	5
> 75 - 95 %	6
> 95 - 100 %	7

Three intersections of the sub-quadrats should be randomly chosen, and the plant species identity and height recorded as a measure of canopy for that quadrat. The overall tallest plant species and height should also be recorded for each quadrat to characterize maximum canopy height. Observers tend to generally think about cover estimates in terms of rounded numbers to the nearest 5 or 10 (K. Johnston, personal observation). Thus, it is very important to have the discussion in the field about whether the actual cover is more or less than the cover class boundary value (e.g., “5” or “25”). If agreed that the cover is less, then it should stay in that category, if it is higher than the boundary number, then it should be bumped up to the next higher cover class.

Alternate Cover Estimate Methods

In addition to the method described above for the cover-class quadrat (“Canopy Cover Method”), there is another method identified here. The Canopy Cover Method recommends a total cover estimate for each species regardless of whether it is in the top canopy layer or not. In some cases, one vegetation species will overlap another, and the above method will capture total cover for both species (Figure 4). As an alternate, some monitoring programs assess overall aerial cover (“Aerial Cover Method”), which does not account for canopy overlap (Figure 4). The results produced are not transferrable between methods, except for total cover / bare ground. The Canopy Cover Method produces more accurate total estimates of cover by species, but the Aerial Cover Method can be quality control checked from visuals such as aerial or on-site photographs. Both produce similar types of data outputs.

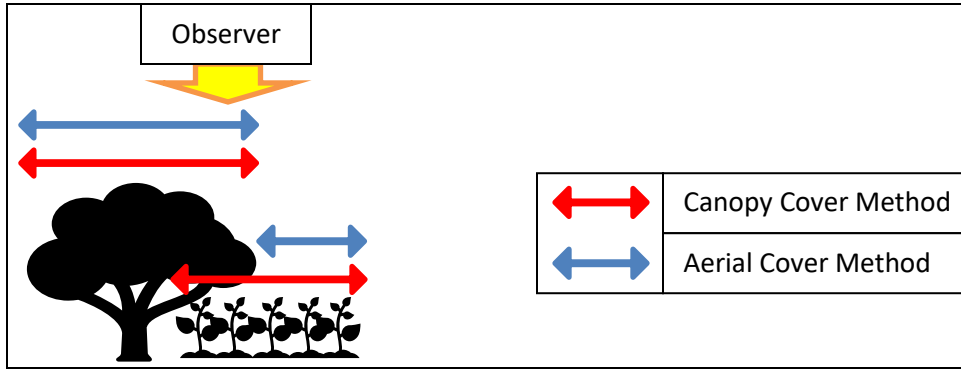


Figure 4. Graphic showing two methods of cover estimation for vegetation.

Additionally, as an alternate to the cover-class method, some programs like to make exact cover estimates in the field of each species. For example, if a plant species had an estimated 17% cover within a quadrat, instead of the 5-25% category being recorded on the data sheet, it would be the exact value (i.e., 17%). These data, while more precise, are often less accurate. They also often require specialized trainings or field calibration days with full teams doing “blind” calibration estimates (independent from one another), and then comparing to make sure all individual estimates are within a comfortable error margin of each other. However, the benefit of this exact cover estimate method is that exact percentages can be analyzed in the data and are then comparable to the cover-class estimates once transformed (which can be done as a post-processing data transformation into the categories). With large data sets, either method can be performed with similar grand mean averages for the analyses, but usually larger error bars for the cover-class estimates.

Laser Quadrat Transects

For all salt marsh habitats, where the average vegetation height is less than 1 m, the laser quadrat method can be utilized to demarcate exact points (Figure 5). The laser quadrat reduces observer bias and can be used to determine average percent cover. Five to ten quadrats can be completed along each transect, depending on the degree of variability of the vegetation along the transect (higher variability = more quadrats). If quadrats are randomly assigned, they should be allocated by a random number generator (Excel is one option) prior to field deployment. If the quadrats are ‘fixed’, then the same meter marking can be used on each transect. A laser quadrat transect datasheet for a transect with seven quadrats is shown in Appendix 3.2E.

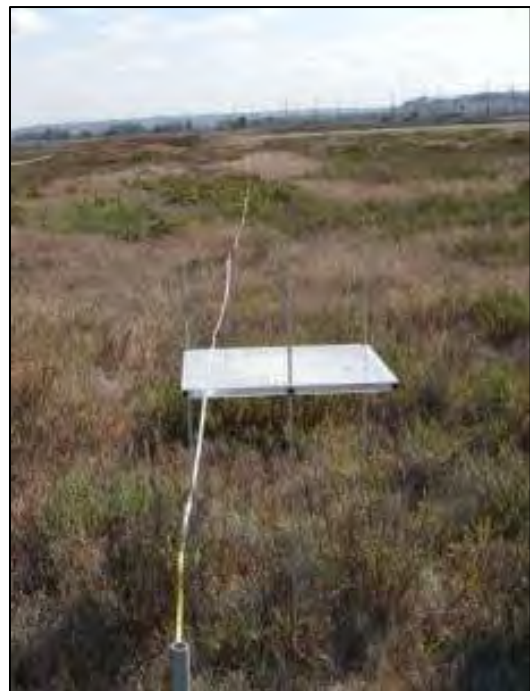


Figure 5. Photo of an example laser quadrat transect.

A portable 0.5 x 0.5 m (0.25 m²) Plexiglas™ board (Figure 2), supported by three independently adjustable legs, is

positioned parallel to the substrate and leveled at each quadrat starting meter point along the transect (the left corner of the Plexiglas™ board should be placed on the transect starting meter point, to maintain consistency throughout the survey) (Shuman and Ambrose 2003, Ambrose et al. 2006, S. Anderson, pers. comm.). The board design is a modified pin-drop cover board with a downward shining laser pointer taking the place of the rod or pin that would make contact and define a single contact point. A laser pointer should be inserted successively into each of the 49 evenly distributed points in a 7 x 7 grid so that the laser beam points in a direction perpendicular to the substratum. This method is much faster than traditional pin-drop methods, does not disturb the architecture of the canopy (particularly important to surveying vegetation with vertical gramminoid-morphology or with interwoven stems and leaves), and is observer-independent. Species should be further delineated as either living or dead. *Note: If the laser beam happens to shine on two independent pieces of vegetation, then the top illuminated species is the only one recorded.*

Within each quadrat, three of the 49 points should be randomly sampled for canopy height (these should be marked in advance with tape to avoid observer bias). At each of the three points, the plant height and species identity are recorded. Additionally, the plant height and species identity should be recorded for the tallest plant within the 0.5 x 0.5 m quadrat area (to nearest cm) as a measure of maximum canopy height.



Laboratory Methods

Not applicable. Vouchers from the field should be pressed for later identification.

Data Entry and QAQC Procedures

Data should be entered in the field using the appropriate data sheet (Appendices 3.2B – E). All required fields should be completed in full, and the data recorder should assign their name at the top of the document(s). Data should be transferred to the appropriate electronic database within three days, and

the hard copies filed in labeled binders. Electronic copies of all data should be housed on an in-house dedicated server and backed up to a cloud-based or off-site server nightly. Hard copies should be saved for five years. Electronic copies should be saved indefinitely.

Quality Assurance and Quality Control (QAQC) procedures should be conducted on all data. QAQC procedures should be conducted by the QA Officer and include a thorough review of all entries, double checking of all formulas or macros, and a confirmation that all data sheets, Chain-of-Custody forms, and field notes are filed appropriately with electronic back-up copies available. QAQC should verify that the entered data match the hard copies of the field data sheets. Any discrepancies should be corrected, and the initial data entry technician notified.

Data Analyses

After data have been entered, corrections made, and QAQC procedure completed, data can be used in multiple analyses. Examples include bar graphs with native and non-native cover comparisons, spatial analyses based on maps of averages across each transect or habitat, species lists or richness, and many more. Individual analyses for each transect type should be completed as follows:

Point-contact and Line-intercept Transects

Point-contact data should be summed as the number of points for each species per transect and then divided by the total number of points per transect to determine percent cover by transect (per species). Line-intercept data should be summed by species and divided by the total length of the transect to determine percent cover for each transect and habitat.

Cover Class Transects

Species data should be analyzed using the median of each Daubenmire cover category and averaged to determine percent cover within each transect and/or habitat. Variability should be represented as standard deviation or error. If using the exact cover estimate method for your analyses, no data transformation is needed (i.e., median of the cover class). However, to compare data using both methods, the exact cover estimate method must then be binned into the cover class categories and the median used similar to above. Data can not be transformed the other way (i.e., turning binned data into exact percentages). With large data sets, either method can be performed with similar grand mean averages for the analyses, but usually larger error bars for the cover-class estimates (K. Johnston and C. Whitcraft, unpublished data).

Laser Quadrat Transects

Percent cover is analyzed as the proportion of points (out of a total of 49) hitting a particular plant species. Plant cover can be averaged by transect and then again by habitat type. Variability should be represented as standard deviation or error.

Health and Safety Precautions

Not applicable.

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APPENDIX 3.2A

	Evaluation Metric	Point-contact	Line-intercept	Cover Class	Laser Quadrat	Notes
	Correlation to L2 CRAM	Attribute 4	Attribute 4	Attribute 4	Attribute 4	----
Personnel Requirements	Specialty Equipment or Clothing Required	No Specialty Items	No Specialty Items	No Specialty Items	Few Specialty Items	Laser quadrat
	Ease of Transport (amount or weight of supplies)	Few Items / Easy	Few Items / Easy	Few Items / Easy	Some Items / Moderate	----
	Ease of Implementation	Moderate	Moderate	Moderate	Moderate	Easy implementation within salt pan habitats
	Expertise / Skill Level	Some Technical Knowledge	Some Technical Knowledge	Some Technical Knowledge	Some Technical Knowledge	Familiarity with species identifications is required
	Number of Personnel	2	2	2	2	----
	Training Requirements	None	None	None	None	Calibration between team members may be necessary for subjective surveys
	Seasonality of Survey Time	Late summer	Late summer	Late summer	Late summer	Seasonality of 'Degraded' / fill habitat areas is site dependent
	Suggested Frequency	Annual	Annual	Annual	Annual	Annual or herbaceous habitats may require more
Survey / Data Quality	Type of Output	Numerical	Numerical	Numerical	Numerical	----
	Active or Passive Monitoring Style	Passive	Passive	Passive	Passive	----
	Specialty Computer Software Required	No	No	No	No	----
	Availability of Online / External Resources	Many	Many	Many	Few	Many online and print media are also available to assist with species identifications
Potential Limitations	Wetland Type Applicability	All	All	All	All	Dependent on vegetation height
	Images or Multi-Media Required	Images Required	Images Required	Images Required	Images Required	----
	Degree of Impact / Disturbance	Low Disturbance	Low Disturbance	Low Disturbance	Low Disturbance	----
	Vegetation Height Limitation	Overhead (~2m)	Overhead (~2m)	Overhead (~2m)	Low Vegetation Only (< 1 m)	----
	Appropriate for Tidal / Wet Habitats	Yes	Yes	Yes	Yes	See category below
	Tide Height	Low Tide Only	Low Tide Only	Low Tide Only	Low Tide Only	Submersion of low-lying vegetation will skew numbers and not allow for accurate estimates
	Regional or Broad Implementation *	Frequently Used	Frequently Used	Almost Always Used	Infrequently Used	<i>* based on monitoring literature review</i>
	Potential for Hazards / Risk	Low to No Risk	Low to No Risk	Low to No Risk	Low to No Risk	----
Restrictions	Special status species	Special status species	Special status species	Special status species	----	

APPENDIX 3.2B

VEGETATION SAMPLING DATA SHEET – POINT CONTACT

Sampling Program Information						NOTES:		
DATE: _____		HABITAT: _____				SITE: WEATHER:		
TIME (start): _____ (end): _____		FID #: _____						
STAFF: _____		PAGE: _____ of _____						
METER	SPECIES	D/L	METER	SPECIES	D/L	METER	SPECIES	D/L
0.20			8.00			15.80		
0.40			8.20			16.00		
0.60			8.40			16.20		
0.80			8.60			16.40		
1.00			8.80			16.60		
1.20			9.00			16.80		
1.40			9.20			17.00		
1.60			9.40			17.20		
1.80			9.60			17.40		
2.00			9.80			17.60		
2.20			10.00			17.80		
2.40			10.20			18.00		
2.60			10.40			18.20		
2.80			10.60			18.40		
3.00			10.80			18.60		
3.20			11.00			18.80		
3.40			11.20			19.00		
3.60			11.40			19.20		
3.80			11.60			19.40		
4.00			11.80			19.60		
4.20			12.00			19.80		
4.40			12.20			20.00		
4.60			12.40			20.20		
4.80			12.60			20.40		
5.00			12.80			20.60		
5.20			13.00			20.80		
5.40			13.20			21.00		
5.60			13.40			21.20		
5.80			13.60			21.40		
6.00			13.80			21.60		
6.20			14.00			21.80		
6.40			14.20			22.00		
6.60			14.40			22.20		
6.80			14.60			22.40		
7.00			14.80			22.60		
7.20			15.00			22.80		
7.40			15.20			23.00		
7.60			15.40			23.20		
7.80			15.60			23.40		

APPENDIX 3.2B

METER	SPECIES	D/L	METER	SPECIES	D/L
23.60			24.40		
23.80			24.60		
24.00			24.80		
24.20			25.00		

Height at 5m:

SPS:

SOIL COND'N:

SOIL TYPE:

Height at 10m:

SPS:

ADDT'L SPECIES:

Height at 15m:

SPS:

TARGET SPECIES:

Height at 20m:

SPS:

CROSS:

WATER?

Y / N

ROAD?

Y / N

MAX HEIGHT:

SPS:

CHAIN OF CUSTODY

NAME	DATE	TIME	ACTION
			Recorded
			Data Folder
			Entered
			QAQC

NOTES:

APPENDIX 3.2C

VEGETATION SAMPLING DATA SHEET - LINE INTERCEPT

Sampling Program Information				NOTES:	
DATE: _____		HABITAT: _____		SITE: WEATHER:	
STAFF: _____		FID #: _____			
TIME (start): _____ (end): _____		PAGE: _____ of _____			

	Start	End	SPECIES	D/L		Start	End	SPECIES	D/L
1					40				
2					41				
3					42				
4					43				
5					44				
6					45				
7					46				
8					47				
9					48				
10					49				
11					50				
12					51				
13					52				
14					53				
15					54				
16					55				
17					56				
18					57				
19					58				
20					59				
21					60				
22					61				
23					62				
24					63				
25					64				
26					65				
27					66				
28					67				
29					68				
30					69				
31					70				
32					71				
33					72				
34					73				
35					74				
36					75				
37					76				
38					77				
39					78				

APPENDIX 3.2C

	Start	End	SPECIES	D/L
79				
80				
81				
82				
83				
84				
85				
86				
87				
88				
89				
90				
91				
92				
93				
94				
95				
96				
97				
98				
99				
100				
101				
102				
103				
104				
105				
106				
107				
108				
109				
110				
111				
112				
113				
114				
115				
116				
117				
118				
119				
120				

SOIL COND'N:

SOIL TYPE:

ADDT'L SPECIES (10m):

TARGET SPECIES:

CROSS: Y / N ROAD? Y / N

Height at 5m:
SPS:

Height at 10m:
SPS:

Height at 15m:
SPS:

Height at 20m:
SPS:

MAX HEIGHT:
SPS:

NOTES:

CHAIN OF CUSTODY			
NAME	DATE	TIME	ACTION
			Recorded
			Data Folder
			Entered
			QAQC

APPENDIX 3.2D

VEGETATION SAMPLING DATA SHEET - 25m % COVER TRANSECT				NOTES:
DATE:	PHOTO: Y / N	HABITAT:		
TIME (start):	(end):	FID #:	SITE:	
FIELD LEAD:	PAGE: _____ of _____		WEATHER:	
DATA RECORDER:		ADDITIONAL STAFF:		
Quadrats on the Right			Cover Classes A:[0-1] B:[1-5] C:[5-25] D:[25-50] E:[50-75] F:[75-95] G:[95-99]	

QUADRAT: 1	METER:	PHOTO: Y / N		
SPECIES	DEAD %	LIVE %		
BARE GROUND				
HEIGHTS	1st	2nd	3rd	Max
HT Species	1st	2nd	3rd	Max
Dead/Live	D / L	D / L	D / L	D / L

QUADRAT: 2	METER:	PHOTO: Y / N		
SPECIES	DEAD %	LIVE %		
BARE GROUND				
HEIGHTS	1st	2nd	3rd	Max
HT Species	1st	2nd	3rd	Max
Dead/Live	D / L	D / L	D / L	D / L

QUADRAT: 3	METER:	PHOTO: Y / N		
SPECIES	DEAD %	LIVE %		
BARE GROUND				
HEIGHTS	1st	2nd	3rd	Max
HT Species	1st	2nd	3rd	Max
Dead/Live	D / L	D / L	D / L	D / L

QUADRAT: 4	METER:	PHOTO: Y / N		
SPECIES	DEAD %	LIVE %		
BARE GROUND				
HEIGHTS	1st	2nd	3rd	Max
HT Species	1st	2nd	3rd	Max
Dead/Live	D / L	D / L	D / L	D / L

QUADRAT: 5	METER:	PHOTO: Y / N		
SPECIES	DEAD %	LIVE %		
BARE GROUND				
HEIGHTS	1st	2nd	3rd	Max
HT Species	1st	2nd	3rd	Max
Dead/Live	D / L	D / L	D / L	D / L

QUADRAT: 6	METER:	PHOTO: Y / N		
SPECIES	DEAD %	LIVE %		
BARE GROUND				
HEIGHTS	1st	2nd	3rd	Max
HT Species	1st	2nd	3rd	Max
Dead/Live	D / L	D / L	D / L	D / L

APPENDIX 3.2D

QUADRAT: 7 METER: PHOTO: Y / N

SPECIES	DEAD %		LIVE %	
BARE GROUND				
HEIGHTS	1st	2nd	3rd	Max
HT Species	1st	2nd	3rd	Max
Dead/Live	D / L	D / L	D / L	D / L

Dominance within 20m:		
Cover:	Native (>50%) <input type="checkbox"/>	Non-Native (<50%) <input type="checkbox"/>

Soil Condition/Texture:

SPECIES W/IN 10m:

_____	_____	_____	_____
_____	_____	_____	_____
_____	_____	_____	_____
_____	_____	_____	_____
_____	_____	_____	_____
_____	_____	_____	_____

TARGET SPECIES:

CROSS CHANNEL?	Y / N	ROAD?	Y / N
----------------	-------	-------	-------

SITE DESCRIPTION:

ADD'L NOTES:

CHAIN OF CUSTODY			
NAME	DATE	TIME	ACTION
			Recorded
			Data Folder
			Entered
			QAQC

APPENDIX 3.2E

VEGETATION SAMPLING DATA SHEET -LASER QUADRAT TRANSECT				NOTES:
DATE:	PHOTO	HABITAT:		
TIME (start):	(end):	FID #:	SITE:	
FIELD LEAD:	PAGE:	of	WEATHER:	
RECORDER:	ADDITIONAL STAFF:			
**Quadrats on the Right*				

QUADRAT: 1	METER:	PHOTO: Y / N		
SPECIES	LIVE	DEAD	TOTALS	
			/	
			/	
			/	
			/	
			/	
			/	
			/	
			/	
			/	
			/	
			/	
BARE GROUND				
HEIGHTS	1st	2nd	3rd	Max
HT Species	1st	2nd	3rd	Max
Dead/Live	D / L	D / L	D / L	D / L

QUADRAT: 2	METER:	PHOTO: Y / N		
SPECIES	LIVE	DEAD	TOTALS	
			/	
			/	
			/	
			/	
			/	
			/	
			/	
			/	
			/	
			/	
			/	
BARE GROUND				
HEIGHTS	1st	2nd	3rd	Max
HT Species	1st	2nd	3rd	Max
Dead/Live	D / L	D / L	D / L	D / L

QUADRAT: 3	METER:	PHOTO: Y / N		
SPECIES	LIVE	DEAD	TOTALS	
			/	
			/	
			/	
			/	
			/	
			/	
			/	
			/	
			/	
			/	
			/	
BARE GROUND				
HEIGHTS	1st	2nd	3rd	Max
HT Species	1st	2nd	3rd	Max
Dead/Live	D / L	D / L	D / L	D / L

QUADRAT: 4	METER:	PHOTO: Y / N		
SPECIES	LIVE	DEAD	TOTALS	
			/	
			/	
			/	
			/	
			/	
			/	
			/	
			/	
			/	
			/	
			/	
BARE GROUND				
HEIGHTS	1st	2nd	3rd	Max
HT Species	1st	2nd	3rd	Max
Dead/Live	D / L	D / L	D / L	D / L

QUADRAT: 5	METER:	PHOTO: Y / N		
SPECIES	LIVE	DEAD	TOTALS	
			/	
			/	
			/	
			/	
			/	
			/	
			/	
			/	
			/	
			/	
			/	
BARE GROUND				
HEIGHTS	1st	2nd	3rd	Max
HT Species	1st	2nd	3rd	Max
Dead/Live	D / L	D / L	D / L	D / L

QUADRAT: 6	METER:	PHOTO: Y / N		
SPECIES	LIVE	DEAD	TOTALS	
			/	
			/	
			/	
			/	
			/	
			/	
			/	
			/	
			/	
			/	
			/	
BARE GROUND				
HEIGHTS	1st	2nd	3rd	Max
HT Species	1st	2nd	3rd	Max
Dead/Live	D / L	D / L	D / L	D / L

APPENDIX 3.2E

QUADRAT: 7

METER:

PHOTO: Y / N

SPECIES	LIVE	DEAD	TOTALS	
			/	
			/	
			/	
			/	
			/	
			/	
			/	
			/	
			/	
BARE GROUND				
HEIGHTS	1st	2nd	3rd	Max
HT Species	1st	2nd	3rd	Max
Dead/Live	D / L	D / L	D / L	D / L

Dominance within 20m:		
Cover:	Native (>50%) <input type="checkbox"/>	Non-Native (<50%) <input type="checkbox"/>

Soil Condition: _____

SPECIES W/IN 10m:

_____	_____	_____	_____
_____	_____	_____	_____
_____	_____	_____	_____
_____	_____	_____	_____
_____	_____	_____	_____

TARGET SPECIES: _____

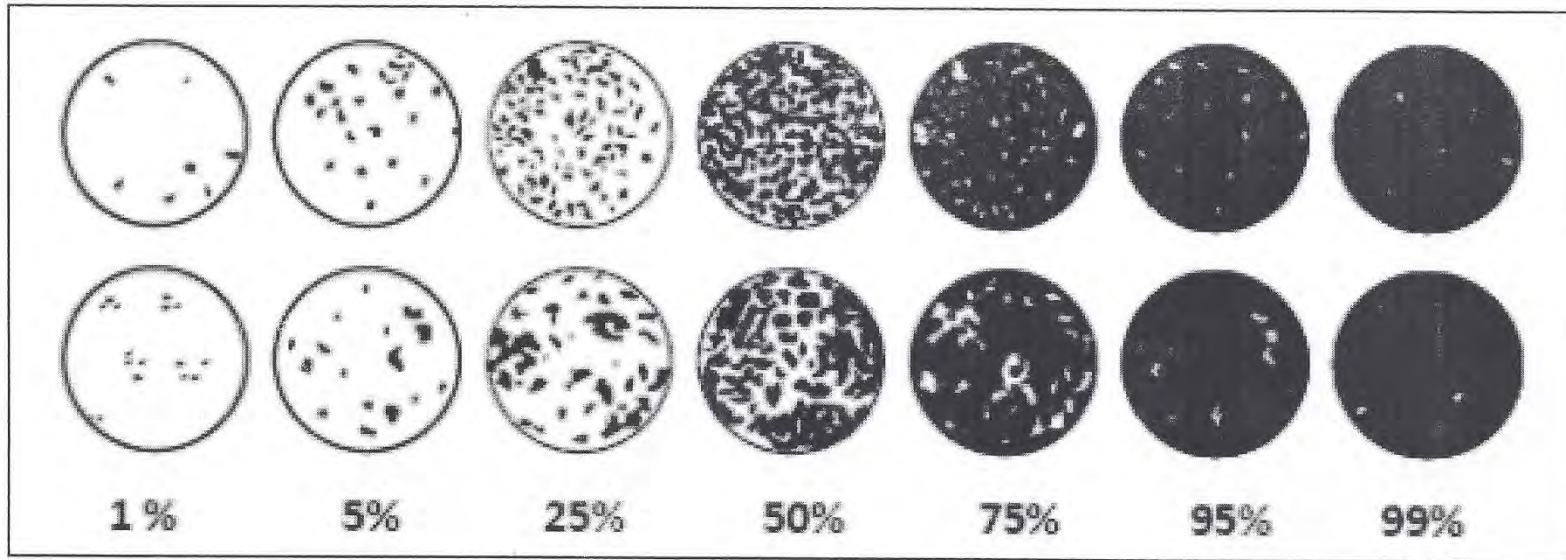
CROSS CHANNEL?	Y / N	ROAD? Y / N
-----------------------	--------------	--------------------

SITE DESCRIPTION:

ADD'L NOTES:

CHAIN OF CUSTODY			
NAME	DATE	TIME	ACTION
			Recorded
			Data Folder
			Entered
			QAQC

APPENDIX 3.2F



Excerpted from Daubenmire 1959.



Standard Operating Procedures (3.3): Vegetation Biomass

March 2021

Prepared for the United States Environmental
Protection Agency



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Standard Operating Procedures: Vegetation Biomass

SOP Identification Number: SOP 3.3 Vegetation Biomass

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Developed by: The Bay Foundation and California State University, Channel Islands

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Protocol Suitability Evaluation

A habitat suitability table containing appropriate estuarine wetland habitat types (of those evaluated) to implement vegetation biomass protocols is displayed in Table 1. All vegetated habitats are appropriate for this survey type. A comparative assessment of cost, effort, and data quality are shown in Table 2. A matrix of additional detailed categorical evaluations of vegetation biomass protocols can be found in Appendix 3.3A.

Table 1. Appropriate habitat types for vegetation biomass survey protocols.

Survey Protocol	Habitat Types					
	Tidal Channel	Mud/sand flat	Emergent salt marsh	Non-tidal salt marsh	Salt pan	'Degraded' / fill
Vegetation Biomass			X	X	X	X

Table 2. Categorical assessment of cost/effort and data quality for vegetation biomass survey protocols.

	Evaluation Metric	Vegetation Biomass	Notes
Time / Effort	Office Preparation Time	10-30 minutes	----
	Equipment Construction Time (one time)	0-10 minutes	----
	Field Time (per transect)	10-30 minutes	Additional time may be necessary along transects with high biomass
	Laboratory Time (per transect)	30-60 minutes	Samples must be sorted, dried, and weighed
	Post-Survey Processing / QAQC Time	10-30 minutes	----
	Minimum Repetition (site-dependent)	Many Repetitions	----
	Relative Cost (equipment and supplies)	< \$15	----
Survey / Data Quality	Accuracy (at a survey area level)	High	----
	Precision (at a survey area level)	Medium	----
	Qualitative-Quantitative Score	Quantitative	----
	Subjectivity-Objectivity Score	Objective	----

Resulting Data Types

The application of vegetation biomass survey protocols will yield quantitative data displayed in species-specific grams per square meter of above ground biomass. These data may be used in conjunction with vegetation cover survey data to extrapolate transect-level biomass per species or identify biomass trends over in relation to external stressors (e.g., low rainfall years, installation of tide gates). These data provide an additional layer of functional assessment when combined with other vegetation SOPs such as vegetation cover (SOP 3.2).

Objective

Long-term monitoring of vegetation is one of the most common methods of evaluating the health and functioning of a wetland system (Zedler 2001). Change in the relative amount of native and non-native plant species may affect the distributions of associated wildlife species. Many different approaches have been used to estimate plant species cover, especially for terrestrial vegetation (see review in Murray et al. 2006). However, vegetation cover alone is often not enough to accurately assess the health of a vegetation community, and aboveground biomass may be used as an indicator metric to quantify net primary productivity of the community (EPA 2002), particularly if harvested annually at the end of the primary growing season.

This method samples above ground vegetation tissue (as dry weight) within a defined area for use in conjunction with vegetation cover data to assess wetland vegetation communities and alliances. Biomass data should be collected during the vegetation cover surveys (SOP 3.2) to optimize time management. Specific protocols were developed by Dr. Sean Anderson at California State University, Channel Islands.

Additional below ground biomass can supplement data even further; however, these methods often require time-intensive collection, lengthy durations of an experiment, or more intensive permit conditions and potential impacts to the plant community.

Equipment

Equipment and supplies needed for this survey include:

1. GPS and camera
2. Transect tape (30m)
3. 10cm x 10cm PVC quadrat
4. Various sized paper bags (large and 'lunch-sized')
5. Grass shears or clippers
6. Permanent ink pen to label bags with transect, date, and time of collection.

Field Preparation

Equipment described above should be collected prior to the field shift. Batteries for all electronic devices should be checked, charged, and/or replaced as needed. Relevant data sheets should be printed and attached to the clipboards. Avoid planning for field survey days that are subsequent to rain events, as samples will require more drying time and may be more likely to rot.

Field Methods

Transects surveyed for biomass should be the same as the vegetation cover surveys (see Vegetation Cover SOP 3.2 for details on assigning transects within wetland habitats). Biomass sampling can be



Figure 1. Transect tape deployed from labeled PVC.



Figure 2: Cylindrical area representing where plant tissue should be collected.

disruptive or impact the vegetation community and is recommended at a frequency of annually (or bi-annually) near the end of the wetland growing season (late summer / early fall; Broome et al 1986; Collins et al 2010). *Note: new biomass quadrat locations must be surveyed each time to account for the impacts of the previous year's surveys.*

After sampling for vegetation cover using quadrats, survey for above ground biomass on a subset of these quadrats (3-5). All biomass within a 10 x 10 cm quadrat should be collected at each quadrat location (S. Anderson, pers. comm. 2010). *Note: special permits may be required for special status or sensitive plant species (and/or they may need to be avoided).* The 100 cm² quadrat should be placed in the center of the

cover quadrat and all live plant material throughout the three-dimensional canopy to the ground should be collected from within this area. Visually, this resembles a rectangular prism within which all live plant material should be cut using the grass shears and collected. Harvested samples should be placed into an appropriately sized bag labeled with the transect/plot number, species, and date, and time. Plant material may be separated by species either in the field or laboratory, as preferred, as long as the bags are appropriately labeled.

Helpful hint: The outer diameter of a 4" PVC pipe also gives an area of approximately 100 cm² and can be used in lieu of the mini-quadrat (Figure 2).

Helpful hint: If the survey site is dominated by two or three species, it is usually faster to segregate plant tissue in the field as you snip the stems. Often laboratory-based sorting can be quite tedious and time-consuming when technicians are presented with numerous cut stems that lack leaves or other distinguishing characteristics.

Laboratory Methods

Wet harvested biomass needs to be dried (using a laboratory oven) immediately upon return to the laboratory to avoid rot. Dry vegetation can sit in a dry, well aerated room for up to one week before processing (or while other samples are drying). Excessively wet samples (e.g., plants from low elevation sites collected after a high tide) should generally be air dried until any visible moisture on the outer plant surfaces or paper bag is gone. This assures that the drying oven does not become overly "steamy" which can lengthen the drying time any potential cause problems with older oven models. It is generally recommended to attempt low tide vegetation collection in those habitats to reduce drying.

Plant biomass should be dried at 80° C for 24-48 hours and then immediately weighed to the nearest 0.1 gram. Note that samples should be weighed before cooling to avoid weight changes due to reabsorbed moisture from the air. Three control (empty) bags of each size should also be dried (and the weights averaged for each size) to calculate the empty bag weight. This weight should be subtracted from the total weight of the plant material plus the bag to determine actual plant weights. *Helpful hint: If you have very little vegetation material in the sample, it is more accurate to weight the plant tissue directly upon the balance without the bag, but care must be taken to clearly denote this on the data sheet to avoid calibration mistakes.*

Data Entry and QAQC Procedures

Data should be entered in the field using the appropriate data sheet (Appendix 3.3B). All required fields should be completed in full, and the data recorder should sign their name at the top of the document(s). Data should be transferred to the appropriate electronic database within three days, and the hard copies filed in labeled binders. Electronic copies of all data should be housed on an in-house dedicated server and backed up to a cloud-based or off-site server nightly. Hard copies should be saved for five years. Electronic copies should be saved indefinitely.

Quality Assurance and Quality Control (QAQC) procedures should be conducted on all data. QAQC procedures should be conducted by the QA Officer and include a thorough review of all entries, double checking of all formulas or macros, and a confirmation that all data sheets, Chain-of-Custody forms, and field notes are filed appropriately with electronic back-up copies available. QAQC should verify that the entered data match the hard copies of the field data sheets. Any discrepancies should be corrected, and the initial data entry technician notified.

Data Analyses

After data have been entered, corrections made, and QAQC procedure completed, data can be used in multiple analyses. Examples include averaging the above ground biomass estimates by species per transect or habitat type and displaying the resulting graphs or assessing the biomass in relation to the cover data to get a total biomass of each species in each transect.

Health and Safety Precautions

Not applicable.

References and Applicable Literature

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Contact Information

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APPENDIX 3.3A

	Evaluation Metric	Vegetation Biomass	Notes
	Correlation to L2 CRAM	Attribute 4	----
Personnel Requirements	Specialty Equipment or Clothing Required	Few Specialty Items	Scissors, plastic bags
	Ease of Transport (amount or weight of supplies)	Few Items / Easy	----
	Ease of Implementation	Easy	May be more difficult in areas with high biomass
	Expertise / Skill Level	Some Technical Knowledge	No expertise is required for field implementation, but lab processing will require familiarity with species identifications. <i>In situ</i> field separation of species is easier than lab post-processing.
	Number of Personnel	2	----
	Training Requirements	None	----
	Seasonality of Survey Time	Fall	Peak growing season
	Suggested Frequency	Annual	Or biannual to reduce disturbance
Survey / Data Quality	Type of Output	Numerical	----
	Active or Passive Monitoring Style	Active	----
	Specialty Computer Software Required	No	----
	Availability of Online / External Resources	Some	----
Potential Limitations	Wetland Type Applicability	All	----
	Images or Multi-Media Required	Images Required	----
	Degree of Impact / Disturbance	High Disturbance	Taking vegetation cuttings and trampling
	Vegetation Height Limitation	Overhead (~2m)	Must be able to access the highest vegetation
	Appropriate for Tidal / Wet Habitats	Yes	----
	Tide Height	Low Tide Only	Tidal inundation may make it difficult to access or identify submerged vegetation
	Regional or Broad Implementation *	Infrequently Used	----
	Potential for Hazards / Risk	Low to No Risk	----
Restrictions	Special Status Species	----	

* based on monitoring literature review

APPENDIX 3.3B

Vegetation Biomass Data Sheet				
Date:		Staff:		
Survey Start Time:		End Time:	Uploaded:	Date:
Habitat:		QAQC:	Date:	
Other Notes:				
Station Information		Station Information		
Transect: _____		Transect: _____		
Meter: _____		Meter: _____		
sp. collected (%): _____		sp. collected (%): _____		
Add'l sp. w/in 5 m: _____		Add'l sp. w/in 5 m: _____		
GPS Coordinates: N 33.		GPS Coordinates: N 33.		
W 118.		W 118.		
Weight weight (g): _____		Weight weight (g): _____		
Dry weight (g): _____		Dry weight (g): _____		
Notes: _____		Notes: _____		
Station Information		Station Information		
Transect: _____		Transect: _____		
Meter: _____		Meter: _____		
sp. collected (%): _____		sp. collected (%): _____		
Add'l sp. w/in 5 m: _____		Add'l sp. w/in 5 m: _____		
GPS Coordinates: N 33.		GPS Coordinates: N 33.		
W 118.		W 118.		
Weight weight (g): _____		Weight weight (g): _____		
Dry weight (g): _____		Dry weight (g): _____		
Notes: _____		Notes: _____		
Station Information		Station Information		
Transect: _____		Transect: _____		
Meter: _____		Meter: _____		
sp. collected (%): _____		sp. collected (%): _____		
Add'l sp. w/in 5 m: _____		Add'l sp. w/in 5 m: _____		
GPS Coordinates: N 33.		GPS Coordinates: N 33.		
W 118.		W 118.		
Weight weight (g): _____		Weight weight (g): _____		
Dry weight (g): _____		Dry weight (g): _____		
Notes: _____		Notes: _____		
Station Information		Station Information		
Transect: _____		Transect: _____		
Meter: _____		Meter: _____		
sp. collected (%): _____		sp. collected (%): _____		
Add'l sp. w/in 5 m: _____		Add'l sp. w/in 5 m: _____		
GPS Coordinates: N 33.		GPS Coordinates: N 33.		
W 118.		W 118.		
Weight weight (g): _____		Weight weight (g): _____		
Dry weight (g): _____		Dry weight (g): _____		
Notes: _____		Notes: _____		



Standard Operating Procedures (3.4): Seed Bank Germination

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Prepared for the United States Environmental
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Standard Operating Procedures: Seed Bank Germination

SOP Identification Number: SOP 3.4 Seed Bank Germination

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Protocol Suitability Evaluation

A habitat suitability table containing appropriate estuarine wetland habitat types (of those evaluated) to implement seed bank germination protocols is displayed in Table 1. A comparative assessment of cost, effort, and data quality are shown in Table 2. A matrix of additional detailed categorical evaluations of seed bank survey protocols can be found in Appendix 3.4A.

Table 1. Appropriate habitat types to implement seed bank survey protocols.

Survey Protocol	Habitat Types					
	Tidal Channel	Mud/sand flat	Emergent salt marsh	Non-tidal salt marsh	Salt pan	'Degraded' / fill
Seed Bank	X (edge)	X (edge)	X	X	X	X

Table 2. Categorical assessment of cost/effort and data quality for seed bank survey protocols.

	Evaluation Metric	Seed Bank	Notes
Time / Effort	Office Preparation Time	10-30 minutes	Site selection and any GPS locations; print data sheets
	Equipment Construction Time (one time)	10-30 minutes	Setting up cores; trays
	Field Time (per transect)	30-60 minutes	----
	Laboratory Time (per transect)	> 60 minutes	Seed germination checks in the greenhouse bi-weekly
	Post-Survey Processing / QAQC Time	30-60 minutes	----
	Minimum Repetition (site-dependent)	Many Repetitions	Germination success data are highly variable
	Relative Cost (equipment and supplies)	< \$15	----
Survey / Data Quality	Accuracy (at a survey area level)	High	----
	Precision (at a survey area level)	Medium	----
	Qualitative-Quantitative Score	Quantitative	----
	Subjectivity-Objectivity Score	Objective	----

Resulting Data Types

The application of seed bank germination survey protocols will yield quantitative data displayed in germinated seedlings per square meter categorized by species and nativity. These data are useful to identify the potential species composition, richness, and density of the seed bank in a given area and can be extrapolated up to habitat type. Additionally, this survey method can inform restoration planning or comparative analyses to other sites. An important consideration is that seed bank studies are ineffective at quantifying species that rely on non-seeding propagation strategies.

Objective

Information about the seed bank of a wetland may be a better predictor of successful wetland functioning than the presence of adult plants (i.e., plant canopy) alone because the presence of a viable and diverse seed bank indicates recent well-functioning ecological and hydrological dynamics of the site (Johnston et al. 2011). Soil seed banks also forecast subsequent adult plant species richness under optimal conditions (S. Anderson, unpublished data). However, it should be noted that a limitation of this method is that it excludes species that do not rely on seeds to propagate.

Specific objectives of seed bank surveys include:

1. Define relative species richness of germinated plant seedlings by habitat type (Figure 1);
2. Determine the potential for future recruitment of plant species within habitat types;
3. Comparison of native and non-native seed banks;
4. Evaluate potential species recruitment / propagation at a transect level under ideal conditions.

Equipment

Equipment and supplies needed for this survey include:

1. GPS with transect locations
2. Digital camera or smartphone with camera application
3. Core (10 cm deep and 8 cm diameter);
Helpful hint: several brands of soup cans are the appropriate dimensions and may be used as a cheap core alternative. They should be replaced when the edges dull and start bending (Figure 2).
4. Plunger; this consists of a plug or disk the size of the core and an attached handle (several options are available at Home Depot; Figure 2).
5. Hand gardening trowel
6. Steam sterilized soil (e.g., Supersoil®) or sterilized playground sand
7. Bucket for soil



Figure 1. Labeled seed bank core with germinated pickleweed.



Figure 2. Seed bank equipment.

8. Greenhouse tray / flat (useful to transport cores from the field and keep them well-organized) capable of holding 20 of the 4" pots (Figure 2). Alternatively, a small, heavy-duty plastic cement mixing trough will also work.
9. 4" nursery pots (Figure 2)
10. Permanent ink pen and duct tape or a paint pen to label the pots
11. Large, shallow tubs (called "masonry mixing tubs" at Home Depot) if automated, misting sprinkler arrays are unavailable.
12. Greenhouse space with a freshwater source
13. Greenhouse datasheets (Appendix 3.4B)
14. Vegetation species ID guide; *Helpful hint: having a seedling guide is recommended as many species look different than adult plants when they have just germinated.*
15. Hand counter (optional)

Field Preparation

Equipment described above should be collected prior to the field shift. Batteries for all electronic devices should be checked and replaced as needed, and relevant data sheets should be printed.

Field Methods

Soil cores should be collected at ten equally spaced points along a subset of the 25 m vegetation transects (see Vegetation Cover SOP 2013). For a habitat-level assessment, survey a minimum of three transects per habitat. Additionally, transects along high tide (wrack) lines or the edges of salt pans are recommended to characterize the areas of highest biodiversity, as most wetland plant species have positively buoyant seeds (S. Anderson, unpublished data) and will accumulate at hydrological discontinuities such as wracklines and channel bends. Soil cores should be collected during fall (October – December), soon after the first rains of the wet season to capture the seed bank at its peak (S. Anderson, unpublished data).

Specific protocols are as follows:

1. Use the duct tape and permanent pen or paint pen to label every nursery pot with the transect number, replicate number (i.e., 1-10), and date collected.
2. Use the core to collect ten individual cylinders of soil approximately 10 cm deep and 8 cm in diameter from each transect (approximately 2.5 m apart). While 10 cm depths are the goal, shallower cores are acceptable, but should never be less than 5 cm in depth.
3. The soil plug must be extracted from the corer by pushing upwards with the plunger from the bottom. Most seeds are in the upper few millimeters of the soil surface, so care must be taken to avoid pushing seeds into and/or burying seeds under the surface layers of soil.
4. Immediately following collection, place each individual core in a 4" nursery pot filled with approximately one-third steam sterilized soil on the bottom such that the uppermost surface of the core is approximately 0.5 cm below the lip of the pot. Carefully fill excess space around the corners of the pot with sterilized soil so the core cannot shift within the pot. Cores must always maintain their original orientation, with the uppermost soil surface oriented towards the top.

5. Place the nursery pots on the tray for transport to the greenhouse. Take care to position them so that they will not spill upon movement and will retain the original orientation of the soil plug throughout the collection and transport process.
6. Should you need to carry the cores over a great distance to return to your vehicle or if your drive back to the laboratory is over rough ground, it is recommended to sprinkle a light dusting of steam sterilized soil over the surfaces of all seed cores. This should minimize any potential for seeds to “disperse” from one core to another due to unexpected jarring. This light soil covering should generally be washed away with the first irrigation.
7. It is also essential to create at least one “control core” per transect. A control core consists of a pot filled only with sterilized soil (to the same overall height as the seed core pots), and appropriately labeled as “control” with the site and date. These controls should serve to detect contamination, either during transportation or (more commonly) in the greenhouse.

Greenhouse Methods

1. Transport soil cores to a greenhouse, taking care to minimize bumps and disturbances to the cores. While a formal greenhouse is not technically required for this procedure, it is essential to have a location that is well lit and slightly warmer than ambient winter conditions to promote rapid germination/growth. Consistency and control over ambient conditions is recommended (e.g., light, heat). There has been success using plastic-covered sheeted areas in well-lit sun rooms at ranger stations/remote sites. Wherever is chosen, it is essential that the germination location is sealed from external seed sources and wind (to avoid contamination of the cores with ambient seeds).
2. Saturate all the cores with fresh water and make sure cores are watered routinely. Two protocols have been used with similar success. Given available infrastructure, one or the other is likely preferable, but should be applied consistently for each individual project, and if repeated over time.
 - a. Option 1: maintain cores in a large tub (Figure 3A) and fill the tub with approximately 1-2 cm of freshwater daily. Water should wick through the bottom holes of the pots and keep the soil moist. If using this method, you must change the pooled water in the trays once every other week (maintaining approximately 1-2 cm of water throughout the growth period) and mist or spray the cores with fresh water several times a week (once daily is recommended).
 - b. Option 2: mist cores heavily (5-10 minutes) twice a day from overhead sprinklers.
3. Germinated seedlings (Figure 3b) should be counted, identified, and photographed every two weeks for up to three months or until all seedlings are identifiable (e.g., flowering). Many species should germinate quickly and be identifiable within the first several weeks of watering. However, the grasses will be difficult to identify until they have flowered/formed seeds. This may take as long as three months. A core that has no germinated seeds after six weeks may be discarded and scored as zero seedlings. Control cores should be maintained for the duration of the seedling census.
4. Record all counts of species on the greenhouse datasheets (Appendix 3.4B).



Figure 3. Photos of a collection of cores arranged in a watering tub (A) and an individual potted core (B) in a greenhouse.

Data Entry and QAQC Procedures

Data should be entered in the field using the appropriate data sheet (Appendix 3.4B). All required fields should be completed in full, and the data recorder should assign their name at the top of the document(s). Data should be transferred to the appropriate electronic database within three days, and the hard copies filed in labeled binders. Electronic copies of all data should be housed on an in-house dedicated server and backed up to a cloud-based or off-site server nightly. Hard copies should be saved for five years. Electronic copies should be saved indefinitely.

The seed bank protocol requires extensive QAQC procedures to be completed before the data are analyzed. After each greenhouse count has been completed and photographs of all cores have been taken, the lab technician lead should take reference photographs of each species identified. The QA Officer should verify the identification of each species for each count based on these photos and reference materials. Additionally, after every other greenhouse count, it is recommended that photos be taken for each soil core sample from both an oblique overview (including transect number on side of pot; Figure 1) and direct overview (Figure 3b) for future count verification and additional QAQC against the greenhouse datasheet (Appendix 3.4B).

Office QAQC procedures should include creating a spreadsheet tab for each counting event, comparing the species tallies for each count (comparing the tabs of the spreadsheet), locating quantity discrepancies between counting events (referencing previous counts), and verifying against past soil core and voucher photographs. This step should be completed by the QA Officer and should involve cross-checking between the tabs, photographs, and datasheets for each count. If germinated seedlings die before the end of the experiment, they should still be counted in the final tallies for that core.

Quality Assurance and Quality Control (QAQC) procedures should then be conducted on all data. QAQC procedures should be conducted by the QA Officer and include a thorough review of all entries, double checking of all formulas or macros, and a confirmation that all data sheets, Chain-of-Custody forms, and field notes are filed appropriately with electronic back-up copies available. QAQC should verify that the entered data match the hard copies of the field data sheets. Any discrepancies should be corrected, and the initial data entry technician notified.

Data Analyses

After data have been entered, corrections made, and QAQC procedures completed, data can be used in multiple analyses. Seed bank germinated seedlings should be identified to species. Cores can be analyzed by number of germinated seedlings per m² and averaged across each transect or habitat type. Additionally, seed bank vegetation species lists can be compared across habitats or areas.

Additional analyses for species diversity (e.g., the Shannon-Weaver Index) may be conducted.

Health and Safety Precautions

Not applicable.

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APPENDIX 3.4A

	Evaluation Metric	Seed Bank	Notes
	Correlation to L2 CRAM	Attribute 4	----
Personnel Requirements	Specialty Equipment or Clothing Required	Few Specialty Items	Soil, nursery pots, soup cans, plunger
	Ease of Transport (amount or weight of supplies)	Some Items / Moderate	Can get heavy if cores are potted in the field
	Ease of Implementation	Easy	----
	Expertise / Skill Level	Some Technical Knowledge	Familiarity with species identifications is required
	Number of Personnel	2 +	----
	Training Requirements	None	----
	Seasonality of Survey Time	early Fall	Peak of the growing season
	Suggested Frequency	Annual	----
Survey / Data Quality	Type of Output	Numerical	----
	Active or Passive Monitoring Style	Active	----
	Specialty Computer Software Required	No	----
	Availability of Online / External Resources	Some	----
Potential Limitations	Wetland Type Applicability	All	----
	Images or Multi-Media Required	Images Required	----
	Degree of Impact / Disturbance	High Disturbance	Soil disturbance
	Vegetation Height Limitation	No Limitations	----
	Appropriate for Tidal / Wet Habitats	Yes	----
	Tide Height	Low Tide Only	----
	Regional or Broad Implementation *	Infrequently Used	----
	Potential for Hazards / Risk	Low to No Risk	----
Restrictions	Special Status Species; Cultural	Soil disturbance may be a restricted activity within some locations	

* based on monitoring literature review



Standard Operating Procedures (3.5): Vegetation Mapping

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Prepared for the United States Environmental
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Standard Operating Procedures: Vegetation Mapping

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Protocol Suitability Evaluation

A habitat suitability table containing appropriate habitat types (of those evaluated) to implement the vegetation mapping protocol is displayed in Table 1. While tidal channel and mudflat habitats are often unvegetated, their delineated areas can still be incorporated at a habitat-level based on elevation and hydrology characteristics. This protocol is appropriate in any habitat type. A comparative assessment of cost, effort, and data quality are shown in Table 2. A matrix of additional detailed categorical evaluations of the vegetation mapping survey protocol can be found in Appendix 3.5A.

