Estuary Marine Protected Area

Monitoring Protocol

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EMPA

Monitoring Program

OCEAN PROTECTION COUNCIL

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Summary

The condition of California's diverse coastal lagoons and estuaries has the potential to significantly influence water quality and ecosystem functioning in nearby coastal habitats, including California's offshore Marine Protected Areas (MPAs). Answering key questions about the efficacy of the estuarine MPA program, stressor management, and resilience to climate change requires long-term investment in a coordinated monitoring program. However, management agencies will only invest in a program that can provide cohesive, interpretable information over time in the face of changing conditions and be applicable across diverse estuary types. To address this challenge, we created a monitoring framework that enables estuaries across California to be systematically assessed, monitored, and linked to offshore MPA performance. The overall goal of the Estuary Marine Protected Area (EMPA) Monitoring Program is to establish a monitoring framework, including data collection, analysis, synthesis, and reporting to determine the health of estuaries in California and the efficacy of MPA designation in estuaries. A key aspect of this program is a focus on ecological functions versus a single type of flora or fauna. This focus on function allows the framework to accommodate different estuary types and assimilate data from diverse existing monitoring programs, while maintaining underlying comparability. In service of assessing functional performance, we have developed standard protocols to assess key estuarine features across different estuaries, coupled with standard data templates and guidance on analysis, synthesis, and reporting, focused on four guiding principles – flexibility, comparability, interpretability, and practicality. Here, we outline the EMPA framework and provide the 14 SOPs that make up the bulk of the framework. The EMPA monitoring framework project represents a cost-effective, highly leveraged approach to building a state-wide monitoring program.

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Section 1. General Overview

The overall goal of the Estuary Marine Protected Area (EMPA) Monitoring Program is to establish a monitoring framework, including data collection, analysis, synthesis, and reporting to determine the health of estuaries in California and the efficacy of MPA designation in estuaries. A key element is the development of a standardized monitoring protocol that can be used not only by the MPA program, but by any program aimed at assessing estuary function, condition, or health to provide data that can be easily compared across systems and between programs.

A robust condition assessment framework for estuaries should be able to accommodate the underlying differences in estuarine structure and form, while still allowing comparable intra-estuary comparisons of health and condition. The purpose of this manual is to develop a standardized approach to monitor and assess the functions (key species abundance, diversity, structure, function, and integrity) of estuarine ecosystems.

Key Principles

In creating the sampling approach, we focused on four key principles:

1. Flexibility: Assessing estuarine condition using a function-based approach

   The protection and maintenance of ecological functions and ecosystem services is the goal of most coastal management programs (e.g., United States Clean Water Act [33 U.S.C. 1251], European Union Water Framework Directive [WFD 2000/60/EC]). Focusing an assessment framework on ecological functions allows for the creation of linkages between assessment results and ecological services, designated beneficial uses for each estuary (State Water Resources Control Board 2012). Furthermore, an assessment framework built to evaluate ecological functions (Box 1) will have greater flexibility of application within a highly heterogeneous state, like California. The species of plants and animals that are the components of and are used as indicators of ecological functions may change between regions and estuarine functional types, but the focal estuarine functions should remain as constant as practical. Focusing assessments on function should allow for relatively consistent evaluation of bar-built estuaries in both their open- and closed-mouth states while also permitting comparisons between bar-built and perennial systems and between estuaries within these classes. Flexibility of the function-based approach will ultimately allow comparative assessment across estuary type, regional differences, anthropogenic impacts, ultimately permitting assessment of management actions and protected area designations.

2. Comparability: Characterizing systems by geomorphic features

   California is a large state with a considerable diversity of coastal wetlands and estuaries, ranging from large seismic fault estuaries like Tomales Bay to small ephemeral bar-built estuaries like San Mateo Creek Lagoon. Different types of estuaries have different hydrodynamics (tidal inundation, freshwater inputs, and density-driven estuarine circulation) and consequently support different types of flora and fauna (e.g., Edgar et al. 2000; Harrison and Whitfield 2006). Variability in the

physical-biological dynamics of these systems is largely a product of the state's Mediterranean climate. During low rainfall (and calm ocean conditions), smaller bar-built estuaries disconnect from the ocean through beach process and bar formation. While each system is unique, there are underlying environmental similarities in watershed size, morphology, and mouth dynamics among estuaries that influence their resident biota and allow them to be grouped together into different typologies (e.g., Elliott and McLusky 2002; Dürr et al. 2011; Mahoney and Bishop 2018). By classifying estuaries by geomorphic forms (embayments/bays, riverine, lagoons, etc.) and focusing on key landscape features (mudflats, marsh, subtidal channels, etc.), users will be able to make comparisons across systems.

3. **Interpretability: Concentrating sampling in a given area rather than diffusely across the site**

   Regional differences in annual precipitation, watershed and coastal geology, and land use drive tremendous variability in estuarine conditions and functions. In order to capture seasonal and interannual variation among and within sites, sampling protocols will concentrate multiple measurements around sampling zones (Box 2). Users will establish a number of permanent sampling zones within their sites in order to concentrate multiple sampling methods (i.e., cluster sampling) in a given area and have the ability to return to the area. This approach is favored over distributing measures more diffusely across the site, as concentrating multiple measures will enhance our ability to interpret data.

4. **Practicality: Accomplishing sampling within three-days**

   To increase the feasibility of this sampling protocol, we have chosen to limit data collection to what can be reasonably accomplished in a three-day sampling campaign. A three-day campaign should reduce personnel costs and allow users to implement the protocol across multiple sites.

Sampling Program Goals

The protocol was designed with the following *sampling program goals* that embody the above principles:

- Characterize floral and faunal communities
- Understand physical drivers that affect estuarine condition
- Sample broadly to be representative of the spatial complexity of the site
- Collect data that allows exploration of stress-response relationships
- Provide information that can be used to relate estuary function to adjacent offshore function

Ecological Indicators and Functions

A function-based assessment will be used to assess the condition of each estuary, where multiple indicators can be used to assess a given ecological function. Given the ecological and hydrological complexity of estuaries, there are a vast number of potential indicators one could use to evaluate the health and condition of these systems. Eleven priority ecological functions of estuaries were identified by the EMPA technical team (see EMPA Technical Memo). The underlying principle is that all estuaries should provide a variety of ecological functions at some ideal rate in the absence of anthropogenic disturbance and alteration. Priority ecological functions were selected to present a mix of true ecological functions (processes with limited direct society value) as well as ecosystem services (processes with direct, often commodifiable, society value; Figure 1.1, Table 1.1, Box 1).

		Indicators												
		Water quality	Water nutrient concentration	General community composition (eDNA)	Sediment characteristics	Benthic infauna abundance/diversity	SAV/macroalgae distribution	Fish abundance/ diversity	Crab abundance/ diversity	Marsh vegetation distribution/ diversity/ invasives	Marsh plain elevation	Sediment accretion rates	Mouth dynamics	General habitat condition
Ecosystem Functions	Nekton Habitat	■	■				■	■	■					
	Primary Production			■	■		■			■	■	■		■
	Secondary Production					■	■			■				
	Protected Species Support	■		■			■	■	■	■	■		■	■
	Nutrient Cycling		■		■	■	■			■		■	■	
	SLR Amelioration									■	■	■	■	■
	Bird Habitat					■	■	■	■	■	■		■	■
	Shellfish Support	■	■	■	■	■	■							
	Nursery Habitat					■	■	■	■				■	
	Support Vascular Plants	■			■		■			■	■	■		■
	Wildlife Support	■	■	■			■			■	■			■

Figure 1.1. A function-based assessment will be used to assess the condition of each estuary, where multiple indicators can be used to assess a given ecological function. Green squares represent the indicators that can be used to evaluate function.

Table 1.1. Each indicator has an associated collection method and Standard Operating Procedure (SOP).

Indicator	Collection Method	SOP
Water quality: PH, temperature, DO, salinity Water elevation	Continuous data sensors	SOP 1
Water quality: Vertical profile of multiple parameters	YSI/handheld water quality sensor	SOP 2
Water and sediment nutrient concentration	Water and sediment grabs - nitrate, nitrite, Total N, Chl a, Total P	SOP 3
General community composition (eDNA)	Water grabs - eDNA	SOP 4
Sediment characteristics (grainsize, total N, TOC)	Sediment cores	SOP 5
Benthic infauna abundance Benthic infauna diversity	Sediment cores	SOP 6
SAV distribution SAV diversity Macroalgae distribution Macroalgae diversity	Percent cover Shoot density Macroinvertebrate abundance	SOP 7
Fish species abundance	BRUV	SOP 8
Fish species abundance Fish species diversity Fish species age class	Seine	SOP 9
Crab species abundance Crab species diversity Crab species size class/biomass	Minnow traps Shrimp pots	SOP 10
Marsh vegetation diversity Marsh vegetation density Invasive marsh vegetation density	Percent cover Canopy height	SOP 11
Marshplain elevation	Topographic surveys	SOP 12
Sediment accretion rates	Feldspar	SOP 13
Mouth dynamics	Trail cameras	SOP 14
General habitat condition	CRAM	www.cramwetlands.org

Box 1. Priority estuarine ecological functions with a brief definition of each.

Nekton Habitat – Support for a variety of resident and transitory fishes and crustacean by providing structure that serves as shelter from predation and providing benthic or water column food sources.

Primary Production – Production of organic material from carbon input to the system that supports development of diverse microbial, algal, and macrophyte (plant) communities

Secondary Production –Transformation of allochthonous and autochthonous organic matter into meiofauna and macrofauna, which in turn are consumed by the resident nekton of the estuary or are exported out to the nearshore coastal zone

Protected Species Support –Provision of the appropriate subtidal, intertidal, and marsh habitat to support one or more life stages of species that are protected by federal, state, or local regulations. Includes physical structure and water quality conditions (salinity, oxygen, pH, etc) to support these organisms.

Nutrient Cycling – Processing of nitrogen, phosphorous, and carbon from their elemental or detrital forms to support primary production by algae and vascular plants. Nutrient cycling is often high in estuaries because of high inputs, density/tidally driven estuarine circulation patterns, and geomorphology

Sea Level Rise Amelioration and Resiliency –Capacity to absorb and protect adjacent uplands from increasing sea level based on the geomorphology and habitat associated with the marine-freshwater-terrestrial interfaces. Intact estuaries provide resiliency to sea level rise by dissipating energy accreting sediment and providing space for habitat growth and transgression.

Bird Habitat – Provision of physical and biological structure for resident and migratory birds to support predator evasion or nesting (via their associated wetlands) and abundant food (via high secondary and tertiary (nekton) productivity)

Shellfish Support – Provision of habitat for establishment and growth of shellfish. Estuaries are obligate habitats for a variety of societally, economically, and ecologically important shellfish species that rely on the basin morphology, mesohaline/oligohaline salinities, and large amounts of primary production only available in estuaries.

Nursery Habitat – Provision of habitat for spawning and nursery support for marine or anadromous species based on the structural complexity and high primary / secondary productivity found in estuaries.

Support of Vascular Plant Communities – support of a diversity of fresh and salt tolerant plant species distributed throughout the system based on the complex geographic and temporal variability in water depth, sediment composition and elevation, salinity gradient and submergent condition.

Wildlife Support – Support for different life stages and access to movement corridors for a variety of marine- and land-based fauna that rely on estuaries to fulfil a portion of their life history needs.

Box 2. Definitions of terms commonly used throughout the document.

Landscape feature – Habitat types or other landforms commonly found in estuaries, such as mudflats, submerged aquatic vegetation (SAV) beds, marshes, channels, etc.

Sampling area – The full, designated area of the estuary that will be monitored and sampled, marked by a polygon on the sampling map. Given the variability in the sizes of estuaries, some sampling areas may include the whole estuary, while a subsection of the estuary will have to be designated as the sampling area for larger estuaries.

Sampling map – A site map created prior to field sampling to delineate the sampling area, sampling stations, and additional sampling points.

Sampling event – The unique time period when sampling occurs, e.g., in one year, there may be two sampling events – spring and fall.

Sampling point –A generic term used to refer to a location where a specific sampling method will be applied.

Sampling zone – Permanent markers or areas will be set up as sampling zones within estuaries in order to concentrate multiple sampling methods (i.e., cluster sampling). Two to three zones will be set up

Section 2. Cataloguing Project Information and Estuary Characterization

A key component to this monitoring framework is the application of consistent tools for collecting essential metadata, such as project and site information. Before monitoring a specific site, users will walk through a series of steps to 1) catalogue project information, 2) compile site information (imagery, reconnaissance information, permits, etc.), and 3) classify estuaries to choose permanent sampling zones within sites (Figure 2.1).

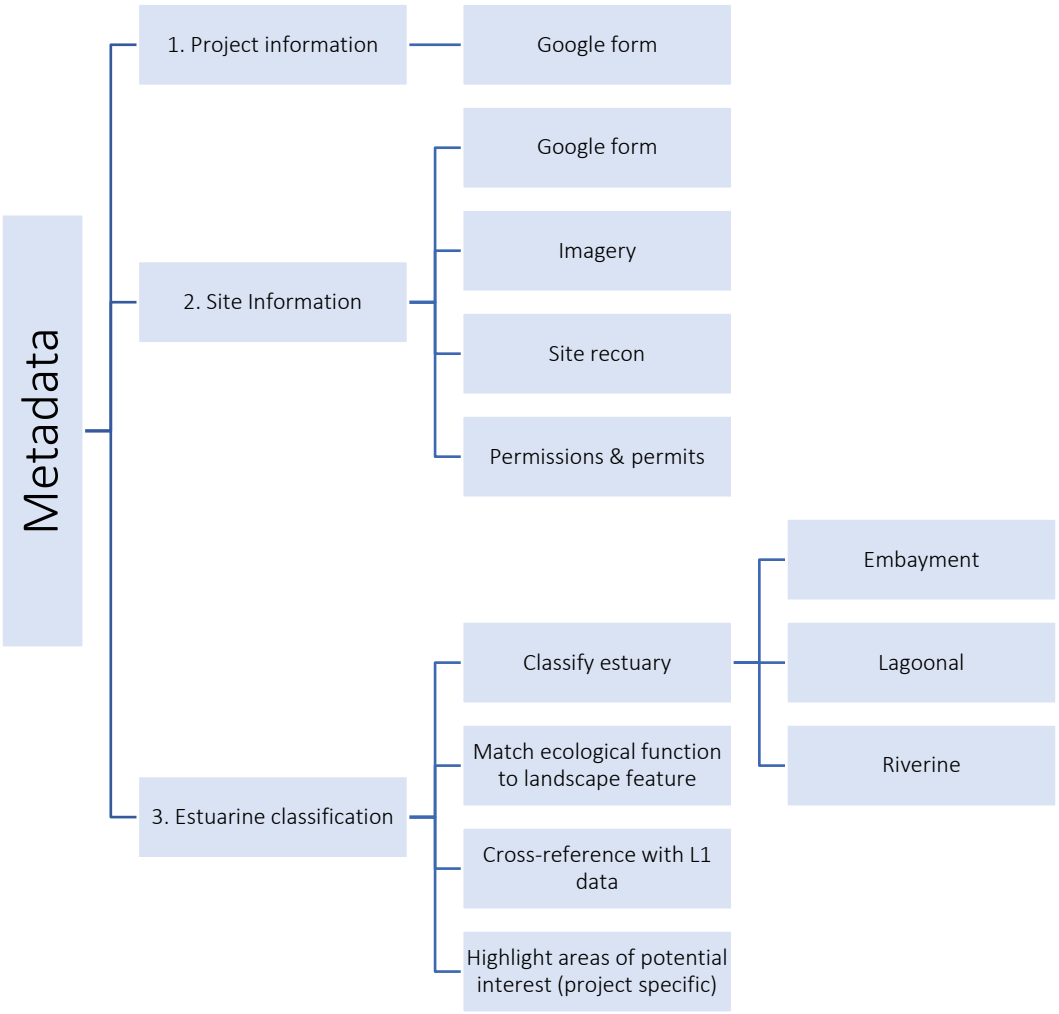


Figure 2.1. *An overview of the metadata collected prior to sampling sites. This information will help users make decisions regarding sampling.*

Project Information

Basic information about each unique project and estuary should be compiled to provide important contextual information for data interpretation, facilitate data compilation and comparison across sampling events and across estuaries, and to enhance repeatability of the protocols. For ease of collection, users will fill out a Google Form to help collect project specific information, which can be found on the project website. The Project Specific Survey[1] should be filled out once per project. This will ensure that project specific information is collected alongside field and lab data. The survey collects general information, for example - project name, purpose and goals, contact information, participating agencies, funding agencies, date of initiation, and project length.

Site Information

To be prepared for monitoring, we encourage all users to collect and catalogue basic site information before entering the field. The Site Metadata Survey[2] allows users to move through a series of questions to make sure 1) general information is collected, such as estuary coordinates, ownership (State Park, Reserve, Public Land, etc.), past and current monitoring, restoration, and research status, 2) permits and permissions are granted, 3) imagery and maps are obtained, and 4) site recon is conducted.

To effectively sample the estuary, the best site imagery should be collected. Site imagery will be crucial for characterizing the estuary, choosing sampling zones for cluster sampling, and marking any additional sampling points of interest. Maps could include SAV[3], aerial imagery, wetland extent, watershed, bathymetry, and topographic or geologic. We recommend the following data sources:

1. Google (Earth, Maps)[4]
2. Navigation charts
3. ArcGIS [5]
4. National Agricultural Imagery Program (NAIP)[6]
5. Statewide topobathy surveys (Coastal Conservancy and Ocean Protection Council)
6. US Fish and Wildlife National Wetland Inventory (NWI)[7]
7. California Aquatic Resource Inventory (CARI)[8]
8. Planet Satellite Imagery (Planet.com)[9]
9. Drone surveys

[1] https://forms.office.com/pages/responsepage.aspx?id=PfKopOEaHEuZAuqhUwKBkCy-6rSJNa5DjdRY9cbIeflURTJCVzA4Ulk2Q1lGWkQ5QlZDWFFKWkVHSiQlQCN0PWcu

[2] https://forms.office.com/pages/responsepage.aspx?id=PfKopOEaHEuZAuqhUwKBkCy-6rSJNa5DjdRY9cbIeflURFE2RUtSMTNaN1NRU0dTVTlHVURNME4yTyQlQCN0PWcu

[3] https://www.pacificfishhabitat.org/data/west-coast-usa-eelgrass-habitat/

[4] https://earth.google.com/web/

[5] https://www.arcgis.com/index.html

[6] https://www.fsa.usda.gov/programs-and-services/aerial-photography/imagery-programs/naip-imagery/

[7] https://www.fws.gov/program/national-wetlands-inventory

[8] https://www.sfei.org/cari

[9] https://www.planet.com/

After permission is granted to access the estuary, we recommend visiting the site to acquire the additional data. Users should confirm any major landscape features (Box 3) or areas of interest; identify access points, hazards, and no-go zones; determine vehicle of craft needs; and make additional observations about seasonal changes, mouth status, and surrounding land-uses.

Estuarine Classification to Identify Sampling Zones

Once the initial site information and base maps are collected for each site, the next step is to identify the sampling zones within each estuary. The overarching goal of this process is to develop a standardized process for the placement of sampling points, which will allow comparison among sites. To do this, users will classify each estuary in order to link ecological functions and landscape features (mudflats, marsh, subtidal channels, etc.; Box 3) for focused sampling efforts.

The initial step in this process is to understand and categorize the nature of the system to be evaluated. This is critical because biotic and abiotic processes in an estuary are influenced by the geomorphic form/type of the estuary, which will constrain the types and magnitudes of ecological functions provided by the system. To assist in some of these decisions, a framework was created that walks users through a classification process (Figure 2.2).

Once the estuary is classified, the second step is for users to identify the main landscape features that will allow them to assess ecological functions, given their specific estuary. The degree of replication needed to accurately capture these processes will vary with the size of the sampling area, but also the diversity of habitats contained within it.

Third, users will cross-reference the identified and chosen landscape features with existing maps of habitat features (level 1 data) from their specific estuary or with their reconnaissance data. With this step the users can ensure that the landscape features they chose are represented in the estuary.

The final step is to highlight any other areas of potential interest. These areas could include temporal or spatial gradients (depth, temperature, salinity, etc.), restoration or sensitive areas, historic points of interest, or hydrologic features (mouth dynamics, tributaries, etc.). The purpose of these final three steps is to pre-determine areas for focused or concentrated sampling.

Step 1: Classify estuary following the California EMPA definitions

1. Refer to Table 2.1 for the definitions
    a. Lagoonal estuaries/bar-built estuaries
    b. Embayment/Bay/perennial estuaries
    c. Riverine estuaries
2. To correctly classify the estuary, users can refer to the classification flowchart for further assistance (Figure 2.2)
3. Classification **should be based on current, contemporary conditions**.
4. The Nature Conservancy classified all 534 California coastal confluences following the Coastal and Marine Ecological Classification Standard (CMECs) guidelines. Users can consult these classifications for further guidance (Heady et al. 2014).

