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California Estuarine Wetland Monitoring Manual (Level 3)

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The Bay Foundation
Southern California Coastal Water Research Project
California State University, Channel Islands

Estuarine Wetland Monitoring Manual

Application and Assessment of USEPA Three-Tiered Monitoring Strategy to Southern 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, our former ability to meet this goal was 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 redundancy as each project developed independent protocols. Major coastal wetland restoration projects are planned throughout the southern California Bight, 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.

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Executive Summary

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. 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. 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.

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. Recommendations were primarily based on scientific evaluations of data quality, cost and effort, expertise requirements, and disturbance.

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. Table ES-1 presents two groupings of Level 3 estuarine wetland protocols, including a minimum protocol recommendation for each parameter evaluated within the scope of this program and a second recommendation for programs with greater resources or which require higher resolution data to inform wetland function or processes.

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 minimum recommended protocols and high resource recommended protocols.

Parameter	Minimum Recommendation	High Resource Recommendation
Water Quality	Level 1 and 2 landscape-scale analyses	Data sonde (SOP 1.1)
Soil Characteristics	None	None
Vegetation	Cover class quadrat (SOP 3.2)	Vegetation mapping (SOP 3.5)
Fish	Beach seine (SOP 4.1)	More replicates and higher frequency
Birds	Point count (SOP 5.1)	More replicates and higher frequency
Mammals	None	None
Invertebrates	None	Benthic invertebrates (SOP 6.1)

Water Quality

Ambient water quality plays an integral role in influencing habitat and species distributions. However, no Level 3 protocol is recommended for programs with limited resources due to the high costs associated with purchasing and maintaining monitoring equipment. As a proxy for on-site monitoring, it is recommended that office-based GIS aerial image analyses and rapid assessments be utilized to evaluate the surrounding landscapes, freshwater inputs, and impairments to dominant hydrology to broadly infer water quality characteristics. However, if adequate resources are available, it is highly recommended to secure a permanent data sonde capable of quantifying at least basic water quality parameters (e.g. pH, temperature, salinity, chlorophyll, depth) over time or to assess discrete events.

Soil Characteristics

Protocols surveying soil characteristics are not recommended for implementation. Intensive monitoring of parameters which influence (i.e. hydrology) and respond directly (i.e. vegetation) to soil characteristics should be sufficient to infer basic soil qualities (i.e. soil texture, organic matter, and salinity range). While there are a range of potential options, including those not explored in this manual (e.g. chemical constituents), soil is not commonly evaluated as part of broad-scale monitoring programs for the reasons listed above and will need to be evaluated on a project-specific basis.

Vegetation

Some form of vegetation monitoring is recommended as a key component for nearly 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.

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.

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. 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.

Invertebrates

While intensive invertebrate assessments provide valuable information about the lower trophic levels within a given wetland area, no Level 3 protocols are recommended as basic assessments due to the high labor, time, and resource costs associated with their implementation. However, sampling benthic invertebrates is recommended if resources are available, as they can be scaled to be more cost efficient and supply valuable supplementary information to water and sediment constituent monitoring. Additionally, broader biomass or productivity may be assessed using the terrestrial invertebrate sampling protocols; however, evaluations of these data do not always cleanly correlate with other sampling parameters.

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. 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) diagnose causes of wetland degradation, (c) develop designs and performance standards for wetland restorations, including compensatory wetland mitigation and restoration trajectories, and (d) 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.

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 for each protocol, describe methodologies and techniques for

28 protocols covering a broad range of monitoring parameters including: ambient water quality, soil characteristics, vegetation, fish, birds, mammals, and invertebrates.

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, and private 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 take into account that are not 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.

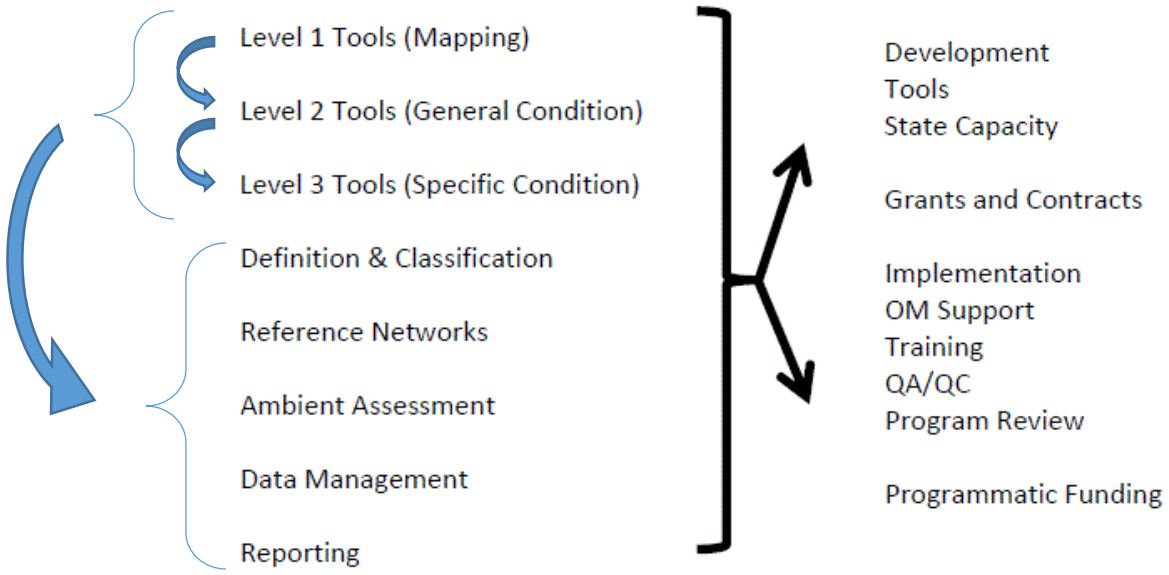

Figure 1. WRAMP strategic elements and framework (modified from WRAMP 2010).

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.

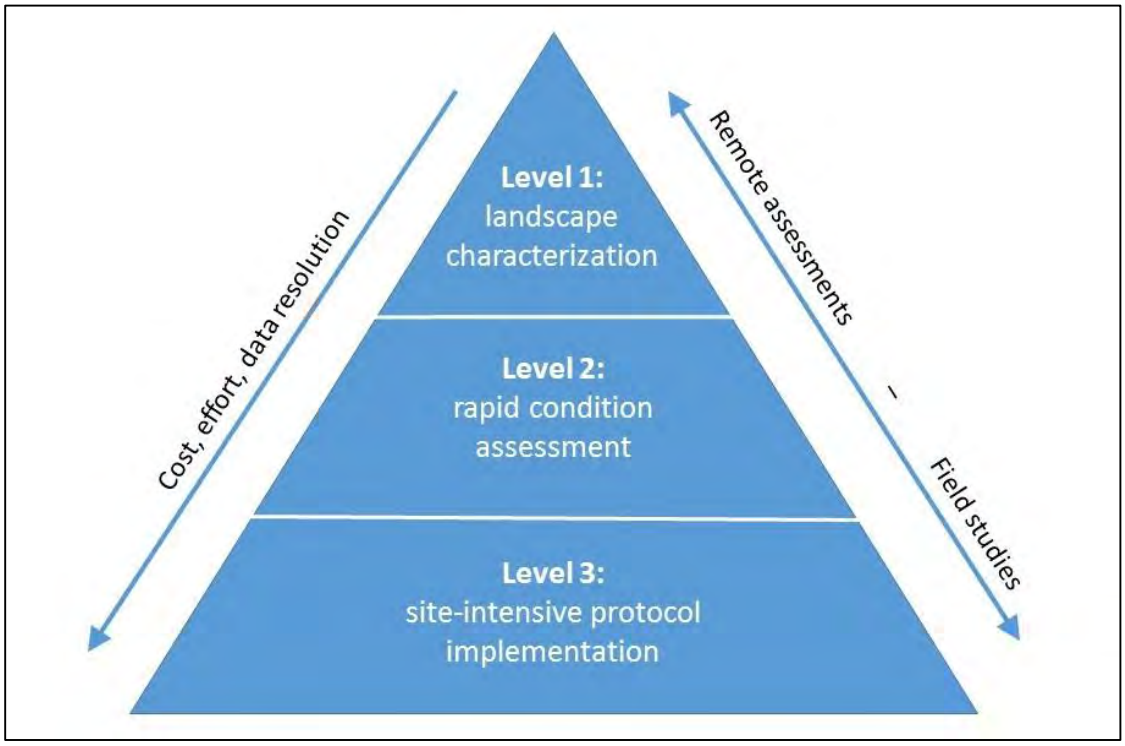

Figure 2. Conceptual model of USEPA three-tiered wetland monitoring structure.

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, it makes compilation and comparison of data across projects difficult. 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 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.

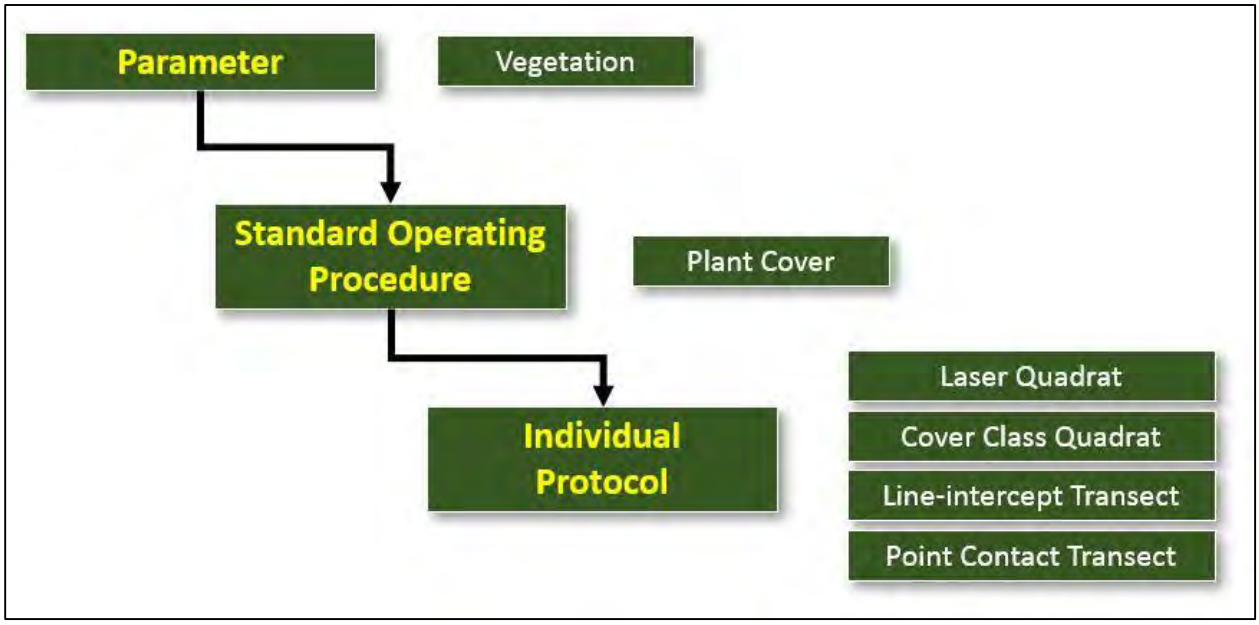

Figure 3. Graphic flow chart of parameter section outline.

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 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 SOP (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 = 16) and protocols (N = 28) 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 the exception of water quality and mammals. 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

Parameter	SOP	Protocol	Appendix
	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	B – 4.1
		Shrimp Trawl	B – 4.1
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 particular 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.

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.

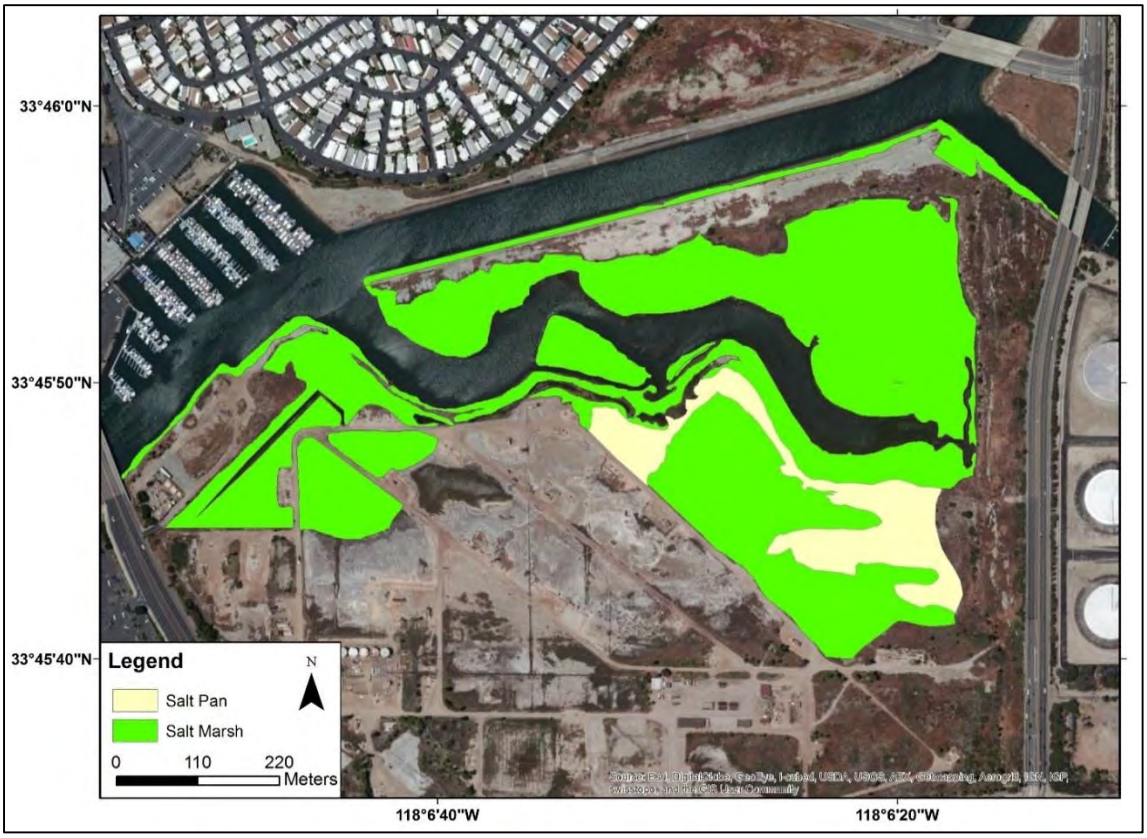

Figure 4. Example of wetland habitat data layers at the Los Cerritos Wetland Complex downloaded from the National Wetlands Inventory database (accessed: June 2013).

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.

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.

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. depressional versus bar-built estuary). 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

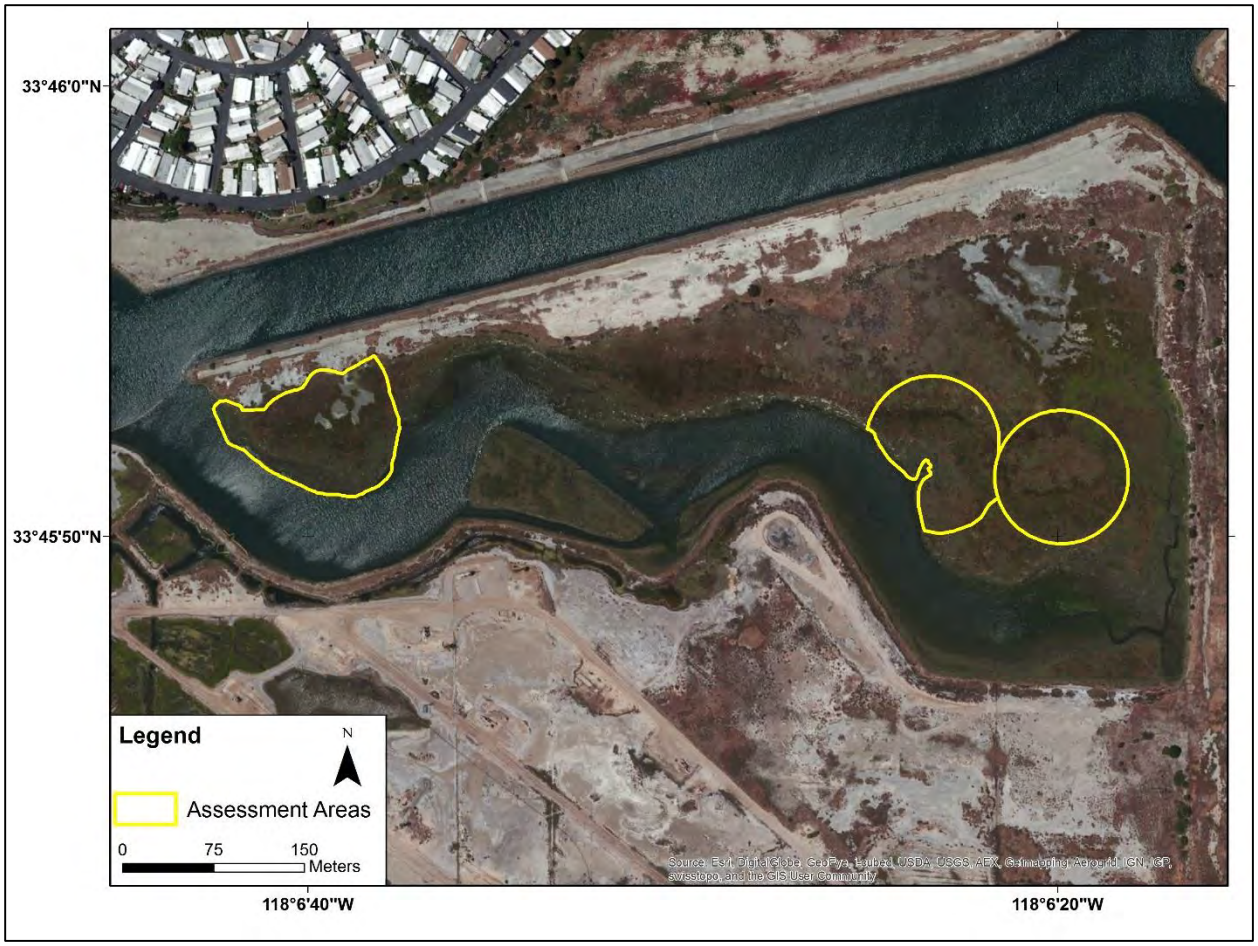

Figure 6. Map of CRAM Assessment Areas at Steamshovel Slough in the Los Cerritos Wetlands Complex created using GIS.

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. 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 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 from anthropogenic or natural events (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, soil, or water quality analysis. The focus of this manual was 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:

1) Refine or validate rapid assessment methods based on a characterization of reference condition and specific functions;
2) Diagnose the causes of wetland degradation;
3) Develop design and performance standards for wetland restoration, including compensatory wetland mitigation; and
4) 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 redundancy 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 more repetition.

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 discreet 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 intensive 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. 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.

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 (e.g. biodiversity) 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 of the over 75 protocols described below were evaluated as part of this program, a representative subset from each parameter were chosen (N = 28). 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
		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

Parameter		Indicator or Metric	Method or Protocol		
			SIMPLE VISUAL ESTIMATE / CATEGORIZATION	STRUCTURED METHOD	TOOL-BASED METHOD
	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 (sps or group-level ID)	-----
		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 (sps or group-level ID)	-----
		distribution	presence of indicator species (e.g. tiger beetle)	habitat or area-based assessments using pitfall traps	-----

Parameter		Indicator or Metric	Method or Protocol		
			SIMPLE VISUAL ESTIMATE / CATEGORIZATION	STRUCTURED METHOD	TOOL-BASED METHOD
BENTHIC INVERTEBRATES	Infauna	density	visual ID, presence; categorical	counts in cores	-----
		compositional diversity	visual ID, presence; categorical; presence; cores w/presence	sps- 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	sps- 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

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). 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).

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). 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 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. 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 fairly simple 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 four 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. non-tidal habitats are not appropriate for a permanent data sonde assessing water quality parameters).

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

	Habitat Types					
Survey Protocol	**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
Pore Water Salinity	X	X	X		X	
Soil Texture	X	X	X	X	X	X
Soil Particle Grain Size	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) 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 collected quantitative data with a high 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 (using sonde)**	**Soil Salinity**	**Pore Water Salinity**	**Soil Texture**	**Soil Particle Grain Size**
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)	10-30 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
	Post-Survey Processing / QAQC Time	> 60 minutes	10-30 minutes	0-10 minutes	0-10 minutes	> 30 minutes
	Minimum Repetition (site-dependent)	Few Repetitions	Many Repetitions	Many Repetitions	Many Repetitions	Once
	Relative Cost (equipment and supplies)	Very High (> $ 4,000)	> $ 50	$ 15 – 50	$ 0	Very high (> $15,000)
Survey / Data Quality	Accuracy (at a survey area level)	High	High	High	Medium	High
	Precision (at a survey area level)	High	High	High	Medium	High
	Qualitative-Quantitative Score	Quantitative	Quantitative	Quantitative	Qualitative	Quantitative
	Subjectivity-Objectivity Score	Objective	Objective	Objective	Subjective	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). Change in the relative presences 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.

Vegetation cover sampling methods will likely vary depending on project goals. As a result, this manual compares four vegetation cover sampling methods including: point-contact transect, line-intercept transect, cover class quadrat, and laser quadrat. Table 8 is a habitat suitability index containing appropriate estuarine wetland habitat types (of those evaluated) to 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.

	Habitat Types					
Survey Protocol	**Tidal Channel**	**Mud/sand flat**	**Emergent salt marsh**	**Non-tidal salt marsh**	**Salt pan**	**'Degraded' / fill**
Algae / SAV	X	X	X			
Point-contact			X	X	X	X
Line-intercept			X	X	X	X
Cover Class			X	X	X	X
Laser Quadrat			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 3.2A 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 prior to restoration activities.

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

	Evaluation Metric	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	> 60 minutes	10-30 minutes
	Equipment Construction Time (one time)	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	10-30 minutes	10-30 minutes	30-60 minutes	10-30 minutes	30-60 minutes	Multiple days	> 60 minutes
	Laboratory Time	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	30-60 minutes	> 60 minutes	10-30 minutes
	Minimum Repetition (site-dependent)	Many Repetitions	Many Repetitions	Many Repetitions	Many Repetitions	Many Repetitions	Many Repetitions	Many Repetitions	Many Repetitions
	Relative Cost (equipment and supplies)	< $15	< $15	< $15	$15 – 50	< $15	< $15	< $15	< $15
Survey / Data Quality	Accuracy (at a survey area level)	Low	Medium	Medium	High	High	High	Low to High	Not Applicable
	Precision (at a survey area level)	Low	Medium	Medium	High	Medium	Medium	Medium	Not Applicable
	Qualitative-Quantitative Score	Quantitative	Quantitative	Quantitative	Quantitative	Quantitative	Quantitative	Qualitative and Quantitative	Not Applicable
	Subjectivity-Objectivity Score	Objective	Objective	Subjective	Objective	Objective	Objective	Subjective	Not Applicable

Fish

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 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 and minnow traps are generally appropriate for shallow, slow-moving water in tide channels or the equivalent habitat, while shrimp 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).

Table 10. Appropriate habitat types for fish beach seine survey protocols.

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

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 trap surveys are appropriate for species-specific targeted surveys (e.g. if the monitoring goals include collecting adult killifish), but the 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 doesn't work 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 accurately sample the fish population.

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

	Evaluation Metric	**Fish Beach Seine**	**Minnow Trap**	**Shrimp Trawl**
Time / Effort	Office Preparation Time	30-60 minutes	0-10 minutes	10-30 minutes
	Equipment Construction Time (one time)	> 60 minutes	0-10 minutes	> 60 minutes
	Field Time (per station)	> 60 minutes	30-60 minutes	10-30 minutes
	Laboratory Time (per station)	0 minutes	0 minutes	0 minutes
	Post-Survey Processing / QAQC Time	10-30 minutes	10-30 minutes	10-30 minutes
	Minimum Repetition (site-dependent)	Few Repetitions	Few Repetitions	Few Repetitions

	Evaluation Metric	Fish Beach Seine	Minnow Trap	Shrimp Trawl
	Relative Cost (equipment and supplies)	> $50	> $50	> $50
Survey / Data Quality	Accuracy (at a survey area level)	Medium	Low	Medium
	Precision (at a survey area level)	Medium	Low	Medium
	Qualitative-Quantitative Score	Quantitative	Quantitative	Quantitative
	Subjectivity-Objectivity Score	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 more accurately represent avifauna populations.

The primary purpose of these observational sampling methods are 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 individual 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) to for each bird abundance and activity protocol.

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

	Habitat Types					
Survey Protocol	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 the majority of 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	5 minutes
	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)	< $15	< $15	< $15
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.

	Habitat Types					
Survey Protocol	**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

Terrestrial invertebrates are a vital component of wetland food webs and are indicators of the overall health of a system (Zedler 2001). Aquatic benthic invertebrate taxa are also 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). 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).

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, Johnston et al. 2011, 2012). 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.

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. The primary purpose of the 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.

Table 16. Appropriate habitat types for invertebrate survey protocols.

	Habitat Types					
Survey Protocol	**Tidal Channel**	**Mud/sand flat**	**Emergent salt marsh**	**Non-tidal salt marsh**	**Salt pan**	**'Degraded' / fill**
Benthic Cores	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

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. The 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

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. Table 18 presents a minimum protocol recommendation for each parameter evaluated within the scope of this program and a second recommendation for programs with greater resources or which require higher resolution data to inform wetland function or processes.