Table 1. Appropriate habitat types to implement vegetation mapping survey protocols.

Survey Protocol	Habitat Types					
	Tidal Channel	Mud/sand flat	Emergent salt marsh	Non-tidal salt marsh	Salt pan	'Degraded' / fill
Vegetation Mapping	X	X	X	X	X	X

Table 2. Categorical assessment of cost/effort and data quality for vegetation mapping survey protocols.

	Evaluation Metric	Vegetation Mapping	Notes
Time / Effort	Office Preparation Time	> 60 minutes	Heads-up digitizing for field verification
	Equipment Construction Time (one time)	Not Applicable	----
	Field Time	Multiple days	Dependent on the complexity and size of the site
	Laboratory Time (per transect)	Not Applicable	----
	Post-Survey Processing / QAQC Time	> 60 minutes	Digitizing maps; very time-intensive, depending on the complexity and size of the site; multiple office days
	Minimum Repetition (site-dependent)	Many Repetitions	Dependent on required level of resolution (more complex areas will require more polygons)
	Relative Cost (equipment and supplies)	> \$100	Specialty digitizing software and a high-resolution GPS is required, and price is variable; see Appendix 3.5A
Survey / Data Quality	Accuracy (at a survey area level)	Low to High	Dependent on required level of resolution and survey effort
	Precision (at a survey area level)	Medium	Precision increases in survey areas with well-defined edges
	Qualitative-Quantitative Score	Qualitative and Quantitative	----
	Subjectivity-Objectivity Score	Subjective	Vegetation alliances and associations are partially based on percent cover estimates

Resulting Data Types

The application of the vegetation mapping survey protocol yields both qualitative and quantitative data displayed as vegetation alliance, association, and habitat category polygons for an entire site. Data visualization outputs in the form of polygons on a map are typically represented qualitatively in categories specified by the user; however, these data can be extracted for quantitative analyses (e.g., acres of individual species, percent cover of exotic vs. native, or acres of habitat type). Current Geographic Information Systems (GIS) such as ESRI ArcPro have functionality that allows users to

perform a variety of quantitative analyses within the software and represent these data in maps (e.g., percent cover by species). These data can be a useful foundation for designing a habitat-based monitoring or restoration plan. Additionally, vegetation mapping data can help identify large-scale temporal vegetation changes to inform adaptive management for problematic or aggressive non-native species.

Objective

The composition and distribution of vegetation species across wetland habitats directly affects many ecosystem functions such as productivity, soil composition, and nitrogen and carbon exchange dynamics (Schwartz et al. 2000, Keer and Zedler 2002). Additionally, the presence and structure of various plant species may serve as a reliable indicator for several biological and physical conditions such as wildlife and invertebrate populations, soil characteristics, and hydrologic regimes (De Boer 1983). As the primary connection between physical factors and biological activity, vegetation responses to impacts and stressors over time and space have become an essential component to effective environmental management and conservation.

The development of high-resolution, standardized vegetation mapping methods by the California Department of Fish and Wildlife (CDFW) Vegetation Classification and Mapping Program (VegCAMP) (CDFW 2014) has increased the accuracy and comparability of vegetation maps across the state of California (<https://wildlife.ca.gov/Data/VegCAMP>). This SOP is based on the VegCAMP methodology and outlines a synthesized vegetation stand delineation strategy based on a combination of aerial imagery, office-based digitization in a mapping software program (e.g., ArcGIS, QGIS, Grass GIS), and *in situ* field verification. Vegetation mapping methods employ “A Manual of California Vegetation Second Edition” (Sawyer et al., 2009; ISBN 978-0-943460-49-9) (Figure 1) as the standard for classification and delineation of most native and many non-native vegetation alliances and associations based on the presence and relative cover of co-dominant species. An updated version of the Manual can also be found through the California Native Plant Society online at: <https://vegetation.cnps.org/>. Vegetation communities may be further grouped into distinct habitat categories to allow for broader analyses of condition and function. In the case of unique or transitional communities not currently recognized by Sawyer et al. (2009), or where no single species is dominant, the methodology can be used to designate new community alliances.

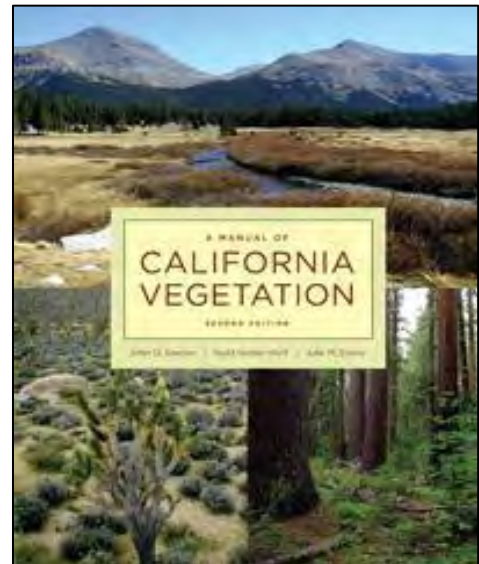


Figure 1. Cover of the *Manual of California Vegetation, 2nd ed.*

As a general note, vegetation mapping is a very time- and labor-intensive survey but yields a robust foundational product capable of informing monitoring plans, small- or large-scale restoration designs, habitat assessments, wetland delineations, and special status species surveys. Survey durations may

range from a few days to crossing several years, depending on the size of the mapped area and the level of resolution desired. When determining the level of resolution desired to implement the vegetation mapping SOP, considerations like monitoring objectives, extent of site, resources available, and/or other supplementary protocols used (i.e., vegetation transect cover) are among examples that should be considered. In particular, resolution and effort will vary based on monitoring goals and objectives. This SOP assumes the user has an informed background in GIS, global positioning systems (GPS), and vegetation identification and surveying; therefore, it is recommended for individuals to attend preparatory classes or workshops before attempting to implement this protocol. A course offered by the California Native Plant Society (CNPS) is recommended (<http://www.cnps.org/cnps/vegetation/workshops.php>).

Equipment

Equipment and supplies needed for this survey include:

1. High resolution aerial imagery (Figure 2). Recently, higher resolution aerial image capture services utilizing drones (e.g., Airphrame) have become more cost efficient and can dramatically shorten survey time/effort. See *Helpful Hint* below.
2. Geographic information system software (e.g., ArcGIS / ArcPro, QGIS, Grass GIS, others)
3. CNPS Relevé and Rapid Assessment Protocols and Datasheets (Appendix 3.5B)
4. GPS or GPS enabled field tablet (mapping grade preferred) with metadata collection functionality or smart forms (e.g., Trimble Terra Sync, Trimble connect, Survey 123, etc.)
5. Camera with GPS capability. On smartphones, the Solocator app is an affordable alternative to GPS-capable camera.
6. “A Manual of California Vegetation” (Sawyer et al., 2009) (MCV)
7. Additional vegetation references [e.g., “Jepson Manual”, “Terrestrial Vegetation of California” (bringing books into the field is optional, but they are recommended office references)]
8. Pre-existing knowledge of local plant species and vegetation cover surveys
9. Additional site-specific background maps [e.g., soils, past vegetation, inundation, elevation, surrounding land use (recommended)]
10. Printed spreadsheet of polygon and attributes (optional) (Example in Appendix 3.5C)

Helpful Hint: High resolution aerial imagery (e.g., ESRI World Imagery, Google maps, and Bing maps) may be sufficient depending on project scope and GIS software. NOAA Coastal Imagery, drone collected imagery, and/or City/County Imagery, if available, may provide higher resolution aerial imagery in some circumstances. When conducting multiple surveys over time for the same area, it is recommended to use aerial imagery from the same source or ensure that the datum and projection of the datasets are comparable.

Helpful Hint: Many mapping software options are available, including, but not limited to pay options (e.g., ArcGIS Pro) and open source options (e.g., QGIS, Grass GIS, others).



Figure 2. High resolution image of the Ballona Wetlands Ecological Reserve, Los Angeles, CA (credit: ESRI).

Field Preparation

Initial office preparation should include the creation of a geodatabase in a projected coordinate system equipped with a non-overlapping, gapless polygon topology. Multiple tools in ESRI ArcGIS and ArcGIS Pro allow for streamlining and automating topology processes. The database should be developed with an attribute table listing all desired attributes to be recorded for each vegetation stand. Required attributes should include vegetation name (alliance or association according to MCV Volume 2), a unique identification number, and estimates of percent cover ranges for general vegetation parameters which may include total vegetated cover, native cover, nonnative cover, tree cover, shrub cover, and herb cover (Figure 3). Other attributes may be collected on a project dependent basis including estimated cover of specific plant species, hydrology, impacts, soil type, and/or land use.

FID	VEGNAME	HABTYPE	HEIGHT	SHRUBCOV	HERBCOV	NATIVE	NON_NATIVE	BAREGROUND
0	Salix lasiolepis alliance	Riparian Scrub and Woodland	3	0	3	5	3	0
1	Cortaderia selloana stand	Pampas Grass	2	0	5	0	5	0

Figure 3. Example of attribute table showing general vegetation parameters quantified into cover classes.

Prior to preliminary field investigations, all aerial images should be reviewed in depth, and vegetation stands with distinct aerial signatures digitized in GIS for field verification and classification (Figure 4). Variations between aerial signatures should be evaluated against multiple criteria including photo attributes such as color, texture, shadows, shape, uniformity, and local site characteristics such as surrounding vegetation, elevation, soil type, and hydrology. Additional site-specific characteristics may also be helpful in discerning differences between vegetation stands. Digital elevation models or other topographic maps may be useful to cross-reference. Depending on project scope, available resources, accessibility, and property size, it may be necessary to assign polygon attributes remotely with selective field validation or *in situ* attribute data collection, but it is recommended to collect on-site data whenever possible. Following office review, maps for each digitized area should either be printed with a supplementary spreadsheet (Appendices 3.5C and D) listing all polygons and attributes for field data collection, or maps should be loaded as a data file into a GPS with data dictionary capabilities. It may also be beneficial to load the digitized map as a background image into the GPS unit to aid with orientation. An increasingly popular alternative to a standard GPS unit is a field-rugged tablet with GPS enabled, or even a standard smartphone with a camera application. Digital maps and backgrounds can be transferred using software tools on most GPS data collection devices.



Figure 4. Example of an aerial image before (A) and after digitization (B).

Equipment described above should be collected prior to the field shift. Batteries for all electronic devices should be checked and recharged as needed, and relevant data sheets should be printed and attached to the clipboards.

Helpful Hint: External power supplies and/or extra fully-charged batteries can extend battery life in the field. Enabling GPS, especially on multi-function devices or smart phones, can quickly drain battery life.

Field Methods

The field verification methods of the vegetation mapping process are designed to provide attribute data for individual vegetation stands, identify the borders of remotely indiscernible vegetation alliances, collect any required species-specific data, and generally validate the accuracy of mapping efforts. Accurate vegetation cover data collected *in situ* are essential to properly classify vegetation alliances and associations in accordance with MCV standards. Depending on the project, there are a range of options for gathering the diversity of plant cover data within vegetation stands which vary in level of

detail and time required; however, most mapping projects should use a combination of methods for highest quality results. Vegetation cover assessment method options are listed below in order from the least detailed and most rapid to the most detailed, requiring the greatest time investment. In all methods, geotagged photos should be collected as often as possible to provide an additional reference source for map updates. See Table 3 for a categorical comparison of each method.

Table 3. Comparison of vegetation sampling methods to quantify attribute data for vegetation polygons.

Survey Type	Time / Labor Requirement	Observer Effect / Margin of Error	Data Resolution	Experience Required
Visual Percent Cover Estimates	Low	Diverse Stands: High Monospecific Stands: Low	Low	Low
CNPS / DFW Relevé and Rapid Assessments	Moderate	Moderate	Moderate	Moderate
Level 3 Vegetation Surveys	High	Low	High	Moderate

1. **Visual Percent Cover Estimates** – This method consists of rapid, walk through cover-class estimates for each vegetation attribute (e.g., total cover, percent non-native) of an entire vegetation stand. Unless a project has a large budget and requires an extremely high level of accuracy, the attribute data for most stands should be collected using this method to minimize costs and time required. Visual estimates should be entered either into Appendix 3.5C or the GPS data dictionary.

Due to the highly subjective nature of this method, its use is not recommended without extensive experience calibrating estimating abilities with more detailed methods (e.g., vegetation cover surveys, see SOP 3.2). It is also important to conduct calibration exercises with all field team members to minimize observer error. See Daubenmire 1959 for basic outline regarding techniques to estimate vegetative cover.

2. **CNPS / DFW Relevé and Rapid Assessment Surveys** – These methods are most useful when the cover of a stand is ambiguous, or finer scale detail and higher quality is desired or required for a particular area. For large areas when it is infeasible to visit the entire site, it may be beneficial to identify the most common, distinct, or characteristic vegetation signatures and perform a more formal survey of the stand’s vegetative cover. Additionally, if available resources have limited the majority of the mapping effort to office digitization and remote estimates, it may be necessary to validate estimates with more detailed assessments of some vegetation stands. In general, the Relevé surveys are plot-based as opposed to being based on the entire stand (Rapid Assessment Surveys) and may be simpler for larger vegetation stands. Detailed protocols and datasheets for each survey are found in Appendix 3.5B or through the following link: <https://wildlife.ca.gov/Data/VegCAMP/Publications-And-Protocols>. Additional information on Relevé and Rapid Assessment Survey workshops and trainings can be found at this link: <http://www.cnps.org/cnps/vegetation/workshops.php>.

3. **Level 3 Vegetation SOP surveys** – While a vegetation map may provide a site-wide snapshot of the major vegetation alliances, many site-intensive monitoring programs also incorporate quantitative fine scale vegetation surveys. SOP 3.2 – Vegetation Cover Surveys – describes each of the vegetation cover surveys in detail. These surveys are the most time intensive but yield the highest quality data and may be helpful when used in conjunction with classifying vegetation alliances. Typically, unless required for independent vegetation monitoring purposes, the higher-intensity monitoring protocols are not used for vegetation mapping as they are too resource intensive. Additional field methods include the delineation of remotely indistinct or difficult to discern vegetation stands (Figure 5). Some stands or species may be very similar in appearance within an aerial image, especially if the image is taken outside of the growing season for the species; therefore, it is necessary to verify the boundary in the field. This process is achieved by simply walking the boundary between vegetation stands with a GPS and incorporating the track into the vegetation map via GIS. Additionally, advanced aerial image analysts may be able to distinguish differences in vegetation communities based on the presence of the C3, C4, and CAM photosynthesis in infrared images. For more obvious transitions, polygon transitions may be hand drawn onto the field map, attribute data recorded in Appendix 3.5C, and subsequently transcribed into GIS. Areas dominated by herbaceous and/or annual species may require field verification over multiple seasons or years.



Figure 5. Example of vegetation stands with indistinct or difficult aerial signatures (A) and the delineation resulting from walking transition boundaries (B).

Laboratory Methods

Not Applicable.

Data Entry and QA/QC Procedures

Data should be entered in the field using the appropriate data sheet (Appendix 3.5C) or GPS data dictionary. All required fields should be completed in full, and the data recorder should assign their name at the top of the document or in file name. Data should be uploaded, transcribed, and/or digitized into the appropriate geodatabase within 24 hours, and if applicable, the hard copies filed in labeled binders. It is also recommended to scan and store copies of raw data sheets electronically. Electronic copies of all data and digitized maps should be housed on an in-house dedicated server and

backed up to a cloud-based or off-site server. Hard copies should be saved for five years. Electronic copies should be saved indefinitely.

Quality Assurance and Quality Control (QAQC) procedures should be conducted on all data. QAQC procedures should be conducted by the QA Officer and include a thorough review of all entries, double checking of all formulas or macros, and a confirmation that all data sheets, and field notes are filed appropriately with electronic back-up copies available. QAQC should verify that the entered data match the hard copies of the field data sheets. Any discrepancies should be corrected, and the initial data entry technician notified.

Additional QAQC for remote-attributed vegetation stands should involve *in situ* verification of vegetation classification utilizing either the Relevé or Rapid Assessment Survey methodologies. Target accuracies for remote attributed data should be 80% or greater. Data not meeting these standards should be re-assessed until the minimum accuracy threshold is met and the GIS and field technician notified.

Data Analyses

After data have been entered, corrections made, and QAQC procedures completed, final vegetation maps may be used independently in multiple analyses or as a base for management and conservation decisions. Some basic analyses include tables identifying acreage by nativity, habitat, or alliance / association by area, then exporting maps of habitat types within a site and the associated table listing the area by habitat type (e.g., Figure 6). More complex analyses assessing temporal variations are possible if vegetation has been mapped for multiple years including maps comparing habitat change by location over time, maps tracking the invasion of non-native species over time (e.g., Figure 7), and graphs showing change in acreage by habitat (e.g., Figure 8) or by vegetation species. However, the applications for vegetation map data are far reaching and include the identification of locations for rare plant conservation, alliances which support special status wildlife, habitat modeling to predict special status species populations, disease probability maps, climate change response scenarios, and the identification of high priority conservation areas (CDFW 2014).

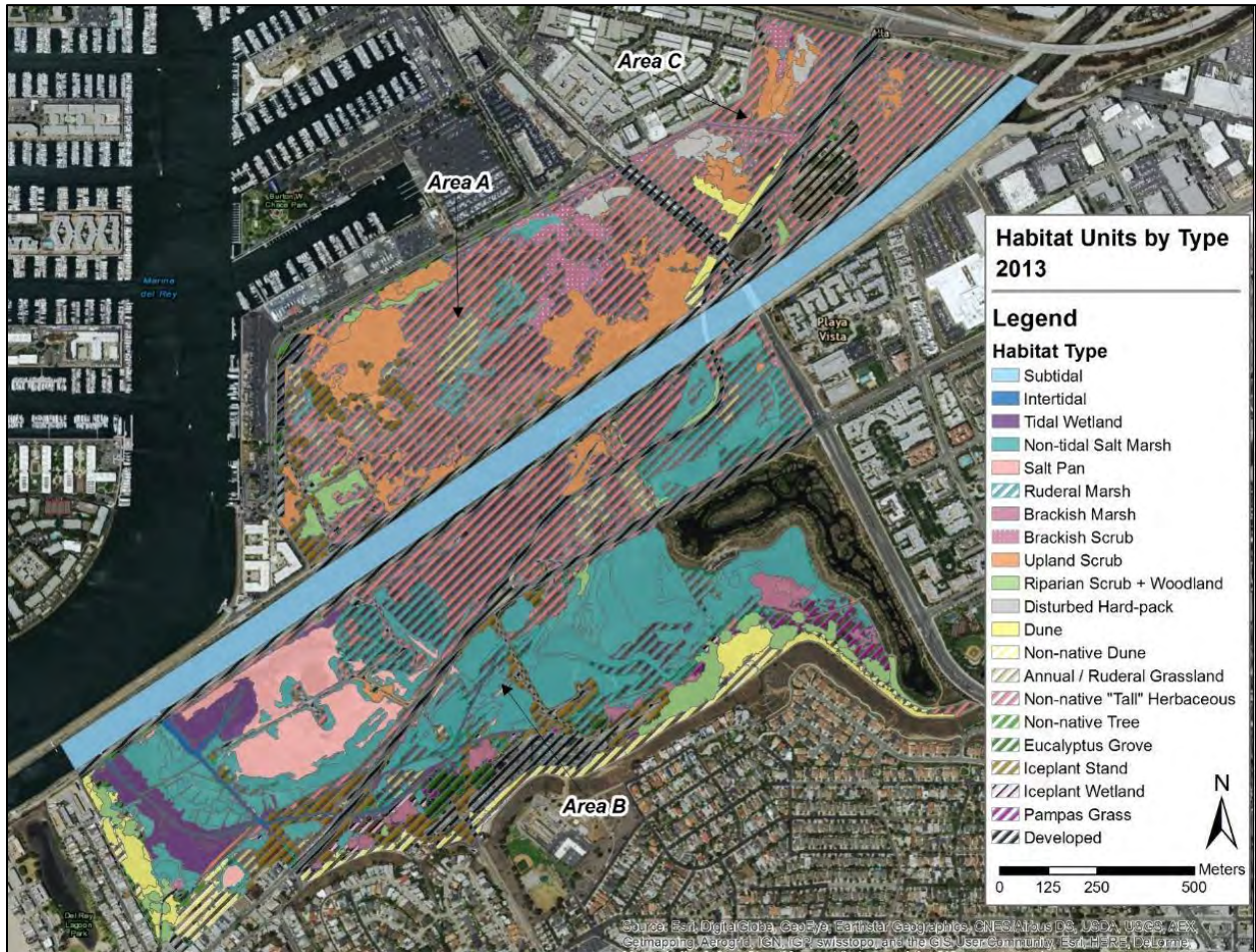


Figure 6. Habitat map (2013) of the Ballona Wetlands Ecological Reserve (replicated from Johnston et al. 2015).



Figure 7. Map depicting *Brassica nigra* change over time (replicated from Johnston et al. 2015).

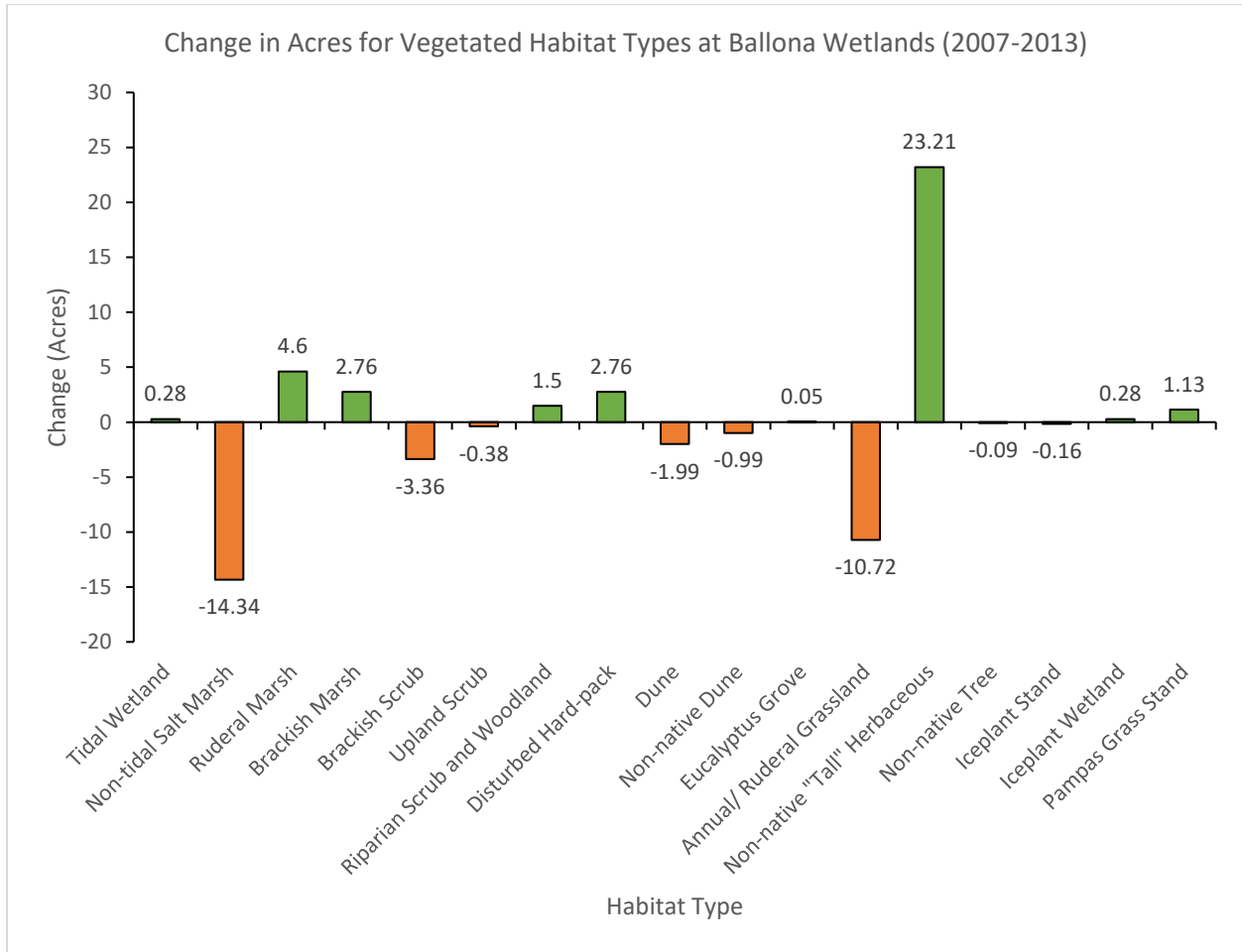


Figure 8. Chart displaying the habitat change in acres between 2007 and 2013 in the Ballona Wetlands Ecological Reserve (replicated from Johnston et al. 2015).

Health and Safety Precautions

Field team members should exercise caution in addition to standard field safety protocols when conducting surveys in remote study areas or those not frequently accessed. Considerations such as difficulty accessing the survey area, difficulty navigating in the survey area, and unhoused people should be made during field preparation.

Applicable Literature

- Baldwin, B.G., Goldman D.H., Keil, D.J., Patterson, R., Rosatti, T.J., Wilken, D.H., eds. *The Jepson Manual: Vascular Plants of California* 2nd ed. University of California Press: Berkeley, CA. 1568 pp.
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- (CDFW) California Department of Fish and Wildlife. "The Vegetation Classification and Mapping Program." Accessed June 2014. <http://www.dfg.ca.gov/biogeodata/vegcamp/>
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- Johnston, K.K., I.D. Medel, R.C. Abbott, M.W. Grubbs, E. Del Giudice-Tuttle, C. Piechowski, M. Wong Yau, and J. Dorsey. 2015. "Ballona Wetlands Ecological Reserve: Comprehensive 5-Year Monitoring Report." Report prepared by The Bay Foundation for the California State Coastal Conservancy. 193 pp.
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Contact Information

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APPENDIX 3.5A

	Evaluation Metric	Seed Bank	Notes
	Correlation to L2 CRAM	Attribute 4	----
Personnel Requirements	Specialty Equipment or Clothing Required	Few Specialty Items	Sub-meter GPS is recommended. Camera or smartphone application with GPS capabilities is also recommended.
	Ease of Transport (amount or weight of supplies)	Few Items / Easy	----
	Ease of Implementation	Difficult	----
	Expertise / Skill Level	High Technical Knowledge	Familiarity with species identifications and Geographic Information Systems software is required
	Number of Personnel	2	Multiple teams of people surveying different areas may reduce survey times
	Training Requirements	Specific Training recommended	Courses are offered through CNPS. Additional information may be found here: http://www.cnps.org/cnps/education/workshops/index.php
	Seasonality of Survey Time	early Fall	Peak of the growing season (will vary if habitats are not coastal wetland)
	Suggested Frequency	Every 5 years	Dependent on monitoring program objectives
Survey / Data Quality	Type of Output	Numerical and Non-numerical	Areas will be categorized into non-numerical vegetation categories but may be analyzed for numerical areas
	Active or Passive Monitoring Style	Passive	----
	Specialty Computer Software Required	Yes	GIS computer software (both pay and open source options available); GIS data collection software recommended (ESRI Survey123, Trimble TerraSync, Trimble Connect, etc.)
	Availability of Online / External Resources	Many resources	Resources are available mainly for the identification of vegetation alliance and association categories. Fewer methodological resources are available
Potential Limitations	Wetland Type Applicability	All	----
	Images or Multi-Media Required	Images Required	----
	Degree of Impact / Disturbance	Low Disturbance	----
	Vegetation Height Limitation	No Limitations	----
	Appropriate for Tidal / Wet Habitats	Yes	----
	Tide Height	Low Tide Only	Submersion of low-lying vegetation will reduce accuracy of cover estimates
	Regional or Broad Implementation *	Frequently Used	----
	Potential for Hazards / Risk	Low to No Risk	----
	Restrictions	Special Status Species	----

* based on monitoring literature review

Appendix 3.5B (CNPS 2007)

CALIFORNIA NATIVE PLANT SOCIETY RELEVÉ PROTOCOL CNPS VEGETATION COMMITTEE October 20, 2000 (Revised 8/23/2007)

Introduction

In *A Manual of California Vegetation* (Sawyer and Keeler-Wolf 1995), CNPS published a Vegetation Sampling Protocol that was developed as a simple quantitative sampling technique applicable to many vegetation types in California. Investigators use an ocular estimation technique called a relevé to classify and map large areas in a limited amount of time.

The relevé method of sampling vegetation was developed in Europe and was largely standardized by the Swiss ecologist Josias Braun-Blanquet. He helped classify much of Europe's vegetation, founded and directed a synecology center in France, and was editor of *Vegetatio* for many years. The relevé was, and is, a method used by many European ecologists, and others around the world. These ecologists refer to themselves as phytosociologists. The use of relevé in the United States has not been extensive with the exception of the US Forest Service.

The relevé is particularly useful when observers are trying to quickly classify the range of diversity of plant cover over large units of land. In general, it is faster than the point intercept technique. One would use this method when developing a classification that could be used to map of a large area of vegetation, for example. This method may also be more useful than the line intercept method when one is trying to validate the accuracy of mapping efforts.

The relevé is generally considered a “semiquantitative” method. It relies on ocular estimates of plant cover rather than on counts of the “hits” of a particular species along a transect line or on precise measurements of cover/biomass by planimetric or weighing techniques.

Selecting a stand to sample:

A stand is the basic physical unit of vegetation in a landscape. It has no set size. Some vegetation stands are very small, such as alpine meadow or tundra types, and some may be several square kilometers in size, such as desert or forest types. A stand is defined by two main unifying characteristics:

- 1) It has compositional integrity. Throughout the site the combination of species is similar. The stand is differentiated from adjacent stands by a discernable boundary that may be abrupt or indistinct, and
- 2) It has structural integrity. It has a similar history or environmental setting that affords relatively similar horizontal and vertical spacing of plant species throughout. For example, a hillside forest originally dominated by the same species that burned on the upper part of the slopes, but not the lower, would be divided into two stands. Likewise, a sparse woodland occupying a slope with very shallow rocky soils would be considered a different stand from an adjacent slope with deeper, moister soil and a denser woodland or forest of the same species.

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The structural and compositional features of a stand are often combined into a term called homogeneity. For an area of vegetated ground to meet the requirements of a stand it must be homogeneous.

Stands to be sampled may be selected by assessment prior to a site visit (e.g. delineated from aerial photos or satellite images), or may be selected on site (during reconnaissance to determine extent and boundaries, location of other similar stands, etc.). Depending on the project goals, you may want to select just one or a few representative stands for sampling (e.g., for developing a classification for a vegetation mapping project), or you may want to sample all of them (e.g., to define a rare vegetation type and/or compare site quality between the few remaining stands).

Selecting a plot to sample within in a stand:

Because most stands are large, it is difficult to summarize the species composition, cover, and structure of an entire stand. We are also usually trying to capture the most information with the least amount of effort. Thus, we are typically forced to select a representative portion to sample.

When sampling a vegetation stand, the main point to remember is to select a sample that, in as many ways possible, is representative of that stand. This means that you are not randomly selecting a plot; on the contrary, you are actively using your own best judgement to find a representative example of the stand.

Selecting a plot requires that you see enough of the stand you are sampling to feel comfortable in choosing a representative plot location. Take a brief walk through the stand and look for variations in species composition and in stand structure. In many cases in hilly or mountainous terrain look for a vantage point from which you can get a representative view of the whole stand. Variations in vegetation that are repeated throughout the stand should be included in your plot. Once you assess the variation within the stand, attempt to find an area that captures the stand's common species composition and structural condition to sample.

Plot Size

All relevés of the same type of vegetation to be analyzed in a study need to be the same size. It wouldn't be fair, for example, to compare a 100 m² plot with a 1000 m² plot as the difference in number of species may be due to the size of the plot, not a difference in the stands.

A minimal area to sample is defined by species/area relationships; as the sampler identifies species present in an area of homogeneous vegetation, the number will increase quickly as more area is surveyed. Plot shape and size are somewhat dependent on the type of vegetation under study. Therefore general guidelines for plot sizes of tree-, shrub-, and herb-dominated upland, and fine-scale herbaceous communities have been established. Sufficient work has been done in temperate vegetation to be confident the following conventions will capture species richness:

- Alpine meadow and montane wet meadow: 100 sq. m
- Herbaceous communities: 10 sq. m plot, 100 sq. m plot or 400 sq. m plot (Consult with CNPS, and use one consistent size)
- Shrublands: 400 sq. m plot
- Forest and woodland communities: 1000 sq. m plot
- Open desert vegetation: 1000 sq. m plot

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Plot Shape

A relevé has no fixed shape, plot shape should reflect the character of the stand. If the stand is about the same size as a relevé, you need to sample the entire stand. If we are sampling a desert wash, streamside riparian, or other linear community our plot dimensions should not go beyond the community's natural ecological boundaries. Thus, a relatively long, narrow plot capturing the vegetation within the stand, but not outside it would be appropriate. Species present along the edges of the plot that are clearly part of the adjacent stand should be excluded.

If we are sampling broad homogeneous stands, we would most likely choose a shape such as a circle (which has the advantage of the edges being equidistant to the center point) or a square (which can be quickly laid out using perpendicular tapes). If we are trying to capture a minor bit of variety in the understory of a forest, for example a bracken fern patch within a ponderosa pine stand, we would want both bracken and non-bracken understory. Thus, a rectangular shape would be appropriate.

GENERAL PLOT INFORMATION

The following items appear on each data sheet and are to be collected for all plots. Where indicated, refer to attached code sheet.

Polygon or Relevé number: Assigned either in the field or in the office prior to sampling.

Date: Date of sampling.

County: County in which located.

USGS Quad: The name of the USGS map the relevé is located on; note series (15' or 7.5').

CNPS Chapter: CNPS chapter, or other organization or agency if source is other than CNPS chapter.

Landowner: Name of landowner or agency acronym if known. Otherwise, list as private.

Contact Person: Name, address, and phone number of individual responsible for data collection.

Observers: Names of individuals assisting. Circle name of recorder.

Plot shape: indicate the sample shape as: square, rectangle, circle, or the entire stand.

Plot size: length of rectangle edges, circle radius, or size of entire stand. NOTE: See page 2 for standard plot sizes.

Study Plot Revisit: If the relevé plot is being revisited for repeated sampling, please circle "Yes".

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Photo interpreter community code: If the sample is in area for which delineation and photo interpretation has already been done, the code which the photointerpreters applied to the polygon. If the sample site has not been photointerpreted, leave blank.

Other polygons of same type (yes or no, if applicable), if yes, mark on map: Other areas within view that appear to have similar vegetation composition. Again, this is most relevant to areas that have been delineated as polygons on aerial photographs as part of a vegetation-mapping project. If one is not working from aerial photographs, draw the areas as on a topographic map.

Is plot representative of whole polygon? (yes or no, if applicable), if no explain: Detail what other vegetation types occur in the polygon, and what the dominant vegetation type is if there is more than one type.

Global Positioning System Readings: Due to the recent availability of very accurate and relatively low cost GPS units, we highly recommend obtaining and using these as a standard piece of sampling equipment. Now that the military intentional imprecision (known as “selective availability”) has been “turned off” (as of July 2000), it is typical for all commercial GPS units these units to be accurate to within 5 m of the actual location. Also note that the GPS units can be set to read in UTM or Latitude and Longitude coordinates and can be easily translated. Thus, the following fields for Latitude, Longitude, and legal description are now optional. In order for all positional data to be comparable within the CNPS vegetation dataset, we request using UTM coordinates set for the NAD 83 projection (see your GPS users manual for instructions for setting coordinates and projections).

Caveat: Although GPS units are valuable tools, they may not function properly due to the occasionally poor alignment of satellites or due to the complexity of certain types of terrain, or vegetation. We thus also recommend that you carry topographic maps and are aware of how to note your position on them in the event of a non-responsive or inaccurate GPS.

UTMN and UTME: Northing and easting coordinates using the Universal Transverse Mercator (UTM) grid as delineated on the USGS topographic map, or using a Global Positioning System.

UTM zone: Universal Transverse Mercator zone. Zone 10S for California west of the 120th longitude; zone 11S for California east of 120th longitude.

Legal Description: Township/Range/Section/Quarter Section/Quarter-Quarter section/Meridian: Legal map location of the site; this is useful for determining ownership of the property. California Meridians are Humboldt, Mt. Diablo, or San Bernardino. (This is optional, see above discussion of GPS units)

Latitude and Longitude: Degrees north latitude and east longitude. This is optional (see above)

Elevation: Recorded in feet or meters. Please indicate units.

Slope: Degrees, read from clinometer or compass, or estimated; averaged over relevé

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Aspect: Degrees from true north (adjust declination), read from a compass or estimated; averaged over relevé.

Macrotopography: Characterize the large-scale topographic position of the relevé. This is the general position of the sample along major topographic features of the area. *See attached code list.*

Microtopography: Characterize the local relief of the relevé. Choose the shape that mimics the lay of the ground along minor topographic features of the area actually within the sample. *See attached code list.*

VEGETATION DESCRIPTION

Dominant layer: Indicate whether the community is dominated by the Low layer (L), Mid-layer (M), or Tall (T) layer.

Preliminary Alliance name: Name of series, stand, or habitat according to CNPS classification (per Sawyer and Keeler-Wolf 1995); if the type is not defined by the CNPS classification, note this in the space.

Dominant Vegetation Group: Use code list to choose group

Phenology: Based on the vegetative condition of the principal species, characterize the phenology of each layer as early (E), peak (P), or late (L).

WETLAND COMMUNITY TYPES

Community type: Indicate if the sample is in a wetland or an upland; note that a site need not be officially delineated as a wetland to qualify as such in this context.

Dominant vegetation form: This is a four letter code which relates the vegetation of the plot to the higher levels of the NBS/NPS National Vegetation Classification System hierarchy. *See attached code list.*

Cowardin class: See “Artificial Keys to Cowardin Systems and Names” (attached). If the plot is located in a wetland, record the proper Cowardin system name. Systems are described in detail in Cowardin et al. 1979. Classification of wetlands and deepwater habitats of the United States. US Dept. of the Interior, Fish and Wildlife Service, Office of Biological Services, Washington, D.C.

Marine: habitats exposed to the waves and currents of the open ocean (subtidal and intertidal habitats).

Estuarine: includes deepwater tidal habitats and adjacent tidal wetlands that are usually semi-enclosed by land but have open, partly obstructed, or sporadic access to the open ocean, and in which ocean water is at least occasionally diluted by freshwater runoff from the land (i.e. estuaries and lagoons).

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Riverine: includes all wetlands and deepwater habitats contained within a channel, excluding any wetland dominated by trees, shrubs, persistent emergent plants, emergent mosses, or lichens. Channels that contain oceanic-derived salts greater than 0.5% are also excluded.

Lacustrine: Includes wetlands and deepwater habitats with all of the following characteristics: 1) situated in a topographic depression or a dammed river channel; 2) lacking trees or shrubs, persistent emergents, emergent mosses or lichens with greater than 30% aerial coverage; and total area exceeds 8 ha (20 acres). Similar areas less than 8 ha are included in the lacustrine system if an active wave-formed or bedrock shoreline feature makes up all or part of the low tide boundary, or if the water in the deepest part of the basin exceeds 2 m (6.6 feet) at low tide. Oceanic derived salinity is always less than 0.5%.

Palustrine: Includes all nontidal wetlands dominated by trees, shrubs, persistent emergents, emergent mosses or lichens, and all such wetlands that occur in tidal areas where salinity derived from oceanic salts is less than 0.5%. Also included are areas lacking vegetation, but with all of the following four characteristics: 1) areas less than 8 ha (20 acres); active wave-formed or bedrock shoreline features lacking; 3) water depth in the deepest part of the basin less than 2 m (6.6 feet) at low water; and 4) salinity due to ocean-derived salts less than 0.5%.

Vertical distance from high water mark of active stream channel: If the plot is in or near a wetland community, record to the nearest meter or foot the estimated vertical distance from the middle of the plot to the average water line of the channel, basin, or other body of water.

Horizontal distance from high water mark of active stream channel: If the plot is in or near a wetland community, record to the nearest meter or foot the estimated horizontal distance from the middle of the plot to the average water line of the channel, basin, or other body of water.

Stream channel form: If the plot is located in or near a community along a stream, river, or dry wash, record the channel form of the waterway. The channel form is considered S (single channeled) if it consists of predominately a single primary channel, M (meandering) if it is a meandering channel, and B (braided) if it consists of multiple channels interwoven or braided.

Adjacent alliance: Adjacent vegetation series, stands or habitats according to CNPS classification; list in order of most extensive to least extensive. Give the name of the alliance, the direction in relation to stand and list up to four species under Description.

Photographs: Write the name or initials of the camera owner and the JPEG numbers for photos taken. Write the camera's view direction from compass bearings. Take four or eight photos (depending on the project) from the same point as the GPS reading (center of a circle or NW corner of rectangle). Using a compass, take the first photo from the north, and rotate clockwise, taking the photos in sequence, N, NE, E etc, or N, E, S, W. Keep camera at same orientation, zoom level, and distance from ground for all four (or eight) photos., You may take photos close to the ground, if for instance, you are photographing a low herbaceous stand. Additional photos of the stand may also be helpful. If using a digital camera or scanning in the image into a computer, relevé numbers and compass directions can be recorded digitally. If using a 35mm camera, please note the roll number, frame number, compass direction, and the initials of the person whose camera is being used. (e.g. Roll 5, #1, to the NW, SE)

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STAND AND ENVIRONMENTAL INFORMATION

Vegetation trend: Based on the regenerating species and relationship to surrounding vegetation, characterize the stand as either increasing (expanding), stable, decreasing, fluctuating, or unknown.

Impacts: Enter codes for potential or existing impacts on the stability of the plant community. Characterize each as either 1. Light, 2. Moderate, or 3. Heavy. *See attached code list.*

Site location and plot description: A concise, but careful description that makes locating and/or revisiting the vegetation stand and plots possible; give landmarks and directions. Used in conjunction with the GPS position recorded earlier, this should enable precise re-location of the plot. Indicate where the GPS reading was taken within the plot. In general, the location of the GPS reading should be on the Northwestern corner of the plot, if the plot is rectangular (or square), or in the center if the plot is circular. It is also helpful to briefly describe the topography, aspect, and vegetation structure of the site. If you can't take the GPS reading at the Northwest corner (an obstacle in the way) then note where the GPS point was taken. If you can't get a GPS reading, then spend extra time marking the plot location as precise as possible on a topo map.

Site history: Briefly describe the history of the stand, including type and year of disturbance (e.g. fire, landslides or avalanching, drought, flood, or pest outbreak). Also note the nature and extent of land use such as grazing, timber harvest, or mining.

Unknown plant specimens: List the numbers of any unknown plant specimens, noting any information such as family or genus (if known), important characters, and whether or not there is adequate material for identification. Do not take samples of plants of which there are only a few individuals or which you think may be rare. Document these plants with photographs.

Additional comments: Feel free to note any additional observations of the site, or deviations from the standard sampling protocol. If additional data were recorded, e.g. if tree diameters were measured, please indicate so here.

SURFACE COVER AND SOIL INFORMATION

Surface cover: Estimate the cover class of each size at or near the ground surface averaged over the plot. Always remember to estimate what you actually see on the surface as opposed to what you think is hiding under, organic litter, big rocks, etc. However, rocks, organic litter, or fine material visible under the canopy of shrubs or trees should be included in the cover estimate.

One way to consider this is to assume that all of the components of surface cover plus the basal cross-section of living plant stems and trunks (at ground level) will add up to 100%. Thus, estimate the cover value of each of the items in the box on the form for surface cover (including the basal area of plant stems) so that they will add up to 100%. Remember that the basal area of plant stems is usually minimal (e.g., if there were 10 trees, each 1 m in diameter at ground level on a 1000 square meter plot, they would cover less than 1% {0.79%} of the plot).

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These data are asked for because certain categories of surface cover of rock and other materials have been shown to correlate with certain vegetation types and are thus likely influencing the type of vegetation that is growing in a given area. These estimates should be made quickly with the main point to keep in mind being a rough estimate of the relative proportions of different coarse fragments on the plot.

Fines: Fine mineral fragments including sand, silt, soil, "dirt" < 2 mm in diameter

Gravel: rounded and angular fragments 0.2-7.5 cm (0.08 -3 in.) diameter

Cobble: rounded and angular fragments >7.5-25 cm (3 -10 in.) in diameter

Stone: rounded and angular coarse fragments >25 cm-60 cm (10 -24 in.) in diameter

Boulder: rounded and angular coarse fragments >60 cm (>24 in.) in diameter

Bedrock: continuous, exposed, non-transported rock

Litter: extent of undecomposed litter on surface of plot (this includes all organic matter, e.g. fallen logs, branches, and twigs down to needles and leaves).

Living stems of vascular plants: basal area of living stems of the plants at ground surface

% Bioturbation: Estimate percent cover of ground disturbance by animals (e.g., small mammal burrowing trails, cow hoof marks) across the entire plot surface.

Soil texture: Record the texture of the upper soil horizon, below the organic layer if one is present. *See attached key and code list.*

Parent Material: Geological parent material of site. *See attached code list.*

VEGETATION DATA

Assessment of Layers

Data are recorded for five layers (tree overstory, tree understory, shrub, herb, and non-vascular). The layer a species occupies is determined by life-form. The estimates need not be overly precise and will vary among vegetation types. A young tree, if shrub sized, is considered an understory tree. A caveat: if several relevés are being sampled within the same vegetation type, it is important to be consistent when assigning layers. Some types will have more than five layers (e.g. two tree layers of different maximum height); this should be indicated in the relevé description.

Species List

The collection of vegetation data continues with making a comprehensive species list of all vascular plants within the relevé. This list is achieved by meandering through the plot to see all

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microhabitats. During list development, observers document each taxon present in each layer in which it occurs separately, recording it on a different line of the data form and noting which layer is represented. This is important for data entry because each layer of each represented taxon will be entered separately. Each individual plant is recorded in only one layer, the layer in which the tallest portion of the individual is found. One should reach a point at which new taxa are added to the list only very slowly, or sporadically. When one has reached that point, the list is probably done.

The following sections explain how to perform the actual relev , the Estimation of Cover Values. The sections prefaced by bold-faced titles explain the technique, and the sections with regular font titles refer to the steps needed to complete the accompanying Field Form.

DBH – see separate field form (optional)

DBH if >10 cm:

The diameter at breast height (dbh) is important in certain studies. It may be recorded next to each tree species name. First indicate the species name by code and then record the number of sprouts/trunks in clonal trees. You should measure the tree dbh of every tree trunk/sprout that has diameter $>$ or $=$ 10 cm at breast height in the plot, and each measurement should be in centimeters (cm) using a dbh tape measure. For trunks that may be fused below breast height and branched at breast height, each trunk at breast height gets a separate measurement.

Also indicate if each tree/clone is in the overstory or understory. Trees in the overstory are generally at canopy level. Trees in the understory are entirely below the general level of the canopy.

If snags are encountered in plot, record the dbh and denote it as dead by circling its dbh measurement. If you are unable to identify the snag to species, put the four letter code “SNAG” in the species column.

Depending on the density of trees in each plot, you can record dbh of trees for every tree trunk in the plot, or you can sub-sample the trunks to estimate dbh for every tree species in relatively dense plots. For woodland/forest plots, sub-sampling is appropriate for half the plot if there are at least 50 trees/resprouts present (e.g., 200 m² sub-sample in riparian and 500 m² sub-sample in upland).

When sub-sampling, make sure to denote this as a sub-sample (note on the data form) and record the sub-sample of dbh’s for each tree species in the appropriate row on the Field Form. Once the data are post-processed and entered into a database, then you will need to record each sub-sampled dbh reading three additional times to come up with a full sample of dbh readings. For example, with a sub-sampled tree dbh of 15 cm, this value of 15 should be entered four times (not just once) when it is entered in the database.

Lifeform and size class: If dbh $<$ 15.2 cm, counts should be made for conifers and hardwoods in two different size classes. Count seedlings (\leq 2.54 cm) and saplings ($>$ 2.54 but $<$ 15.2 cm). First estimate if there are more than 50 seedlings in one half (50% subsample) of the plot. If so, then do counts of seedlings and saplings in five sub-plots of 2x2 m squares. If the plot shape is a

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circle, place one square in the center of the plot, and four other squares 10 m to the N, S, E, and W of the plot center. If there are less than 50 seedlings in the 50% subsample plot, then record counts for that subsample instead.