Table 2.1. California EMPA definitions for estuary type – Lagoonal, Embayment/Bay, and Riverine.

Estuarine type	Definition	Examples
Lagoonal (bar-built estuary BBE)	**Frequently closed:** A (frequently) closed system being one that is closed at or above mean high tide (e.g., closed, perched, dune dammed) for a period of time in 8 of 10 years or a similar fraction of July - October air photos.	Navarro River, Carmel River, Ventura River, Arroyo de la Cruz, Pajaro River, Malibu Creek, Goleta Slough
Embayment/Bay	**If not (frequently) closed:** A system that is large and deep (> 3meters below MLW) and a) dominated by inherited space from tectonics or postglacial flooding (e.g., Tomales Bay, Drakes Bay) or b) maintained deep by regular dredging (e.g., Newport Bay, Batiquitos Lagoon).	Morro Bay, Newport Bay, Bolinas Lagoon, Drakes Estero, Batiquitos Lagoon (artificial), Moro Cojo Slough (artificial)
Riverine	**If not (frequently) closed**: A system that is shallow (relative to size) and dominated by riverine processes.	Klamath River, Big River, 10-Mile River

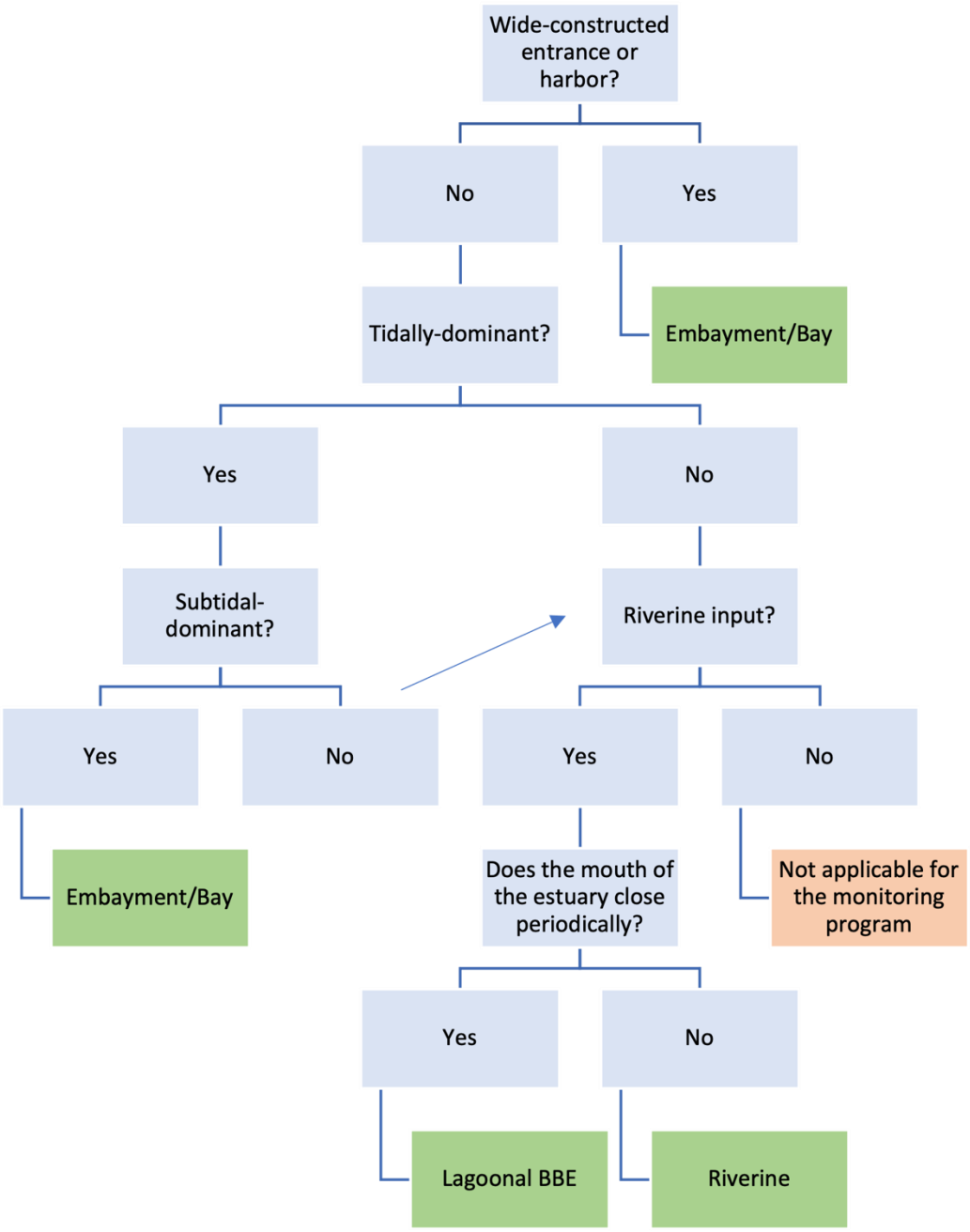


Figure 2.2. Using the definitions of estuary type, users can follow this flowchart to help make additional classification decisions. All decisions should be made based on current, contemporary conditions. The use of 'dominant' throughout the flowchart refers to base-conditions: those conditions outside of storm-events (e.g., the dominant water source in the absence of rain or storm events).

Step 2: Match ecological functions to major landscape features (Table 2.2)

1. Once the estuary is classified, users will consult the function-feature table for their given estuary type to identify the major landscape features (Box 3) at their sites.
    a. The objective of the function-feature table is to help users identify the main landscape features to sample that will allow them to best assess their priority ecological functions within the three estuary types. This is used to guide sampling locations, where sampling a feature informs functional evaluation.
    b. A key component is to highlight the features that best assess the function. A low prioritization does not mean there is a lack of the function within the estuary, rather that it is not the most important feature to sample (with regard to that function).
    c. Due to geomorphic differences in estuary type, the tables take into account temporal variability by factoring temporal dependency (seasonal and mouth closure patterns) into scoring criteria.
    d. This monitoring protocol focuses on cluster sampling in order to meet our goal of feasibility. The three-day sampling campaign will focus sampling at three sampling zones. These zones should encompass the major landscape features identified in this step.
    e. For more information on how the function-feature tables were created, see Appendix I.
2. How to use the function-feature tables.
    a. First, users should prioritize ecological functions (the first column) based on project goals, management priorities, stressor monitoring, etc.
        i. For the purpose of the EMPA project, we have prioritized all eleven ecological functions.
    b. Next, users will identify the highest priority landscape features in their estuary type by matching them with the prioritized ecological functions.
3. Specific ecological functions may not be able to be monitored during a mouth closure in riverine and lagoonal systems due to the immersion of key landscape features. For example, the following ecological functions may change during open versus closed mouth conditions:
    a. Nekton habitat
    b. Shellfish support
    c. Nursery habitat
    d. Bird habitat

Box 3. Description of selected landscape features that will guide sampling zone selection. Most landscape feature definitions were derived from the Coastal and Marine Ecological Classification Standard (CMECs 2012).

Beach/dune – Beach: A gently sloping zone formed by unconsolidated material at the shoreline, typically with a concave profile. This zone extends landward from the low-water line to either (a) the place where there is a definite change in material or physiographic form (such as a cliff) or (b) the line of permanent vegetation; Dune: an active accumulation of sand (formed by wind action) with some elevation; dunes occur on a beach or further inland (CMECs 2012).

Unvegetated marsh (salt panne and pools) – Shallow depressions or flats, often occurring in and adjacent to marshes in the high intertidal that zone that receive saltwater inflow on an infrequent basis. They often are unvegetated and can have encrustations of salt left by evaporation (CMECs 2012).

Freshwater pools – Seasonal pool of water that provide habitat for distinctive plants and animals, typically located on the marsh.

High marsh –Marshes dominated by herbaceous, emergent vegetation and forb-like dwarf shrubs; areas are infrequently flooded by tides and characterized by distinctive patterns of halophytic vegetation. Low shrubs may be present, but they are not dominant (CMECs 2012). The high marsh typically is the last vegetation zone in the marsh before the upland/terrestrial habitat.

Low marsh – The flat, often thick, accumulation of peat that supports emergent marsh vegetation. It is commonly dissected by tidal creeks, and it is occasionally buried and re-exposed through the action of beach erosion and new inlet development (CMECs 2012). The low marsh is dominated by plant species that can tolerate high inundation, such as *Spartina foliosa* and *Sarcocornia pacifica.*

Intertidal channel – Areas of the channel that are exposed and flooded by tides.

Subtidal channel – Areas of the channel that are predominantly submerged

Shellfish reef – Areas dominated by the ridge- or mound- like structures formed by the colonization and growth of shellfish that are attached (cemented) to a substrate of live and dead conspecifics (CMECs 2012).

Mudflat – An extensive, nearly horizontal, barren (or sparsely vegetated) tract of land that is alternately covered and uncovered by the tideTidal flats consist of unconsolidated sediment (mostly clays, silts and/or sand, and organic materials) (CMECs 2012).

Submerged Aquatic Vegetation (SAV) – Aquatic vascular vegetation beds dominated by submerged, rooted, vascular species (such as seagrasses) or submerged or rooted floating freshwater tidal vascular vegetation (CMECs 2012). In this monitoring program, we do not include kelp as SAV.

Table 2.2. Function-feature table. First, users should prioritize ecological functions (the first column) based on project goals, management priorities, stressor monitoring, etc. Next, users will identify the highest priority landscape features in their estuary type by matching them with the prioritized ecological functions. A low prioritization does not mean there is a lack of the function within the estuary, rather that it is not the most important feature to sample (with regard to that function).

Functions	Landscape Features									
	Beach/ dune	Un-vegetated marsh (salt pannes and pools)	Fresh-water pools *lagoonal and riverine only	High marsh	Low marsh	Intertidal channel	Subtidal channel	Shellfish reef	Mudflat	SAV
Nekton Habitat										
Primary Production										
Secondary Production										
Protected Species Support										
Nutrient Cycling										
SLR Amelioration										
Bird Habitat										
Shellfish Support										
Nursery Habitat										
Support Vascular Plants										
Wildlife Support										

Key: Not a priority | Low Priority | Medium Priority | High Priority

Step 3: Cross-reference identified landscape features with estuary reconnaissance or existing maps (Level 1 data).

1. Once users have consulted the function-feature tables, users will cross-reference the identified and chosen landscape features (from step 2) with Level 1 data from their specific estuary.
2. By consulting Level 1 data, users can ensure that the landscape features they chose are represented in the estuary.
    a. Level 1 data sources could include
        i. Pacific Marine & Estuarine Fish Habitat Partnership
            1. Estuarine Biotic Habitat (PMEP)
            2. Eelgrass habitat and extent
        ii. U.S. Fish & Wildlife National Wetlands Inventory (NWI)
        iii. EcoAtlas
        iv. Southern California Wetlands Recovery Project (SCWRP)
        v. North Pacific Landscape Conservation Cooperative (NPLCC)
3. Users should determine whether each pre-defined landscape feature listed in Table 2.2 is present or absent in the estuary.
    a. Table 2.3 shows an example of a level 1 table.
    b. Table 2.4 allows users to relate the "EMPA landscape features" to other commonly used landscape features.
    c. Users can move on to Step 4 if the landscape features identified in step 2 are present in the estuary.
        i. If no landscape features are present in the estuary, then users should return to the function-feature tables and pick the next priority landscape features.
4. Users can either highlight the landscape features on their imagery and maps (previously collected) or make note of the landscape features in order to use them in choosing sampling points (section 3).

Step 4: Highlight areas of potential interest or temporal patterns

1. The final step in the process is to highlight any other areas within the estuary that are potential areas of interest. These features could include-
2. Gradients (depth, salinity, temperature)
3. Historic features or attributes
4. Anthropogenic features (rip rap walls, train/road abutments or supports)
5. Restoration areas
6. Mouth closure zone
7. Designated sensitive habitat areas
8. Different tributary confluence locations

Table 2.3. A Landscape Feature table created for each estuary in the initial EMPA Monitoring Program implementation effort. First, users should determine if the landscape features identified in step 2 from the function-feature tables are present (green) or absent (red) in their estuary. Next, users can either highlight the landscape features on their imagery and maps (previously collected) or make note of the landscape features in order to use them in choosing sampling points (section 3).

Estuaries	Landscape Features (present/absent)									
	Beach/ dune[1]	Un-vegetated marsh	Fresh-water pools	High marsh	Low marsh	Intertidal channel	Subtidal channels	Shellfish reef	mudflat	SAV[2]
Ten Mile River								NA		
Big River								NA		
Navarro River								NA		
Drakes Estero								NA		
Bolinas Lagoon								NA		
Pajaro River								NA		
Moro Cojo Slough								NA		
Carmel River		NA[3]						NA		NA
Arroyo de la Cruz		NA						NA		
Morro Bay		NA						NA		
Goleta Slough								NA		
Ventura River								NA		
Malibu Lagoon								NA		
Newport Bay								NA		
Batiquitos Lagoon								NA		

[1]Beach/dune – Estuaries that are artificially opened or have a widened and hardened entrance were marked as absent (red).
[2]SAV – Most available data is for eelgrass extent only, therefore there may be SAV beds in estuaries marked absent.
[3]NA – There is no available data for this landscape feature.

Table 2.4. EMPA landscape features compared to level 1 data habitat names.

EMPA Landscape Features	Feature Names by Website/Organization				
	PMEP	EcoAtlas	NWI	SCCWRP	NPLCC
Beach/dune		Beach, Dune, and Rocky Shore			
Unvegetated marsh (Salt panne)		Tidal Flat and Marsh Panne		Estuarine Unvegetated Wetland	
Fresh-water pools		Pond and associated vegetation	Freshwater Pond		
High marsh (brackish)	Brackish Emergent Tidal Marsh, Brackish Tidal Scrub-Shrub Wetland				
Low marsh (tidal)	Emergent Tidal Marsh	Tidal Marsh			Emergent Tidal Marsh
Intertidal channel			System: Marine, Subsystem: Intertidal		
Subtidal channel		Subtidal water	System: Estuarine, Subsystem: Subtidal		
Shellfish reef					
Mudflat		Tidal Flat and Marsh Panne			
SAV	Eelgrass Maximum Observed Extent				Aquatic vegetation bed, aquatic bed

Section 3. Creating Sampling Maps

After the specific estuary characteristics are collected, users will use the site information, imagery, and estuarine classification to help them designate the sampling area(s) within the system and allocate sampling zones and points for each sampling method. The steps outlined in Appendix II provide a detailed explanation for choosing and marking sampling points on the map for each sampling method (Table 1.1; for further information regarding the objectives and protocols for each method, refer to Section 5, SOP 1-14).

Section 4. Example Field Plan

We recommend the following field plan. The size of the estuary, as well as the number of personnel may influence your plan. Users should use their best judgement.

Day -28 - Loggers

1. Deploy data sensors; SOP 1
2. Deploy temperature loggers; SOP 1
3. Feldspar plots; SOP 13
4. Trail camera; SOP 14

Day 1 – Zone 1 + SAV assessment

1. Cluster sampling
2. SAV assessment; SOP 7
3. Deploy crab traps at zone 1; SOP 10
4. Marsh vegetation survey near Zone 1; SOP 11
5. Topographic survey near Zone 1; SOP 12

Day 2 – Zone 2

1. Cluster sampling
2. Retrieve crab traps from zone 1; SOP 10
3. Deploy crab traps at zone 2 and 3; SOP 10
4. Marsh vegetation survey near Zone 2; SOP 11
5. Topographic survey near Zone 2; SOP 12

Day 3 – Zone 3 + retrieve instruments

1. Cluster sampling
2. Retrieve crab traps from zone 2 and 3; SOP 10
3. Marsh vegetation survey near Zone 3; SOP 11
4. Topographic survey near Zone 3; SOP 12

Day TBD in Fall – CRAM

Day 90, 180, 270, 365 – exchange in-situ monitoring instruments

Day 365 - Extract feldspar brownie; SOP 13

Section 5. Standard Operating Procedures

Standard operating procedures (SOP) provide a general overview and suggested replication and sampling frequency for each sampling method. The intent of the schedule is to best document the various environmental parameters. Replication and sampling should be adjusted based on sampling maps and site recon. Minimum replication is listed in Table 5.1. SOPs include measured parameters, required materials, and general field collection methods. Recommended field data sheets and data templates for data archiving and storage can be found on the EMPA website[10]. Each SOP can be found in a corresponding appendix.

The transfer of invasive species and pathogens should be considered for each member of the team when moving from site to site. Protocols to prevent such transfer should be in place before sampling. See Appendix III for resources to avoid transmission of invasive species.

[10] https://empa.sccwrp.org

Table 5.1. Suggested temporal sampling frequency and number of replicates per estuary for each sample method, based on an estuary with 3 sampling zones.

SOP	Method	Replication	Continuous	Monthly	Seasonal	Annual	Periodic
SOP 1: Continuous monitoring	VanEssen CTD-Driver with the PME MiniDot	1-2	x				
	Rugged Troll	1	x				
	HOBO TidbiT	6-8	x				
SOP 2: Discrete monitoring – Point water quality measurements	YSI	6-8		x	x		
SOP 3: Water and sediment quality – nutrient concentrations	Freshwater nutrient replicates	1			x		
	Freshwater nutrient Field Blank (FB)	1			x		
	Estuary ambient nutrient replicates	3			x		
	Estuary nutrient Field Blank (FB)	1			x		
	Sediment nutrient samples	6			x		
SOP 4: eDNA	eDNA water samples	9			x	x	
	eDNA surface sediment samples	9			x	x	
	eDNA benthic core sediment samples	9			x	x	
SOP 5: Sediment grainsize	Sediment grainsize core	9				x	
SOP 6: Benthic invertebrates	Subtidal benthic core	3			x	x	
	Intertidal benthic core	3			x	x	
SOP 7: SAV & macroalgal surveys	Transects	Dependent on # of beds		x	x		
SOP 8: Fish - BRUV	BRUV	3-6		x	x		
SOP 9: Fish – Seine & cast net	Seines	9		x	x		
SOP 10: Crab traps	Shrimp pots	3			x	x	
	Minnow traps (3 top and 3 bottom)	6			x	x	
SOP 11: Marsh plain vegetation surveys	Transects (2 per monitoring station)	Minimum 6				x	
SOP 12: Topographic surveys	GPS	Varies					x
SOP 13: Sediment accretion rates	Feldspar	1-3					x
SOP 14: Trail cameras	Trail cameras	1	x	x			
CRAM	CRAM	Depends on estuary size					x

Section 6. Sample Processing, Shipping, and Storing

The following protocol is adapted from: Southern California Bight 2008 Regional Marine Monitoring Survey (Bight '08). 2008. Estuarine eutrophication assessment laboratory manual. Southern California Coastal Water Research Project[11].

Samples Collected

Six types of samples will be collected during the sampling survey:

1. Freshwater loading samples for nutrient analysis (optional, SOP 3)
2. Estuary water samples for nutrient analysis (SOP 3)
3. Sediment samples for nutrient analysis (SOP 3)
4. Water samples and sediment tubes for eDNA (SOP 4)
5. Sediment cores for grainsize (SOP 5)
6. Benthic invertebrates (SOP 6)

Each of these sample types must be shipped to an analytical lab within the holding time.

A representative will receive coolers from designated field teams at the end of the field day. The representative should check that all samples have been collected. Table 6.1 provides a comprehensive list of the samples that should be received.