In addition to the recommended Level 3 protocols listed in Table 18, Level 2 CRAM 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 18.

Table 18. Final suite of minimum recommended protocols and high resource recommended protocols.

Parameter	Minimum Recommendation	High Resource Recommendation
Water Quality	Level 1 and 2 landscape-scale analyses	Data sonde (SOP 1.1)
Soil Characteristics	None	None
Vegetation	Cover class quadrat (SOP 3.2)	Vegetation mapping (SOP 3.5)
Fish	Beach seine (SOP 4.1)	More replicates and higher frequency
Birds	Point count (SOP 5.1)	More replicates and higher frequency
Mammals	None	None
Invertebrates	None	Benthic invertebrates (SOP 6.1)

Water Quality

Ambient water quality plays an integral role in influencing habitat and species distributions. However, no Level 3 protocol is recommended for programs with limited resources due to the high costs associated with purchasing and maintaining monitoring equipment. As a proxy for on-site monitoring, it is recommended that office-based GIS aerial image analyses and rapid assessments be utilized to evaluate the surrounding landscapes, freshwater inputs, and impairments to dominant hydrology to broadly infer water quality characteristics. However, if adequate resources are available, it is highly recommended to secure a permanent data sonde capable of quantifying at least basic water quality parameters (e.g. pH, temperature, salinity, chlorophyll, depth) over time or to assess discrete events.

Soil Characteristics

Protocols surveying soil characteristics are not recommended for implementation. Intensive monitoring of parameters which influence (i.e. hydrology) and respond directly (i.e. vegetation) to soil characteristics should be sufficient to infer basic soil qualities (i.e. soil texture, organic matter, and salinity range). While there are a range of potential options, including those not explored in this manual (e.g. chemical constituents), soil is not commonly evaluated as part of broad-scale monitoring programs for the reasons listed above and will need to be evaluated on a project-specific basis.

Vegetation

Some form of vegetation monitoring is recommended as a key component for nearly 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.

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.

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. 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.

Invertebrates

While intensive invertebrate assessments provide valuable information about the lower trophic levels within a given wetland area, no Level 3 protocols are recommended as basic assessments due to the high labor, time, and resource costs associated with their implementation. However, sampling benthic invertebrates is recommended if more resources are available, as they can be scaled to be more cost efficient and supply valuable supplementary information to water and sediment constituent monitoring. Additionally, broader biomass or productivity may be assessed using the terrestrial invertebrate sampling protocols; however, evaluations of these data do not always cleanly correlate with other sampling parameters.

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.

Future Directions

Opportunities to add Level 3 protocols and evaluations to this manual should be investigated. Additionally, the next step for the Level 3 program development will crosswalk pre-existing monitoring program data sets to assess their comparability given slight differences in protocol implementation, sampling frequencies, or constraints. Cross-program data transfer and the opportunity to synthesize the datasets in an online database management system 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:
Continuous Water Quality Monitoring – Data Sonde

SOP Identification: SOP 1.1 Continuous Water Quality – Data Sonde
Date of Issue: 30 June 2015
Date of Last Revision: 22 June 2015
Developed by: The Bay Foundation
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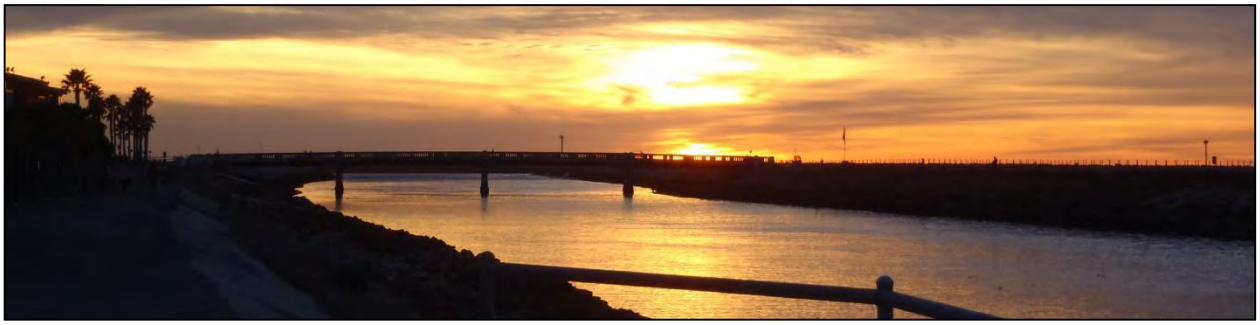

Protocol Suitability Evaluation

A habitat suitability table containing appropriate estuarine wetland habitat types (of those evaluated) to implement 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 receiving full tidal or partial tidal inundation that would allow for a submerged or partially-submerged sonde.

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

	Habitat Types					
Survey Protocol	**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)	10-30 minutes	Depending on field location and hiking time (site-dependent); 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 (> $4,000)	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 (e.g. pH, salinity, temperature, depth, dissolved oxygen) displayed as discreet readings for each monitoring interval (e.g. every 15 or 30 minutes). These 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.

Objective

Water quality measurements may be used as indicators of both human health concerns and the overall chemical and physical 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 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. 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 to identify the overall general water quality condition of that area and identify gross problematic events (e.g. periods of low dissolved oxygen).

Due to the different maintenance requirements of various water quality multi-probe sondes, general guidelines are presented below. These should apply reasonably well to most multi-probe sondes, with a focus on the specific calibration needs for the YSI 6600 multi-probe (Figure 1).

Water Quality Parameters

Water quality parameters identified in this SOP include:

- Temperature, salinity, conductivity, dissolved oxygen, depth, pH, turbidity, and chlorophyll.

Figure 1. Photo of YSI 6600 V2 multi-probe with guard (courtesy YSI 2012).

Field Methods: Installation

Methods for sonde deployment will vary by site. Considerations include (modified from USGS 2012):

1. Sampling intent (e.g. for full tidal range, probe should be placed in deepest part of tidal channel to capture both highest and lowest tides)
2. Sonde location (e.g. river, tidal channel, or subtidal habitat)
3. Method of access (e.g. boat on high tide vs. foot on low tide)
4. Substrate type (soft substrates may require additional reinforcements)
5. Potential threats to instrument (e.g. biofouling, logs, *Ulva sp.*, boats)

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

For most purposes, installation includes driving a post (stake or PVC) into the substrate to which the sonde is attached, either in a protected PVC tube (Figure 2, preferable) or directly to the post. The PVC tube should have large slits or holes cut into the bottom of the tube (similar to the guard on the YSI 6600) to allow natural water movement on and around the probes.

The elevation of the base of the probes in the field should be measured using surveying equipment so that water depth data can be converted from relative depth to elevation or tidal datum to allow for comparison amongst sites and to predicted tides. It is critical that the probe be returned to the same location after each download so that water depth data can be converted to a tidal datum.

Field Methods: Programming Sonde

Before deploying a sonde for the first time, the settings must be activated by directly connecting to a handheld data logger (e.g. YSI 650MDS) or computer equipped with interface software (e.g. YSI EcoWatch® or EcoWatch Lite® if using Windows 7) to set the specifications of the logging record (e.g. time and date range, logging frequency, file name). Use specific instructions provided by the instrument's user manual (see below for YSI programming instructions). Sampling frequency typically can be modified based on user needs. One sample every 15 or 30 minutes is recommended.

1. Make sure the date and time are correct.
2. Check the internal battery voltage. If the battery voltage is below 10 you should replace the batteries before the instrument is redeployed. *Helpful hint*: Many sondes also display remaining battery usage, but this may vary depending on water temperature and sampling frequency.
3. Program parameters:
    a. File Name: Choose a file name that includes the site name, station, and deployment date (e.g. BWER_4_10Aug2012).
    b. Start Logging: This should be the date and time that the instrument will begin logging. The time needs to be at least 15 minutes in the future to allow time for the file to save.

c. Stop Logging: This should be the date and time that the instrument will stop logging. It is a good idea to set up the log file for at least 1 month past the proposed scheduled date to check the equipment, in case there is a problem or delay.
d. Logging Interval: This should be set to 15 or 30 minutes.
e. Parameters in Log File: These are the parameters that will be recorded in the log file. Standard parameters to select are: Temp (^{o}C), pH, SpCond (mS/cm), Salinity (ppt), DO / LDO (mg/l), DO/LDO %, Internal Battery (volts), Turbidity (NTUs).

4. Ensure all probes are enabled and each parameter is set to record.
5. Once the parameters and other options are selected you must save the settings and enable the multi-probe to start logging.

Field Methods: Download, Cleaning, and Maintenance

Probe download and calibration (below) should occur every two to four weeks. Batteries should be recharged once monthly or replaced at least every other month. Remove the sonde from the housing and transport it to the laboratory for maintenance and calibration (this can also be done in the field if the laboratory is far from the project site). Check for the following in the field:

- Overall integrity of sonde housing;
- Corrosion (parts may need to be replaced; zinc washers may help reduce corrosion between metal contact points);
- Biofouling (Figure 3; nylons and/or copper mesh or tape around the sensor may help).

Figure 3. Biofouling on YSI probes.

The downloading procedure should begin by connecting the cable and laptop or handheld computer to the multi-probe sonde. Follow instructions provided in your User Manual to download data to the laptop. Once the file is downloaded it should be checked to make sure it is complete and that there are no errors or problems with the file (e.g. blank parameter values, misaligned or mislabeled column headings). It will then be checked for quality control (see QAQC below).

Cleaning should occur using the following: old toothbrush, soft bristlebrush or bottlebrush, Kim wipes, sponge, DI water, and cotton swabs. Special care should be taken to not scratch the DO membrane / LDO cap, or the conductivity sensor. Do not manually rotate the self-cleaning brush unit at any time. During cleaning, inspect the DO membrane for scratches, bubbles, wrinkles, ripples or holes. If any of these exist, replace the DO membrane (refer to the sonde manual). Note that the pH probe is extremely fragile. *Helpful hint:* the DO membrane and wipers need to be replaced the most frequently.

Check DO anode and membrane for discoloration or degradation. Also check to ensure that the wipers fully circulate, are clean, and park 180 degrees from the sensor. *Helpful hint:* the flat glass pH probe is recommended for use over the bulb pH probe as the bulb is very fragile and susceptible to breaking during cleaning. The flat glass pH probe takes an additional 30 seconds to record, but has the same degree of accuracy.

All cleaning and maintenance should take place in a shaded location or, preferably, in a laboratory.

Field Methods: Calibration

The probe should be calibrated before the initial deployment and after each download, or approximately every two to four weeks:

1. Use calibration instructions provided with the sonde.
2. Before and in between calibrations, thoroughly rinse sensors and probes with distilled water.
3. If the value of the standard changes with temperature or elevation, calibrate using the appropriate value.
4. All calibrations and/or maintenance should be recorded. Appendix 1.1B provides the appropriate calibration sheet to be filled out each time the sonde is removed, downloaded, and calibrated. Entries should include the date, serial number of the equipment, calibrations performed, outcome of calibrations, notes of problems or failed calibrations, notes of actions taken, any replacement of parts or maintenance, expiration dates and lot numbers of all standards/parts/electrolyte solutions used, and the barometric pressure (can be found at www.noaa.gov or from a personal handheld reader) at the time of calibration. Appendix 1.1C provides some helpful troubleshooting tips not found in the YSI User Manual.
5. After calibration, take a simultaneous reading against a second, quality assurance multi-probe.

Once the data are downloaded and the sonde is calibrated, refer to “Programming” to program the sonde for re-deployment.

Data Entry and QAQC Procedures

Data output from the sondes is downloaded 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 parameter that did not meet calibration standards for that particular deployment period or are outside the instrument’s accuracy range (Appendix 1.1D);
2. Depth readings and subsequent data below zero should be deleted (exposed sonde);
3. Depth data should be converted to official tidal datum (e.g. NAVD88 or MHHW);
4. Outliers or unusual spikes in data points should be identified and potentially removed pending additional assessment (e.g. temperature readings indicating dramatic shifts in very little time);

5. Data should be thoroughly evaluated and possibly removed when the battery voltage is below approximately 10.

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 of both calibration forms and raw data should be saved indefinitely.

Data Analyses

After corrections have been made, data may be used in multiple analyses. Examples include graphing the data over time (Figure 4), grouping the data by hour or day to look at broader trends over time, 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).

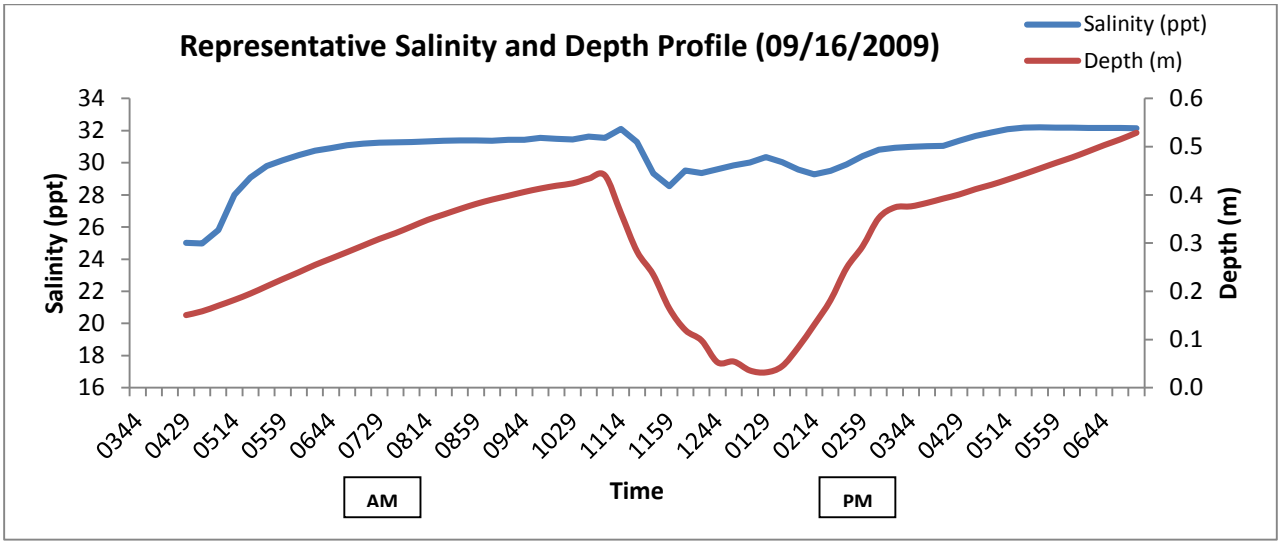

Figure 4. Salinity and depth profile across a range of tidal conditions from the Ballona Wetlands data sonde on 16 September 2009 (reproduced from Johnston et al. 2011).

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 of time. 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.

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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	----
	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 1.1B

CALIBRATION WORK SHEET

Date of Calibration: _____ Sonde ID: _____

Technician: _____

RP DO membrane changed? Y N

RP DO membrane o-ring changed? Y N

Note: Wait 3 to 6 hours before calibrating for unattended 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 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 ÷ 7.6 = 100.0%

Record Calibration Values	Standard	Pre Cal / Post Cal
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	_____	____/____

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 _____

Notes:

Modified from Xylem instrumentation, 2013.

Appendix 1.1C

Symptoms	Probable Cause	Action
Data set collected is incomplete (stopped logging prior to pick-up)	Batteries died during unattended sample	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	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	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	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	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	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	1. Soak probe in pH 4 standard for 3 - 45 minutes
	Defective probe	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%: ±1% of reading or 1% air saturation, whichever is greater; 200 to 500%: ±15% of reading
Conductivity 6560 Sensor*	0 to 100 mS/cm	0.001 to 0.1 mS/cm (range dependent)	±0.5% of reading + 0.001 mS/cm
Salinity	0 to 70 ppt	0.01 ppt	±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	±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

Specifications for YSI 6600 series sonde probes modified from Xylem instrumentation, 2013.

Standard Operating Procedures:
Soil Salinity, Texture, and Pore Water

SOP Identification Number: SOP 2.1 Soil Salinity, Texture, and Pore Water
Date of Issue: 30 June 2015
Date of Last Revision: 18 June 2015
Developed by: The Bay Foundation
Protocols reviewed by:

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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.

	Habitat Types					
Survey Protocol	**Tidal Channel**	**Mud/sand flat**	**Emergent salt marsh**	**Non-tidal salt marsh**	**Salt pan**	**'Degraded' / fill**
Soil Salinity			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 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 the overall soil conditions 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:

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

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 and graduated cylinder (if available)
11. Pens and markers
12. 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. 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 as 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.

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 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). An additional option includes geospatially-allocating grid points throughout a targeted survey area.

Soil Salinity (use on dry soils):

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

Pore Water (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.

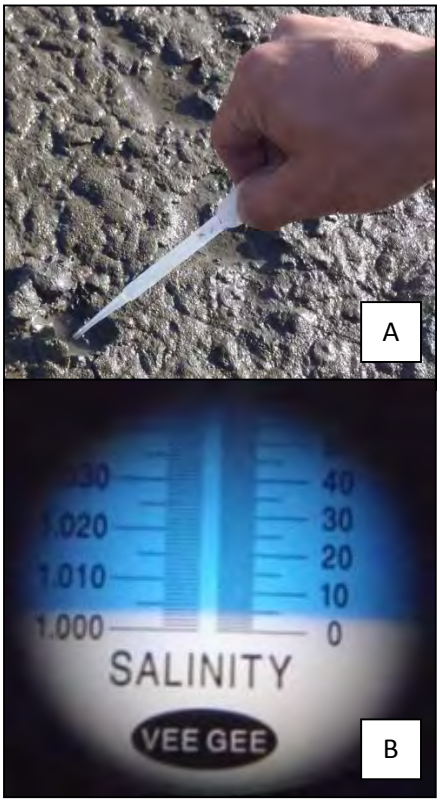

Figure 3. Eyedropper removing pore water from a shallow depression (A) and reading the refractometer (B).

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)

Bring samples back to the lab for processing:

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 rolling pin, hammer, empty glass jar, etc.
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.
5. Add one part soil by volume to one part distilled water by volume (Zedler 2001), e.g. 100 mL of soil sample should be mixed with 100 mL of distilled water.
6. 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).

7. Let samples sit, undisturbed, for 24-48 hours to allow salts to fully dissolve and create a less turbid sample (Figure 4).
8. 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.
9. 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:* A graduated cylinder with a slightly larger diameter than the conductivity probe will reduce the amount of sample water required (Figure 5).
10. When complete, discard the sample, wash the refractometer and sample jar with distilled water and air dry.

Figure 4. Eyedropper collecting water to test salinity.

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-

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

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 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 2.1A

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/H20)**	____ 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/H20)**	____ 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**

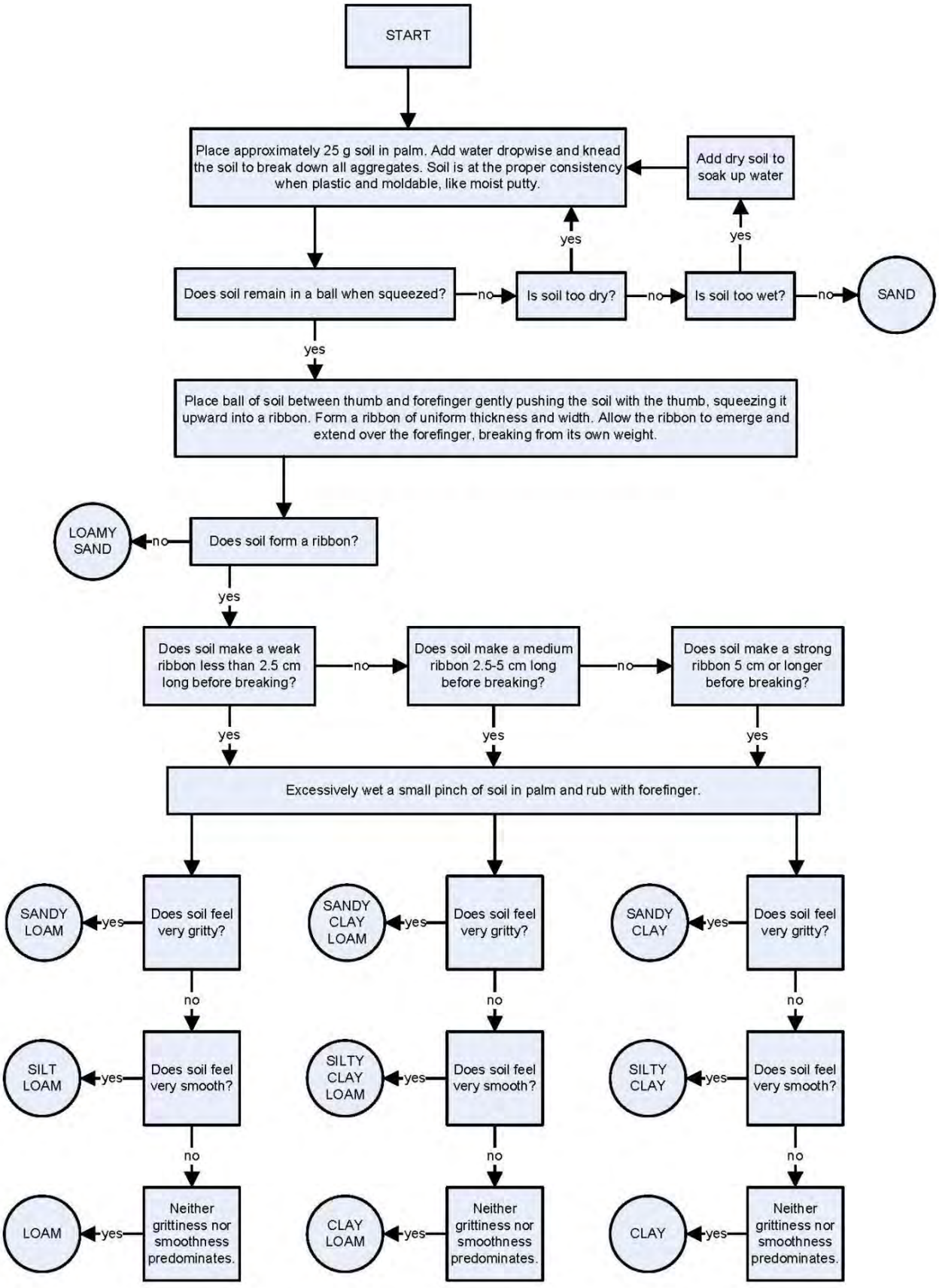
Salinity Measurements & Station Info ***Sampling (circle one): Soil OR Pore Water***

Station ID	____________	**GPS Coordinates:**		**General Notes:**
Time Collected	_____:_____	**1**		
Time Read	_____:_____	**2**		
Saturated (Y/N)	____________	**3**		
Dilution (soil / H2O)	____ mL /_____ mL	**Dilution (soil/H20)**	____ 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/H20)**	____ 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 2.1B



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:
Soil Grain Size and Organic Content

SOP Identification Number: SOP 2.2 Soil Grain Size
Date of Issue: 30 June 2015
Date of Last Revision: 23 June 2015
Prepared by: Loyola Marymount University | Seaver College of Science and Engineering and The Bay Foundation
Protocols reviewed by:
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Vincent Graves, Loyola Marymount University
Courtney McCammon, Loyola Marymount University

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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.

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

	Habitat Types					
Survey Protocol	**Tidal Channel**	**Mud/sand flat**	**Emergent salt marsh**	**Non-tidal salt marsh**	**Salt pan**	**'Degraded' / fill**
Soil Particle Grain Size	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	Notes
Time / Effort	Office Preparation Time	0-10 minutes	Print data sheets
	Equipment Construction Time (one time)	10-20 minutes	Assemble equipment
	Field Time (per transect)	30-60 minutes	Collect soil samples
	Laboratory Time (per sample)	> 280 minutes	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	Download LISST data; enter data sheet results
	Minimum Repetition (site-dependent)	Once	----
	Relative Cost (equipment and supplies)	> $15,000	LISST Particle Analyzer, drying oven, furnace, supplies
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 soil particle size survey protocols will yield qualitative data displayed in percentage of sand and fines (silt and clay). 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. This SOP is based specifically on methods related to the LISST-Portable Diffraction Particle Size Analyzer. See Appendix 2.2B for a summary procedural flow chart.

Equipment

Equipment and supplies needed for this survey include:

Equipment will need to be washed for multiple soil samples:

1. LISST-Portable Laser Diffraction Particle Size Analyzer (Figure 1)
2. Mortar and pestle
3. Turkey baster
4. 1000 mL beaker
5. 500 mL beaker
6. 2 – 100 mL beaker
7. 25 mL beaker
8. Eye dropper
9. 5-liter rectangular basin
10. 5 gallon bucket
11. 62 micron mesh screen (#230)
12. DI water (~300 mL)
13. Soil sample (30 mL)
14. DI squirt bottle
15. Scoopula
16. Drying oven
17. Aluminum foil
18. Pen or marker
19. Duct or lab tape for labeling

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

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.

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

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 trawl or knife. Make a square on the soil surface with each side measuring approximately 15 cm in length (Figure 2). *Helpful hint*: using the gardening trawl 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.

Laboratory Methods

Steps 1-24 are for sample preparation:

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 80° Celsius for two or more days until sample is fully dry. With a piece of lab tape, label the 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. 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.

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

Figure 4. Homogenized sample with mortar and pestle.

6. Measure 30 mL of homogenized sample (Figure 5) and pour into crucible (Figure 6). 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.

Figures 5. 30 mL of homogenized sample measured.

Figure 6. Homogenized sample (30 mL) placed in crucible.