Estimating Cover:

There are many ways to estimate cover. Many people who have been in the cover estimation “business” for a long time can do so quickly and confidently without any props and devices. However, to a novice, it may seem incomprehensible and foolhardy to stand in a meadow of 50 different species of plants and systematically be able to list by cover value each one without actually “measuring” them in some way.

Of course, our minds make thousands of estimates of various types every week. We trust that estimating plant cover can be done by anyone with an open mind and an “eye for nature.” It’s just another technique to learn.

It is very helpful to work initially with other people who know and are learning the technique. In such a group setting, typically a set of justifications for each person’s estimate is made and a “meeting of the minds” is reached. This consensus approach and the concomitant calibration of each person’s internal scales is a very important part of the training for any cover estimate project.

An underlying point to remember is that estimates must provide some level of reliable values that are within acceptable bounds of accuracy. If we require an accuracy level that is beyond the realm of possibility, we will soon reject the method for one more quantitative and repeatable. As with any scientific measurement, the requirement for accuracy in the vegetation data is closely related to the accuracy of the information needed to provide a useful summary of it. Put into more immediate perspective - **to allow useful and repeatable analysis of vegetation data, one does not need to estimate down to the exact percent value the cover of a given plant species in a given stand.**

This point relates to two facts: there is inherent variability of species cover in any environment. For example, you would not expect to always have 23% *Pinus ponderosa*, 14% *Calocedrus decurrens*, and 11% *Pinus lambertiana* over an understory of 40% *Chamaebatia foliosa*, 3% *Clarkia unguiculata*, and 5% *Galium bolanderi* to define the Ponderosa pine-Incense cedar/mountain misery/bolander bedstraw plant community. Anyone who has looked at plant composition with a discerning eye can see that plants don’t space themselves in an environment by such precise rules. Thus, we can safely estimate the representation of species in a stand by relatively broad cover classes (such as <1%, 1-5 %, 5-25%, etc.) rather than precise percentages.

The data analysis we commonly use to classify vegetation into different associations and series (TWINSPAN and various cluster analysis programs, for example) is likewise forgiving. When analyzed by quantitative multivariate statistics information on species cover responds to coarse differences in cover and presence and absence of species, but not to subtle percentage point differences. This has been proven time and again through quantitative analysis of vegetation classification. Many of the world’s plant ecologists estimate cover rather than measure it precisely. Some of the seminal works in vegetation ecology have been based on cover estimates taken by discerning eyes.

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With this as a preamble, below we offer some suggestions on estimating cover that have proven helpful. These are simply “tricks” to facilitate estimation, some work better for different situations. You may come up with other methods of estimation that may seem more intuitive, and are equally reliable in certain settings. All values on the relevé protocol that require a cover class estimate, including coarse fragment and vegetation layer information, may rely on these techniques. Just make the appropriate substitutions (using the coarse fragment example substitute, bedrock, stone, cobbles, gravel, and litter for vegetation).

Method 1: The invisible point-intercept transect:

This method works well in relatively low, open vegetation types such as grasslands and scrubs where you can see over the major stand components. For those who have worked with the original CNPS line intercept methodology it’s like counting hits along an imaginary line at regular intervals of the 50 m tape. Here’s how it goes:

Envision an imaginary transect line starting from your vantage point and running for 50 m (or however many meters you wish, as long as you are still ending up within the same stand of vegetation you’re sampling - never keep counting outside of your homogeneous stand). Now “walk” your eye along this tape for 50 m and visually “take a point” every 0.5 m. Don’t worry about precision, just try to “walk” your eye along the line and stop every 0.5 m or at any other regular interval until you reach its end and mentally tally what species you hit. Once you come up with a number of hits for each major species in one imaginary transect, take another transect in another direction and estimate the number of hits on that one. Do this several times (usually 3-4 is enough if you are in a homogeneous stand), then average your results.

This can go quickly in simple environments and in environments where the major species are easily discernable (chaparral, bunch-grassland, coastal scrub, desert scrub). Your average number of hits need not be a total of 100 as in the original transect method, but could be 50 along a 25 m imaginary line (in which case you would multiply by two to get your estimated cover), or 25 along a 12.5 m line (multiply average by 4), etc.

Method 2: Subdivision of sample plot into quadrants:

Many plots, whether they are square, circular, or rectangular, may be “quartered” and have each quadrant’s plant cover estimated separately. If the plot is a given even number of square meters (such as 100, 400, or 1000 m²) then you know that a quarter of that amount is also an easily measurable number. If you can estimate the average size of the plants in each of the quarters (e.g, small pinyon pines may be 5 m² (2.2m x 2.2m), creosote bush may be 2m² (or 1.41 m x 1.41 m), burrobush may be 0.5m²) then you simply count the number of plants in each size class and multiply by their estimated size for the cover in a given quadrant. Then you average the 4 quadrants together for your average cover value.

This method works well in vegetation with open-to-dense cover of low species such as grasses or low shrubs, in open woodlands, and desert scrubs.

Method 3; “Squash” all plants into a continuous cover in one corner of the plot :

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Another way to estimate how much of the plot is covered by a particular species is to mentally group (or “march”, or “squash”) all members of that species into a corner of the plot and estimate the area they cover. Then calculate that area as a percentage of the total plot area. This technique works well in herb and shrub dominated plots but is not very useful in areas with trees.

Method 4: How to estimate tree cover:

Cover estimates of tall trees is one of the most difficult tasks for a beginning relevé sampler. However it is possible to do this with consistency and reliability using the following guidelines.

1. Have regular sized and shaped plots that you can easily subdivide.
2. Estimate average crown spread of each tree species separately by pacing the crown diameter of representative examples of trees of each species and then roughly calculating the crown area of each representative species.
3. Add together the estimated crown area of each individual of each species of tree on the plot for your total cover.

Method 5: The process of elimination technique:

This method is generally good for estimating cover on sparsely vegetated areas where bare ground, rocks, or cobbles cover more area than vegetation. In such a situation it would be advisable to first estimate how much of the ground is not covered by plants and then subdivide the portion that is covered by plants into rough percentages proportional to the different plant species present. For example, in a desert scrub the total plot not covered by plants may be estimated at 80%. Of the 20% covered by plants, half is desert sunflower (10% cover), a quarter is California buckwheat (5% cover), an eighth brittlebush (2.5% cover), and the rest divided up between 10 species of herbs and small shrubs (all less than 1% cover).

Any of these techniques may be used in combination with one another for a system of checks and balances, or in stands that have characteristics lending themselves for a different technique for each layer of vegetation.

In a relevé, cover estimates, using the techniques described above, are made for each taxon as it is recorded on the species list. Estimates are made for each layer in which the taxon was recorded. For example, if individuals of coast live oak occur in the tree overstory (canopy trees) and tree understory (seedlings and saplings), an estimate is made for both layers should be recorded.

In a traditional relevé, cover is estimated in “cover classes,” not percentages, because of the variability of plant populations over time and from one point to another, even within a small stand. This protocol uses the following 6 cover classes:

Cover Class 1: the taxon in that layer covers < 1 % of the plot area

Cover Class 2: the taxon in that layer covers 1 % - 5 % of the plot area

Cover Class 3a: the taxon in that layer covers >5 - 15 % of the plot area

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Cover Class 3b: the taxon in that layer covers >15 - 25 % of the plot area

Cover Class 4: the taxon in that layer covers >25 - 50 % of the plot area

Cover Class 5: the taxon in that layer covers >50 - 75 % of the plot area

Cover Class 6: the taxon in that layer covers > 75% of the plot area

Percentages (optional)

This CNPS protocol also encourages observers to estimate percentages if they feel confident in their estimation abilities. This optional step allows the data to be compared more easily to data collected using different methods, such as a line or point intercept. It also instills confidence in the cover estimate of borderline species that are close calls between two cover classes (e.g., a cover class 2 at 5% as opposed to a cover class 3 at 6%). It is particularly useful for calculating cover by the process of elimination techniques and for estimating total vegetation cover (see below) and coarse fragment cover.

Overall Cover of Vegetation

In addition to cover of individual taxa described above, total cover is also estimated for each vegetation layer. This is done using the same cover classes as described above but combines all taxa of a given category. They can be calculated from the species percent cover estimates, but please make sure to disregard overlap of species within each layer. These estimates should be absolute aerial cover, or the “bird’s eye view” of the vegetation cover, in which each category cannot be over 100%.

To come up with a specific number estimate for percent cover, first use the cover intervals, used in the species cover estimates, as a reference aid to get a generalized cover estimate: While keeping these intervals in mind, you can then refine your estimate to a specific percentage for each category below.

% Overstory Conifer/Hardwood Tree: The total aerial cover (canopy closure) of all live tree species that are specifically in the overstory or are emerging, disregarding overlap of individual trees. Estimate conifer and hardwood covers separately. Please note: These cover values should not include the coverage of suppressed understory trees.

%Low-Medium Tree: The total aerial cover (canopy closure) of all live understory low to medium height tree species, disregarding overlap of individual trees and shrubs. This category contains recruits of overstory tree species (with seedlings and saplings in the understory) and understory tree species that typically do not make up the overstory canopy (e.g. trees that typically do not attain a height >10m).

% Shrub: The total aerial cover (canopy closure) of all live shrub species disregarding overlap of individual shrubs.

% Herb: The total aerial cover (canopy closure) of all herbaceous species, disregarding overlap of individual herbs.

% Total Vascular plants: The total aerial cover of all vegetation. This is an estimate of the absolute vegetation cover, disregarding overlap of the various tree, shrub, and/or herb layers.

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% Total Non-vascular plants: The total cover of all lichens and bryophytes (mosses, liverworts, hornworts) on substrate surfaces (not standing or inclined trees).

Modal height for conifer/hardwood tree, shrub, and herbaceous categories (optional)

If height values are important in your vegetation survey project, provide an ocular estimate of height for each category listed. Record an average height value per each category by estimating the mean height for each group. Please use the following height intervals to record a height class: 01=<1/2m, 02=1/2-1m, 03=1-2m, 04=2-5m, 05=5-10m, 06=10-15m, 07=15-20m, 08=20-35m, 09=35-50m, 10=>50m.

Caveats

Please consult with the members of the vegetation committee for advice and feedback on proposed vegetation surveys prior on initiating projects.

Notes on the Order and Division of Labor for Data Collection: As with every procedure, there are always more and less efficient ways to collect the information requested. Although we respect each field crews' option to choose in what order they collect the data, we suggest the following general rules:

- Work with teams of two for each plot collected.
- Both team members can determine the plot shape and size and lay out the tapes and mark the edges for the plot boundary (see below).
- The two person teams can also divide up tasks of data collection with one member collecting location, environmental (slope, aspect, geology, soil texture, etc.) and plot description information while the other begins the species list. Thus, two clipboards are useful and data sheets that are at first separated (not stapled).
- Following the making of the initial species list and collection of location and environmental data both team members convene to do the estimation of plant cover by species followed by the estimation of total vegetation cover and cover by layer.
- Following that process, the estimation of cover by the up to 10 height strata classes and the listing of the diagnostic species for each is done collaboratively.
- This is followed by the estimation of the coarse fragment information, again done collaboratively.

For egalitarian and familiarization purposes we suggest that the roles be switched regularly between the team members and that if multiple teams are being used in a larger project, that each team member switches frequently between teams, building all-important calibration, and camaraderie among the whole group.

Suggestions for Laying out Plots: If you are laying out a circular plot, work with two or more people. One person stands at the center of the plot and holds the tape case while the other walks the end of the tape out to the appointed distance (radius 5.6 for 100 m² circle, radius 11.3 m for a 400 m² circle, and radius 17.6 m for a 1000m² circle). The walker then fixes the tape end with a pin flag and walks back to the center where he/she instructs the center person to walk in the opposite direction of the already laid out tape radius, stretching the rest of the tape to an equal

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length (another 11.3 or 17.6 m) to the opposite edge of the plot, where he/she affixes it with another pin flag. This process is again repeated with another tape laid out perpendicular to the first so that an “+” shape is created. The margins of the circle can be further delineated by measuring to the center of the circle with an optical tape measure (rangefinder) and marking mid points between the four ends of the crossed tapes.

When laying out square or rectangular plots work with two or more people per team. If doing a rectangle, determine the long axis of the plot first and have one person be stationed at the zero m end of the tape while the other person walks the unrolling tape case out to the appropriate length. The stationary end person can guide the walker, keeping them moving in a straight line. Once that tape is laid out and the far end staked, the team lays out another tape perpendicular to the first, either at one end, using the same type of process. This establishes the width of the rectangle (or square). Using an optical rangefinder and pin-flags, or colored flagging the team can further mark additional points along the other parallel long axis and short axis of the plot (every 5 m for shorter plots or every 10 m for longer plots is suggested) so that the entire plot boundary can be easily visualized.

References:

Barbour M.G., J.H. Burk, and W.D. Pitts 1987. Terrestrial Plant Ecology, Second Edition. Benjamin/Cummings Publishing Co. Menlo Park, CA. 634 pages.

Sawyer and Keeler-Wolf. 1995. Manual of California Vegetation. California Native Plant Society, Sacramento, CA. 471 pages

The Nature Conservancy and Environmental Systems Research Institute. 1994. Final Draft, Standardized National Vegetation Classification System. Prepared for United States Department of the Interior, National Biological Survey, and National Park Service. Arlington, VA.

Complete document available at the following website:

<http://biology.usgs.gov/npsveg/fieldmethods.html>

Suggested Equipment:

Equipment List: Prices as of May 2000, toll free orders from Forestry Suppliers (1-800-647-5368) (item numbers in parentheses)

Chaining pins, surveyor steel (#39167)	\$21.50
Fiberglass tapes 2 - 165'/50 m (#39972)	\$42.90
Logbook cover 8 ½ " x 12" (#53200)	\$23.95
Perforated flagging (#57960)	\$1.95
UTM Coordinate Grid (#45019)	\$16.95
Rangefinder, 10-75m (#38973)	\$51.60
Silva Compass w/ clinometer (#37036)	\$43.90
Garmin GPS 12XL (#39095, #39111)	\$244.90

Appendix 3.5B
CALIFORNIA NATIVE PLANT SOCIETY RELEVANT FIELD FORM CODE LIST (revised 3/0107)
(CNPS 2007)

MACRO TOPOGRAPHY

- 00 Bench
- 01 Ridge top (interfluvial)
- 02 Upper 1/3 of slope
- 03 Middle 1/3 of slope
- 04 Lower 1/3 of slope (lowslope)
- 05 Toeslope (alluvial fan/bajada)
- 06 Bottom/plain
- 07 Basin/wetland
- 08 Draw
- 09 Other
- 10 Terrace (former shoreline or floodplain)
- 11 Entire slope
- 12 Wash (channel bed)
- 13 Badland (complex of draws & interfluvial)
- 14 Mesa/plateau
- 15 Dune/sandfield
- 16 Pediment
- 17 Backslope (cliff)

MICRO TOPOGRAPHY

- 01 Convex or rounded
- 02 Linear or even
- 03 Concave or depression
- 04 Undulating pattern
- 05 Hummock or Swale pattern
- 06 Mounded
- 07 Other

SITE IMPACTS

- 01 Development
- 02 ORV activity
- 03 Agriculture
- 04 Grazing
- 05 Competition from exotics
- 06 Logging
- 07 Insufficient population/stand size
- 08 Altered flood/tidal regime
- 09 Mining
- 10 Hybridization
- 11 Groundwater pumping
- 12 Dam/inundation
- 13 Other
- 14 Surface water diversion
- 15 Road/trail construction/maint.
- 16 Biocides
- 17 Pollution
- 18 Unknown
- 19 Vandalism/dumping/litter
- 20 Foot traffic/trampling
- 21 Improper burning regime
- 22 Over collecting/poaching
- 23 Erosion/runoff
- 24 Altered thermal regime
- 25 Landfill
- 26 Degrading water quality
- 27 Wood cutting
- 28 Military operations
- 29 Recreational use (non ORV)
- 30 Nest parasitism
- 31 Non-native predators
- 32 Rip-rap, bank protection
- 33 Channelization (human caused)
- 34 Feral pigs
- 35 Burros
- 36 Rills
- 37 Phytogenic mounding
- 38 Sudden oak death syndrome (SODS)

PARENT MATERIAL

- IGTU Igneous (type unknown)
- VOLC General volcanic extrusives
- RHYO Rhyolite
- ANDE Andesite
- BASA Basalt
- ASHT Ash (of any origin)
- OBSI Obsidian
- PUMI Pumice
- PYFL Pyroclastic flow
- VOFL Volcanic flow
- VOMU Volcanic mud
- INTR General igneous intrusives
- GRAN Granitic (generic)
- MONZ Monzonite
- QUDI Quartz diorite
- DIOR Diorite
- GABB Gabbro
- DIAB Diabase
- PERI Peridotite
- METU Metamorphic (type unknown)
- GNBG Gneiss/biotite gneiss
- SERP Serpentine
- SCHI Schist
- SESC Semi-schist
- PHYL Phyllite
- SLAT Slate
- HORN Hornfels
- BLUE Blue schist
- MARB Marble
- SETU Sedimentary (type unknown)
- BREC Breccia (non-volcanic)
- CONG Conglomerate
- FANG Fanglomerate
- SAND Sandstone
- SHAL Shale
- SILT Siltstone
- CACO Calcareous conglomerate
- CASA Calcareous sandstone
- CASH Calcareous shale
- CASI Calcareous siltstone
- DOLO Dolomite
- LIME Limestone
- CALU Calcareous (origin unknown)
- CHER Chert
- FRME Franciscan melange
- GREE Greenstone
- ULTU Ultramafic (type unknown)
- MIIG Mixed igneous
- MIME Mixed metamorphic
- MISE Mixed sedimentary
- MIRT Mix of two or more rock types
- GLTI Glacial till, mixed origin, moraine
- LALA Large landslide (unconsolidated)
- DUNE Sand dunes
- LOSS Loess
- CLAL Clayey alluvium
- GRAL Gravelly alluvium
- MIAL Mixed alluvium
- SAAL Sandy alluvium (most alluvial fans and washes)
- SIAL Silty alluvium
- OTHE Other than on list

SOIL TEXTURE

- COSA Coarse sand
- MESN Medium sand
- FINS Fine sand
- COLS Coarse, loamy sand
- MELS Medium to very fine, loamy sand
- MCSL Moderately coarse, sandy loam
- MESA Medium to very fine, sandy loam
- MELO Medium loam
- MESL Medium silt loam
- MESI Medium silt
- MFCL Moderately fine clay loam
- MFSA Moderately fine sandy clay loam
- MFSL Moderately fine silty clay loam
- FISA Fine sandy clay
- FISC Fine silty clay
- FICL Fine clay
- SAND Sand (class unknown)
- LOAM Loam (class unknown)
- CLAY Clay (class unknown)
- UNKN Unknown
- PEAT Peat
- MUCK Muck

DOMINANT VEGETATION GROUP

Trees:

- TBSE Temperate broad-leaved seasonal evergreen forest
- TNLE Temperate or subpolar needle-leaved evergreen forest
- CDF Cold-deciduous forest
- MNDF Mixed needle-leaved evergreen-cold deciduous forest
- TBEW Temperate broad-leaved evergreen woodland
- TNEW Temperate or subpolar needle-leaved evergreen woodland
- EXEW Extremely xeromorphic evergreen woodland
- CDW Cold-deciduous woodland
- EXDW Extremely xeromorphic deciduous woodland
- MBED Mixed broad-leaved evergreen-cold deciduous woodland
- MNDW Mixed needle-leaved evergreen-cold deciduous woodland

Shrubs:

- TBES Temperate broad-leaved evergreen shrubland
- NLES Needle-leaved evergreen shrubland
- MIES Microphyllous evergreen shrubland
- EXDS Extremely xeromorphic deciduous shrubland
- CDS Cold-deciduous shrubland
- MEDS Mixed evergreen-deciduous shrubland
- XMED Extremely xeromorphic mixed evergreen-deciduous shrubland

Dwarf Shrubland:

- NMED Needle-leaved or microphyllous evergreen dwarf shrubland
- XEDS Extremely xeromorphic evergreen dwarf shrubland
- DDDS Drought-deciduous dwarf shrubland
- MEDD Mixed evergreen cold-deciduous dwarf shrubland

Herbaceous:

- TSPG Temperate or subpolar grassland
- TGST Temperate or subpolar grassland with sparse tree
- TGSS Temperate or subpolar grassland with sparse shrublayer
- TGSD Temperate or subpolar grassland with sparse dwarf shrub layer
- TFV Temperate or subpolar forb vegetation
- THRV Temperate or subpolar hydromorphic rooted vegetation
- TAGF Temperate or subpolar annual grassland or forb vegetation

Sparse Vegetation:

- SVSD Sparsely vegetated sand dunes
- SVCS Sparsely vegetated consolidated substrates

Simplified Key to Soil Texture
Appendix 9.5B
 (Adapted from Brewer and McCann 1982)
(CNPS 2007)

Place about three teaspoons of soil in the palm of your hand. Take out any particles ≥ 3 mm in size.

A. Does soil remain in ball when squeezed in your hand palm?

Yes, soil does remain in a ball when squeezed..... **B**

No, soil does not remain in a ball when squeezed..... **sand**

Very coarse texture..... SAND Sand (class unknown)
 Moderately coarse texture..... COSA Coarse sand
 Moderately fine texture..... MESN Medium sand
 FISN Fine sand

B. Add a small amount of water until the soil feels like putty. Squeeze the ball between your thumb and forefinger, attempting to make a ribbon that you push up over your finger. Does soil make a ribbon?

Yes, soil makes a ribbon; though it may be very short..... **C**

No, soil does not make a ribbon..... **loamy sand**

Very gritty with coarse particles..... COLS Coarse, loamy sand
 Moderately to slightly gritty with medium to fine particles..... MELS Medium to very fine, loamy sand

C. Does ribbon extends more than one inch?

Yes, soil extends > 1 inch..... **D**

No, soil does not extend > 1 inch..... Add excess water

Soil feels gritty..... **loam or sandy loam**

Very gritty with coarse particles..... LOAM Loam (class unknown)
 Moderately gritty with medium to fine particles..... MCSL Moderately coarse, sandy loam
 Slightly gritty MESA Medium to very fine, sandy loam
 MELO Medium loam

Soil feels smooth..... **silt loam**

MESIL medium silt loam

D. Does soil extend more than 2 inches?

Yes, ribbon extends more than 2 inches, and does not crack if bent into a ring..... **E**

No, soil breaks when 1–2 inches long; cracks if bent into a ring..... Add excess water

Soil feels gritty..... **sandy clay loam or clay loam**

Very gritty..... MFSA Moderately fine sandy clay loam
 Slightly gritty..... MFCL Moderately fine clay loam

Soil feels smooth..... **silty clay loam or silt**

Moderately fine texture..... MFSL Moderately fine silty clay loam
 Very fine texture..... MESI Medium silt

E. Soil makes a ribbon 2+ inches long; does not crack when bent into a ring..... Add excess water

Soil feels gritty..... **sandy clay or clay**

Very gritty..... CLAY Clay (class unknown)
 Slightly gritty..... FISA Fine sandy clay
 FICL Fine clay

Soil feels smooth..... **silty clay**

FISC Fine silty clay

UNKN = UNKNOWN

PEAT = PEAT

MUCK = MUCK

Appendix 3.5B
Artificial Key to the Systems and Classes
(CNPS 2007)
Key to the Systems

1. Water regime influenced by oceanic tides, and salinity due to ocean-derived salts 0.5% or greater.
 2. Semi-enclosed by land, but with open, partly obstructed or sporadic access to the ocean. Halinity wide-ranging because of evaporation or mixing of seawater with runoff from land ESTUARINE
 - 2'. Little or no obstruction to open ocean present. Halinity usually euhaline; little mixing of water with runoff from land 3
 3. Emergents, trees, or shrubs present ESTUARINE
 - 3'. Emergents, trees, or shrubs absent. MARINE
- 1'. Water regime not influenced by ocean tides, or if influenced by oceanic tides, salinity less than 0.5%
 4. Persistent emergents, trees, shrubs, or emergent mosses cover 30% or more of the area PALUSTRINE
 - 4'. Persistent emergents, trees, shrubs, or emergent mosses cover less than 30% of substrate but nonpersistent emergents may be widespread during some seasons of year 5
 5. Situated in a channel; water, when present, usually flowing RIVERINE
 - 5'. Situated in a basin, catchment, or on level or sloping ground; water usually not flowing. 6
 6. Area 8 ha (20 acres) or greater LACUSTRINE
 - 6'. Area less than 8 ha 7
 7. Wave-formed or bedrock shoreline feature present or water depth 2 m (6.6 feet) or more LACUSTRINE
 - 7'. No wave-formed or bedrock shoreline feature present and water > 2 m deep PALUSTRINE

Key to the Classes

1. During the growing season of most years, aerial cover by vegetation is less than 30%.
 2. Substrate a ridge or mound formed by colonization of sedentary invertebrates (corals, oysters, tube worms) REEF
 - 2'. Substrate of rock or various-sized sediments often occupied by invertebrates but not formed by colonization of sedentary invertebrates 3
 3. Water regime subtidal, permanently flooded, intermittently exposed, or semipermanently flooded. Substrate usually not soil 4
 4. Substrate of bedrock, boulders, or stones occurring singly or in combination covers 75% or more of the area ROCK BOTTOM
 - 4'. Substrate of organic material, mud, sand, gravel, or cobbles with less than 75% areal cover of stones, boulders, or bedrock. UNCONSOLIDATED BOTTOM
 - 3'. Water regime irregularly exposed, regularly flooded, irregularly flooded, seasonally flooded, temporarily flooded, intermittently flooded, saturated, or artificially flooded. Substrate often a soil 5
 5. Contained within a channel that does not have permanent flowing water (i.e., Intermittent Subsystem of Riverine System or Intertidal Subsystem of Estuarine System) STREAMBED
 - 5'. Contained in a channel with perennial water or not contained in a channel 6
 6. Substrate of bedrock, boulders, or stones occurring singly or in combination covers 75% or more of the area ROCKY SHORE
 - 6'. Substrate of organic material, mud, sand, gravel, or cobbles; with less than 75% of the cover consisting of stones, boulders, or bedrock. UNCONSOLIDATED SHORE
 - 1'. During the growing season of most years, percentage of area covered by vegetation 30% or greater.
 7. Vegetation composed of pioneering annuals or seedling perennials, often not hydrophytes, occurring only at time of substrate exposure 8
 8. Contained within a channel that does not have permanent flowing water. STREAMBED (VEGETATED)
 - 8'. Contained within a channel with permanent water, or not contained in a channel UNCONSOLIDATED SHORE (VEGETATED)
 - 7'. Vegetation composed of algae, bryophytes, lichens, or vascular plants that are usually hydrophytic perennials 9
 9. Vegetation composed predominantly of nonvascular species 10
 10. Vegetation macrophytic algae, mosses, or lichens growing in water or the splash zone of shores AQUATIC BED
 - 10'. Vegetation mosses or lichens usually growing on organic soils and always outside the splash zone of shores MOSS-LICHEN WETLAND
 - 9'. Vegetation composed predominantly of vascular species 11
 11. Vegetation herbaceous 12
 12. Vegetation emergents. EMERGENT WETLAND
 - 12'. Vegetation submergent, floating-leaved, or floating. AQUATIC BED
 - 11'. Vegetation trees or shrubs 13
 13. Dominants less than 6 m (20 feet) tall SCRUB-SHRUB WETLAND
 - 13'. Dominants 6 m tall or taller FORESTED WETLAND

CNPS and CDFW Combined Vegetation Rapid Assessment and Relevé Field Form
(Revised February 27, 2014)

For Office Use Final database #:	Final vegetation type: Alliance _____ Association _____
--	---

I. LOCATIONAL/ENVIRONMENTAL DESCRIPTION

Stand ID: _____	Date: _____	Name of recorder: _____
		Other surveyors: _____

GPS name: _____ **Datum:** NAD83 or _____ For Relevé: **Bearing** °, left axis at SW point _____ of **Long / Short** side
UTME _____ **UTMN** _____ **Zone:** 10 / 11 (circle one) **Error:** ± _____ ft / m / pdop

GPS within stand? Yes / No If No, cite from GPS to stand: distance (m) _____ bearing ° _____ inclination ° _____
and record projected UTM: **UTME** _____ **UTMN** _____

Elevation: _____ ft / m **Camera Name/Photograph #'s:** _____

Stand Size (acres): <1, 1-5, >5 | **Plot Size (m²):** 10 / 100 / 400 / 1000 | **Plot Shape** ___ x ___ ft / m or **Circle Radius** ___ ft / m
Exposure, Actual °: _____ NE NW SE SW Flat Variable All | **Steepness, Actual °:** _____ 0° 1-5° 5-25° >25

Topography: Macro: top upper mid lower bottom | **Micro:** convex flat concave undulating
Geology code: _____ **Soil Texture code:** _____ | **Upland** or **Wetland/Riparian** (circle one)

% Surface cover: _____ (Incl. outcrops) (>60cm diam) (25-60cm) (7.5-25cm) (2mm-7.5cm) (Incl sand, mud)
H20: BA Stems: _____ Litter: _____ Bedrock: _____ Boulder: _____ Stone: _____ Cobble: _____ Gravel: _____ Fines: _____ =100%

% Current year bioturbation _____ **Past bioturbation present?** Yes / No | **% Hoof punch** _____
Fire evidence: Yes / No (circle one) If yes, describe in Site history section, including date of fire, if known.

Site history, stand age, comments: _____

Disturbance code / Intensity (L,M,H): _____ / _____ / _____ / _____ / _____ / _____ / _____ "Other" _____ / _____

II. HABITAT AND VEGETATION DESCRIPTION

Tree DBH: T1 (<1" dbh), T2 (1-6" dbh), T3 (6-11" dbh), T4 (11-24" dbh), T5 (>24" dbh), T6 multi-layered (T3 or T4 layer under T5, >60% cover)

Shrub: S1 seedling (<3 yr. old), S2 young (<1% dead), S3 mature (1-25% dead), S4 decadent (>25% dead)

Herb: H1 (<12" plant ht.), H2 (>12" ht.) **Desert Riparian Tree/Shrub:** 1 (<2ft. stem ht.), 2 (2-10ft. ht.), 3 (10-20ft. ht.), 4 (>20ft. ht.)

Desert Palm/Joshua Tree: 1 (<1.5" base diameter), 2 (1.5-6" diam.), 3 (>6" diam.) **% NonVasc cover:** _____ **% Vasc Veg cover:** _____

% Cover: Conifer tree / Hardwood tree: _____ / _____ **Regenerating Tree:** _____ **Shrub:** _____ **Herbaceous:** _____

Height Class: Conifer tree / Hardwood tree: _____ / _____ **Regenerating Tree:** _____ **Shrub:** _____ **Herbaceous:** _____

Height classes: 01=<1/2m 02=1/2-1m 03=1-2m 04=2-5m 05=5-10m 06=10-15m 07=15-20m 08=20-35m 09=35-50m 10=>50m

Species, Stratum, and % cover. Stratum categories: T=Tree, S= Shrub, H= Herb, E = SEedling, A = SApling, N= Non-vascular.
% cover intervals for reference: <1%, 1-5%, >5-15%, >15-25%, >25-50%, >50-75%, 75%.

Strata	Species	% cover	C	Strata	Species	% cover	C

Unusual species: _____

III. INTERPRETATION OF STAND

Field-assessed vegetation alliance name: _____

Field-assessed association name (optional): _____

Adjacent alliances/direction: _____ / _____, _____ / _____

Confidence in alliance identification: L M H **Explain:** _____

Phenology (E,P,L): Herb Shrub Tree **Other identification or mapping information:** _____

Appendix 3.5D



Source: Esri, DigitalGlobe, GeoEye, iStock, USDA, USGS, AeroGRID, IGN, IGP, swisstopo, and the GIS User Community



Standard Operating Procedures (3.6): Seed Collection and Germination

March 2021

Prepared for the United States Environmental
Protection Agency



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Standard Operating Procedures: Seed Collection and Germination

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Protocol Suitability Evaluation

A habitat suitability table containing appropriate estuarine wetland habitat types (of those evaluated) to implement seed collection and germination protocols is displayed in Table 1. Specifically, the protocols focus on common wetland species and adjacent transitional habitat species. A comparative assessment of cost, effort, and data quality are shown in Table 2. A matrix of additional detailed categorical evaluations of seed collection and germination protocols can be found in Appendix 3.6A.

Table 1. Appropriate habitat types to implement seed collection and germination protocols.

Survey Protocol	Habitat Types					
	Tidal Channel	Mud/sand flat	Emergent salt marsh	Non-tidal salt marsh	Salt pan	'Degraded' / fill
Seed Collection			X	X	X	X

Table 2. Categorical assessment of cost/effort and data quality for seed collection and germination protocols.

	Evaluation Metric	Seed Collection	Notes
Time / Effort	Office Preparation Time	10-30 minutes	Site selection and any GPS locations; print data sheets
	Equipment Construction Time (one time)	10-30 minutes	To gather supplies
	Field Time	> 60 minutes	Dependent on quantity of seeds to be collected and number of locations
	Laboratory Time (per transect)	> 60 minutes	Seed cleaning, processing, and watering in greenhouse
	Post-Survey Processing / QAQC Time	10-30 minutes	----
	Minimum Repetition (site-dependent)	Many Repetitions	Germination success data are highly variable
	Relative Cost (equipment and supplies)	< \$15	----
Survey / Data Quality	Accuracy (at a survey area level) *	Not Applicable	----
	Precision (at a survey area level) *	Not Applicable	----
	Qualitative-Quantitative Score *	Not Applicable	----
	Subjectivity-Objectivity Score *	Not Applicable	----

**Seed collection and germination protocols are not a traditional survey method and do not yield specific data*

Resulting Data Types

Seed bank collection and germination protocols do not qualify as a survey type and do not yield any specific data, but rather, present a methodological approach to the direct collection and propagation of native plant species. The application of these protocols will help increase the probability of success when collecting and germinating native plant species. Many of these protocols were written concurrently, and are similar to, those found in Barton et al. 2016.

Objective

The majority of wetland restoration projects incorporate a vegetation plan, or protocol, that dictates how the specified marsh plain, as well as surrounding transitional and upland areas, will be re-vegetated during the restoration process. To facilitate re-vegetation efforts during wetland restoration projects, native seeds are often collected, stored, and propagated. Collection and use of local seeds and cuttings in restoration projects is preferred to use of nursery stock as locally collected individuals are best adapted to local environmental conditions (Vander Mijnsbrugge, Bischoff, and Smith 2010), will maintain local genetic information, may improve the long-term sustainability of the site, and may enrich the diversity of the wetland plant community (Zedler 2001). As wetland complexes naturally support a variety of brackish, freshwater, dune, and salt marsh plant species, restoration plant palettes attempt to mimic natural diversity and incorporate plants from a variety of habitat types (Johnston et al. 2012).

This document outlines the basic seed collection and germination strategies to be employed within southern California estuarine and adjacent upland habitats. For more detailed information on specific plant species, see Barton et al. 2016, published in the Bulletin of Southern California Academy of Sciences, which lists available information for 84 native plant species common to southern California restoration efforts.

Equipment

Equipment and supplies needed for seed collection, cleaning, and germination varies depending on the specific species of interest. The following equipment is recommended:

Field Equipment:

- Collecting bins or paper bags
- Sealable plastic bags
- Pens/pencils/markers
- Paper clips/binder clips
- Field Data Collection Sheet (Appendix 3.6B)
- Clipboard
- Background documentation on species locations (e.g., reports, vegetation maps) (recommended)
- Mesh screens/sieves (optional)
- Tarp(s) (optional)
- Gloves (optional)
- Gardening shears (optional)
- Jepson manual (optional)

Lab/Greenhouse Equipment:

- Sieves of varying sizes (ranging from 2 mm- 500 um)
- Paper envelopes
- Freezer and oven
- Refrigerator

- Growing medium (species specific)
- Sterile petri dishes (species specific)
- Ethylene source (ethephon or sliced apple) (species specific)
- Hydrogen peroxide (H₂O₂) (species specific)
- Nail clippers (species specific)
- Mothballs (species specific)

Field Preparation

Prior to seed collection, a list of target plant species should be developed. The field equipment necessary (from the list above) to collect seeds from the plants on the list should be gathered before the field shift. Species-specific scientific documents, reports, and maps should be studied prior to collection to identify areas where target species are most likely to occur.

Field Methods

Pre-Collection:

First, using flowers, seed, stems, leaves, and/or root structures, verify that the parent plant is the desired species. If unable to identify a species in the field, take a voucher specimen, with flowers, seeds, and stems if possible, to key out in the office following the identification techniques and strategies described in *The Jepson Manual* 2nd ed. (Baldwin et al. 2012). Once the plant has been confirmed as the target species, carefully examine seeds to assess seed maturity (Figure 1). Avoid collection of



Figure 1. *Isocoma menziesii* plant with seeds of varying degrees of maturity. Yellow and amber flowers (center) have immature seeds. White, fluffy flower heads (bottom, left and top, center) have ripe, tan seeds.

immature seed, as premature collection may result in low seed viability (John et al. 2010). Generally, seeds are considered ripe if one or more of the following conditions is met: seed capsules are dry and dark tan/brown in color, seed capsules detach easily from the parent plant, and/or fruit is soft and detaches easily from the parent plant. For many common wetland species, more detailed descriptions of mature seeds are listed in Barton et al. 2016. See Appendix 3.6C for a list of species-specific collection times. While most seeds should be collected when ripe, seeds or inflorescences from certain dehiscent species, particularly those that explosively release ripe seeds, should be collected early (Teel 2011). The proper procedure for doing so is outlined in the 'Dehiscent seeds/inflorescences' section below.

To maximize the range of genetic diversity represented in the collection, seed should be collected from a large number of parent plants, ideally 50-100, if possible (John et al. 2010). When collecting, it is advantageous to sample populations, or individuals, that grow in distinct environmental conditions as these individuals likely exhibit genetic variability. Effort should be made to sample as randomly and evenly from the plant population as possible (Rancho Santa Ana Botanic Garden 2014). Additionally, if a

species is known to be dioecious (e.g., *Croton californicus*, *Baccharis* spp., *Salix* spp. *Populus fremontii*, *Distichlis spicata*, *Monanthochloe littoralis*, *Atriplex lentiformis*), care should be taken to ensure that sufficient collections from both male and female plants are made (Clarke et al. 2007). It should be noted that when collecting seeds, less intense, more frequent seed harvests are preferable to infrequent, intense harvests (Rancho Santa Ana Botanic Garden 2014). To practice safe harvesting, take no more than 5% of seed from a given species/geographic area (Zedler 2001).

The lack of published information regarding collection and propagation for many native species often forces restoration managers to rely on information from the genus or other closely related species or prompts exploratory studies (Dreesen and Harrington 1997). While Barton et al. 2016 lists collection and germination information for many common species included in southern California estuarine wetland restoration plant palettes, knowledge gaps exist for many listed species. In these instances, consult literature for the genus or family when possible. Additionally, frequent visits to collection sites are suggested to assess seed stage (i.e., ripe, unripe). More specifically, if multiple scouting trips are made, it is advisable to note the percentage of seed that is early/unripe, ripe, and exhausted per species per date. Detailed field notes are essential for the successful collection of seeds. Noting and analyzing this information will help managers focus on the ideal collection window for each plant species.

Seed Collection

Once the seeds of a target species are deemed ripe, the collection process can begin. Collection / isolation of seed varies based on plant anatomy. Observe the plant and note if the species has berries or dry fruits, dehiscent or indehiscent seeds, and note if seeds are in seed heads or seed clusters. Once this information has been determined, and the plant has been classified, find the appropriate guild below and use the subsequent information to aid collection.

Moist/Wet Fruits/Berries

Hand pluck fruits (Rancho Santa Ana Botanic Garden 2014). Place fruit into a sealed plastic bag labeled with species name, date of collection, and location of collection.

Dehiscent seeds/inflorescences

If seeds are wind-dispersed, cut entire stalk/inflorescence from plant with gardening shears in the field prior to seed maturation. Store developing seed heads or stalks inside a covered box or paper bag so that when released, ripe seeds will remain in the vessel for easy collection (Teel 2011). Alternatively, cloth bags can be secured around ripening stalks in the field. Dispersed seed will be captured by the bag. Bag will need to be checked for seed periodically and recollected at a later date (Rancho Santa Ana Botanic Garden 2014).

Seed heads

Cut entire stalk off plant. Place stalks in paper bag and shake to release seed (light crushing of the seed heads may be required) (Teel 2011). Alternatively, shake ripe seed directly onto a tarp or collection bag underneath the target plant (Rancho Santa Ana Botanic Garden 2014).

Tight Seed Clusters

For tight seed clusters, such as *Baccharis salicifolia* (Figure 2), remove entire seed cluster from plant. Remove as much flower material/chaff as possible. Use of sieves can be helpful.

Data Collection/Field Notes

Information about collections should be recorded on appropriate data sheet (Appendix 3.6B). Record all relevant information. Additionally, properly label individual paper collection bags or envelopes. Indicating the species name, date of collection, and location of collection. It is advisable to bring paperclips and/or binder clips into the field to ensure that collection bags are properly sealed and to prevent unnecessary seed loss/mixing.



Figure 2. *Baccharis salicifolia* plant and seed. Photo courtesy: RSABG.org.

Laboratory Methods

Cleaning Seeds

Once back in the lab, seed cleaning can begin. Seed cleaning removes floral parts, seed coats, pods, fleshy fruit material, or other debris from seeds (Jorgensen and Stevens 2004). Before beginning the cleaning process, identify which of the following guilds the species of interest falls into:

Moist/Wet Fruits/Berries

Place collected fruit in a sealed plastic bag. Ensure bag is well sealed and then mash the berries. Let fruit decay until the pulp is fairly watery (this process will usually take a few days) (Teel 2011). During this time, store fruit in a cool, shady place as overheating can damage seed (John et al. 2010). Rinse the pulp from the seeds in a large bowl of fresh water. Pulp should float, and the seed will sink. Repeat the process until the seeds are clean. To disinfect clean seed, use a diluted hydrogen peroxide solution (1 H₂O₂: 5 H₂O) (Teel 2011). Dry seed at room temperature, unless otherwise noted (e.g., Barton et al. 2016). Once seed is thoroughly dry, it is ready for storage.

Dehiscent seeds/inflorescences

If dehiscent inflorescences are collected early or bagged in the field as suggested above, ripe seeds or seed capsules will be released directly into the storage bag. If seed is contained in a capsule, gently crush the capsule by hand or with a rolling pin to remove the seed. Rub seeds over a sieve to remove excess chaff (Figure 3). Use stacked sieves of varying sizes to expedite the process (Figure 4). To use this technique, stack a sieve with larger openings (e.g., 1-2 mm) over a sieve with smaller pores (e.g., 500-750 µm). Rub plant material over the tower to remove both large and fine chaff from seeds. Ideally, seeds will be isolated in the middle of the tower. This methodology can be adapted based on exact seed size or sieve availability. Once seeds are isolated, only keep seeds that look ripe (i.e., dark brown/tan in color, healthy looking). Discard sickly or deformed seeds.



Figure 3. *Encelia californica* seeds and chaff over a single sieve.



Figure 4. Stacked sieves of decreasing screen size.

Seed heads

If seed is contained in a capsule, crush capsules to isolate the seed. Removal of woody capsules, as seen in *Abronia* spp., may be aided with the use of generic nail clippers (P.M. Drennan, personal communication) (Figure 5). To separate seeds from chaff, pour bag over an appropriately sized sieve for your specific seed (Teel 2011). Rub seeds over a sieve to remove remaining chaff. Stacking sieves into a tower, as described above, may expedite the process. Only retain seeds that look healthy and ripe.



Figure 5. *Abronia maritima* capsules and seed photo. Photo courtesy: RSABG.org.

Tight Seed Clusters

Gently crush capsules by hand or with a rolling pin over an appropriately sized sieve. Sift chaff/seed mixture with a sieve to remove chaff and isolate seeds (Teel 2011). Use a sieve tower if desired. Again, only retain seeds that appear ripe and healthy. For more detailed procedures on seed cleaning for specific species, see Barton et al. 2016.

Storing Seeds

For the greatest germination yield, storage time should be minimized and use of newer seeds should be prioritized. While seed longevity varies by genus and/or species, a number of seeds are known to be short-lived. For example, seeds of *Lycium californicum*, *Limonium californicum*, and *Heteromeles arbutifolia* are viable for a year at most. While seeds of other species (e.g., *Atriplex* spp., *Astragalus* spp., and *Lupinus chamissonis*) will remain viable for much longer (i.e., 4-10 years), the germination rate of seeds in long-term storage will likely decline over time. See Appendix 3.6D for more information regarding seed longevity. The longevity of certain seeds can be increased if specific storage rules are followed for the species and/or general seed storage rules are applied. After cleaning seeds and organizing them into appropriately labeled paper envelopes/bags, Vierhelig suggests storing seed packets in a large, sealed, collective container with a number of mothballs for 1-2 days to kill remaining insects and their eggs (Vierhelig 2014). To further increase longevity, keep seed dry and store in a

stable environment with low temperature and humidity (Jorgensen and Stevens 2004). Certain species will store better if kept at lower temperatures in a refrigerator or freezer. See 'Seed Storage' in Barton et al. 2016 to see suggested storage temperatures and other species-specific storage information. In addition to reducing germination rate, long-term storage will often induce seed coat or embryo dormancy, and seeds may need to be treated prior to planting to break dormancy.

Greenhouse Methods

Seedlings of a variety of marsh angiosperm species have been successfully grown in greenhouses. Transplanting greenhouse-grown seedlings is an effective re-vegetation strategy and often offers restoration ecologists a greater degree of success than simple seeding. Seedlings of appropriate size can be transplanted to the restoration site (Broome, Seneca, and Woodhouse 1988).

Germination Considerations

Successful propagation of native marsh and dune vegetation species requires a deep understanding of seed germination ecology. Naturally, seed germination is dependent upon a number of evolutionary and ecological factors, factors which generally must be observed, and often replicated, in the lab or greenhouse to successfully grow propagules. These factors include but are not limited to the following: germination timing/seasonality, environmental conditions, such as temperature, moisture, soil salinity, and light availability, seed age, and dormancy state, both at the time of maturation and dispersal (Baskin and Baskin 2014).

For many species, germination is only possible during a particular season or for a small fraction of the year. For instance, it is ideal to plant *Atriplex lentiformis* in winter. For other species, germination is possible almost year-round (Baskin and Baskin 2014). Understanding germination timing is important to determine the best environmental conditions to promote germination in the greenhouse or lab.

Understanding germination timing will in turn often indicate what temperature, or range of temperatures, best promote germination. Further, the germination rate of certain species is enhanced with simulated temperature fluctuations, rather than constant temperatures. While response to fluctuating temperatures is species-specific, a few generalities exist. Both small seeded species and forbs tend to respond well to fluctuating temperatures while larger seeded species and graminoid species do not show as marked a preference for temperature fluctuations (Liu et al. 2013). If information regarding the necessary conditions or procedures to promote germination is not readily available, it is advisable to run simple tests/experiments using a variety of the possible treatments.

Dormancy Considerations

Much in the same way that environmental germination requirements should be mimicked in the greenhouse, if a species is known to have dormant seeds, understanding which environmental conditions are necessary to naturally break seed dormancy, and thus must be manipulated in the greenhouse, is vital. If a species undergoes seed coat or embryo dormancy at any point in its life cycle, its seeds will need to be treated prior to sowing to break dormancy (Vierheilig 2014). A variety of methods can be used to break dormancy and prepare seeds for planting. These methods include:

scarification, submersion in hot water, treatment with dry heat, exposure to fire, acid, mulch treatment, cold stratification, warm stratification, and exposure to light. Unfortunately, there is not a uniform method to break seed dormancy. Instead, methods vary based on the life history of the species. Species that typically germinate in early spring after a cold and/or rainy winter, such as *Platanus racemosa*, will often need cold, moist stratification to break dormancy to mimic natural wintering. Other species, such as *Acemispson glaber* require heat treatment to break dormancy.

Please see Barton et al. 2016 for detailed seed treatment information. Please note that this information is incomplete due to gaps in published literature and some experimentation may be necessary. However, treating seeds to break dormancy, is not enough to guarantee germination. Germination requirements must also be considered.

Germination Techniques and Methods

To promote or ensure germination, seed dormancy must be broken (if applicable) and seeds must be sown in an appropriate set of environmental conditions (Baskin and Baskin 2014). To grow seedlings, clean, viable seeds should be planted in mixtures of sand, top soil, and peat moss or vermiculite (Broome, Seneca, and Woodhouse 1988). To achieve the greatest germination rate, the exact composition of the mixture should be tailored to the individual plant species of interest. Life history and preferred habitat of the species should be considered when determining optimal soil conditions. For instance, *Abronia maritima*, which naturally occurs on sandy dunes, should be sown in soil consisting largely of sand, or other coarse grains. Similarly, seeds of halophytic species should be sown in mediums that contain an appropriate level of salt or allowed to sprout directly in a saline solution, while salt intolerant species should not be sown in such conditions.