Table 6.1. *List of samples from each field team*

Sample Type	#	Samples	Container
Freshwater loading samples	1	Nutrient replicates	50 mL HDPE bottles
	1	Nutrient Field Blank (FB)	
Estuary Ambient Water Samples	3	Water nutrient replicates	50 mL HDPE bottles
	1	Nutrient Field Blank (FB)	
Sediment nutrient samples	6	Sediment nutrient replicates	Whirlpak
eDNA	9	eDNA water samples	500 mL Nalgene
	9	eDNA surface sediment samples	1.5mL tubes
	9	eDNA benthic core sediment samples	1.5mL tubes
Sediment grain size	9	Sediment grainsize core	Whirlpak
Benthic invertebrates	3	Subtidal benthic invertebrate core	950 mL Nalgene
	3	Intertidal benthic invertebrate core	500 mL Nalgene

[11] https://www.sccwrp.org/about/research-areas/regional-monitoring/southern-california-bight-regional-monitoring-program/bight-program-documents/bight-2008/

Protocol for Receiving and Storing Samples

Samples will be picked up the day of collection. Samples will be stored on ice in coolers in the field and will need to be stored upon return to the lab until analysis. Samples should be frozen and cataloged the day they are received. Below is a description of the procedure for cataloging and storing samples when they arrive from the field, an example copy of the Sample Checklist is provided in Table 6.2:

1. Open the cooler and note on the Sample Checklist if there is ice, if the ice has melted appreciably, and if the samples are cool.
2. Begin to remove samples from the cooler, starting with the water samples.
3. For each sample type-
    a. Check that all samples written on the sample check list (Table 6.2) are present in the cooler. Place a small check for each sample as it is received.
    b. Note if any of the samples leaked, bottles are damaged, or if the labels fell off on the Sample Checklist.
    c. Check that the volume in each of the sample bottles is not greater than 3/4 the total volume of the bottle.
    d. Place all water samples in the freezer until they can be shipped for analysis.
4. Prepare and ship samples to designated parties (Table 6.3)

Table 6.2. Sample Checklist

Estuary/Estuaries:	
Samples Picked Up By:	Samples Processed By:
Date:	Time:

Ice: ☐ Solid Ice ☐ Ice/Water Mixture ☐ Water Only/Cold ☐ Water Only/Warm

Sample Status:

Sample Type	Arrival Temperature: F = Frozen C = Cold W = Warm	Labels On? (Y/N)	Damage? (Y/N)	Leakage? (Y/N)	Storage: F = Frozen/ R = Refrig	Total # Samples
Freshwater loading samples						
Estuary water nutrient samples						
Sediment nutrient samples						
eDNA water samples						
eDNA surface sediment						
eDNA benthic core sediment						
Sediment grainsize core						
Benthic subtidal invertebrates						
Benthic intertidal invertebrate						

*Write N/A if samples are not present during this sampling period

Shipping Samples for Analysis

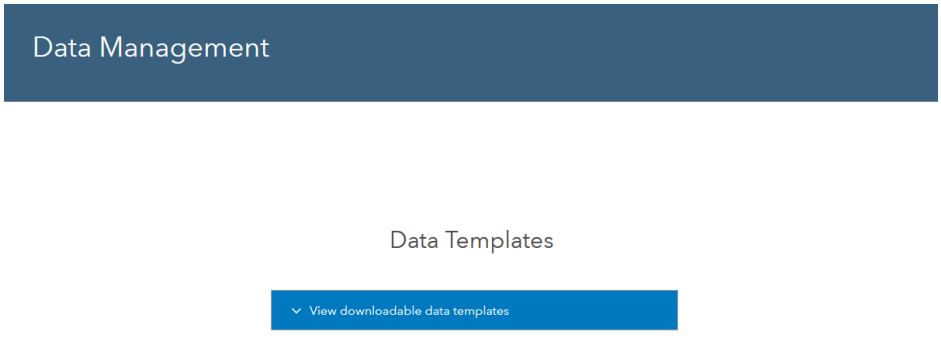
Users should select and notify laboratories before collecting samples. The head of each laboratory should be notified via email the week before samples ship to make sure that samples will be received when they arrive and will not sit around and thaw. Wait for a confirmation that the lab is ready to receive the samples before you ship.

Samples should be shipped following the below protocols. All samples should be shipped on ice, except benthic invertebrate cores. Benthic invertebrate cores should be shipped in ethanol, which will require a hazardous material certification.

1. Samples should be shipped for overnight delivery.
2. Prepare sample Chain of Custody (CoC; examples can be found on the EMPA website)
3. Get 10-20 lbs of ice (wet or dry).
4. Wrap sample bottles in bubble wrap.
5. Using coolers, pack dry ice on the sides of the bubble wrapped samples.
6. Fill cooler the rest of the way with bubble wrap or other packing material so samples can't slip around easily.
7. Place CoCs into a gallon ziploc bag and place on top of samples in the cooler.
8. Close cooler and tape shut with packing tape.
9. Print labels from www.fedex.com.
10. Service type should be priority overnight, our packaging, and estimate the other factors they indicate.
11. Print mailing label and affix to cooler.
12. Schedule priority overnight delivery for the same day.

Section 7. Data Submission

Once the samples have been collected or processed, each SOP is submitted to the EMPA data portal individually using the EMPA data templates, listed on the website under 'Data Management' and 'Data Templates'.



Each data template consists of a series of tabs or tables. Every data template has the following tabs -

1. 'Instructions' – Lists the instructions for templates and contact information
2. 'Protocol metadata' – Collects the contact information of the person submitting the data
3. 'Sample metadata' – The metadata associated with each row of data (latitude, latitude, observations, etc.)
4. 'Data' – The data that is collected
5. 'Glossary' – Provides the associated metadata for each column header by defining each column and indicating the accepted format (integer, text, numeric, etc.)
6. 'Lookup lists' – The accepted values for each column

If specific SOPs have an associated lab component, then there will be separate data templates for field and lab data. The field data templates must be submitted prior to the lab templates. These include –

1. SOP 3 – Nutrients
2. SOP 4 – eDNA
3. SOP5 – Sediment grain size
4. SOP 6 – Benthic infauna
5. SOP 8 – BRUV

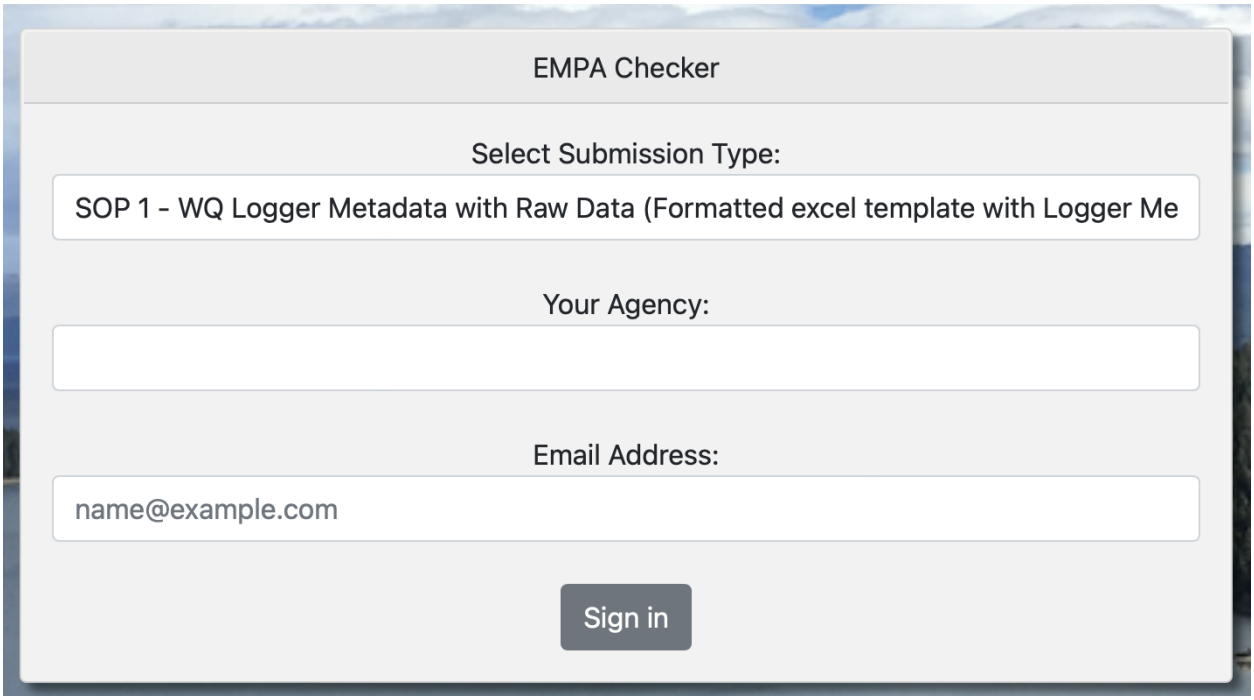
Each data template has associated 'Lookup lists,' which can be found on the EMPA website as a drop-down menu. Additionally, 'Lookup lists' are appended as tabs to the EMPA data templates when downloaded from the website.

Once the data templates are filled out, users will submit their data directly to the EMPA data portal using the EMPA checker tool[12]. The checker tool provides an automated process for data QA/QC. Training materials for using the checker tool are provided on the EMPA website under 'Data Management' and 'Training.'

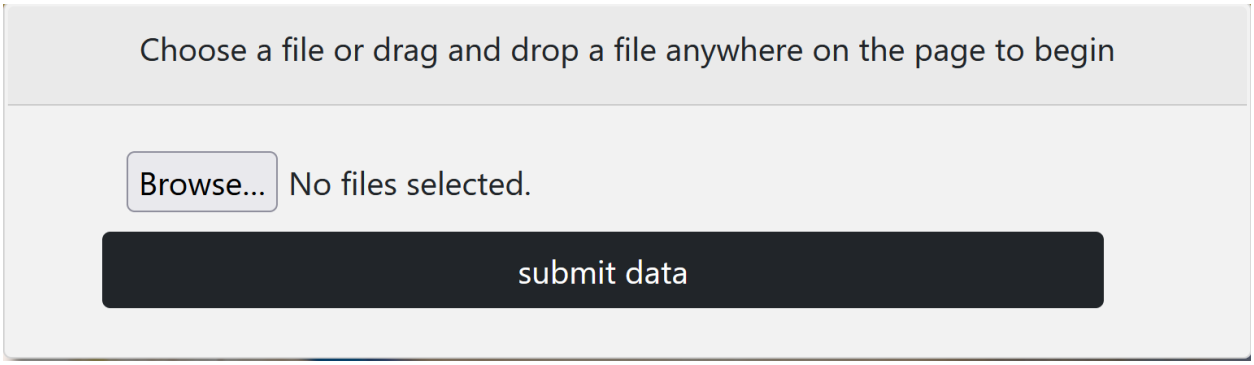
To submit data –

1. Login
    a. Select the submission type
        i. For a more detailed explanation of SOP 1 submission types, see the SOP 1 manual.
    b. Enter the email that you want the confirmation receipt to go to
    c. Select the agency the data is affiliated with

[12] https://empachecker.sccwrp.org/checker/



2. Upload data
    a. Once signed in, use the *Browse* button to select the file to submit
        i. NOTE – At this time, the data portal can only accept files less than 40,000 rows. If your data is greater than 40,000 rows, parse your data file into multiple files.
    b. Hit 'submit data'

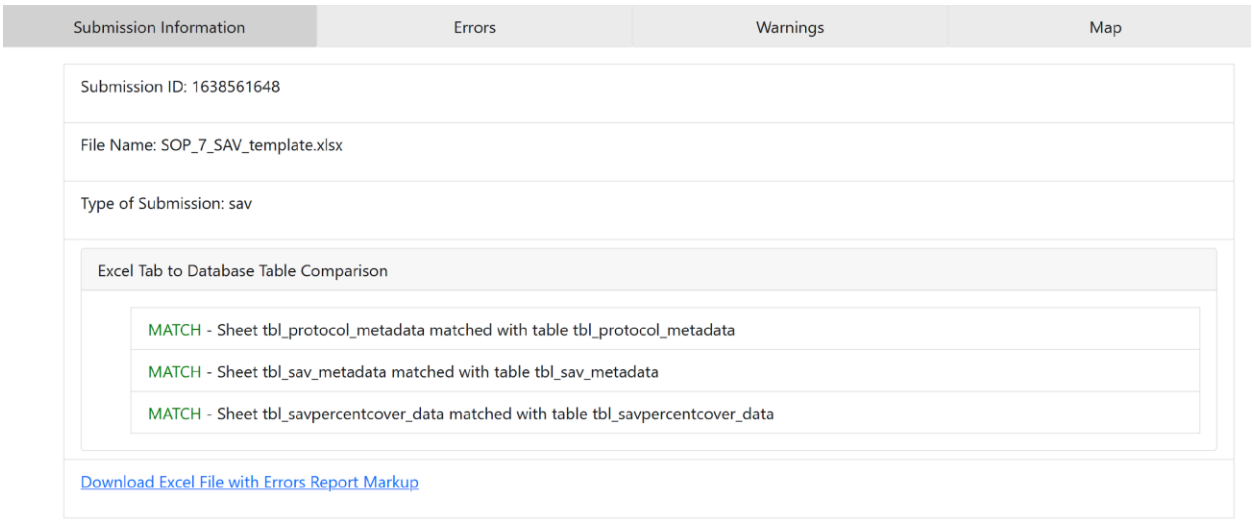


3. The checker application
    a. The checker application will then go through a series of checks to prevent errors in the final dataset
    b. The checker will produce two types of messages
        i. *Errors* – these will prevent you from submitting data

ii. *Warnings* – these will flag portions of the data and let you know that the data entered is unexpected, but *will pass* upon 'Final Submit'

c. The checker will perform a series checks
   i. Core checks - these errors prevent data from entering the database
   ii. Custom checks – these errors ensure cleaner data
   iii. You will most likely have to fix the core errors, resubmit the data, and then fix custom errors

4. After uploading the data file, you'll be taken to the landing page with the submission info.
   a. This page allows you to access the errors in two manners.
      i. You can toggle along the top of the page and click on 'Errors' and 'Warnings'
      ii. You can download the 'Excel File with Errors Report Markup' at the bottom of the page



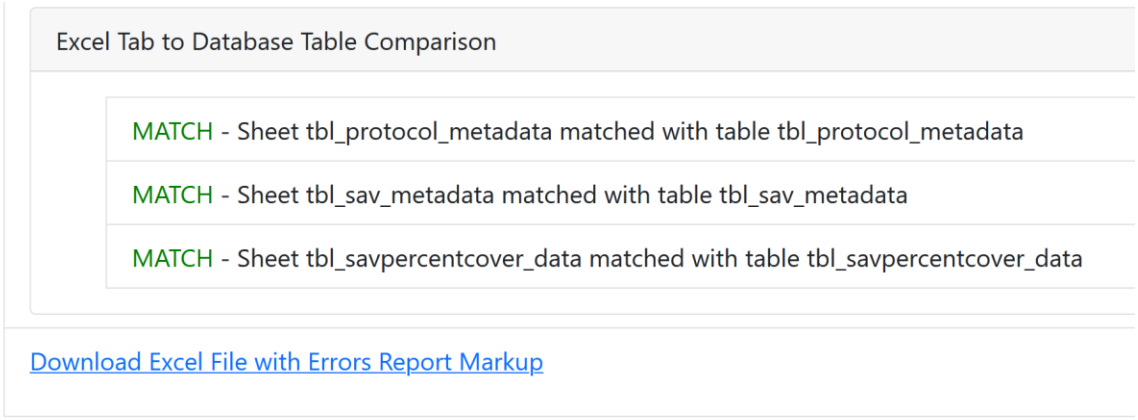
   b. If you are not brought to the landing page or the app crashes, this means there is a critical error. If this happens, the EMPA team will receive an automatic email notification. Even though we are notified, it is still advised that you send us an email with the file attempted for submission. SCCWRP will get back to you so we can get the data submitted with no issue

   c. You can send/forward the email to empa-im@sccwrp.org along with the data file

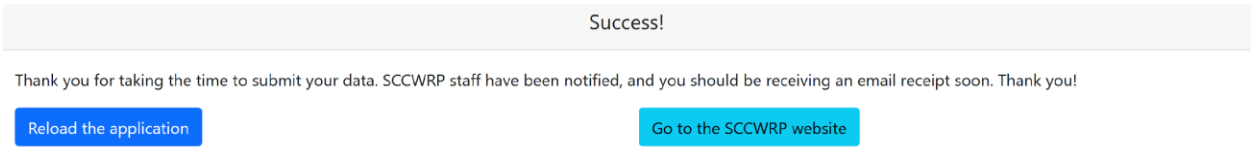
5. Final submission

   a. When the data is ready for submission, a green 'Final Submit' button will appear.

   b. Be sure to check out any warnings, double check the data before submitting, and then hit the 'Final Submit' button.



c. You will receive a 'Success' notification and an email receipt with the data and time of submission.



Section 8. Data Download

EMPA data can be directly downloaded from the website.[13]

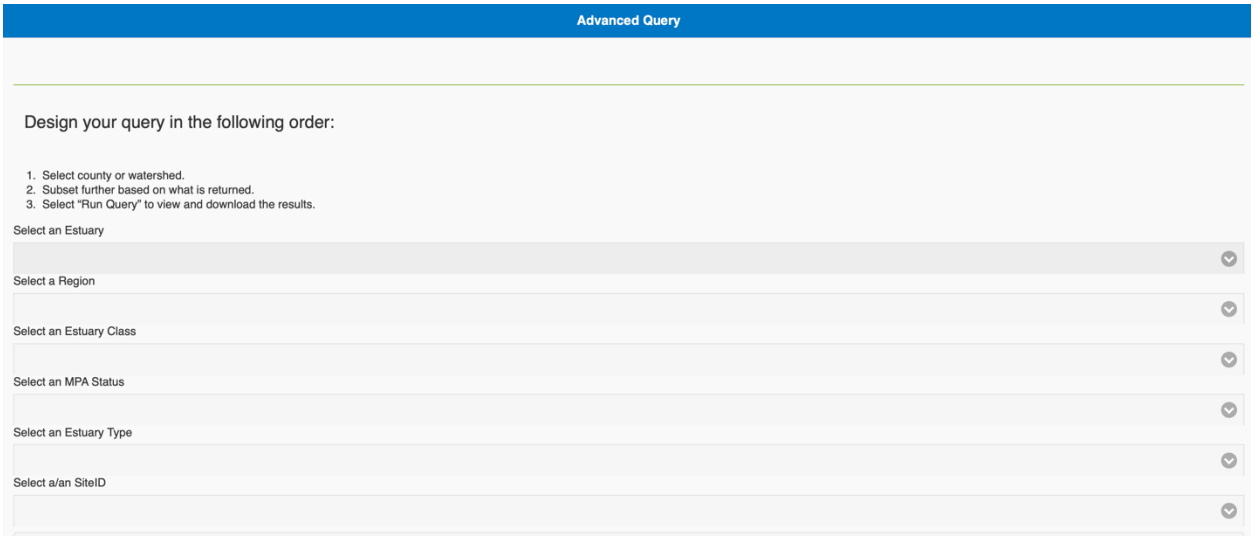
The data download features for the EMPA project are still under development, and therefore, for most up to date information and instructions use the website.

As of December 2022, data can be downloaded from the website using the 'Data Download' button, which will redirect users to a query tool.

Data can be downloaded using the following queries:

1. Estuary (name of estuary)
2. Region (north, central, south)
3. Estuary Class (embayment, riverine, lagoonal)
4. MPA status (MPA, NON-MPA)
5. Estuary type (perennially open, temporarily closed)

[13] http://empa.sccwrp.org



Once users chose their query, then each dataset can be selected for download. Each dataset is broken up by SOP (section 5). Users have the option to download the data and the metadata or just the associated data.

Appendices

Contents

Appendix I: Setting the function-feature table criteria

The main objective of the function-feature table is to help users identify the main landscape features to sample that will allow them to best assess their priority ecological functions within the three estuary types. This is used to guide sampling locations, where sampling a feature informs functional evaluation.

We created a scoring criterion based on four principles (fidelity, exclusivity, dependency, and consistency) to score each function and feature relationship. We provided our team with instructions and a scoring rubric to score the tables based on their own best professional judgment. The final function-feature table was created by averaging the scores across each team member for each function-feature pair.

Instructions given to technical team:

Premise:

1. The objective of the function-feature table is to help users identify the main landscape features to sample that will allow them to best assess their priority ecological functions within the three estuary types. This is used to guide sampling locations, where sampling a feature informs functional evaluation.
2. A key component is to highlight the features that best assess the function. A low prioritization does not mean there is a lack of the function within the estuary, rather that it is not the most important feature to sample (with regard to that function).
3. Due to geomorphic differences in estuary type, the tables will take into account temporal variability by 1) assessing estuary types independently (embayment, riverine, and lagoonal) and 2) factoring in temporal dependency (seasonal and mouth closure patterns) into scoring criteria.