9. With tongs and asbestos glove(s), place the crucible into furnace at 500-550° Celsius for 15 minutes to burn off any remaining organic matter (Figure 7).
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 8).

Figure 7. Place crucible carefully in furnace.

Figure 8. 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.
13. 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.

14. 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) (Figure 9).
15. Fill beaker (minimum 150 mL) with water. Pour water from 1000 mL beaker into 400 mL sample beaker until approximately ½ to ⅔ full. Homogenize sample by stirring with metal scoopula to re-suspend settled soils. Let sit for 1-2 minutes.
16. Wet 62 micron mesh sieve with water.

Figure 9. To reduce loss to dust, sample gently transferred to beaker with water.

17. Pour soil from beaker slowly through the 62 micron sieve into a large clean plastic tub (Figure 10). 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 (Figure 11). 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).

Figure 10. Slowly pour sample through sieve. Sample in the plastic bin are the fines.

Figure 11. Note how sand particles remain in beaker.

18. Use wash bottle to rinse off sides of beaker, add more water until beaker is about ½ to ⅔ 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 amount of required rinses (typically 6 – 10) increases for samples with higher proportions of fines.
19. 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 the vast majority of the fines have passed through the sieve into the tub.

20. 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 12). 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.

Figure 12. Trapped sand removed from sieve and returned to beaker.

21. 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).
22. The soils remaining in the plastic tub are composed of fine-grained particles (silt and clay) (Figure 13). Pour the contents of the entire tub into the 2-gallon plastic bucket (large enough to accommodate sample) (Figure 14). Use wash bottle and scoopula to ensure all soil is transferred and none is left on bottom of tub.

Figure 13. Transfer fines from large bin to tall bucket for stirring.

Figure 14. 2-gallon bucket for stirring fines.

Figure 15. Magnetic stirring cross (bar is also appropriate) (courtesy: Bel-Art Scienceware)

23. Place round magnetic stirring bar (cross PTFE-coded magnetic stirring bar is recommended; Figure 15) 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.
24. 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.

Steps 25-36 are specific to the LISST Particle Size Analyzer:

25. Set the 100 mL beaker aside. Plug in and turn on the LISST Laser Diffraction Particle Size Analyzer (Figure 16) 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 (Figure 17). Remove the plain circular insert in the mixing chamber lid and replace with ultrasonic probe insert (Figure 18).
26. From the main menu on the LISST, tap the "Measure" button.

Figure 16. LISST-Portable Laser Diffraction Particle Analyzer (courtesy Sequoia Scientific).

Figure 17. Yellow drainage hose and bucket.

Figure 18. Sonic probe and adapter.

27. 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.
28. 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.
29. 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.
30. In the menu screen comment box, add FID number and necessary comments. This information will display in the data report.
31. 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%.

32. 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 (Figure 19).

Figure 19. LISST displays as results as histogram (left) and tabular format (right).

33. 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.
34. Store the LISST Particle Size Analyzer with the drain open to allow ventilation of the chamber.
35. When rinsing all materials used throughout the process, use a wet paper towel to ensure all small particles are removed.
36. Download data to computer using LISST software or to an excel file.

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):

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

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):

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

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

Use the USGS soil texture classification chart (Figure 20) 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 (green line), 45% silt (blue line), and 10% sand (red line) would be classified as 'silty clay'. These broad categorizations are the same as the soil texture analyses from SOP 2.1.

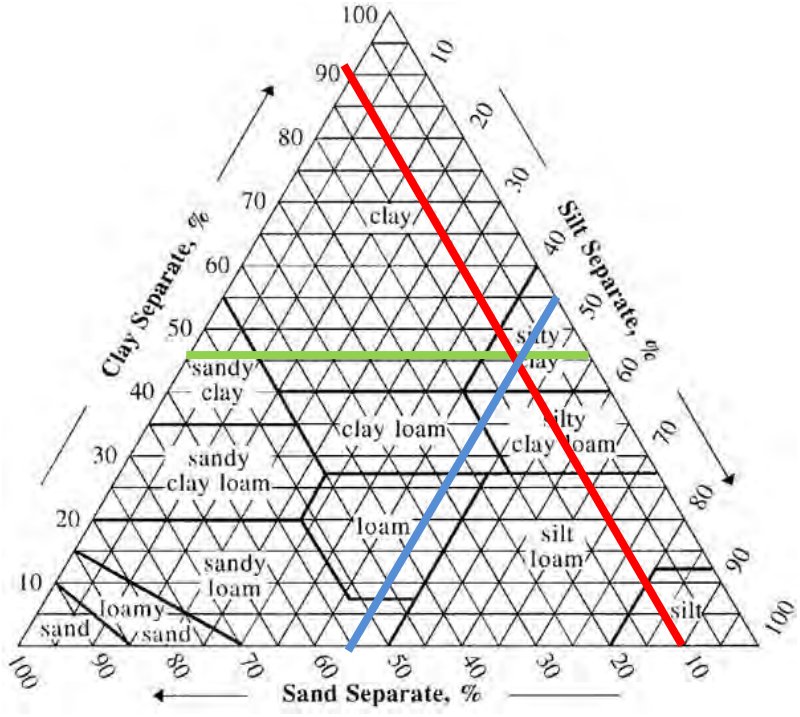

Figure 20. Soil texture classification chart (courtesy: USGS).

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.

References and Applicable Literature

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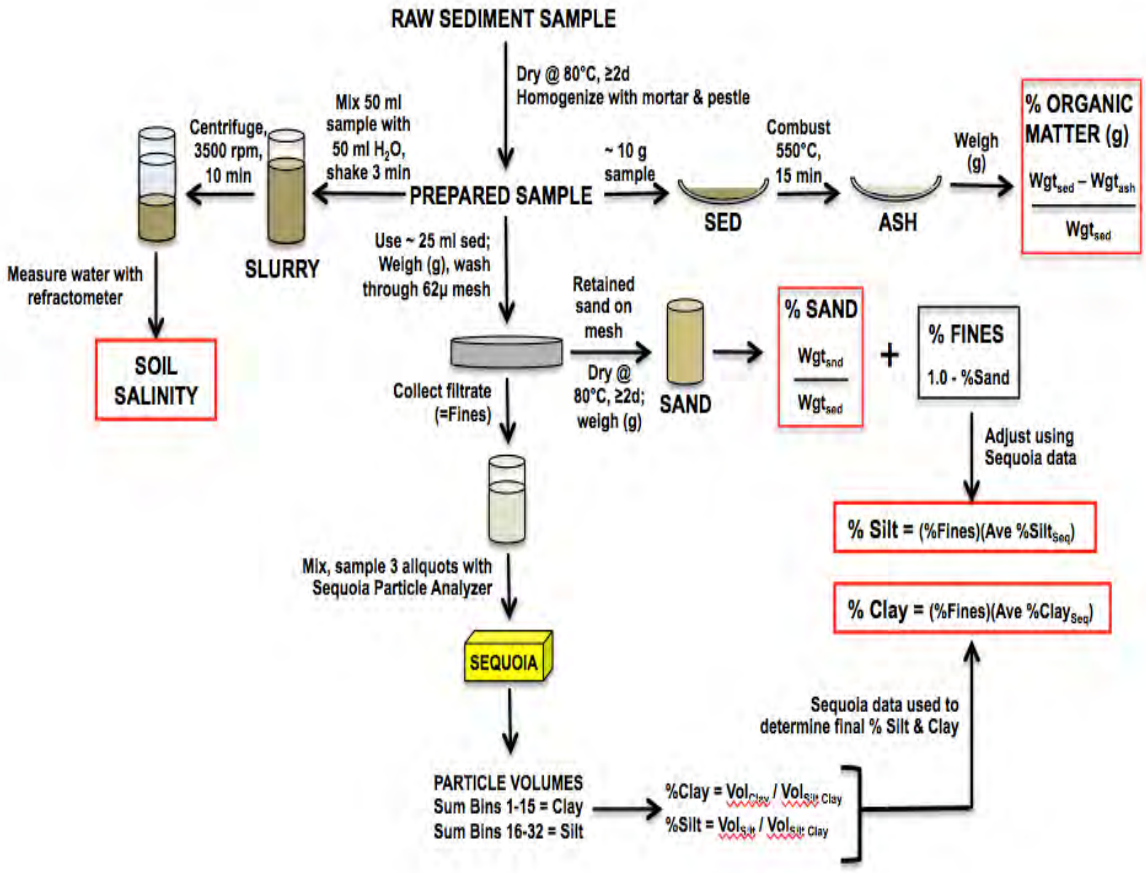
Appendix 2.2A

	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 table*

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:
Algae and Submerged Aquatic Vegetation

SOP Identification: SOP 3.1 Algae and SAV
Date of Issue: 30 June 2015
Date of Last Revision: 22 June 2015
Developed by: The Bay Foundation, Southern California Coastal Water Research Project (Bight '08 Program)
Protocols reviewed by:
Karina Johnston, The Bay Foundation
Christine Whitcraft, California State University, Long Beach
Ivan Medel, The Bay Foundation
Charles Piechowski, The Bay Foundation

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Disclaimer: Any use of trade, product, or firm names in this publication is for descriptive purposes only and does not imply endorsement by contributing agencies.

Protocol Suitability Evaluation

A habitat suitability table containing appropriate estuarine wetland habitat types (of those evaluated) to implement algae 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 implement algae and SAV protocols can be found in Appendix 3.1A.

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

	Habitat Types					
Survey Protocol	**Tidal Channel**	**Mud / sand flat**	**Emergent salt marsh**	**Non-tidal salt marsh**	**Salt pan**	**'Degraded' / fill**
Algae and Submerged Aquatic Vegetation	X	X	X			

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

	Evaluation Metric	Algae and Submerged Aquatic Vegetation	Notes
Time / Effort	Office Preparation Time	10-30 minutes	Site selection and any GPS locations
	Equipment Construction Time (one time)	30-60 minutes	Will need to construct quadrat with PVC and twine
	Field Time (per transect)	10-30 minutes	----
	Laboratory Time (per transect)	30-60 minutes	Cleaning and weighing algae biomass
	Post-Survey Processing / QAQC Time	10-30 minutes	----
	Minimum Repetition (site-dependent)	Few Repetitions	Algal cover may vary across tidal channel areas
	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	Quantitative	----
	Subjectivity-Objectivity Score	Objective	----

Resulting Data Types

The application of algae 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. 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 identify potential areas of eutrophication within estuaries.

Objective

Algae and submerged aquatic vegetation surveys provide important information about primary productivity within a system and given trophic structure (Figure 1). Algae abundance and growth can also be useful indicators of eutrophication and tidal flushing (Zedler 2001, Hughes et al. 2010).

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 a given 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 condition through cooperative regional-scale monitoring. 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.

SOPs are described based on standardized methods conducted by Johnston et al. (2011, 2012) and developed by the Bight Eutrophication program (2008).

Equipment

Equipment and supplies needed for this survey include:

1. GPS
2. Transect tape
3. 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)
4. Sealable bags
5. Small PVC cylinder (6 in diameter)
6. Data sheet (Appendix 3.1B)

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.

Field Methods

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 concluded after approximately three hours.

Figure 3. Transect deployment adjacent to the tide channel.

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 3; 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 4). This area has been demonstrated to be representative of macroalgae accumulation in southern California estuaries (Kennison et al. 2003).

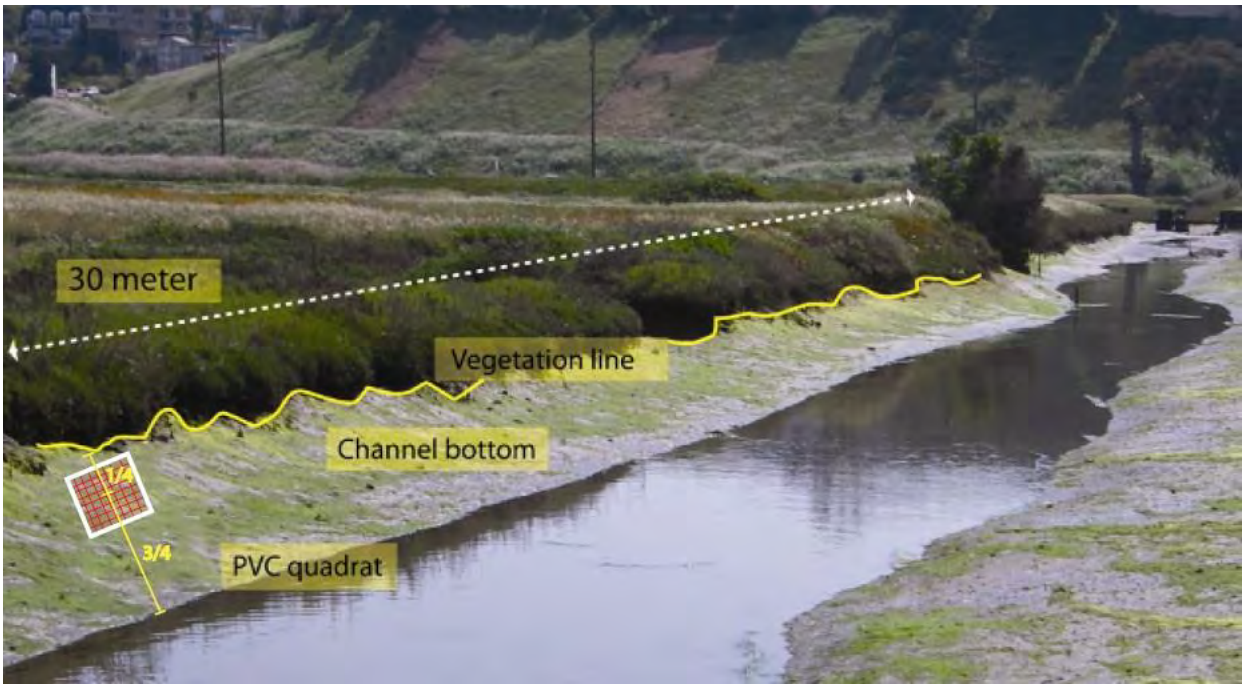

Figure 4. Diagram of algae/SAV transect showing placement of quadrat and vegetation line (replicated from Johnston et al. 2011). Note: diagram is not drawn to scale.

Percent cover should be measured at ten randomly chosen points along the transect using a random number generator. Place the 0.25 m^2 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 Submerged Aquatic Vegetation datasheet may be used for notating supplementary invertebrates (e.g. *Cerithidea californica*) and trash presence or absence.

Laboratory Methods

In the laboratory, 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/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.

For biomass data, one, 6-inch PVC pipe equates to an area of 0.072963725 m^2. To extrapolate up to grams per meter squared, enter the resulting individual weight (g) of each biomass sample into the following equation:

Weight of sample (g) x (1 / 0.072963725 m^2) = grams per m^2

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.

Health and Safety Precautions

Not applicable.

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APPENDIX 3.1A

	Evaluation Metric	Submerged Aquatic Vegetation and Algae	Notes
	Correlation to L2 CRAM	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	Hard-soled wetsuit booties work well in tidal channels
	Ease of Transport (amount or weight of supplies)	Few Items / Easy	----
	Ease of Implementation	Easy	----
	Expertise / Skill Level	Some Technical Knowledge	Familiarity with species identifications is required
	Number of Personnel	2	Includes one data recorder and one surveyor
	Training Requirements	None	----
	Seasonality of Survey Time	Every season	Spring, summer, fall, winter
	Suggested Frequency	Quarterly	----
Survey / Data Quality	Type of Output	Numerical	----
	Active or Passive Monitoring Style	Passive	----
	Specialty Computer Software Required	No	----
	Availability of Online / External Resources	Some	Other suggested use documents are available
Potential Limitations	Wetland Type Applicability	All	----
	Images or Multi-Media Required	Images Required	----
	Degree of Impact / Disturbance	Moderate Disturbance	Walking through channels will disturb sediments
	Vegetation Height Limitation	Not Applicable	----
	Appropriate for Tidal / Wet Habitats	Yes	----
	Tide Height	Low Tide Only	----
	Regional or Broad Implementation *	Almost Always Used	----
	Potential for Hazards / Risk	Low to No Risk	----
	Restrictions	None	----

** based on monitoring literature review table*

APPENDIX 3.1A
(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 Postion: 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 Marcoalgae: 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:
Vegetation Cover Surveys

SOP Identification Number: SOP 3.2 Vegetation Cover Surveys
Date of Issue: 30 June 2015
Date of Last Revision: 22 June 2015
Developed by: The Bay Foundation
Protocols reviewed by:

Karina Johnston, The Bay Foundation
Jeff Crooks, Tijuana River National Estuarine Research Reserve
Ivan Medel, The Bay Foundation
Charles Piechowski, The Bay Foundation
Sean Anderson, California State University, Channel Islands

Suggested citation: TBF. 2015. Vegetation Cover Surveys Standard Operating Procedures. Unpublished protocols. The Bay Foundation, Los Angeles, CA.

Disclaimer: Any use of trade, product, or firm names in this publication is for descriptive purposes only and does not imply endorsement by contributing agencies.

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.

	Habitat Types					
Survey Protocol	**Tidal Channel**	**Mud/sand flat**	**Emergent salt marsh**	**Non-tidal salt marsh**	**Salt pan**	**'Degraded' / fill**
Point-contact			X	X	X	X
Line-intercept			X	X	X	X
Cover Class			X	X	X	X
Laser Quadrat			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	Not applicable, unless post QAQC for species identification is necessary
	Post-Survey Processing / QAQC Time	10-30 minutes	10-30 minutes	10-30 minutes	10-30 minutes	----
	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 appropriate calibrations and trainings

Resulting Data Types

Each of the four cover protocols provide variable quantitative vegetation cover estimates at the transect-level that can be extrapolated up to a habitat- or site-level. 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
- 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)
- GPS with extra batteries
- Digital camera

- Two, 1m PVC pipes to permanently mark the beginning and ends of the transects (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)
- 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) taped holes for with 16 sub-quadrants 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 once annually during the peak biomass season for each habitat type (e.g. estuarine wetland in late summer; Zedler 2001).

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 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, and then permanently identified using thin, UV-resistant PVC piping and a waterproof tag (Figure 3) 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). 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.

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

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.

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 1; 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 m^2 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 1. 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.

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. 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.

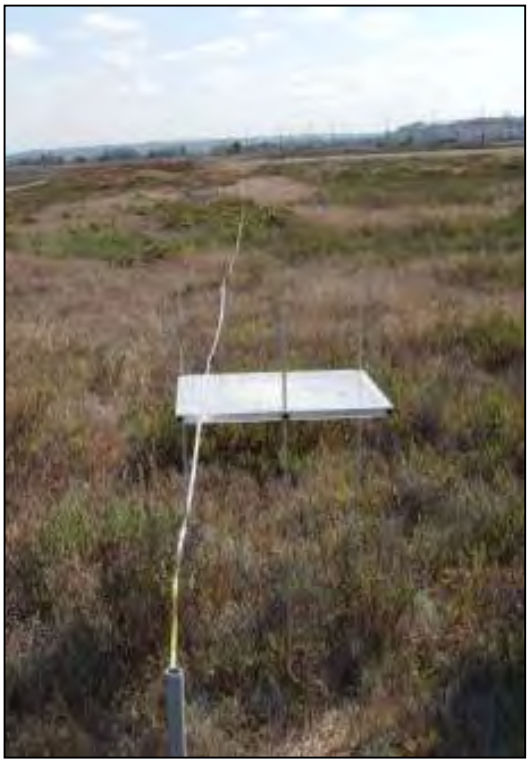

Figure 4. Photo of an example laser quadrat transect.

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 (Figure 4). 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.

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 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 Transects

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.

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 of 'bare ground'
	Regional or Broad Implementation *	Frequently Used	Frequently Used	Almost Always Used	Infrequently Used	----
	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	----

** based on monitoring literature review*

APPENDIX 3.2B

VEGETATION SAMPLING DATA SHEET – POINT CONTACT

Sampling Program Information

DATE: HABITAT:

TIME (start): (end): FID #: _____

STAFF: PAGE: _____ of _____

NOTES:

SITE:

WEATHER:

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:

Height at 10m:
SPS:

Height at 15m:
SPS:

Height at 20m:
SPS:

MAX HEIGHT:
SPS:

NOTES:

SOIL COND'N:	
SOIL TYPE:	

ADDT'L SPECIES:

TARGET SPECIES:

CROSS:
WATER? **Y / N** **ROAD?** **Y / N**

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

APPENDIX 3.2C

VEGETATION SAMPLING DATA SHEET - LINE INTERCEPT

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

	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%) ☐	Non-Native (<50%) ☐

Soil Condition/ Texture:

SPECIES W/IN 10m:

TARGET SPECIES:

CROSS CHANNEL?	Y / N	ROAD?	Y / N

SITE DESCRIPTION:

ADDT'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%) ☐	Non-Native (<50%) ☐

Soil Condition:

SPECIES W/IN 10m:

TARGET SPECIES:

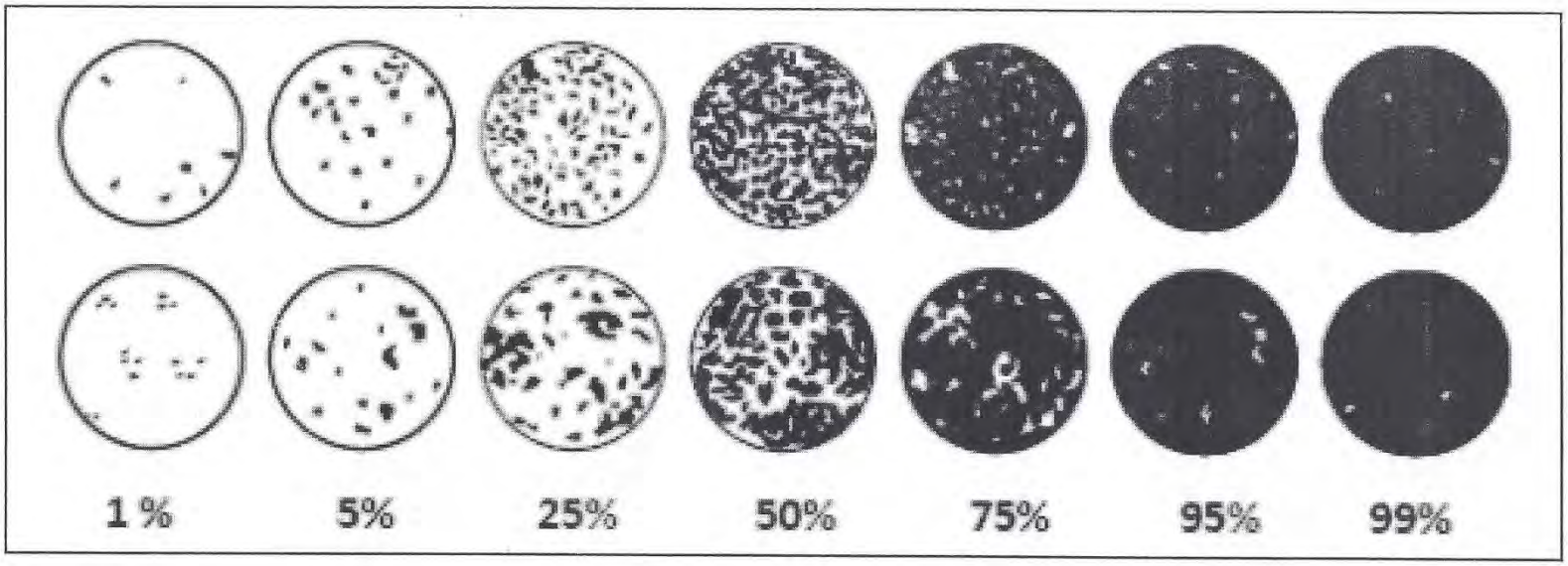
CROSS CHANNEL? Y / N	ROAD? Y / N

SITE DESCRIPTION:

ADDT'L NOTES:

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

APPENDIX 3.2F



Excerpted from Daubenmire 1959.

Standard Operating Procedures:
Vegetation Biomass

SOP Identification Number: SOP 3.3 Vegetation Biomass
Date of Issue: 30 June 2015
Date of Last Revision: 22 June 2015
Developed by: The Bay Foundation and California State University, Channel Islands
Protocols reviewed by:

Karina Johnston, The Bay Foundation
Sean Anderson, California State University Channel Islands
Christine Whitcraft, California State University, Long Beach
Ivan Medel, The Bay Foundation
Charles Piechowski, The Bay Foundation

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Disclaimer: Any use of trade, product, or firm names in this publication is for descriptive purposes only and does not imply endorsement by contributing agencies.

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. 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.

	Habitat Types					
Survey Protocol	**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).

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, or lengthy durations of an experiment.

Figure 1. Transect tape deployed from labeled PVC.

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 and replaced as needed, and relevant data sheets should be printed and attached to the clipboards.

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 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;

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

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). 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.

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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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
	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:	

APPENDIX 3.3A

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:
Seed Bank Germination

SOP Identification Number: SOP 3.4 Seed Bank Germination
Date of Issue: 30 June 2015
Date of Last Revision: 22 June 2015
Developed by: California State University, Channel Islands and The Bay Foundation
Protocols reviewed by:

Sean Anderson, California State University, Channel Islands
Karina Johnston, The Bay Foundation
Ivan Medel, The Bay Foundation
Charles Piechowski, The Bay Foundation
Eric Zahn, Tidal Influence

Suggested citation: TBF. 2015. Vegetation – Seed Bank Standard Operating Procedures. Unpublished protocols. The Bay Foundation, Los Angeles, CA.