If germination studies need to be performed, it is preferable that they are conducted shortly after collection, within 7-10 days, to ensure that seeds have not entered dormancy. While a germination data sheet has not been generated as part of this SOP, when designed it should include the following items:

- Species name
- Dormancy treatments performed
- Date seed planted
- Planting medium
- Percentage germination at varying time points (e.g., 1, 2, and 3 weeks post-planting)

Data Entry and QAQC Procedures

Data should be entered in the laboratory using the appropriate data sheet (Appendix 3.6B). All required fields should be completed in full, and the data recorder should assign their name at the top of the document(s). Data should be transferred to the appropriate electronic database within three days, and the hard copies filed in labeled binders. Electronic copies of all data should be housed on an in-house dedicated server and backed up to a cloud-based or off-site server nightly. Hard copies should be saved for five years. Electronic copies should be saved indefinitely.

Data Analyses

Results of germination experiments should be carefully analyzed. Suggestions for analyses include: assessing the percentage of seed germination as a function of collection location, species, or growing medium, analyzing the percent germination as a function of time in storage, and analyzing the percent germination as a function of dormancy treatment(s).

Results Summary:

Table 3 is an example of information collected and summary results of several species of seed collections made at the Ballona Wetlands Ecological Reserve in spring and summer 2014.

Table 3. Example of summary results for several species collected at the Ballona Wetlands Ecological Reserve.

Seed Information	Seed Maturity	Field Information				
Species Name	# Plants Sampled	Date(s)	Early	Ripe	Late	BWER Area
<i>Baccharis pilularis (female)</i>	1	1-Jun	X	X	X	FW marsh
<i>Baccharis salicifolia (female)</i>	3	1-Jun		X		FW marsh
<i>Camissonia cheiranthifolia</i>	3	11-Jul	X	X		BWER Dunes
<i>Encelia californica</i>	10	19-May		X		FBW Dunes
<i>Eriogonum fasciculatum</i>	2	19-May	X		X	FBW Dunes
<i>Frankenia salina</i>	3	11-Jul	X	X		BWER Dunes
<i>Heliotropium curassavicum</i>	15-20	1-Jun	X	X	X	FBW dunes
<i>Juncus acutus</i>	1	11-Jul		X	X	FW marsh
<i>Lupinus chamissonis</i>	20	19-May	X			FBW Dunes

Health and Safety Precautions

While the plants on the southern California plant palette are safe for human handling, individuals should exercise caution in the field as certain native and non-native marsh species are known to be toxic. For more information on a specific species, reference Calflora.org, which lists toxicity ratings for all plant species (Calpoison.org 2014; “Calflora: Information on California Plants for Education, Research, and Conservation” 2014).

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APPENDIX 3.6A

	Evaluation Metric	Seed Collection and Germination	Notes
	Correlation to L2 CRAM	Not Applicable	----
Personnel Requirements	Specialty Equipment or Clothing Required	Many Specialty Items	Most specialty items are related to greenhouse processing and cleaning methods
	Ease of Transport (amount or weight of supplies)	Some Items / Moderate	----
	Ease of Implementation	Moderate	----
	Expertise / Skill Level	Some Technical Knowledge	Familiarity with species identifications is required
	Number of Personnel	2	----
	Training Requirements	None	----
	Seasonality of Survey Time	Year round	Species dependent
	Suggested Frequency	As needed	----
Survey / Data Quality	Type of Output	Not Applicable	Seed collection and germination protocols are not a traditional survey method and do not yield specific data
	Active or Passive Monitoring Style	Active	----
	Specialty Computer Software Required	Not Applicable	----
	Availability of Online / External Resources	Many resources	----
Potential Limitations	Wetland Type Applicability	All	----
	Images or Multi-Media Required	Images Required	----
	Degree of Impact / Disturbance	Moderate Disturbance	----
	Vegetation Height Limitation	Overhead (~2m)	Must be able to reach seeds
	Appropriate for Tidal / Wet Habitats	Yes	----
	Tide Height	Low Tide Only	----
	Regional or Broad Implementation *	Frequently Used	Especially for restoration project
	Potential for Hazards / Risk	Low to No Risk	----
Restrictions	Special Status Species	----	

* based on monitoring literature review

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Appendix 3.6D

Scientific Name	Start	End	Seed Collection Details (copy of draft palette)
<i>Isomeris arborea</i>	Feb	Nov	Flowers several times/year (except Dec-Jan). Ready for collection when capsules turn brown and are crisp. Strip mature fruits from plants by hand. Break apart pods by hand to remove seeds. A hammermill or coater blender can also be used for this step.
<i>Juncus bufonius</i>	Mar	May	Collect Mar-May. Seed capsules quickly dehisce, seeds should be collected quickly after plant death. Shake mature flowers to collect tiny seed
<i>Stipa lepida</i> [Nassella l.]	Mar	May	Collect seed heads in spring when flowers fade. Allow seed heads to dry on plants, remove and harvest seed. Clean prior to storage
<i>Plantago erecta</i>	Apr	May	The tiny capsules dehisce when mature, usually from April-May. Dehiscent inflorescences can be collected early into a paper bag and left to dry. Use sieve to clean.
<i>Lupinus chamissonis</i>	Apr	Jun	Collect seed April 1- June 30th. Remove seeds from receptacles, no further cleaning required.
<i>Melica imperfecta</i>	Apr	Jun	Collect seeds April 15th- June 1st.
<i>Acmispon glaber</i> [<i>Lotus scoparius</i>]	May	Jul	Collect seeds May-July. When ripe, strip seed pods from stems by hand. Avoid breaking seeds during thrashing. Rub pods with wooden block over #16 (medium) screen. Seeds should be removed from seed pods. Remove excess chaff with seed blower.
<i>Astragalus tener var. titi</i>	May	Jul	Collect May- June. Extract seeds from fruits by hand. Thresh seeds over soil sieve large enough to let seeds fall through. Extracted seeds should be run through a seed blower to remove parasitized or aborted seed.
<i>Baccharis salicifolia</i>	May	Jul	Dioecious. Collect seeds May-June. Collect ripe fruits by hand or shaking seeds onto canvases/tarps. Fruits can be rubbed with fingers or over a screen to remove the pappus. For cuttings, use a stem as long as your arm and as wide as your finger. Cut the bottom of the stem at an angle, strip off leaves, push the stem into soil, leaving at least 2 buds above the surface. New leaves will sprout in ~2 months.
<i>Abronia maritima</i>	May	Aug	Plants produce flowers and seeds throughout the year, with the majority of flower and seed production occurring in late spring/summer. Rub fruits over a medium screen, use a seed blower unit to remove chaff from sieved seeds.
<i>Abronia umbellata</i>	May	Aug	Plants produce flowers and seeds throughout the year, with the majority of flower and seed production occurring in late spring/summer. Rub fruits over a medium screen, use a seed blower unit to remove chaff from sieved seeds.
<i>Eriogonum fasciculatum</i>	May	Aug	May-Aug (best Jun-Jul). Collect inflorescences as they begin to brown and turn rusty in color. Seeds may be separated or left in the flowers. Push seeds through a screen to remove chaff
<i>Salvia apiana</i>	May	Aug	Propagates more easily from seed than cuttings. Collect seeds as capsules begin to dry, but before seeds are released. Shake seeds from seed heads and/or use a sieve to isolate seeds. Dry clean seeds for a few days before transferring to refrigerator. For cuttings, gather soft wood before flowering. Cuttings should be 3-4 inches long. Remove lower leaves, dip cutting in growth medium
<i>Suaeda taxifolia</i>	Jun	Jul	Collect seed by stripping flowers with seed from inflorescences (Jun-Jul)
<i>Mimulus aurantiacus</i>	Jun	Aug	Seeds are collected Jun 1st- August 1st. Rub seed capsules over a sieve
<i>Salvia mellifera</i>	Jun	Aug	Seeds collected Jun- Aug, after inflorescences with calyces are dry and brown. Mature seeds can be collected by clipping, stripping, or shaking seed heads. Seed should be dried and passed through a sieve. Use of a blower is recommended
<i>Atriplex watsonii</i>	Jun	Sep	Seed collection: Jun-Sep
<i>Monanthochloe littoralis</i> (<i>Distichlis littoralis</i>)	Jun	Sep	Dioecious. Seeds small and difficult to collect. Seeds should be acquired over summer from Jun-Sep.
<i>Artemisia douglasiana</i>	Jun	Oct	Seed is ready to harvest when it can be easily removed from the heads by shaking. Clip the seed stalks and bag the material for air drying. Seeds can be threshed by rubbing the inflorescence through a screen and separating chaff with a blower.
<i>Grindelia camporum</i>	Jun	Oct	Harvest seed in June and again in October. Clip seed heads or shake/rub mature seeds from seed heads into a collection bag. To clean, rub seed heads over sieve. Remove chaff using additional sieves or an air separator. Air dry in oven at 203 F (room temp ok).
<i>Hordeum brachyantherum</i>	Jun	July	Collect seeds June 1st to July 31st. Mature inflorescences are light brown. Seed easily removed when stalks are hand stripped. No additional cleaning required
<i>Salix exigua</i>	Jun	July	Dioecious. Harvest when catkins change from green to yellow-brown in June-July and capsules begin to open. Seeds are then easily stripped from branches (15-36 seeds/capsule). Dried seeds will separate from cottony catkins when shaken. For cuttings, branches must be cut before seed is dispersed and placed into water buckets.
<i>Heliotropium curassavicum</i>	Jun		Seeds ripen from base of stalk toward tip.
<i>Cressa truxillensis</i>	Jul	Aug	Produces mature seeds from late summer into early autumn.
<i>Rosa californica</i>	Jul	Aug	Hips can be collected as soon as they are ripe, in late summer or early fall. Achenes extracted by macerating hips in water and removing floating seeds
<i>Stipa cernua</i> [Nassella c.]	Jul	Aug	Seed can be harvested by hand or with a flow-vac or combine. Dry seeds in paper bags kept in warm conditions.
<i>Triglochin maritime</i>	Jul	Sep	Collect seeds between July 17- Sept. 23rd. Mature inflorescences are brown. Seed Cleaning: Rub dry fruits between fingers to extract the seeds.
<i>Iva axillaris</i>	Jul	Oct	Collect late in the season. Seeds can be hand stripped or beaten into a hopper/open container. Flower material should be rubbed over a sieve/screen and run through a blower to remove chaff
<i>Croton californicus</i>	Jul	Nov	Dioecious. Collect July 15th- November 17th, shake chaff away from seeds by hand. Seeds encased in seed pods, pods will need to be removed. Mature seeds are round and brown with tan spots.
<i>Vulpia microstachys</i>	Jul	Sept*	As it is an annual, only regenerates from seed. Unknown when seeds from S. California plants mature (intermountain varieties mature in late July-- late Sept.)

Appendix 3.6D

Scientific Name	Start	End	Seed Collection Details (copy of draft palette)
<i>Astragalus pycnostachyus</i> var. <i>lanosissimus</i>	Jul		A. sinuatus seeds mature and should be collected in late July (http://www.centerforplantconservation.org/collection/cpc_viewprofile.asp?CPCNum=491)
<i>Astragalus trichopodus</i>	Jul		Other plants in genus, specifically A. sinuatus, have seeds that mature in late July
<i>Schoenoplectus acutus</i>	Aug	Sep	Seeds mature late August- September. Because they are easily dispersed by wind, it is important to collect seeds close to the time of maturity. Seeds must be seperated from the panicle and cleaned.
<i>Achillea millefolium</i>	Aug	Oct	Cut entire inflorescences, collect in paper bags. Keep seed in a well ventilated area while drying and before cleaning. Clean seeds with a hammermill, screen, and fanning mill
<i>Oenathera elata</i>	Aug	Oct	Collect seed from spring cultivars in October and from winter cultivars in September. Bag seed heads and allow them to dry on plant or collect early and allow to ripen in paper bags.
<i>Jaumea carnosa</i>	Aug	Nov	Collect seed Sept. 9- Nov. 11 while fruits are swollen and green. Seeds are linear achenes with longitudinal stripes. Rub seeds over #12 sieve to clean.
<i>Suaeda moquinii</i>	Aug	Nov	Seeds ready for collection when they are hard, black, and shiny. The calyces will be brown and crumbly. Seeds can be stripped from plant by hand. Seeds should be spread out to dry before being processed/stored
<i>Baccharis pilularis</i>	Aug	Dec	Collect Sept-Dec. Dioecious. Seeds can be collected by hand into open breathable bags or branches can be shaken with open tubs or tarps placed underneath branches. Fruit should be spread out to dry in a well ventilated room or in the sun. Dried heads and achenes can be rubbed between palms or over a screen to remove the pappus and phyllaries.
<i>Atriplex lentiformis</i>	Sep	Jan	Dioecious. Seeds should be collected in the fall and winter. Produces large amounts of seed
<i>Atriplex californica</i>	Sep	Oct	Seeds are collected from Sep- Oct. Gently rub over #18 sieve. Blow off as much chaff as possible with a seed blower.
<i>Atriplex patula/triangularis</i>	Sep	Oct	Seed collection: Sep-Oct. Collect seed as flowers mature. Fully mature fruit can be shaken or hand stripped from the branches and collected in bags or baskets or onto a canvas spread below the bush. Seeds will often remain on bushes until April, so later collecting is possible
<i>Frankenia salina</i>	Sep	Oct	Collect: September 16th- October 21st. Collect mature flowers. Rub entire flower head over #25 sieve. Use of gloves when handling the plant is advised as plant can be spiky.
<i>Suaeda nigra</i>	Sep	Oct	Collect seeds from mid-September- October. Seeds ready for collection when they are hard, black, and shiny. The calyces will be brown and crumbly. Seeds can be stripped from plant by hand. Seeds should be spread out to dry before being processed/stored.
<i>Batis maritima</i>	Sep	Nov	Collect seed Sept-Nov (best early Oct-early Nov), as fruits mature and turn from green to white. Dried fruits should fragment easily, exposing seed
<i>Distichlis spicata</i>	Sep	Nov	Dioecious. Collect September 11th- November 4th. Mature inflorescences are panicles, 2-8 cm long. Seed is 2 mm long and brownish-gray at maturity. Rub seeds over #18 sieve to clean.
<i>Limonium californicum</i>	Sep	Nov	Collect seed Sept. 9- Nov 17th (Oct best). Collect entire flower heads, flower should detach easily when ripe. Rub flower heads over #20 sieve to clean
<i>Salicornia bigelovii</i>	Sep	Nov	Entire nflorescences should be collected and air dried. Seeds strip easily from inflorescences after drying.
<i>Artemisia tridentata</i> (?)	Sep	early winter	Strip the entire inflorescence by hand. Seeds will need to be cleaned and chaff removed.
<i>Heteromeles arbutifolia</i>	Oct	Jan	Collect seeds from Oct- Jan. Fruits should be clipped/stripped from branches when bright red. Soak berries in water to ferment slightly (over-soaking can be damaging). Separate seeds from the pulp (flotation will help remove pulp). Allow seeds to dry before storing.
<i>Ambrosia psilostachya</i>	Oct	Dec	Fruits form and seeds disseminate October-December.
<i>Isocoma menziesii</i> var. <i>vernonoides</i>	Oct	Dec	Harvest seed mid-October to mid-December. Collect achenes golden in color, as seeds are usually eaten by time achenes turn brown. Shake ripe heads over open containers to collect achenes. Alternatively, remove ripe heads and keep in porous bags. For I. acradenia Wall and Macdonald recommend rubbing flowers over a large screen, using a seed blower, and sieving over a #18 screen to separate seeds from bracts
<i>Salicornia pacifica</i> [<i>S. virginica</i>]	Oct	Dec	Collect inflorescences Oct- Dec (Nov-early Dec is best). Collect when tips of plants are purple. Dry seeds on screen up to 3 mo.
<i>Suaeda esteroa</i>	Oct	Dec	Collect seeds Oct-Dec (best Nov/early Dec). Seeds can be harvested by collecting whole inflorescence or stripping flowers with seeds from inflorescence. Seeds remain in flower until senescence. After cleaning, seeds should be dried
<i>Platanus racemosa</i>	Oct	spring	Collect seedpods October- early spring by cutting them directly from the tree. Cut off the stem and break seedpod open. Let the seeds dry 2-3 days. If the seed is not ripe yet, it will be difficult to break open
<i>Artemisia californica</i>	Dec	Jan	Collect seeds in December and January. Strip entire inflorescence by hand. Seeds will need to be cleaned.
<i>Salix lasiolepis</i>	Dec	Jan	Dioecious. Seeds can be hand harvested when capsules begin opening. Hardwood cuttings collected Dec 15- Jan 31st. Cuttings should be kept moist and cool. When ready to process, dip in mild bleach soln.
<i>Atriplex canescens</i>			Collect utricles when mature August-September in northern territories and October- March in southern.
<i>Baccharis glutinosa</i> [<i>B. douglasii</i>]			
<i>Baccharis sarothroides</i>			Dioecious. Seeds can be collected by hand into open breathable bags or branches can be shaken above open tubs or tarps
<i>Brickellia californica</i>			Long, narrow achenes. Dark brown in color when mature.
<i>Camissonia cheiranthifolia</i>			

Appendix 3.6D

Scientific Name	Start	End	Seed Collection Details (copy of draft palette)
<i>Elymus triticoides</i> [<i>Leymus triticoides</i>]			
<i>Encelia californica</i>			Achenes are densely compressed, wedge shaped. Edges are long-ciliate and faces are flabrous or short-hairy. Seeds should be dark brown at maturity. Collection timing is critical as achenes are easily blown from plant after reaching maturity.
<i>Eriogonum parvifolium</i>			Other members of Eriogonum genus: flower July-August; fruit is a hard, dry, three sided achene. Achenes can be hand stripped from plants.
<i>Euthamia occidentalis</i>			
<i>Hazardia squarrosa</i>			Fruit: 5–8 mm, 5-angled, glabrous; pappus 7–12 mm, white to red-brown in color
<i>Hordeum depressum</i>			No information found, use H. brachyantherum information as a rough guide
<i>Isolepis cernua</i> [<i>Scirpus cernuus</i>]			
<i>Juncus acutus</i> ssp. <i>Leopoldii</i>			
<i>Juncus balticus</i>			
<i>Juncus mexicanus</i>			
<i>Lasthenia glabrata</i> var. <i>coulteri</i>			
<i>Lycium californicum</i>			Seeds (berries) best collected within 2 weeks of setting, otherwise birds will eat the majority. Must be picked by hand
<i>Malacothamnus fasciculatus</i>			
<i>Phacelia ramosissima</i>			Collect seed when flowers are dry and brown. Strip seed from mature inflorescences directly into collection bag.
<i>Pluchea odorata</i>			
<i>Populus fremontii</i>			Dioecious.
<i>Potentilla anserina</i> ssp. <i>Pacifica</i>			Seeds should be dried on the plant, then collected. If using, root ball divisions should be made in spring.
<i>Pseudognaphalium californicum</i> [<i>Gnaphalium c.</i>]			
<i>Rumex salicifolia</i>			
<i>Salicornia subterminalis</i>			Collect inflorescences and air dry, seeds fall out of remaining fragments
<i>Sambucus nigra</i> [<i>S. mexicana</i>]			
<i>Schoenoplectus californicus</i> [<i>Scirpus c.</i>]			Collect, dry, and store seeds in brown paper bags or burlap bags. Seeds and seed heads need to be cleaned in a seed cleaner.
<i>Spartina foliosa</i>			Multiple harvests may increase probability of collected good seeds prior to dispersal or herbivory loss. Seed should be refrigerated dry for 2-4 weeks and then refrigerated in the dark in salt or fresh water.

Appendix 3.6E

Species	Seed Longevity	Storage Details (copy of draft palette)
<i>Lycium californicum</i>	1 year	Store in cool place. Will begin to mold after 1 week at room temp or 3-4 weeks at 40 F. separated seeds can be stored up to a year
<i>Limonium californicum</i>	1 year (ideally)	Dry, store at cool temperatures. Best if used w/in 1 year (Zedler 2001)
<i>Salicornia bigelovii</i>	1 year (ideally)	Should be stored in cool temperatures. Viability is reduced after 1 year.
<i>Heteromeles arbutifolia</i>	1 year (or less)	Seeds have limited longevity at room temperature. Store at cool temperatures, probably orthodox in storage behavior. Shelf life of less than one year.
<i>Baccharis sarothroides</i>	1+ year	Cleaned seeds can be stored dry over winter (and possibly longer)
<i>Atriplex californica</i>	10 years	Keep dry, store at room temperature. Atriplex seeds can be stored for 10+ years in tightly closed containers in a shed/warehouse
<i>Atriplex patula/triangularis</i>	10 years	Store at cool temperatures. Atriplex seeds can be stored for 10+ years in tightly closed containers in a shed/warehouse
<i>Atriplex watsonii</i>	10 years	Atriplex seeds can be stored for 10+ years in tightly closed containers in a shed/warehouse
<i>Salvia mellifera</i>	1-2 years	Store in cool, dry conditions. storage in a warehouse- 41.3% germination rate. Longevity increased in cold. 1-2 years ambient temps
<i>Isocoma menziesii</i> var. <i>vernoides</i>	1-2 years (or less)	Store in cool, dry conditions to increase longevity. Seeds are relatively short lived. After 2 years of storage, only 9.6% of seeds germinated
<i>Frankenia salina</i>	2 years (or less)	Keep seeds dry at room temperature. Seeds last up to 2 years.
<i>Batis maritima</i>	2+ years	Refrigerate (viability is 2+ years)
<i>Salicornia pacifica</i> [S. <i>virginica</i>]	2+ years	Seeds viable for 2+ years
<i>Suaeda esteroa</i>	2+ years	Stored at 5C. Seed germinability remains high after 2 years
<i>Rosa californica</i>	2-4 years	Seeds stored dry in sealed vials will retain viability for 2-4 years
<i>Salix lasiolepis</i>	3 years (freezer)	Can be stored for up to 10 days at room temperature or up to 1 mo in wet, refrigerated containers. Seed viability increases at cold temperatures (-10 C) and can be as long as 3 yrs- must use double 3 mil polyethylene bags
<i>Salix exigua</i>	3-4 years (freezer)	Dried to 6-10% of dry weight. Can be stored under constant humidity. Longevity: 1-5 C- 6 months, subfreezing (-10 or -20) can last up to 44 months.
<i>Achillea millefolium</i>	3-5 years	
<i>Atriplex lentiformis</i>	3-6 years	Seeds can be stored 3-6 years, have been successfully stored for 5 years (6 years is recorded max)
<i>Malacothamnus fasciculatus</i>	50+ (genus)	
<i>Atriplex canescens</i>	5-7 years	Reported good for 5-7 years in sealed containers at 21C. Dewinging may increase storability.
<i>Abronia maritima</i>	6 years	not indicated
<i>Acmispon glaber</i> [Lotus <i>scoparius</i>]	Long-lived	Long lived in soil seed bank and in cool, dry storage. Dried to low moisture content and stored in vacuum vials
<i>Hordeum brachyantherum</i>	Long-lived (4-5 years)	Keep dry in refrigerator
<i>Hordeum depressum</i>	Long-lived (4-5 years)	
<i>Melica imperfecta</i>	Long-lived (4-5 years)	Dry seeds should be refrigerated
<i>Stipa cernua</i> [Nassella c.]	Long-lived (4-5 years)	After drying and cleaning, seal in paper bags and store at 40 F and 40% RH.
<i>Stipa lepida</i> [Nassella l.]	Long-lived (4-5 years)	not indicated
<i>Lupinus chamissonis</i>	long-lived (many years)	Store dry seeds at room temperature. Longevity not noted
<i>Astragalus pycnostachyus</i> var. <i>lanosissimus</i>	long-lived (many years)	
<i>Astragalus tener</i> var. <i>titi</i>	long-lived (many years)	Maternal samples packaged in glassine envelopes and dried to equilibrium at 14% rel humidity. After 3 weeks, transfer seeds to heavy duty foil/plastic pouches at keep at 18 C
<i>Astragalus trichopodus</i>	long-lived (many years)	
<i>Iva axillaris</i>	Short-lived	Seeds may be relatively short lived if dry stored. Seeds should be stored submerged in water inside lumite screen bags
<i>Potentilla anserina</i> ssp. <i>Pacifica</i>	Short-lived	May be stored short term (longevity not noted)
<i>Spartina foliosa</i>	Short-lived, 4 months	Viability decreases after 4 months
<i>Abronia umbellata</i>		
<i>Ambrosia psilostachya</i>		
<i>Artemisia californica</i>		
<i>Artemisia douglasiana</i>		
<i>Artemisia tridentata</i> (?)		
<i>Baccharis glutinosa</i> [B. <i>douglasii</i>]		
<i>Baccharis pilularis</i>		Cleaned dry seeds can be stored at 1.7-4.5 C in airtight containers
<i>Baccharis salicifolia</i>		
<i>Brickellia californica</i>		
<i>Camissonia cheiranthifolia</i>		
<i>Cressa truxillensis</i>		
<i>Croton californicus</i>		
<i>Distichlis spicata</i>		Seeds are kept dry and stored in refrigerator
<i>Elymus triticoides</i> [Leymus <i>triticoides</i>]		
<i>Encelia californica</i>		
<i>Eriogonum fasciculatum</i>		Store in a cool, dry place
<i>Eriogonum parvifolium</i>		
<i>Euthamia occidentalis</i>		
<i>Grindelia camporum</i>		
<i>Hazardia squarrosa</i>		
<i>Heliotropium curassavicum</i>		
<i>Isolepis cernua</i> [Scirpus <i>cernuus</i>]		
<i>Isomeris arborea</i>		
<i>Jaumea carnosa</i>		Store with perlite to remove moisture at room temperature
<i>Juncus acutus</i> ssp. <i>Leopoldii</i>		
<i>Juncus balticus</i>		
<i>Juncus bufonius</i>		
<i>Juncus mexicanus</i>		
<i>Lasthenia glabrata</i> var. <i>coulteri</i>		

Appendix 3.6E

<i>Mimulus aurantiacus</i>		Store dry in refrigerator
<i>Monanthochloe littoralis (Distichlis littoralis)</i>		air dried, and stored in cool temp. stolon cuttings root well in moist soil.
<i>Oenothera elata</i>		
<i>Phacelia ramosissima</i>		
<i>Plantago erecta</i>		Cool, dry storage
<i>Platanus racemosa</i>		
<i>Pluchea odorata</i>		
<i>Populus fremontii</i>		
<i>Pseudognaphalium californicum</i> [<i>Gnaphalium c.</i>]		
<i>Rumex salicifolia</i>		
<i>Salicornia subterminalis</i>		Store in cool temperatures.
<i>Salvia apiana</i>		
<i>Sambucus nigra [S. mexicana]</i>		
<i>Schoenoplectus acutus</i>		
<i>Schoenoplectus californicus</i> [<i>Scirpus c.</i>]		
<i>Suaeda moquinii</i>		
<i>Suaeda nigra</i>		
<i>Suaeda taxifolia</i>		Store in cool temperatures
<i>Triglochin maritime</i>		Storage Conditions: Seeds are kept dry and stored at room temperature.
<i>Vulpia microstachys</i>		



Standard Operating Procedures (4.1): Fish Beach Seine

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Protocol Suitability Evaluation

A habitat suitability table containing appropriate estuarine wetland habitat types (of those evaluated) to implement the fish beach seine protocol is displayed in Table 1. Small seines may also be appropriate in emergent salt marsh, but they are difficult to implement with vegetation present. A comparative assessment of cost, effort, and data quality are shown in Table 2. A matrix of additional detailed categorical evaluations of the fish beach seine protocol can be found in Appendix 4.1A.

Table 1. Appropriate habitat types for fish beach seine survey protocols.

Survey Protocol	Habitat Types					
	Tidal Channel	Mud/sand flat	Emergent salt marsh	Non-tidal salt marsh	Salt pan	'Degraded' / fill
Fish Beach Seine	X	X				

Table 2. Categorical assessment of cost/effort and data quality for fish beach seine survey protocols.

		Evaluation Metric	Fish Beach Seine	Notes
Time / Effort	Office Preparation Time		30-60 minutes	Gather equipment, site selection
	Equipment Construction Time (one time)		> 60 minutes	Build the seine and blocking nets (unless already put together), including placing weights and floats and tying to posts
	Field Time (per station)		> 60 minutes	Depending on the number of fish and size of the station, each station may take 1-3 hours
	Laboratory Time (per station)		0 minutes	Not applicable, unless post quality control checks on species identifications are necessary
	Post-Survey Processing / QAQC Time		10-30 minutes	----
	Minimum Repetition (site-dependent)		Few Repetitions	As fish are highly mobile and variable, repetitions are encouraged but are often time/effort limited
	Relative Cost (equipment and supplies)		> \$1,000	Seines and blocking nets (may be expensive); wheelbarrow or other transportation
Survey / Data Quality	Accuracy (at a survey area level)		Medium	----
	Precision (at a survey area level)		Medium	Somewhat species-dependent; less effective for benthic species
	Qualitative-Quantitative Score		Quantitative	----
	Subjectivity-Objectivity Score		Objective	----

Resulting Data Types

The application of the fish beach seine survey protocol will yield quantitative data displayed as abundances by species or size frequency distributions across multiple time scales (e.g., seasonally, annually). These data are useful to identify the potential fish species composition / richness of particular wetlands, sub-areas, or habitats and to potentially identify the uses or functions of a particular wetland area by specific fish species (e.g., nursery). Data can be displayed as size frequency or abundance graphs, species presence tables, or at a higher-level using diversity indices. These data can also be used for multivariate community composition analyses. This survey protocol may also provide quantitative data on larger nektonic or demersal macroinvertebrate species such as shrimp or crabs (individuals must be larger than the net size to be captured).

Objective

Defining the fish assemblage of a wetland can be difficult, due to the highly mobile nature of the fauna. Fish are often among the first organisms to rapidly colonize restored habitats (Zedler 2001, Johnston et al. 2011). Wetlands act as nursery habitat for commercially and recreationally important species such as halibut (Beck et al. 2001), and are an assessable component of food web complexity, vertebrate diversity, overarching water quality conditions, and/or anthropogenic stressors (WRP 2006). For example, indicator fish such as the federally endangered tidewater goby prefer tidally restricted or calmer, brackish conditions (Swenson 1997).

The primary purpose of this sampling method is to quantitatively assess the distribution, relative abundances, species richness, and diversity of fish in intertidal wetland habitats using beach seines. While each type of fish sampling equipment (e.g., seines, trawls, enclosure traps, etc.) exhibits some degree of preferential capture or limitations to specific fauna, beach seines are generally appropriate for shallow, slow-moving water in tide channels or the equivalent habitat. As such, the geometry of the site (channel width) and tides should be the central factor in planning these surveys. Another goal of this SOP is to use a consistent method to develop quantitative, transferrable data for California wetland fish.

Monitoring methods have consistently used beach seines to quantify fish abundances, but studies such as Steele et al. (2006) have shown that slight variations in sampling protocols can create substantial differences; therefore, consistent methods between survey programs are essential. Additional survey methods may be employed to assess broader fish species richness or targeted species, including Gobiidae or highly mobile species.



Equipment

Equipment and supplies needed for this survey include:

1. GPS and extra batteries
2. Rulers or fish measuring board (Figure 1)
3. Fish seine net (1.8 m depth by 6 m width with 3.2 mm mesh delta style knotless nylon netting); must have floats at the top and a lead line at the base
 - a. Note: size of the net can be varied if needed for additional depth or if the goal is to catch smaller fish; abundance data



Figure 1. Round stingray being measured with a fish ruler (top), halibut being measured by fish measuring board (bottom).

from monitoring programs with different sized mesh may not be transferrable.

4. Two blocking nets (1.8 m depth by longer than the width of longest channel); must have floats at the top and a lead line at the base. The length of the net should be at least a few meters longer than the longest channel to be surveyed due to bowing from tidal currents and extra net to secure at the ends.

Helpful hint: Larger nets are more difficult, logistically, for access and mobility to stations. If possible, a small wagon or dual-wheel wheelbarrow is recommended for transport.

5. Wetsuit (optional) or chest waders (if water is < 1.2 m)
6. Neoprene dive/surf booties and gloves
7. Aquarium nets (at least two, more are preferable)
8. Buckets and plastic containers (2-4 large buckets are recommended, with multiple other containers of varying sizes)
9. Camera and extra batteries or phone with camera application
10. Ice or MS22 if these are required for Institutional Animal Care and Use Committee (IACUC) or similar fish handling protocols
11. Scale (optional) Note: weighing of fish can be quite time consuming, may result in additional mortality and can be calculated fairly accurately using standard length; alternately, a subset of fish may be weighed, and the data extrapolated.
12. Datasheets (Appendix 4.1B)
13. Tarp (optional for protection of the transport vehicle) and to lay out at a station, if desired
14. Pliers or wire clippers for handling stingrays or spined fish

Helpful hint: If night fishing is part of the project or site monitoring goal, additional equipment will be necessary, including flashlights, headlamps, lanterns, and/or glowsticks.

Field Preparation

Site selection for fishing stations should follow guidelines developed for the SONGS Wetland Monitoring Program (CCC 2006). Many programs throughout California follow those guidelines, which were based on protocols developed by Dr. Joy Zedler. These guidelines recommend sampling multiple stations per estuary (e.g., 10 for a large wetland, fewer for a smaller site) spaced a minimum of 100 m apart to decrease chances for spatial autocorrelation. The stations should cover the range of tidal conditions of the estuary (e.g., creeks, channels, and/or basins). For extremely wide channels or basins, a separate field deployment configuration for the nets is recommended. Mouths of tidal channels may be challenging when experiencing higher tidal flows, due to the dragging of the nets.

Equipment described above should be collected prior to the field shift. Batteries for all electronic devices should be checked and replaced as needed, and relevant data sheets should be printed and attached to the clipboards. *Helpful hint: Waterproof paper can help reduce potential damage to field data sheets, though it is more expensive.*

Field Methods

June and September are recommended as the targeted survey months to coincide with peak fish abundances, but additional survey times (e.g., March or December) may be added by individual site needs or if additional time may be allocated (Zedler 2001). Sampling should not be conducted within 72 hours of a rain event due to the shift in freshwater conditions.

Station and seining protocols [recommended from SONGS Wetland Monitoring Program (CCC 2006)]:

1. Photograph each station before beginning (preferably with a GPS-enabled camera or smart phone from the center of the blocking nets facing across the channel).
2. For each station, position two blocking nets approximately six meters apart on the channel bank with the smaller seine in the middle. *Helpful hint: arrange the nets on the ground so upon deployment they will unfurl continuously from the bank without tangling. This may involve unrolling them prior to deployment and having one person at each net deploying the lead line.*
3. Deploy blocking nets (Alternative 1) (this configuration is recommended for small channels and creeks, or anything approximately < 30 m):
 - a. Blocking nets should be deployed perpendicular to the shore and across the entire channel (Figure 2) to help prevent fish from escaping the survey area (Nordby and Zedler 1991, Steele et al. 2006, WRP 2006). Deploy both blocking nets slowly and simultaneously, at a rate of approximately one meter every few seconds (variable based on sediment composition). It is essential that the lead line (weighted line at the bottom of the net) remains in constant contact with the bottom substrate.
 - i. Blocking nets can be deployed either by walking them across the channel or in a two-person kayak with one person paddling and the other facing backwards and unfurling the net from the back of the kayak.



Figure 2. Deployed blocking nets across a wetland channel.

- b. After the blocking nets are deployed, walk back across the lead line to ensure consistent contact across the bottom of the channel, and check to make sure the float line is above the water surface.
4. Deploy blocking nets (Alternative 2) [this configuration is recommended for large (wide) channels or basins, or open water]:
 - a. Blocking nets should be deployed with the person on each side walking straight out into the water approximately six meters (simultaneously), and then beginning to have each person who is deploying the blocking nets begin to walk towards each other until they intersect and overlap (Johnston et al. 2019, Figure 3). Deploy both blocking nets slowly and simultaneously, at a rate of approximately one meter every few seconds (variable based on sediment composition). It is essential that the lead line (weighted line at the bottom of the net) remains in constant contact with the bottom substrate. Once the blocking nets intersect, they should be joined such that there are no gaps between them for fish to escape.
 - i. Someone should remain at the intersection of the nets to keep them joined (wrapping or holding the poles together is effective and pushing them into the sediment slightly) and to keep the lead lines pressed to the bottom.



Figure 3. Deployed blocking nets and walking out a fish seine across a large wetland channel (> 50 m).

5. Complete five replicate seine pulls across the channel (parallel to the shoreline for Alternative 1 or within the “triangle” of Alternative 2) in between the previously deployed blocking nets by

pulling the poles slowly and steadily with the lead line in constant contact with the substrate. Five replicates allow for most individual fish to be collected (Steele et al. 2006, CCC 2006).

- a. Upon completion of each individual seine pull, bring the seine onto shore (lead line first with float line immediately following until the net is folded like a taco to prevent fish from escaping).
 - b. Place fish immediately into large buckets of water labeled with the seine number (i.e., 1-5). Take care to transfer the mid-water fish (e.g., topsmelt, anchovies) as quickly as possible to the buckets as these fish tend to be most susceptible to asphyxiation (Skinner et al. 1962). To avoid mortality, conduct counts of each seine immediately after completion, and some species may be counted and immediately released back into the channels. *Helpful hint: try to get the fish back in the water as soon as possible, especially mid-water fish, but do not release within or adjacent to deployed nets.*
 - c. Thoroughly search each seine multiple times. *Helpful hint: large chunks of algae can be discarded outside of the survey area after they are thoroughly searched for small or cryptic fish. This will allow the seine to be checked faster and with less repetitive effort.*
6. Retrieve the blocking nets (Figure 4) with nested, inward arcs and transfer those fish to buckets (see 5a-c). Maintain as close proximity as possible with the two sets of nets without overlapping. Tap the poles along the bottom and maintain lead line contact.



Figure 4. Retrieving blocking nets in a wetland channel.

Fish identification and measuring protocols:

1. Transfer fish immediately from the nets (Figure 5) into buckets filled with seawater to be measured and identified to species using fish field guides (Miller and Lea 1972, Allen et al. 2006). Appendix 4.1C is an abbreviated fish guide for southern California tidal marshes.

2. If there are fewer than 30 individuals of a species, all fish standard lengths (most anterior part of the upper or lower jaw to caudal peduncle) should be measured to the nearest millimeter (Merkel and Woodfield 2007, City of Los Angeles 2005, Figure 6). If more than 30 individuals of a given species are collected in each seine, there are two options for measuring (Alternative 1 and 2, below).
 - a. Alternative 1: Only the first 30 “randomly” selected individuals of each species will be measured. The remaining fish of that species (> 30) should be counted and held for release in the buckets. This method is not preferred because there is no way to truly determine a random assortment of fish sizes. Grabbing fish at “random” can lead to selection of larger individuals. Small handheld nets swung around without looking into the buckets can assist in reducing selectiveness.
 - b. Alternative 2: Measure every fish of a given species of interest (e.g., flatfish, special status species, warm water, or specialized species, etc.).



Figure 5. Searching through the pulled seine for fish transfer into buckets.



Figure 6. Measuring captured fish.

3. Fish that are too small (e.g., gobies ≤ 10 mm) to accurately identify in the field should be labeled as juveniles.
4. After being counted and measured, fish should be transferred to a release bucket (or released immediately – see seining protocol 5b).
5. Once a seine has been fully counted and measured, the fish may be released outside of the immediate station area (to avoid recapture). Repeat steps 1-5 for all five seines and the blocking nets.
6. (Optional) record macroinvertebrate catch data. Programs should be consistent in this level of data collection across surveys. Many wetland monitoring programs count and record macroinvertebrates of specific species such as crabs (e.g., *Cancer* spp., *Portunus* spp., etc.), invasive species (e.g., oriental shrimp, *Palaemon macrodactylus*), or other species of concern.
7. Complete the datasheet including start time, duration of survey, cloud cover, and precipitation (Appendix 4.1B).

Laboratory Methods

Not applicable.

Data Entry and QAQC Procedures

Data should be entered in the field using the appropriate data sheet (Appendix 4.1B). All required fields should be completed in full, and the data recorder should assign their name at the top of the document(s). Data should be transferred to the appropriate electronic database within three days, and the hard copies filed in labeled binders. Electronic copies of all data should be housed on an in-house

dedicated server and backed up to a cloud-based or off-site server nightly. Hard copies should be saved for five years. Electronic copies should be saved indefinitely.

Quality Assurance and Quality Control (QAQC) procedures should be conducted on all data. QAQC procedures should be conducted by the QA Officer and include a thorough review of all entries, double checking of all formulas or macros, and a confirmation that all data sheets, Chain-of-Custody forms, and field notes are filed appropriately with electronic back-up copies available. QAQC should verify that the entered data match the hard copies of the field data sheets. Any discrepancies should be corrected, and the initial data entry technician notified.

Data Analyses

After data have been entered, corrections made, and QAQC procedure completed, data can be used in multiple analyses. Examples include abundances by species (Figure 7), size frequency distributions, species presence or abundance by station or wetland, etc. If length-weight ratios are developed for individual species, then biomass estimates can be conducted.

Health and Safety Precautions

Care should be taken when handling species with spines (e.g., sculpin, stingrays) or sharp teeth (e.g., lizardfish). Additionally, appropriate attire and clothing should be worn for comfort and warmth in exposure to cold water for extended periods of time, e.g., wetsuit or waders (Figures 8 and 9). Bivalves or other invertebrates with sharp features can also be present in survey areas.

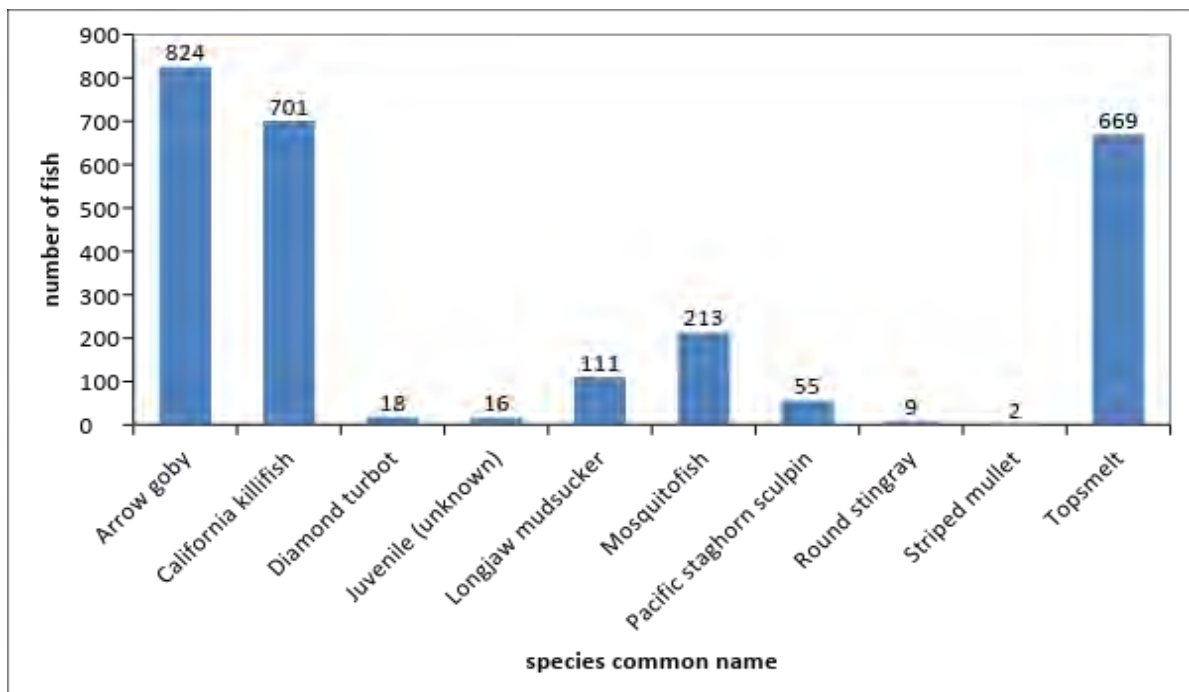


Figure 7. Total counts of each species of fish caught in the beach seine surveys across all stations (N = 6) throughout the first Baseline year (note: data are not intended for comparison, just reference to potential visualization type).



Figure 8. Photograph of fish seine being deployed at Malibu Lagoon.



Figure 9. Photograph of fish measuring board used to assess length of caught fish.

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APPENDIX 4.1A

	Evaluation Metric	Fish Beach Seine	Notes
	Correlation to L2 CRAM	Attribute 2	Hydrology-dependent
Personnel Requirements	Specialty Equipment or Clothing Required	Many Specialty Items	Fish seines and blocking nets, aquarium nets, wetsuits
	Ease of Transport (amount or weight of supplies)	Many or Heavy Items / Difficult	See above (nets can be very heavy / bulky)
	Ease of Implementation	Difficult	Time consuming and a high level of coordination is required for successful implementation; field training is recommended
	Expertise / Skill Level	Some Technical Knowledge	Familiarity with species identifications is required
	Number of Personnel	> 3	----
	Training Requirements	None	----
	Seasonality of Survey Time	Spring and Fall	Both seasons are required to capture the breadth of fish activity and species diversity
	Suggested Frequency	Semi-annual	Or more frequent, project-dependent
Survey / Data Quality	Type of Output	Numerical	----
	Active or Passive Monitoring Style	Active	----
	Specialty Computer Software Required	No	----
	Availability of Online / External Resources	Many	----
Potential Limitations	Wetland Type Applicability	Bar-built and Estuarine	Must have tidal influence or prolonged water exposure
	Images or Multi-Media Required	Images Required	Photos are also helpful for species identifications
	Degree of Impact / Disturbance	High Disturbance	Walking and dragging nets through tidal channels will disturb sediments
	Vegetation Height Limitation	Not Applicable	----
	Appropriate for Tidal / Wet Habitats	Yes	----
	Tide Height	Medium to High Tide Only	Implementation within flood and ebb tides may be possible in full tidal environments
	Regional or Broad Implementation *	Almost Always Used	----
	Potential for Hazards / Risk	Medium Risk	----
	Restrictions	Special Status Species	----

* based on monitoring literature review

APPENDIX 4.1B

FISH SAMPLING DATA SHEET

Sampling Program Information	
DATE:	GEAR:
STATION:	PAGE: <u> </u> of <u> </u>
STAFF:	
WEATHER:	

Rep	Start time / Stop time	Haul length
1	/	
2	/	
3	/	
4	/	
5	/	

#	REP	SPECIES	SL (mm)	WT (g)	#	REP	SPECIES	SL (mm)	WT (g)
1					36				
2					37				
3					38				
4					39				
5					40				
6					41				
7					42				
8					43				
9					44				
10					45				
11					46				
12					47				
13					48				
14					49				
15					50				
16					51				
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24					59				
25					60				
26					61				
27					62				
28					63				
29					64				
30					65				
31					66				
32					67				
33					68				
34					69				
35					70				

COMMENTS:

** measure first 30 of each species
** additional counts on back

APPENDIX 4.1B

#	REP	SPECIES	SL (mm)	WT (g)	#	REP	SPECIES	SL (mm)	WT (g)
71					116				
72					117				
73					118				
74					119				
75					120				
76									
77									
78									
79									
80									
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110									
111									
112									
113									
114									
115									

1. SPECIES: _____
2. 30 individuals measured? _____ (y / n)
3. COUNT (> 30 but < 100) _____ (#)
4. BATCH WT (> 30 but < 100) _____ (g)
5. BATCH WT (remaining) _____ (g)

1. SPECIES: _____
2. 30 individuals measured? _____ (y / n)
3. COUNT (> 30 but < 100) _____ (#)
4. BATCH WT (> 30 but < 100) _____ (g)
5. BATCH WT (remaining) _____ (g)

1. SPECIES: _____
2. 30 individuals measured? _____ (y / n)
3. COUNT (> 30 but < 100) _____ (#)
4. BATCH WT (> 30 but < 100) _____ (g)
5. BATCH WT (remaining) _____ (g)

1. SPECIES: _____
2. 30 individuals measured? _____ (y / n)
3. COUNT (> 30 but < 100) _____ (#)
4. BATCH WT (> 30 but < 100) _____ (g)
5. BATCH WT (remaining) _____ (g)

1. SPECIES: _____
2. 30 individuals measured? _____ (y / n)
3. COUNT (> 30 but < 100) _____ (#)
4. BATCH WT (> 30 but < 100) _____ (g)
5. BATCH WT (remaining) _____ (g)

1. SPECIES: _____
2. 30 individuals measured? _____ (y / n)
3. COUNT (> 30 but < 100) _____ (#)
4. BATCH WT (> 30 but < 100) _____ (g)
5. BATCH WT (remaining) _____ (g)

1. SPECIES: _____
2. 30 individuals measured? _____ (y / n)
3. COUNT (> 30 but < 100) _____ (#)
4. BATCH WT (> 30 but < 100) _____ (g)
5. BATCH WT (remaining) _____ (g)

1. SPECIES: _____
2. 30 individuals measured? _____ (y / n)
3. COUNT (> 30 but < 100) _____ (#)
4. BATCH WT (> 30 but < 100) _____ (g)
5. BATCH WT (remaining) _____ (g)

NOTES:

Appendix 4.1C

CLIO

Clevelandia ios

Arrow goby

Dorsal spines (total): 4 - 5; Dorsal soft rays (total): 15 - 17; Anal spines: 0; Anal soft rays: 14 - 17. Caudal rounded



FUPA

Fundulus parvipinnis

California killifish

Max 11cm; squarish tail fin; small pelvic fin, long anal w/ 11-13 rays: olive-green above, and a yellowish brown below



HYGU

Hypsopsetta guttulata

Diamond turbot

Max 46.0 cm; flattened/compressed body; triangular shape



PACA

Paralichthys californicus

California halibut

Max 152.0 cm; typically weighs 6 to 50 pounds (3 to 23 kg); flattened/compressed body; both eyes on one side of head



MUCE

Mugil cephalus
Striped mullet

bluish-gray/greenish above, silver along the sides, white on ventral surface; 6-7 black horizontal bars along sides; no lateral line; pectoral fins high on shoulders, pelvic fins abdominal



ATAF

Atherinops affinis
Topsmelt

silver, w/shiny silver lateral band; blue or green coloration dorsally; gills = golden-yellow; eyes small and beady; top lip folded down; long pelvic fins



GIMI

Gillichthys mirabilis
Longjaw mudsucker

Max 21.0 cm; first dorsal fin is relatively small, with 4-8 spines; second dorsal fin is larger, with 10-17 rays



GAAF

Gambusia affinis
Mosquitofish

small and stout, dull grey, robust fish with a rounded tail and a terminal and upward-pointing mouth



Appendix 4.1C

LEAR

Leptocottus armatus

Pacific staghorn sculpin

Max 46.0 cm, spines just anterior of gills, stripes on fins, slightly dorsally flattened

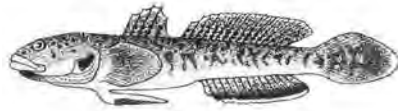


ILGI

Ilypnus gilberti

Cheekspot goby

Max 6.4 cm; shadow spot anterior to pectoral fin



URHA

Urobatis halleri

Round stingray

nearly round pectoral fin disc; brown or grayish above; pale yellow spots or reticulations; underside white to yellowish; tail short and stout, with a long, thick, serrated stinging spine



POLA

Poecilia latipinna

Sailfin molly

body oblong; head small and dorsally flattened, w/small, upturned mouth; caudal peduncle broad & large, rounded, and sometimes tipped with black





Standard Operating Procedures (4.2): Fish Minnow Trap and Enclosure Trap

March 2021

Prepared for the United States Environmental
Protection Agency



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Standard Operating Procedures: Fish Minnow Trap and Enclosure Trap

SOP Identification: SOP 4.2 Fish Minnow Trap and Enclosure Trap

Date of Original Issue: 29 March 2021

Date of Last Revision: 29 March 2021

Developed by: The Bay Foundation

Protocols reviewed by:

Karina Johnston, The Bay Foundation

Jeff Crooks, Tijuana River National Estuarine Research Reserve

Christine Whitcraft, California State University, Long Beach

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Protocol Suitability Evaluation

A habitat suitability table containing appropriate estuarine wetland habitat types (of those evaluated) to implement the fish minnow trap and enclosure trap protocol is displayed in Table 1. Both protocols require consistent tidal or flooded influence; thus, emergent salt marsh is possible, but only during high tide conditions. A comparative assessment of cost, effort, and data quality are shown in Table 2. A matrix of additional detailed categorical evaluations of the fish minnow trap and enclosure trap protocol can be found in Appendix 4.1A.