Criteria:

Using your best professional judgment, rank each function to feature relationship based on the following four principles -

1. Fidelity - The frequency by which the feature supports the function
2. Exclusivity - The degree to which the function depends on the feature to the exclusion of other features
3. Dependency - The strength of the relationship between the feature and the function, which could be further supported by data, documentation, etc.
4. Consistency - The amount of temporal dependency between the function and the feature; e.g. an estuary that opens and closes may lose features based on the mouth state

Scoring:

0 - No relationship or uncertainty between function and feature OR feature not present in wetland type
1 - Weak relationship between function and feature OR intermittent relationship
2 - Middling relationship between function and feature
3 - Strong and consistent relationship between function and feature

For each function-feature pair, add up each category to get a total score for the pair

0-3 Not a priority
4-6 Low priority
7-9 Medium priority
10-12 High priority

Average scores produced by technical team:

Embayment		**Landscape features**									
Functions	**Criteria**	Beach/ dune	Un-vegetated marsh	Fresh-water pools	High marsh	Low marsh	Intertidal channel	Subtidal channel	Shellfish reef	Mudflat	SAV
Nekton Habitat	Fidelity	0	0.25	1	0.5	1.75	3	3	2.25	1.75	2.25
	Exclusivity	0	0	0.75	0.25	1	2.5	3	1.5	1.25	1.5
	Dependency	0	0.25	0.75	0.25	1.75	3	3	2.25	1.75	2.5
	Consistency	0	0.5	0.5	0.75	2	2.75	2.75	2.5	2.25	2.5
	Total	0	1	3	1.75	6.5	11.25	11.75	8.5	7	8.75
Primary Production	Fidelity	0.5	0.75	1	3	3	2.5	2.5	1	1.5	3
	Exclusivity	0.5	0.75	0.75	1.25	1.25	2	2	0.75	1	2.5
	Dependency	0.5	0.75	1.25	2.5	2.75	2.5	2.25	1	1.25	3
	Consistency	0.5	1.25	1.5	2.5	2.75	2.5	2.25	0.75	1.5	2.5
	Total	2	3.5	4.5	9.25	9.75	9.5	9	3.5	5.25	11
Secondary Production	Fidelity	1	1.25	1	2.5	2.75	3	3	3	3	2.75
	Exclusivity	1	1	0.75	1.25	1.25	2	2	1.5	1.25	2
	Dependency	1	1	1.25	2	2.25	3	3	3	2.25	2.75
	Consistency	1.25	2	1	2.5	2.5	2.75	2.5	2.5	1.25	2.75
	Total	4.25	5.25	4	8.25	8.75	10.75	10.5	10	7.75	10.25
Protected Species Support	Fidelity	2.25	0.75	1.5	3	2.75	2.25	2.25	2.75	2.25	2.75
	Exclusivity	1.25	0.5	1.5	2	1.25	0.75	1	2.5	1	2.5
	Dependency	2	1	1.75	2.5	2.25	2	2.5	2.5	1.75	2.75
	Consistency	2.25	0.75	1.5	2.25	2.25	1.75	2.5	2.25	2	2.75

	Total	7.75	3	6.25	9.75	8.5	6.75	8.25	10	7	10.75
Nutrient Cycling	Fidelity	0.5	1.25	1.5	2.75	3	3	2.75	2.75	2.25	3
	Exclusivity	0.5	1	1	2	2	1.75	1.75	1.5	1.75	2
	Dependency	0.5	1.5	1.25	2.5	2.75	2.75	2.75	2.75	2	2.75
	Consistency	0.5	1.25	1.25	2.75	2.5	3	2.75	2.5	2.5	2.25
	Total	2	5	5	10	10.25	10.5	10	9.5	8.5	10
SLR Amelioration	Fidelity	3	0.75	0	3	1.75	1	1	1.75	2	1.25
	Exclusivity	2.75	0.5	0	2.5	1.75	1	1	1.25	1	1
	Dependency	3	0.75	0	2.75	1.75	1	1	1.75	2	1.25
	Consistency	3	1.25	0	2.5	2	1.5	2	1.75	2	1.25
	Total	11.75	3.25	0	10.75	7.25	4.5	5	6.5	7	4.75
Bird Habitat	Fidelity	2.75	2.75	1.25	3	2.75	2.5	2	2	2.75	1.25
	Exclusivity	1.75	1.25	1	2.5	1.75	1	1.25	1	1.5	1
	Dependency	2.25	2	1.5	2.75	2.5	1.75	1.75	1	2.25	1
	Consistency	2.75	2.5	1.25	2.5	2.5	2.25	2	1.25	2.25	1
	Total	9.5	8.5	5	10.75	9.5	7.5	7	5.25	8.75	4.25
Shellfish Support	Fidelity	0	0	0.75	0	2.25	3	3	3	3	2.75
	Exclusivity	0	0	0.75	0	1	1.25	1.25	3	2	1.25
	Dependency	0	0	0.5	0	1.25	2.25	2.25	3	3	2
	Consistency	0	0	0.25	0	1.5	1.75	2	3	3	1.75
	Total	0	0	2.25	0	6	8.25	8.5	12	11	7.75
Nursery Habitat	Fidelity	0	0	1.75	0.5	2.5	3	3	2.5	0.75	3
	Exclusivity	0	0	1.25	0.5	1.5	1.75	2.25	1.5	0.75	2.75
	Dependency	0	0	1.5	0.5	2.25	2.25	2.5	2.5	1	3
	Consistency	0	0	1.5	0.5	2	2.75	2.5	2	0.75	2.75
	Total	0	0	6	2	8.25	9.75	10.25	8.5	3.25	11.5
Support Vascular Plants	Fidelity	2.75	0	1	3	3	1.75	0.5	0	0	3
	Exclusivity	1.5	0	0.75	2.75	2.5	1	0.5	0	0	3
	Dependency	2.25	0	1	3	3	1.25	0.5	0	0	3
	Consistency	2.5	0	1.5	3	3	2.25	0.5	0	0	2.75

	Total	9	0	4.25	11.75	11.5	6.25	2	0	0	11.75
Wildlife Support	Fidelity	2.75	1.25	1.5	3	3	2	1.25	0.5	2	1.25
	Exclusivity	1.5	1	1	2	2	1	0.75	0.5	1	0.75
	Dependency	2.75	1	1.75	3	3	2	1.25	0.5	1	1.25
	Consistency	2	1.5	1.5	2.75	2.75	2	1.25	0.5	1.75	1.25
	Total	9	4.75	5.75	10.75	10.75	7	4.5	2	5.75	4.5

Final table:

	Landscape features									
Functions	**Beach/ dune**	**Un-vegetated marsh** (salt pannes and pools)	**Fresh-water pools** *lagoonal and riverine only	**High marsh**	**Low marsh**	**Intertidal channel**	**Subtidal channel**	**Shellfish reef**	**Mudflat**	**SAV**
Nekton Habitat										
Primary Production										
Secondary Production										
Protected Species Support										
Nutrient Cycling										
SLR Amelioration										
Bird Habitat										
Shellfish Support										
Nursery Habitat										
Support Vascular Plants										
Wildlife Support										

Key	Not a priority	Low Priority	Medium Priority	High Priority

Appendix II: Creating sampling maps

After the specific estuary characteristics are collected, users will use the site information, imagery, and estuarine classification to help them designate the sampling area(s) within the system and allocate sampling zones and points for each sampling method. The steps below provide a detailed explanation for choosing and marking sampling points on the map for each sampling method (Table 1.1; for further information regarding the objectives and protocols for each method, refer to Section 5, SOP 1-14). Figure 3.1 provides an overview of steps to mark up the map.

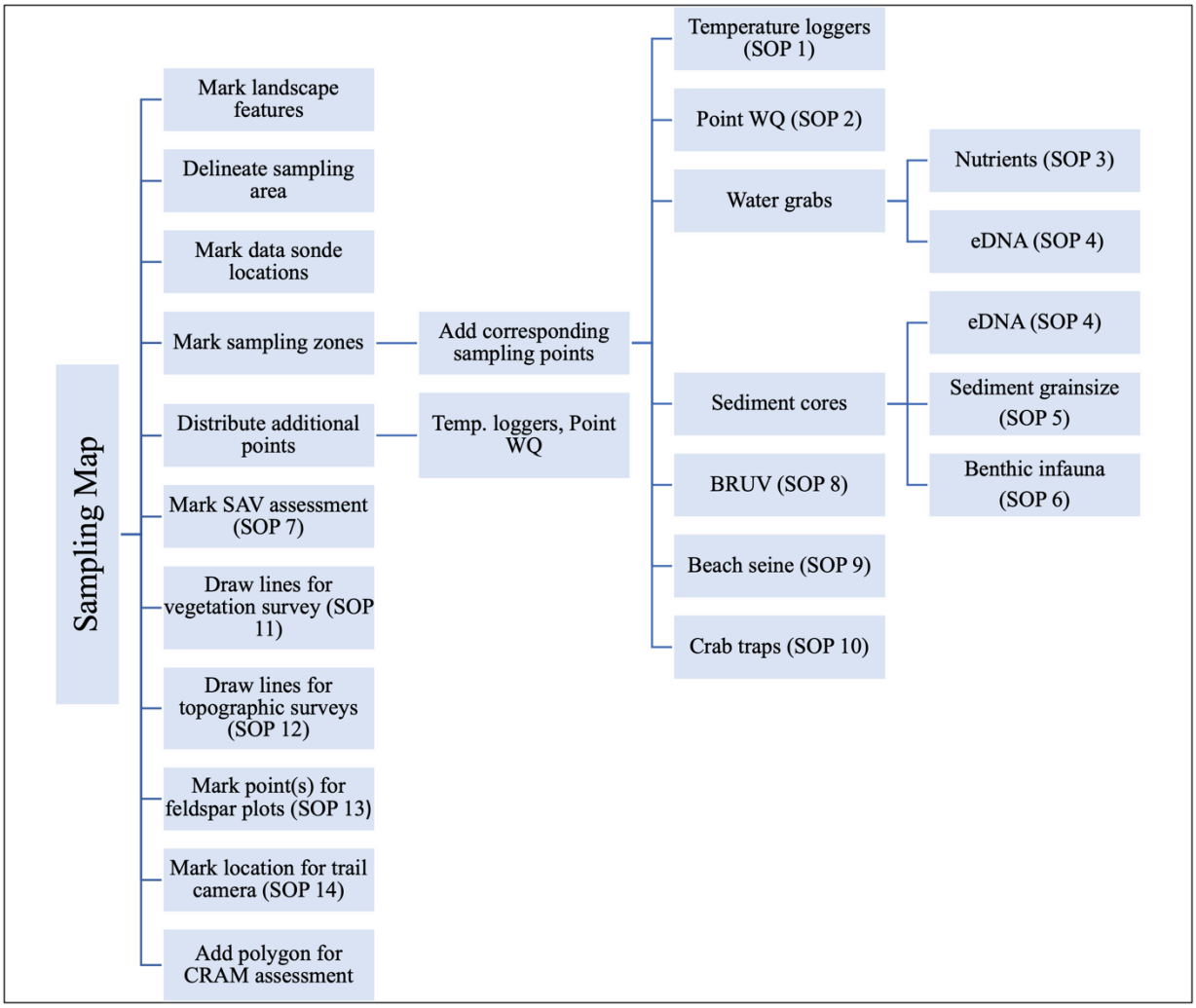


Figure 3.1. Sampling maps will be created prior to sampling in the field in order to ensure that sampling focuses on ecological functions and project goals.

Using PowerPoint or another imaging editing software, users will mark-up site maps to create a rough estimate of the sampling points. We provide an example of Newport Bay that includes our chosen sampling zones, sampling points, and rationale for choosing each one.

1. Mark the major landscape features as identified in the function-feature table (Table 2.2) and cross-referenced with the Landscape Feature table (Table 2.3) on the map
    a. Users can use whatever method works best for them
        i. Physically marking landscape features on the map by circling or drawing an X near the features or
        ii. Writing the key landscape features down near the map for reference
2. Delineate the sampling area by drawing a polygon marking the lateral boundary and upstream extent.
    a. The sampling area should be chosen based on the important features that define the estuary (Table 2.2).
        i. Embayment
            1. Given the size of these systems, it is most likely that a subsection of the estuary will have to be designated as the sampling area.
            2. With their connectivity to the ocean and higher salinity waters, these systems may exhibit stratification and density-driven estuarine circulation, so capturing salinity, DO, and turbidity gradients may be important.
            3. These types of systems would be expected to support SAV, soft sediment fauna, and salt marshes and have a relatively simple network of channelization.
        ii. Lagoonal
            1. It's important to identify and include the central and primary basin in the sampling area.
            2. A lagoonal system may have complicated mouth dynamics, so the beach berm should be included if the system includes partial or full closure.
        iii. Riverine
            1. It's important to identify the primary channel and include enough area along the channel to sample along the salinity gradient.
    b. Total sampling area (size of polygon) may change per site due to
        i. Total size of the estuary
        ii. Channel width and length (e.g., personnel may decide to collect water samples at the head and the mouth of estuary)
        iii. Landscape features
        iv. Multiple sampling areas may be designated based on
            1. Project objectives
            2. Landscape features
            3. Important features (restoration areas, historic features, etc.)
3. Determine the position and number of data sensors to deploy (SOP 1)
    a. First, mark pre-existing data sensors on the sampling map, as determined while compiling site characteristics and information (section 2).
    b. The number of sensors should be based on
        i. Number of channels
            1. Identify major channels and rank by relative size
            2. Prioritize sampling locations along largest tidal channels
        ii. Size of open bay or harbor

1. In an embayment system, users may prioritize a sensor in the open bay, if
    a. Bay conditions are important for project objectives
    b. Bay conditions heavily influence upstream conditions

iii. Security of gear
iv. Feasibility of deployment

4. Mark two to three sampling zones
    a. These zones will allow for multiple sampling methods to be clustered together to conduct comprehensive sampling and the ability to return seasonally and annually to these areas.
    b. Number of sampling zone will depend on
        i. Project objectives
        ii. Size of estuary
        iii. Landscape features
            1. The location of the landscape features may influence the number of zones. Key landscape features, as identified from the function-feature tables, may be distributed throughout the site or clustered together.
    c. At least 2 sampling zones should be included, which focus on different landscape features
        i. 1 sampling zone should be close to the mouth, ideally located within SAV or near another downstream feature, like a beach, oyster reef, or mudflat
        ii. 1 sampling zone should be along the primary channel, adjacent to the marsh plain and further upstream
        iii. An optional, but recommended, third sampling zone should be along a secondary channel or tributary
            1. This third zone could focus on an area of interest, like a restoration zone
    d. The zones should be numbered based on location to the mouth.
        i. Zone #1 should be closest to the mouth of the estuary.
    e. At each zone, cluster sampling will include
        i. Temperature logger (SOP 1)
        ii. Point water quality measurement (SOP 2)
        iii. Water grab for nutrients (SOP 3)
        iv. Water grab and sediment for eDNA (SOP 4)
        v. Sediment core for sediment grainsize (SOP 5)
        vi. Sediment core for benthic infauna (SOP 6)
        vii. BRUV (SOP 8)
        viii. Beach seine (SOP 9)
        ix. Crab traps (SOP 10)
        x. Topographic survey (SOP 12)
        xi. Users should use their own discretion if some methods aren't fitting for a specific sampling zone due to the landscape
5. Distribute additional points throughout the sampling area
    a. For some methods, we recommend sampling other areas beyond the sampling zones.

i. Temperature loggers (SOP 1) and point water quality measurements (SOP 2) should be distributed equally throughout the sampling area.
    1. These methods will be paired together in order to calibrate across instruments
    2. Targeted replication between 6-8 locations (including sampling zones)
    3. Locations should include
        a. Identified gradients, such as salinity or depth
        b. Major sublittoral landscape features (e.g., SAV)

6. Mark the location for the SAV assessment (SOP 7)
    a. SAV should be clearly marked on the sampling map
        i. If multiple SAV areas…
            1. Determine the SAV locations that should be sampled
                a. Proximity to other key landscape features
                b. Project objectives
                c. Areas of interest
            2. Decide if sampling should
                a. Include the full bed
                b. A subset of beds in order to sample multiple beds
7. Draw lines for the vegetation surveys (SOP 11)
    a. The primary objective of the vegetation surveys is to provide a standard approach to evaluate plant community parameters in tidal marshes
    b. Surveys should be in wetland habitats, including
        i. the backshore (i.e., upland transition zone)
        ii. marsh plains
        iii. channel margins
        iv. tidal pannes and ponds
        v. foreshore (i.e., bay-ward transition zones from the marsh plain to open tidal mud flats).
    c. Vegetation surveys should be adjacent to zones in order to return to areas for annual or seasonal monitoring
8. Mark points for topographic surveys (SOP 12)
    a. Topographic surveys should cover the major marsh platforms in the estuary
    b. Surveys should start at low elevation and move to high elevation
    c. Points should include
        i. Sampling zones
        ii. Vegetation survey
            1. A GPS point will be taken at every vegetation quadrat
        iii. Channel margins
            1. Upstream
            2. Mouth
        iv. Backshore
9. Mark points for feldspar plots (SOP 13)
    a. Feldspar areas should be paired with vegetation transects
    b. Locations should be on the marsh platform
    c. Depending on the estuary, 1 to 3 plots should be deployed

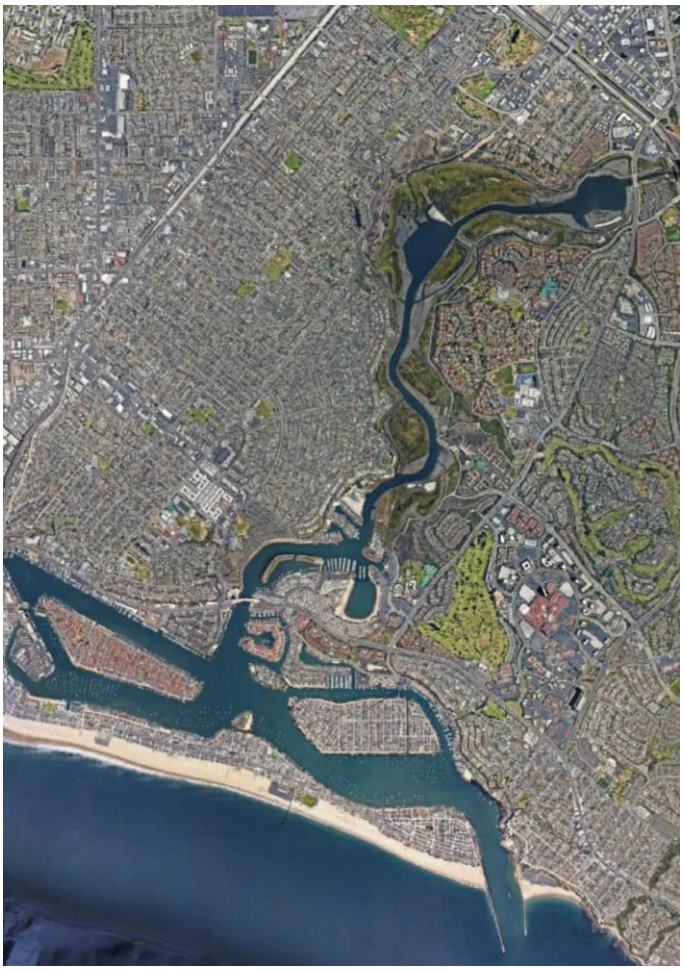
        i. If the system is dynamic with many marsh plains, higher replication should be used to capture potential variation
10. Mark a potential location for a trail camera (SOP 14)
    a. Trail cameras will be used to monitor and capture estuarine mouth dynamics through pictures
    b. Cameras will be deployed if
        i. The system is lagoonal and frequently closes
        ii. There is an area that ensures the safety of the camera from
            1. Theft
            2. Vandalism
            3. Weather damage (wind, swell, etc.)
11. Draw a polygon for CRAM assessment locations
    a. The objective of CRAM is to assess the condition of the wetland, therefore CRAM areas should include major landscape features

Refer to https://www.cramwetlands.org for more detail

Southern California sampling map example

We provide a step-by step guide for collecting site information and classifying the estuary (section 2), as well as marking sampling points on sampling maps (section 3) in Newport Bay.

First, we obtained overhead imagery from Google Earth for Newport Bay.



Next, we conducted site recon, where we went to Newport Bay in order to scope out potential sampling zones and identify hazards or no-go areas. Based on our recon, we identified three access points, which would give us easy accessibility to potential sampling zones and points, listed below. We also decided to focus our sampling in the back portion of Newport Bay because the front bay is armored. In general, Newport Bay is accessible on foot. We may need a kayak for water quality point measurements, but this could be done with waders at a lower tide.

1. Lower Castaways Park
    a. This access point was near an active oyster restoration site, as well as a seagrass bed. The active restoration suggests that we should avoid putting a sampling zone near this location in order to avoid excess disturbance. However, we may want to monitor water quality nearby (YSI, SOP 2).
2. Back Bay Science Center
    a. The Back Bay Science Center provides us access to the back bay via the Mountain to Sea Trail, where the majority of marsh habitat is located.
    b. Along this trail, there are many access points to the main channel, as well as the tributary channels.
3. Newport Bay MPA
    a. This location marks another access point via car, further in the back bay. From here we may have to paddle or kayak to collect other water quality points.