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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.

	Habitat Types					
Survey Protocol	**Tidal Channel**	**Mud/sand flat**	**Emergent salt marsh**	**Non-tidal salt marsh**	**Salt pan**	**'Degraded' / fill**
Seed Bank			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. However, it should be noted 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 spread.

Specific objectives of seed bank surveys include:

1. Define relative species richness of germinated plant seedlings (Figure 1) by habitat type;
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.

Figure 1. Labeled seed bank core with germinated pickleweed.

Equipment

Equipment and supplies needed for this survey include:

1. GPS with transect locations
2. Digital camera
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 trawl
6. Steam sterilized soil (e.g. Supersoil ®)
7. Bucket for soil
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)

Figure 2. Seed bank equipment.

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 some 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, Johnston et al. 2011) 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. The vast majority of 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 upward.
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 be over rough ground, it is recommend 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 make 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 ability to seal the germination location from external seed sources and wind exists (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 equal success. Given available infrastructure, one or the other is likely preferable, but should be applied consistently for each individual project.
    a. Option 1: maintain cores in a large tub (Figure 3A) and fill the tub with approximately 1 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 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 minute duration) twice a day from overhead sprinklers.
3. Germinated seedlings (Figure 3b) should be counted, identified, and photographed every 2 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 recruits. 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).

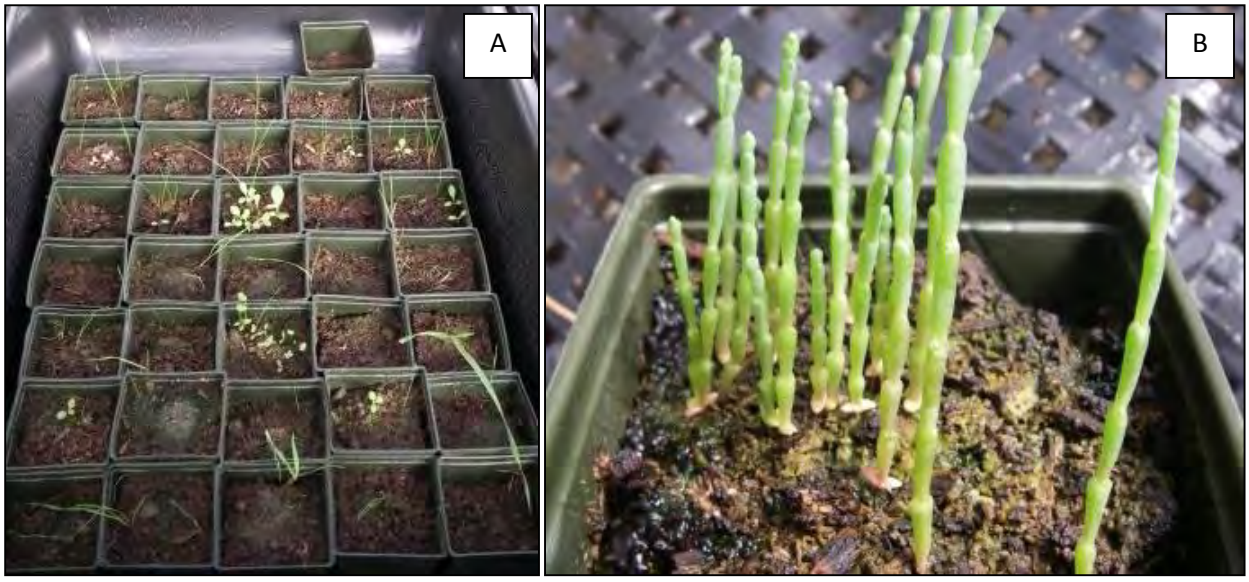

Figure 3. Photos of a collection of cores arranged in a watering tub (A) and an individual potted core (B) in a greenhouse.

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, photos should 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 m^2 and averaged across each transect or habitat type (Figure 4). Additionally, seed bank vegetation species lists can be compared across habitats or areas.

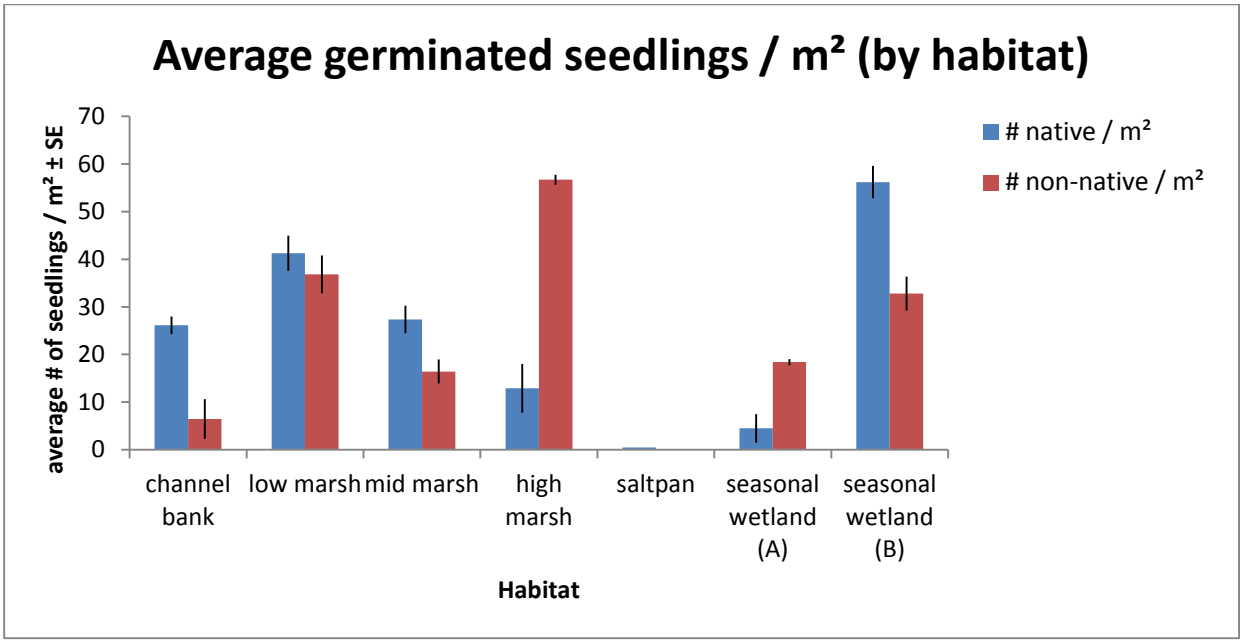

Figure 4. Example graph of average number of native and non-native germinated seedlings per m^2 by habitat (Johnston et al. 2011).

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

Health and Safety Precautions

Not applicable.

References and Applicable Literature

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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*

APPENDIX 3.4B

Date: ______________________	Counter:	Entered:
	Recorder:	Photos Uploaded:
End Time_________ Start Time: ________	Photographer/s:	QAQC'd:

Transect	**Core**	**Sprouts**	**Unk. Grass**	**Unk. Salicornia**	**SAPA**	**Unk. Aster**	**Unk. Forb (not Sali)**	**JACA**	**FRSA**	**CRTR**	**POMO**	**PAIN**	**MEIN**	**Cusa (?)**	**Other**	**Notes (Include No Seeds)**

Standard Operating Procedures:
Vegetation Mapping

SOP Identification Number: SOP 3.5 Vegetation Mapping
Date of Issue: 30 June 2015
Date of Last Revision: 22 June 2015
Developed by: The Bay Foundation
Protocols reviewed by:

Amanda McCarthy, WRA
Edith Read, E. Read and Associates
Ivan Medel, The Bay Foundation
Karina Johnston, The Bay Foundation

Suggested citation: TBF. 2015. Vegetation mapping standard operating procedures. Unpublished protocols. The Bay Foundation, Los Angeles, CA.

Disclaimer: Any use of trade, product, or firm names in this publication is for descriptive purposes only and does not imply endorsement by contributing agencies.

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. 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.

	Habitat Types					
Survey Protocol	**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 size
	Laboratory Time (per transect)	Not Applicable	----
	Post-Survey Processing / QAQC Time	> 60 minutes	Digitizing maps; very time-intensive, depending on the complexity
	Minimum Repetition (site-dependent)	Many Repetitions	Dependent on required level of resolution
	Relative Cost (equipment and supplies)	< $15	Specialty digitizing software 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
	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 will yield both qualitative and quantitative data displayed in vegetation alliance, association, and habitat category polygons for an entire site. The specific data output is qualitative because the polygons are identified categorically, but it is possible to extract quantitative analyses (e.g. acres of individual species or habitat types). These data can be a useful foundation for designing a habitat-based monitoring 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 within the last decade 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. This SOP is based on the VegCAMP methodology and outlines a synthesized vegetation stand delineation strategy based on a combination of aerial imagery, office digitization (commonly in ArcGIS), and *in situ* field verification. Vegetation mapping methods employ A Manual of California Vegetation (Sawyer et al., 2009) (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 online at explorer.natureserve.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.

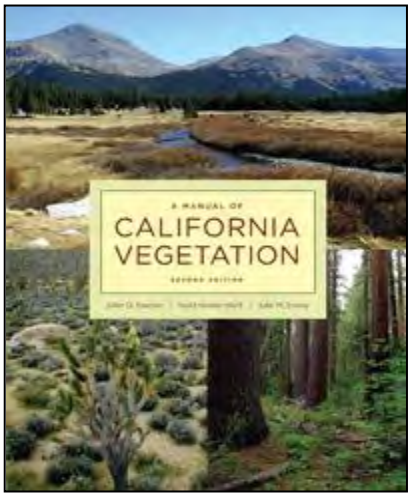

Figure 1. Cover of the *Manual of California Vegetation, 2nd ed.*

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, restoration designs, habitat assessments, wetland delineations, and special status species surveys. Survey durations may range from a few days to several years depending on the size of the mapped area and the level of resolution desired. This SOP assumes the user has an informed background in geographic information systems (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 image [Bing and Google maps may be sufficient depending on project scope, 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
2. Geographic information system software (ArcGIS recommended)
3. CNPS Relevé and Rapid Assessment Protocols and Datasheets (Appendix 3.5B)
4. GPS with data dictionary functionality (sub-meter preferred)
5. A Manual of California Vegetation (MCV)
6. Additional vegetation references [e.g. The Jepson Manual, Terrestrial Vegetation of California (bringing books into the field is optional, but they are recommended office references)]
7. Pre-existing knowledge of local plant species and vegetation cover surveys
8. Additional site-specific background maps [e.g. soils, past vegetation, inundation, elevation, surrounding land use (recommended)]
9. Printed spreadsheet of polygon and attributes (optional) (Example in Appendix 3.5C)

Figure 2. High resolution image of the Ballona Wetlands Ecological Reserve, Los Angeles, CA.

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. The database should be developed with an attribute table which lists all desired attributes which will 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 non-natives, hydrology, impacts, soil type, and/or land use.

Table

2013_TBF_ELEV_Matching

FID	VEGNAME	HABTYPE	HEIGHT	SHRUBCOV	HERBCOV	NATIVE	NON_NATIVE	BAREGROUND
0	Salix lasiolepsis 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. Depending on project scope, available resources, 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.

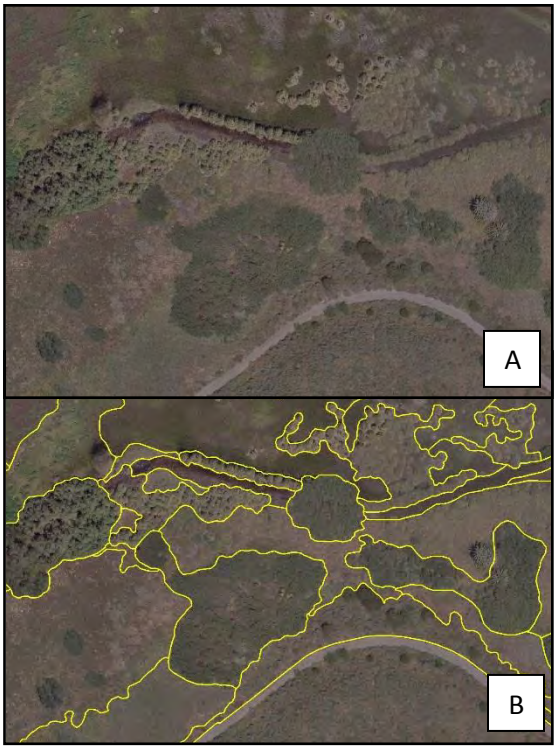

Figure 4. Example of an aerial image before (A) and after digitization (B).

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

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, and, in general, validate the accuracy of mapping efforts. Accurate vegetation cover data 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. 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. laser quadrat vegetation cover survey, see SOP 3.2). It is also important to have calibration exercises with all field team members to account for 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 at http://www.dfg.ca.gov/biogeodata/vegcamp/veg_publications_protocols.asp. Additional information on Relevé and Rapid Assessment Survey workshops and trainings can be found at: 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.

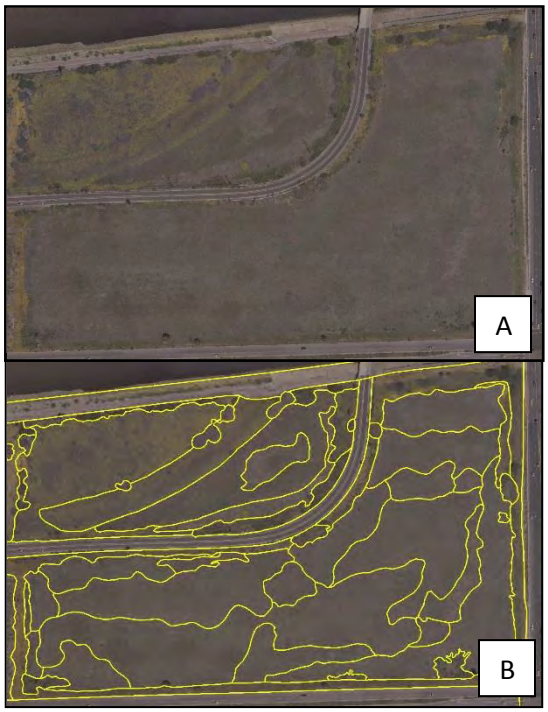

Figure 5. Example of vegetation stands with indistinct or difficult aerial signatures (A) and the delineation resulting from walking transition boundaries (B).

Laboratory Methods

Not Applicable.

Data Entry and QAQC 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 GIS geodatabase within 24 hours, and if applicable, the hard copies filed in labeled binders. 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 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.

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 habitat or alliance/association by area, then exporting maps of habitat types within a site (Figure 6) and the associated table listing the area by habitat type (Table 4). 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 (Figure 6), maps tracking the invasion of non-native species over time (Figure 7), and graphs showing change in acreage by habitat (Figure 8) or 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).

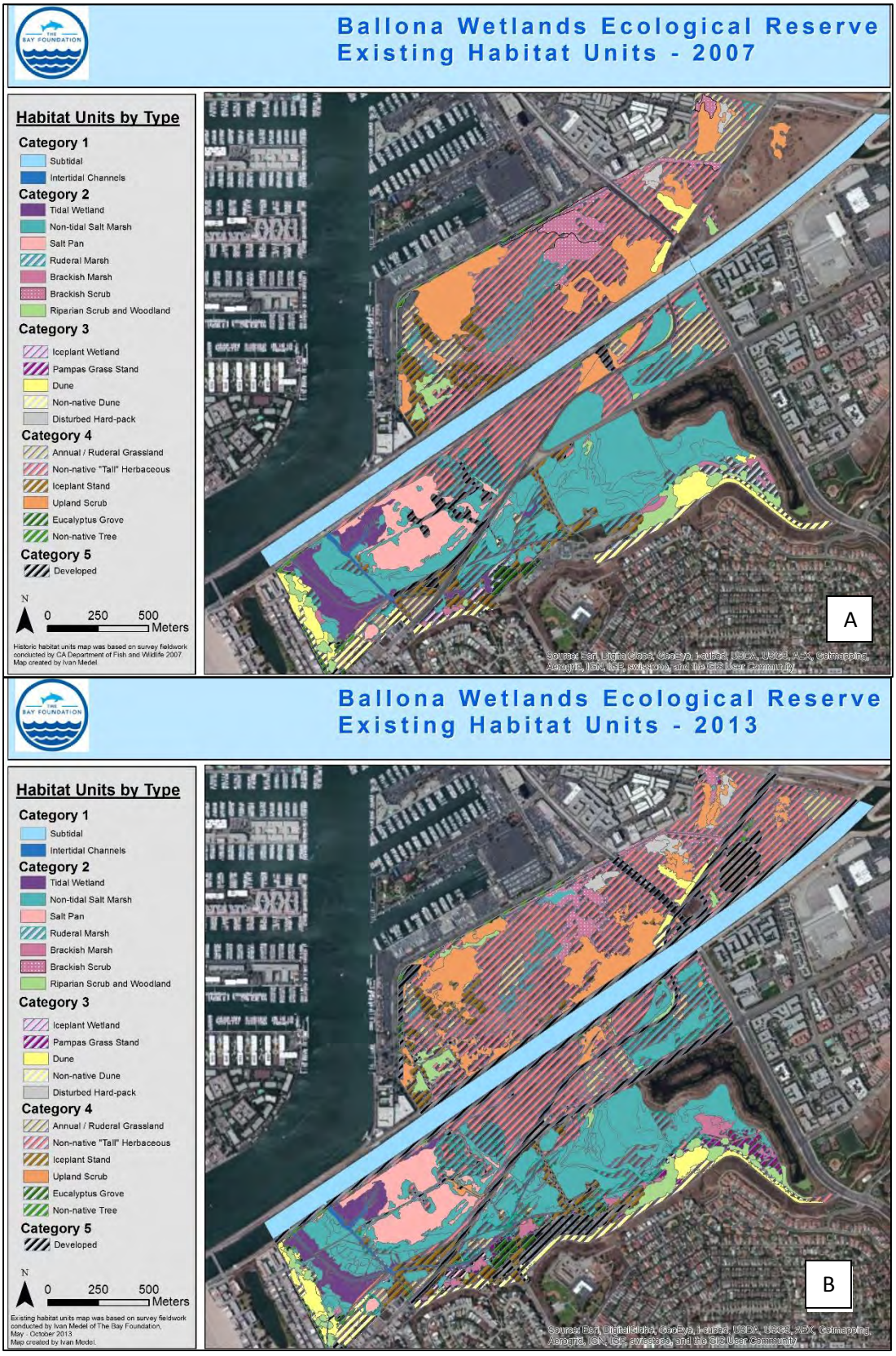

Figure 6. Habitat maps from 2007 (A) and 2013 (B) of the Ballona Wetlands Ecological Reserve. Maps indicate change over time (see also Table 4).

Table 4. Habitat types summarized by acreage

Habitat Type	Acres
Subtidal	53.69
Intertidal	3.49
Tidal Wetland	18.23
Non-tidal Salt Marsh	85.64
Ruderal Marsh	39.55
Brackish Marsh	6.51
Brackish Scrub	10.56
Salt Pan	22.81
Riparian Scrub and Woodland	15.46
Iceplant Wetland	2.04
Pampas Grass	5.80
Dune	10.65
Non-native Dune	14.34
Disturbed Hard-pack	4.96
Annual / Ruderal Grassland	14.64
Non-native "Tall" Herbaceous	159.21
Iceplant Stand	27.77
Upland Scrub	41.91
Non-native Tree	5.56
Eucalyptus Grove	3.40
Developed	65.14
TOTAL	**611.35**

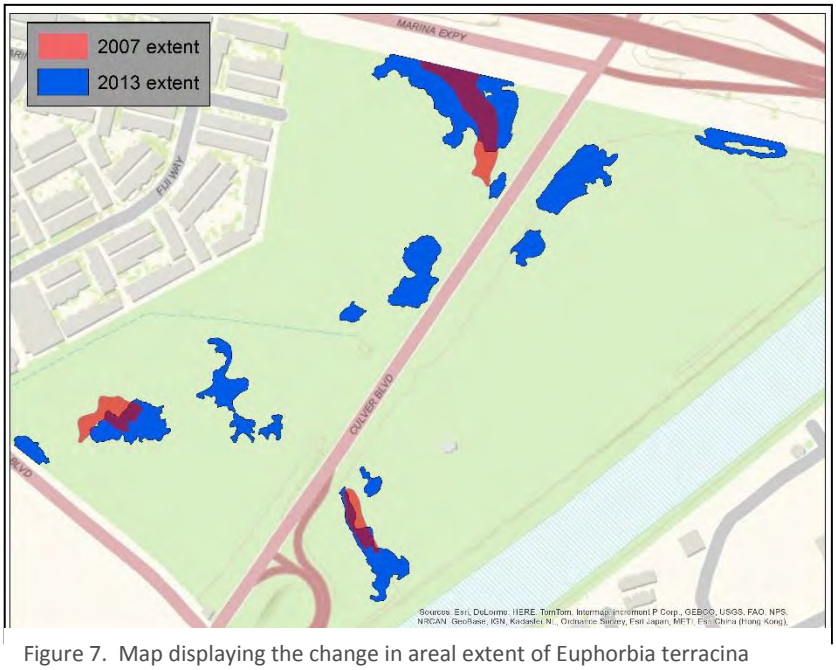

Figure 7. Map displaying the change in areal extent of Euphorbia terracina between 2007 and 2013.

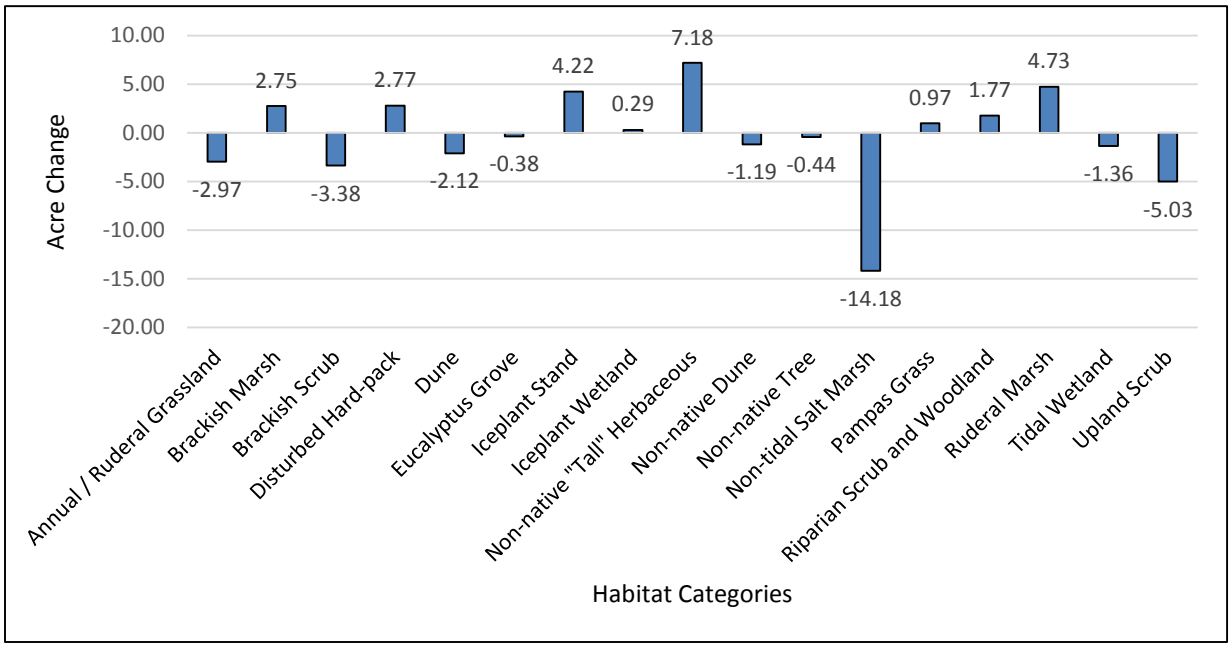

Figure 8. Chart displaying the habitat change in acres between 2007 and 2013 in the Ballona Wetlands Ecological Reserve.

Health and Safety Precautions

Not Applicable

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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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 required
	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
	Suggested Frequency	Every 5 years	----
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	ESRI ArcMap is recommended
	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 <u>compositional</u> 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 <u>structural</u> 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.