Table 1. Appropriate habitat types for fish beach seine survey protocols.

Survey Protocol	Habitat Types					
	Tidal Channel	Mud/sand flat	Emergent salt marsh	Non-tidal salt marsh	Salt pan	'Degraded' / fill
Minnow Trap	X	X	X (high tide)			
Enclosure Trap	X	X	X (high tide)			

Table 2. Categorical assessment of cost/effort and data quality for fish minnow trap and enclosure trap survey protocols.

	Evaluation Metric	Minnow Trap	Enclosure Trap	Notes
Time / Effort	Office Preparation Time	30-60 minutes	30-60 minutes	Gather equipment, site selection
	Equipment Construction Time (one time)	10-30 minutes	> 60 minutes	Minnow traps largely pre-constructed; enclosure traps require assembling
	Field Time (per station)	10-30 minutes	> 60 minutes	For both, more fish will require more time
	Laboratory Time (per station)	0 minutes	0 minutes	Not applicable, unless post quality control checks on species identifications are necessary
	Post-Survey Processing / QAQC Time	10-30 minutes	10-30 minutes	----
	Minimum Repetition (site-dependent)	Many Repetitions	Many Repetitions	As fish are highly mobile and variable, repetitions are encouraged but may be time/effort limited
	Relative Cost (equipment and supplies)	> \$100	> \$100	Minnow traps can be purchased constructed; enclosure traps will need to be made by hand
Survey / Data Quality	Accuracy (at a survey area level)	Low	Medium-Low	----
	Precision (at a survey area level)	Medium	Medium	Heavily species-dependent for both protocols
	Qualitative-Quantitative Score	Quantitative	Quantitative	Relative to time deployed for minnow traps
	Subjectivity-Objectivity Score	Objective	Objective	----

Resulting Data Types

The application of the fish minnow trap and enclosure trap protocol will yield quantitative data displayed as abundances by species or size frequency distributions across multiple time scales. These data are useful to identify targeted species or guilds within particular wetlands, sub-areas, or habitats and to potentially identify the uses or functions of a particular wetland area by specific fish species (e.g., nursery). Both survey methods are targeted towards specific guilds of fish.

Objective

Defining the fish assemblage of a wetland can be difficult, due to the highly mobile nature of the fauna. Fish are often among the first organisms to rapidly colonize restored habitats (Zedler 2001). Wetlands act as nursery habitat for commercially and recreationally important species such as halibut (Beck et al. 2001), and are an assessable component of food web complexity, vertebrate diversity, overarching water quality conditions, and/or anthropogenic stressors (WRP 2006). For example, indicator fish such as the federally endangered tidewater goby prefer tidally restricted or calmer, brackish conditions (Swenson 1997).

The primary purpose of this sampling method is to quantitatively assess the distribution and relative abundances of targeted fish guilds in intertidal wetland habitats using two protocols. While each type of fish sampling equipment exhibits some degree of preferential capture or limitations to specific fauna, minnow traps (Figure 1) tend to capture highly mobile fish species, whereas enclosure traps are targeted towards fish associated with benthos. As such, the general understanding of the fish community and location are key factors in planning these surveys. Minnow traps have been highly successful at capturing typical marsh fish species attracted to bait (e.g., California killifish), while enclosure traps are generally better at capturing fish who reside in benthos or burrows in sediment (e.g., Gobiidae). Additional survey methods are recommended to assess broader fish species richness or diversity (e.g., fish beach seine SOP 4.1).

Equipment

Equipment and supplies needed for both surveys include:

1. GPS and extra batteries
2. Rulers or fish measuring board
3. Aquarium nets (at least two, more are preferable)
4. Buckets and plastic containers (at least two large buckets are recommended, with multiple other containers of varying sizes)
5. Camera and extra batteries or phone with camera application
6. Scale (optional) Note: weighing of fish can be quite time consuming, may result in additional mortality and can be calculated fairly accurately using standard length; alternately, a subset of fish may be weighed, and the data extrapolated.
7. Datasheets (Appendix 4.1B)
8. Tarp (optional for protection of the transport vehicle) and to lay out at a station, if desired



Figure 1. Deployed minnow trap.

Helpful hint: If night fishing is part of the project or site monitoring goal, additional equipment will be necessary, including flashlights, headlamps, lanterns, and/or glowsticks.

Additional supplies for minnow trap survey:

1. Minnow traps and clip (however many the monitoring program requires to be deployed simultaneously; Figure 2)
 - a. Minnow traps can be purchased from any fishing store or online and are relatively cheap (\$15)
2. Line or rope to attach to shore
3. PCV stake to attach line
4. Pantyhose (optional)
5. Dog food / cat food
 - a. Other bait can also used (e.g., squid or shrimp) but should be consistent among sites
6. Marking flags (optional)
7. Zip ties (optional)

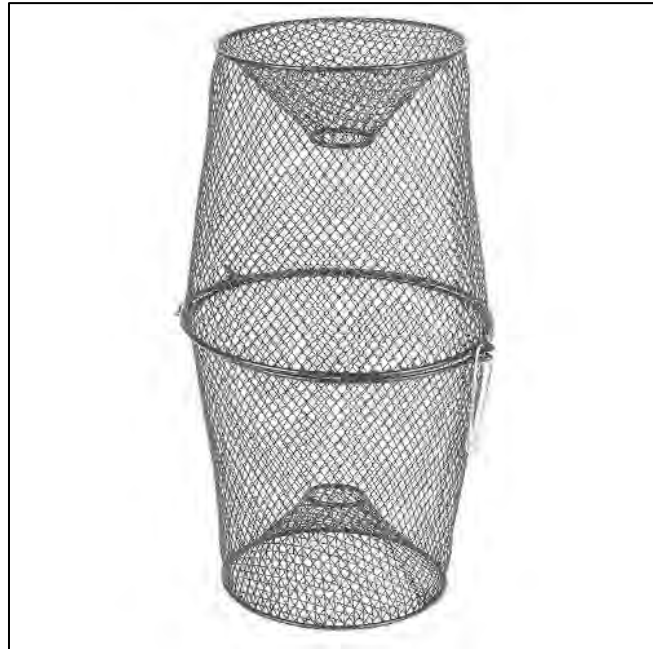


Figure 2. Minnow trap.

Additional supplies for enclosure trap survey (Figure 3): *

1. 3 mm thick sheets of translucent (white) polypropylene plastic (0.9 m in height); two plastic sheets may be needed (standard size is 2.4 x 1.2 m)
2. Lead weights (1.8 kg)
3. Wetsuits or waders / dive or surf booties
4. BINKE nets (benthic ichthyofauna net for coral/kelp environments); see Anderson and Carr 1998 or recommendations in Steele et al. 2006 for specifics. Long-handled large aquarium nets are an alternative option than building a custom BINKE net; however, aquarium nets are far less effective at capturing fish and will require more effort (repetitions) to capture most fish.
5. Long-handled aquarium dip nets (large mouth)

** Supplies recommendations for enclosure traps follow those from Steele et al. 2006 for the 0.5 m² sampling area (best recommendation for combination of effectiveness and logistics) or the 0.43 m² sampling area, which only will require one sheet of plastic to construct. For 0.5 m², the final trap dimensions should be 0.80 m diameter and 2.5 m circumference.*



Figure 3. Enclosure trap being deployed (left), and BINKE net (right).

Field Preparation

Site selection for fishing stations should follow guidelines developed for the SONGS Wetland Monitoring Program (CCC 2006). Many programs throughout California follow those guidelines, which were based on protocols developed by Dr. Joy Zedler. These guidelines recommend sampling multiple stations per estuary spaced a minimum of 100 m apart to decrease chances for spatial autocorrelation. The stations should cover the range of tidal conditions of the estuary (e.g., creeks, channels, and/or basins).

While minnow traps come largely assembled (just need to be clipped or locked), enclosure traps require construction and may vary depending on the monitoring program goals. Enclosure traps should be assembled in the lab prior to field deployment, following protocols and recommendations from Steele et al. 2006. Enclosure traps, also known as drop traps, throw traps, and drop samplers, are bottomless containers of various sizes and shapes that rapidly enclose a known volume of water, trapping mobile animals within them (Steele et al. 2006). The trapped animals can then be removed with nets. Enclosure traps have a higher catch efficiency than most traditional fishing gear (e.g., seines, trawls) (Steele et al. 2006).

Equipment described above should be collected prior to the field shift. Batteries for all electronic devices should be checked and replaced as needed, and relevant data sheets should be printed and attached to the clipboards. *Helpful hint: Waterproof paper can help reduce potential damage to field data sheets, though it is more expensive.*

Field Methods

June and September are recommended as the targeted survey months to coincide with peak fish abundances, but additional survey times (e.g., March or December) may be added by individual site

needs or if additional time may be allocated (Zedler 2001). Sampling should not be conducted within 72 hours of a rain event due to the shift in freshwater conditions.

Minnow trap protocols:

1. Cut approximately 6 in (or 15 cm) sections from the pantyhose, and tie one end if both ends are open. Place one handful of dog or cat food in the pantyhose pouch.
2. Fit two sides of the minnow trap together, but do not clip shut. Lower the filled pantyhose pouch into the assembled minnow trap with enough of the open end to tie a knot remaining outside the minnow trap once it is clipped shut. *Helpful hint: while pantyhose are not required, they are recommended so that the bait does not break apart or is just eaten from the outside of the trap.*
3. Close the minnow trap securely with the clip and tie the pantyhose pouch to the clip with the remaining extra pantyhose on the outside of the minnow trap. *Helpful hint: Zip ties may also be used to ensure the minnow trap securely remains shut through the collection process, but scissors or a knife must be available to open the traps once collected.*
4. Cut a line or rope long enough to ensure the minnow trap will be submerged in the desired location but can also be accessible on land (usually about 4-5 meters). Tie one end of the line using a bowline knot to the clip. The minnow trap is now ready for deployment.
5. Bundle the minnow trap line in one hand and have the minnow trap ready to toss in the other. Toss the minnow trap in the desired location, ensuring that the trap lay horizontally so that both open holes are available for fish to swim into (Figure 4).
6. Tie the remaining line onto a PVC stake or marker flag using a clove hitch, and place marker flag into the ground. The duration of the minnow trap remaining in the field depends on your location, tidal conditions, and monitoring program objectives.
7. Once the minnow traps are ready for collection, pull the minnow traps up, unclip the opening (or cut the zip tie if used) and empty the fish into a bucket of water.
8. Rinse minnow traps and line with fresh water and throw away pantyhose pouch.



Figure 4. Deployed minnow trap.

Note: if the survey area is tidal, a medium-high tide is desirable. If the minnow trap is deployed during low tide, the tide may be too low for fish to reach the entrance hole, or potential hypoxia may expose the collected subjects to stressful or fatal conditions. In tidal areas, the maximum duration of time a minnow trap should be deployed is about six hours. Shorter time frames (3-4 hours) may prevent overcrowding in the minnow trap, or predation.

Enclosure trap protocols (follow methods developed by Steele et al. 2006 for the SONGS monitoring program, CCC 2006):

1. Construct the enclosure trap and BINKE nets in advance of fieldwork (see Steele et al. 2006)
2. Deploy enclosure trap by tossing into appropriate habitat area, allowing to sink, and then pressing firmly into the mud. Enclosure trap should be deployed in water at a lower depth than the maximum height of the plastic, such that the fish cannot escape over the lip of the trap. Pressing the trap into the mud will ensure that benthic fish are retained in the trap (Figure 3).
3. Use the BINKE net to sweep the entire enclosure area, trying to stick as close to the walls of the enclosure trap as possible, and pressing it several centimeters into the sediment before snapping closed and removing. Try to shift the sediment out as much as possible before transferring fish to the buckets.
4. Repeat the BINKE net sweeps until you have 1-3 fishless net sweeps (be consistent; the number should be determined by program objectives, effort, and density of fish in each wetland area)
5. Replicate stations (depends on monitoring program objectives, but due to a high level of variability, replication is recommended)

Note: previous studies have determined that burrow-dwelling fish species are still effectively captured using the enclosure trap method (Steele et al. 2006). No chemicals are recommended by this protocol.

Fish identification and measuring protocols:

1. Transfer fish immediately from the minnow traps or enclosure traps into buckets filled with seawater. Identify to species and measure each fish using fish field guides (Miller and Lea 1972, Allen et al. 2006). Appendix 4.1C is an abbreviated fish guide for southern California tidal marshes.
2. If there are fewer than 30 individuals of a species, all fish standard lengths (most anterior part of the upper or lower jaw to caudal peduncle) should be measured to the nearest millimeter (Merkel and Woodfield 2007, City of Los Angeles 2005). If more than 30 individuals of a given species are collected in a single deployment of either survey method, there are two options for measuring (Alternative 1 and 2, below).
 - a. Alternative 1: Only the first 30 “randomly” selected individuals of each species will be measured. The remaining fish of that species (> 30) should be counted and held for release in the buckets. This method is not preferred because there is no way to truly determine a random assortment of fish sizes. Grabbing fish at “random” can lead to selection of larger individuals. Small handheld nets swung around without looking into the buckets can assist in reducing selectiveness.

- b. Alternative 2: Measure every fish of a given species of interest (e.g., flatfish, special status species, warm water, or specialized species, etc.).
3. Fish that are too small (e.g., gobies ≤ 10 mm) to accurately identify in the field should be labeled as juveniles.
4. After being counted and measured, fish should be transferred to a release bucket (or released immediately) (Figure 5).
5. Once a trap has been fully counted and measured, any retained fish may be released outside of the immediate station area (to avoid recapture). Some surveyors choose to hold fish in the release bucket until all fishing is completed, ensuring that the bucket is not too hot or holding too many fish, but this can increase mortality. Repeat steps 1-5 for all stations.
6. (Optional) record macroinvertebrate catch data. Programs should be consistent in this level of data collection across surveys. Many wetland monitoring programs count and record macroinvertebrates of specific species such as crabs (e.g., *Cancer* spp., *Portunus* spp., etc.), invasive species (e.g., oriental shrimp, *Palaemonetes* sp.), or other species of concern.
7. Complete the datasheet including start time, duration of survey, cloud cover, and precipitation (Appendix 4.1B).

Laboratory Methods

Not applicable.

Data Entry and QAQC Procedures

Data should be entered in the field using the appropriate data sheet (Appendix 4.1B). All required fields should be completed in full, and the data recorder should assign their name at the top of the document(s). Data should be transferred to the appropriate electronic database within three days, and the hard copies filed in labeled binders. Electronic copies of all data should be housed on an in-house dedicated server and backed up to a cloud-based or off-site server nightly. Hard copies should be saved for five years. Electronic copies should be saved indefinitely.

Quality Assurance and Quality Control (QAQC) procedures should be conducted on all data. QAQC procedures should be conducted by the QA Officer and include a thorough review of all entries, double checking of all formulas or macros, and a confirmation that all data sheets, Chain-of-Custody forms, and field notes are filed appropriately with electronic back-up copies available. QAQC should verify that the entered data match the hard copies of the field data sheets. Any discrepancies should be corrected, and the initial data entry technician notified.

Data Analyses

After data have been entered, corrections made, and QAQC procedure completed, data can be used in multiple analyses. Examples include abundances by species, size frequency distributions, species presence or abundance by station or wetland, etc. If length-weight ratios are developed for individual species, then biomass estimates can be conducted. Note that survey methods collect disparate data. While multiple combinations of survey methods may contribute to an overall master species list, the

data should not be compared quantitatively for abundances. Individual stations (e.g., for minnow traps) may be compared to each other or over time, if consistent effort (deployment time) was used.

Health and Safety Precautions

Care should be taken when handling species with spines (e.g., sculpin, stingrays) or sharp teeth (e.g., lizardfish). Additionally, appropriate attire and clothing should be worn for comfort and warmth in exposure to cold water for extended periods of time, e.g., wetsuit or waders. Bivalves or other invertebrates with sharp features can also be present in survey areas.



Figure 5. Surveyors measuring and counting fish from deployed trap.

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Contact Information

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APPENDIX 4.1A

	Evaluation Metric	Minnow Trap	Enclosure Trap	Notes
	Correlation to L2 CRAM	Attribute 2	Attribute 2	Hydrology-dependent
Personnel Requirements	Specialty Equipment or Clothing Required	Many Specialty Items	Many Specialty Items	Minnow traps, aquarium nets, enclosure traps, BINKE nets, fish measuring board
	Ease of Transport (amount or weight of supplies)	Many or Heavy Items / Difficult	Many or Heavy Items / Difficult	Both sampling methods require bulky supplies, but not heavy
	Ease of Implementation	Easy	Medium	Enclosure traps may require field calibration
	Expertise / Skill Level	Some Technical Knowledge	Some Technical Knowledge	Familiarity with species identifications is required
	Number of Personnel	2 +	> 2	----
	Training Requirements	None	None	Recommended that someone with enclosure trap experience join the field team the first time
	Seasonality of Survey Time	Spring and Fall	Spring and Fall	Both seasons are required to capture the breadth of fish activity and species diversity; spring will capture juveniles
	Suggested Frequency	Semi-annual	Semi-annual	Or more frequent, project-dependent
Survey / Data Quality	Type of Output	Numerical	Numerical	----
	Active or Passive Monitoring Style	Active	Active	----
	Specialty Computer Software Required	No	No	----
	Availability of Online / External Resources	Many	Many	----
Potential Limitations	Wetland Type Applicability	Bar-built and Estuarine	Bar-built and Estuarine	Must have tidal influence or prolonged water exposure
	Images or Multi-Media Required	Images Required	Images Required	Photos are also helpful for species identifications
	Degree of Impact / Disturbance	Low Disturbance	Medium Disturbance	Walking and dragging nets through tidal channels will disturb sediments
	Vegetation Height Limitation	Not Applicable	Not Applicable	Both protocols effective in SAV environments
	Appropriate for Tidal / Wet Habitats	Yes	Yes	----
	Tide Height	Medium to High Tide Only	Medium Tide	Implementation within flood and ebb tides may be possible in full tidal environments; enclosure traps are height limited, minnow traps must stay submerged
	Regional or Broad Implementation *	Occasionally Used	Occasionally Used	----
	Potential for Hazards / Risk	Low Risk	Low Risk	----
	Restrictions	Special Status Species	Special Status Species	----

* based on monitoring literature review

APPENDIX 4.1B

FISH SAMPLING DATA SHEET

Sampling Program Information	
DATE:	GEAR:
STATION:	PAGE: <u> </u> of <u> </u>
STAFF:	
WEATHER:	

Rep	Start time / Stop time	Haul length
1	/	
2	/	
3	/	
4	/	
5	/	

#	REP	SPECIES	SL (mm)	WT (g)	#	REP	SPECIES	SL (mm)	WT (g)
1					36				
2					37				
3					38				
4					39				
5					40				
6					41				
7					42				
8					43				
9					44				
10					45				
11					46				
12					47				
13					48				
14					49				
15					50				
16					51				
17					52				
18					53				
19					54				
20					55				
21					56				
22					57				
23					58				
24					59				
25					60				
26					61				
27					62				
28					63				
29					64				
30					65				
31					66				
32					67				
33					68				
34					69				
35					70				

COMMENTS:

** measure first 30 of each species
** additional counts on back

APPENDIX 4.1B

#	REP	SPECIES	SL (mm)	WT (g)	#	REP	SPECIES	SL (mm)	WT (g)
71					116				
72					117				
73					118				
74					119				
75					120				
76									
77									
78									
79									
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108									
109									
110									
111									
112									
113									
114									
115									

1. SPECIES: _____
2. 30 individuals measured? _____ (y / n)
3. COUNT (> 30 but < 100) _____ (#)
4. BATCH WT (> 30 but < 100) _____ (g)
5. BATCH WT (remaining) _____ (g)

1. SPECIES: _____
2. 30 individuals measured? _____ (y / n)
3. COUNT (> 30 but < 100) _____ (#)
4. BATCH WT (> 30 but < 100) _____ (g)
5. BATCH WT (remaining) _____ (g)

1. SPECIES: _____
2. 30 individuals measured? _____ (y / n)
3. COUNT (> 30 but < 100) _____ (#)
4. BATCH WT (> 30 but < 100) _____ (g)
5. BATCH WT (remaining) _____ (g)

1. SPECIES: _____
2. 30 individuals measured? _____ (y / n)
3. COUNT (> 30 but < 100) _____ (#)
4. BATCH WT (> 30 but < 100) _____ (g)
5. BATCH WT (remaining) _____ (g)

1. SPECIES: _____
2. 30 individuals measured? _____ (y / n)
3. COUNT (> 30 but < 100) _____ (#)
4. BATCH WT (> 30 but < 100) _____ (g)
5. BATCH WT (remaining) _____ (g)









1. SPECIES: _____
2. 30 individuals measured? _____ (y / n)
3. COUNT (> 30 but < 100) _____ (#)
4. BATCH WT (> 30 but < 100) _____ (g)
5. BATCH WT (remaining) _____ (g)

1. SPECIES: _____
2. 30 individuals measured? _____ (y / n)
3. COUNT (> 30 but < 100) _____ (#)
4. BATCH WT (> 30 but < 100) _____ (g)
5. BATCH WT (remaining) _____ (g)

1. SPECIES: _____
2. 30 individuals measured? _____ (y / n)
3. COUNT (> 30 but < 100) _____ (#)
4. BATCH WT (> 30 but < 100) _____ (g)
5. BATCH WT (remaining) _____ (g)

NOTES:

Appendix 4.1C

<p style="text-align: center;">CLIO</p> <p style="text-align: center;"><i>Clevelandia ios</i></p> <p style="text-align: center;">Arrow goby</p> <p>Dorsal spines (total): 4 - 5; Dorsal soft rays (total): 15 - 17; Anal spines: 0; Anal soft rays: 14 - 17. Caudal rounded</p> 	<p style="text-align: center;">FUPA</p> <p style="text-align: center;"><i>Fundulus parvipinnis</i></p> <p style="text-align: center;">California killifish</p> <p>Max 11cm; squarish tail fin; small pelvic fin, long anal w/ 11-13 rays: olive-green above, and a yellowish brown below</p> 	<p style="text-align: center;">HYGU</p> <p style="text-align: center;"><i>Hypsopsetta guttulata</i></p> <p style="text-align: center;">Diamond turbot</p> <p>Max 46.0 cm; flattened/compressed body; triangular shape</p> 	<p style="text-align: center;">PACA</p> <p style="text-align: center;"><i>Paralichthys californicus</i></p> <p style="text-align: center;">California halibut</p> <p>Max 152.0 cm; typically weighs 6 to 50 pounds (3 to 23 kg); flattened/compressed body; both eyes on one side of head</p> 
<p style="text-align: center;">MUCE</p> <p style="text-align: center;"><i>Mugil cephalus</i></p> <p style="text-align: center;">Striped mullet</p> <p>bluish-gray/greenish above, silver along the sides, white on ventral surface; 6-7 black horizontal bars along sides; no lateral line; pectoral fins high on shoulders, pelvic fins abdominal</p> 	<p style="text-align: center;">ATAF</p> <p style="text-align: center;"><i>Atherinops affinis</i></p> <p style="text-align: center;">Topsmelt</p> <p>silver, w/shiny silver lateral band; blue or green coloration dorsally; gills = golden-yellow; eyes small and beady; top lip folded down; long pelvic fins</p> 	<p style="text-align: center;">GIMI</p> <p style="text-align: center;"><i>Gillichthys mirabilis</i></p> <p style="text-align: center;">Longjaw mudsucker</p> <p>Max 21.0 cm; first dorsal fin is relatively small, with 4-8 spines; second dorsal fin is larger, with 10-17 rays</p> 	<p style="text-align: center;">GAAF</p> <p style="text-align: center;"><i>Gambusia affinis</i></p> <p style="text-align: center;">Mosquitofish</p> <p>small and stout, dull grey, robust fish with a rounded tail and a terminal and upward-pointing mouth</p> 

Appendix 4.1C

LEAR

Leptocottus armatus

Pacific staghorn sculpin

Max 46.0 cm, spines just anterior of gills, stripes on fins, slightly dorsally flattened

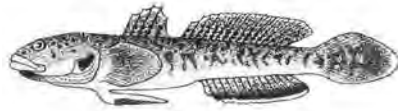


ILGI

Ilypnus gilberti

Cheekspot goby

Max 6.4 cm; shadow spot anterior to pectoral fin



URHA

Urobatis halleri

Round stingray

nearly round pectoral fin disc; brown or grayish above; pale yellow spots or reticulations; underside white to yellowish; tail short and stout, with a long, thick, serrated stinging spine



POLA

Poecilia latipinna

Sailfin molly

body oblong; head small and dorsally flattened, w/small, upturned mouth; caudal peduncle broad & large, rounded, and sometimes tipped with black





Standard Operating Procedures (4.3): Fish Cameras – Baited Remote Underwater Video

March 2021

Prepared for the United States Environmental
Protection Agency



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Standard Operating Procedures: Fish Cameras – Baited Remote Underwater Video (BRUV)

SOP Identification: SOP 4.3 Fish Cameras

Date of Original Issue: 29 March 2021

Date of Last Revision: 29 March 2021

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Protocols reviewed by:

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Karina Johnston, The Bay Foundation

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Protocol Suitability Evaluation

A habitat suitability table containing appropriate estuarine wetland habitat types (of those evaluated) to implement the fish camera Baited Remote Underwater Video (BRUV) protocol is displayed in Table 1. Some camera trapping may also be appropriate in emergent salt marsh but only at high tides. A comparative assessment of cost, effort, and data quality are shown in Table 2. A matrix of additional detailed categorical evaluations of fish cameras can be found in Appendix 4.3A.

Table 1. Appropriate habitat types for fish camera trap survey protocols.

Survey Protocol	Habitat Types					
	Tidal Channel	Mud/sand flat	Emergent salt marsh	Non-tidal salt marsh	Salt pan	'Degraded' / fill
Fish Camera (BRUV)	X	X				

Table 2. Categorical assessment of cost/effort and data quality for fish beach seine survey protocols.

	Evaluation Metric	Fish Camera (BRUV)	Notes
Time / Effort	Office Preparation Time	30-60 minutes	Gather equipment, site selection
	Equipment Construction Time (one time)	10 minutes	Assemble the bait and camera set up
	Field Time (per station)	2 + hours	Depending on the accessibility, each station may take more than 2 hours of video time
	Laboratory Time (per station)	0 minutes	Not applicable, unless post quality control checks on videos are necessary
	Post-Survey Processing / QAQC Time	Variable	Watching time will depend on the length of video taken and will decrease with experience of the data collector
	Minimum Repetition (site-dependent)	Few Repetitions	As fish are highly mobile and variable, repetitions are encouraged but are often time/effort limited; typically, 2 – 3 video sessions per site per sampling period are conducted
	Relative Cost (equipment and supplies)	> \$500	Cameras, housings, SD cards, batteries, PVC, bait, stockings
Survey / Data Quality	Accuracy (at a survey area level)	Medium	----
	Precision (at a survey area level)	Medium	----
	Qualitative-Quantitative Score	Quantitative	----
	Subjectivity-Objectivity Score	Objective	----

Resulting Data Types

The application of the fish camera trap survey protocol will yield quantitative data displayed as counts (MaxN –see discussion below) by species or size frequency distributions across multiple time scales (e.g., seasonally, annually). These data are useful to identify the potential species composition / richness of specific wetlands, sub-areas, or habitats and to potentially identify the uses or functions of a particular wetland area by specific fish species (e.g., nursery). Data can be displayed as size frequency or MaxN abundance graphs, species presence tables, multivariate charts (e.g., MDS or CAP), or at a higher level using diversity indices.

Objective

Defining the fish assemblage of a wetland can be difficult, due to the highly mobile nature of the fauna. Characterizing wetland fish assemblages is often a primary goal of monitoring efforts, but besides the mobility, there are often inherent biases associated with sampling methodologies. Therefore, it is often advantageous to use a variety of methodologies to characterize the fish communities as fully as possible. The primary purpose of this sampling method is to quantitatively assess the distribution, relative abundances, species richness, and diversity of fish in intertidal or subtidal wetland habitats using cameras. Fish cameras are a potentially useful addition to many sampling programs and can be co-deployed with stationary gear such as minnow traps.

Baited cameras are highly effective at attracting scavengers and subsequent predators (Wilson and Smith 1984, Henriques et al. 2002, Davis et al. 2019). Additionally, they are non-extractive, cost-effective, and particularly useful when other forms of sampling are challenging due to uneven substrate (e.g., shell hash or oyster beds), fish handling restrictions, or other reasons (Dorman et al. 2012). Baited video monitoring and MaxN calculation have been successfully used to document fish assemblages within many structured coastal systems (Mallet 2014).

Equipment

Equipment and supplies needed for this survey include:

1. GoPro cameras and battery extenders (Figure 1)
2. Go Pro attachments (Figure 2) and waterproof housings (Figure 1)
3. Micro SD card, 64-GB (4) and adapter
4. PVC supports
 - a. PVC pipe, 6 ft, white (4)
 - b. PVC pipe, 0.5 m, clear (4)
 - c. PVC primer and cement, with T-fitting (4)
5. Stockings or similar mesh material
6. Defrosted bait (fish, shrimp, etc.)
7. Zipties, electrical tape
8. Scissors
9. Screwdrivers to loosen the attachment gear
10. Wetsuit (optional)
11. Neoprene dive/surf booties and gloves



Figure 1. Go Pro camera (bottom left) with the battery extender (top left) and the waterproof case that attached to the PVC apparatus (middle); baited camera PVC T-frame setup (right).

12. Map of sites
13. Buckets and plastic containers for carrying gear
14. Field notebooks or data sheets for recording field notes (see Appendix 4.3B)
15. External hard drive or similar (for video storage)



Figure 2. Go Pro attachment gear to hold cameras onto the PVC structure.

Field Preparation

Site selection for fishing stations will vary with the question being asked. Generally, the placement of BRUVs within an estuary can be more widely spaced than other methods because they sample a broader range of species than many other techniques (e.g., Dorman et al. 2021). Following guidelines in the SONGS protocols for spacing (CCC 2006), it is recommended that multiple BRUV stations per estuary be spaced a minimum of 100 m apart to decrease chances for spatial autocorrelation. The stations should cover the range of tidal conditions of the estuary (e.g., creeks, channels, and/or basins).

Equipment described above should be collected prior to the field shift. BRUV stations should be constructed prior to field deployment. Batteries for all electronic devices should be charged, checked, and replaced as needed, and relevant data sheets should be printed and attached to the clipboards.

Field Methods

While the exact timing will depend on the monitoring objectives or research questions, using cameras at the time of peak fish abundance for the location is recommended. In southern California, this is often June and September as the targeted survey months to coincide with peak fish abundances. Additional survey times (e.g., March or December) may be added by individual site needs or if additional time may be allocated (Zedler 2001). Sampling should not be conducted within 72 hours of a rain event and other constraints may need to be considered (e.g., bird nesting season).

Individual set ups for the camera may vary based on substrate type, flow rates, and gear available. The general goal of the camera set up is to use a horizontal clear support to hold bait a known distance from the camera close to the substrate (if the goal is to capture benthic or demersal fish species). The clear

horizontal support has been demonstrated to be less noticed by fish. A white PVC bar underneath the baited arm may help secure the frame.

An upright support should be visible above the water to help relocate the camera post-deployment. Attachments for the GoPro camera should be set up in a way so the GoPro sits just above the horizontal clear pipe, and its view will be directly parallel with the horizontal clear pipe. If using older units, the GoPros may be paired with extendable battery packs to allow each of the units to collect videos for at least an hour and a half. Newer GoPros have extended battery life and can easily film for two hours.

The bait used for each camera should be store-bought bait or seafood (e.g., large frozen shrimp) placed in a pair of nylon pantyhose then tied to the clear plastic pipe approximately two-thirds down the pipe away from the camera but still clearly in view (Figure 3). The apparatus is inserted into the sediment so that the camera will be just above the bottom in a stable position, and then left out for 1-2 hours at each treatment of each site. Literature suggests collecting at least one hour of videos at each treatment per site per season (four seasons per year) over the course of two years (Bacheler and Shertzer 2015).

Tide selection of deployment will depend on the project needs but should be standardized across the monitoring or research program (e.g., always rising low-to-mid tide when target area is at least minimally covered the entire time) among sampling stations and sampling timing.



Figure 3. Photograph of mullet in an estuary at a baited BRUV.

Station protocols:

1. Randomly select station locations within the study area (Figure 4).
2. Secure GoPro cameras to PVC T-frames (Figure 1).
3. Fasten GoPro to attachments (base + 1 larger attachment + 1 smaller attachment), and secure camera to the arm of the T-frame using zipties. Cover zipties with electrical tape.
4. Chop shrimp and squid (or other available bait) and place in a bag made of nylon. Place bait approximately 0.3 m from GoPro, ensuring that its position is visible on camera.
5. Samples should be collected at low-to-mid tide when sites are minimally submerged. At each site, place the base of the T-frame into the sediment a fixed distance from the shore, facing away from shore (towards deeper water). Record for 1-2 hours (be consistent), or the maximum recording time with the battery available on the camera or extension pack.
6. Collect deployed cameras. Transfer files to external hard drive using SD card converter. Ensure all cameras are cleared and that both cameras and battery packs are charged before the next sampling date.



Figure 4. Example of camera placement locations from Upper Newport Bay.

Laboratory (Office) Methods

Analyze each video sample using a media player (e.g., VLC media player, open source) at up to 3x speed. For each fish passing through the frame of view (Figure 5), record ID to the lowest taxonomic level possible, and the time it enters and leaves the field of view. Abundance should be estimated by species as MaxNspecies, the maximum number of individuals present in the field of view at one time. Total time on camera for each species, as well as species richness and MaxN should be reported for each sample.

For each data sheet (i.e., each treatment within a site on a specific day, Appendix 4.3B):

- Calculate the amount of time each fish spends on camera (time out of frame – time in frame)
 - Note: Time calculations in Excel may return incorrect results depending on the format of the cells. They should be formatted as a time (Format cells > Custom > h:mm:ss).
- Add up quantities: (1) by species and (2) total
- Total number of different species observed = species richness (S)
- Total number of individuals observed = abundance (N)
- Additionally, calculate total amount of time used by each species as a different metric (Note: abundance may not be “true” abundances because the individual may re-enter the frame multiple times).
- Compare average and standard error by treatment

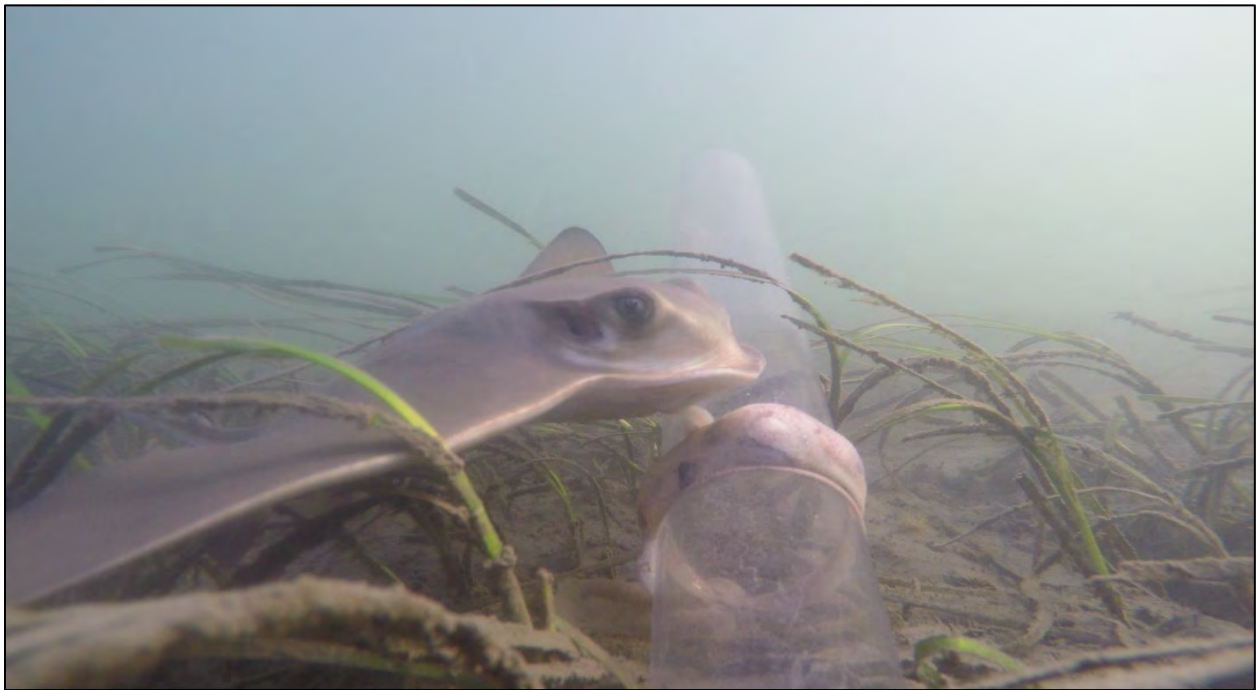


Figure 5. Bat ray appearing in a frame from a BRUV in Upper Newport Bay (credit: Marjorie Howard).

Data Entry and QAQC Procedures

Data should be entered in the field in a field notebook as described above. Back in the laboratory or office, data should be entered using the appropriate database (e.g., Appendix 4.3C). All required fields should be completed in full, and the data recorder should assign their name at the top of the document(s). Data should be transferred to the appropriate electronic database within three days, and the hard copies filed in labeled binders. Electronic copies of all data and videos should be housed on an in-house dedicated server and backed up to a cloud-based or off-site server nightly. Hard copies should be saved for five years. Electronic copies should be saved indefinitely.

Quality Assurance and Quality Control (QAQC) procedures should be conducted on all data. QAQC procedures should be conducted by the QA Officer and include a thorough review of all entries, double checking of all formulas or macros, and a confirmation that all data sheets, Chain-of-Custody forms, and field notes are filed appropriately with electronic back-up copies available. QAQC should verify that the entered data match the hard copies of the field data sheets. Any discrepancies should be corrected, and the initial data entry technician notified.

Data Analyses

After data have been entered, corrections made, and QAQC procedure completed, data can be used in multiple analyses. Examples include abundances by MaxN within site (Figure 6) or multivariate community analysis (Figure 7). Figure 7 displays a CAP analysis, with each point representing a sampling point and all the fish seen in the associated video. Distance between points corresponds to differences among community composition.

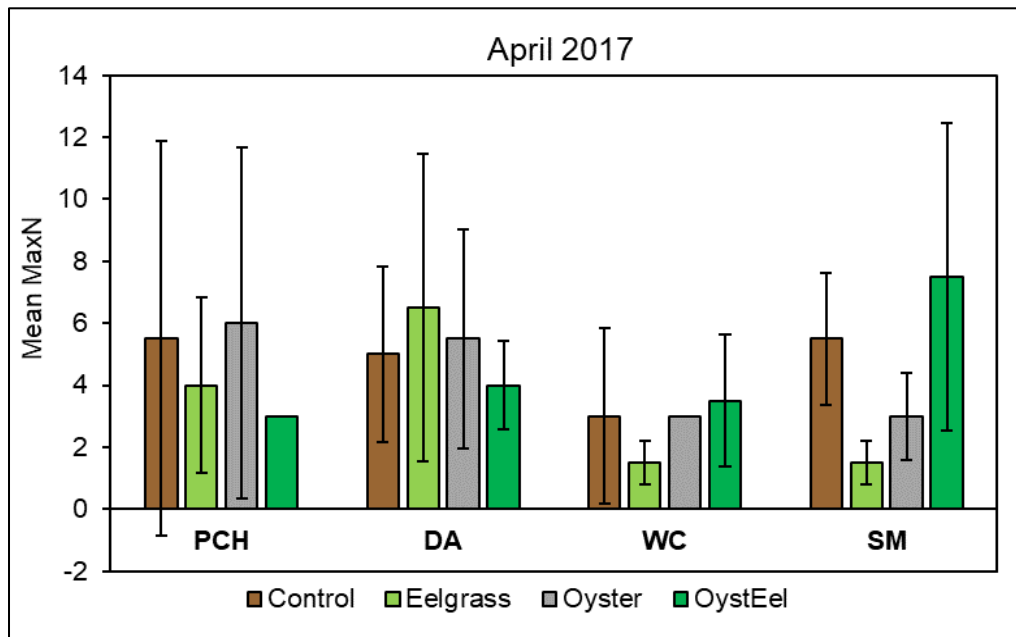


Figure 6. MaxN per site and treatment (replicated from Howard et al. 2019).

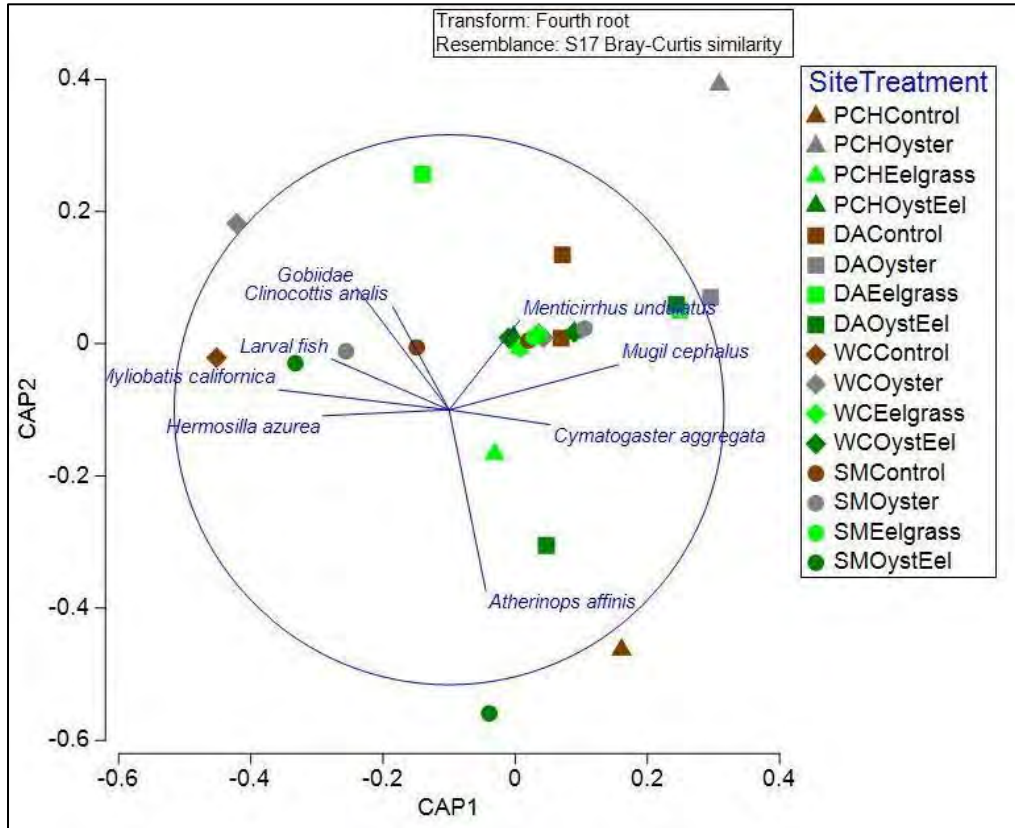


Figure 7. CAP multivariate plot of fish communities among treatments and locations (replicated from Howard et al. 2019).

Health and Safety Precautions

Care should be taken when setting up the cameras as deploying involves walking in habitats that have species with spines (e.g., sculpin, stingrays) or sharp terrain (e.g., shells or rocks). Additionally, appropriate attire and clothing should be worn for comfort and warmth in exposure to cold water for extended periods of time, e.g., wetsuit.

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APPENDIX 4.3A

	Evaluation Metric	BRUV	Notes
	Correlation to L2 CRAM	Attribute 2	Hydrology-dependent
Personnel Requirements	Specialty Equipment or Clothing Required	Many Specialty Items	Cameras, supports, wetsuits, etc.
	Ease of Transport (amount or weight of supplies)	Many or Heavy Items / Difficult	See above; much less challenging than fish seines
	Ease of Implementation	Moderate	Time consuming and a high level of coordination is required for successful implementation
	Expertise / Skill Level	Some Technical Knowledge	Familiarity with species identifications is required
	Number of Personnel	2	Depends on number of sites
	Training Requirements	None	----
	Seasonality of Survey Time	Spring and Fall	Both seasons are required to capture the breadth of fish activity and species diversity
	Suggested Frequency	Semi-annual	Or more frequent, project-dependent
Survey / Data Quality	Type of Output	Numerical	----
	Active or Passive Monitoring Style	Active	----
	Specialty Computer Software Required	Yes	Video player or video editing software
	Availability of Online / External Resources	Many	----
Potential Limitations	Wetland Type Applicability	Bar-built and Estuarine	Must have tidal influence or prolonged water exposure
	Images or Multi-Media Required	Images Required	Videos are the data product
	Degree of Impact / Disturbance	Low Disturbance	Walking to deploy and retrieve cameras will disturb sediments
	Vegetation Height Limitation	Not Applicable	----
	Appropriate for Tidal / Wet Habitats	Yes	----
	Tide Height	Medium to High Tide Only	Implementation within flood and ebb tides may be possible in full tidal environments
	Regional or Broad Implementation *	Sometimes Used	----
	Potential for Hazards / Risk	Medium Risk	----
Restrictions	Special Status Species	Although no collection occurs, special status species may still be present in survey area	

* based on monitoring literature review

APPENDIX 4.3B

Timepoint 1: Time on camera								
	Date 1	Date 2	Date 1	Date 2	Date 1	Date 2	Date 1	Date 2
Species	Control		Oyster		Eelgrass		Paired	
Species 1								
Species 2								
Species 3								
...								
...								
...								
Total	0:00	0:00	0:00	0:00	0:00	0:00	0:00	0:00
Mean	0:00		0:00		0:00		0:00	
St Dev	0		0		0		0	

Timepoint 1: MaxN								
	Date 1	Date 2	Date 1	Date 2	Date 1	Date 2	Date 1	Date 2
Species	Control		Oyster		Eelgrass		Paired	
Species 1								
Species 2								
Species 3								
...								
...								
...								
Total	0:00	0:00	0:00	0:00	0:00	0:00	0:00	0:00
Mean	0:00		0:00		0:00		0:00	
St Dev	0		0		0		0	

Timepoint 1: Species Richness								
	Date 1	Date 2	Date 1	Date 2	Date 1	Date 2	Date 1	Date 2
Species	Control		Oyster		Eelgrass		Paired	
S	0	0	0	0	0	0	0	0
Mean	0		0		0		0	
St Dev	0		0		0		0	

APPENDIX 4.3C

Site Shellmaker
Treatment
Date 6/1/2019
Camera # Lowe 5
Total run time SUM(B8:B#)

File name	Total Video Clip Length (min)	Fish ID	Time in frame	Time out of frame	Time on camera (sec)	MaxN	Notes
GOPR####		round ray	7:15	7:24	9	1	
		round ray	7:25	8:23	58		
		round ray	8:28	8:51	23		

Species	Time on camera	Time (min)	MaxN
round ray		90	8.88
Species 2	SUM(F#,F#,F#...)	...	MAX(MaxNsp2)
Species 3	SUM(F#,F#,F#...)	...	MAX(MaxNsp3)
...
...
...