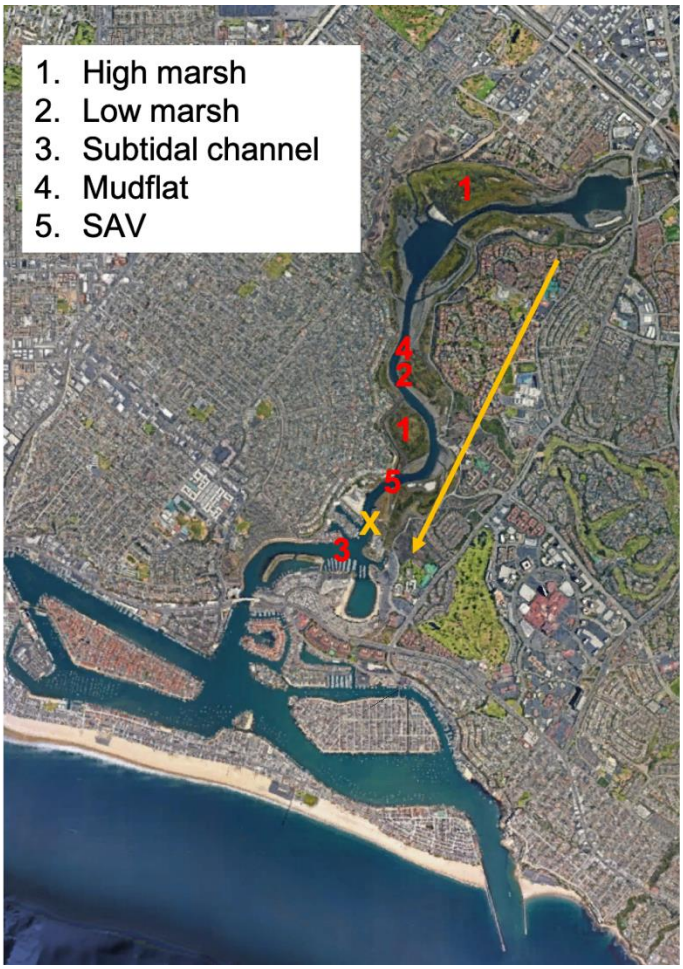
After site recon, we followed the four-step process to estuarine classification. We marked our decisions on our sampling map.

Step 1: Estuarine classification – Embayment – We classified Newport Bay as an Embayment based on the definitions in Table 2.1 and the flowchart in Figure 2.2. Newport Bay has a 'Wide-constructed entrance or harbor.'

Step 2: Feature-function table – We used Table 2.2 to pull out the important landscape features in Newport Bay. The key features we should focus on putting our sampling zones near are the high marsh, low marsh, subtidal channel, mudflat, and SAV (marked with red numbers).

Step 3: Level 1 table – We consulted Table 2.3 to make sure the five key landscape features were present in Newport Bay. All five were present.

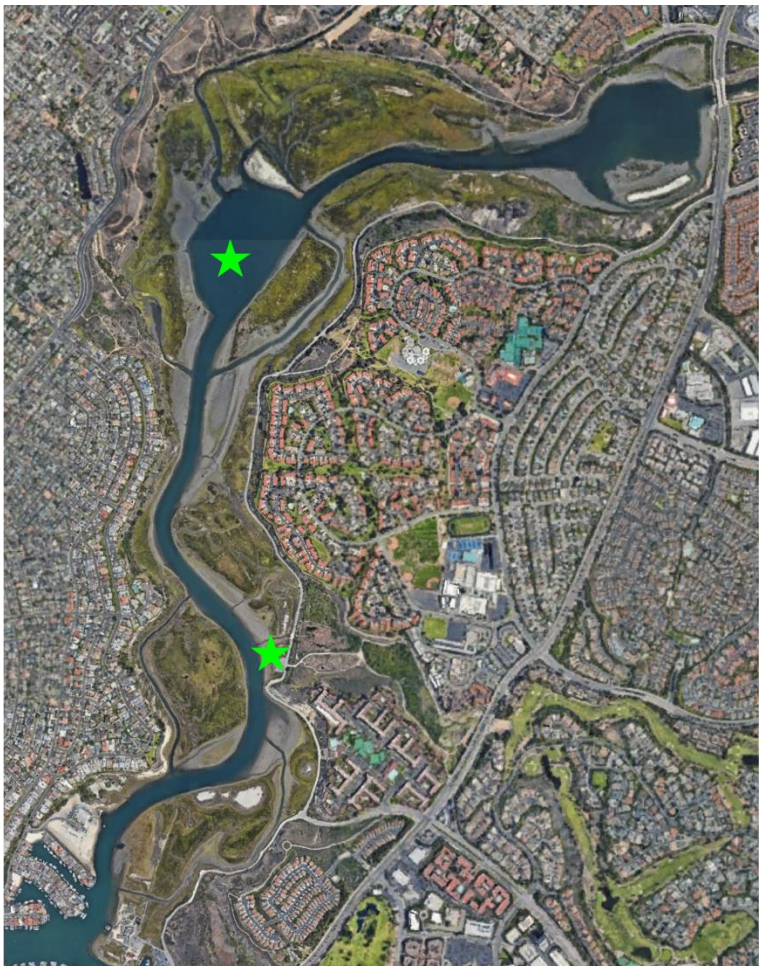
Step 4: Points of interest – In Newport Bay, there is a strong salinity gradient in the bay (marked with a yellow arrow), as well as an eelgrass restoration (marked with a yellow X). We marked these on the sampling map in order to remind ourselves to capture these areas of interest.


1. High marsh
2. Low marsh
3. Subtidal channel
4. Mudflat
5. SAV
1
4
2
1
5
X
3

Using all this site information, we added the sampling locations onto the sampling map.

First, we marked the specific sampling area on the map with a red polygon. Since Newport Bay is a large embayment, we chose to focus on the back bay, where the majority of landscape features were located that would allow us to assess ecosystem function. The front bay is armored and mostly harbor, therefore it would not provide us a good estimate of estuarine condition.

Next, we chose two locations for data sensors (marked with green stars). We chose to deploy two data sensors in Newport Bay in order to capture conditions along the main channel. One sensor was located near the south end of the sampling area and one sensor was located near the north end of the sampling area. This arrangement captures one of our points of interest – the salinity gradient.



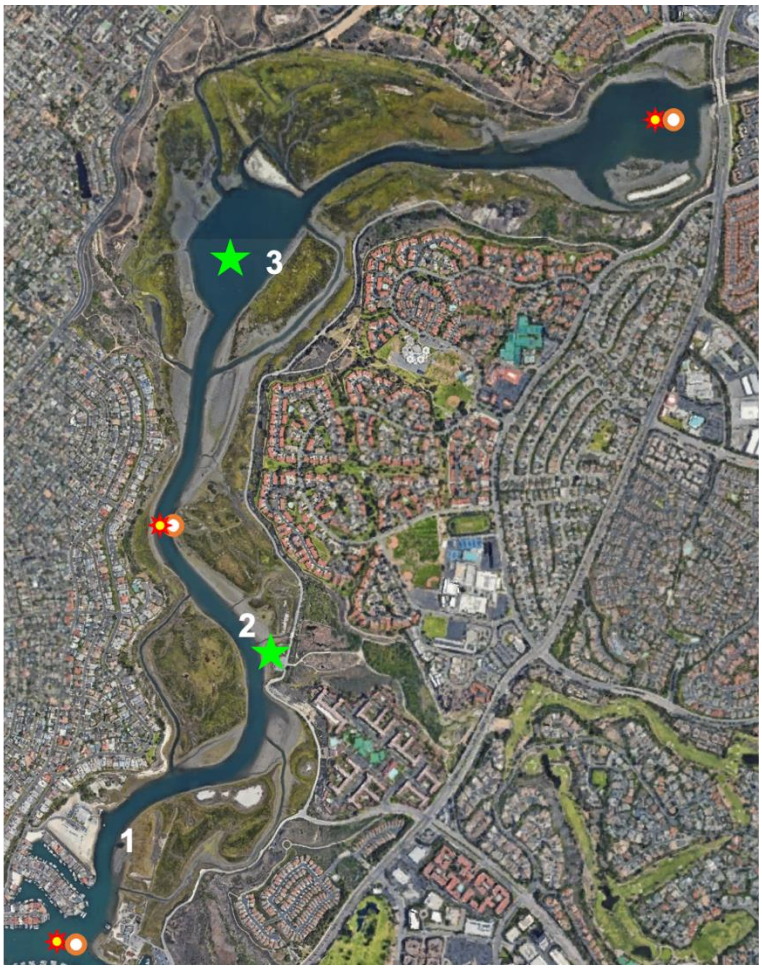
We then chose the three sampling zones in Newport Bay (marked with white numbers). Sampling zones are the locations where multiple sampling methods will be clustered together in order to conduct comprehensive sampling and enhance our ability to return seasonally and annually to these areas. We picked three sampling locations along the main channel adjacent to or within our key landscape features (high marsh, low marsh, subtidal channel, mudflat, and SAV).

At each zone, cluster sampling will include:

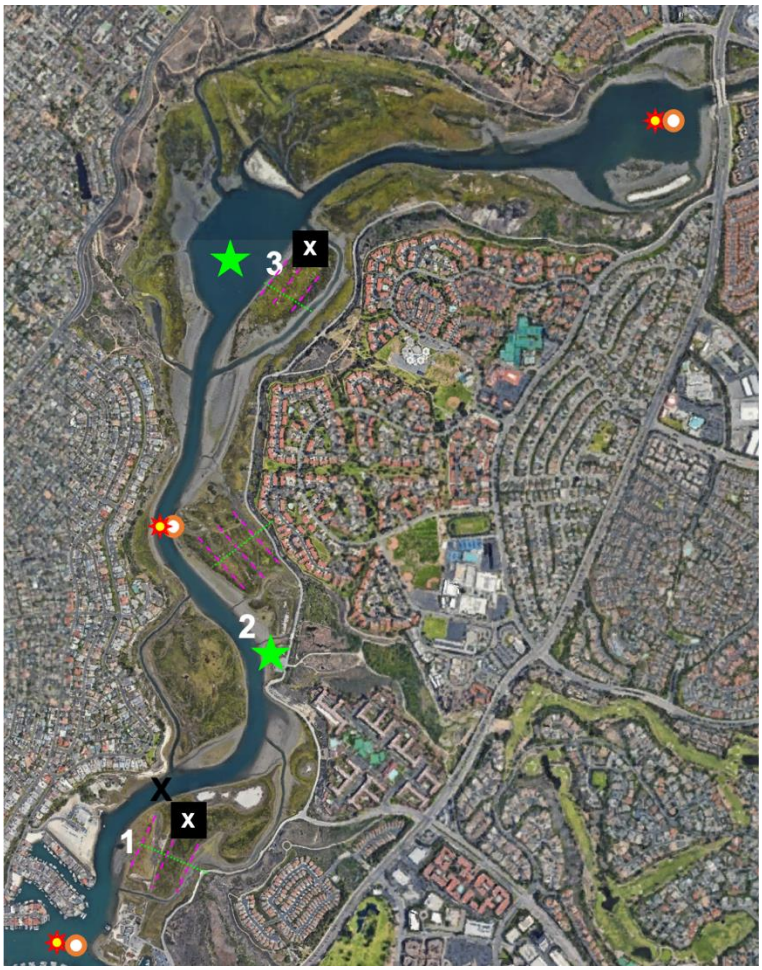
1. Temperature logger (SOP 1)
2. Point water quality measurement (SOP 2)
3. Water grab for nutrients (SOP 3)
4. Water grab and sediment for eDNA (SOP 4)
5. Sediment core for sediment grainsize (SOP 5)

6. Sediment core for benthic infauna (SOP 6)
7. BRUV (SOP 8)
8. Beach seine (SOP 9)
9. Cast net (SOP 9)
10. Crab traps (SOP 10)
11. Topographic survey (SOP 12)

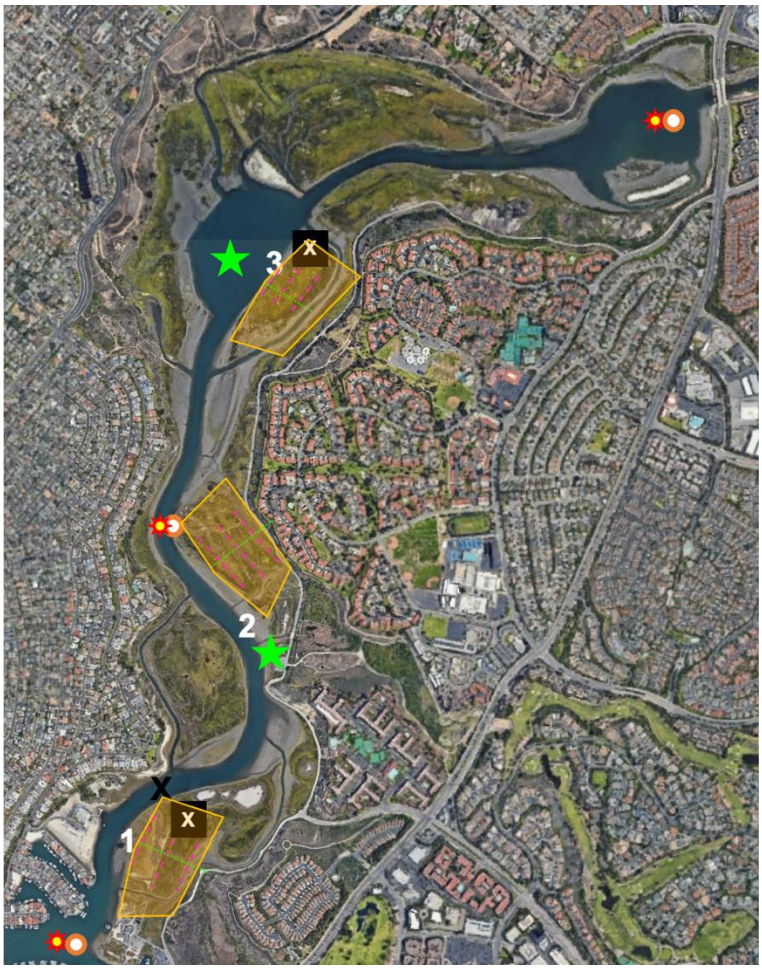
Additional, temperature loggers (red and yellow suns) and point water quality measurements (orange circles) were added in order to further sample along the main channel.



Next, we marked locations for the SAV assessments (SOP 7), vegetation surveys (SOP 11), topographic surveys (SOP 12), and feldspar plots (SOP 13). There was one location where SAV was known to be present within the sampling area (marked with a black X). Since our sampling zones were near large marsh plains, we marked vegetation surveys adjacent to each sampling zone. Due to the size of the marsh plain, we estimate three transects per zone (marked with pink lines). In addition to taking GPS points at all other sampling locations, topographic surveys will be conducted along elevation gradients to capture the change in elevation across the marsh plain from the channel edge up to the upland transition. We include these surveys adjacent to our sampling zones and perpendicular to the vegetation surveys (marked with green lines). We added two feldspar plots in the marsh plains adjacent to sampling zone 1 and 3 (marked with a white X) because we hypothesize that these marsh plains may experience different accretion rates due differences in their location in the bay (sampling zone 1 is closer to the mouth).



Lastly, we marked potential areas for CRAM (yellow polygons). Since Newport Bay has many marsh habitats along the main channel, there are many places that a CRAM assessment could be done. However, we chose the three locations next to our sampling zones, where both the vegetation surveys and topographic surveys will be completed. This will allow us to crosswalk the CRAM scores with our other data.


3
X
2
X
1

Appendix III: Resources for Avoiding Introduction of Invasive Species

The following is an adaptation of an excerpt taken from an EMAP-based Quality Assurance Project Plan developed by the California Department of Fish and Game Aquatic Bioassessment Laboratory (2008).

Organisms of concern in the U.S. include, but may not be limited to, Eurasian watermilfoil (Myriophyllum spicatum), New Zealand mud snail (Potamopyrgus antipodarum), zebra mussel (Dreissena polymorpha), Myxobolus cerebralis (the sporozoan parasite that causes salmonid whirling disease), and Batrachochytrium dendrobatidis (a chytrid fungus that threatens amphibian populations).

Field crews must be aware of regional species of concern and take appropriate precautions to avoid transfer of these species. Crews should make every attempt to be apprised of the most up-to-date information regarding the emergence of new species of concern, as well as new advances in approaches to hygiene and decontamination to prevent the spread of such organisms (e.g., Hosea and Finlayson, 2005; Schisler et al., 2008).

In between sites, all field crews should bleach their waders and field shoes to prevent tracking species between marshes.

When boats are required for data collection the field teams will make every effort to reduce or eliminate the spread of invasive species. Protocols will include:

- Load the boat on the trailer and inspect the boat, motor, and trailer for evidence of weeds and other macrophytes.
- Clean the boat, motor, and trailer as completely as possible before leaving the launch site.
- Inspect all equipment for pieces of macrophyte or other organisms and remove as much as possible before packing the nets for transport.
- Be sure to clean up all waste material at the launch site and dispose of or transport it out of the site if a trash can is not available.

There are several online resources regarding invasive species, including information on cleaning and disinfecting gear:

USDA Forest Service - Preventing Accidental Introductions of Freshwater Invasive Species
www.fs.fed.us/invasivespecies/documents/Aquatic_is_prevention.pdf

California Department of Fish and Game
www.dfg.ca.gov

U.S. Geological Survey Nonindigenous Aquatic Species: general information about freshwater invasive species
http://nas.er.usgs.gov

Protect your Waters - Co-sponsored by the U.S. Fish and Wildlife Service
www.protectyourwaters.net/hitchhikers

The California State Water Resources Control Board Aquatic Invasive Species website
www.swrcb.ca.gov/water_issues/programs/swamp/ais

Standard Operating Procedures (SOPs)

Contents

SOP 1: Continuous environmental monitoring

Protocol:

Southern California Bight 2008 Regional Marine Monitoring Survey (Bight'08). 2008. Estuarine Eutrophication Assessment Field Operations Manual. Southern California Coastal Water Research Project. https://www.sccwrp.org/about/research-areas/regional-monitoring/southern-california-bight-regional-monitoring-program/bight-program-documents/bight-2008/

TBF. 2015. Continuous Water Quality Data Sonde Standard Operating Procedures. Unpublished protocols. The Bay Foundation, Los Angeles, CA. https://www.santamonicabay.org/explore/library/reports/california-estuarine-wetlands-monitoring-manual-level-3/

SCCWRP. 2015. Hydrologic and Geomorphic Changes to Southern California Estuaries and Lagoons During Episodic Events Associated with the 2015-2016 El Niño: Insight to Potential Future Response to Sea Level Rise. Project Field Sampling Protocols.

Objective: This SOP describes a multi-instrument array containing conductivity (salinity), temperature, depth, and dissolved oxygen sensors deployed within a primary channel in an estuary to identify the general water quality condition of that area. Additionally, temperature loggers can be deployed at areas of interest in each estuary to capture variability associated with key landscape features (SAV, beach, subtidal mud, etc.) and depth (surface and benthic). Deployment and recovery of instruments typically requires two people. Some sites will be accessible by foot, other sites may require use of a small boat or kayak.

Users should follow calibration and maintenance instructions for their instruments. These instruments should be programmed in **UTC (GMT)** and set to measure parameters every 6 minutes to match NOAA meteorological data intervals. If possible, they should be set up to start the 6-minute interval on the hour or a subsequent 6-minute increment (e.g., 11:00, 11:06, 11:12…). With routine maintenance, these instruments can be deployed for long periods of time, though they will need to be removed from the water to download the data and for maintenance (removal of biofouling, re-calibration, etc.) approximately every one to three months.

Field Methods – Continuous Monitoring Stations

Within the sampling area, one or two locations will be selected as the main continuous monitoring station, depending on location, depth, and substrate. At a minimum, one instrument array should be deployed near-bottom in the main channel of an estuary. If possible, an additional instrument array should be deployed near-surface at the same location to capture stratification in deeper estuaries. In shallower estuaries, the second array should be deployed near-bottom at a separate location in the main channel. These sites will be selected for the potential to capture the variability in water column conditions, while accounting for site accessibility and minimizing potential vandalization. Water depth should be sufficient to keep the bottom sensors wet during the spring low tides or when the beach berm breaches. Use of existing support structures (e.g., bridges abutments, railroad trestles, etc.) should also be

considered. The location of the continuous monitoring array locations should be recorded with a GPS unit. For seasonally tidal lagoons, every effort should be made to install the instruments in the same locations for the spring and fall monitoring. Instruments can be kept in situ, but every one to three months, the data should be downloaded, the copper cleaned, and the batteries replaced (if applicable).

The following criteria should be used to select the site for deployment of the instruments:

- Logistics of instrument deployment, maintenance, safety and vandalism/theft.
- Existing site where historical continuous data exist.
- Area that characterizes the variability in water characteristics.
- Maximum depth. Every effort should be made to ensure that the instruments remain under water at low water levels.
    - The main sensor array should be prioritized in a central, deep location. Sensors should be deployed on the surface and the bottom in order to capture estuarine stratification.
    - If funds are limited, then sensors should be deployed at the bottom with a temperature logger deployed at the surface.

Visibility, Security, and other Loss/Theft Considerations

In addition to considering the ideal location for relevant data and ease of retrieval, each deployment should also consider factors that affect the possibility of loss or theft of the instruments. If an estuary is heavily traffic by the public, certain precautions can be taking to limiting disturbance of the data, including:

- limiting visibility of the deployment setup
    - all infrastructure always submerged
    - using existing infrastructure to attach to
- increasing security
    - using capped and locked PVC French drains (Figure 5.1.1)
    - utilizing hose clamps and other attachments that require tools (not just tape and zipties)
    - placing in an area that is hard to access by the public

There are also precautions that can be taken to avoid non-human loss of the instruments and disturbance of data, including:

- avoiding snags from debris coming downstream
    - using existing infrastructure to attach to
    - limiting use of line/floats that can get tangled
- decreasing likelihood of fouling using copper and other anti-fouling techniques

Each of these factors should be considered and weighted, and compromises made to balance the ideal data collection location with the minimization of the possibility of loss of the instrument or disturbance of the data.