Appendix 3.5B
(CNPS 2007)

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 releves 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 he 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, of 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, of 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

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 relevé, 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 m^2 sub-sample in riparian and 500 m^2 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 (≤ 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

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 mutivariate 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 m^2) 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 m^2 (2.2m x 2.2m), creosote bush may be $2m^2$ (or 1.41 m x 1.41 m), burrobush may be $0.5m^2$) 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 to 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 (radium 5.6 for 100 m^2 circle, radius 11.3 m for a 400 m^2 circle, and radius 17.6 m for a 1000m^2 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

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 (CNPS 2007)

CALIFORNIA NATIVE PLANT SOCIETY RELEVÉ FIELD FORM CODE LIST (revised 3/0107)

MACRO TOPOGRAPHY

00 Bench
01 Ridge top (interfluve)
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 & interfluves)
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
FISN	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-leafed evergreen forest
CDF	Cold-deciduous forest
MNDF	Mixed needle-leafed 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-leafed evergreen-cold deciduous woodland

Shrubs:

TBES	Temperate broad-leaved evergreen shrubland
NLES	Needle-leafed evergreen shrubland
MIES	Microphyllus 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-leafed 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

Appendix 3.5B
(CNPS 2007)

Simplified Key to Soil Texture

(Adapted from Brewer and McCann 1982)

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**

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

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

CLAY Clay (class unknown)
Very gritty…… FISA Fine sandy clay
Slightly gritty…….. 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 UTMs: UTME ____ ____ ____ ____ ____ ____ UTMN ____ ____ ____ ____ ____ ____

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

Stand Size (acres)**: <1, 1-5, >5 | Plot Size** (m^2)**: 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:**

CNPS and CDFW Combined Vegetation Rapid Assessment and Relevé Field Form

RELEVE SPECIES SHEET (Revised 2/27/2014)

Page _______ of Polygon/Stand #: ____________

Stratum categories: T = Tree, S = Shrub, H = Herb, E = SEedling, A = SApling, and N=Non-vascular
% Cover Intervals for reference: r = trace, + = <1%, 1-5%, >5-15%, >15-25%, >25-50%, >50-75%, >75%

Strata	Vascular plant name or lichen/bryophyte	% Cover	Collection	Final species determination (or DBH)

Appendix 3.5C

FID	VEGNAME	HETEROGEN	HEIGHT	SHRUBCOV	HERBCOV	ARUNDO	EUTE	RICO	GLCO	CAED	BRNI	NATIVE	NON_NATIVE	BAREGROUND	COMMENTS
0	Salix lasiolepsis alliance														
1	Salix lasiolepsis alliance														
2	Cortaderia selloana stand														
3	Cortaderia selloana stand														
4	Cortaderia selloana - Salicornia pacifica mu														
5	Typha spp. alliance														
6	Cortaderia selloana stand														
7	Salix lasiolepsis alliance														
8	Glebionis coronaria mu														
9	Baccharis salicifolia alliance														
10	Clematis lingusticifolia mu														
11	Salicornia pacifica alliance														
12	Ricinus communis - Raphanus sativus mu														
13	Bromus diandrus - Avena spp. stand														
14	Encelia californica - Artemesia californica association														
15	Artemesia californica alliance														
16	Isocoma menziesii alliance														
17	Schoenoplectus (S. americanus, Bulboschoenus maritimus, B. robustus) mu														
18	Salicornia pacifica alliance														
19	Salicornia pacifica - Symphyotrichum subulatum mu														

Appendix 3.5D

Standard Operating Procedures:
Seed Collection and Germination

SOP Identification Number: SOP 3.6 Seed Collection and Germination
Date of Issue: 30 June 2015
Date of Last Revision: 23 June 2015
Developed by: The Bay Foundation and California State University, Long Beach
Protocol reviewed by:

Michelle Barton, California State University, Long Beach
Ivan Medel, The Bay Foundation
Karina Johnston, The Bay Foundation
Christine Whitcraft, California State University, Long Beach
Phillippa Drennan, Loyola Marymount University

Suggested citation: TBF. 2015. Vegetation – Seed Bank Standard Operating Procedures. Unpublished protocols. The Bay Foundation, Los Angeles, CA.

Disclaimer: Any use of trade, product, or firm names in this publication is for descriptive purposes only and do not imply endorsement by contributing agencies.

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.

	Habitat Types					
Survey Protocol	**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.

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 a specific plant species, see Appendix 3.6B, which lists available information for 84 native plant species common to southern California restoration efforts or the associated publication (Barton et al. 2015, *in review*).

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 3.6C)
- 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
- Refrigerator

- Oven
- Growing medium (species specific)
- Sterile petri dishes (species specific)
- Ethylene source (ethephon or sliced apple) (species specific)
- Hydrogen peroxide (H_20_2) (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:

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.

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 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 a number of common wetland species, more detailed descriptions of mature seeds are listed in Appendix 3.6B. See Appendix 3.6D 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 Appendix 3.6B 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 of 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.

Figure 2. *Baccharis salicifolia* plant and seed. Photo courtesy: RSABG.org.

Data Collection/Field Notes

Information about collections should be recorded on appropriate data sheet (Appendix 3.6C). 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.

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 H_20_2: 5 H_20) (Teel 2011). Dry seed at room temperature, unless otherwise noted in Appendix 3.6B. 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 sees that appear ripe and healthy. For more detailed procedures on seed cleaning for specific species, see 'Seed Collection' and 'Seed Germination' columns in Appendix 3.6B.

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 in 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 3.6E for more information regarding seed longevity. The longevity of certain seeds can be increased if specific storage rules are

followed for the species (see Appendix 3.6B) 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 (Vierheilig 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' column in Appendix 3.6B 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 *Acmpispon glaber* require heat treatment to break dormancy.

Please see Appendix 3.6B 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

In order 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**
Baccharis pilularis (female)	1	1-Jun	X	X	X	FW marsh
Baccharis salicifolia (female)	3	1-Jun		X		FW marsh
Camissonia cheiranthifolia	3	11-Jul	X	X		BWER Dunes
Encelia californica	10	19-May		X		FBW Dunes
Eriogonum fasciculatum	2	19-May	X		X	FBW Dunes
Frankenia salina	3	11-Jul	X	X		BWER Dunes
Heliotropium curassavicum	15-20	1-Jun	X	X	X	FBW dunes
Juncus acutus	1	11-Jul		X	X	FW marsh
Lupinus chamissonis	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*

Appendix 3.6B

Scientific Name	Common Name	Collection Type	Life Form	CNPS Rare Plant Rank	CNPS State Rank	CNPS Global Rank	Low Marsh	Mid Marsh	High Marsh	Salt Pan	Low Transition	High Transition	Grass	Scrub	Fresh Water	Salt Tolerant	Fruit/Seed Characteristics	Seed Collection/Cleaning	Seed Germination	Seed Storage
Abronia maritima	Red sand verbena	Seed	Perennial forb	4.2	S3?	G4?						x				x	Flowers produce single seeded achenes	Plants produce flowers and seeds throughout the year, with the majority of flower and seed production occuring in late spring/summer. Rub fruits over a medium screen, use a seed blower unit to remove chaff from sieved seeds.	Remove achenes from anthrocarp. Place achenes on filter paper in sterile petri dishes with ethephon (or other ethylene source). Effective ethylene concentration 10-100 umol. Incubate achenes in a chamber with alternating 12 h periods of light (27 C) and dark (20 C). Requires a sandy substrate.	
Abronia umbellate	Pink sand verbena	Seed	Perennial forb									x				x	Flowers produce single seeded achenes	Plants produce flowers and seeds throughout the year, with the majority of flower and seed production occuring in late spring/summer. Rub fruits over a medium screen, use a seed blower unit to remove chaff from sieved seeds.	Some seed lots require cold pre-treatment, others may not. Literature indicates that germination requirements may differ from year to year. For best results, sow clean seeds in the top 1" of a sandy growing medium.	
Achillea millefolium	Common yarrow	Seed, Salvage	Perennial forb									x	x				Flowers April to October (other sources say May-June). Seeds, brown disk achenes, mature late summer-early fall	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	Seeds should be lightly covered with growing medium (milled sphagnum, peat, perlite, vermiculite w/ oscmocote). 90-100% germination.	Longevity: 3-5 yrs.
Acmispon glaber [Lotus scoparius]	Deer vetch	Seed, Salvage	Evergreen shrub									x	x	x		x	Seed pods are narrow, bean-shaped, curved, and 1-2 mm long. Indehiscent pods ripen in 4-6 weeks. Pods are brown and dry when seeds are mature. Can be olive green at maturity.	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.	The hard seeds require heat or mechanical scarification to break dormancy. Heat treatment yields highest germination: cover cleaned seeds with boiling water and leave to soak or heat in an oven at 120 C for 5 minutes.	Long lived in soil seed bank and in cool, dry storage. Seed should be dried to low moisture content and stored in vacuum vials.
Ambrosia psilostachya	Western ragweed	Seed, Cuttings	Perennial forb														Flowering peaks in late fall. Achenes are quite tiny.	Fruits form and seeds disseminate October-December.		
Artemisia californica	California sagebrush	Seed, Salvage	Evergreen shrub											x			Artemisia spp. seeds are very small achenes that generally mature in early fall or winter. Seeds are normally wind dispersed. This plant relies on wildfire for seed germination and burned plants can crown-sprout and keep growing.	Collect seeds in December and January. Strip entire inflorescence by hand. Seeds will need to be cleaned.	Seeds will germinate when fresh. Stored seeds need to be exposed to light and may require cold stratification. Other sources imply that pregermination treatment is not necessary for Artemisia spp.	
Artemisia douglasiana	Mugwort	Seed	Perennial forb								x	x				x	Flowers Jun- Oct. <1 mm, glabrous fruit. Seeds are small, ellipsoid, hairless achenes without ribs or angles.	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.	Germinates naturally at relatively cool temps.	
Artemisia tridentata (?)	Tall sagebrush	unk	Perennial forb										x	x		x	Seeds of Artemisia spp. are very small achenes that generally mature in early fall or winter.	Strip the entire inflorescence by hand. Seeds will need to be cleaned and chaff removed.	No pregermination treatment necessary for Artemisia spp.	
Astragalus pycnostachyus var. lanosissimus	Ventura marsh milk vetch	unk	Perennial forb	1B.1	S1	G2T1		x	x	x	x					x	Approximately 2 seeds/fruit	A. sinuatus seeds mature and should be collected in late July (http://www.centerforplantconservation.org/collection/cpc_viewprofile.asp?CPCNum=491)	Hard seed coat may require scarification prior to germination	
Astragalus tener var. titi	Coastal dunes milk vetch	unk	Annual forb	1B.1	S1	G2T1			x	x	x	x		x		x		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.	If stored for an extended period of time, hard seed coat may require scarification to initiate germination. 95% germination success rate on 0.5% agar plates with 11 hours light at 20 C and 13 hours dark at 12 C.	Maternal samples packaged in glassine envelopes and dried to equilibrium at 14% relative humidity. After 3 weeks, transfer seeds to heavy duty foil/plastic pouches and keep at 18 C
Astragalus trichopodus	Santa Barbara milk vetch		Perennial forb	CBR									x			x		Other plants in genus, specifically A. sinuatus, have seeds that mature in late July		
Atriplex californica	California saltbush	Seed	Perennial forb						x		x	x		x		x	Mature fruit is an utricle w/ a membranous pericarp bearing 1 seed. Seeds are black, shiny, hard, round, and flat; 2 mm at maturity	Seeds are collected from Sep- Oct. Gently rub over #18 sieve. Blow off as much chaff as possible with a seed blower.	Pre-planting: soak in water for 24 hours. Rinse following the soak. Seeds germinate 10 days after sowing in peat moss, perlite, nutrients, gypsum, and dolomitic lime. 86% germination rate following these specifications.	Keep dry, store at room temperature. Atriplex seeds can be stored for 10+ years in tightly closed containers in a shed/warehouse

Appendix 3.6B

Scientific Name	Common Name	Collection Type	Life Form	CNPS Rare Plant Rank	CNPS State Rank	CNPS Global Rank	Low Marsh	Mid Marsh	High Marsh	Salt Pan	Low Transition	High Transition	Grass	Scrub	Fresh Water	Salt Tolerant	Fruit/Seed Characteristics	Seed Collection/Cleaning	Seed Germination	Seed Storage
Atriplex canescens	Fourwing saltbush	Seed	Perennial shrub											x		x	Flowers bloom May-July and are tan to greenish-brown. Fruit: 4 winged utricle, 5-23 mm wide, cream to white in color. Seed: 3-10 mm long and 5-10 mm wide with a brown, papery inner seed coat.	Collect utricles when mature August-September in northern territories and October- March in southern.	Early collections may require a 60 min. soak in sulfuric acid or a pre-chill at 5C for 12 weeks. High substrate moisture desirable. Germination is inhibited by lack of aeration. De-winging improves germination, but potassium nitrate does not. Best temperatures for germination: 18-24C in California. Species known to have a high percentage of empty seed; smallest fruits have the highest percent of filled seed.	Reported good for 5-7 years when stored in sealed containers at 21C. De-winging may increase storability. Refrigeration does not increase storability.
Atriplex lentiformis	Large saltbush	Seed	Evergreen shrub											x		x		Dioecious. Seeds should be collected in the fall and winter. Produces large amounts of seed	Winter is the best time to plant seeds.	Seeds can be stored 3-6 years. High ambient temperatures known to decrease germination rates. Other seeds from the Atriplex genus have been successfully stored for 10+ years in tightly closed containers in a shed/warehouse
Atriplex patula/triangularis	Fat hen	Seed	Annual forb						x		x					x		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	Readily propagated from seed. In the field, germinates in late spring (March - May).	Store at cool temperatures. Atriplex seeds can be stored for 10+ years in tightly closed containers in a shed/warehouse
Atriplex watsonii	Watson's saltbush	Seed	Perennial forb						x		x	x				x	Flowers: Mar-Jul	Seed collection: Jun-Sep	Readily propagated from seed. Readily germinates and establishes in field. Cuttings also work well.	Atriplex seeds can be stored for 10+ years in tightly closed containers in a shed/warehouse
Baccharis glutinosa [B. douglasii]	Saltmarsh Baccharis	Seed, Cuttings, Salvage	Perennial forb					x	x		x					x				
Baccharis pilularis	Coyote brush	Seed? Cuttings, Salvage	Evergreen shrub									x		x			Plants produce many single-sex heads with many small creamy to white disk flowers. The tiny achenes have a ring of long, unbranched pappus bristles. Achene dispersal: begins in November and continues through the fall. Mature seeds are dark brown.	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 phylliaries.	Seeds germinate w/o treatment (Vierhelig). Generally seeds are sown fall- early spring	Cleaned dry seeds should be stored at 1.7-4.5 C in airtight containers. Seeds of the genus Baccharis are not long-lived.
Baccharis salicifolia	Mule fat	Seed, Cuttings, Salvage	Evergreen shrub											x			Clustered white flowers, inflorescence less than 1". Seeds are tiny.	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.	No pretreatment in necessary. Increase yield by sowing seed or taking cuttings in the summer	Seeds of the genus Baccharis are not long-lived
Baccharis sarothroides	Broom baccharis	Seed, Cuttings, Salvage	Perennial shrub											x		x		Dioecious. Seeds can be collected by hand into open breathable bags or branches can be shaken above open tubs or tarps		Cleaned seeds can be stored dry over winter (and possibly longer), but generally seeds of the genus Baccharis are not long-lived
Batis maritima	Saltwort	Limited Success With Cuttings, Even More Limited With Seed	Evergreen shrub	CBR				x	x	x	x					x	Yellow-green inflorescences, with greenish-white fruits. Fruiting occurs from Jul-Oct	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	Cuttings grow well with 1. potting soil or 2. sand and vermiculite or 3. without potting hormone (Zedler 2001)	When refrigerated, viability is 2+ years.
Brickellia californica	California bricklebush	Seed	Evergreen shrub									x		x		x	The flowers and achenes are creamy white and arranged in drooping clusters	Long, narrow achenes. Dark brown in color when mature.		
Camissonia cheiranthifolia	Beach evening primrose	Seed	Perennial forb										x			x	Flowers April-August. Dark brown, teardrop-shaped seeds. Shiny and smooth in appearance.			

Appendix 3.6B

Scientific Name	Common Name	Collection Type	Life Form	CNPS Rare Plant Rank	CNPS State Rank	CNPS Global Rank	Low Marsh	Mid Marsh	High Marsh	Salt Pan	Low Transition	High Transition	Grass	Scrub	Fresh Water	Salt Tolerant	Fruit/Seed Characteristics	Seed Collection/Cleaning	Seed Germination	Seed Storage
Cressa truxillensis	Alkali weed	Seeds (slow)- don't salvage	Perennial forb														Flowers May-Aug. Fruits are small hairy capsules, 1/8" long. Seeds are pinkish in color and broadly egg-shaped	Produces mature seeds from late summer into early autumn.	Establishes readily from plugs with rhizomes	
Croton californicus	California croton	Seed (slow)	Perennial forb									x	x	x		x	Flowers develop into compact, greenish seed pods	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.	Pre-planting: soak seeds for 24 hours in water and cold stratify for 30 days. Should germinate 30 days after sowing.	
Distichlis spicata	Salt grass	Seed, Salvage	Perennial graminoid					x	x		x					x	Flowers Apr-Oct. Inflorescence spikes range in color from green to purple. Seed is likely dormant at time of dispersal.	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.	Establishes readily from rhizome cuttings including a node. Establishes well in restoration sites. Seed germination highest after wet stratification/ a fluctuating inundation regime and with low salinity (Elsey-Quirk, 2009)	Keep seeds dry and cool (refrigerate)
Elymus triticoides [Leymus triticoides]	Creeping wild rye	Seed, Salvage	Perennial graminoid									x	x		x	x				
Encelia californica	California brittlebush	Seed, Salvage	Evergreen shrub										x	x			Small, yellow sunflowers bloom February- July	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.	No seed treatment is necessary. To break dormancy, pre-soak seeds	Store in a standard refrigerator (5-10 C)
Eriogonum fasciculatum	California buckwheat	Seed, Salvage	Perennial forb											x			Whitish pink clusters, 3 mm diameter, flowers Mar-Jun	May-Aug (best Jun-Jul). Collect inflorescences as they begin to brown and turn rusty in color. Seeds may be seperated or left in the flowers. Push seeds through a screen to remove chaff	Seeds germinate well in flats. Seeds should be planted in fall-early winter. Light improves germination rate.	Store in a cool, dry place
Eriogonum parvifolium	Coast buckwheat	Seed, Salvage	Perennial forb											x			After blooming, seed heads ripen and turn rusty brown	Other members of Eriogonum genus: flower July-August; fruit is a hard, dry, three sided achene. Achenes can be hand stripped from plants.	Seeds from genus germinate well w/o pre-treatment.	
Euthamia occidentalis	Western goldentop	Seed, Salvage	Perennial forb					x	x		x					x				
Frankenia salina	Alkali heath	Seed, Salvage	Perennial forb					x	x	x	x	x				x	Small, 5 petaled flowers bloom from Apr-Nov. Naturally, seed remains in flower until senescence. F. salina has ellipsoid seed capsules (8 mm) that contain seeds. Seeds are 1 mm long, brownish black, and ovular in shape with pointed tips at maturity.	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.	Plants grow well from seedlings or rooted cuttings. Germination naturally promoted by low salinity and high temps in spring. Cuttings grow best with water as medium (Zedler Handbook 2001). Seeds need no pretreatment	Store clean, dry seeds in cool conditions. Storage at room temperature is okay. Seeds last up to 2 years.
Grindelia camporum	Valley gum weed	Seed	Perennial forb											x		x	Flowers from May to November. At maturity, disk flowers will be dry and brown. Species has wind-borne, dandelion-like achenes with featherlike tufts. Small, long, and flat achenes can be easily removed from the receptacle	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).	Pre-treat seeds by soaking in water under continuous light OR use two-stage cold stratification at 32 F followed by 59 F in the dark (Zafar 1994)	
Hazardia squarrosa	Common hazardia	Seed	Perennial shrub										x	x		x	Inflorescences with yellow flowers. Seeds oblong to lanceolate.	Fruit: 5–8 mm, 5-angled, glabrous; pappus 7–12 mm, white to red-brown in color		
Heliotropism curassavicum	Seaside heliotrope	Seed, do not salvage	Perennial forb					x	x	x	x					x	2-4 terminal inflorescence spikes per stem. Ends of inflorescences are coiled while in flower	Seeds ripen from base of stalk toward tip.		
Heteromeles arbutifolia	Toyon	Seed, do not salvage	Evergreen shrub										x	x	x		Summer-flowering. Seeds ripen from October- January	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.	Chill for 3 months at 3-5 C, germinate at 23 C	Limited shelf life (less than a year). Storage at room temperature further limits seed longevity. To optimize longevity, store at cool temperatures, as seeds are probably orthodox in storage behavior.

Appendix 3.6B

Scientific Name	Common Name	Collection Type	Life Form	CNPS Rare Plant Rank	CNPS State Rank	CNPS Global Rank	Low Marsh	Mid Marsh	High Marsh	Salt Pan	Low Transition	High Transition	Grass	Scrub	Fresh Water	Salt Tolerant	Fruit/Seed Characteristics	Seed Collection/Cleaning	Seed Germination	Seed Storage
Hordeum brachyantherum	Meadow barley	unk	Perennial graminoid								x	x			x	x	Inflorescences	Collect seeds June 1st to July 31st. Mature inflorescences are light brown. Seed easily removed when stalks are hand stripped. No additional cleaning required	No pre-treatment required. Sow seeds May 1st. Seeds should germinate 21 days after sowing. Germination rate: 60%.	Keep dry in refrigerator
Hordeum depressum	Alkali barley	unk	Annual graminoid								x	x			x	x	Inflorescences	No information found, use H. brachyantherum information as a rough guide	No information found, use H. brachyantherum information as a rough guide	No information found, use H. brachyantherum information as a rough guide
Isocoma menziesii var. vernonoides	Coastal goldenbush	Seed, Salvage	Evergreen shrub									x	x	x		x	Bloom period: April- November. Seeds mature in the fall when the pappus becomes fluffy and achenes detach easily from the receptacle. Achenes are tan-colored, about six times longer than wide, wider on the plumose end, and usually have lengthwise striations. The top of the achene has a ring of white bristles	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	No pre-treatment required. Seedlings emerge in early winter w/ appropriate rainfall. Cuttings root well	Store in cool, dry conditions to increase longevity. Seeds are relatively short lived. After 2 years of storage, only 9.6% of seeds germinated
Isolepis cernua [Scirpus cernuus]	Low bulrush	Seed, Salvage	Annual graminoid				x	x							x	x				
Isomeris arborea	Bladder pod	Seed	Perennial shrub											x		x	Fruits are capsules. Mature fruits will often split at the seam, revealing seeds	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.	Species does not require high soil moisture to germinate.	
Iva axillaris	Poverty weed	Seed, Salvage	Perennial forb														Flowers May-Sept. Each flower head produces 1-2 seeds. Achenes are 2 mm long, turnip-shaped, light, and buoyant.	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	Scarification is not effective. Cold stratification may be (studies needed). Seedling emergence isgenerally in cool season	Seeds may be relatively short lived if dry stored. Seeds should be stored submerged in water inside lumite screen bags
Jaumea carnosa	Fleshy jaumea	Seed, Salvage	Perennial forb				x	x								x	Flowers May-Sep. Yellow composite flowers, 2 cm in diameter.	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.	Cuttings grow well in all mediums tried (Zedler 2001). Seeds germinate readily in moist soil.	Store with perlite to remove moisture at room temperature
Juncus acutus ssp. Leopoldii	Spiny rush	Seed, Salvage	Perennial graminoid	4.2	S3.2	G5T5											2-4 mm flowers, 6 anthers in lateral inflorescences		Grows readily from seed in moderate salinities. Clones can be dug entire and transplanted	
Juncus balticus	Baltic rush	Seed, Salvage	Perennial graminoid				x	x	x		x					x				
Juncus bufonius	Toad rush	Seed, salvage	Annual graminoid						x	x	x					x	Small greenish white flowers, 1-2 mm in diameter. Flowers Feb-Apr	Collect Mar-May. Seed capsules quickly dehisce, seeds should be collected quickly after plant death. Shake mature flowers to collect tiny seed	Seeds germinate readily in low salinity soils	
Juncus mexicanus	Mexican rush	Seed, Salvage	Perennial graminoid						x		x					x				
Lasthenia glabrata var. coulteri	Coulter's goldfields	Seed, do not salvage	Annual forb	1B.1	S2.1	G4T3				x										
Limonium californicum	Sea lavender	Seed (slow)	Perennial forb					x	x		x					x	Blooms April- September. Small lavender flowers. Seed is 3 mm long narrow ellipse. Dark brown/red at maturity.	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	Propagates readily from seed or plugs	Dry, store at cool temperatures. Best if seed used w/in 1 year (Zedler 2001)
Lupinus chamissonis	Dune bush lupine	Seed, do not salvage	Evergreen shrub											x			Purple flowers bloom in spring. The fruit is a hairy legume pod 2.5-3.5 centimeters long. Mature seeds are dark brown and speckled (3-4 mm).	Collect seed April 1- June 30th. Remove seeds from receptacles, no further cleaning required.	Pre-planting: scarify using sandpaper for 5 minutes. Then, soak seeds in heated water over night (repeat for seeds that do not imbibe). Sow in growing medium mid-October. Should germinate 3 days later.	Store dry seeds at room temperature. Longevity not noted