Standard Operating Procedures (5.1): Bird Abundance and Activity

March 2021

Prepared for the United States Environmental
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Standard Operating Procedures: Bird Abundance and Activity

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Protocol Suitability Evaluation

A habitat suitability table containing appropriate estuarine wetland habitat types (of those evaluated) to implement bird abundance and activity protocols is displayed in Table 1 (i.e., site-wide, box count, and point count). A comparative assessment of cost, effort, and data quality are shown in Table 2. A matrix of additional detailed categorical evaluations of bird abundance and activity protocols can be found in Appendix 5.1A.

Table 1. Appropriate habitat types for bird abundance and activity protocols.

Survey Protocol	Habitat Types					
	Tidal Channel	Mud/sand flat	Emergent salt marsh	Non-tidal salt marsh	Salt pan	'Degraded' / fill
Site-wide	X	X	X	X	X	X
Box Count	X	X	X	X	X	X
Point Count	X	X	X	X	X	X

Table 2. Categorical assessment of cost/effort and data quality for bird abundance and activity protocols.

	Evaluation Metric	Site-wide	Box Count	Point Count	Notes
Time / Effort	Office Preparation Time	10-30 minutes	10-30 minutes	10-30 minutes	This may include printing maps and datasheets, prepping site locations, etc.
	Equipment Construction Time (one time)	Not Applicable	Not Applicable	Not Applicable	----
	Field Time (per unit)	> 120 minutes	5 minutes per station	5 minutes per station	Site-wide survey times can range from a few hours to multiple days depending on survey area
	Laboratory Time	Not Applicable	Not Applicable	Not Applicable	----
	Post-Survey Processing / QAQC Time	> 120 minutes	10-30 minutes	10-30 minutes	Site-wide surveys may require multiple days if all bird sightings are digitized into GIS to allow geospatial analyses
	Minimum Repetition (site-dependent)	Few Repetitions	Many Repetitions	Many Repetitions	Fewer repetitions for box count and point count may be conducted in salt pan or lower diversity habitat areas
	Relative Cost (equipment and supplies)	< \$150	< \$150	< \$150	Only initial cost is binoculars and / or a spotting scope (higher cost)
Survey / Data Quality	Accuracy (at a survey area level)	High	Medium	Medium	Some level of observer-dependence; expertise
	Precision (at a survey area level)	Medium	Medium	Medium	Some level of observer-dependence; expertise
	Qualitative-Quantitative Score	Quantitative	Quantitative	Quantitative	----
	Subjectivity-Objectivity Score	Objective	Objective	Objective	----

Resulting Data Types

The application of bird survey protocols will yield quantitative data displayed in number of birds (or species richness) per unit area categorized by species, guild, and/or activity. Each protocol type will

result in different data outputs and should be determined by the goals of the monitoring program. These data are useful to characterize representative avian assemblages and spatial distributions within a particular site. These data may be used to identify changes in bird populations or guilds across time and space and in response to physical phenomena (e.g., rain, tides) or restoration activities. For some monitoring programs, supplemental specialized surveys such as protocol-level surveys for rare birds or nesting surveys may be necessary and are not covered in SOP 4.1 Bird Abundance and Activity.

Objective

The presence and distribution of avifauna within an ecosystem is often used as an index of habitat quality due to their diet and vulnerability to environmental conditions (Conway 2008). Bird communities are in constant flux. Turnover, especially at isolated sites, can be high with new species colonizing and rare species becoming extirpated (Cooper 2006). Regular, repeated surveys help maintain a clear picture of bird communities on a site. Additionally, sites with high habitat variability may employ multiple survey types to represent avifauna populations more accurately.

The primary purpose of these observational sampling methods is to develop maps of species presence and distributions including information on rare species, to quantify a snapshot of the avian community, and/or to supplement historical or volunteer data. Additionally, recording activity of each species will allow for an assessment of higher ecological function of the survey area. Bird surveys are conducted as an integral part of most monitoring programs, though individual programs have variations on specific survey details. Specific protocols are recommended here that are transferrable between programs.

Equipment

Equipment and supplies needed for these surveys varies depending on the specific type of avifauna survey to be conducted. Three monitoring protocols are discussed, including site-wide, box count, and point count surveys. Several pieces of equipment are used in all bird surveys, including:

- Binoculars or a spotting scope (Figure 1)
- Watch or timer
- GPS (recommended)
- Pens and/or pencils
- Species code list (Appendix 5.1B); comprehensive American Ornithologists Union Checklist for North American Birds may be found at (field identification books also recommended): http://www.birdpop.org/docs/misc/Alpha_codes_eng.pdf
- Camera or phone with camera application (recommended for field verification of location or vegetation community. Note: photos that are not high resolution and zoomed in on the bird are difficult to use for subsequent bird identification)



Figure 1. Spotting scope.

The site-wide surveys also require:

- Site maps displaying the entire area which should be surveyed. These maps will also serve as the datasheet as bird sightings will be recorded in the location they are identified. A representative example can be found in Appendix 5.1C.

The box count surveys also require:

- Site maps with 100 x 150 m grids outlining the survey area overlain on appropriate habitat types (Appendix 5.1D)
- Datasheet(s) (Appendix 5.1E)

The point count surveys also use:

- Site maps identifying survey points (Appendix 5.1F)
- Datasheet (Appendix 5.1E)
- Range finder (recommended)

Helpful hint: as an alternative to printed maps, data may be recorded using a GPS or other electronic field device, if preferred. This would reduce errors in data transference through digitization methods but is slightly more time intensive in the field (e.g., entering fields into the GPS survey form). Survey point and box count maps can be digitized using a Geographic Information System (GIS) or Google Earth file.

Field Preparation

Pre-defined maps of the survey areas appropriate for the survey type (e.g., Appendices 5.1C, D, and F) should be printed (see details in Field Methods section). Alternately, a GIS database should be developed with an attribute table listing all desired attributes to be recorded for each individual or group of birds. Required attributes should include species, count, activity, and others as required for program-specific information. Batteries for all electronic devices should be checked and replaced as needed, and relevant data sheets should be printed and attached to the clipboards. Bird activity and habitat codes can be found on the datasheet (Appendix 5.1A). A camera or phone application that can take high resolution photographs is recommended for field verification of species, as needed, or to document activity (Figure 2).

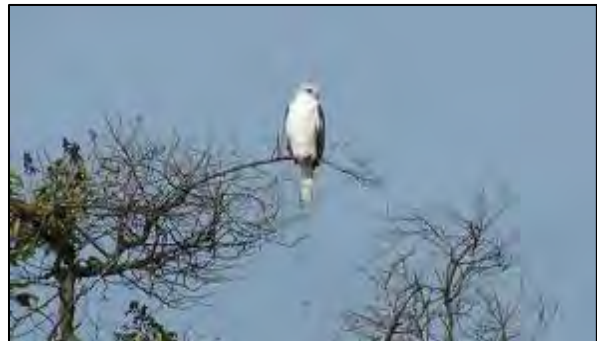


Figure 2. Roosting White-tailed Kite (WTKI) (photo: Dan Cooper 2009).

Additionally, for box count and point count surveys, the survey locations should be identified in the office. For box count surveys, Geographic Information System (GIS) should be used to create as many 150 x 100 m grid cells as possible within the survey area (e.g., Appendix 5.1D), including a space requirement of a 200 m gap between each box to reduce the potential for double counting. Each box should be assigned a number and a subset chosen for monitoring that best represent the wetland habitat variety, and to maximize the number of boxes that will fit within the study area.

Selections of which protocol to use and monitoring sites should be informed by the monitoring program goals and objectives (e.g., ambient monitoring, tracking restoration success, etc.), site access, impacts, and other considerations. Generally, it is recommended that surveys are conducted in at least 10% of the available habitat. Similarly, fixed points for the point count surveys should be spread across the proposed survey area and selected to broadly cover the habitat type fairly evenly, while maximizing the number of points that are at least 200 m from each other, and at least 150 m from the urban/non-wetland edge. Site-wide surveys are more effort-intensive, cover more area (possibility of vegetation trampling), but are more comprehensive.

Field Methods

Surveys should be conducted during both high and low tides twice annually in fall/winter and spring (Bache 2009, Johnston et al. 2011, 2012). Seasonality of surveys may also be dependent on the individual site/project goals or targeted during breeding seasons and may be replicated quarterly. Additional surveys are recommended if time and funds allow, due to the high level of variability of avian assemblages (Merkel & Associates 2009; Figure 3). Fall surveys (October is recommended) will capture migrating birds, and spring surveys (April is recommended) will survey birds during the breeding season. If conducting three times per year, April, August, and January (or mid-winter) is recommended. High tide surveys are designed to capture the presence of waterfowl and dabbling ducks. Low tide surveys target shorebirds and other species. One additional set of surveys in the seasonal wetland and seasonally ponded habitats should be conducted sometime during the wet season after the rains begin (e.g., once sometime between late October to early March).

Data recorded during surveys should include wetland name or location, date, start time, end time, tide, weather, area/grid or point, species, numbers of each species, habitat conditions, and bird behavior (see datasheet in

Appendix 5.1C for box count and point count surveys). All surveys will capture a seasonal snapshot of avian activity and distribution within the study areas. As such, birds are identified and enumerated quickly while moving throughout the study area.

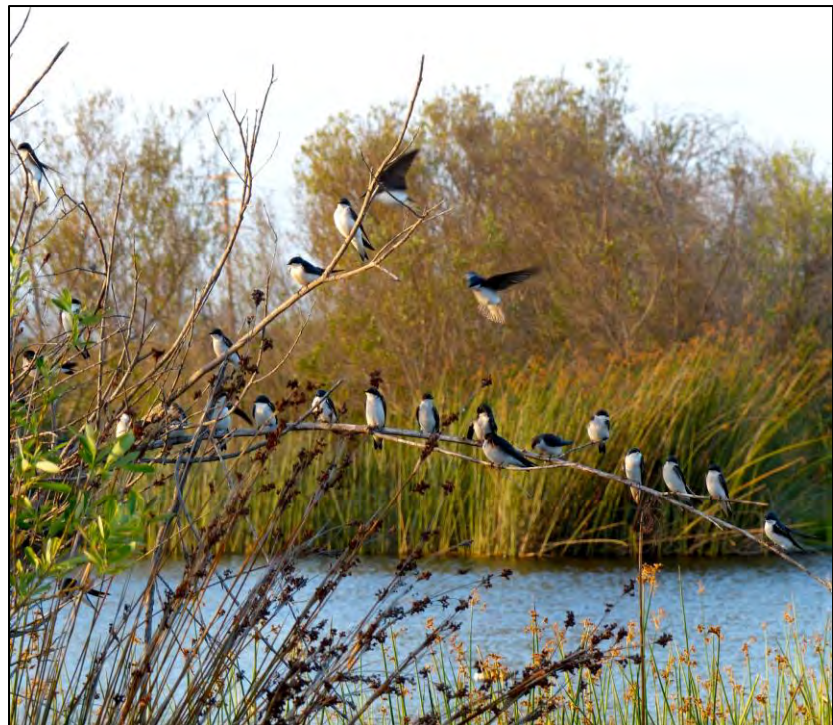


Figure 3. Flock of tree swallows in a freshwater marsh system (photo: Neysa Frechette, Friends of Ballona Wetlands).

Morning surveys should be conducted between 0600 and 1130, when birds are most vocal and easily detected (Johnston et al. 2011, 2012) with the second, afternoon survey performed any time after 1400 to capture either the following low or high tide, and to detect a separate bird community than present in the morning. Existing or pre-defined paths should be followed during each survey to ensure consistency and reduce disturbance and impacts. Surveys should not be conducted in fog, rain, or winds greater than 15 mph, which may reduce visual acuity and are known to decrease bird counts (Ralph et al. 1995, Conway 2008, Page et al. 2011).



Figure 4. Great blue heron (GBHE) roosting along a rip-rap channel bank.

Birds observed on levees or other anthropogenic structures should be recorded as such (Figure 4). Each species is recorded using a standardized four-letter code for the common name used in the American Ornithologists Union Checklist for North American Birds (e.g., RTHA = red-tailed hawk, RPHE = ring-necked pheasant; Appendix 5.1B). If uncertain of the species of a bird, observers are to record the lowest taxonomic classification identifiable. For example, “dabbling duck”, or “duck” if the species or foraging guild is unknown, while providing any conspicuous identification details (e.g., rufous crest) and a photo if possible. Observers are also to record the

habitat and behavior for each bird or group of birds observed (codes are in Appendix 5.1C). Species and breeding dispositions (e.g., paired, singing, mating, and other behavior) of all birds encountered should be noted. Observations should be terminated for the day if adverse weather conditions arise which may reduce accuracy of the counts, or the ability to perform a normal count (e.g., wind, heat, cold). High water may alter the pathway taken during the low tide count, which should be noted on data sheets.

Site-wide Surveys

The study site may be partitioned in advance into 100 x 150 m grids overlaid on a site map for each set of wetland habitats using GIS so that an observer can reference the location in which birds are detected for the site-wide surveys, and to conduct box count surveys concurrently (Page et al. SONGS Wetland Monitoring Plan, 2011). Site-wide surveys employ spot-mapping survey methods wherein an ornithologist or birder walks the entire site or a selected area over multiple days (depending on site size) following timing requirements listed in ‘field methods’ and records the locations of all birds on an aerial photo (Appendix 5.1C) or on a GPS or other electronic device. In cases of a large flock (e.g., foraging swallows), rough outlines can be drawn around the position of the flock, noting species composition make-up and number of individuals. Completed spot-maps should then be digitized or transcribed within three days. Surveys should attempt to minimize double counting, with individuals suspected of being the same noted as such on the data sheets.

All birds within the survey areas should be counted (also include individuals that have originated or ended their flight within the study area). High flying birds overhead that do not land can be noted on the datasheet in the extra note column but should not be incorporated into analyses (Figure 5). Only birds within the study area on or near the ground/vegetation (e.g., Figure 6) should be counted. However, if of interest to the monitoring program, this may vary and other notes may be added; for example, birds of prey that are foraging overhead but fail to find prey during the survey time (e.g., kiting and soaring birds).



Figure 5. Red tailed hawk flying above and passing over survey area (photo: Neysa Frechette, Friends of Ballona Wetlands).



Figure 6. Cedar waxwings (left) and white crowned sparrow (right) perched on vegetation (photos: Neysa Frechette, Friends of Ballona Wetlands).

Box Count Surveys

Box count surveys follow guidelines developed for the SONGS Wetland Monitoring Program (CCC 2006). Using the pre-drawn survey grids, each grid cell, or “box” should be a minimum of 200 m from another to reduce the risk of double counting (Appendix 5.1D). Representative boxes spread throughout the site can adequately cover habitat types and reduce survey time. Survey boxes should be non-randomly placed (i.e., fixed locations) to cover all representative habitat areas selected by habitat maps, aerial photographs, and/or ground surveys. For larger sites, survey boxes may be selected using a random

number generator. Boxes should be placed throughout wetland or adjacent targeted habitats, but not on open water.

Once the locations of survey boxes are determined, “active boxes” to be surveyed during a given count may then be identified such that no two boxes are located closer than 200 m apart. These active boxes should be subjected to repeated counts during each survey period (i.e., certain boxes will never be counted, and certain ones will always be counted). Box count surveys should be limited to exactly five minutes within each box for consistency. All birds within the box (including individuals that have originated or ended their flight in or out of a box) should be recorded on the datasheet (Appendix 5.1E). High flying birds overhead that do not land can be noted on the datasheet in the extra “notes” column but should not be incorporated into analyses. Only birds within the study area on or near the ground/vegetation should be counted, and any bird seen moving from one active cell to another, or suspected of doing so, should be noted (these may be counted for just a single cell, or for both, depending on the analysis used).

Point Count Surveys

Field methods for point count surveys are similar to box count surveys, however, a 150 m radius around selected points (Appendix 5.1F) is used to define survey areas instead of grid cells. Points should be separated by at least 200 m to reduce double counting and should be at least 150 m from the edge of the habitat, typically a street or retaining wall, or non-wetland / urban habitat. Points should be printed on a site map for each set of wetland habitats using GIS software (Page et al. SONGS Wetland Monitoring Plan, 2011), or they can be navigated to using a handheld GPS. Survey points should be non-randomly placed to cover all representative habitat areas selected by habitat maps, aerial photographs, and/or ground surveys. For larger sites, survey points may be selected using a random number generator; however, most study sites are relatively small, and only a few survey points (typically five or fewer) should fit into each one. Points should be placed throughout wetland habitats, but not on open water. Survey points may be permanently marked, if desired and permitted (e.g., PVC pipe).

Surveys at each point should be limited to exactly five minutes. All birds within a 150 m radius should be recorded (also include individuals that have originated or ended their flight within the study area) (Appendix 5.1E). High flying birds overhead that do not land can be noted on the datasheet in the extra “notes” column but should not be incorporated into analyses. As with the above survey method, individual birds already counted and then seen flying into another point count circle should be noted as having done this, to avoid double-counting. Only birds within the study area should be counted. *Helpful Hint: Use a range finder to verify whether specific individuals are within the 150 m radius.*

Laboratory Methods

Not applicable.

Data QAQC Procedures

Data should be entered in the field using the appropriate data sheets (Appendices 5.1C and E), maps, and/or GPS or another electronic device. All required fields should be completed in full, and the data

recorder will assign their name at the top of the document(s). Data should be transferred to the appropriate electronic database within three days, and the hard copies filed in labeled binders. Electronic copies of all data should be housed on an in-house dedicated server and backed up to a cloud-based or off-site server nightly. Hard copies should be saved for five years. Electronic copies should be saved indefinitely.

Quality Assurance and Quality Control (QAQC) procedures should be conducted on all data. QAQC procedures should be conducted by the QA Officer and include a thorough review of all entries, double checking of all formulas or macros, and a confirmation that all data sheets, Chain-of-Custody forms, and field notes are filed appropriately with electronic back-up copies available. QAQC should verify that the entered data match the hard copies of the field data sheets. Any discrepancies should be corrected, and the initial data entry technician notified.

Data Entry and Analysis

After data have passed QAQC procedures, any new bird species should be added to the master bird list in the bird database. Data can be summarized by species richness, total bird abundance, or density per grid cell. If desired, completed spot maps can be digitized (if not field digitized) to assess habitat use of different species, or data can be analyzed by grid, area, or habitat.

Once summarized, bird survey data can be used in multiple analyses. Examples include:

1. Changes in bird composition over time, season, and/or since restoration action;
2. Differences in bird composition between high and low tide;
3. Spatial distribution of birds throughout and between study sites;
4. Differences in bird activity (Figure 7, or habitat use) by guild, season and/or study site;
5. Photo and data documentation of rare or special status species (Figure 8).

Health and Safety Precautions

Not applicable.



Figure 7. Common yellowthroat foraging (photo: Neysa Frechette, Friends of Ballona Wetlands).



Figure 8. Western snowy plover foraging along wrack line (photo: R. Abbott).

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APPENDIX 5.1A

	Evaluation Metric	Site-wide	Box Count	Point Count	Notes
Personnel Requirements	Correlation to L2 CRAM	Not Applicable	Not Applicable	Not Applicable	Feeding activity is loosely correlated to vegetation
	Specialty Equipment or Clothing Required	Few Specialty Items	Few Specialty Items	Few Specialty Items	Binoculars, spotting scope, and/or range finder
	Ease of Transport (amount or weight of supplies)	Few items / Easy	Few items / Easy	Few items / Easy	----
	Ease of Implementation	Easy	Easy	Easy	----
	Expertise / Skill Level	Some Technical Knowledge	Some Technical Knowledge	Some Technical Knowledge	Familiarity with bird species identifications is required; species identification books are also recommended
	Number of Personnel	1-2	1	1	Additional personnel may also be used for data recording
	Training Requirements	None	None	None	Familiarity with avian species is important
	Seasonality of Survey Time	Spring and Fall	Spring and Fall	Spring and Fall	Frequency may be increased on a project- or site-level as needed. Seasonality should capture both the wintering and breeding guilds in a given area.
Survey / Data Quality	Suggested Frequency	Semi-annual	Semi-annual	Semi-annual	----
	Type of Output	Geospatial and numerical	Geospatial and numerical	Geospatial and numerical	----
	Active or Passive Monitoring Style	Passive	Passive	Passive	----
	Specialty Computer Software Required	Yes	None	None	Google Earth may be used for digitizing site-wide surveys, but specialty GIS software is necessary to conduct analyses
Potential Limitations	Availability of Online / External Resources	Some	Some	Some	----
	Wetland Type Applicability	All	All	All	Appropriate for any non-open water habitat
	Images or Multi-Media Required	Images Required	Images Required	Images Required	----
	Degree of Impact / Disturbance	Low Disturbance	No Disturbance	No Disturbance	----
	Vegetation Height Limitation	No Limitations	No Limitations	No Limitations	----
	Appropriate for Tidal / Wet Habitats	Yes	Yes	Yes	----
	Tide Height	All tides	All tides	All tides	Different species will be targeted for different tide heights
	Regional or Broad Implementation *	Almost always used	Almost always used	Almost always used	* based on monitoring literature review
	Potential for Hazards / Risk	Low to No Risk	Low to No Risk	Low to No Risk	----
Restrictions	Special Status Species	Special Status Species	Special Status Species	----	

Appendix 5.1B

Example species code list: American Ornithologists Union Checklist for North American Birds

Bird Code	Common	Scientific Name
ALHU	Allen's Hummingbird	Selasphorus sasin
AMCO	American Coot	Fulica americana
AMCR	American Crow	Corvus brachyrhynchos
AMGO	American Goldfinch	Spinus tristis
AMKE	American Kestrel	Falco sparverius
AMPI	American Pipit	Anthus rubescens
AMRO	American Robin	Turdus migratorius
AMWI	American Wigeon	Anas americana
ANHU	Anna's Hummingbird	Calypte anna
ATFL	Ash-throated Flycatcher	Myiarchus cinerascens
AUWA	Audubon Warbler	Setophaga coronata auduboni
BAWW	Black-and-white Warbler	Mniotilta varia
BBPL	Black-bellied Plover	Pluvialis squatarola
BCNH	Black-crowned night Heron	Nycticorax nycticorax
BEKI	Belted Kingfisher	Megaceryle alcyon
BEWR	Bewick's Wren	Thryomanes bewickii
BGGN	Blue - grey Gnatcatcher	Polioptila caerulea
BHCO	Brown Headed Cowbird	Molothrus ater
BHGR	Black-headed Grosbeak	Pheucticus melanocephalus
BLPH	Black Phoebe	Sayornis nigricans
BLSK	Black Skimmer	Rynchops niger
BANO	Barn Owl	Tyto alba
BNST	Black-necked Stilt	Himantopus mexicanus
BARS	Barn Swallow	Hirundo rustica
BOBO	Bobolink	Dolichonyx oryzivorus
BOGU	Bonaparte's Gull	Chroicocephalus philadelphia
BRBL	Brewer's Blackbird	Euphagus cyanocephalus
BRPE	Brown Pelican	Pelecanus occidentalis
BSSP	Belding's Savannah Sparrow	Passerculus s. beldingi
BTYW	Black-throated Grey Warbler	Setophaga nigrescens
BUFF	Bufflehead	Bucephala albeola
BUOR	Bullock's Oriole	Icterus bullockii
BUOW	Burrowing Owl	Athene cunicularia
BUSH	Bushtit	Psaltriparus minimus
CAGN	California Gnatcatcher	Polioptila californica
CANG	Canada Goose	Branta canadensis
CAKI	Cassin's Kingbird	Tyrannus vociferans
CALT	California Towhee	Melospiza crissalis

Appendix 5.1B

Bird Code	Common	Scientific Name
CATE	Caspian Tern	Hydroprogne caspia
CATH	California Thrasher	Toxostoma redivivum
CHSP	Chipping Sparrow	Spizella passerina
CITE	Cinnamon Teal	Anas cyanoptera
CLSW	Cliff Swallow	Petrochelidon pyrrhonota
COHA	Cooper's Hawk	Accipiter cooperii
COHU	Costa's Hummingbird	Calypte costae
COPO	Common Poorwill	Phalaenoptilus nuttallii
CORA	Common Raven	Corvus corax
COYE	Common Yellowthroat	Geothlypis trichas
DCCO	Double-crested Cormorant	Phalacrocorax auritus
DOWO	Downy Woodpecker	Picoides pubescens
DUNL	Dunlin	Calidris alpina
ELTE	Elegant Tern	Thalasseus elegans
EUST	European Starling	Sturnus vulgaris
FOSP	Fox Sparrow	Passerella iliaca
GADW	Gadwall	Anas strepera
GBHE	Great Blue Heron	Ardea herodias
GCSP	Golden-crowned Sparrow	Zonotrichia atricapilla
GHOW	Great-horned Owl	Bubo virginianus
GREG	Great Egret	Ardea alba
GRHE	Green Heron	Butorides virescens
GRSP	Grasshopper Sparrow	Ammodramus savannarum
GRYE	Greater Yellowlegs	Tringa melanoleuca
GTGR	Great-tailed Grackle	Quiscalus mexicanus
GWTE	Green-winged Teal	Anas crecca
HETH	Hermit Thrush	Catharus guttatus
HOFI	House Finch	Haemorhous mexicanus
HOOR	Hooded Oriole	Icterus cucullatus
HOSP	House Sparrow	Passer domesticus
HOWR	House Wren	Troglodytes aedon
KILL	Killdeer	Charadrius vociferus
LAGO	Lawrence's Goldfinch	Spinus lawrencei
LBCU	Long-billed Curlew	Numenius americanus
LBDO	Long-billed Dowitcher	Limnodromus scolopaceus
LBVI	Least Bell's Vireo	Vireo b. pusillus
LEGO	Lesser Goldfinch	Spinus psaltria
LESA	Least Sandpiper	Calidris minutilla
LETE	Least Tern	Sternula antillarum
LISP	Lincoln's Sparrow	Melospiza lincolnii

Appendix 5.1B

Bird Code	Common	Scientific Name
LOSH	Loggerhead Shrike	Lanius ludovicianus
LIBU	Lazuli Bunting	Emberiza pusilla
MAGO	Marbled Godwit	Limosa fedoa
MALL	Mallard	Anas platyrhynchos
MAWR	Marsh Wren	Cistothorus palustris
MERL	Merlin	Falco columbarius
MODO	Mourning Dove	Zenaida macroura
MYWA	Myrtle Warbler	Setophaga coronata coronata
NAWA	Nashville Warbler	Oreothlypis ruficapilla
NOHA	northern Harrier	Circus cyaneus
NOMO	Northern Mockingbird	Mimus polyglottos
NRWS	Northern Rough-winged Swallow	Stelgidopteryx serripennis
NUWO	Nuttall's Woodpecker	Picoides nuttallii
OCWA	Orange-crowned Warbler	Oreothlypis celata
ORBI	Orange Bishop	Euplectes franciscanus
ORJU	Oregon Junco	Junco h. oregonus
PAWA	Palm Warbler	Setophaga palmarum
PBGR	Pied-billed Grebe	Podilymbus podiceps
PEFA	Peregrine Falcon	Falco peregrinus
PSFL	Pacific-slope Flycatcher	Empidonax difficilis
RBGU	Ring-billed Gull	Larus delawarensis
RCKI	Ruby-crowned Kinglet	Regulus calendula
ROPI	Rock Pigeon	Columba livia
RSFL	Red-shafted Flicker	Colaptes a. cafer
RSHA	Red-shouldered Hawk	Buteo lineatus
RTHA	Red tailed Hawk	Buteo jamaicensis
RUDU	Ruddy Duck	Oxyura jamaicensis
RWBL	Red-winged Blackbird	Agelaius phoeniceus
SAFI	Saffron Finch	Sicalis flaveola
SAPH	Say's Phoebe	Sayornis saya
SEPL	Semipalmated Plover	Charadrius semipalmatus
SNEG	Snowy Egret	Egretta thula
SORA	Sora	Porzana carolina
SOSA	Solitary Sandpiper	Tringa solitaria
SOSP	Song Sparrow	Melospiza melodia
SPSA	Spotted Sandpiper	Actitis macularius
SPTO	Spotted Towhee	Pipilo maculatus
SSHA	Sharp-shinned Hawk	Accipiter striatus
SAVS	Savannah Sparrow	Passerculus sandwichensis
SWTH	Swainson's Thrush	Catharus ustulatus

Appendix 5.1B

Bird Code	Common	Scientific Name
TOWA	Townsend's Warbler	Setophaga townsendi
TRES	Tree Swallow	Tachycineta bicolor
VASW	Vaux's Swift	Chaetura vauxi
VEFL	Vermilion Flycatcher	Pyrocephalus rubinus
VESP	Vesper Sparrow	Pooecetes gramineus
VGSW	Violet-green Swallow	Tachycineta thalassina
WCSP	White-crowned Sparrow	Zonotrichia leucophrys
WEKI	Western Kingbird	Tyrannus verticalis
WEME	Western Meadowlark	Sturnella neglecta
WESA	Western Sandpiper	Calidris mauri
WETA	Western Tanager	Piranga ludoviciana
WFIB	White-faced Ibis	Plegadis chihi
WHIM	Whimbrel	Numenius phaeopus
WILL	Willet	Tringa semipalmata
WISN	Wilson's Snipe	Gallinago delicata
WIWA	Wilson's Warbler	Cardellina pusilla
WREN	Wrentit	Chamaea fasciata
WESJ	Western Scrub-Jay	Aphelocoma californica
WTKI	White-tailed Kite	Elanus leucurus
WTSW	White-throated Swift	Aeronautes saxatalis
YHBL	Yellow-headed Blackbird	Xanthocephalus xanthocephalus
YEWA	Yellow Warbler	Setophaga petechia

Appendix 5.1D

Example box count locations map



APPENDIX 5.1E

Bird Area Survey Datasheet

Date:

Tide:

Observers	Start Tide/End Tide /	Start Time/End Time /	Temp	Wind	%Cloud cover	Precipitation
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Habitat/Environment: MF = mudflat (exposed during low tide), MP = marsh plain, BD = bare dirt, OW = open water, SH = shallow water, UL = upland, LV = levee or dike PO = pond or pooled water, AE = aerial, CE = channel edge, CW = in channel water, SC = dry or seasonal channel, UNK = unknown, note if on manmade structure

Behavior: FO = foraging, RO = roosting, CA = calling, FL = flyover, SW = swimming, PR = preening, AL = alert, UN = unknown, CD = courtship display, CN = carrying nest material, CF = carrying food, AG = aggressive display

Grid	Species	Number	Habitat/Environment	Behavior	Notes/Time
			MF MP BD OW SH UL LV PO AE CE CW SC UNK	FO RO CA FL SW PR AL UN CD CN CF AG	
			MF MP BD OW SH UL LV PO AE CE CW SC UNK	FO RO CA FL SW PR AL UN CD CN CF AG	
			MF MP BD OW SH UL LV PO AE CE CW SC UNK	FO RO CA FL SW PR AL UN CD CN CF AG	
			MF MP BD OW SH UL LV PO AE CE CW SC UNK	FO RO CA FL SW PR AL UN CD CN CF AG	
			MF MP BD OW SH UL LV PO AE CE CW SC UNK	FO RO CA FL SW PR AL UN CD CN CF AG	
			MF MP BD OW SH UL LV PO AE CE CW SC UNK	FO RO CA FL SW PR AL UN CD CN CF AG	
			MF MP BD OW SH UL LV PO AE CE CW SC UNK	FO RO CA FL SW PR AL UN CD CN CF AG	
			MF MP BD OW SH UL LV PO AE CE CW SC UNK	FO RO CA FL SW PR AL UN CD CN CF AG	
			MF MP BD OW SH UL LV PO AE CE CW SC UNK	FO RO CA FL SW PR AL UN CD CN CF AG	
			MF MP BD OW SH UL LV PO AE CE CW SC UNK	FO RO CA FL SW PR AL UN CD CN CF AG	
			MF MP BD OW SH UL LV PO AE CE CW SC UNK	FO RO CA FL SW PR AL UN CD CN CF AG	
			MF MP BD OW SH UL LV PO AE CE CW SC UNK	FO RO CA FL SW PR AL UN CD CN CF AG	
			MF MP BD OW SH UL LV PO AE CE CW SC UNK	FO RO CA FL SW PR AL UN CD CN CF AG	
			MF MP BD OW SH UL LV PO AE CE CW SC UNK	FO RO CA FL SW PR AL UN CD CN CF AG	
			MF MP BD OW SH UL LV PO AE CE CW SC UNK	FO RO CA FL SW PR AL UN CD CN CF AG	

APPENDIX 5.1E

Grid	Species	Number	Habitat/Environment	Behavior	Notes/Time
			MF MP BD OW SH UL LV PO AE CE CW SC UNK	FO RO CA FL SW PR AL UN CD CN CF AG	
			MF MP BD OW SH UL LV PO AE CE CW SC UNK	FO RO CA FL SW PR AL UN CD CN CF AG	
			MF MP BD OW SH UL LV PO AE CE CW SC UNK	FO RO CA FL SW PR AL UN CD CN CF AG	
			MF MP BD OW SH UL LV PO AE CE CW SC UNK	FO RO CA FL SW PR AL UN CD CN CF AG	
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			MF MP BD OW SH UL LV PO AE CE CW SC UNK	FO RO CA FL SW PR AL UN CD CN CF AG	
			MF MP BD OW SH UL LV PO AE CE CW SC UNK	FO RO CA FL SW PR AL UN CD CN CF AG	
			MF MP BD OW SH UL LV PO AE CE CW SC UNK	FO RO CA FL SW PR AL UN CD CN CF AG	

APPENDIX 5.1F

Example point count survey locations map





Standard Operating Procedures (6.1): Benthic Invertebrates

March 2021

Prepared for the United States Environmental
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Protocol Suitability Evaluation

A habitat suitability table containing appropriate estuarine wetland habitat types (of those evaluated) to implement benthic invertebrate protocols is displayed in Table 1. For emergent salt marsh habitats, benthic invertebrate protocols are usually applicable in areas receiving at least partial tidal inundation, though the protocol could be used in other areas. A comparative assessment of cost, effort, and data quality are shown in Table 2. A matrix of additional detailed categorical evaluations of benthic invertebrate protocols can be found in Appendix 6.1A.

Table 1. Appropriate habitat types for benthic invertebrate survey protocols.

Survey Protocol	Habitat Types					
	Tidal Channel	Mud/sand flat	Emergent salt marsh	Non-tidal salt marsh	Salt pan	'Degraded' / fill
Benthic Cores	X	X	X	X	X	X

Table 2. Categorical assessment of cost/effort and data quality for benthic invertebrate survey protocols.

	Evaluation Metric	Benthic Cores	Notes
Time / Effort	Office Preparation Time	10-30 minutes	Identification of sampling locations and equipment collection
	Equipment Construction Time (one time)	30-60 minutes	Construction of both large and small cores
	Field Time (per station)	> 60 minutes	Coring and sieving for each station can take between 45-90 minutes, depending on the sediment grain size, number of surveyors, and the salt water source for rinsing
	Laboratory Time (per station)	> 60 minutes	Dependent on invertebrate community and familiarity with groups and taxa
	Post-Survey Processing / QAQC Time	> 30 minutes	----
	Minimum Repetition (site-dependent)	Few Repetitions	----
	Relative Cost (equipment and supplies)	\$ 15-50	More expensive if samples are analyzed by professional taxonomists (laboratory)
Survey / Data Quality	Accuracy (at a survey area level)	Medium	----
	Precision (at a survey area level)	Low	Highly variable based on core placement
	Qualitative-Quantitative Score	Quantitative	----
	Subjectivity-Objectivity Score	Objective	----

Resulting Data Types

The application of benthic invertebrate survey protocols will yield quantitative data displayed in abundances by taxa or density of benthic infauna, recorded as the number of individuals per meter squared for each station. Benthic invertebrate survey protocols are intended to account for the presence of both large (e.g., bivalves, mollusks) and small (e.g., polychaetes, arthropods) infauna. These data may be used in conjunction with existing pollution tolerance indices to identify the abundance of pollution tolerant species as an indicator of habitat health (more common for freshwater species).

Objective

Benthic invertebrate taxa are useful ecological indicators because they provide a reflection of the state of the environment, especially at the transition from water to land and can indicate local biodiversity (Hilty and Merenlender 2000, Johnston et al. 2011, 2012). Long-term changes are often assessed by looking at the invertebrate community at a higher taxonomic level or by evaluating the community as a whole (Hodkinson and Jackson 2005, Johnston et al. 2011, 2012). The presence or absence of certain infauna (i.e., burrows into and lives in bottom sediments) or epifauna (i.e., lives on the surface of bottom sediments) within tidal channels can serve as indicators of water quality, anthropogenic stressors to the estuary, and the potential to support other trophic levels (WRP 2006); these benthic communities provide essential ecosystem services and support (Schreiber 1981).

The primary purpose of this sampling method is to assess the benthic invertebrate community by collecting data on the density and distribution of infauna within wetland tidal channels. Taxa will be assessed by sorting to the lowest taxonomic level possible including recognizable taxonomic units (RTU). This is discussed below, and for more details, see Monitoring Manual Version 2.0 (Johnston et al. 2021). While some protocols allow sorting to a higher taxonomic classification (e.g., order) to facilitate the use of student and volunteer (non-professional taxonomic identification) assistance, it is possible to determine morpho-species even without confirmed taxonomic assignments. Depending upon available funds, if lower level taxonomic classification is required, samples may also be sent to a qualified benthic invertebrate laboratory.

Equipment

General equipment and supplies needed for benthic invertebrate surveys (Figure 1) include:

Collection:

- Sediment corers (a.k.a. Clam gun for large cores); see 'Field Methods' for sizing details
- Labeled and extra sealable bags (1 gallon)
- Pencils, permanent markers, and station data sheets (Appendix 6.1B)
- GPS with extra batteries
- Aerial Photo with stations and core locations or GPS points
- Waders (or surf/dive booties with a thick sole)
- 5-gallon buckets (3-4 recommended)
- Handheld multi-parameter water quality meter
- Cooler (to keep samples cold if an extended time period is expected between collection and preservation)



Figure 1. General items required for benthic invertebrate sampling.

Sieving and Sorting:

- Sample Data Tracking Sheet
- Number 35 (0.5 mm for small cores) or Number 50 (0.3 mm for large cores) sieve. *Note: Choice of sieve size will affect abundance and type of invertebrates collected and ability to compare among studies and samples. For more details, see Monitoring Manual Version 2.0.*
- Glass jars, lids, and labels
- Pencils, thick permanent marker
- Waterproof paper (optional)
- 16 oz. Nalgene squirt bottles filled with DI water
- Dissecting forceps, spatula
- Benthic Sieve Bucket (optional; same mesh size as sieve)
- 70% Ethyl alcohol or 8% buffered formalin with Rose Bengal dye (Be careful, read Safety Data Sheets, SDS: <https://www.osha.gov/hazcom>). *Note: Rose Bengal dye can interfere with identification of some species. Ethyl alcohol can bleach animals and make identification more challenging. Formalin will prevent use for genetics.*
- Dissecting microscope with illuminator



Field Preparation

Equipment described above should be collected prior to the field shift. Batteries for all electronic devices should be checked and replaced as needed, and relevant data sheets should be printed and attached to the clipboards. *Helpful hint: Label and organize bags by station and channel location before going into the field.*

Field Methods – Station Selection and Frequency

It is important to note that specific techniques of benthic invertebrate protocols are typically unique for each monitoring program and are often targeted to particular organisms. This SOP suggests standardized protocols. Zedler (2001) recommends collecting benthic invertebrates quarterly. If that sampling frequency is not possible, then seasons should be chosen to align with monitoring program objectives, ongoing datasets, or regional studies. Some southern California monitoring programs collect data in fall to coincide with fish sampling and to avoid avian nesting season, though spring should also be considered if impacts can be minimized. Some monitoring programs (Johnston et al. 2011, 2012) suggest semi-annual sampling, once at the beginning of the wet season (September / October) and once after the wet season in approximately May (or early summer, if only collecting once). Sampling should

not occur within 72 hours of a rain event, as the freshwater input will affect abundances of certain taxa (Zedler 2001). For more details, see *Monitoring Manual Version 2.0*.

Samples from intertidal zones (e.g., mudflats) should be collected during low tides when the sediment is exposed. Samples from channels should be collected during mid to low tides when the sediment is partially exposed as the channel zones may not ever be completely exposed. Again, this may vary with sampling program. Most programs prefer some water in the channel to help rinse the sediment from the sieves.

Sampling stations should be chosen (fixed) to be representative of the tidal channel and mudflat habitats of the wetland. Note: if feasible, test samples from the inundated marsh plain may also be collected. Each station consists of a cross-section transect of the tidal channel. Large and small core samples should be taken from the left, right, and thalweg of the channel [facing the outflow (Figure 2)]. The thalweg is defined as the lowest portion of the channel, and does not necessarily fall directly in the middle of the channel.

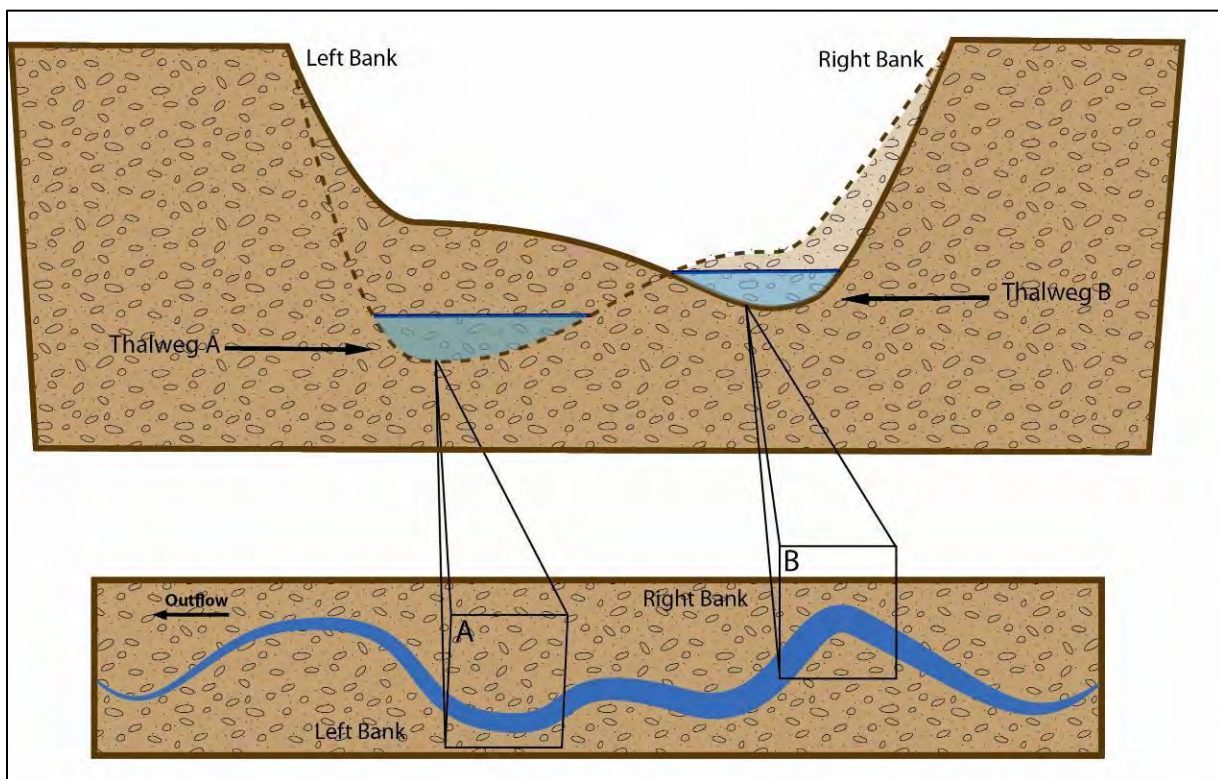


Figure 2. Depiction of cross-section transect of a tidal channel. The figure is not directly representative of a particular benthic survey station. Note: the thalweg is the deepest portion of the channel and not the midpoint.

Field Methods – Station Protocols

Readings for water temperature, salinity, dissolved oxygen (% and mg/L), and pH should be taken with a handheld multi-probe sonde at each station before entering the water (for details, refer to SOP 1.1 – continuous sonde monitoring). *Helpful hint: reaching from the shore using a pole or other device may help avoid trampling. Water should not be disturbed prior to water quality readings.*



Figure 3. Example of large core sediment extraction.

Core size and depth should be chosen to be consistent with existing datasets and/or published literature on macrobenthos from the site and nearby marshes (Levin et al. 1998, Talley and Levin 1999, Levin and Talley 2002, Levin and Currin 2005). Most (78–89%) of the macrofauna in southern California *Spartina foliosa* marshes are found in the top 2 cm of sediment (Levin et al. 1998). Deeper dwelling infauna (e.g., bivalves and shrimp) will need to be collected using a handheld, 10 cm diameter corer pushed into the sediment to a depth of approximately 30 cm (Figure 3). One core should be taken at the left, right, and thalweg of the channel (facing the outflow) (Figure 4). Core size/area will need to be recorded so that values for abundance can be adjusted for core area.

Smaller invertebrate infauna (e.g., polychaetes and amphipods) are often found in the top 5cm of sediment (Zedler 2001). Small infauna should be collected using a 6 cm diameter corer pushed into the sediment to a depth of at least 2 cm but ideally for certain organisms and depending on available processing time, 5 or 6 cm (Figure 5). Three small cores should be collected and composited from the left, right, and thalweg of the channel (Figure 4). Each set of composited cores covers an area of 0.00848 m².

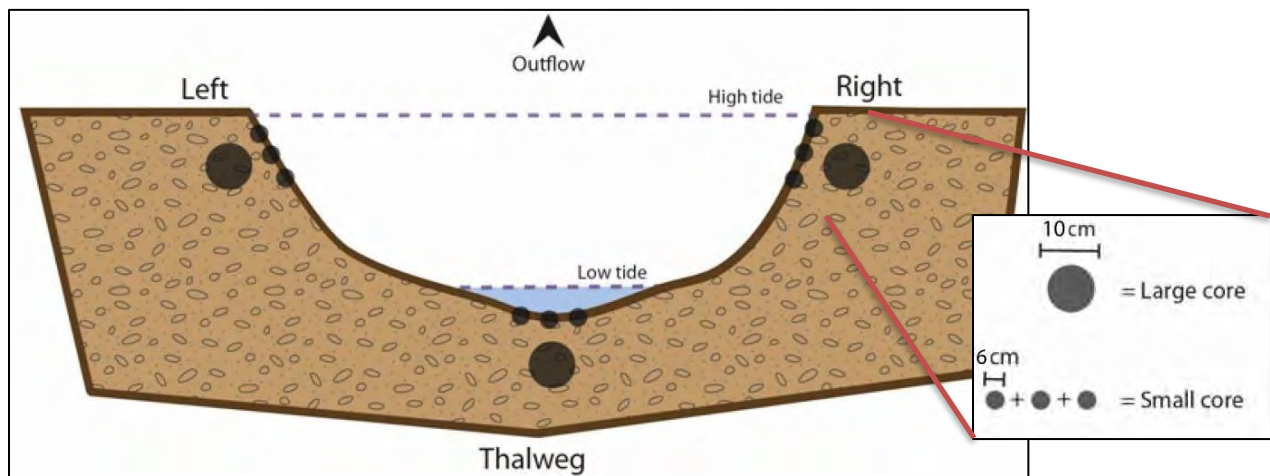


Figure 4. Diagram of benthic infauna core sizes and locations. Note: figure not drawn to scale.