Figure 5.1.1 Example of French drain setup.

Instruments and Materials

We recommend the following instruments to capture the needed parameters, and the necessary materials associated with each:

- VanEssen CTD-Diver: Conductivity, Temperature, Depth*
    - Copper shield (VanEssen)
- PME MiniDOT logger: Dissolved Oxygen
    - Copper mesh (from PME or other supplier)
    - Two AA lithium batteries
    - Small desiccant
- Onset HOBO Pendant MX Temp: Temperature:
- In-Situ Inc Rugged Troll 100: Depth*
- Chlorophyll is also a standard metric used to assess ecosystem condition. An additional sensor would be required to assess Chlorophyll A concentrations.

*Depth will be chronicled using two probes. Depth on the CTD will be collected over the month deployment period and will represent relative depth of water at the sample location. Depth measurements can be compared to long term Rugged Troll water elevations to estimate water elevation at the CTD. A Rugged Troll pressure transducer will be deployed at a permanent location and elevation to track long term water elevation variability and periods of marsh inundation. The Rugged Troll elevation (of sampling port) will need to be accurately measured relative to local benchmarks or landforms. To get a more accurate depth, Real Time Kinematic GPS surveys will be used to establish vertical connections (elevation differences) among the

monitoring site components, for which leveling is impractical or impossible (SCCWRP 2015). We suggest following protocols from the NOAA technical report "Accurate Elevations for Sea Level Change Sentinel Sites", NOAA Technical Report NOS NGS/ CO-OPs NERRS 1 (Sections 6 and 7). Section 6 explains the steps of establishing high accuracy vertical connections between the network and the National Spatial Reference System (NSRS), through either leveling or static GPS techniques. Section 7 discusses leveling techniques, specifically for connecting a water level sensor and Surface Elevation Tables and presents guidelines for Real-Time Kinematic (RTK) GPS techniques]. RTK GPS should be obtained at least two measurements at the top of the sensor post separated in time by at least 20 minutes (single measurements may have errors of 2-5 cm vertically, but multiple measurements made with different satellite configurations will reduce the standard error of the elevation estimate). Distance between the top of the post and the sensor port will need to be documented.

The EMPA data portal can convert absolute pressure read off the Van Essen CTD-Diver and Rugged Troll, as long as users provide accurate elevation and length measurements in the associated logger metadata.

- Latitude and longitude of the sensor array
- Elevation reading of the top of the array (top of the French drain pipe or float), ideally taken with an RTK unit
- Length of the line from the top of the array to the bottom of the sensor

Possible Deployment Setups

Shallower locations that are easily accessed: deploy the CTD and MiniDOT sensors together around 0.25 meters off the bottom. Ideally, they should be at about the same depth, but be sure they do not touch the bottom substrate. Two possible options are shown left in Figure 5.1.2.

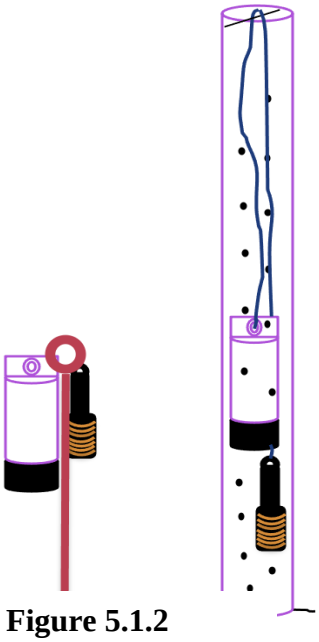


Figure 5.1.2

●Post, stake, sand anchor, or PVC with holes drilled in it (French drain)
●Van Essen CTD
●PME MiniDOT
●Rugged Troll
●Zipties to secure them

If the water column is deep enough and location is secure, attach a line and a float to the bottom post, and attach a temperature logger on the line near the surface. Right, Figure 5.1.3.

●Surface float
●Line
●HOBO temperature logger
●Zipties

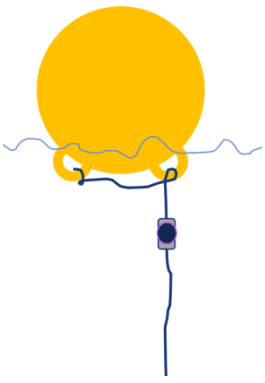


Figure 5.1.3

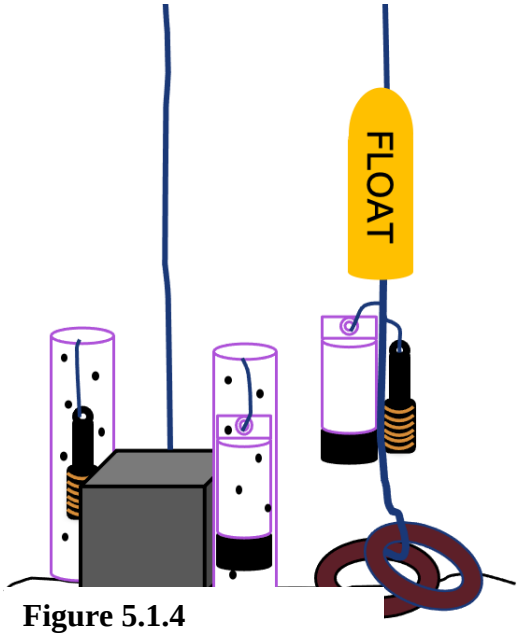


Figure 5.1.4

Deeper locations should have the instrument arrays attached to a weight so they can be deployed from a kayak or boat on the surface. Be sure the instruments will not be sitting in the substrate. These deeper locations are also ideal for both a bottom and surface instrument array to capture *stratification*. Two possible options shown in Figure 5.1.4, left.

- Cement milk crate, flower pot, or iron weight
- Line
- Small PVC sections with holes (to attach instruments to weight)
- Subsurface float (to hold instruments on the line upright)
- PME MiniDOT
- VanEssen CTD
- Rugged Troll
- Zipties

Deep location bottom instruments should be paired with a set of surface instruments, if theft is not a factor and funds are available. (If funds are not available, prioritize a deep deployment with a temperature logger at the surface.) The surface CTD and MiniDOT sensors should be deployed side-by-side, about 0.25 meters below the surface. See Figure 5.1.5, right.

- Surface float
- Line
- PME MiniDOT
- VanEssen CTD
- Zipties

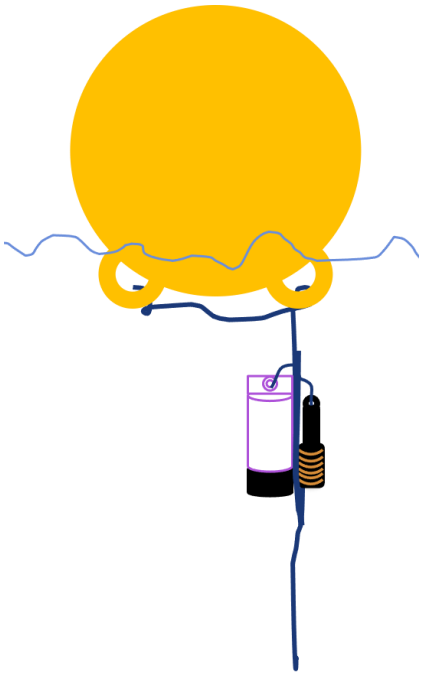


Figure 5.1.5

An example of sensors deployed in the field inside of PVC pipes in a shallow system is shown in Figure 5.1.6.

Figure 5.1.6 Example of sensors inside protective PVC tubes (SCCWRP 2015).

Instrument Information and Setup

Van Essen CTD-Diver DI271 and Baro-Diver
Manual https://www.vanessen.com/images/PDFs/Diver-ProductManual-en.pdf

The Van Essen CTD-Diver is a small instrument that measures conductivity (C) temperature (T) and depth by way of absolute pressure (D). In order to deliver a depth measurement, the CTD-Diver pressure measurements must be corrected for atmospheric pressure changes with barometric pressure data from a nearby weather zone (within 15 kilometers, at a similar elevation), or with data from a Van Essen Baro-Diver (or similar pressure sensor) deployed out of water nearby. To get depth into elevation, follow notes regarding depth measurements in the manual linked above. The CTD-Diver is made of a ceramic housing that can withstand brackish and seawater and can be deployed up to 10 meters deep. It should be deployed with the optional copper shield to help minimize fouling.

Programming
The program Diver-Office can be requested from the VanEssen website:
https://www.vanessen.com/download-diver-office/

Setting up for Deployment (Similar for both CTD-Diver and Baro-Diver)

1. Unscrew small end cap and connect logger to the computer using the provided optical connector
2. Open Diver-Office and create a new project named for the estuary. Set these project settings:
    a. Select the project folder where data should be saved and edit the export settings if needed. This can be changed later.
    b. Set Project Time settings to Coordinated Universal Time (UTC). This will automatically be applied to all sensors saved within this project.
    c. Set Vertical Reference Datum to NAVD88
    d. Merge Time Series should stay set to manual
    e. *These project settings will be saved automatically
3. Click on the Diver button in the top menu to connect to the CTD-Diver or Baro-Diver and set the following configurations:

a. Change the Monitoring Point name to the zone where it will be deployed in the estuary
b. Sample Method: Fixed-length memory
c. Record interval: 6 Minute
d. Conductivity Range: 120 mS/cm (only for CTD)
e. Conductivity type: 1. Conductivity (only for CTD)
f. *Click Program button in the top menu to save these settings

4. Click Start in the top menu and choose the following:
    a. Future Start: choose the closest time on a 6-minute interval that the logger will be deployed in the water
    b. Check the box for Sync Diver Time with Project Time
    c. Start (note: this will automatically clear any data on the logger)

Downloading Data

1. Connect logger to computer and open Diver-Office
2. Click the Open button in the menu and open the project where the logger data should be uploaded
3. Click the Diver button to connect to the logger
4. Click Stop in the Diver window menu (and click Yes in the pop-up warning)
5. Click Data in the top menu to download the data from the logger
6. Click Start to erase memory and redeploy at the exact same settings and location

Cleaning, Testing and Calibrating

The CTD-Diver comes factory-calibrated. The conductivity sensor should be checked regularly and can be field-calibrated with calibration solution if needed, as described on page 18 of the manual. If fouling does occur, it can be soaked and cleaned with white vinegar and a soft cloth. Abrasives, brushes or powerful water pressure should not be used. Be sure to rinse with fresh water after cleaning.

PME MiniDOT Logger

Manual: https://www.pme.com/wp-content/uploads/PME-miniDOT-Manual-2020.pdf

The MiniDOT logger from Precision Measurement Engineering uses a fluorescence method to measure oxygen concentration in water and also measures temperature. It requires two AA batteries (ideally lithium) and should be deployed with a copper mesh covering the sensing foil end. When installing the copper mesh, be sure to only remove three of the screws on the end to secure the copper back on; removing all six end screws will compromise the sensor.

Programming

The MiniDOT logger acts as an external drive and houses the programs needed to operate.

Setting up for Deployment

1. Unscrew the logger and connect to the computer with the USB cable
2. The logger should automatically open as an external drive/disk, depending on computer settings.
3. Open miniDOTControl and click Connect button

4. Measurement quality should be above 0.7
5. Set Sample Interval to 6 minutes
6. Unplug the USB from the logger, LED just below the connector should blink green 5 times

Deployment

1. On the logger itself, flip the switch to Record prior to putting the logger in the water. Attempt to turn the logger on close to a 6 minute interval (i.e. 11:00, 11:06, 11:12, etc.)

Downloading Data

1. Open logger and flip switch to Halt, LED should brink red 5 times
2. Connect the miniDOT to the computer and open external disk folder
3. Copy the folder (named for the serial number) to your computer
4. Optional: open miniDOTConcatenate
    a. Enter surface elevation or barometric pressure at deployment (if known, if not just leave blank – can be changed later)
    b. Enter approximate salinity (or leave blank, can be changed later)
    c. Select the folder containing the data
    d. Click concatenate
5. Optional plotting of data: open miniDOTPlot
    a. Follow same steps for miniDOTConcatenate above, and click Plot

Cleaning, Testing and Calibrating

The sensing foil on the miniDOT is very delicate, do not press or rub too hard. If needed, soak in household vinegar and use a q-tip to clean it off and then rinse with water. They can be tested for accuracy using an aquarium bubbler and a bucket and ice, as described in the manual. MiniDOTs can only be calibrated at PME.

In-Situ Inc. Rugged Troll 100

Manual https://in-situ.com/pub/media/support/documents/Rugged_TROLL_Manual.pdf

The Rugged Troll is a non-vented instrument that measures absolute pressure and temperature. This can be converted to depth and then elevation using techniques described above.

Programming

The program Win-Situ 5 can be downloaded on the In-Situ website here: https://in-situ.com/us/pub/media/support/documents/WS5Setup.5.7.6.1.msi_.zip

Setting up for Deployment

1. Connect the device to the computer and open Win-Situ 5
2. If it doesn't automatically connect to the right com port, change the default communication settings in file > connect
3. Click My Data tab in top right
    a. Click on Site Data folder, then click file > new > site
    b. Add a new site for each estuary, adding coordinates if wanted, and click OK

4. Go to the Device Setup Tab
    a. Name for the site location and set name
    b. Manually set the time to UTC
5. Go to the Logging Tab
    a. Click new log in the bottom left
    b. Select site and add a name for the log, according to zone in the estuary, click next
    c. Make sure all parameters are selected, and the units should be pressure in mBar, temperature is degrees Celsius, and depth in meters
    d. Choose Linear long-term monitoring
    e. Set to every 6 minutes
    f. Schedule start for the nearest 6-minute interval to deployment in the water, do not set a stop condition
    g. Set output to be Surface Water: Depth
    h. Choose brackish water for specific gravity value
    i. Confirm summary and click OK to start

Downloading Data

1. Connect device to computer and open the program
2. In the bottom of the logging tab, click stop
3. Click the download button to save the data to the computer
4. Delete the log if re-deploying

Cleaning, Testing and Calibrating

Rugged Trolls can be calibrated using Win-Situ software as described in the manual or sent back to the company for factory calibration. The instrument can be cleaned with household vinegar and a soft brush.

Field Methods – Additional Temperature Loggers

Temperature logger locations should be chosen on sampling maps, targeting key landscape features:

a. Up-stream channel
b. Down-stream channel
c. Small tidal creeks
d. SAV
e. Subtidal – mud, sand, open

Deployment Setup

Temperature loggers can be deployed in different arrays based on the project questions and objectives.

To capture stratification, sensors can be deployed on float lines at both the surface and the bottom of the line, as in Figure 5.1.3.

If systems are shallow and experience dynamic mouth conditions, such as lagoonal systems, additional temperature loggers can be deployed on PVC poles (Figure 5.1.7). These loggers will be deployed in subtidal to intertidal zones, where they should be submerged the majority of the time.

1. *Before field* - Drill through the PVC pole to make 2 holes near top of PVC post
2. Hammer PVC pole into substrate, holes up
3. Attach temperature logger to PVC post with zipties

Figure 5.1.7 Hobo tidbit deployed on PVC in shallow estuary.

Instrument Information and Setup

Onset HOBO Pendant MX Temp (MX2201)

Manual: https://www.onsetcomp.com/files/manual_pdfs/21536-L%20MX2201%20and%20MX2202%20Manual.pdf

The HOBO Pendant MX Temp is a small, Bluetooth-enabled temperature logger. Under certain settings, it can be connected to and uploaded from about 10 meters away.

Programming
To connect to the instrument, download the HOBO Connect app on a mobile device. *In order for data to be collected in UTC, the mobile device time zone must be set to UTC, otherwise leave it in local time zone and be sure to change it later

Setting up for Deployment

1. Be sure Bluetooth and location are enabled on your mobile device and open the app
2. Push the dot on the center of the instrument, until both small LEDs on the front blink, it should appear in the devices list
3. Select the listed device to connect
    a. Click the edit button in the bottom menu
    b. Change name to zone in the estuary, and name a group for each estuary
    c. Set logging interval to 6 minutes
    d. Start logging: on next logging interval
    e. Stop logging: when memory fills

f. Logging mode: fixed-normal
g. Maintain visual alarms until: Sensors in limits
h. Show LED: ON
i. Bluetooth always on: OFF
j. Push save, on the devices tab it should update to show device status as "awaiting delayed start"

Downloading Data

1. Enable Bluetooth and location and open the app
2. Push the dot on the logger to start Bluetooth
3. Select the device in the list
4. Click the three vertical dot menu in the top right
5. Click stop and yes in the pop-up warning
6. Click the readout button in the bottom left

Data Clipping and General Practices

Certain best practices can be used to minimize the need to clip and remove data.

- Ensure the instrument is secure and does not move about the water column (either secured 0.25m above bottom or secured 0.25m below surface on a float)
- Minimize time of out-of-water data logging
    - try to time the start of data logging when the instrument will already be in the water when possible
    - stop logging and upload as soon as the sensors is removed from the water
- swap in the instruments as often as possible
    - minimizes possibility of long-term data loss
    - swap with a "fresh" instrument when possible
- clean and calibrate instruments often, ideally after/before each deployment
- Utilize copper and other anti-fouling techniques whenever possible
- Minimize possibility of disturbance by considering the suggestions above in *Visibility, Security, and other Loss/Theft Considerations*

Data uploading

Logger data files can be uploaded to the EMPA data portal via the **EMPA checker tool**.

To upload data, users can choose two methods –

1. Users can upload data together with the metadata. Users can format the raw data logger files into a standard template and then upload the metadata alongside the data.
    a. To do this, users download a standard data template named 'SOP 1 WQ Logger Template (Metadata together with data recorded by the instrument)' under the 'Data Templates' drop down on the EMPA website.
2. Alternatively, users can choose to upload the raw files directly from the sensors
    a. Users must first upload metadata.
        i. To do this, users download a standard data template named 'SOP 1 WQ Logger Metadata Template'.

    b. Then users can upload the raw files directly into the checker, choosing the submission type ‘SOP 1 – WQ Logger Raw Data (Data file straight from the instrument)’.
3. Metadata must exist in the database for raw files to be accepted, including
    a. Sensor type (CTD, MiniDot, rugged troll, tidbit)
    b. Sensorid (the serial number of the sensor)
    c. Samplecollectiontimestampstart (the time the logger started recording)
    d. Samplecollectiontimestampend (the time the logger stopped recording)
    e. Latitude
    f. Longitude
    g. Elevation_line (the elevation of the top of the sensor array)
    h. Length_line (the length of the line to the bottom of the sensor)
4. The data checker can only handle 40,000 rows at a single upload. Data files should be parsed to stay under 40,000 rows and submitted as separate submissions.

SOP 2: Discrete environmental monitoring – Point water quality measurements

Objective: This SOP describes the use of a handheld multi-parameter meter device to collect point water quality metrics (e.g., YSI). Ideally, these point water quality measurements will follow the upstream-downstream gradient and include both bottom, middle, and surface measures. Users should follow calibration and maintenance instructions for their handheld device.

Water Quality Parameters: Temperature, salinity, conductivity, pH, TDS (if available on the meter)

Materials:

1. Handheld multi-parameter, multi-meter device with meter-marked cord

Field Methods:

1. The number of Point WQ surveys will be pre-selected on sampling maps and can be paired with temperature loggers (SOP 1); see sampling map section (section 3 and Appendix II) for more information.
    a. Points will follow upstream-downstream gradients in main channels
    b. If back channels are present, points should also be sampled
    c. Sampling points should include key landscape features
        i. SAV
        ii. Mudflat
        iii. Beach/Mouth of estuary
2. At each point, 3 readings should be taken with actual depths and time recorded on the data sheet –
    a. Depth
        i. Surface
        ii. Mid
        iii. Bottom
    b. Water temperature
    c. Air temperature
    d. Salinity
    e. pH
    f. TDS
    g. Conductivity
    h. Any other parameters taken by your handheld device
3. Users should be familiar with operating instructions of the handheld device to obtain correct and calibrated data.
4. On the field datasheet, record the sampling zone, tide, weather, latitude, longitude, profile, depth, water and air temperature, salinity, pH, TDS, and conductivity for each measurement.

SOP 3: Water and sediment quality – nutrient concentrations

Protocol:

Southern California Bight 2008 Regional Marine Monitoring Survey (Bight'08). 2008. Estuarine Eutrophication Assessment Field Operations Manual. Southern California Coastal Water Research Project. https://www.sccwrp.org/about/research-areas/regional-monitoring/southern-california-bight-regional-monitoring-program/bight-program-documents/bight-2008/

Objective: The primary objective of this SOP describes the collection of water and sediment samples to assess nutrient concentrations. Water grabs will be concurrent with the water-quality point measurements (SOP 2) at the sampling zones, and sediment grabs will be concurrent with the intertidal and subtidal sediment grainsize grabs (SOP 5).