Appendix 3.6B

Scientific Name	Common Name	Collection Type	Life Form	CNPS Rare Plant Rank	CNPS State Rank	CNPS Global Rank	Low Marsh	Mid Marsh	High Marsh	Salt Pan	Low Transition	High Transition	Grass	Scrub	Fresh Water	Salt Tolerant	Fruit/Seed Characteristics	Seed Collection/Cleaning	Seed Germination	Seed Storage
Lycium californicum	California boxthorn	Seed (slow), salvage	Evergreen shrub								x	x				x	Lavender-white flowers, 5-10 mm in diamete. Flowers 1 month after first rains of season.	Seeds (berries) best collected within 2 weeks of setting, otherwise birds will eat the majority. Must be picked by hand	Cuttings root easily and root quickly. Seeds should be soaked in water for at least 12 hours and then transferred to moist soil. Germination rates are low (5-10%) in greenhouses	Store in a cool place. Will begin to mold after 1 week at room temp or 3-4 weeks at 40 F. Seeds can be stored up to a year
Malacothamnus fasciculatus	Bushmallow	Seed, do not salvage unless young	Perennial shrub											x		x			Propagation from cuttings offers easy, rapid establishment of bushmallows (and 90% survival)	Herbarium specimens of the Malacaothamnus genus found to be viable 50 years after collection
Melica imperfecta	Small-flowered melic grass	Seed, Salvage	Perennial graminoid										x	x			Small tan-purple flowers. Mature inflorescences are brown, seeds are tan.	Collect seeds April 15th- June 1st.	Plant has irregular germination patterns and a low germination rate, 30%. Literature is inconsistent, certain sources suggest soaking seeds overnight in fresh water and cold stratifying for 2 weeks in peat. Conversely, Emery 1987 feels no pretreatment is necessary.	Dry seeds should be refrigerated
Mimulus aurantiacus	Sticky monkey	Seed, don't salvage	Evergreen shrub										x	x			Flowers March-November. Mature capsules are brown and contain tiny, black seeds.	Seeds are collected Jun 1st- August 1st. Rub seed capsules over a sieve	No pre-treatment needed, 50% germination rate	Store dry in refrigerator
Monanthochloe littoralis (Distichlis littoralis)	Shore grass	Seed, divisions	Perennial graminoid						x	x						x	Flowers are inconspicuous spikelets, nearly concealed by leaves	Dioecious. Seeds small and difficult to collect. Seeds should be acquired over summer from Jun-Sep.	Propagate from seed or cuttings.	Seeds should be air dried, and stored in cool temp. Stolon cuttings root well in moist soil.
Oenathera elata	Hookers evening primrose	Seed, salvage	Perennial forb										x			x	Seeds are irregularly shaped and stacked in capsules. Capsules are small, brown, and woody at maturity. Each has four chambers with two rows of small seeds. Spring cultivars of the genus flower in August, winter cultivars flower in July.	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.	Surface sow (1 mm deep) to allow sufficient light. Should germinate 15-30 days later.	
Phacelia ramosissima	Branching phacelia	Seed	Perennial forb									x	x	x		x	Fruits are capsules that contain 8-12, 1-2 mm long pitted seeds	Collect seed when flowers are dry and brown. Strip seed from mature inflorescences directly into collection bag.		
Plantago erecta	Foothill plantain	Seed	Annual forb										x	x		x	Dehiscent- ballistic seed dispersal	The tiny capsules dehisce when mature, usually from April-May. Dehiscing inflorescences can be collected early into a paper bag and left to dry. Use sieve to clean.	Non-dormant, no pre-treatment needed. With ample water, will germinate from Sep-Dec in varying temperatures	Cool, dry storage
Platanus racemosa	Western sycamore	Seed, do not salvage	Deciduous tree											x	x		Seedpods are brown at maturity.	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	Cold moist stratification mimics wintering best. Plants seed when the air is between 55-100 F.	
Pluchea odorata	Marsh fleabane	Seed, Salvage	Perennial forb					x	x		x					x				
Populus fremontii	Fremont cottonwood	Seeds, cuttings, do not salvage	Deciduous tree											x	x		Capsules contain seeds with long, silky hairs. Female flowers are green and become increasingly cottony as they mature.	Dioecious.		
Potentilla anserina ssp. Pacifica	Silverweed	not sure re: sp, others easy from seed	Perennial forb					x	x		x	x				x	Fruits are oval, flat, and reddish-brown	Seeds should be dried on the plant, then collected. If using, root ball divisions should be made in spring.	Non-dormant. Should be planted in full sun in lightly packed soil. Keep moist.	May be stored short term (longevity not noted in literature)
Pseudognaphalium californicum [Gnaphalium c.]	California cudweed	Seed	Perennial forb														Flower heads are crowded, spike-like, and densely arranged on the stem. Fruits have bristly, tuft-like projections that are shed at maturity		Germination stimulated by presence of charred wood or aqueous extracts of charred wood	
Rosa californica	California wild rose	Seed, Salvage	Evergreen shrub									x	x	x		x	Blooms May- August. After blooming, rose hips (seed bearing fruit) remain on the plant. Rose hips turn red in color as they mature. Rose hips contain multiple seeds.	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	Seeds from rose hips germinate slowly, cold stratification helps.	Seeds stored dry in sealed vials will retain viability for 2-4 years
Rumex salicifolia	Willow dock	Seed, Salvage	Perennial forb					x	x		x					x				

Appendix 3.6B

Scientific Name	Common Name	Collection Type	Life Form	CNPS Rare Plant Rank	CNPS State Rank	CNPS Global Rank	Low Marsh	Mid Marsh	High Marsh	Salt Pan	Low Transition	High Transition	Grass	Scrub	Fresh Water	Salt Tolerant	Fruit/Seed Characteristics	Seed Collection/Cleaning	Seed Germination	Seed Storage
Salicornia bigelovii	Dwarf pickleweed	Seed (slow), salvage	Annual forb	CBR					x	x						x	Seed ripens early in the fall regardless of sowing date	Entire nflorescences should be collected and air dried. Seeds strip easily from inflorescences after drying.	Can be irrigated with seawater, root zone salinity (top 15 cm of soil) should be kept at a salinity of 70-75 g for high yields	Should be stored in cool temperatures. Viability is reduced after 1 year.
Salicornia pacifica [S. virginica]	Common pickleweed	Seed (slow), salvage	Perennial forb				x	x								x	Sequentially hermaphroditic. Mature seeds are pinkish white, puberulent, and 0.5-1 mm long.	Collect inflorescences Oct- Dec (Nov-early Dec is best). Collect when tips of plants are purple. Dry seeds on screen up to 3 mo.	Cuttings grow well in either potting soil or sand and vermiculite without rooting hormone (Zedler 2001). Seed is highly germinable. *Grows aggressively (Zedler 2001)	Seeds viable for 2+ years
Salicornia subterminalis	Parish's pickleweed	Seed (slow), salvage	Perennial forb				x	x								x		Collect inflorescences and air dry, seeds fall out of remaining fragments	Seeds are highly germinable, promoted by lower salinities. Cuttings will root with care, but not well	Store in cool temperatures.
Salix exigua	Narrow-leaved willow	Seeds/cuttings, salvage	Deciduous shrub												x		Staminate catkin. Fruit: glabrous capsules, 4-7 mm long ovals. Fruit ripens June-July. Produces small seeds normally dispersed via wind and water (w/ pappus).	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.	Narrow leaf willow seeds are non-dormant; seeds usually germinate w/in 24 hours	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.
Salix lasiolepis	Arroyo willow	Seeds/cuttings, salvage	Deciduous shrub												x		Catkins.	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.	Seeds may be planted w/o cleaning. As the function of the hairs is not fully understood, it is recommended to keep them attached	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
Salvia apiana	White sage	Seeds/cuttings, salvage	Perennial shrub											x		x	Flowers bloom April- July.	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	Salvia seeds are initially dormant. To break dormancy, seeds require scarification and possibly stratification to germinate. Seed should be sown in early fall. Seeds may respond to light, so plant in surface soil (1/8- 1/4" deep). After planting, soak flats in water. Cuttings do better if kept warm (68-86 F).	
Salvia mellifera	Black sage	Seeds/cuttings, salvage	Perennial shrub											x		x	Up to 4 seeds/flower. Dry calyces are gravity dispersed in June-August. Seeds are 1 mm x 2 mm	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	Physiological dormancy. Seeds must be exposed to light or components of fire (charred wood, smoke, KNO3)	After 1 year of storage in cool, dry conditions- 41.3% germination rate. Longevity increased in cold conditions.
Sambucus nigra [S. mexicana]	Black elderberry	Seed/cuttings, salvage	Deciduous shrub												x					
Schoenoplectus acutus	California tule	unk	Perennial graminoid												x			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.	Germination rates increase with cold stratification of 2C for 30-74 days.	
Schoenoplectus californicus [Scirpus c.]	California bulrush	Seed, Salvage	0												x	x		Collect, dry, and store seeds in brown paper bags or burlap bags. Seeds and seed heads need to be cleaned in a seed cleaner.	Plant clean seed in the fall. Plant in a clean, weed-free, moist seed bed or 1x1x2 inch pots, 1/4" under the soil surface. (Flooded or ponded soils will increase mortality). Keep soil surface moist at a temperature of 100 F. Seeds will germinate in a couple weeks. Plants are ready for transplant after 100-120 days. Genus may require exposure to ethylene to germinate (see Baskin 2003)	
Spartina foliosa	Cord grass	Seed (slow), Divisions	Perennial graminoid				x									x	Green inflorescences. Flowers in late summer	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.	Plants propagate well from 3 to 6 inch plugs with rooted shoots.	Viability decreases after 4 months

Appendix 3.6B

Scientific Name	Common Name	Collection Type	Life Form	CNPS Rare Plant Rank	CNPS State Rank	CNPS Global Rank	Low Marsh	Mid Marsh	High Marsh	Salt Pan	Low Transition	High Transition	Grass	Scrub	Fresh Water	Salt Tolerant	Fruit/Seed Characteristics	Seed Collection/Cleaning	Seed Germination	Seed Storage
Stipa cernua [Nassella c.]	Nodding needlegrass	Seed, Salvage	Perennial graminoid										x	x			Seed matures in mid-to-late spring, collection possible for 2-3 weeks. Alternate source says to collect July-Aug.	Seed can be harvested by hand or with a flow-vac or combine. Dry seeds in paper bags kept in warm conditions.		After drying and cleaning, seal in paper bags and store at 40 F and 40% RH.
Stipa lepida [Nassella l.]	Foothill needlegrass	Seed, Salvage	Perennial graminoid										x	x			Yellow flowers, brown fruit. Blooms in early spring	Collect seed heads in spring when flowers fade. Allow seed heads to dry on plants, remove and harvest seed. Clean prior to storage	From seed: sow outdoors in the fall. Salvage: divide the root ball	not indicated
Suaeda esteroa	Estero sea-blite	Seed (slow)	Perennial forb	1B.2	S2	G3		x	x							x	1-2 mm round green flower clusters, bloom Jul-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	At restoration sites, seedlings establish well	Stored at 5C. Seed germinability remains high after 2 years
Suaeda moquinii	Seepweed	Seed	Perennial forb								x	x				x	Flowers August- November. It's fruit is a small utricle (<1 mm). Seeds enclosed in a calyx.	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	pre-chill recommended	
Suaeda nigra	Black seablite	unk	0													x	Seeds: small, lenticular to spheric; seed coat can be smooth, finely dotted, warty, net-like, or prickly, margin occasionally winged. 0.5–2 mm long, lenticular, shiny, black. Achenes dispersed with persistent calyx or bracts.	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.	pre-chill recommended	
Suaeda taxifolia	Woolly seablite	Seed	Evergreen shrub	4.2	S2S3	G3?											Radial green flowers	Collect seed by stripping flowers with seed from inflorescences (Jun-Jul)	Propagates well from cuttings grown in moist sandy soil. Seeds germinate readily with freshwater	Store in cool temperatures
Triglochin maritima	Seaside arrowgrass	unk, no experience	Perennial forb				x	x	x							x		Collect seeds between July 17- Sept. 23rd. Mature inflorescences are brown. Seed Cleaning: Rub dry fruits between fingers to extract the seeds.		Storage Conditions: Seeds are kept dry and stored at room temperature.
Vulpia microstachys	Small fescue	Seed	Annual forb										x	x		x	Flowers May-July.	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.)	Appears to require neither scarification or stratification. Seeds germinate w/o pretreatment (Young and Young 1986)	

Appendic 3.6C

Seed Collection Field Sheet		
Date:	Site:	Survey Start Time:
Staff:	Weather:	Survey End Time:

Species Name	# Plants Sampled	#Seeds Collected	Fruiting Stage of plants (e.g. early, ripe, late)	Site Conditions	Description of Local Habitat	Other Notes

Appendic 3.6C

Appendix 2: Seed Collection Field Sheet		
Date:	Site:	Survey Start Time:
Staff:	Weather:	Survey End Time:

Species Name	# Plants Sampled	#Seeds Collected	Fruiting Stage of plants (e.g. early, ripe, late)	Site Conditions	Description of Local Habitat	Other Notes

Appendix 3.6D

Scientific Name	Start	End	Seed Collection Details (copy of draft palette)
Isomeris arborea	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.
Juncus bufonius	Mar	May	Collect Mar-May. Seed capsules quickly dehisce, seeds should be collected quickly after plant death. Shake mature flowers to collect tiny seed
Stipa lepida [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
Plantago erecta	Apr	May	The tiny capsules dehisce when mature, usually from April-May. Dehiscing inflorescences can be collected early into a paper bag and left to dry. Use sieve to clean.
Lupinus chamissonis	Apr	Jun	Collect seed April 1- June 30th. Remove seeds from receptacles, no further cleaning required.
Melica imperfecta	Apr	Jun	Collect seeds April 15th- June 1st.
Acmispon glaber [Lotus scoparius]	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.
Astragalus tener var. titi	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.
Baccharis salicifolia	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.
Abronia maritima	May	Aug	Plants produce flowers and seeds throughout the year, with the majority of flower and seed production occuring in late spring/summer. Rub fruits over a medium screen, use a seed blower unit to remove chaff from sieved seeds.
Abronia umbellate	May	Aug	Plants produce flowers and seeds throughout the year, with the majority of flower and seed production occuring in late spring/summer. Rub fruits over a medium screen, use a seed blower unit to remove chaff from sieved seeds.
Eriogonum fasciculatum	May	Aug	May-Aug (best Jun-Jul). Collect inflorescences as they begin to brown and turn rusty in color. Seeds may be seperated or left in the flowers. Push seeds through a screen to remove chaff
Salvia apiana	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
Suaeda taxifolia	Jun	Jul	Collect seed by stripping flowers with seed from inflorescences (Jun-Jul)
Mimulus aurantiacus	Jun	Aug	Seeds are collected Jun 1st- August 1st. Rub seed capsules over a sieve
Salvia mellifera	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
Atriplex watsonii	Jun	Sep	Seed collection: Jun-Sep
Monanthochloe littoralis (Distichlis littoralis)	Jun	Sep	Dioecious. Seeds small and difficult to collect. Seeds should be acquired over summer from Jun-Sep.
Artemisia douglasiana	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.
Grindelia camporum	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).
Hordeum brachyantherum	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
Salix exigua	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.
Heliotropium curassavicum	Jun		Seeds ripen from base of stalk toward tip.
Cressa truxillensis	Jul	Aug	Produces mature seeds from late summer into early autumn.
Rosa californica	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
Stipa cernua [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.
Triglochin maritime	Jul	Sep	Collect seeds between July 17- Sept. 23rd. Mature inflorescences are brown. Seed Cleaning: Rub dry fruits between fingers to extract the seeds.
Iva axillaris	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
Croton californicus	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.
Vulpia microstachys	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)
Astragalus pycnostachyus var. lanosissimus	Jul		A. sinuatus seeds mature and should be collected in late July (http://www.centerforplantconservation.org/collection/cpc_viewprofile.asp?CPCNum=491)
Astragalus trichopodus	Jul		Other plants in genus, specifically A. sinuatus, have seeds that mature in late July
Schoenoplectus acutus	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.
Achillea millefolium	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
Oenathera elata	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.
Jaumea carnosa	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.
Suaeda moquinii	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
Baccharis pilularis	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 phylliaries.
Atriplex lentiformis	Sep	Jan	Dioecious. Seeds should be collected in the fall and winter. Produces large amounts of seed
Atriplex californica	Sep	Oct	Seeds are collected from Sep- Oct. Gently rub over #18 sieve. Blow off as much chaff as possible with a seed blower.
Atriplex patula/triangularis	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
Frankenia salina	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.
Suaeda nigra	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.
Batis maritima	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
Distichlis spicata	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.
Limonium californicum	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
Salicornia bigelovii	Sep	Nov	Entire nflorescences should be collected and air dried. Seeds strip easily from inflorescences after drying.
Artemisia tridentata (?)	Sep	early winter	Strip the entire inflorescence by hand. Seeds will need to be cleaned and chaff removed.
Heteromeles arbutifolia	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.
Ambrosia psilostachya	Oct	Dec	Fruits form and seeds disseminate October-December.
Isocoma menziesii var. vernonoides	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
Salicornia pacifica [S. virginica]	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.
Suaeda esteroa	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
Platanus racemosa	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
Artemisia californica	Dec	Jan	Collect seeds in December and January. Strip entire inflorescence by hand. Seeds will need to be cleaned.
Salix lasiolepis	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.
Atriplex canescens			Collect utricles when mature August-September in northern territories and October- March in southern.
Baccharis glutinosa [B. douglasii]			
Baccharis sarothroides			Dioecious. Seeds can be collected by hand into open breathable bags or branches can be shaken above open tubs or tarps
Brickellia californica			Long, narrow achenes. Dark brown in color when mature.
Camissonia cheiranthifolia			

Appendix 3.6D

Scientific Name	Start	End	Seed Collection Details (copy of draft palette)
Elymus triticoides [Leymus triticoides]			
Encelia californica			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.
Eriogonum parvifolium			Other members of Eriogonum genus: flower July-August; fruit is a hard, dry, three sided achene. Achenes can be hand stripped from plants.
Euthamia occidentalis			
Hazardia squarrosa			Fruit: 5–8 mm, 5-angled, glabrous; pappus 7–12 mm, white to red-brown in color
Hordeum depressum			No information found, use H. brachyantherum information as a rough guide
Isolepis cernua [Scirpus cernuus]			
Juncus acutus ssp. Leopoldii			
Juncus balticus			
Juncus mexicanus			
Lasthenia glabrata var. coulteri			
Lycium californicum			Seeds (berries) best collected within 2 weeks of setting, otherwise birds will eat the majority. Must be picked by hand
Malacothamnus fasciculatus			
Phacelia ramosissima			Collect seed when flowers are dry and brown. Strip seed from mature inflorescences directly into collection bag.
Pluchea odorata			
Populus fremontii			Dioecious.
Potentilla anserina ssp. Pacifica			Seeds should be dried on the plant, then collected. If using, root ball divisions should be made in spring.
Pseudognaphalium californicum [Gnaphalium c.]			
Rumex salicifolia			
Salicornia subterminalis			Collect inflorescences and air dry, seeds fall out of remaining fragments
Sambucus nigra [S. mexicana]			
Schoenoplectus californicus [Scirpus c.]			Collect, dry, and store seeds in brown paper bags or burlap bags. Seeds and seed heads need to be cleaned in a seed cleaner.
Spartina foliosa			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.

Appendic 3.6E

Species	Seed Longevity	Storage Details (copy of draft palette)
Lycium californicum	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
Limonium californicum	1 year (ideally)	Dry, store at cool temperatures. Best if used w/in 1 year (Zedler 2001)
Salicornia bigelovii	1 year (ideally)	Should be stored in cool termperatures. Viability is reduced after 1 year.
Heteromeles arbutifolia	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.
Baccharis sarothroides	1+ year	Cleaned seeds can be stored dry over winter (and possibly longer)
Atriplex californica	10 years	Keep dry, store at room temperature. Atriplex seeds can be stored for 10+ years in tightly closed containers in a shed/warehouse
Atriplex patula/triangularis	10 years	Store at cool temperatures. Atriplex seeds can be stored for 10+ years in tightly closed containers in a shed/warehouse
Atriplex watsonii	10 years	Atriplex seeds can be stored for 10+ years in tightly closed containers in a shed/warehouse
Salvia mellifera	1-2 years	Store in cool, dry conditions. storange in a warehouse- 41.3% germination rate. Longevity increased in cold. 1-2 years ambient temps
Isocoma menziesii var. vernonoides	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
Frankenia salina	2 years (or less)	Keep seeds dry at room temperature. Seeds last up to 2 years.
Batis maritima	2+ years	Refrigerate (viability is 2+ years)
Salicornia pacifica [S. virginica]	2+ years	Seeds viable for 2+ years
Suaeda esteroa	2+ years	Stored at 5C. Seed germinability remains high after 2 years
Rosa californica	2-4 years	Seeds stored dry in sealed vials will retain viability for 2-4 years
Salix lasiolepis	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**
Salix exigua	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.
Achillea millefolium	3-5 years	
Atriplex lentiformis	3-6 years	Seeds can be stored 3-6 years, have been successfully stored for 5 years (6 years is recorded max)
Malacothamnus fasciculatus	50+ (genus)	
Atriplex canescens	5-7 years	Reported good for 5-7 years in sealed containers at 21C. Dewinging may increase storability.
Abronia maritima	6 years	not indicated
Acmispon glaber [Lotus scoparius]	Long-lived	Long lived in soil seed bank and in cool, dry storage. Dried to low moisture content and stored in **vacuum vials**
Hordeum brachyantherum	Long-lived (4-5 years)	Keep dry in refrigerator
Hordeum depressum	Long-lived (4-5 years)	
Melica imperfecta	Long-lived (4-5 years)	Dry seeds should be refrigerated
Stipa cernua [Nassella c.]	Long-lived (4-5 years)	After drying and cleaning, seal in paper bags and store at 40 F and 40% RH.
Stipa lepida [Nassella l.]	Long-lived (4-5 years)	not indicated
Lupinus chamissonis	long-lived (many years)	Store dry seeds at room temperature. Longevity not noted
Astragalus pycnostachyus var. lanosissimus	long-lived (many years)	
Astragalus tener var. titi	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
Astragalus trichopodus	long-lived (many years)	
Iva axillaris	Short-lived	Seeds may be relatively short lived if dry stored. Seeds should be stored submerged in water inside **lumite screen bags**
Potentilla anserina ssp. Pacifica	Short-lived	May be stored short term (longevity not noted)
Spartina foliosa	Short-lived, 4 months	Viability decreases after 4 months
Abronia umbellate		
Ambrosia psilostachya		
Artemisia californica		
Artemisia douglasiana		
Artemisia tridentata (?)		
Baccharis glutinosa [B. douglasii]		
Baccharis pilularis		Clearned dry seeds can be stored at 1.7-4.5 C in airtight containers
Baccharis salicifolia		
Brickellia californica		
Camissonia cheiranthifolia		
Cressa truxillensis		
Croton californicus		
Distichlis spicata		Seeds are kept dry and stored in refrigerator
Elymus triticoides [Leymus triticoides]		
Encelia californica		
Eriogonum fasciculatum		Store in a cool, dry place
Eriogonum parvifolium		
Euthamia occidentalis		
Grindelia camporum		
Hazardia squarrosa		
Heliotropium curassavicum		
Isolepis cernua [Scirpus cernuus]		
Isomeris arborea		
Jaumea carnosa		Store with **perlite** to remove moisture at room temperature
Juncus acutus ssp. Leopoldii		
Juncus balticus		
Juncus bufonius		
Juncus mexicanus		
Lasthenia glabrata var. coulteri		

Appendic 3.6E

Mimulus aurantiacus		Store dry in refrigerator
Monanthochloe littoralis (Distichlis littoralis)		air dried, and stored in cool temp. stolon cuttings root well in moist soil.
Oenathera elata		
Phacelia ramosissima		
Plantago erecta		Cool, dry storage
Platanus racemosa		
Pluchea odorata		
Populus fremontii		
Pseudognaphalium californicum [Gnaphalium c.]		
Rumex salicifolia		
Salicornia subterminalis		Store in cool temperatures.
Salvia apiana		
Sambucus nigra [S. mexicana]		
Schoenoplectus acutus		
Schoenoplectus californicus [Scirpus c.]		
Suaeda moquinii		
Suaeda nigra		
Suaeda taxifolia		Store in cool temperatures
Triglochin maritime		Storage Conditions: Seeds are kept dry and stored at room temperature.
Vulpia microstachys		

Standard Operating Procedures:
Fish Beach Seine

SOP Identification: SOP 4.1 Fish Beach Seine
Date of Issue: 30 June 2015
Date of Last Revision: 18 June 2015
Developed by: 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 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.

	Habitat Types					
Survey Protocol	**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)
	Field Time (per station)	> 60 minutes	Depending on the number of fish, 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)	> $50	Seines and blocking nets
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 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 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.

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 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). For example, indicator fish such as the 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 tide 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 significant differences; therefore consistent methods between survey programs is essential. Additional survey methods should be employed to assess broader fish species richness including Gobiidae or highly mobile species.

Equipment

Equipment and supplies needed for this survey include:

1. GPS and extra batteries
2. Fish seine net (1.8 m depth by 6 m width with 3.2 mm mesh delta style knotless nylon netting)
3. Two blocking nets (1.8 m depth by width of longest channel). *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.
4. Wetsuit (optional) or chest waders (if water is < 1.2 m)
5. Neoprene dive/surf booties and gloves
6. Aquarium nets (at least two)
7. Buckets and plastic containers
8. Rulers or a fish measuring board (Figure 1)
9. Camera and extra batteries
10. Scale (optional) Weighing of fish can be very time consuming, may result in additional mortality and can be

Figure 1. Round stingray being measured with a fish ruler.

calculated fairly accurately using standard length
11. Datasheets (Appendix 4.1B)
12. Tarp (optional for protection of the transport vehicle)
13. Pliers or wire clippers for handling stingrays

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). These guidelines recommend sampling multiple stations per estuary (e.g. 10 for a large wetland) spaced a minimum of 100 m apart for independence. 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. 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

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.

Station and seining protocols:

1. Photograph each station before beginning (preferably with a GPS-enabled camera from the center of the blocking nets facing across the channel).
2. For each station, position two blocking nets approximately 6 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:
    a. Blocking nets should be deployed perpendicular to the shore and across the entire channel (Figure 2) to prevent fish from escaping the survey area (Nordby and Zedler

Figure 2. Deployed blocking nets in a wetland channel.

1991, Steele et al. 2006b, 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.