Samples can either be wet-sieved in the field (see below) or carried back to the laboratory for preservation and then sieved. In this case, this choice of method will impact the type and abundance of invertebrates captured in the samples. Qualitatively, wet-sieving results in loss of the more mobile organisms like polychaetes and oligochaetes.

Laboratory Sieving – The samples, placed in appropriate jars, should be kept cool in the field until being transported back to the laboratory. Once back in the laboratory (within 12 hours), the cores should be preserved with 8% buffered formalin with or without Rose Bengal (see note above). If not immediately processed, reserved samples should be stored in formalin approximately 2-5 days, and then transferred to ethanol before being processed. Longer-term storage in ethanol is appropriate up to several months (with checks to make sure the ethanol has not evaporated). At time of processing in an appropriate laboratory hood, formalin should be poured off through appropriate sieve (see note above) into waste storage jar. Once formalin is poured off, DI water can be used to rinse sediment through the sieve until the DI water runs clear out of the bottom of sieve. Material retained on the sieve is then sorted as discussed below.

Field Methods – Wet-sieving

The samples should undergo a wet-sieving process in a bucket filled with salt water, to separate infauna from sediment. Small cores should be processed using a 0.5 mm mesh sieve, and large cores should be processed using a 2.5 mm mesh sieve. As noted above, sieve size will have a significant impact on organisms retained (Johnston et al. 2021).

It is important to perform all rinsing using salt water to maintain the correct osmotic pressure for the invertebrates (or brackish water from the surveyed channel). Once wet-sieved, the remaining material on the screen of the sieve (organisms, large sediment, and debris) should be carefully transferred using forceps into labeled, screw top glass jars. One option is to identify the larger organisms in the field, and if this can be done with confidence, these organisms can be released instead of brought back to the laboratory. The sieves should then be rinsed and scrubbed, to avoid cross contamination. *Helpful hint: large rocks may be discarded after being thoroughly inspected for benthic invertebrates and noted on the data sheets.* If a salt water source is present in the laboratory, it is recommended that samples undergo an initial wet sieving process to remove the bulk of the sediment and debris followed by the final sieving and labeling process being performed in the controlled laboratory environment.



Figure 5. Small core pushed into the sediment.

Laboratory Methods – Wet Sieved samples

Following the final wet-sieving process, all samples should be transferred to labeled glass jars. The jars should be filled with sample material to 50-70% capacity, leaving at least 30% uncovered space for further processing. The jar should then be filled with salt water leaving 10% available open space. If more than one jar is needed to hold the entire sample, label as follows: 1 of 2, 2 of 2, etc. Each label should include the station ID, sample location within the channel (i.e., left, right, thalweg), date, and the split number (as applicable). *Helpful hint:* a label written in pencil on waterproof paper and placed inside the jar provides a failsafe against losing or damaging the outer label.

In the laboratory, jars should be initially preserved with a 10% formalin saltwater solution. Between two and five days after fixation, the formalin should be removed, properly disposed of, and the samples and jars should be rinsed with tap water. Samples should then be transferred back to the formalin-free jars and filled with a 70% ethyl alcohol (ethanol) solution to a level that completely immerses the sample. Samples should remain stored in the ethanol solution until sorting and analysis.

Sorting Methods – All Samples

To facilitate sorting, samples should be placed on white plastic plates and divided into small sorting trays using an illuminator, dissecting scope, spatula, and forceps. Benthic invertebrates should be sorted into the following categories: bivalves (subdivided into ridged and smooth clams, razor clams, and mussels), *C. californica*, other gastropods, worms, and amphipods (Figure 6; WRA 2004). All shelled organisms should be recorded as dead or alive, determined by the presence of muscle tissue in the bivalves. Each gastropod should be checked for an intact operculum. All unknown invertebrates (heads only to prevent overcounting and to facilitate identification) should be placed in vials and labeled for later taxonomic identification. Several examples of each taxon should be photographed, labeled, and preserved in a 70% ethanol solution as voucher specimens. The presence of wood and algae should be noted, as well as general grain size of the remaining rocky substrate (e.g., sand or pebbles). If present, algae and sea grass should be collected and placed in small aluminum pie tins. Tins should then be placed in a dehydrator for 24 hours, weighed, and the value recorded to determine dry algal weight per sample.

Taxonomic Identification – For more details, see *Monitoring Manual Version 2.0 (Johnston et al. 2021)*.

Trying to identify small marine animals can be challenging, time-consuming as well as require additional training and expertise in taxonomy. In addition, the dirt on sieved organisms often sticks onto the specimens thereby masking key features such as hairs, chaetae, bristles, spicules, plates, and many other features. Second, animals that are fixed in formalin or alcohol often lose color, a key feature for some organisms. In this manual, it is recommended to identify organisms to the lowest possible taxonomic group or parataxonomic sorting of samples to recognizable taxonomic units (RTUs). RTUs are also called morphospecies, morphotypes, and are generally considered to be a sufficiently reliable and conservative approach in ecological biodiversity studies or conservation biology (e.g., Krell 2004). It should be noted that evaluations of RTUs or morphospecies show many overestimations of species numbers. Use of multivariate or benthic indices may overcome some of the challenges.



Figure 6. Large core benthic invertebrate sample sorted in the lab showing bivalves (A) (D), *C. californica* (C), and other gastropods (B).

Data Entry and QAQC Procedures

Data should be entered in the field using the appropriate data sheet (Appendix 6.1B). All required fields should be completed in full, and the data recorder should assign their name at the top of the document(s). Data should be transferred to the appropriate electronic database within three days, and the hard copies filed in labeled binders. Electronic copies of all data should be housed on an in-house dedicated server and backed up to a cloud-based or off-site server nightly. Hard copies should be saved for five years. Electronic copies should be saved indefinitely.

Quality Assurance and Quality Control (QAQC) procedures should be conducted on all data. QAQC procedures should be conducted by the QA Officer and include a thorough review of all entries, double checking of all formulas or macros, and a confirmation that all data sheets, Chain-of-Custody forms, and field notes are filed appropriately with electronic back-up copies available. QAQC should verify that the entered data match the hard copies of the field data sheets. Additionally, every 30th sample should be sorted and recounted and all voucher specimens should be double checked for QAQC purposes. Any discrepancies should be corrected, and the initial data entry or sorting technician notified.

Data Analyses

After data have been entered, corrections made, and QAQC procedure completed, data can be used in multiple analyses. The resulting data can be analyzed to determine the density of benthic infauna, recorded as the number of individuals per meter squared for each station. Data can be combined for

each portion of the creek sampled (i.e., left, right, and thalweg), and analyzed separately for both large and small cores. Using the recommended protocols above, each station will sample a total area of 0.023562 m² for the large cores and 0.02544 m² for the small cores.

Presence and relative abundance of general taxonomic groups may be calculated for each location. Examples of additional analyses include abundance graphs by group or taxa or maps of distributions of each taxonomic group. For more detailed recommendations for data analyses, especially combining data between or among monitoring programs, see Johnston et al. 2021.

Health and Safety Precautions

When handling formalin, a respirator mask, latex gloves, and protective eyewear should be worn. Any formalin that comes into contact with skin should be rinsed immediately for 15 – 20 minutes to avoid irritation or other adverse effects. In the event of prolonged exposure or burning, seek immediate medical attention. Additionally, individual laboratory health and safety precautions should be always followed (e.g., closed-toed shoes, recognition of where the closest emergency equipment is located, etc). Safety Data Sheets for all chemicals should always be followed (<https://www.osha.gov/hazcom>).

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APPENDIX 6.1A

	Evaluation Metric	Benthic Cores	Notes
	Correlation to L2 CRAM	Not Applicable	Benthic invertebrate community is tied to hydrology and water circulation patterns
Personnel Requirements	Specialty Equipment or Clothing Required	Many Specialty Items	Large and small cores, sieves, and formalin
	Ease of Transport (amount or weight of supplies)	Many or Heavy Items / Difficult	See above, plus buckets
	Ease of Implementation	Difficult	For time-intensive coring efforts and sediment sieving
	Expertise / Skill Level	Some Technical Knowledge	No technical knowledge required if samples are sent to a lab for processing
	Number of Personnel	> 2	---
	Training Requirements	None	---
	Seasonality of Survey Time	Early Summer and Fall (beginning of wet season)	Both seasons are required to capture the breadth of benthic invertebrate species diversity; or late spring/early summer if only one sampling event is conducted; must not collect samples within 72 hours of a rain event
	Suggested Frequency	Semi-annual	---
Survey / Data Quality	Type of Output	Numerical	---
	Active or Passive Monitoring Style	Active	---
	Specialty Computer Software Required	No	---
	Availability of Online / External Resources	Many	Invertebrate guides are recommended for in-house processing
Potential Limitations	Wetland Type Applicability	Estuarine and Bar-built	Must have tidal influence or ponded water
	Images or Multi-Media Required	Images Required	Particularly for the voucher database
	Degree of Impact / Disturbance	High Disturbance	Walking and coring in tidal channels will severely disturb sediments
	Vegetation Height Limitation	Not Applicable	---
	Appropriate for Tidal / Wet Habitats	Yes	---
	Tide Height	Low to Mid-Tide	Depending on site, implementation during flood and ebb tides may be advisable to facilitate easier sample processing
	Regional or Broad Implementation *	Almost Always Used	---
	Potential for Hazards / Risk	Medium Risk	Caution must be exercised when using formalin and handling sharp inverts
Restrictions	Special Status Species	---	

* based on monitoring literature review

APPENDIX 6.1B

BENTHIC INVERT SAMPLING DATASHEET

Sampling Program Information	
DATE:	LOCATION:
TIME (start): (end):	WEATHER:
STAFF:	PAGE: ___ of ___
GPS LAT:	GPS LONG:

YSI PROBE MEASUREMENTS	
Time	___ : ___ am / pm (circle one)
Temp	___ °C
Turbidity	___ TDS g/L
Salinity	___ ppt
DO	___ % _____ mg/L
pH	___ pH
Notes:	

SEDIMENT INFORMATION	
Soil type: _____	Algae: YES NO
Soil moisture: _____	Species: _____
Soil color: _____	Thickness: _____ mm Notes: _____

SAMPLE COLLECTION - SMALL PVC	
Number of samples collected: <input type="text"/>	(3 cores per sample)
# jars (Sample 1): _____	Bank: LEFT RIGHT MID Notes: _____
# jars (Sample 2): _____	Bank: LEFT RIGHT MID Notes: _____
# jars (Sample 3): _____	Bank: LEFT RIGHT MID Notes: _____
Number of jars (total): <input type="text"/>	Notes: _____

SAMPLE COLLECTION - LARGE CORE	
Number of samples collected: <input type="text"/>	(1 core per sample)
# jars (Sample 1): _____	Bank: LEFT RIGHT MID Notes: _____
# jars (Sample 2): _____	Bank: LEFT RIGHT MID Notes: _____
# jars (Sample 3): _____	Bank: LEFT RIGHT MID Notes: _____
Number of jars (total): <input type="text"/>	



Standard Operating Procedures (6.2): Terrestrial Invertebrates

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Prepared for the United States Environmental
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Protocol Suitability Evaluation

A habitat suitability table containing appropriate estuarine wetland habitat types (of those evaluated) to implement terrestrial invertebrate protocols is displayed in Table 1. The protocols (especially epigeal – close to the ground) are difficult, if not infeasible, in tidal habitats. A comparative assessment of cost, effort, and data quality are shown in Table 2. A matrix of additional detailed categorical evaluations of terrestrial invertebrate survey protocols can be found in Appendix 6.2A.

Table 1. Appropriate habitat types for terrestrial invertebrate survey protocols.

Survey Protocol	Habitat Types					
	Tidal Channel	Mud/sand flat	Emergent salt marsh	Non-tidal salt marsh	Salt pan	'Degraded' / fill
Aerial traps			X	X	X	X
Pitfall traps (non-tidal)				X	X	X
Pitfall traps (tidal)		X	X			

Table 2. Categorical assessment of cost/effort and data quality for terrestrial invertebrate survey protocols.

	Evaluation Metric	Aerial traps	Pitfall (non-tidal)	Pitfall (tidal)	Notes
Time / Effort	Office Preparation Time	10-30 minutes	10-30 minutes	10-30 minutes	Need to prepare and label all Sticky Traps, cups, and identify survey locations
	Equipment Construction Time (one time)	0-10 minutes	10-30 minutes	10-30 minutes	Building tomato cages
	Field Time (per transect)	0-10 minutes	10-30 minutes	10-30 minutes	May be less time with 3+ people or unconsolidated soils; protocols may be implemented concurrently
	Laboratory Time (per transect)	> 60 minutes	> 60 minutes	> 60 minutes	Dependent on familiarity with species identifications and quantity of invertebrates; more than an hour if identifying to a low-level taxa (e.g., genus- or species-level)
	Post-Survey Processing / QAQC Time	10-30 minutes	30-60 minutes	30-60 minutes	----
	Minimum Repetition (site-dependent)	Many Repetitions	Many Repetitions	Many Repetitions	Invertebrate communities are highly variable
	Relative Cost (equipment and supplies)	> \$15	> \$15	> \$15	----
Survey / Data Quality	Accuracy (at a survey area level)	Medium	Medium	Medium	----
	Precision (at a survey area level)	Low	Medium	Medium	----
	Qualitative-Quantitative Score	Quantitative	Quantitative	Quantitative	----
	Subjectivity-Objectivity Score	Objective	Objective	Objective	----

Resulting Data Types

The application of terrestrial invertebrate survey protocols will yield quantitative data displayed in biomass or productivity per square meter per transect for flying invertebrates. Data can be extrapolated up to habitat type. Pitfall invertebrate surveys are also quantitative and are useful to identify the

potential species composition, richness, and density of epigeal invertebrates in a given area; they can also be analyzed as biomass or productivity for a given area over time.

Objective

Terrestrial invertebrates are a vital component of wetland food webs and are indicators of the overall health of a system (Zedler 2001). Invertebrate-related ecosystem function has traditionally been measured by enumerating and identifying insects to the species level to calculate compositional biodiversity. In practice, such approaches are exceedingly costly, require extensive periods of sample interrogation, and therefore have resulting processing times on the order of many months to years for monitoring efforts with robust/frequent sampling plans. Logistically, simpler and more rapid measures that more directly describe functions or rates of arthropod productivity may be better indicators of ecosystem health (Anderson 2009). The high diversity of coastal arthropods, a lack of existing, complete baseline inventories, and the growing dearth of qualified invertebrate taxonomists also make traditional high-resolution taxonomically-focused terrestrial invertebrate assessments in this habitat expensive and challenging.

The primary purpose of this sampling method is to document aerial and epigeal (above soil surface) arthropod productivity (as biomass per unit area, or biomass per day) for each habitat or area by extrapolation from enumerated arthropods via length-fresh weight regressions. Taxa should be assessed in the pitfall traps by sorting to a higher taxonomic classification (e.g., order) or recognizable taxonomic units (RTUs) to facilitate the use of student and volunteer (non-professional taxonomic identification) assistance, but they can also be sorted to lower taxa by taxonomists. To meet previously identified concerns of local resource managers, these sampling methods include specific steps/elements to minimize any impacts upon non-target taxa (e.g., birds encountering sticky traps, coyotes ingesting pitfall traps). Sticky traps are routinely surrounded by tomato cages to deter birds from contacting the adhesive trap surface and have no statistical effect on the arthropod biomass accumulated by those sticky traps (Anderson 2010); similarly, plastic covers suspended just above pitfall traps deter ancillary catch of herpetofauna and small mammals in pitfall traps.

Equipment

General equipment and supplies needed for any terrestrial invertebrate surveys include:

- Plastic wrap
- Permanent ink pen (e.g., Sharpie) and duct or lab tape (for labeling the cups on the pitfall surveys)
- Bucket to hold supplies and pulled traps
- Datasheet(s) (Appendices 6.2B & C)
- GPS

Additional equipment and supplies for the aerial arthropod surveys includes:



Figure 1. Deployed and labeled aerial arthropod sticky trap.

- Sticky Strip yellow plastic insect traps (“Stiky Traps®” Tanglefoot-covered, Bioquip catalog #2873). Traps are supplied in 6 x 12 inch sheets and should be cut in half to produce 6 x 6 in sheets (or approximately 15 x 15 cm) with an area of 0.021 m² (Figure 1).
- Razor blade, box cutter, or utility knife to cut Sticky Traps in half. *Helpful Hint: use a dedicated, sharp blade. The Tanglefoot will get onto your blade and limit the value of that cutter for other uses. Cutting traps in half is also facilitated with a hard, straight edge such as a wooden ruler to guide the cutting blade. As with the blades, a dedicated guide capable of becoming contaminated with Tanglefoot is suggested. While scissors will work to cut a single trap, use of scissors is not recommended, as they rapidly clog with Tanglefoot and cease to function, leading to imprecise trap cutting.*
- Galvanized wire hoop Sticky Trap holders (Bioquip catalog #2874)
- Tomato cages, prepared in advance with ½ inch wide metalized bird-deterrent mylar tape (e.g., TheTape Depot catalog #71858SLO001) attached (tied or stapled to itself) approximately every decimeter around the circumference of the cage (Figure 2).

Equipment and supplies for the epigeal pitfall surveys include:

- Marine-friendly, less-toxic antifreeze
- Plastic cups (preferred model; Solo Product# TP9-9oz.) with a 9cm diameter rim. *Helpful Hint: while the depth of the cup can be variable, the Solo TP-9oz. cups with a depth of 7.2 cm are preferred for consistency.* While deeper cups may be used, they require additional soil disturbance and do not yield any significant improvement in performance. These larger cups also entice field technicians to put excessive amounts of antifreeze into each cup, necessitating additional coolant use.
- Small plastic plates big enough to extend over the edge of the cups. *Helpful Hint: While any style/color plate will work, opaque colored plates which obscure the antifreeze-containing traps and reduce the attractiveness to curious carnivores are preferred.*
- *Alternative to plastic cups and plates:* 50 mL centrifuge vials with leak-proof screw cap lids. Note: the opening will be much narrower with less likelihood of consistent invertebrate capture. This method is one of the alternate tidal survey options (“Method 2”).
- Rubber bands
- Hand gardening trowel



Figure 2. Deployed tomato cage with metallic ribbon and green wire.

- Nails, screws, or coated wire (14 gauge, in ~20 cm segments)
- Fabric and garden staples (optional with alternate “Method 1” and “Method 2”)

Laboratory equipment and supplies:

- 500 μm Geological Sieve (= ASTM Sieve Size 35; = Tyler Mesh Size 32) or 300 μm for intertidal
- Tweezers, scoops, small spatulas and/or additional laboratory utensils
- Dissecting scope and light source
- Invertebrate identification books and/or manual (e.g., PIRatE and TBF Coastal Salt Marsh and Coastal Strand Pitfall Invertebrate Key V3.0, 2014)
- Small ruler or calipers
- Hand counter (optional)
- Petri dish(es)
- Squirt bottle filled with 70% ethanol and funnel (optional)
- Glass vials or jars and Parafilm (Model# PM-996) for storage. *Helpful Hint:* the smallest size container for long-term sample preservation is desirable. This will vary depending upon your site, but a safe initial purchase will be 4 oz. wide-mouth glass jars (for larger individuals or abundant captures) and 20 mL vials (for depauperate captures)
- Laboratory labeling tape (colored – optional)
- Magnifying glass or hand lens

Field Preparation

The tomato cages should be prepared in advance by using the small gardening wire to wrap strategically through the largest holes (if present) to reduce the possibility of a bird flying into the tomato cage. Distance between the wires should be approximately 15 cm (6 inches) or less. Additionally, several small pieces of the metallic ribbon should be tied or stapled to itself around both the top and middle of the tomato cage (Figure 2). These will also act as bird deterrents. The direct from factory Sticky Traps should be cut in half prior to field deployment, and both Sticky Traps and pitfall trap cups should be labeled in advance with location (e.g., site name and transect number), deployment date, and replicate (e.g., 1-3 for each transect).

Equipment described should be collected prior to the field shift. Batteries for all electronic devices should be checked and replaced as needed, and relevant data sheets should be printed.

Field Methods: Aerial Arthropod Traps

Aerial arthropod traps for any given vegetation transect should be deployed in replicate. Specific transect selection should follow the same randomly allocated vegetation transects within each of the marsh habitat types (see Vegetation Cover SOP 3.2 for details on randomly allocating transect locations). Traps should be placed in conjunction with the pitfall traps (within 1 m, see Figure 3, below).

1. Label each trap using a permanent ink pen with the individual transect number, date deployed, and replicate (i.e., 1, 2, or 3) along the transect (Figure 1).

2. Deploy three sticky traps equidistant along 30 m transects, which extend 2.5 meters past the start and end of the 25 m vegetation transects (Figure 3). Note: when conditions are particularly windy (i.e., >40 KPH or 25 MPH) for extended periods of time, the plastic Sticky Traps may crack at the wire holder. It is best to avoid deploying traps under these conditions. But if trap deployment cannot be forestalled, deploy an additional replicate (n=4) may be deployed to assure that the sample size is not limited by wind impacts to the traps.
 - a. Each Sticky Trap should be placed so the lower edge of the sheet is approximately 5-10 cm above the uppermost surface of the substrate (e.g., the soil surface of unvegetated salt pans) or vegetation canopy (Figure 1). In cases of short or sparse vegetation, the insect trap should be set approximately 10 cm above the bare ground to avoid potential inundation or entanglement with blowing plant stems (Ambrose et al. 2006, Anderson 2009). Traps should never be placed such that the lower trap edge is suspended more than 15 cm above the highest surface. Placing traps too high will significantly reduce the diversity and abundance of the capture and artificially lower productivity estimates. Assure that any stray plant stems are trimmed such that wind gusts will not blow vegetation onto the Sticky Trap surface.
 - b. If birds are present in the sampling area, place a tomato cage with reflective tape over the deployed sticky trap to deter bird activity. As with plant stems, assure that the reflective tape will not contact the trap surface before concluding deployment.
3. Leave traps out for four days (deployment times of 3-6 days produce statistically indistinguishable results when standardized for days of deployment; Anderson 2009). While four days is the default deployment time, the ultimate goal is to accrue maximum saturation of the trap surface area by arthropods. Using an *a priori* assumption or previous field survey site knowledge, deployment should tend towards three days where arthropods fairly are abundant. In situations where arthropods are scarce, deployment should tend towards six days.
4. Upon collection, wrap the traps with clear plastic film (i.e., "Saran™ Wrap") and return them to the lab for processing. Care should be taken to stretch the plastic film taut and maintain a smooth surface over both faces of the trap (a wrinkle-free plastic covering will greatly speed the subsequent lab processing of the traps). This clear film prevents additional items or sediment from getting stuck upon the trap surface, allows traps to be stacked without sticking to one



Figure 3. Deployed invertebrate transect. Grey PVC pole is the end of a vegetation transect.

another, and allows rapid processing in the laboratory.

5. Traps should normally be processed within 10 days of collection (see Laboratory Methods section), however if the trap surface was wet (e.g., collected in heavy fog or dew) when collected and filmed, processing should occur immediately (within three days). Wet traps/arthropods will decay rapidly, particularly during warm summertime conditions.



Figure 4. Deployed pitfall trap with antifreeze (top) and covered by a plastic plate (bottom).

Field Methods: Pitfall Traps

Epigeal pitfall traps should be placed along the same randomly allocated vegetation transects within each of the marsh habitat types (see Vegetation Cover SOP for details on randomly allocating transect locations). Pitfall traps should be placed in conjunction with the aerial Sticky Traps (within 1m, see above). Generally, the pitfall traps using the cup method should not be deployed in the lower marsh/intertidal zone (see “centrifuge method”, below for intertidal deployment strategies). These two methods can be extrapolated up by area for analyses but may not provide complimentary data.

1. Label the side of each trap (cup) using a permanent ink pen on a strip of duct tape with the individual transect number, date deployed, and replicate (i.e., 1, 2, or 3) along the transect.
2. Deploy three to four pitfall traps equidistantly spaced along 30 m transects, which extend 2.5 meters past the start and end of the 25 m vegetation transects. Be consistent across all transects and normalize to survey area based on the number of traps.

- a. Dig a small hole in the surface of the sediment to the depth of and slightly wider than the rim of the cup using a hand trowel. Place excavated soil to the side for use momentarily.
- b. Sink the cup into the excavated hole. The rim should rest 1-3 millimeters lower than the surrounding soil surface but avoid spilling sediment into the trap itself. Should any sediment fall into the cup, remove the cup, empty the soil, and repeat the deployment.
- c. Pack the extra (removed) sediment into the space between the edge of the excavated hole and the cup’s rim to create an unbroken soil surface such that invertebrates will experience no gaps/cracks before the encounter the rim of the cup itself (Figure 4).
Helpful hint: stack two cups and insert them together into the hole, adjust the sediment, then pull the top cup out, leaving the bottom cup clean (devoid of sediment) and flush with the soil surface.
- d. Pour 1-2 cm of antifreeze into the base of the cup to act as a euthanizing medium which will not evaporate under excessive summertime/direct sunlight conditions.
- e. Cover the cup with the plastic plate suspended 2-4 cm above the soil surface by pushing

the nails/screws/wire through the plate and into the sediment until the appropriate height is reached, allowing invertebrates access but deterring larger animal tampering.

3. Leave traps out for four days (deployment times of 3-6 days produce statistically indistinguishable results when standardized for days of deployment; see notes for Sticky Trap deployment duration; Anderson 2009).
4. Upon collection, pull the cups out of the soil, replace the soil, cover the traps with Parafilm or a clear plastic film secured with a rubber band, and return to the lab for processing. Care should be taken to avoid spilling the samples or the antifreeze.
5. Traps should be processed within 3-5 days of collection (see Laboratory Methods).

Field Methods: Pitfall Traps in Intertidal Zones (Alternate Deployment Method)

As indicated in the previous pitfall deployment methods, it is difficult or infeasible to use the aforementioned procedure in the lower marsh areas and intertidal zones. These pitfall traps would be completely inundated with water from the incoming and outgoing tides, spilling the contents into the marsh. Due to the difficulty of collecting data, this zone is often overlooked. There are two potential methods to collect quantitative data of terrestrial invertebrates in the intertidal zones.

The first method (see “Method 1: Cup Removal,” below) is very similar to the non-tidal habitat pitfall trap deployment but requires much more significant effort regarding timing around the tides (pulling and placing daily or semi-daily). Care should be taken to account for the exact deployment times to allow for cross-habitat evaluations of biomass or productivity. Only the revisions to the standard deployment method are included below and should be combined with the pitfall trap deployment methods found above.

The second method (see “Method 2: Vial Deployment,” below) is essentially a combination of the two deployment methods previously discussed for pitfall traps but uses a different trap and smaller holes. It also requires an extra deployment step. Similarly to “Method 1,” only the revisions to the standard deployment methods are included below.

Method 1: Cup Removal

Revisions to 2a: Begin to deploy traps while the tides are falling (deploy highest elevation areas first and follow tides down the elevation gradient). To maximize deployed time, begin trap placement as soon as the soil is no longer completely submerged. Place each cup using the same strategies as the non-tidal pitfall methods. Dig a small hole in the surface of the sediment to the depth of and slightly wider than the rim of the cup rim using a hand trowel. Place excavated soil to the side for use momentarily.

Revisions to 2c: If the cups begin to rise due to the soil still being saturated with water, use small stakes to hold them into the ground (Fabric and Garden Staples work well).

Revisions to 3: Try to leave the traps out for 4-6 hours in the same tidal period or until the tide rises to the elevation of the transect, then cover and remove. Replace as described in “revisions to 2a,” above.

Repeat daily or semi-daily matching the tide pattern; try to achieve a similar deployment time as the 3-6 day time frame of the standard pitfall deployment method. It is helpful to have an in-depth understanding of the local field conditions regarding inundation times within the survey area.

Method 2: Vial Deployment

Revisions to 1: Additionally, on the first deployment day, fill each vial to the rim with water to minimize the air in the container; then, screw the lid on tightly.

Revisions to 2c: Additionally, use stakes (Fabric and Garden Staples) to help hold the vials down in the ground and to prevent the traps from rising with the incoming tide. Leave the traps deployed (closed and full of water) until the following day. This minimizes the disturbance from creating the holes.

Revisions to 3: Once the tide has fallen below the elevation of the transect, return to the survey area, remove the stakes and water from the vial, and replace the vial in the ground with antifreeze (uncovered). If the vials rise from soil saturation, use the stakes to hold them down. Try to leave the traps out for 4-6 hours in the same tidal period or until the tide rises to the elevation of the transect, then cover and remove. Replace as described in “revisions to 2a,” above. Repeat daily or semi-daily matching the tide pattern; try to achieve a similar deployment time as the 3-6 day time frame of the standard pitfall deployment method. It is helpful to have an in-depth understanding of the local field conditions regarding inundation times within the survey area.

Laboratory Methods: Aerial Arthropod Traps

Processing of the aerial traps (Figure 5) follows methods developed by Dr. Sean Anderson, California State University Channel Islands / Pacific Institute for RestorATion Ecology (PIRatE Lab):

1. All individual invertebrates should be counted and classed by size (anterior-posterior length) into one of five operationally-determined categories: <0.5 mm, 0.5-2 mm, 2-5 mm, 5-10 mm, or >10 mm and recorded on the appropriate datasheet (Appendix 6.2B). *Helpful hint:* for traps with high numbers of individuals, use a permanent ink pen to divide up the trap into quarters or other convenient subdivisions and count each subdivision separately. It may be beneficial to use a magnifying glass to count the smaller invertebrates.



Figure 5. Aerial sticky trap ready for processing.

2. Aerial arthropod biomass is estimated by extrapolation based on weight and number of individuals per size class, according to the following formula and length-fresh weight regressions by size class (S. Anderson, pers. comm. 2009):

$$(\# \text{ of arthropods in size class } Y) \times (\text{fresh weight regression multiplier for size class } Y \text{ in g}) \\ \times (\text{trap area in m}^2) \times (\text{duration in days}) = \text{productivity of size class } Y$$

3. Multiply the number of arthropods in a given size category by the average fresh weights and sum to produce total productivity in the form of grams of arthropods per m² per day.
4. Each Sticky Trap (front and back together) is considered a single trap (i.e., a single spatial plane through which insects passed).
5. Multipliers for estimating arthropod productivity:
 - a. <0.5mm: mean individual fresh weight = 0.0000079g
 - b. 0.5-2mm: mean individual fresh weight = 0.0002738g
 - c. 2-5mm: mean individual fresh weight = 0.0009839g
 - d. 5-10mm: mean individual fresh weight = 0.0081993g
 - e. >10mm: mean individual fresh weight = 0.097621g

Laboratory Methods: Pitfall Traps

Processing of the pitfall traps (Figure 6) follows methods developed by Dr. Sean Anderson, California State University Channel Islands / PIRatE lab and The Bay Foundation:

1. Separate all individual invertebrates from the antifreeze by pouring all material out of the sampling cup through a 500 µm sieve. If analyzing terrestrial invertebrates from the intertidal habitats, use the 300 µm sieve. *Helpful hint:* if done using a funnel, the first pour of the antifreeze can be reused to reduce waste. Antifreeze may be reused for an extended period if care is taken to avoid excessive accumulation of dirt and other contaminants.
2. Repeatedly rinse remaining sample with distilled water until only debris (too large to fit through the sieve) and invertebrates remain in the sieve. Keep in mind, the water that comes through the sieve is considered biological waste and should be disposed of according to individual laboratory hazardous waste disposal protocols. As such, care should be taken to minimize excessive rinsing.
3. If ancillary catch is present in the sample (e.g., juvenile lizard), it should be stored as a voucher specimen for the site or disposed of at the discretion of the



Figure 6. Representative pitfall sample in ethanol.

project manager (after first being measured, photographed, and identified). Such ancillary catch may require a formalin preservation in contrast to the normal ethanol-based archiving.

- Using tweezers, scoops, small spatulas and other laboratory utensils, separate invertebrates from debris, rocks, and remaining sediment. *Helpful hint:* remove the largest debris first, check for attached invertebrates, and dispose of properly before pulling inverts off the sieve mesh.
- Place invertebrates into label glass vials and cover completely with 70% ethanol. Seal vial with a layer of parafilm.

Identification of the invertebrates:

- All individual invertebrates should be placed in petri dishes (Figure 6) and grouped into the lowest possible taxa (to a minimum of Order, but higher resolution if possible) using invertebrate identification books, manuals (e.g., PIRatE Coastal Salt Marsh and Coastal Strand Pitfall Invertebrate Key V2.0, 2013), and online identification resources (e.g., www.bugguide.net). Dissecting scopes (or higher power scopes) and light sources are recommended to identify minute anatomical features of each taxonomic group (Figures 7 and 8). Larger specimens may be identified using a small magnifying glass.
- The number of individuals in each taxon should be counted. In addition, a representative size class estimate (approximate mean) and a maximum size should be recorded for each group (see Appendix 6.2C for a copy of the datasheet).



Figure 7. Charles Piechowski using a dissection scope to identify and measure pitfall invertebrates.



Figure 8. Photo of a scavenger beetle taken under a dissection scope (Photo: Maria Wong).

- Completed samples are placed back into a glass vial, covered with 70% ethanol, and labeled as complete along with sampler technician's name and completion date.
- Epigeal invertebrate biomass is estimated by extrapolation based on weight and number of individuals per size class, according to the following formula and length-fresh weight regressions by size class (S. Anderson, pers. comm. 2009):

$$(\text{\# of arthropods in size class Y}) \times (\text{fresh weight regression multiplier for size class Y}) \times (\text{area}) \times (\text{duration}) = \text{productivity of size class Y}$$

10. Multiply the number of arthropods in a given size category by the average fresh weights and sum to produce total productivity in the form of grams of arthropods per m² per day.

Data Entry and QAQC Procedures

Data should be entered in the laboratory using the appropriate data sheet (Appendices 6.2B and 6.2C). All required fields should be completed in full, and the data recorder should assign their name at the top of the document(s). Data should be transferred to the appropriate electronic database within three days, and the hard copies filed in labeled binders. Electronic copies of all data should be housed on an in-house dedicated server and backed up to a cloud-based or off-site server nightly. Hard copies should be saved for five years. Electronic copies should be saved indefinitely.

Quality Assurance and Quality Control (QAQC) procedures should be conducted on all data. QAQC procedures should be conducted by the QA Officer and include a thorough review of all entries, double checking of all formulas or macros, and a confirmation that all data sheets, Chain-of-Custody forms, and field notes are filed appropriately with electronic back-up copies available. QAQC should verify that the entered data match the hard copies of the field data sheets. Any discrepancies should be corrected, and the initial data entry technician notified.

Extensive QAQC should be conducted on every twentieth completed pitfall and flying arthropod sample to ensure accuracy of taxonomic identifications and size class estimates. The sample should be reprocessed, discrepancies corrected, and the initial technician notified. Additional QAQC of samples sorted by that technician should be repeated at the discretion of the QA Officer, and the technician may be required to go through the laboratory training again.

Data Analyses

After data have been entered, corrections made, and QAQC procedure completed, data can be used in multiple analyses. Examples include graphs of biomass or productivity by habitat or assessments of individual transect or area biomass and productivity. Each pitfall trap should be analyzed independently.

Health and Safety Precautions

Extreme caution should be taken to ensure no anti-freeze is spilled on wetland soils or disposed of improperly in the laboratory.

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APPENDIX 6.2A

	Evaluation Metric	Aerial traps	Pitfall traps (non-tidal)	Pitfall traps (tidal)	Notes
	Correlation to L2 CRAM	Not Applicable	Not Applicable	Not Applicable	Loosely tied to biotic metrics
Personnel Requirements	Specialty Equipment or Clothing Required	Few Specialty Items	Many Specialty Items	Many Specialty Items	Sticky traps, microscope, tomato cages, antifreeze
	Ease of Transport (amount or weight of supplies)	Many or Heavy Items / Difficult	Many or Heavy Items / Difficult	Many or Heavy Items / Difficult	Primarily for the tomato cages and collection of the processed samples, which can be bulky
	Ease of Implementation	Easy	Easy	Difficult	Tidal requires frequent checks
	Expertise / Skill Level	Some Technical Knowledge	High Technical Knowledge	High Technical Knowledge	No technical knowledge required for field implementation; Familiarity with species identifications is required for laboratory processing
	Number of Personnel	2	2+	2+	Two personnel are fine, more increases speed
	Training Requirements	Some	Some	Some	Familiarity with taxonomic identifications as required; may be necessary for laboratory processing
	Seasonality of Survey Time	During peak productivity	During peak productivity	During peak productivity	May be performed in conjunction with vegetation surveys to capture site conditions concurrently
	Suggested Frequency	Annual	Annual	Annual	Or semi-annual; project-dependent
Survey / Data Quality	Type of Output	Numerical	Numerical	Numerical	----
	Active or Passive Monitoring Style	Active	Active	Active	----
	Specialty Computer Software Required	No	No	No	----
	Availability of Online / External Resources	Some	Some	Some	----
Potential Limitations	Wetland Type Applicability	All	All	All	----
	Images or Multi-Media Required	Images Suggested	Images Required	Images Required	Voucher photographs recommended
	Degree of Impact / Disturbance	Low Disturbance	Moderate Disturbance	Moderate Disturbance	Soil disturbance will be required
	Vegetation Height Limitation	Overhead	None	None	Must be able to place the sticky trap above highest vegetation
	Appropriate for Tidal / Wet Habitats	Yes	No	Yes	See tide height for aerial surveys
	Tide Height	< 2 feet	Not Applicable	Full	High tide level must be below sticky trap
	Regional or Broad Implementation *	Infrequently Used	Infrequently Used	Infrequently Used	<i>* based on monitoring literature review</i>
	Potential for Hazards / Risk	Medium Risk	Medium Risk	Medium Risk	Tanglefoot and antifreeze
	Restrictions	Special Status Species	Special Status Species	Special Status Species	----

APPENDIX 6.2B

FLYING INVERT DATASHEET

Sampling Program Information			
DATE:	STAFF:		FID:
TIME (start):	(end):	SAMPLE DATE:	

TRAP 1 (front)	SIZE CLASS	COUNT	SPECIES: _____	COUNT: _____
	(~0.5 mm):	<input type="text"/>	SPECIES: _____	COUNT: _____
	<2 mm:	<input type="text"/>	SPECIES: _____	COUNT: _____
	2-5mm:	<input type="text"/>	SPECIES: _____	COUNT: _____
	5-10mm:	<input type="text"/>	SPECIES: _____	COUNT: _____
	>10 mm:	<input type="text"/>	SPECIES: _____	COUNT: _____
TRAP 1 (back)	SIZE CLASS	COUNT	SPECIES: _____	COUNT: _____
	(~0.5 mm):	<input type="text"/>	SPECIES: _____	COUNT: _____
	<2 mm:	<input type="text"/>	SPECIES: _____	COUNT: _____
	2-5mm:	<input type="text"/>	SPECIES: _____	COUNT: _____
	5-10mm:	<input type="text"/>	SPECIES: _____	COUNT: _____
	>10 mm:	<input type="text"/>	SPECIES: _____	COUNT: _____
Morphic Species				

TRAP 2 (front)	SIZE CLASS	COUNT	SPECIES: _____	COUNT: _____
	(~0.5 mm):	<input type="text"/>	SPECIES: _____	COUNT: _____
	<2 mm:	<input type="text"/>	SPECIES: _____	COUNT: _____
	2-5mm:	<input type="text"/>	SPECIES: _____	COUNT: _____
	5-10mm:	<input type="text"/>	SPECIES: _____	COUNT: _____
	>10 mm:	<input type="text"/>	SPECIES: _____	COUNT: _____
TRAP 2 (back)	SIZE CLASS	COUNT	SPECIES: _____	COUNT: _____
	(~0.5 mm):	<input type="text"/>	SPECIES: _____	COUNT: _____
	<2 mm:	<input type="text"/>	SPECIES: _____	COUNT: _____
	2-5mm:	<input type="text"/>	SPECIES: _____	COUNT: _____
	5-10mm:	<input type="text"/>	SPECIES: _____	COUNT: _____
	>10 mm:	<input type="text"/>	SPECIES: _____	COUNT: _____
Morphic Species				

TRAP 3 (front)	SIZE CLASS	COUNT	SPECIES: _____	COUNT: _____
	(~0.5 mm):	<input type="text"/>	SPECIES: _____	COUNT: _____
	<2 mm:	<input type="text"/>	SPECIES: _____	COUNT: _____
	2-5mm:	<input type="text"/>	SPECIES: _____	COUNT: _____
	5-10mm:	<input type="text"/>	SPECIES: _____	COUNT: _____
	>10 mm:	<input type="text"/>	SPECIES: _____	COUNT: _____
TRAP 3 (back)	SIZE CLASS	COUNT	SPECIES: _____	COUNT: _____
	(~0.5 mm):	<input type="text"/>	SPECIES: _____	COUNT: _____
	<2 mm:	<input type="text"/>	SPECIES: _____	COUNT: _____
	2-5mm:	<input type="text"/>	SPECIES: _____	COUNT: _____
	5-10mm:	<input type="text"/>	SPECIES: _____	COUNT: _____
	>10 mm:	<input type="text"/>	SPECIES: _____	COUNT: _____
Morphic Species				



Standard Operating Procedures (7.1): California Rapid Assessment Method

March 2021

Prepared for the United States Environmental
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Protocol Suitability Evaluation

A habitat suitability table containing appropriate coastal wetland habitat types (of those evaluated) to implement several California Rapid Assessment Method (CRAM) modules is displayed in Table 1. A comparative assessment of cost, effort, and data quality are shown in Table 2. A matrix of additional detailed categorical evaluations CRAM protocols can be found in Appendix 7.1A.

Table 1. Appropriate habitat types for CRAM survey protocols.

Survey Protocol	Habitat Types					
	Tidal Channel	Mud/sand flat	Emergent salt marsh	Non-tidal salt marsh	Salt pan	'Degraded' / fill
Estuarine CRAM	X (partial)	X (partial)	X			
Bar-Built CRAM	X (partial)	X (partial)	X	X		
Depressional CRAM				X		X
Slope CRAM				X		X
Playa CRAM					X	
Vernal Pool CRAM				X		X

Table 2. Categorical assessment of cost/effort and data quality for CRAM survey protocols.

	Evaluation Metric	CRAM Survey	Notes
Time / Effort	Office Preparation Time (per AA)	> 60 minutes	Identification of CRAM Assessment Area locations, background research, and Attributes 1 and 2 (using maps)
	Equipment Construction Time (one time)	Not Applicable	----
	Field Time (per AA)	> 120 minutes	One AA usually takes approximately three hours to complete
	Laboratory Time (per AA)	Not Applicable	----
	Post-Survey Processing / QAQC Time	10-30 minutes	Mainly data entry and raw score computation
	Minimum Repetition (site-dependent)	Few Repetitions	Depends on size of site, variability of Assessment Area scores and quantity of hydrologic sub-units
	Relative Cost (equipment and supplies)	< \$15	----
Survey / Data Quality	Accuracy (at a survey area level)	Medium	----
	Precision (at a survey area level)	High	----
	Qualitative-Quantitative Score	Qualitative	----
	Subjectivity-Objectivity Score	Subjective	----

Resulting Data Types

The application of CRAM survey protocols will yield a quantitative final “Index” score between 25 and 100 for each individual Assessment Area (AA). Additionally, in each AA, scores will be recorded for a variety of metrics and attributes which can be analyzed independently or as part of the final score. Resulting data may be averaged for multiple AAs within the same hydrologic unit to provide a broad-scale condition score which may be compared to statewide quartiles as an assessment of regional or

project-level health. As CRAM scores are standardized, they may be compared across wetland types. For more details on CRAM assessment and analyses, refer to the CRAM Technical Bulletin (2019).

Objective

The following description of the summary and objectives of CRAM surveys are directly cited from the CRAM User Manual (CWMW 2012a):

“The overall goal of CRAM is to provide rapid, scientifically defensible, standardized, cost-effective assessments of the status and trends in the condition of wetlands and the performance of related policies, programs and projects throughout California...

A consortium of local, state and federal authorities has been developing new tools to increase the State’s capacity to monitor its wetlands. Level 2 consists of rapid assessment of wetland condition in relation to the broadest suite possible of ecological and social services and beneficial uses. CRAM is being developed as a cost-effective and scientifically defensible Level 2 method for monitoring the conditions of wetlands throughout California. The CRAM web site (www.cramwetlands.org) provides access to an electronic version of this manual, training materials, eCRAM, and the CRAM database. CRAM results can be uploaded to the database, viewed, and retrieved via the CRAM web site using eCRAM. CRAM, eCRAM, and the supporting web sites are public and non-proprietary...

CRAM enables two or more trained practitioners working together in the field for one half day or less to assess the overall health of a wetland by choosing the best-fit set of narrative descriptions of observable conditions ranging from the worst commonly observed to the best achievable for the type of wetland being assessed. Metrics are organized into four main attributes: (landscape context and buffer, hydrology, physical structure, and biotic structure) for each of six major types of wetlands recognized by CRAM (riverine wetlands, lacustrine wetlands, depressional wetlands, slope wetlands, playas, and estuarine wetlands).”

More details on CRAM technical resources, manuals, SOPs, and documents can be found on the website: <https://www.cramwetlands.org/documents>.

Equipment

Equipment and supplies needed for this survey include:

1. GPS
2. Camera or camera app on a phone (e.g., “Solocator”)
3. Range finder (preferable) or two 100 m transect tapes
4. CRAM Field Guide (required) and User Manual (optional)
5. Datasheets (Appendix 7.1B) and site maps with scale showing assessment area (an example can be found in Appendix 7.1C)

6. Meter stick to measure vegetation heights

Field Preparation

CRAM surveys for any of the wetland modules should only be conducted by trained practitioners who have received the corresponding CRAM training prior to any field work. For more information see www.cramwetlands.org. Batteries for all electronic devices should be checked and replaced as needed, and relevant data sheets should be printed and attached to the clipboards. Note that Assessment Areas (AA)'s should be defined *a priori* using mapping software (e.g., Google Earth, ArcGIS Pro, QGIS, etc.). Follow the User Manual requirements for defining an AA. Maps should be printed of each AA (Appendix 7.1C), including a scale bar, and attached to the datasheets. The final location of AAs can be adjusted in the field.

The following list describes the overarching steps for using CRAM (CWMW 2012a, pp 15):

- Step 1.* Assemble background information about the management of the wetland.
- Step 2.* Classify the wetland using CRAM typology.
- Step 3.* Verify the appropriate season and other timing aspects of the field assessment.
- Step 4.* Estimate the boundary of the AA in the office (subject to field verification).
- Step 5.* Conduct the office assessment of stressors and on-site conditions of the AA.
- Step 6.* Conduct the field assessment of stressors and on-site conditions of the AA (see below).
- Step 7.* Complete CRAM assessment scores and QA/QC Procedures.
- Step 8.* Upload CRAM results into statewide information data management system.

For details about each of the steps and what they entail, refer to the User Manual (CWMW 2012) or the corresponding Field Book (e.g., CWMW 2012b and CWMW 2012c).

Field Methods

Detailed field methods should follow protocols described in the User Manual (CWMW 2012) and the Field Book that corresponds with the type of wetland being surveyed for CRAM (e.g., CWMW 2012b, CWMW 2012c). Appendix 7.1B contains an example copy of the Estuarine CRAM datasheets, and Appendix 7.1C is an example of appropriate maps for one AA.

Helpful hint: In addition to the protocols in the field manual, marking the centroid of the AA with a PVC pipe will assist in finding the site again, and in permanently marking the location.



Figure 1. Example georeferenced photograph.

Additionally, geotagged and watermarked photographs using a phone application such as “Solocator” can help take georeferenced bearing photographs (Figure 1).

Laboratory Methods

Not applicable.

Data Entry and QAQC Procedures

Under no circumstances should anyone modify procedures to establish CRAM AAs, combine any aspects of two or more CRAM modules, or modify CRAM Attributes, Metrics, Metric descriptors, scoring tables, or procedures for calculating scores (CWMW 2019). Doing so will invalidate the CRAM assessment.

Data should be entered in the field using the appropriate data sheets (e.g., Appendix 7.1B). All required fields should be completed in full, and the data recorder should assign their name at the top of the document(s). Data should be transferred to the appropriate electronic database within three days, and the hard copies filed in labeled binders. It is also recommended to scan and store copies of raw data sheets electronically. Electronic copies of all data should be housed on an in-house dedicated server and backed up to a cloud-based or off-site server. Hard copies should be saved for five years. Electronic copies should be saved indefinitely.

Quality Assurance and Quality Control (QAQC) procedures should be conducted on all data. QAQC procedures should be conducted by the QA Officer and include a thorough review of all entries, double checking of all formulas or macros, and a confirmation that all data sheets and field notes are filed appropriately with electronic back-up copies available. QAQC should also verify that the entered data match the hard copies of the field data sheets. Any discrepancies should be corrected, and the initial data entry technician notified. QAQC’ed CRAM data should also be entered into the eCRAM public database through: www.cramwetlands.org. Only scores that are consistent with all QAQC requirements should be used to support regulatory and grant funding decisions; entry into eCRAM, for modules supported by eCRAM, is strongly encouraged (CWMW 2019).