Water and Sediment Quality Parameters: nitrate, nitrite, ammonia, phosphate, urea, total nitrogen (TN), and total organic carbon (TOC)

Materials:

1. 1 liter amber high-density polyethylene bottles (HDPE) or 50 cc syringe for water sample grab
2. Pre-labeled 120 ML whirlpaks/Ziplocs with location (subtidal, intertidal), sediment nutrients, site ID, and date
3. Nitrile gloves
4. MCE 0.45 µm filter
5. 50 mL syringe
6. distilled deionized water
7. 50 mL HDPE bottle or 50 mL falcon tubes
8. Garden trowel
9. Labels
10. Cooler and ice packs

Field Methods – Water Samples:

Sampling for ambient water nutrients will only occur at the three sampling zones (if more than three zones, include all zones). The location at which the water sample is collected should be done last to minimize the amount of time the water sample sits before being processed. Water samples can be collected into an amber 1L HDPE bottle and placed on ice and filtered at a later time (but not more than 2-3 hours after collection). It can take between 5 and 20 minutes to filter a single sample depending on how much suspended material is in the water column.

Alternatively, we recommend collecting water with a clean plastic syringe, and then squeezing the water through a disposable 0.45uM filter into the 60mL collection bottle on site. For turbid waters, more than one filter may be needed. Fill 60 mL sample to the 50mL line, close lid and store in ice chest till ready to freeze.

Collecting water samples

1. Walk down to the edge of the water
2. Put on Nitrile gloves
3. Wade to a water depth of 30 cm
    a. Alternatively, access sampling point via kayak.
4. Record the local temperature and salinity on the data sheets using a hand-held meter (YSI)
    a. Alternatively, pair nutrient samples with handheld readings in SOP 2.
5. Fill one 1-liter amber high-density polyethylene bottles (HDPE) or clean syringe full of surface water at ~10 cm depth from the surface
    a. Try not to disturb the sediments when you are collecting water, water should not be overly turbid when you collect the water column samples.
    b. Place closed pre-labeled, 1 Liter Amber HDPE sample bottle under the water surface and open the bottle underwater at approximately 10 cm (one hand span) below the surface.
    c. Close the bottle underwater and bring to surface (or fill syringe).
    d. Shake and discard water downstream (this is your first rinse).
    e. Fill, rinse and discard water three times total.
    f. After the third rinse, open bottle under water, fill, close underwater and return the bottle to surface.
6. Take the water sample bottles (or syringe) up to the edge of the vegetation.
7. Find a shady place to begin filtering the water column samples or store water on ice until samples can be cleanly filtered.

Collecting freshwater loading samples

1. An additional water sample should be taken at the main freshwater tributary to the estuary
2. Field Crew should walk upstream to collect one bottle of freshwater (one replicate)
3. Repeat the above collection steps (*Collecting water samples*)

Filtering samples for nutrient analysis

1. Wear gloves.
2. Start with the Field Blanks (FB)
    a. Open MCE 0.45 μm filter
    b. Pull plunger out of a clean 60 mL syringe
    c. Affix MCE filter on the end of the syringe
    d. Pour approximately 5 mL of distilled deionized water (DDI) into syringe. Invert the syringe a few times to rinse it and the rubber stopper of the plunger. Discard rinse water. Rinse a total of three times.
    e. Fill the syringe with DDI water
    f. Put the plunger back in the syringe, take off the filter to push out the air trapped in the syringe (do not touch the end of the filter where the water comes out). Put the filter back on the syringe.
    g. Push ~10 mL through the filter and discard the rinse water

h. Open the 60mL FB bottle
i. Rinse three times with blank water, discarding rinse water each time
j. Fill 60mL FB bottle no more than 2/3 full with blank water.
k. Refill the syringe with DDI water as necessary
l. Store all the field blanks on ice in the dark.
m. Note on the Data Sheet that the blanks were collected, as well as the collection time and date.

3. You can now start preparing the nutrient samples.
    a. Rigorously shake amber water column sample bottle.
    b. Open MCE 0.45 μm filter
    c. Pull plunger out of a clean 60 mL syringe
    d. Affix MCE filter on the end of the syringe
    e. Put a few milliliters of sample water into the syringe, rinse and discard rinse water; repeat for a total of 3 rinses
    f. Rinse the rubber stopper end of the plunger with sample water too.
    g. After the third rinse, fill syringe with sample water from the sample bottle
    h. Put the plunger back in the syringe
    i. Remove the filter (do not touch the end of the filter where the water comes out) and push the plunger into the syringe all the air is expelled.
    j. Put the filter back on the syringe.
    k. Push approximately 10 mL through the filter discarding this water to rinse the filter.
    l. Open a 50 mL HDPE bottle or falcon tubr pre-labeled for dissolved nutrients
    m. Expel 5-10 mL of sample water into the bottle, rinse bottle and cap, discard rinse water; repeat for 3 rinses total
    n. Slowly add ~25-40 mL of water into the nutrient bottle.
        i. Bottle should be no more two-thirds full. This is necessary because water will expand when frozen.
    o. Cap tightly, record all information on the sample bottle label and data sheet, including collection time and date.
    p. Cover label with plastic tape, and place in the cooler on ice.
    q. A new filter should be used for every sample, but syringes can be reused within the same estuary.
4. All samples should be put on ice and brought back to the lab for analysis or storage.

All nutrient bottles should be labeled with Nutrients, the site ID, date, sampling zone, location, and time. Labels can be pre-downloaded here.

Field Methods – Sediment Samples:

Sampling for sediment nutrients will occur at the three sampling zones (if more than three zones, include all zones). Sediment nutrient samples will be paired with sediment grainsize samples (SOP 5). However, sediment nutrients will only be collected in the intertidal and subtidal zone at each sampling zone.

1. Two sediment samples will be taken at each sampling zone.

a. Two samples will be adjacent to the sediment grainsize grabs
    i. One core at or near the bottom of the channel, depending on the size of the channel (adjacent to subtidal sediment grainsize grab, SOP 5)
        1. Using the high tide mark as a reference point, collect cores ~0.5m below mean low water at the edge of the channel
        2. For lagoonal or BBE, samples should be taken in areas of the lagoon that are inundated most of the time.
            a. Areas that are predominantly sandy/rocky should be avoided
            b. If these areas are unknown, users can focus sampling at ~0.5m below the water surface at a point proximate to the shoreline.
    ii. One core at the channel edge (adjacent to intertidal sediment grainsize grab, SOP 5), at the edge of the marsh plain, where the vegetation starts.
2. At each collection spot, identify a ~5 x 5 cm area without vegetation near the sediment grainsize grab
3. Insert a garden trowel into the top 20cm of the soil
4. Extract the trowel filled with sediment
5. Extrude the sediment into labeled whirlpak/ Ziploc
6. Clean trowel by rinsing with estuary water and then reuse the syringe for subsequent samples
7. All samples should be put on ice and brought back to the lab for analysis or storage.

SOP 4: eDNA

Field

Protocol:

California Molecular Methods Workgroup SOP. DNA sampling SOP for water. *https://docs.google.com/document/d/1Zh6bwnF4Epd9K1Hbk0YUTS6Nx87yluLYJ3gsmRJBTqM/edit*

Objective: This SOP describes the capture and preservation of suspended environmental DNA (eDNA) in aquatic environments.

Materials:

1. Sterilized bottle (500ml Nalgene or similar)
2. Standard filter (0.45um, cellulose nitrate [CN])
3. 10 cm x 10 cm sediment core
4. 1.5ml Eppendorf tubes
5. Cooler
6. Ice

Overview:

1. Three types of eDNA samples will be collected
    a. Water samples
        i. 3 samples collected at each zone (9 bottles per estuary)
    b. Surface sediment samples
        i. 3 samples collected at each zone (9 Eppendorf tubes per estuary)
        ii. Samples should be collected superficially and near intertidal benthic cores (SOP 6)
    c. Sediment samples from benthic intertidal cores
        i. 3 samples collected at each zone (9 Eppendorf tubes per estuary)
        ii. Samples should be collected from a homogenized, intertidal benthic cores (SOP 6)
2. Due to field logistics, all samples will be frozen on dry ice in the field and then shipped to UCLA on dry ice for processing.
    a. Water samples will be frozen prior to filtering

Field methods: Water column

This protocol is considered applicable for the capture and preservation of suspended environmental DNA (eDNA) in aquatic environments.

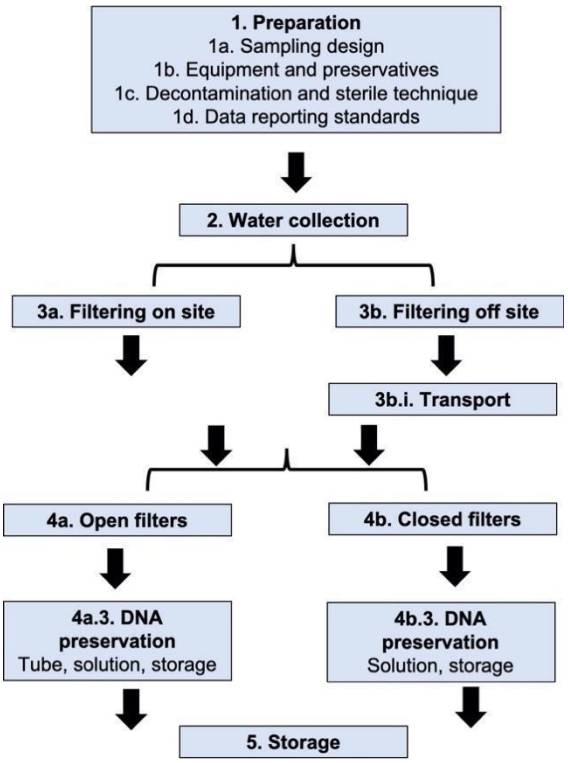


Figure 5.4.1. Workflow of sample collection and filtration suggested by the California Molecular Methods Workgroup SOP. For more detail regarding each step, specific materials, and detailed protocols, visit https://docs.google.com/document/d/1Zh6bwnF4Epd9K1Hbk0YUTS6Nx87yluLYJ3gsmRJBTqM/edit

All steps have been refined to fit the scope of the EMPA project.

1. Preparation
    a. Sampling design
        i. Sample container - Sterilized bottle (500ml Nalgene or similar)
        ii. Filter choice
            1. Pore size: Dependent on application.
                a. Recommended 0.45um filter
            2. Composition: Cellulose nitrate (CN)
            3. Sterility: When possible, an enclosed filter unit (e.g. Sterivex) is preferred over an open filter unit (e.g. Stericup or Swinnex). All filters should be sterile and DNA-free.
        iii. Volume to collect/filter
            1. Volume to collect is determined by a combination of detection sensitivity, resources to filter/analyze multiple replicates, spatial coverage, oligotrophic/eutrophic conditions, etc.
            2. More replicates and larger volumes will provide greater probability of detection.
            3. Recommended: collect 500ml samples.
        iv. Replicates

1. Replicates help increase detection probability and support statistical comparisons.
2. Default: 3 replicates.
3. If resources are limited, replicates may be combined for analyses.

v. Field blanks

1. The number of field blanks collected is determined by both cost and importance of detecting contamination at regular intervals to protect impacted samples.
2. Default: 1 field blank per sampling outing.

b. Preservatives
   i. Ice – Due to limitations in the field, we preserved the samples on ice until filtering could occur in the lab.
c. Sterilization and sterile technique (please see the additional California Molecular Methods Workgroup SOPs).
d. Data reporting standards (please see the additional California Molecular Methods Workgroup SOPs).

2. Water collection
   a. eDNA water grabs will be taken at each sampling zone.
      i. 3 replicates should be taken at each zone.
      ii. 9 total bottles for the estuary and at least 1 field blank with DDI
   b. Grab sample:
      i. Rinse sterile bottle 3x with sample water, discarding rinse water downstream or offshore.
      ii. Submerge bottle in water to collect sample, ideally with sampling pole to minimize risk of contamination.
   c. Pre-label bottles
      i. eDNA bottles should be labeled with eDNA, site ID, date, sampling zone, location, and replicate # or FB
      ii. Labels can be pre-downloaded.
3. Filtering on site
   a. Proceed to filtration steps below.
4. Filtering off-site*
   a. Unfiltered water samples should be frozen on ice and stored in the dark.
   b. Filtration should occur within 4-8 hours of sample collection. Record storage conditions and time between sample collection and filtration.
5. Open filter (Stericup or Swinnex)
   a. Load filter onto filter holder using sterile tweezers.
   b. Invert sample gently to homogenize. Filter sample until volume is completed or filter clogs. Pass ~10cc air through filter to dry. Record volume filtered. Using sterile tweezers, remove filter from filter holder, gently roll filter using clean forceps; two sets of forceps can be used if necessary. Place filter in sterile tube (Falcon or Eppendorf appropriate for size) for preservation.
   c. If using liquid preservation, the filter must be thoroughly submerged in the preservation solution. Label the tube and place it in a labeled bag.
6. Closed filter (Sterivex)
   a. Invert sample gently to homogenize.

    b. Filter sample until volume is completed or filter clogs. Pass ~10ml air through filter to dry. Record volume filtered.
    c. If using liquid preservation, load preservation solution into the filter unit using a sterile pipette, ensuring the membrane is fully covered, and place the filter and housing in a labeled bag.
7. Storage**
    a. Keep tubes with filters (with or without preservation solution) on ice, dry ice, or liquid nitrogen until they are transported to -20°C or -80°C freezer for storage.

*We recommend filtering off-site following collection. <u>If filtering is not possible due to remote sampling, then samples should be frozen on dry-ice and transported to the lab for freezing until filtering.</u>
**Filtering before storage will help preserve the integrity of the eDNA. The freeze-thaw cycle is known to degrade DNA, especially extracellular DNA which we are targeting in the water samples. Additionally, thawing large volumes of water will take a long time and could result in more DNA decay.

<u>Field methods: Surface sediment</u>

1. Sediment eDNA samples will be taken at each sampling zone.
    a. 3 replicates should be taken at each zone.
    b. 9 total tubes for the estuary
2. Sediment samples will be taken in the intertidal zone adjacent to sediment grainsize (SOP 5) and intertidal benthic faunal (SOP 6) cores
3. At each collection spot, identify a ~5 x 5 cm area without vegetation near the benthic core
4. Wearing gloves, scrape a 1.5ml Eppendorf tube along the top sediment, scooping the sediment into the tube.
5. Tubes should be labeled as triplicates in order to pair location with replicate
    a. For example, eDNA tubes from UCLA are labeled with the following nomenclature
        i. K-1045-B2-@, K-1045-B2-#, K-1045-B2-&
        ii. B2 represents a single location, and @, #, and & represent the 3 replicates
    b. Record the sample code on the field datasheet and Estuary Checklist
6. All samples should be brought back to lab and frozen for later analysis

<u>Field methods: Sediment from intertidal benthic cores</u>

1. Sediment eDNA samples will be taken at each sampling zone.
    a. 3 replicates should be taken at each zone.
    b. 9 total tubes for the estuary
2. Take an additional intertidal benthic infauna core following methods outlined in SOP 6
3. Homogenize the sediment from the core in a bucket
4. Wearing gloves, insert 3, 1.5ml Eppendorf tube into the homogenized sediment (from the core), scooping the sediment into the tube.
5. Label tubes as described above and record the sample code on the field datasheet and estuary checklist

6. All samples should be brought back to lab and frozen for later analysis

Lab

Protocol:

CALeDNA Methods for Researchers. CALeDNA Library Preparation.
https://ucedna.com/methods-for-researchers

Objective: This SOP describes the extraction and library preparation of environmental DNA (eDNA) from filtered water and sediment samples.

Table of Contents:

Materials:

General

1. Fume hood
2. Bunsen burner
3. Microcentrifuge
4. Vortex
5. Pipettes and pipette tips
6. 20% bleach
7. 70% ethanol
8. 100% ethanol
9. DNA Away
10. PCR water

11. 1.5 – 2.0uL microcentrifuge tubes
12. PCR strip tubes or PCR plates
13. Magnetic plate

eDNA Extraction

14. Qiagen DNeasy Powersoil Kit
15. Forceps
16. Scissors
17. Spatula
18. SpeedVac (or other vacuum concentrator)

Metabarcoding PCR

19. Qiagen Multiplex Mix 2x
20. Forward and reverse primers (see primer information below)
21. Thermocycler

Gel Electrophoresis and Extraction
Note: Methods may vary by lab.

22. Agarose
23. 1x TBE buffer
24. Glass flask
25. Small beaker
26. Gel box
27. Gel mold and comb
28. Midori green stain (or other fluorescent DNA stain)
29. 6x loading dye
30. 100bp ladder
31. UV transilluminator
32. QIAquick Gel Extraction Kit

Bead Purification

33. AMPure beads

Indexing PCR

34. Kapa Hifi HotStart ReadyMix
35. Nextera DNA CD Indexes (96 indexes, 96 samples)
36. Thermocycler

Quantification

37. Qubit dsDNA BR Assay Kit
38. Qubit fluorometer

Library Pooling

39. SpeedVac (or other vacuum concentrator)

Overview:

1. eDNA will be extracted from water filters and sediment samples.
    a. For multiple water filters generated from the same sample, a pooling step may be performed.
2. Extracted eDNA will undergo PCR for 3-6 metabarcoding primers.
3. PCR products will be run on gels to check for success of PCR.
    a. If higher molecular weight secondary products are present, a size selection step using gel extraction may be performed.
        i. If gel extraction is performed, the first bead cleaning step can be skipped.
4. PCR products will be pooled by marker, purified, and quantified.
5. Purified PCR products will be indexed for Next Generation Sequencing.
6. Indexed PCR products will be run on gels to check for success of PCR.
7. Indexed PCR products will be purified, quantified, and pooled into a library.
8. Sequence library and analyze resulting data through Anacapa bioinformatic pipeline.
    a. The protocols for these steps are being actively developed and will not be discussed in detail in this SOP.

Lab Methods: eDNA Extraction

1. Preparation
    a. Preparing workspace
        i. Clean benches with 20% bleach, 70% ethanol, and DNA Away.
        ii. Set up Bunsen burner and containers of 20% bleach and 100% ethanol.
        iii. Sterilize forceps, scissors, and spatula by soaking in 20% bleach, then 100% ethanol, then lighting over flame.
    b. Allow samples to fully thaw on ice before use.
2. Extraction from water filter
    a. Cut filter into strips using sterilized scissors and place into PowerBead Tube from Qiagen DNeasy PowerSoil Kit.
    b. Follow Qiagen DNeasy PowerSoil Kit protocol (Qiagen 2017) recommended by kit handbook, with the following modification:
        i. During the incubation step immediately after the addition of Solution C6, leave tube lid open for 2 minutes, and close tube for the remaining 3 minutes of the incubation time.
3. Extraction from soil
    a. Transfer 0.25-0.3 g of soil to PowerBead Tube from Qiagen DNeasy PowerSoil Kit using sterilized spatula.
    b. Follow Qiagen DNeasy PowerSoil Kit protocol (Qiagen 2017) recommended by kit handbook, with the following modification:
        i. During the incubation step immediately after the addition of Solution C6, leave tube lid open for 2 minutes, and close tube for the remaining 3 minutes of the incubation time.
4. Pooling of eDNA from multiple water filters
    a. If a single water sample required multiple filters to process, the eDNA extracted from each filter may be pooled.
    b. Concentrate pooled sample to 100uL volume using SpeedVac or other DNA concentration method.

5. Storage
    a. Store sample tubes in -20°C or -80°C low-DNA freezer.

Lab Methods: Metabarcoding PCR

1. Preparation
    a. Preparing workspace
        i. Sterilize fume hood with UV light for 10 minutes.
        ii. Clean fume hood and benches with 20% bleach, 70% ethanol, and DNA Away.
        iii. Clean all materials entering hood with 20% bleach, 70% ethanol, and DNA Away, with the exception of pipettes (only ethanol) and labeled sample tubes (only bleach).
        iv. Wear protective sleeves when working in sterilized hood.
    b. Determining primer set
        i. Use the following table to select primer set based on desired target.
            1. Italics represent Illumina adapter sequence.