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. Complete five replicate seine pulls across the channel (parallel to the shoreline) in between the previously-deployed blocking nets by pulling the poles slowly and steadily with the lead line in constant contact with the substrate.
    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) as quickly as possible to the buckets as these fish tend to be most susceptible to asphyxiation (Skinner et al. 1962).
    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.
5. Retrieve the blocking nets (Figure 3) with nested, inward arcs and transfer those fish to buckets.

Figure 3. Retrieving blocking nets in a wetland channel.

Fish identification and measuring protocols:

1. Transfer fish immediately from the nets (Figure 4) 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; Figure 5) 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 given seine, only the first 30 randomly selected individuals of each species should be measured. The remaining fish of that species (> 30) should be counted and held for release in the buckets.

Figure 4. Searching though the pulled seine for fish transfer into buckets.

3. Fish that are too small (typically those ≤ 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.
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.
7. Complete the datasheet including start time, duration of survey, cloud cover, and precipitation (Appendix 4.1B).

Figure 5. California killifish with approximate standard length designated as yellow bracket.

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 6) or size frequency distributions (Figure 7).

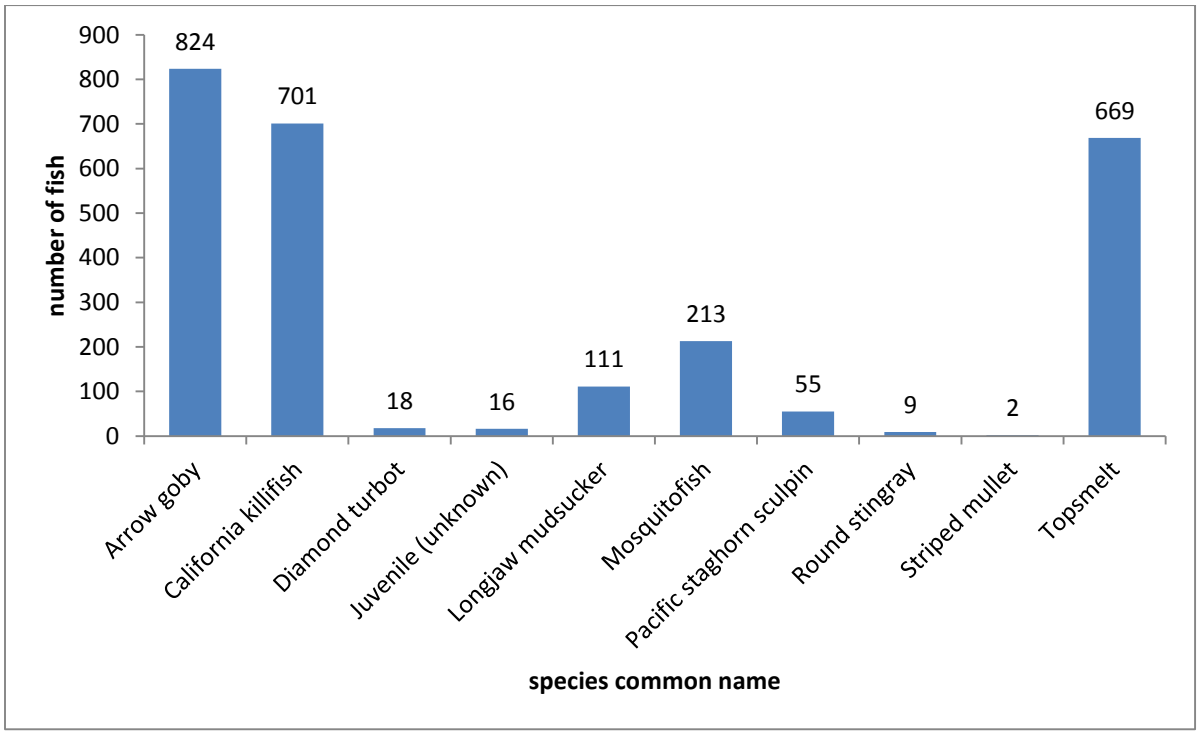

Figure 6. Total counts of each species of fish caught in the beach seine surveys across all stations throughout the first Baseline year.

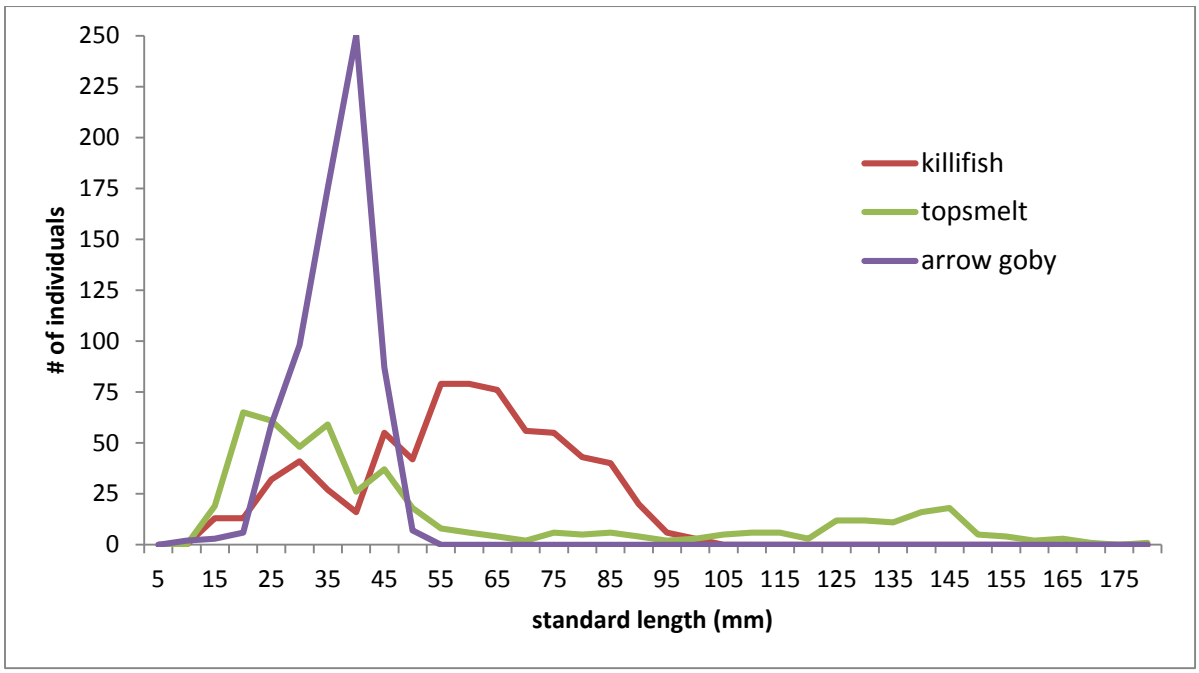

Figure 7. Size frequency distribution for killifish, topsmelt, and arrow gobies.

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 warn for comfort and warmth in exposure to cold water for extended periods of time, e.g. wetsuit or waders.

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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
	Ease of Implementation	Difficult	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	> 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
	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 - BALLONA

Sampling Program Information	
DATE:	GEAR:
STATION:	PAGE: __of__
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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25					60				
26					61				
27					62				
28					63				
29					64				
30					65				
31					66				
32					67				
33					68				
34					69				
35					70				

**** measure first 30 of each species**
**** additional counts on back**

COMMENTS:

APPENDIX 4.1A

#	REP	SPECIES	SL (mm)	WT (g)
71				
72				
73				
74				
75				
76				
77				
78				
79				
80				
81				
82				
83				
84				
85				
86				
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111				
112				
113				
114				
115				

#	REP	SPECIES	SL (mm)	WT (g)
116				
117				
118				
119				
120				

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

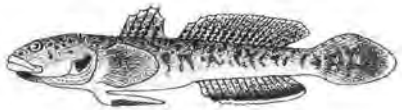
GAAF

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	ILGI	URHA	POLA
Leptocottus armatus	*Ilypnus gilberti*	*Urobatis halleri*	*Poecilia latipinna*
Pacific staghorn sculpin	**Cheekspot goby**	**Round stingray**	**Sailfin molly**
Max 46.0 cm, spines just anterior of gills, stripes on fins, slightly dorsally flattened	Max 6.4 cm; shadow spot anterior to pectoral fin	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	body oblong; head small and dorsally flattened, w/small, upturned mouth; caudal peduncle broad & large, rounded, and sometimes tipped with black
			

Standard Operating Procedures:
Bird Abundance and Activity

SOP Identification Number: SOP 5.1 Bird Abundance-Activity
Date of Issue: 30 June 2015
Date of Last Revision: 22 June 2015
Developed by: The Bay Foundation and Cooper Ecological Monitoring
Protocols reviewed by:

Dan Cooper, Cooper Ecological Monitoring
Karina Johnston, The Bay Foundation
Sean Anderson, California State University, Channel Islands
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Christopher Soleck, Southern California Coastal Water Research Program
Ivan Medel, The Bay Foundation
Charles Piechowski, 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 bird abundance and activity 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 bird abundance and activity protocols can be found in Appendix 5.1A.

Table 1. Appropriate habitat types for bird abundance and activity protocols.

	Habitat Types					
Survey Protocol	**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	5 minutes	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)	< $15	< $15	< $15	----
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	----

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. 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 across time and space and in response to physical phenomena (e.g. rain, tides).

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, 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 more accurately represent avifauna populations.

The primary purpose of these observational sampling methods are to develop maps of species presence and distributions including information on rare species, as well as to supplement historical or volunteer data. Additionally, recording 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 individual program has variations on the specific details of the surveys. 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. Several pieces of equipment are used in all vegetation cover 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: http://www.birdpop.org/docs/misc/Alpha_codes_eng.pdf

Figure 1. Photo of 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 100m x 150m 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)

Field Preparation

Pre-defined maps of the survey areas appropriate for the survey type (Appendices 5.1C, D, and F) should be printed (see details in Field Methods section). 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).

Figure 2. Great blue heron along a rip-rap channel bank.

Additionally, for box count and point count surveys, the survey locations should be identified in the office. For box count surveys, a Geographic Information System (GIS) should be used to create as many 150 x 100 m grid cells as possible within the survey area (Appendix 5.1D) and 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. 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.

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). 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. 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.

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 3. Roosting White-tailed Kite (WTKI) (photo: Dan Cooper 2009).

Birds observed on levees or other anthropogenic structures should be recorded as such. 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 (Figure 3). 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. DRAFT SONGS Wetland Monitoring Plan, 2011). Site-wide surveys employ spot-mapping survey methods wherein an ornithologist or birder walks the entire site 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). In cases of a large flock (e.g. foraging swallows), rough outlines should 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. Only birds within the study area on or near the ground/vegetation should be counted.

Box Count Surveys

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 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. DRAFT SONGS Wetland Monitoring Plan, 2011). 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.

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. *Helpful Hint*: Use a range finder to verify whether particular individuals are within the 150 m radius. Only birds within the study area should be counted.

Laboratory Methods

Not applicable.

Data QAQC Procedures

Data should be entered in the field using the appropriate data sheets (Appendices 5.1C and E) and maps. 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 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 behaviors (or habitat use) by guild, season and/or study site.

Health and Safety Precautions

Not applicable.

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APPENDIX 5.1A

	Evaluation Metric	Site-wide	Box Count	Point Count	Notes
	Correlation to L2 CRAM	Not Applicable	Not Applicable	Not Applicable	Feeding activity is loosely correlated to vegetation
Personnel Requirements	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 species identifications is required
	Number of Personnel	1-2	1	1	Additional personnel may also be used for data recording
	Training Requirements	None	None	None	----
	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.
	Suggested Frequency	Semi-annual	Semi-annual	Semi-annual	----
Survey / Data Quality	Type of Output	Geospatial and numerical	Numerical	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 software is necessary to conduct analyses
	Availability of Online / External Resources	Some	Some	Some	----
Potential Limitations	Wetland Type Applicability	All	All	All	----
	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	----
	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	----

** based on monitoring literature review*

Appendix 5.1C

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	Melozone 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

06 Apr 2010 0836

Appendix 5.1C

Aud. cottontail-15

Appendix 5.1D

APPENDIX 5.1B

Bird Area Survey Datasheet **Date:** **Tide:**

Observers	Start Tide/End Tide	Start Time/End Time	Temp	Wind	%Cloud cover	Precipitation
	/	/				

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	
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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	
			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 5.1B

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	

APPENDIX 5.1F

Standard Operating Procedures:
Benthic Invertebrates

SOP Identification Number: SOP 6.1 Benthic Invertebrates
Date of Issue: 30 June 2015
Date of Last Revision: 23 June 2015
Developed by: The Bay Foundation
Protocols reviewed by:

Karina Johnston, The Bay Foundation
Ivan Medel, The Bay Foundation
Mark Page, University of California, Santa Barbara
Christopher Soleck, Southern California Coastal Water Research Program
Christine Whitcraft, California State University, Long Beach
Charles Piechowski, 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 benthic invertebrate protocols is displayed in Table 1. For emergent salt marsh habitats, benthic invertebrate protocols are only applicable in areas receiving at least partial tidal inundation. 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.

	Habitat Types					
Survey Protocol	**Tidal Channel**	**Mud/sand flat**	**Emergent salt marsh**	**Non-tidal salt marsh**	**Salt pan**	**'Degraded' / fill**
Benthic Cores	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 a higher taxonomic classification (e.g. order) to facilitate the use of student and volunteer (non-professional taxonomic identification) assistance. 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
- Waders (or surf/dive booties with a thick sole)
- 5 gallon buckets (3-4 recommended)
- Handheld multi-parameter water quality meter (e.g. YSI 600)
- 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) sieve
- 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 with Rose Bengal dye (Be careful, read MSDS)
- Formalin
- 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 & 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 early summer is preferred. 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).

Samples should be collected during medium to low tides when the sediment is partially exposed. 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.

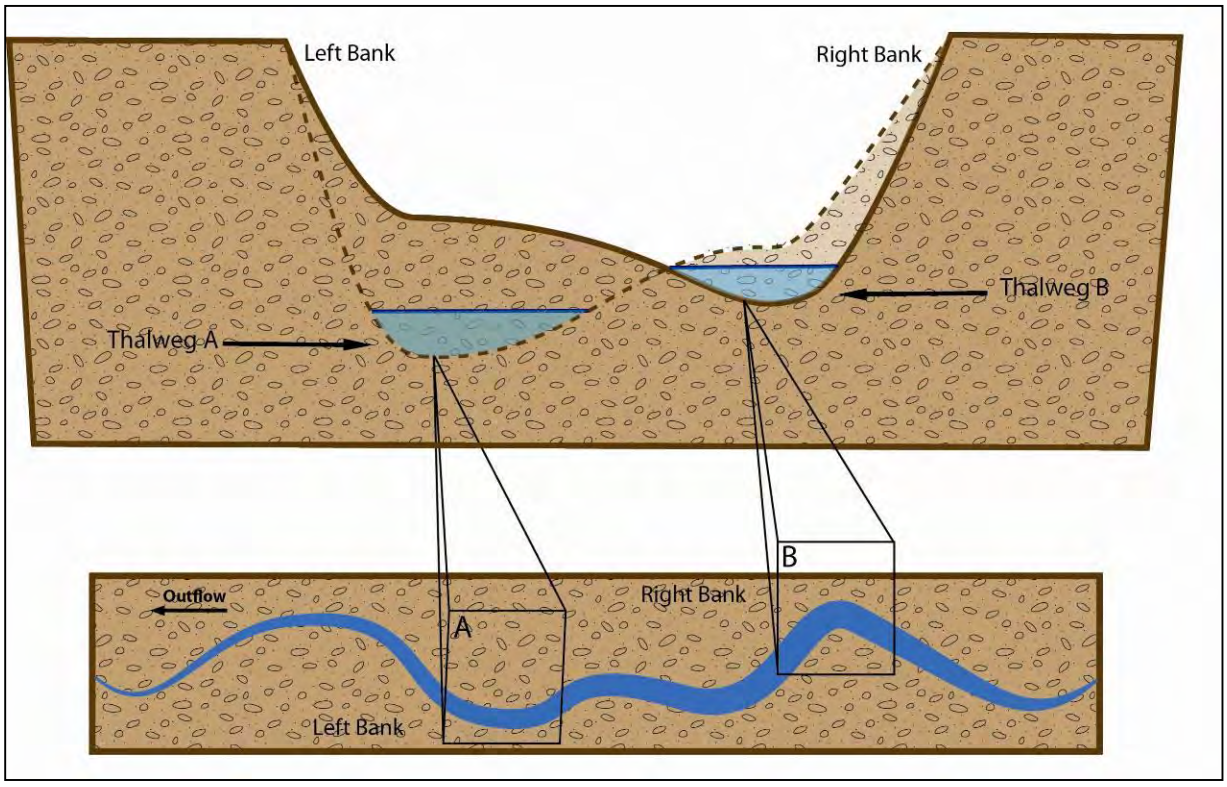

Figure 2. Depiction of cross-section transect of a tidal channel. The figure is not directly representative of a particular benthic survey station. Note: the thalweg is the deepest portion of the channel and not the midpoint.

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).

Figure 3. Example of large core sediment extraction.

Deeper dwelling infauna (e.g. bivalves and shrimp) should 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). Each core will cover an area of 0.007854 m^2.

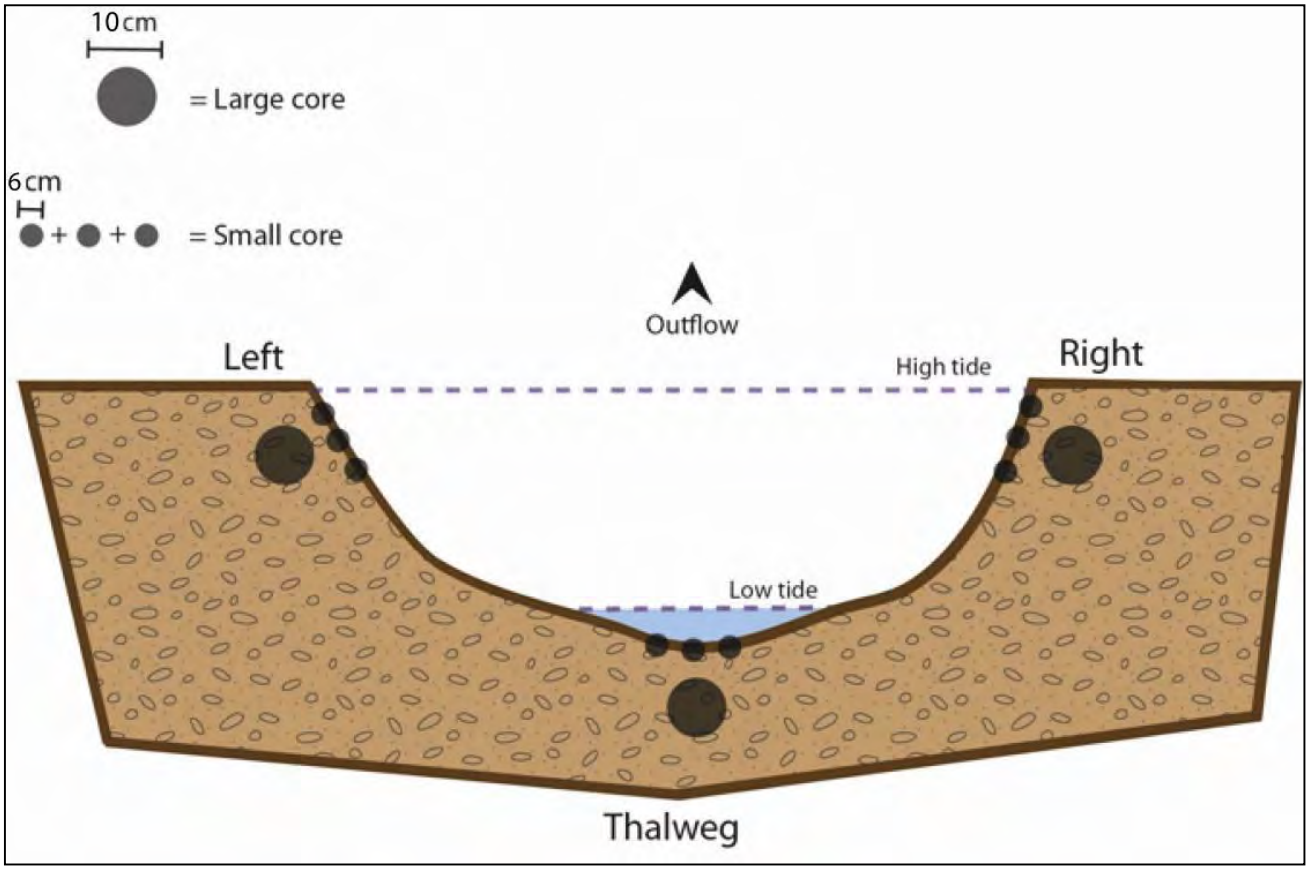

Figure 4. Diagram of benthic infauna core sizes and locations. Note: figure not drawn to scale.

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 5 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 m^2.

Figure 5. Small core pushed into the sediment.

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.

It is important to perform all rinsing using salt water to maintain the correct osmotic pressure for the invertebrates. 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. 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. However, 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.

Laboratory Methods

Following the final 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.

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 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% ethyl alcohol (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.

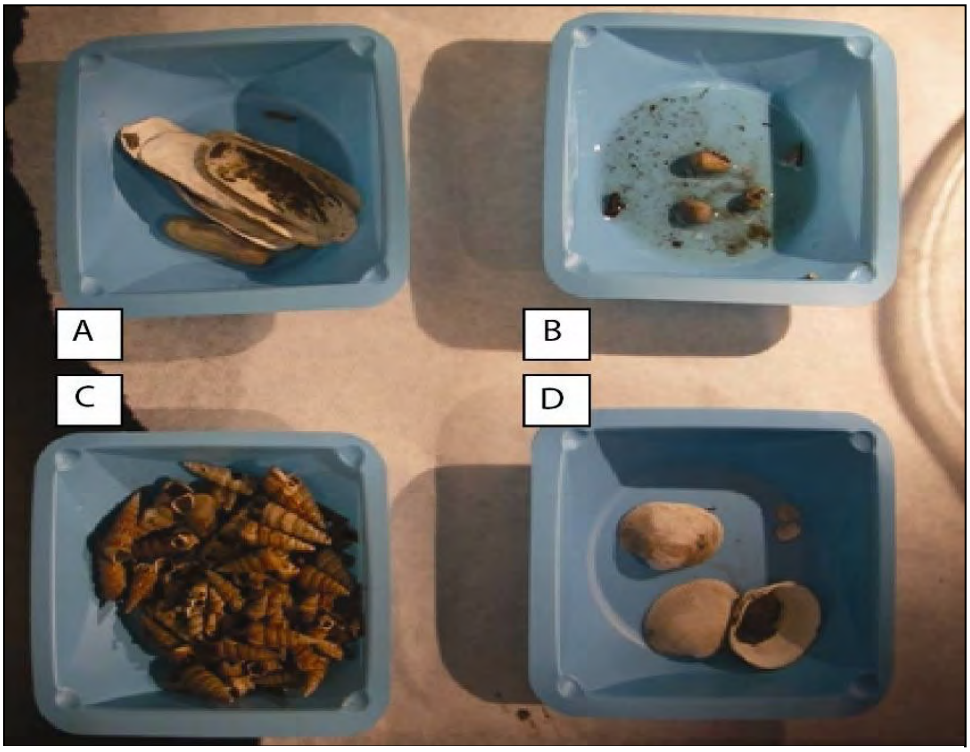

Figure 6. Large core benthic invertebrate sample sorted in the lab showing bivalves (A) (D), *C. californica* (C), and other gastropods (B).

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 m^2 for the large cores and 0.02544 m^2 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.

Health and Safety Precautions

When handling formalin, a respirator mask, latex gloves, and protective eye wear 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 followed at all times (e.g. closed-toed shoes, recognition of where closest emergency equipment is located, etc).

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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
	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 Only	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 _____ ºC
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: _____ (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): _____ Notes:
_____

SAMPLE COLLECTION - LARGE CORE

Number of samples collected: _____ (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): _____

Standard Operating Procedures: Terrestrial Invertebrates

SOP Identification: SOP 6.2 Terrestrial Invertebrates
Date of Issue: 30 June 2015
Date of Last Revision: 22 June 2015
Developed by: The Bay Foundation; California State University, Channel Islands; and California State University, Long Beach
Protocols developed and reviewed by:

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Karina Johnston, The Bay Foundation
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Ivan Medel, The Bay Foundation
Charles Piechowski, The Bay Foundation
Eric Zahn, Tidal Influence
Kyra Barboza, California State University, Long Beach
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 terrestrial invertebrate protocols is displayed in Table 1. The protocols (especially epigeal) 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.

	Habitat Types					
Survey Protocol	**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 Stiky 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, Johnston et al. 2011, 2012). 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.

The primary purpose of this sampling method 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. Taxa should be assessed in the pitfall traps by sorting to a higher taxonomic classification (e.g. order) 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 previous 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

Figure 1. Deployed and labeled aerial arthropod sticky trap.

Additional equipment and supplies for the aerial arthropod surveys includes:

- 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 inch sheets (or approximately 15 cm x 15 cm) with an area of 0.021 m^2 (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, we do not recommend use of scissors 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-deterring 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).

Figure 2. Deployed tomato cage with metallic ribbon and green wire.

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, we prefer to utilize the Solo TP-9oz. cups with a depth of 7.2 cm. 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.
- *Alternate 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 trawl
- 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. Particular transect selection should follow the same randomly allocated vegetation transects within each of the marsh habitat types (see Vegetation Cover SOP for details on randomly allocating transect locations). Traps should be placed in conjunction with the pitfall traps (within 1m, 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.