Data Analyses

CRAM is intended for cost-effective ambient and project-specific monitoring and assessment that can be performed on different scales, ranging from an individual wetland to a watershed or a larger region. It can be used to develop a picture of reference condition for a particular wetland type or to create a landscape-level profile of the conditions of different wetlands within a region of interest. This information can then be used in planning wetland protection and restoration activities. CRAM is intended to assess the overall condition of wetlands and streams (i.e., functional capacity); CRAM does not measure functions (CWMW 2019). In many cases, CRAM must be used in conjunction with Level 1 and 3 methods to provide the needed breadth and depth of assessment.

Additional CRAM applications could include (CWMW 2012a):

- *Preliminary* assessments to determine the need for more traditional intensive analysis or monitoring;
- Providing *supplemental* information during the evaluation of wetland condition to aid in regulatory review under Section 401 and 404 of the Clean Water Act, the Coastal Zone Management Act, Section 1600 of the Fish and Game code, or local government wetland regulations;
- *Comparing* to ambient or reference systems; and
- *Assisting* in the monitoring and assessment of restoration or mitigation projects by providing a rapid means of checking progress along restoration trajectories.

Data can be evaluated by combining metric scores into an attribute score. The four attribute scores are averaged for an Index score for a given AA. Multiple AA Index scores can be averaged for a general condition assessment of that particular wetland habitat area. Care should be taken to use the data only as recommended by the User Manual (CWMW 2012a) and the Technical Bulletin (CWMW 2019) and not for purposes such as mitigation requirements. Refer to CWMW 2019 for appropriate confidence intervals.

Health and Safety Precautions

Not applicable.

References and Applicable Literature

California Wetlands Monitoring Workgroup (CWMW). 2012a. California Rapid Assessment Method (CRAM) for Wetlands and Riparian Areas, Version 6.0 pp. 95

California Wetlands Monitoring Workgroup (CWMW). 2012b. "Perennial Estuarine Wetlands Field Book." *California Rapid Assessment Method for Wetlands*. Version 6.0.

California Wetlands Monitoring Workgroup (CWMW). 2012c. "Depressional Wetlands Field Book." *California Rapid Assessment Method for Wetlands and Riparian Areas*. Version 6.0.

California Wetland Monitoring Workgroup (CWMW). 2019. Using the California Rapid Assessment Method (CRAM) for Project Assessment as an Element of Regulatory, Grant, and other Management Programs. Technical Bulletin – Version 2.0, 85 pp.

Fetscher, A.E., Collins, J., Grenier, L., Clark, R., Sutula, M. 2005. "Quality Assurance Project Plan for the Development of a Wetland Rapid Assessment Method in California: Phase II". *Prepared for the United States Environmental Protection Agency*.

Contact Information

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APPENDIX 7.1A

	Evaluation Metric	CRAM Survey	Notes
	Correlation to L2 CRAM	All Attributes	(same protocol)
Personnel Requirements	Specialty Equipment or Clothing Required	No Specialty Items	----
	Ease of Transport (amount or weight of supplies)	Some Items / Moderate	Only basic items are necessary (e.g., GPS, datasheet, clipboard)
	Ease of Implementation	Moderate	Depends on complexity of Assessment Area; usually approximately three hours per AA
	Expertise / Skill Level	Specific Training Required	Registration for CRAM trainings may be found at http://www.cramwetlands.org/training
	Number of Personnel	2 or more	Due to some subjectiveness of the survey methods, more scientific opinions will yield a higher degree of accuracy and reduce the subjectivity
	Training Requirements	CRAM certification training	Registration for CRAM trainings may be found at http://www.cramwetlands.org/training
	Seasonality of Survey Time	Spring and Fall	Varies based on the individual wetland module being applied.
	Suggested Frequency	Semi-annual	Dependent on the monitoring program objectives
Survey / Data Quality	Type of Output	Numerical	----
	Active or Passive Monitoring Style	Passive	----
	Specialty Computer Software Required	No	Mapping software (open source) is recommended for ease in mapping steps, though not required
	Availability of Online / External Resources	Many	Most materials may be found at http://www.cramwetlands.org/
Potential Limitations	Wetland Type Applicability	All	Specific modules are available for individual wetland types
	Images or Multi-Media Required	Images Required	----
	Degree of Impact / Disturbance	Low Disturbance	----
	Vegetation Height Limitation	No Limitations	----
	Appropriate for Tidal / Wet Habitats	Yes	----
	Tide Height	Low tide preferred	Must be able to view attributes within intertidal habitat areas
	Regional or Broad Implementation *	Almost Always Used	----
	Potential for Hazards / Risk	Low to No Risk	----
Restrictions	Special Status Species	----	

* based on monitoring literature review

APPENDIX 7.1B

Basic Information Sheet: Perennial Estuarine Wetlands

Assessment Area Name:					
Project Name:					
Assessment Area ID #:					
Project Site ID #:				Date:	
Assessment Team Members for This AA					
Center of AA:					
Latitude:			Longitude:		
Wetland Sub-type:					
<input type="checkbox"/> Perennial Saline		<input type="checkbox"/> Perennial Non-saline			
AA Category:					
<input type="checkbox"/> Restoration <input type="checkbox"/> Mitigation <input type="checkbox"/> Impacted <input type="checkbox"/> Ambient <input type="checkbox"/> Reference <input type="checkbox"/> Training					
<input type="checkbox"/> Other:					
What best describes the tidal stage over the course of the time spent in the field?					
Note: It is recommended that the assessment be conducted during low tide.					
<input type="checkbox"/> high tide		<input type="checkbox"/> low tide			
Photo Identification Numbers and Description:					
	Photo ID No.	Description	Latitude	Longitude	Datum
1		North			
2		South			
3		East			
4		West			
5					
6					
7					
8					
9					
10					

Site Location Description:

Comments:

Scoring Sheet: Perennial Estuarine Wetlands

AA Name:			Date:			
Attribute 1: Buffer and Landscape Context (pp. 8-14)			Comments			
Aquatic Area Abundance (D)	Alpha.	Numeric				
Buffer (based on sub-metrics A-C)						
<i>Buffer submetric A:</i> <i>Percent of AA with Buffer</i>			Alpha.	Numeric		
<i>Buffer submetric B:</i> <i>Average Buffer Width</i>						
<i>Buffer submetric C:</i> <i>Buffer Condition</i>						
Raw Attribute Score = $D + [C \times (A \times B)^{1/2}]^{1/2}$			Final Attribute Score = (Raw Score/24) x 100			
Attribute 2: Hydrology Attribute (pp. 15-19)						
Water Source	Alpha.	Numeric				
Hydroperiod						
Hydrologic Connectivity						
Raw Attribute Score = sum of numeric scores			Final Attribute Score = (Raw Score/36) x 100			
Attribute 3: Physical Structure Attribute (pp. 20-25)						
Structural Patch Richness	Alpha.	Numeric				
Topographic Complexity						
Raw Attribute Score = sum of numeric scores			Final Attribute Score = (Raw Score/24) x 100			
Attribute 4: Biotic Structure Attribute (pp. 26-34)						
Plant Community Composition (based on sub-metrics A-C)						
<i>Plant Community submetric A:</i> <i>Number of plant layers</i>	Alpha.	Numeric				
<i>Plant Community submetric B:</i> <i>Number of Co-dominant species</i>						
<i>Plant Community submetric C:</i> <i>Percent Invasion</i>						
Plant Community Composition <i>(numeric average of submetrics A-C)</i>						
Horizontal Interspersion						
Vertical Biotic Structure						
Raw Attribute Score = sum of numeric scores			Final Attribute Score = (Raw Score/36) x 100			
Overall AA Score (average of four final Attribute Scores)						

Worksheet for Aquatic Area Abundance Metric for Estuarine Wetlands

Percentage of Transect Lines that Contains an Aquatic Feature of Any Kind	
Segment Direction	Percentage of Transect Length That is an Aquatic Feature
North	
South	
East	
West	
Average Percentage of Transect Length that is an Aquatic Feature	

Percent of AA with Buffer Worksheet.

In the space provided below make a quick sketch of the AA, or perform the assessment directly on the aerial imagery; indicate where buffer is present, estimate the percentage of the AA perimeter providing buffer functions, and record the estimate amount in the space provided.

Percent of AA with Buffer: _____ %

Worksheet for calculating average buffer width of AA

Line	Buffer Width (m)
A	
B	
C	
D	
E	
F	
G	
H	
Average Buffer Width *Round to the nearest integer*	

Structural Patch Type Worksheet for Estuarine Wetlands

Circle each type of patch that is observed in the AA and enter the total number of observed patches in the worksheet below.

STRUCTURAL PATCH TYPE (circle for presence)	Estuarine
Minimum Patch Size	3 m²
Abundant wrackline or organic debris in channel, on floodplain, or across depressional wetland plain	1
Animal mounds and burrows	1
Bank slumps or undercut banks in channels or along shoreline	1
Debris jams	1
Filamentous macroalgae or algal mats	1
Large Woody Debris	1
Non-vegetated flats or bare ground (sandflats, mudflats, gravel flats, etc.)	1
Pannes or pools on floodplain	1
Plant hummocks and/or sediment mounds	1
Point bars and in-channel bars	1
Pools or depressions in channels (wet or dry channels)	1
Secondary channels	1
Shellfish beds (living)	1
Soil cracks	1
Standing snags (at least 3 m tall)	1
Submerged vegetation	1
Total Possible	16
No. Observed Patch Types (enter here and use in Table 14 below)	

Worksheet for AA Topographic Complexity

At two locations in the AA, make a sketch of the profile from the AA boundary to AA boundary. Try to capture the major channels, slopes and intervening micro-topographic relief. Based on these sketches and the profiles in Figure 8, choose a description in Table 16 that best describes the overall topographic complexity of the AA.

North to South

East to West

Plant Community Metric Worksheet: Co-dominant species richness
 (A dominant species represents $\geq 10\%$ *relative* cover)

Special Note:

* *Combine the counts of co-dominant species from all layers to identify the total species count. Each plant species is only counted once when calculating the Number of Co-dominant Species and Percent Invasion submetric scores, regardless of the numbers of layers in which it occurs.*

Floating or Canopy-forming	Invasive?	Short (<0.3 m)	Invasive?
Medium (0.3 – 0.75 m)	Invasive?	Tall (0.75 – 1.5 m)	Invasive?
Very Tall (>1.5 m)	Invasive?		
		Total number of co-dominant species for all layers combined (enter here and use in Table 18)	
		Percent Invasion *Round to the nearest whole number (integer)* (enter here and use in Table 18)	

Horizontal Interspersion Complexity Worksheet.

Use the spaces below to make a quick sketch of the AA in plan view, outlining the major plant zones (this should take no longer than 10 minutes). Assign the zones names and record them on the right. Each zone should comprise as least 5% of the AA. Based on the sketch, choose a single profile from Figure 10 that best represents the AA overall.

	<p>Assigned zones:</p> <p>1)</p> <p>2)</p> <p>3)</p> <p>4)</p> <p>5)</p> <p>6)</p>
--	---

Table 21: Wetland disturbances and conversions.

Has a major disturbance occurred at this wetland?	Yes	No		
If yes, was it a flood, fire, landslide, or other?	flood	fire	landslide	other
If yes, then how severe is the disturbance?	likely to affect site next 5 or more years	likely to affect site next 3-5 years	likely to affect site next 1-2 years	
Has this wetland been converted from another type? If yes, then what was the previous type?	depressional	vernal pool	vernal pool system	
	non-confined riverine	confined riverine	seasonal estuarine	
	perennial saline estuarine	perennial non-saline estuarine	wet meadow	
	lacustrine	seep or spring	playa	

Stressor Checklist Worksheet

HYDROLOGY ATTRIBUTE (WITHIN 50 M OF AA)	Present	Significant negative effect on AA
Point Source (PS) discharges (POTW, other non-stormwater discharge)		
Non-point Source (Non-PS) discharges (urban runoff, farm drainage)		
Flow diversions or unnatural inflows		
Dams (reservoirs, detention basins, recharge basins)		
Flow obstructions (culverts, paved stream crossings)		
Weir/drop structure, tide gates		
Dredged inlet/channel		
Engineered channel (riprap, armored channel bank, bed)		
Dike/levees		
Groundwater extraction		
Ditches (borrow, agricultural drainage, mosquito control, etc.)		
Actively managed hydrology		
Comments		

PHYSICAL STRUCTURE ATTRIBUTE (WITHIN 50 M OF AA)	Present	Significant negative effect on AA
Filling or dumping of sediment or soils (N/A for restoration areas)		
Grading/ compaction (N/A for restoration areas)		
Plowing/Discing (N/A for restoration areas)		
Resource extraction (sediment, gravel, oil and/or gas)		
Vegetation management		
Excessive sediment or organic debris from watershed		
Excessive runoff from watershed		
Nutrient impaired (PS or Non-PS pollution)		
Heavy metal impaired (PS or Non-PS pollution)		
Pesticides or trace organics impaired (PS or Non-PS pollution)		
Bacteria and pathogens impaired (PS or Non-PS pollution)		
Trash or refuse		
Comments		

BIOTIC STRUCTURE ATTRIBUTE (WITHIN 50 M OF AA)	Present	Significant negative effect on AA
Mowing, grazing, excessive herbivory (within AA)		
Excessive human visitation		
Predation and habitat destruction by non-native vertebrates (e.g., <i>Virginia opossum</i> and domestic predators, such as feral pets)		
Tree cutting/sapling removal		
Removal of woody debris		
Treatment of non-native and nuisance plant species		
Pesticide application or vector control		
Biological resource extraction or stocking (fisheries, aquaculture)		
Excessive organic debris in matrix (for vernal pools)		
Lack of vegetation management to conserve natural resources		
Lack of treatment of invasive plants adjacent to AA or buffer		
Comments		

BUFFER AND LANDSCAPE CONTEXT ATTRIBUTE (WITHIN 500 M OF AA)	Present	Significant negative effect on AA
Urban residential		
Industrial/commercial		
Military training/Air traffic		
Dams (or other major flow regulation or disruption)		
Dryland farming		
Intensive row-crop agriculture		
Orchards/nurseries		
Commercial feedlots		
Dairies		
Ranching (enclosed livestock grazing or horse paddock or feedlot)		
Transportation corridor		
Rangeland (livestock rangeland also managed for native vegetation)		
Sports fields and urban parklands (golf courses, soccer fields, etc.)		
Passive recreation (bird-watching, hiking, etc.)		
Active recreation (off-road vehicles, mountain biking, hunting, fishing)		
Physical resource extraction (rock, sediment, oil/gas)		
Biological resource extraction (aquaculture, commercial fisheries)		
Comments		

APPENDIX 7.1C





Standard Operating Procedures (7.2): Photo Point

March 2021

Prepared for the United States Environmental
Protection Agency



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Standard Operating Procedures: Photo Point

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Protocol Suitability Evaluation

A habitat suitability table containing appropriate estuarine wetland habitat types (of those evaluated) to implement Photo-point protocols is displayed in Table 1. This protocol is appropriate in any habitat type. A comparative assessment of cost, effort, and data quality are shown in Table 2. A matrix of additional detailed categorical evaluations of Photo-point survey protocols can be found in Appendix 7.1A.

Table 1. Appropriate habitat types for photo-point protocols.

Survey Protocol	Habitat Types					
	Tidal Channel	Mud/sand flat	Emergent salt marsh	Non-tidal salt marsh	Salt pan	'Degraded' / fill
Photo-point	X	X	X	X	X	X

Table 2. Categorical assessment of cost/effort and data quality for Photo-point survey protocols.

	Evaluation Metric	Photo-point	Notes
Time / Effort	Office Preparation Time (per AA)	0-10 minutes	Print data sheets and site maps
	Equipment Construction Time (one time)	0-10 minutes	Charge camera and check GPS handheld batteries; or charge GPS-enabled camera
	Field Time (per location)	0-10 minutes	Depending on field location and hiking time (site-dependent)
	Laboratory Time (per location)	0 minutes	---
	Post-Survey Processing / QAQC Time	10-20 minutes	Download photos and label file names with standardized format
	Minimum Repetition (site-dependent)	Few repetitions	Locations should be chosen to target the best possible views and attempt to capture change over time; project goal-dependent
	Relative Cost (equipment and supplies)	> \$100	One-time expense for camera and handheld GPS; or GPS-enabled camera; (<i>optional</i>) GPS-camera application on phone; (<i>optional</i>) external power supply
Survey / Data Quality	Accuracy (at a survey area level)	High	---
	Precision (at a survey area level)	High	---
	Qualitative-Quantitative Score	Qualitative	---
	Subjectivity-Objectivity Score	Objective	---

Resulting Data Types

The application of photo-point survey protocols will yield qualitative data displayed as photographic site images over time. These data are useful to identify seasonal site changes or project-level changes (e.g., restoration activities and post-restoration vegetation community expansion). The photographs can be part of a larger database or serve to assist in the development of sampling plans or targeted restoration activities. They can also be useful as stock reference photographs over time.

Objective

The primary purpose of this sampling method is to capture broad changes in the landscape and vegetation communities over seasons or years or to visually track restoration trajectories over time. This method collects georeferenced photos for use in site management (e.g., invasive species tracking) and long-term data collection. Each year (or seasonally), photograph(s) taken at fixed locations and bearings (e.g., Figure 1) are taken to ensure comparability. Panoramic photographs taken at a fixed start bearing can provide supplemental, landscape view, photograph comparison over time. If annually, the targeted time is during mid- to late summer during the peak wetland growing season, or during the appropriate peak growing season for the habitat being surveyed (e.g., spring for annuals).

Additional photo monitoring should be done before and after significant geo-morphological changes caused by natural or anthropogenic events (e.g., tsunamis, restorations), and in conjunction with other site-specific monitoring techniques (e.g., vegetation cover sampling and CRAM; refer to those specific SOPs for method details). Photo monitoring should be timed to capture seasonal vegetation changes, baseline conditions, and restoration progression over time. This SOP is modified from the US Geological Survey protocols (SCC 2005, USGS 2012) and additional monitoring programs.



Figure 1: Example of panorama photograph

Equipment

Equipment and supplies needed for this survey include:

1. GPS-enabled digital camera (preferred) or digital camera; *Helpful hint: most smart phones allow for GPS-enabled, high-resolution, digital photos and applications like “Solocator” allow metadata (location, bearing, user, date/time) to be embedded in photographs ensuring a quality control process. Ideally, users should replicate the same camera settings over time (i.e., photo resolution, size, exposure, etc.).*
2. A GPS is required if the digital camera is not GPS-enabled. GPS equipped with compass and photo point coordinates. The compass headings can be entered into the saved individual GPS coordinate points.

3. External power supply for GPS-enabled camera or extra batteries for camera and GPS combination. *Helpful hint: When using GPS-enabled cameras on mobile devices, especially applications like “Solocator”, power supply can be depleted faster. Having an external power supply with a charger cable is recommended for long field data collection days or multiple days.*
4. Aerial map(s) of site with photo-point locations and compass bearings
5. Field notebook or data sheet (Appendix 7.2B), which includes the tide schedule for the day, GPS coordinates, and printouts of the previous year’s photos
6. Tripod (*optional*). The minimum requirement is a height measurement for the height of the photo being taken or the eye level of the photographer.
7. Compass (*optional and typically integrated with GPS or GPS-enabled cameras*)

Field Preparation

Datasheets should be modified prior to each field excursion to incorporate a recent photo reference from each location, as well as the GPS coordinates and site identifications for each of the stations that will be surveyed.

Equipment described above should be collected prior to the field shift. Batteries for all electronic devices should be checked and replaced or charged as needed, and relevant photo and data sheets should be printed. If using a photo application on a GPS-enabled phone camera, ensure application is downloaded prior to the field shift.



Field Methods

1. Photo points should always be taken at or around low tide, and the time and tide height should be recorded on the datasheet.
2. Locate the photo-points in the field using the previous year (or season’s) images, latitude and longitude coordinates, and compass bearings. Replicate the exact position if possible. *Helpful hint: Permanent field markers can be set up for a site that is frequently visited.*
3. Take a series of images (or a single image if that is more appropriate – site-dependent) at each GPS and mapped location point. This point should represent the same location, height, bearing, number of photographs, vertical angle and panorama as past surveys. Use previous photos, notes, and recorded compass bearings to verify the location. If this is the first time at a particular photo point location, record all of the new information on the datasheet (e.g., direction, number of photos, compass heading range, GPS location, camera orientation, etc; (Appendix 7.2B). *Helpful hint: For the first photo at a particular location, include a large stationary object or non-moving point of reference for ease of future replication.*

4. For panorama photo points, usually three to six photos are taken to capture a 180° panorama from a location. Additionally, many GPS-enabled cameras and/or phones have auto stitching as an included feature. This should be standardized and noted on the datasheet (e.g., four photos covering a 180° area). Depending on project need or site characteristics, a range of photos may be taken, from a single photo to a 360° panorama, as long as the number of photos and bearing are recorded on the data sheet.
5. Set the camera to 'landscape' setting and try to get an equal amount of land and sky in the photos ('portrait' may be more appropriate in some instances, and should be noted on the datasheet). This will allow the inclusion of hilltops or important features closer to the location of the camera to be incorporated in the panorama. If a slightly raised view is used to provide additional information or a better view, this should also be noted (with the height added) on the data sheet. Ideally, once a photo point has been established the effort for future photo points should be aimed at replicating the location, orientation, and bearing.
6. Double check that the date, site, GPS location, point number (or ID number), compass headings, number of photos, photo number, and any additional important notes are recorded for each panorama on the data sheet and notes correspond to the file information of the camera. These data are important when merging and georeferencing the photos.



Laboratory Methods

Not applicable.

Data Entry, Post-processing, and QAQC Procedures

Photograph data (e.g., times, locations, numbers of photos) should be entered in the field using the appropriate data sheet (Appendix 7.2B). All required fields should be completed in full, and the data recorder should fill in their name at the top of the document(s). Data and photographs should be downloaded or transferred to the appropriate electronic database the day of collection, and the hard copies of the datasheets should be filed in labeled binders. Post-processing of panorama photos should be noted on datasheets and in the label of the new photo in the electronic photo database.

Specific data management suggestions include:

1. Download images from the camera and place in appropriate file location. Photographs should be labeled exactly as: "SITE_PHOTO ID_Photo-point-survey_DATE". The words "Photo-point-survey" in the label should be written out.
2. Post-processing may involve creating a mosaic of multiple photos into a single panorama using any photo editing software. One method is the "photomerge" tool in Adobe Photoshop (V CS2 or higher), but there are many software options for Mac or PC. Photo file names for panoramas should include a note (e.g., "panorama") within the image label.
3. With the wide availability of photo enhancing and modification features, careful consideration should be made to limit alterations to original photos. Cropping, tilt, and exposure can be used limitedly to better prepare photos for comparison or to correct minor errors in photo collection. Enhancing color, applying pre-set filters, and other modifications to photos is not recommended, and in some cases should be discouraged (e.g., restoration tracking).

Electronic copies of all data and photographs should be housed on an in-house dedicated server and backed up to a cloud-based or off-site server nightly. Hard copies should be saved for five years or more depending on the requirements of monitoring protocols. Electronic copies should be saved indefinitely. Quality Assurance and Quality Control (QAQC) procedures should be conducted on all data. QAQC procedures should be conducted by the QA Officer and include a review of all entries and a confirmation that all data sheets, Chain-of-Custody forms, and field notes are filed appropriately with electronic back-up copies available. QAQC should verify that the entered data match the hard copies of the field data sheets. Any discrepancies should be corrected, and the initial data entry technician notified.

Data Analyses

After multiple seasons or years, photographs can be used as qualitative assessments of broad-scale changes to an environment or vegetation community, tracking restoration progress, or to assess if invasive vegetation communities should be targeted for management actions (Figure 2).



Figure 2. Example of comparative photos taken in fall 2012 (A) and spring 2013 (B).

Health and Safety Precautions

Not applicable.

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APPENDIX 7.2A

	Evaluation Metric	Photo-point	Notes
	Correlation to L2 CRAM	Not applicable	---
Personnel Requirements	Specialty Equipment or Clothing Required	Few Specialty Items	GPS camera, GPS handheld, compass
	Ease of Transport (amount or weight of supplies)	Few Items / Easy	
	Ease of Implementation	Easy	Depends on field location and hiking time (site-dependent)
	Expertise / Skill Level	None Required	---
	Number of Personnel	1	---
	Training Requirements	None Required	---
	Seasonality of Survey Time	All Seasons	---
	Suggested Frequency	Semi-annual	Four times to capture seasonal variation or before/after site impacts or restoration activities
Survey / Data Quality	Type of Output	Non-numerical	---
	Active or Passive Monitoring Style	Passive	---
	Specialty Computer Software Required	No	May use a photograph-stitching software to combine panorama photographs
	Availability of Online / External Resources	Some	Camera and GPS manuals may be useful
Potential Limitations	Wetland Type Applicability	All	---
	Images or Multi-Media Required	Images Required	---
	Degree of Impact / Disturbance	No / Low Disturbance	Depending on the site; may be outside of project area / wetland habitats
	Vegetation Height Limitation	No Limitations	---
	Appropriate for Tidal / Wet Habitats	Yes	---
	Tide Height	Any tide	Low tide is preferred for maximum potential visibility
	Regional or Broad Implementation *	Frequently Used	---
	Potential for Hazards / Risk	Low to No Risk	---
Restrictions	Special Status Species	---	

* based on monitoring literature review

APPENDIX 7.2B

Photo Point Data Sheet			
Date:	Photographer:		
Survey Start Time:	End Time:	Uploaded:	Date:
Staff:	QAQC:		Date:
Other Notes:			

Photo/Station Information	Photo/Station Information
Station ID: _____	Station ID: _____
Camera: _____	Camera: _____
Photo Number(s): _____	Photo Number(s): _____
Time Taken: _____	Time Taken: _____
GPS Coordinates: _____	GPS Coordinates: _____
N 33. _____	N 33. _____
W 118. _____	W 118. _____
Bearing: _____	Bearing: _____
Notes/Orientation: _____	Notes/Orientation: _____

Photo/Station Information	Photo/Station Information
Station ID: _____	Station ID: _____
Camera: _____	Camera: _____
Photo Number(s): _____	Photo Number(s): _____
Time Taken: _____	Time Taken: _____
GPS Coordinates: _____	GPS Coordinates: _____
N 33. _____	N 33. _____
W 118. _____	W 118. _____
Bearing: _____	Bearing: _____
Notes/Orientation: _____	Notes/Orientation: _____

Photo/Station Information	Photo/Station Information
Station ID: _____	Station ID: _____
Camera: _____	Camera: _____
Photo Number(s): _____	Photo Number(s): _____
Time Taken: _____	Time Taken: _____
GPS Coordinates: _____	GPS Coordinates: _____
N 33. _____	N 33. _____
W 118. _____	W 118. _____
Bearing: _____	Bearing: _____
Notes/Orientation: _____	Notes/Orientation: _____

Photo/Station Information	Photo/Station Information
Station ID: _____	Station ID: _____
Camera: _____	Camera: _____
Photo Number(s): _____	Photo Number(s): _____
Time Taken: _____	Time Taken: _____
GPS Coordinates: _____	GPS Coordinates: _____
N 33. _____	N 33. _____
W 118. _____	W 118. _____
Bearing: _____	Bearing: _____
Notes/Orientation: _____	Notes/Orientation: _____



Standard Operating Procedures (8.1): Motion Wildlife Camera Surveys

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Prepared for the United States Environmental
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Standard Operating Procedures: Motion Wildlife Camera Surveys

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Protocol Suitability Evaluation

A habitat suitability table containing appropriate estuarine wetland habitat types (of those evaluated) to implement the motion wildlife camera survey protocol is displayed in Table 1. While cameras should not be placed directly in habitats with a high tidal range (due to the potential for lens flooding), they can be positioned to capture those habitat types (e.g., view towards tidal channels). A comparative assessment of cost, effort, and data quality are shown in Table 2. A matrix of additional detailed categorical evaluations of the motion wildlife camera survey protocol can be found in Appendix 8.1A.

Table 1. Appropriate habitat types to implement the motion wildlife camera survey protocol.

Survey Protocol	Habitat Types					
	Tidal Channel	Mud/sand flat	Emergent salt marsh	Non-tidal salt marsh	Salt pan	'Degraded' / fill
Wildlife Camera	X	X	X	X	X	X

Table 2. Categorical assessment of cost/effort and data quality for the motion wildlife camera survey protocol.

	Evaluation Metric	Wildlife Camera	Notes
Time / Effort	Office Preparation Time	10-30 minutes	Print data sheets, maps, GPS locations
	Equipment Construction Time (one time)	> 30 minutes	Construct stakes and camera housing
	Field Time (per station)	30-60 minutes	Depending on field location and hiking time (site-dependent) as well as the difficulty of setting up the housing in the field
	Laboratory Time (per transect)	0 minutes	---
	Post-Survey Processing / QAQC Time	30-60 minutes	Download images and label
	Minimum Repetition (site-dependent)	Many Repetitions	Several stations can capture a broad area; however, may need many 'capture nights'
	Relative Cost (equipment and supplies)	> \$50	Motion-activated camera, GPS, tools, housing
Survey / Data Quality	Accuracy (at a survey area level)	High	---
	Precision (at a survey area level)	Medium	---
	Qualitative-Quantitative Score	Qualitative	---
	Subjectivity-Objectivity Score	Objective	---

Resulting Data Types

The application of the motion wildlife camera survey protocol will yield qualitative data displayed as images visually confirming the presence of medium or large wildlife. These images can then be processed into quantitative data displayed as relative frequency of sightings per time of day, or direction of travel. Data are useful to identify broad-scale species distributions and ranges across an entire site.

Objective

Mammalian species and other medium and large fauna fill a wide range of ecological roles and are a central component to maintaining balance within an ecosystem (IUCN 2014). From seed dispersal to the regulation of invertebrate and smaller mammal populations, the presence and abundance of large mammals may act as indicators of general ecosystem health (Jones and Safi 2011).

Documenting the presence and relative abundances of larger wildlife can be difficult due to their high mobility, acute senses, nocturnal behavior, or general aversion to human interaction; however, the use of motion activated cameras provides a non-invasive, cost-effective method to capture medium and large wildlife presence (Moruzzi et al. 2002).



Figure 1. Example of photographs confirming the presence of a raccoon and coyote.

The primary purpose of this sampling method is to visually confirm the presence of medium or large wildlife species residing within an area (Figure 1). While the goal of deploying motion activated cameras is typically aimed at gathering data on medium to large sized mammals, it is not uncommon to capture data on various wildlife species, e.g., birds or reptiles. In many cases it may be possible to document habitat-specific use relationships (e.g., feeding, Figure 2). Behavior and interaction as well as estimated relative abundances can be assessed if distinguishing marks can be utilized to identify recaptured individuals. Additionally, this method can be used to assess movement of different species within or between specific geographical locations. To address vandalism issues, these methods include deterrent measures for high volume human presence areas, but additional efforts may be necessary.



Figure 2. Photograph of a great blue heron feeding.

Equipment

Equipment and supplies needed for this survey include:

1. Motion activated camera(s) (Figure 3)
2. 16 GB (or larger) SD card for each camera
3. Batteries
4. Digital camera
5. Human deterrents and camera housing (as needed, Figure 4). As an alternative, surrounding vegetation may also be used to camouflage the cameras.
 - a. Keys and locks
 - b. Two 2 x 4 in stakes
 - c. Screws & power drill
 - d. Rubber mallet
 - e. Chains
 - f. Cinder Blocks
6. Maps and/or GPS (recommended)
7. Datasheets (recommended; Appendix 8.1B)
8. Plug-in remote control (depending on model)
9. Bait (optional)
10. Informational signage (optional – to avoid tampering in areas with high human use traffic, it may be necessary to state “For scientific survey purposes – Please do not touch”)

Field Preparation

Survey implementation methodologies will vary between targeted surveys and general presence/absence studies. Prior to deploying motion camera traps, it is essential to evaluate the purpose of your survey, study site, and monitoring goals to inform optimum camera deployment location and configuration. The methods outlined in this SOP should be used primarily for general surveys but may be modified for species-specific assessments. For targeted surveys, background research should be conducted on habitat preference, movement patterns, and eating habits. Additionally, numerous studies have been conducted utilizing and evaluating various camera array configurations which should also be referenced prior to deploying wildlife cameras (Kucera and Barrett 1993, Moruzi et al. 2002, Sarmiento et al. 2009, Ikeda et al. 2012).

For previously deployed cameras, if needed, ensure either a map or GPS point showing the camera’s exact location is prepared. For highest quality results, the user’s manual for each motion activated camera model should be read to become familiarized with its specifications and capabilities.



Figure 3. Motion activated wildlife camera secured to cinder blocks and a 2 x 4” stake.



Figure 4. Supplies used to reduce the potential for camera theft or vandalism.

Equipment described above should be collected prior to the field shift. Batteries for all electronic devices should be checked and replaced as needed.

Field Methods

Specific field methods for camera options and placement including sensor sensitivity, multiple photos, location, heights, angles, and multiple camera array configurations will affect the quality and type of data obtained. Methods listed below describe general survey protocols which attempt to maximize the probability of capturing the broadest quantity of species and individuals. Targeted species-specific surveys may have different placement criteria and methodologies.

Specific survey implementation steps:

1. Identify optimal camera trap placement locations by locating the confluence of several game trails. Optimum picture quality range may vary depending on specific camera model, but a 1 – 5 meter distance placement from the camera to the trail will produce quality results for most models. Placement distance and height may vary for species specific surveys, QA/QC test photos should be taken at each station to ensure proper placement properties (see Step 7).
2. Depending on the level of deterrence needed to mitigate tampering, cameras may be attached securely using chain or steel cables to cinder blocks and a 2 x 4" stake hammered into the ground. Cameras with appropriate housings may also be securely mounted to trees or fence posts. Figure 5 illustrates the steps required to attach camera models to a cinder block.

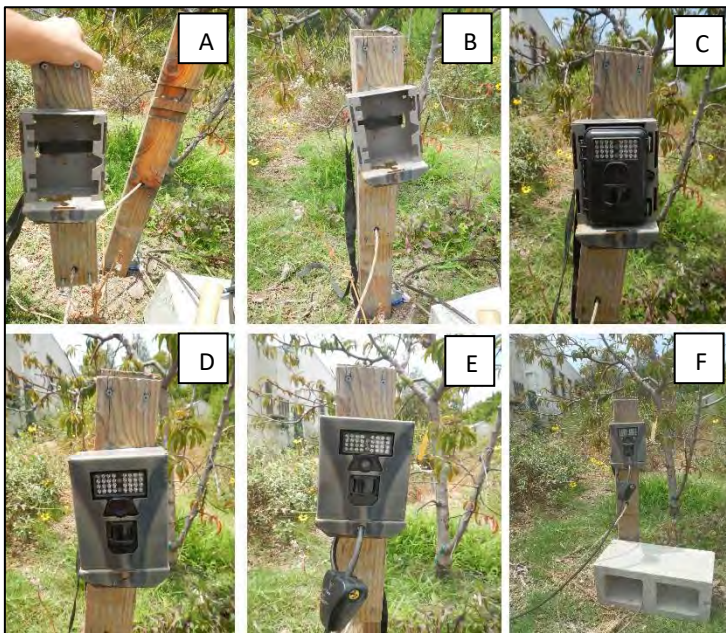


Figure 5. Steps (A-F) to create a full (deterrent) camera housing setup.

- a. Two pieces of 2 x 4" wooden stake (one end hammered into the ground, a tapered edge should be cut for ease) attached via a locking cable strung through holes drilled in-line on each stake. The locking cable should be long enough to be strung through at least one cinder block. The outer camera housing should be secured to the non-grounded stake via a strap.
- b. Secure both stakes together using screws. The strap holding the camera housing should be pinched between both stakes.
- c. Set the camera settings and place camera into the attached housing case.

- d. Cover the camera with the front of the housing case (also see Figure 3).
- e. Secure both housing cases using a sliding cable bike lock.
- f. Completed camera trap deterrent housing setup.

3. To maximize the probability of capturing various-sized species, place the camera at a height of 25 – 45 cm above ground and angle it slightly towards the ground. This technique will ensure the presence of both larger animals (e.g., coyotes and large birds) and smaller rodent sized mammals (e.g., squirrels and rats) are captured. Heights and angle of view may be adjusted for species-specific surveys. To ensure the successful placement, location, angle, and height, it may be necessary to conduct a pilot survey for several days.
4. Consider the vegetation when placing the cameras. If permitted, remove any vegetation (such as nearby grasses) which may wave in front of the camera and activate the motion sensor inadvertently. Note: motion sensor for some models may activate up to 45 feet away.
5. Place empty SD card in the camera unit. Place new batteries or ensure batteries have sufficient power to operate the camera trap for the duration of the deployment period (typically one week to ensure SD memory does not reach capacity, but larger SD cards and stronger batteries may extend the time if frequency of access is an issue). Note: A pilot survey will determine the frequency of capture rate of each camera and will allow for adjustments as needed.
6. Program camera settings to the highest resolution and to capture three-burst photos every time the camera is triggered (Figure 6). The three-photo burst setting will provide additional information required to identify species, individual, activity, and direction of travel which may not be possible with a single photograph.
7. Test photos should be captured for quality assurance purposes. Set the camera to capture images and trigger the camera yourself by walking in front of the motion detection range. Turn the camera off and transfer the SD card to a digital camera. View the images to ensure proper camera placement and settings.
8. As the final step, set the camera to begin taking pictures and close up and lock the housing.



Figure 6. Three shot burst photo sequence. Note: The first photograph would not provide enough information to confirm the coyote is feeding on the bait.

Laboratory Methods

Not applicable.

Data Entry and QAQC Procedures

Photos should be downloaded from the SD card immediately upon returning to the office and should be properly labelled with site location, date, and status of baiting (e.g., BW4_09.15.13_UNBAITED.jpeg).

The level of detail extracted from each photo to be entered into a spreadsheet will be project

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dependent. Extracted detail may range from a simple confirmation of species presence by area to the identification of individuals, inference of activities, and/or direction of travel. Electronic copies of all data should be housed on an in-house dedicated server and backed up to a cloud-based or off-site server nightly. Hard copies should be saved for five years. Electronic copies should be saved indefinitely.

Quality Assurance and Quality Control (QAQC) procedures should be conducted on all data. QAQC procedures should be conducted by the QA Officer and include a thorough review of all entries and confirmation that all data sheets, Chain-of-Custody forms, and field notes are filed appropriately with electronic back-up copies available. QAQC should verify that entered data match the hard copies of the field data sheets. Any discrepancies should be corrected, and the initial data entry technician notified.

Data Analyses

After data have been entered, corrections made, and QAQC procedures completed, data can be used in multiple analyses. Depending on project scope and purpose, possible analyses using camera trap data may include the confirmation of species presence by area or location (Table 1 and Figure 7), pie charts and associated Chi-squared tables displaying the movement patterns by direction of travel for each species (Figure 8 and Table 2), and/or histograms displaying the relative frequency of species sightings during specific time ranges.

Table 1. List of species recorded by each camera trap. Note: Asterisk (*) denotes non-native species.

Common Name	Scientific Name	Area A					Area B				
		A-Middle	A-2	A-3	A-West	A-East	B-Dune	B-Hole	B-Channel	B-FBW	B-Riparian
California ground squirrel	<i>Spermophilus beecheyi</i>						X	X		X	
Cottontail	<i>Sylvilagus audubonii</i>		X	X	X	X	X	X	X	X	X
Coyote	<i>Canis latrans</i>		X	X	X		X		X		
Raccoon	<i>Procyon lotor</i>						X	X		X	
Rat *	<i>Rattus sp.</i>										
Striped skunk	<i>Mephitis mephitis</i>						X				
Virginia opossum *	<i>Didelphis virginiana</i>						X	X		X	X
Domestic cat *	<i>Felis catus</i>						X	X		X	X
Domestic dog *	<i>Canis familiaris</i>		X			X		X		X	
Human	<i>Homo sapien</i>		X	X		X	X	X		X	

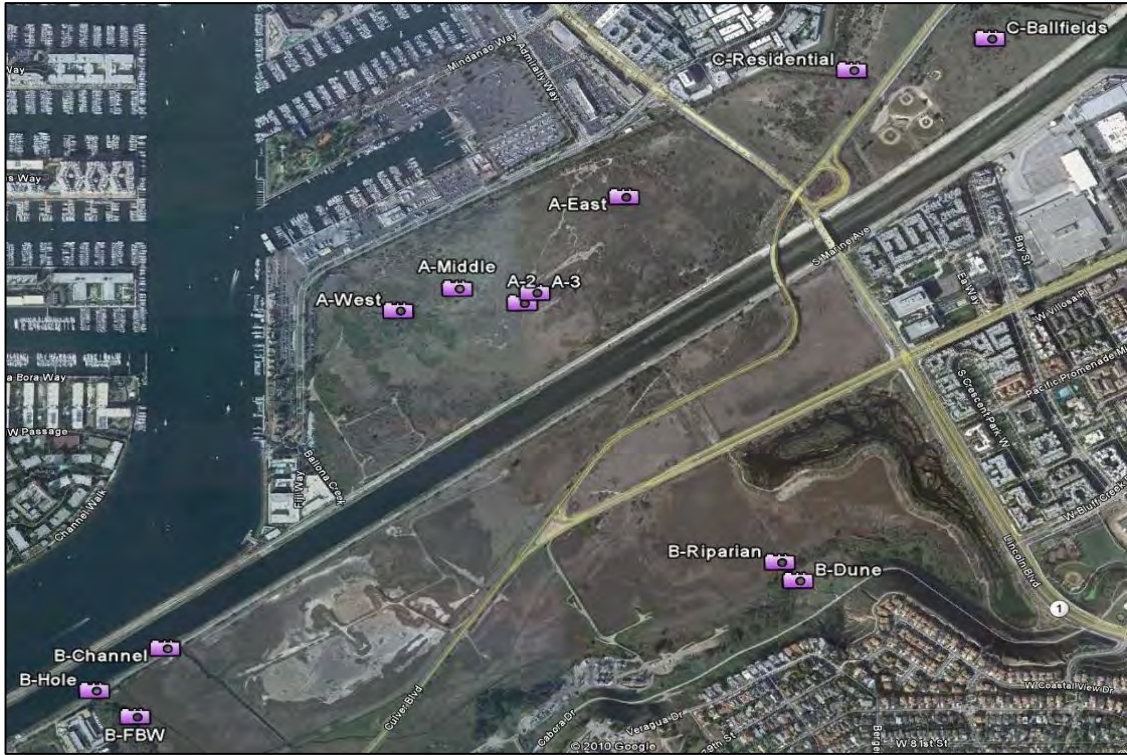


Figure 7. Map showing the location of camera traps.

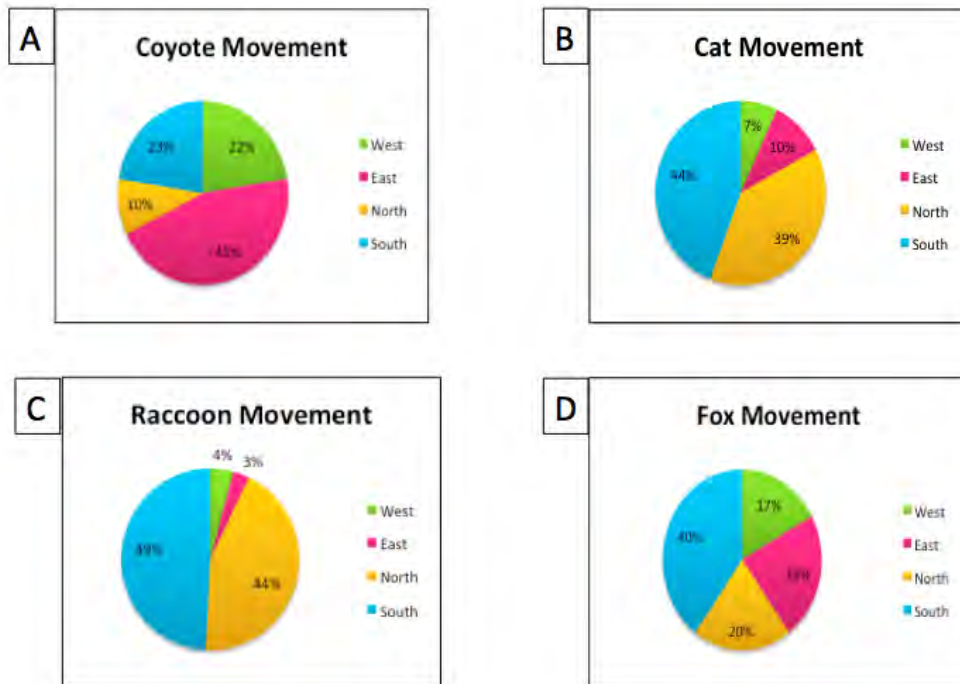


Figure 8. Pie charts displaying movement pattern by direction of travel for recorded coyotes (A), cats (B), raccoons (C), and foxes (D) (McCammon 2014).

Table 2. Chi-squared analysis of animal movement pattern by direction of travel (McCammon 2014).

Direction	Animal				
	Coyote	Fox	Cat	Raccoon	Skunk
West	16	32	42	7	4
East	32	43	61	5	2
North	7	38	231	73	3
South	16	75	264	82	8
p-value	< 0.001	< 0.001	< 0.001	< 0.001	0.1806

Health and Safety Precautions

In areas suspected of containing larger predatory wildlife, extreme caution should be exercised when carrying bait for camera traps. Be familiar with animals that may potentially be present within the study area and the proper responses if confronted with one.

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APPENDIX 8.1A

	Evaluation Metric	Wildlife Camera	Notes
	Correlation to L2 CRAM	Not Applicable	Functions loosely tied to Attribute 4 and the patch type metric
Personnel Requirements	Specialty Equipment or Clothing Required	Many Specialty Items	Motion-activated camera, GPS, housing, supplies, tools
	Ease of Transport (amount or weight of supplies)	Many or Heavy Items / Difficult	Site-dependent; open spaces may require cinder blocks, 2 x 4"
	Ease of Implementation	Moderate	Depending on field location and hiking time (site-dependent)
	Expertise / Skill Level	Some Technical Knowledge	Knowledge of camera operating instructions is required
	Number of Personnel	2+	---
	Training Requirements	None	Knowledge of camera set-up
	Seasonality of Survey Time	Year round	---
	Suggested Frequency	Annual	Or periodically to capture seasonal differences; goal-dependent
Survey / Data Quality	Type of Output	Non-numerical	---
	Active or Passive Monitoring Style	Passive / Active	May require the manipulation of vegetation to provide clear field of vision; otherwise, passive
	Specialty Computer Software Required	No	More advanced image analyses may require specialty software
	Availability of Online / External Resources	Yes	Minimal suggested use documents exist for survey purposes; however, ample instructional manuals are available for camera use
Potential Limitations	Wetland Type Applicability	All	---
	Images or Multi-Media Required	Images Required	Video is also possible
	Degree of Impact / Disturbance	Low disturbance	Moderate disturbance may be necessary at some sample locations
	Vegetation Height Limitation	No Limitations	Camera trap methods may however be limited within areas with of high vegetation density
	Appropriate for Tidal / Wet Habitats	Yes	---
	Tide Height	Low Tide Only	Most camera models are not water resistant and are not applicable in fully tidal habitats; however, they may be placed to photographically capture those habitat types
	Regional or Broad Implementation *	Frequently Used	---
	Potential for Hazards / Risk	Moderate risk	Caution must be exercised while carrying or placing bait
	Restrictions	Special Status Species	---

* based on monitoring literature review

Appendix 8.1B

Motion Wildlife Camera Surveys	
Survey Area / Habitat (e.g., "A / seasonal wetland"):	
Staff:	Comments:
Weather:	Entered (name): QAQC (name):
Station ID:	
GPS Coords:	Location Description:
Date Deployed:	Time Deployed:
Date Pulled:	Time Pulled:
Baited: Yes No	Photo Settings:
Housing Included: Yes No	Timing:
Notes (incl. deterrents implemented):	
Station ID:	
GPS Coords:	Location Description:
Date Deployed:	Time Deployed:
Date Pulled:	Time Pulled:
Baited: Yes No	Photo Settings:
Housing Included: Yes No	Timing:
Notes (incl. deterrents implemented):	
Station ID:	
GPS Coords:	Location Description:
Date Deployed:	Time Deployed:
Date Pulled:	Time Pulled:
Baited: Yes No	Photo Settings:
Housing Included: Yes No	Timing:
Notes (incl. deterrents implemented):	
Station ID:	
GPS Coords:	Location Description:
Date Deployed:	Time Deployed:
Date Pulled:	Time Pulled:
Baited: Yes No	Photo Settings:
Housing Included: Yes No	Timing:
Notes (incl. deterrents implemented):	
Station ID:	
GPS Coords:	Location Description:
Date Deployed:	Time Deployed:
Date Pulled:	Time Pulled:
Baited: Yes No	Photo Settings:
Housing Included: Yes No	Timing:
Notes (incl. deterrents implemented):	