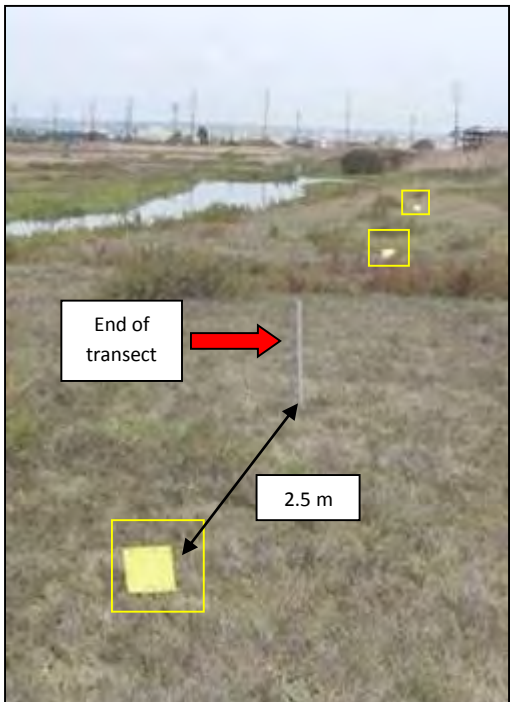

Figure 3. Deployed insect transect. Yellow boxes indicate traps along the transect. Note: the tomato cages have been pulled.

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 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 3 days). Wet traps/arthropods will decay rapidly, particularly during warm summertime conditions.

Figure 4. Deployed pitfall trap with anti-freeze (A) and covered by a plastic plate (B).

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):

   (# of arthropods in size class Y) x (fresh weight regression multiplier for size class Y in g) x (trap area in m^2) x (duration in days) = 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 m^2 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/Pacific Institute for RestorATion Ecology 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. We have been able to reuse antifreeze 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.

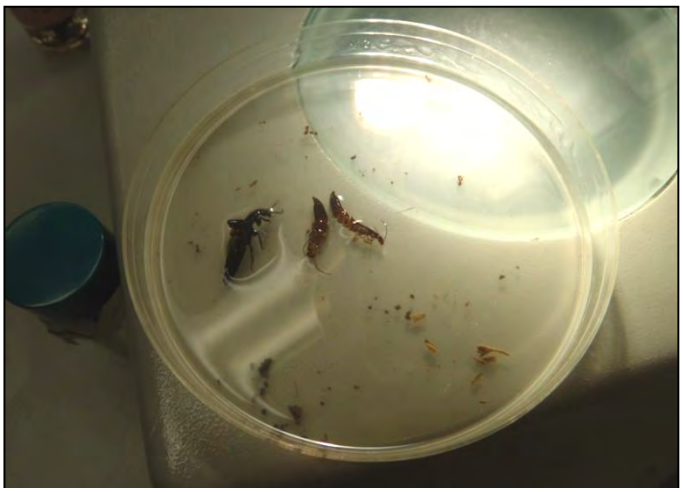

Figure 6. Representative pitfall sample in ethanol.

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 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.
4. 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.

5. Place invertebrates into label glass vials, and cover completely with 70% ethanol. Seal vial with a layer of parafilm.

Identification of the invertebrates:

6. 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.

Figure 7. Charles Piechowski using a dissecting scope to identify and measure pitfall invertebrates.

7. 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 8. Photo of a scavenger beetle taken under a dissecting scope (Photo: Maria Wong).

8. 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.

9. 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):

(# of arthropods in size class Y) x (fresh weight regression multiplier for size class Y) x (area) x (duration) = 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 m^2 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.

References and Applicable Literature

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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, miscroscope, 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 is 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	----
	Potential for Hazards / Risk	Medium Risk	Medium Risk	Medium Risk	Tanglefoot and antifreeze
	Restrictions	Special Status Species	Special Status Species	Special Status Species	----

** based on monitoring literature review*

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):		SPECIES:	COUNT:
<2 mm:		SPECIES:	COUNT:
2-5mm:		SPECIES:	COUNT:
5-10mm:		SPECIES:	COUNT:
>10 mm:		SPECIES:	COUNT:

TRAP 1 (back)

SIZE CLASS	COUNT	SPECIES:	COUNT:
(~0.5 mm):		SPECIES:	COUNT:
<2 mm:		SPECIES:	COUNT:
2-5mm:		SPECIES:	COUNT:
5-10mm:		SPECIES:	COUNT:
>10 mm:		SPECIES:	COUNT:

Morphic Species

TRAP 2 (front)

SIZE CLASS	COUNT	SPECIES:	COUNT:
(~0.5 mm):		SPECIES:	COUNT:
<2 mm:		SPECIES:	COUNT:
2-5mm:		SPECIES:	COUNT:
5-10mm:		SPECIES:	COUNT:
>10 mm:		SPECIES:	COUNT:

TRAP 2 (back)

SIZE CLASS	COUNT	SPECIES:	COUNT:
(~0.5 mm):		SPECIES:	COUNT:
<2 mm:		SPECIES:	COUNT:
2-5mm:		SPECIES:	COUNT:
5-10mm:		SPECIES:	COUNT:
>10 mm:		SPECIES:	COUNT:

Morphic Species

TRAP 3 (front)

SIZE CLASS	COUNT	SPECIES:	COUNT:
(~0.5 mm):		SPECIES:	COUNT:
<2 mm:		SPECIES:	COUNT:
2-5mm:		SPECIES:	COUNT:
5-10mm:		SPECIES:	COUNT:
>10 mm:		SPECIES:	COUNT:

TRAP 3 (back)

SIZE CLASS	COUNT	SPECIES:	COUNT:
(~0.5 mm):		SPECIES:	COUNT:
<2 mm:		SPECIES:	COUNT:
2-5mm:		SPECIES:	COUNT:
5-10mm:		SPECIES:	COUNT:
>10 mm:		SPECIES:	COUNT:

Morphic Species

APPENDIX 6.2C

Terrestrial Invertebrate Pitfall Trap

<u>Staff:</u> <u>Date:</u> <u>Time</u>

Start: End:

Date Collected	FID	Taxonomy					Max Length	Average Length (mm)	Count	Notes	Voucher #
		Order	Superfamily	Family	Genus	Species					

Standard Operating Procedures:
California Rapid Assessment Method (CRAM)

SOP Identification Number: SOP 7.1 Level 2 CRAM
Date of Issue: 30 June 2015
Date of Last Revision: 18 June 2015
Developed by: The Bay Foundation
Protocols reviewed by:

Karina Johnston, The Bay Foundation
Christopher Soleck, Southern California Coastal Water Research Project
Ivan Medel, The Bay Foundation
Charlie Piechowski, The Bay Foundation

Suggested citation: TBF. 2015. California Rapid Assessment Method (CRAM) Standard Operating Procedures. Unpublished protocols. The Bay Foundation, Los Angeles, CA.

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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 of implement CRAM protocols can be found in Appendix 7.1A.

Table 1. Appropriate habitat types for CRAM survey protocols.

	Habitat Types					
Survey Protocol	**Tidal Channel**	**Mud/sand flat**	**Emergent salt marsh**	**Non-tidal salt marsh**	**Salt pan**	**'Degraded' / fill**
Estuarine CRAM			X			
Bar-Built CRAM			X	X		
Depressional CRAM				X		X
Playa CRAM					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 2-3 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 metric final 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.

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…
>
> In essence, 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)."

Equipment

Equipment and supplies needed for this survey include:

1. GPS
2. Camera
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

It is important to note that CRAM surveys for any of the wetland modules should only be conducted by surveyors who have received the corresponding CRAM certification prior to any field work. 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 such as Google Earth. Follow the User Manual rules for defining an AA. Maps should be printed of each AA (Appendix 7.1C), including a scale bar, and attached to the datasheets.

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 and 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 (Figure 1) will assist in finding the site again, and in permanently marking the location.

Figure 1. PVC pipe marking the centroid of an AA.

Laboratory Methods

Not applicable.

Data Entry and QAQC Procedures

Data should be entered in the field using the appropriate data sheet (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. 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 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.

Data Analyses

CRAM is intended for cost-effective ambient 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.

Additional 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; 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 averaging metrics for a total AA score or by averaging AA's for a general health 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 not for purposes such as mitigation requirements.

Health and Safety Precautions

Not applicable.

References and Applicable Literature

Anderson, Sean, Bryan Castro, Robert Rodriguez, and Maria Wong-Yau. 2013. PIRatE Coastal Salt Marsh and Coastal Strand Pitfall Invertebrate Key for the Southern California Bight: Santa Barbara, Ventura, and Los Angeles Counties. Version 2.0. Pacific Institute for RestorATion Ecology Publication No. 4. California State University Channel Islands, Camarillo, California. 48pp.

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APPENDIX 7.1A

	Evaluation Metric	CRAM Survey	Notes
	Correlation to L2 CRAM	All Attributes	----
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
	Expertise / Skill Level	Specific Training Required	Registration for CRAM trainings may be found at http://www.cramwetlands.org/training
	Number of Personnel	2	Due to the subjectiveness of the survey methods, more 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	----
	Suggested Frequency	Semi-annual	----
Survey / Data Quality	Type of Output	Numerical	----
	Active or Passive Monitoring Style	Passive	----
	Specialty Computer Software Required	No	----
	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 structures 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*

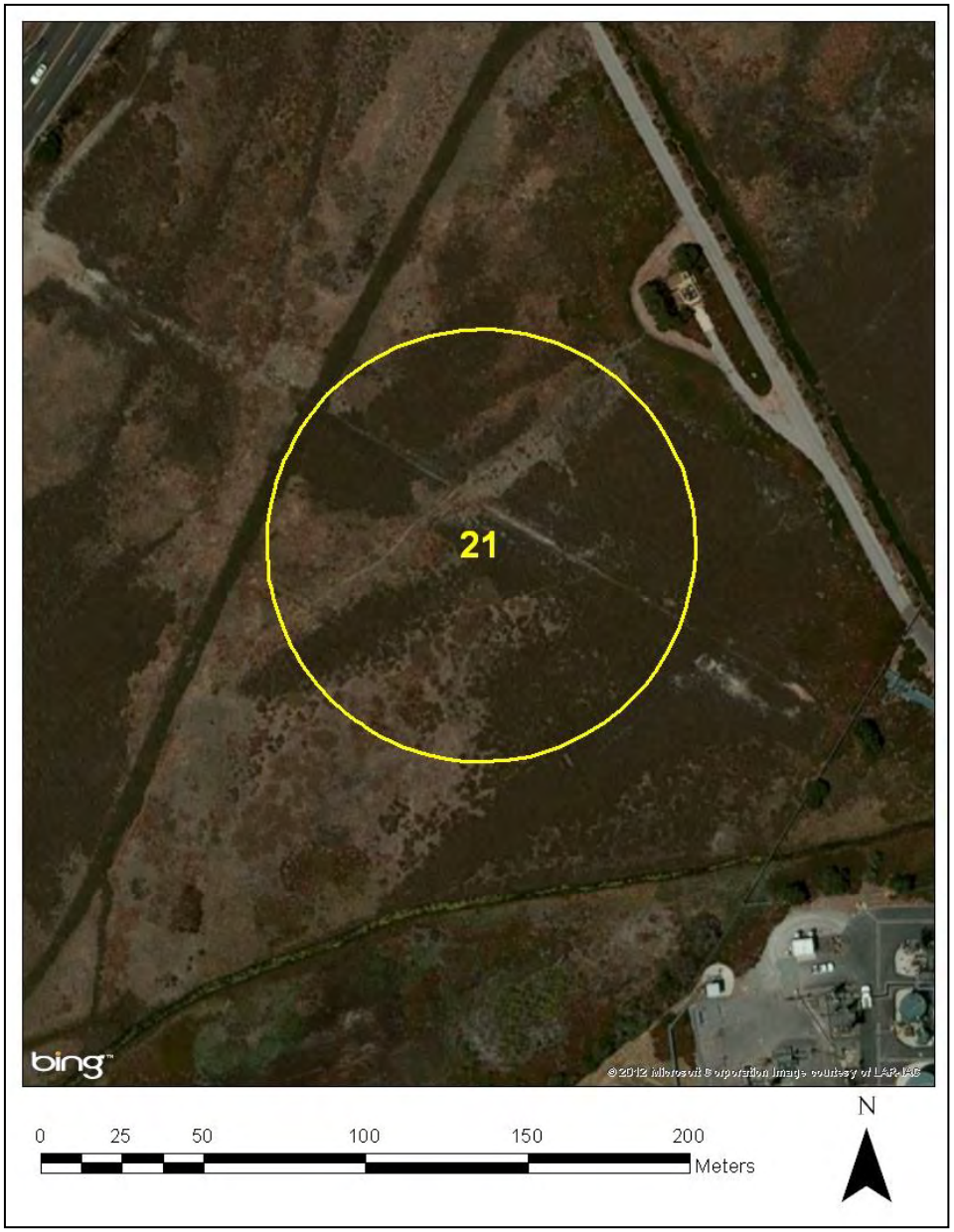
APPENDIX 7.1B

Scoring Sheet: Perennial Estuarine Wetlands

AA Name:				**(m/d/y)**			
Attribute 1: Buffer and Landscape Context				**Comments**			
(D) Aquatic Area Connectivity Score		Alpha.	Numeric				
Buffer (based on sub-metrics A-C)							
(A submetric) Score for Buffer: Percent of AA with Buffer	Alpha.	Numeric					
(B submetric) Score for Buffer: Average Buffer Width							
(C submetric) Score for Buffer: Buffer Condition							
Raw Attribute Score = $D+[C \times (A \times B)^{1/2}]^{1/2}$ (use numerical value to nearest whole integer)				**Final Attribute Score = (Raw Score/24) x 100**			
Attribute 2: Hydrology Attribute							
		Alpha.	Numeric				
Water Source							
Hydroperiod							
Hydrologic Connectivity							
Raw Attribute Score = sum of numeric scores				**Final Attribute Score = (Raw Score/36) x 100**			
Attribute 3: Physical Structure Attribute							
		Alpha.	Numeric				
Structural Patch Richness							
Topographic Complexity							
Raw Attribute Score = sum of numeric scores				**Final Attribute Score = (Raw Score/24) x 100**			
Attribute 4: Biotic Structure Attribute							
Plant Community Composition (based on sub-metrics A-C)							
	Alpha.	Numeric					
Plant Community submetric A: Number of plant layers							
Plant Community submetric B: Number of Co-dominant species							
Plant Community submetric C: Percent Invasion							
Plant Community Composition (*average of submetrics A-C rounded to nearest whole integer*)							
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)							

Excerpted from CWMW 2012a.

APPENDIX 7.1C



Standard Operating Procedures:
Photo Point

SOP Identification: SOP 7.2 Level 2 Photo Point
Date of Issue: 30 June 2015
Date of Last Revision: 22 June 2015
Developed by: The Bay Foundation
Protocols reviewed by:

Karina Johnston, The Bay Foundation
Mark Abramson, The Bay Foundation
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Rodney Abbott, 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 Photo-point 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 Photo-point survey protocols can be found in Appendix 7.1A.

Table 1. Appropriate habitat types for Photo-point protocols.

	Habitat Types					
Survey Protocol	**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
	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)	> $50	One-time expense for camera; handheld GPS
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), a set of panorama photographs (e.g. Figure 1) is taken at permanent locations and bearings to ensure comparable photos. If annually, the targeted time is during mid- to late summer during the peak wetland growing season.

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). 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. Digital camera (high resolution); *Helpful hint:* There are many good panorama applications for smart phones, including Photosynth and Auto Stitch for the iphone, and Camera 360 Ultimate for Android. These may be good alternate options (if high enough resolution, generally > 3MB) to a digital camera and can save time on post processing.
2. GPS equipped with compass and photo point coordinates. The compass headings can be entered into the saved individual GPS coordinate points.
3. Extra batteries for camera and GPS
4. Tripod (if possible). The minimum requirement is a height measurement for the height of the photo being taken or the eye level of the photographer.
5. Compass (can be integrated with GPS)
6. Aerial map(s) of site with Photo-point locations and compass bearings

7. 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

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 ID's 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 as needed, and relevant photo and data sheets should be printed.

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.
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 reference.
4. Usually three to six photos are taken to capture a 180^{o} panorama from a location. This should be standardized and noted on the datasheet (e.g. 4 photos covering a 180^{o} area). Depending on project need or site characteristics, a range of photos may be taken, from a single photo to a 360^{o} 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.
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.

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. 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 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.

References and Applicable Literature

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Johnston, K.K., E. Del Giudice-Tuttle, I.D. Medel, C. Piechowski, D.S. Cooper, J. Dorsey, and S. Anderson. 2012. "The Ballona Wetlands Ecological Reserve Baseline Assessment Program: 2010-2011 Report." Santa Monica Bay Restoration Commission. Report Prepared for the California State Coastal Conservancy, Los Angeles, California. 215 pp.

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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:	N 33.	GPS Coordinates:	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:	N 33.	GPS Coordinates:	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:	N 33.	GPS Coordinates:	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:	N 33.	GPS Coordinates:	N 33.
	W 118.		W 118.
Bearing:		Bearing:	
Notes/Orientation:		Notes/Orientation:	

Standard Operating Procedures: Motion Wildlife Camera Surveys

SOP Identification Number: SOP 8.1 Motion Wildlife Camera Surveys
Date of Issue: 30 June 2015
Date of Last Revision: 22 June 2015
Developed by: The Bay Foundation
Protocols reviewed by:

Ivan Medel, The Bay Foundation
Karina Johnston, The Bay Foundation
Courtney McCammon, Loyola Marymount University
Rodney Abbott, 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 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.

	Habitat Types					
Survey Protocol	**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

Figure 1. Example of photographs confirming the presence of a raccoon and coyote.

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 2. Photograph of a great blue heron feeding.

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.

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 inch 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")

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.

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, Sarmento 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.

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.

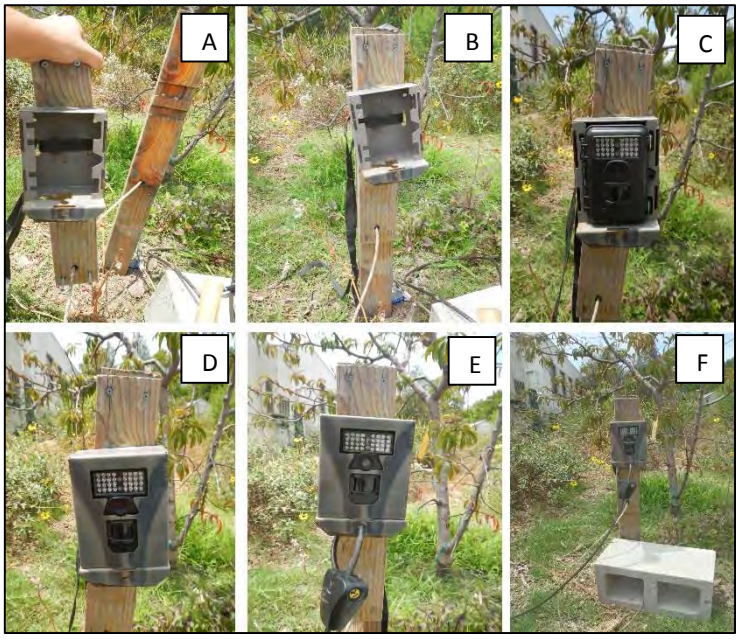

Figure 5. Steps (A-F) to create a full (deterrent) camera housing setup.

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

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.

		Area A					Area B				
Common Name	**Scientific Name**	**A-Middle**	**A-2**	**A-3**	**A-West**	**A-East**	**B-Dune**	**B-Hole**	**B-Channel**	**B-FBW**	**B-Riparian**
California ground squirrel	*Spermophilus beecheyi*						X	X		X	
Cottontail	*Sylvilagus audubonnii*		X	X	X	X	X	X	X	X	X
Coyote	*Canis latrans*		X	X	X		X		X		
Raccoon	*Procyon lotor*						X	X		X	
Rat *	*Rattus* sp.										
Striped skunk	*Mephitis mephitis*						X				
Virginia opossum *	*Didelphis virginiana*						X	X		X	X
Domestic cat *	*Felis cattus*						X	X		X	X
Domestic dog *	*Canis familiaris*		X			X		X		X	
Human	*Homo sapien*		X	X		X	X	X		X	

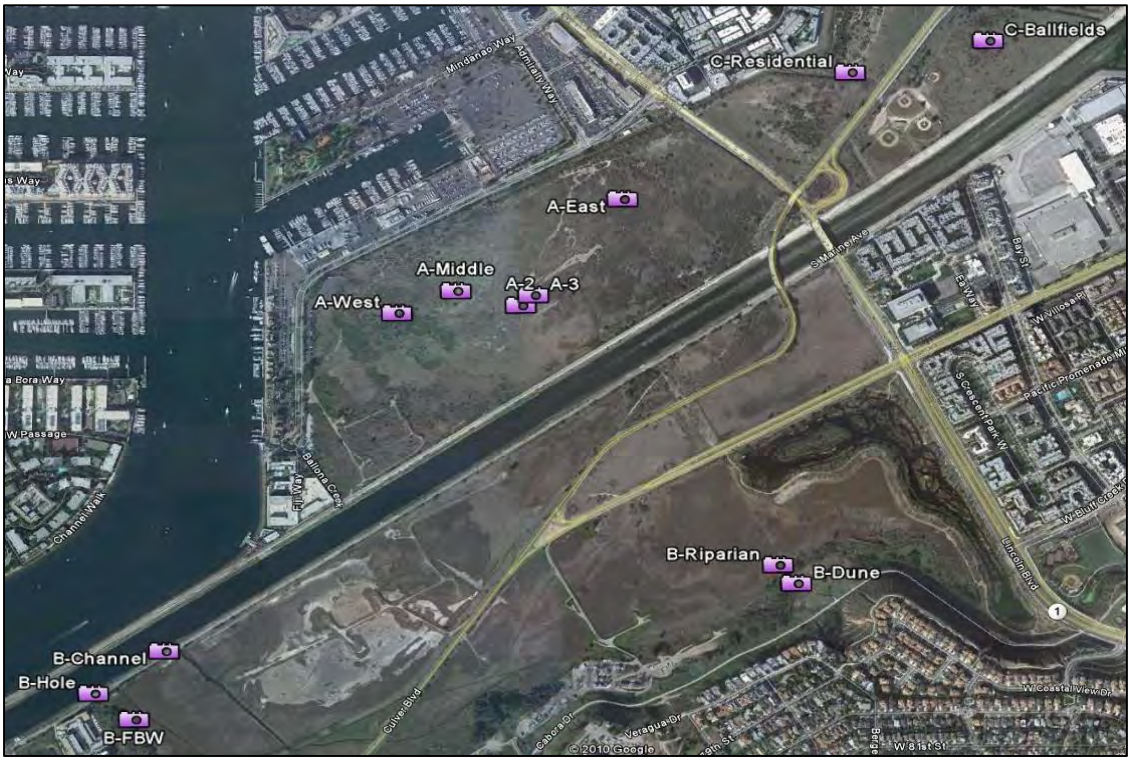

Figure 7. Map showing the location of camera traps.

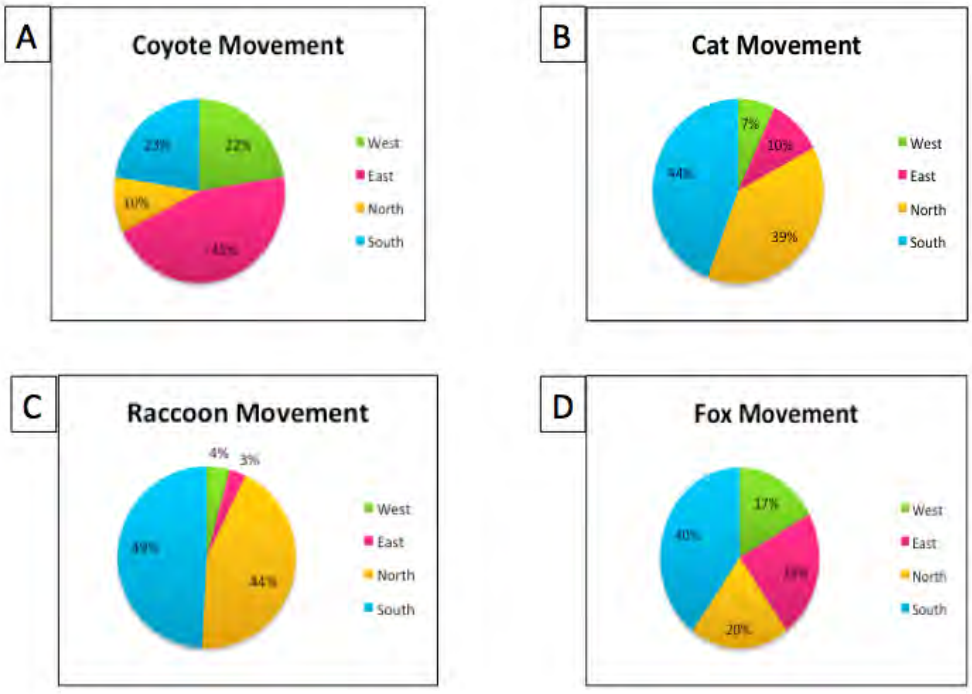

Figure 8. Pie charts displaying movement pattern by direction of travel for recorded cats (A), Coyotes (B), Foxes (C), and Raccoons (D) (McCammon 2014).

Table 2. Chi-squared analysis of animal movement pattern by direction of travel (McCammon 2014).

	Animal				
Direction	**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):		