Gene	**Target**	**Primer Name**		**Sequence**	**Reference**
16S	Bacteria and archaea	Forward	515F	*TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG*GTGYCAGCMGCCGCGGTAA	Caporaso et al. 2012
		Reverse	806R	*GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG*GGACTACNVGGGTWTCTAAT	
18S	Eukaryotes	Forward	1380F	*TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG*GTACACACCGCCCGTC	Amaral-Zettler et al. 2009
		Reverse	1510R	*GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG*TGATCCTTCTGCAGGTTCACCTAC	
Fungal ITS	Fungi	Forward	ITS5	*TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG*GGAAGTAAAAGTCGTAACAAGG	White et al. 1980
		Reverse	5.8S	*GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG*CAAGAGATCCGTTGTTGAAAGTT	Epp et al. 2012

CO1	Animals	Forward	m1COIintF	*TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG*GGWACWGGWTGAACWGTWTAYCCYCC	Leray et al. 2013
		Reverse	jgHCO2198	*GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG*TAIACYTCIGGRTGICCRAARAAYCA	
Plant ITS2	Plants and algae	Forward	ITS-S2F	*TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG*ATGCGATACTTGGTGTGAAT	Gu et al. 2013
		Reverse	ITS-S3R	*GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG*GACGCTTCTCCAGACTACAAT	
12S	Fish	Forward	MiFish-U forward	*TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG*GTCGGTAAAACTCGTGCCAGC	Miya et al. 2015
		Reverse	MiFish-U reverse	*GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG*CATAGTGGGGTATCTAATCCCAGTTTG	

Table 5.4.1. Primer information for metabarcoding PCR as recommended by CALeDNA protocol. This table includes the gene name, target organism group, forward and reverse primer names, sequence (including Illumina adapter in italics), and sources of each primer set used in metabarcoding. Details on primer sources can be found at the end of this document.

c. Calculating reaction mix
    i. The following reaction mix is recommended for the MiFish, CO1, and plant ITS2 primer sets:

Reagent	**1x reaction (uL)**	**Nx reactions (uL)**
Qiagen Multiplex Mix	12.50	N x 12.50
10uM F primer	1.00	N x 1.00
10uM R primer	1.00	N x 1.00
PCR water	7.50	N x 7.50

Master mix	22.00	N x 22.00
DNA template	3.00	---
Total volume	25.00	---

Table 5.4.2. Reaction mix for metabarcoding PCR with 12S, CO1, and plant ITS2 primers. This table includes the names and volumes of reagents to be included in the PCR master mix, the volumes of master mix and DNA template to be aliquoted into PCR tubes, and the total PCR reaction volume. N represents the number of samples to be processed, including positive and negative controls. Multiply 1x reaction volumes with N for total volume of each reagent to include in PCR master mix.

ii. The following reaction mix is recommended for the 16s, 18s, and fungal ITS primer sets:

Metabarcoding PCR Mix (16S, 18S, fungal ITS primers)		
Reagent	**1x reaction (uL)**	**Nx reactions (uL)**
Qiagen Multiplex Mix	7.50	N x 7.50
10uM F primer	0.15	N x 0.15
10uM R primer	0.15	N x 0.15
Molecular grade H2O	6.20	N x 0.15
Master mix	14.00	N x 14.00
DNA template	1.00	---
Total volume	15.00	---

Table 5.4.3. Reaction mix for metabarcoding PCR with 16S, 18S, and fungal ITS primers as recommended by CALeDNA protocol. This table includes the names and volumes of reagents to be included in the PCR master mix, the volumes of master mix and DNA template to be aliquoted into PCR tubes, and the total PCR reaction volume. N represents the number of samples to be processed, including positive and negative controls. Multiply 1x reaction volumes with N for total volume of each reagent to include in PCR master mix.

iii. Add 10% volume to calculations to accommodate pipette error.

d. Allow samples, Qiagen Multiplex Mix, PCR water, and primers to fully thaw before use.

2. Prepare PCR master mix
   a. Gently vortex Qiagen Multiplex Mix and primers.
   b. Pipette calculated amounts of Qiagen Multiplex Mix, primers, and PCR water into a microcentrifuge tube and vortex.
3. Prepare PCR reaction

a. Aliquot 1x reaction volume of master mix into each PCR strip tube or well of PCR plate.
b. Add DNA template to each strip tube or well of plate and mix by pipette or vortex to combine with master mix.

4. Thermocycler settings
    a. Use the following thermocycler programs for each primer set:

Metabarcoding PCR Thermocycler Settings				
Marker	**Cycles**	**Step**	**Temperature**	**Time**
16S, 18S, fungal ITS	1	Activation	95°C	15 min
	13*	Denaturation	94°C	30 sec
		Annealing	69.5°C (-1.5°C/cycle)	30 sec
		Extension	72°C	60 sec
	35	Denaturation	94°C	30 sec
		Annealing	50°C	30 sec
		Extension	72°C	60 sec
	1	Extension	72°C	10 min
		Hold	10°C	forever
CO1	1	Activation	95°C	15 min
	13*	Denaturation	94°C	30 sec
		Annealing	69.5°C (-1.5°C/cycle)	90 sec
		Extension	72°C	90 sec
	40	Denaturation	94°C	30 sec

		Annealing	50°C	30 sec
		Extension	72°C	60 sec
	1	Extension	72°C	10 min
		Hold	10°C	forever
ITS2	1	Activation	95°C	15 min
	13*	Denaturation	94°C	30 sec
		Annealing	70°C (-2.0°C/ycle)	30 sec
		Extension	72°C	60 sec
	50	Denaturation	95°C	30 sec
		Annealing	55°C	30 sec
		Extension	72°C	60 sec
	1	Extension	72°C	10 min
		Hold	10°C	forever
12S	1	Activation	95°C	15 min
	13*	Denaturation	94°C	30 sec
		Annealing	69.5°C (-1.5°C per cycle)	30 sec
		Extension	72°C	90 sec

	25	Denaturation	94°C	30 sec
		Annealing	50°C	30 sec
		Extension	72°C	45 sec
	1	Extension	72°C	10 min
		Hold	10°C	forever

Table 5.4.4. Thermocycler settings for metabarcoding PCR as recommended by CALeDNA protocol. This table includes the temperature, hold time, and number of cycles for each step of the thermocycler protocols for the six metabarcoding primer sets. Touchdown steps are labeled with an asterisk (*) and include the number of degrees to be lowered per cycle.

5. Storage
    a. Store sample tubes or plate in -20°C high-DNA freezer.

<u>Lab Methods: Gel Electrophoresis and Extraction</u>

This protocol applies to both gel electrophoresis steps outlined in the SOP overview.

1. Preparation
    a. Preparing workspace
        i. Clean benches with 20% bleach and 70% ethanol.
    b. Allow samples to fully thaw before use.
2. Mix 1.5% agarose
    a. Calculate weight of agarose needed for the amount of solution desired and add to flask. 1.5g of agarose is needed for every 100mL of 1.5% agarose solution.
        i. e.g. For 250mL of solution, 3.75g of agarose is needed (250mL solution needed / 100mL = 2.5, 1.5g x 2.5 = 3.75g agarose needed).
    b. Add 1x TBE buffer to flask according to amount of solution desired.
        i. e.g. for 250mL of solution, 250mL 1x TBE buffer is needed.
        ii. If flask is uncapped, add 20-30mL extra buffer to accommodate evaporation of buffer in microwave.
    c. Swirl solution and microwave for 1 minute until solution is clear.
        i. If flask is capped, loosen cap to avoid pressure build up in microwave.
        ii. If solution is not clear after microwaving, swirl and microwave for an additional minute. Repeat until solution is clear.
3. Cast gel
    a. NOTE: Amounts of reagents described in this step are based on the use of a 3” x 2” microscope slide as a gel mold.
    b. Mix 12-13uL agarose solution and 1uL Midori green stain in small beaker.
    c. Pour contents of beaker onto gel mold.

    d. Place gel comb ¾” down from top of gel, keeping wells parallel to top and bottom of gel mold.
    e. Let cool for 15-20 minutes until gel is solidified.
    f. Carefully remove gel comb.
4. Load gel
    a. Place gel in gel box. Orient wells so loaded product will travel from negative to positive electrode, with top and bottom of gel parallel to top and bottom of gel box.
    b. Fill gel box with 0.5x TBE buffer until gel is covered.
    c. Mix 1uL of 6x loading dye with 3uL of each PCR product.
    d. Pipette 4uL of loading dye and PCR product mix into wells of gel, leaving first well empty.
    e. Pipette 3uL of 100bp ladder into first empty well.
5. Run gel
    a. Place lid on gel box. Make sure negative electrode is connected to negative lead and positive electrode to positive lead.
    b. Attach leads to power supply. Make sure negative lead is connected to negative voltage output and the positive lead to the positive voltage output.
    c. Run gel at 100V for 25-30 minutes.
        i. Do not let product run off gel - check gel progress every 5 minutes.
6. Image gel
    a. Transfer gel to UV transilluminator, sliding gel off mold.
    b. Close transilluminator door and turn on UV light.
    c. Check that each product has a band of the correct size.
        i. If samples have multiple bands, gel extraction may be performed. This may occur with the MiFish and plant ITS primers.
    d. If the transilluminator is connected to a camera and printer, print an image of the gel and label accordingly.
7. Gel extraction
    a. Follow QIAquick Gel Extraction Kit protocol (Qiagen 2020) recommended by kit handbook.
8. Storage
    a. Store sample tubes or plate resulting from gel extraction in -20°C high-DNA freezer.

Lab Methods: Bead Purification

This protocol applies to both bead purification steps outlined in the SOP overview.

1. Preparation
    a. Preparing workspace
        i. Clean benches with 20% bleach and 70% ethanol.
    b. Determining amount of AMPure XP solution needed
        i. Calculate the amount of AMPure XP solution needed for each reaction through the following equation:
            1. (Volume AMPure XP per reaction) = 1.8 x (reaction volume)
    c. Allow samples to fully thaw before use.

d. Allow AMPure XP solution to reach room temperature before use. Invert tube until solution is fully homogenized.

2. Follow AMPure XP protocol (Beckman Coulter 2016) recommended by product handbook, with the following modifications:
    a. Prepare fresh 70% ethanol solution daily.
    b. During the removal of the supernatant before the first ethanol wash step, remove as much supernatant as possible, avoiding beads. Multiple attempts with small volume pipettes may be necessary.
    c. After the final ethanol wash step, monitor beads closely to avoid drying out. If beads appear to be drying out, proceed immediately to elution step.
        i. If beads have dried out, resuspending of beads during the elution step may take longer.
3. Storage
    b. Store sample tubes or plate in -20°C high-DNA freezer.

Lab Methods: DNA Quantification

This protocol applies to both DNA quantification steps outlined in the SOP overview.

1. Preparation
    a. Preparing workspace
        i. Clean benches with 20% bleach and 70% ethanol.
    b. Allow samples to fully thaw before use.
2. Follow Qubit dsDNA BR Assay Kit protocol (Invitrogen 2022) recommended by kit handbook, with the following modifications:
    a. Use 2uL sample volume for quantification.
    b. Keep samples on magnetic plate and let sit for 2min with caps open before aliquoting.
    c. Keep samples in dark during 2 minute incubation period.

Lab Methods: Indexing PCR

1. Preparation
    a. Preparing workspace
        i. Clean benches with 20% bleach and 70% ethanol.
    b. Assigning sample indices
        i. Kit comes with 12 unique i7 indices and 8 unique i5 indices; assign unique combinations of i7 and i5 indices to each sample.
    c. Calculating reaction mixes
        i. Each index will have its own reaction mix:

Reagent	**1x reaction (uL)**	**Nx reactions (uL)**
Kapa HiFi HotStart ReadyMix	6.25	N x 6.25
Index primer (i7 or i5)	0.625	N x 0.625
Total volume	6.875	---

Table 5.4.5. Reaction mix for indexing PCR as recommended by CALeDNA protocol. This table includes the names and volumes of reagents to be included in the PCR reaction mix. N represents the number of samples to be processed. Multiply 1x reaction volumes with N for total volume of each reagent to include in PCR reaction mix.

ii. Add 10% volume to calculations to accommodate pipette error.

d. Calculating volume of template and water
   i. Each reaction should have 10ng of DNA template. Based on the concentration measured during the first Qubit quantification step, calculate the volume of sample needed for 10ng of DNA.
   ii. The total volume of DNA template and PCR water in each reaction should be 11.25uL. Subtract the volume of sample needed for 10ng of DNA from 11.25uL to calculate the volume of PCR water needed for the reaction.

e. Allow samples, Kapa HiFi HotStart ReadyMix, index primers, and PCR water to fully thaw before use.

2. Prepare PCR reaction mix
   a. Gently vortex Kapa HiFi HotStart ReadyMix and Nextera indices.
   b. Pipette calculated amounts of Kapa HiFi HotStart ReadyMix and Nextera indices into microcentrifuge tube and vortex.
3. Prepare PCR reaction
   a. Place samples on magnetic plate.
   b. Aliquot calculated volumes of water and DNA template into each strip tube or well of PCR plate.
   c. According to assigned indices for each sample, pipette 6.875uL of i7 index reaction mix and 6.875uL of i5 index reaction mix into each strip tube or well of PCR plate.
   d. Gently vortex then spin down strip tubes or PCR plate.
4. Thermocycler settings
   a. Use the following thermocycler program:

Indexing PCR Thermocycler Settings			
Cycles	**Step**	**Temperature**	**Time**
1	Activation	95°C	5 min
8 - 12	Denaturation	98°C	20 sec
	Annealing	56°C	30 sec
	Extension	72°C	3 min
1	Extension	72°C	5 min

	Hold	8°C	forever

Table 5.4.6. Thermocycler settings for indexing PCR as recommended by CALeDNA protocol. This table includes the temperature, hold time, and number of cycles for each step of the thermocycler protocol.

5. Storage
    a. Store sample tubes or plate in -20°C high-DNA freezer.

Lab Methods: Library Pooling

1. Preparation
    a. Preparing workspace
        i. Clean benches with 20% bleach and 70% ethanol.
    b. Calculating sample volume to pool
        i. Use CALeDNA pooling template (Meyer 2018) to calculate amount of each sample to pool.
            1. A final volume of 300uL is recommended.
    c. Allow samples to fully thaw before use.
2. Pool samples according to template
    a. Briefly vortex and spin down samples; place on magnetic plate.
    b. Pipette sample volumes into 2.0uL sample tube according to amount specified by CALeDNA pooling template.
3. Concentrate sample
    a. If sample volume is greater than desired final volume, concentrate sample using SpeedVac.
4. Storage
    a. Transfer half volume of library into separate tube for storage; the remaining volume will be submitted for sequencing.
    b. Store sample tubes in -20°C high-DNA freezer.

Bioinformatic Methods: Library Sequencing

1. The protocols for this step are in active development and will not be discussed in detail in this SOP. A general outline is provided below.
2. Determine sequencing system and number of reads required.
    a. MiSeq is recommended to accommodate the length of reads generated by the included suite of metabarcoding primers.
    b. Each library will generate on average 150,000 reads. This estimate is based on MiFish libraries, and should be used as a rule of thumb rather than an exact value.
3. Send library to sequencing facility for analysis.
    a. The following facilities have been used in the past for eDNA library sequencing:
        i. UCLA Technology Center for Genomics and Bioinformatics
        ii. UC Berkeley Vincent Coates Genome Sequencing Laboratory
        iii. UCSC Paleogenomics Laboratory
    b. A quality control check is recommended if offered by sequencing facility.

Bioinformatic Methods: Analysis Pipeline

1. The protocols for this step are in active development and will not be discussed in detail in this SOP. A general outline is provided below.
2. Run library sequences through Anacapa (CALeDNA 2018) bioinformatic pipeline.
    a. Other eDNA analysis pipelines may be used, but this pipeline was developed with the CALeDNA methods employed by this SOP in mind.

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SOP 5: Sediment cores – sediment grain size analysis

Protocol (for laboratory only):

TBF. 2021. Soil Grain Size and Organic Content Standard Operating Procedures. Unpublished protocols. The Bay Foundation, Loyola Marymount University, California State University Long Beach, and Tijuana River National Estuarine Research Reserve. Los Angeles, CA. https://cms.santamonicabay.org/wp-content/uploads/2021/03/L3-Estuarine-Wetland-Manual-V.2_FINAL_web.pdf

Objective: This SOP provides a standard approach to collecting sediment samples for soil grain size and organic content analysis. Described here are the field protocols for two different types of laboratory analysis. If there are funds for sediment analysis or if sediment is in short supply, analysis via laser system provides more precise data on finer sediment sizes classes. If funds are limited, laser is not available, and laboratory analysis is possible, analysis via hydrometer method provides accurate data on larger grain size class divisions.

Parameters: sediment grain size

Hydrometer Grain Size

Materials:

1. Trowel
2. Ziplocs

Field Methods:

1. Three sediment samples will be taken at each sampling zone.
    a. Two samples will be adjacent to benthic faunal cores
        i. One sample at or near the bottom of the channel, depending on the size of the channel (adjacent to subtidal benthic core, SOP 6)
            1. Using the high tide mark as a reference point, collect samples ~0.5m below mean low water at the edge of the channel
            2. For lagoonal or BBE, samples should be taken in areas of the lagoon that are inundated most of the time.
                a. Areas that are predominantly sandy/rocky should be avoided
                b. If these areas are unknown, users can focus sampling at ~0.5m below the water surface at a point proximate to the shoreline.
        ii. One sample at the channel edge (adjacent to intertidal benthic core, SOP 6), at the edge of the marsh plain, where the vegetation starts.
    b. One sample on the marsh plain
2. At each collection spot, identify a ~5 x 5 cm area without vegetation near the benthic core
3. Insert trowel 2 cm into the sediment and take one large sample.
4. Place the sediment in the Ziploc. Repeat so two large scoops are in the bags.

5. Pre-label Ziplocs with location (subtidal, intertidal, marsh plain), sediment grain size, siteID, and date
6. Clean trowel by rinsing with saltwater and then reuse the trowel for subsequent samples
7. All samples brought back to lab

Laboratory Methods:

1. Hydrometer Methods (Modified from Bouyoucos 1962)

Bouyoucos, G.J. 1962. Hydrometer method improved for making particle size analyses of soil. Agronomy Journal 54: 464-465.

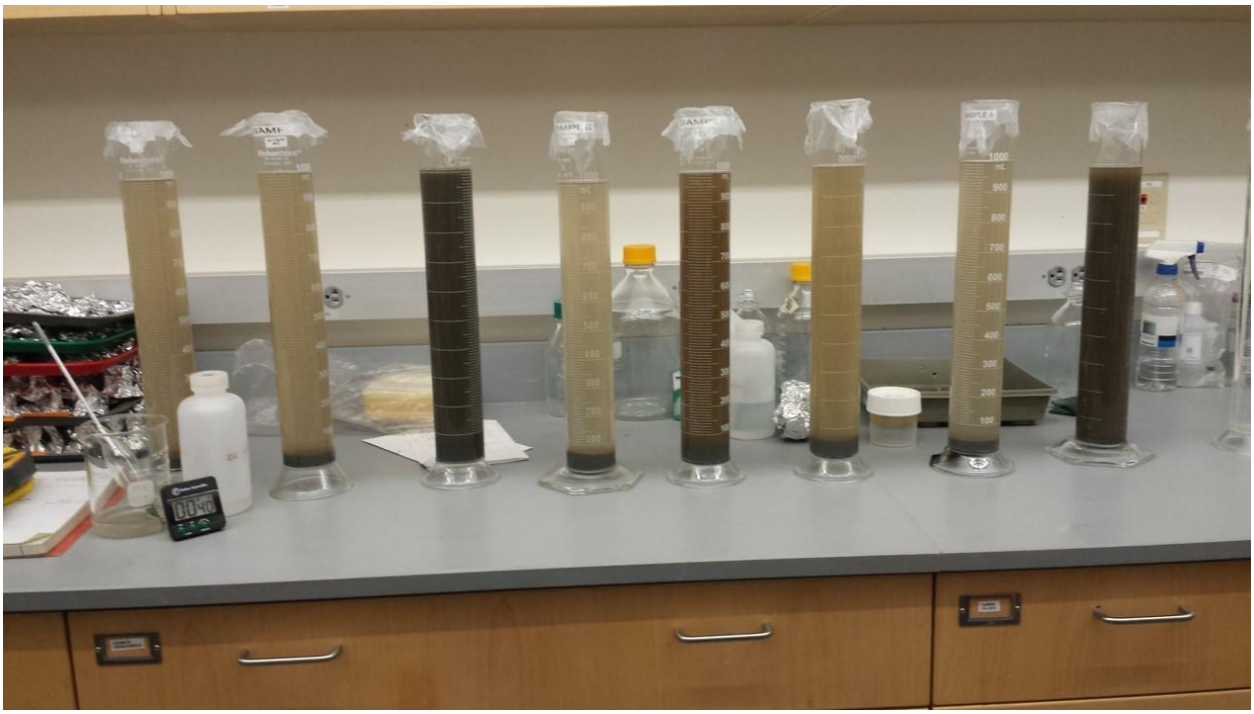


Figure 5.5.1 Graduated cylinders with a range of sediment samples in processing for grain size using hydrometer method. Clear samples are sandier while darker samples have more silt and clay. Photo: C. Whitcraft

Using this method, up to 20 samples can be done at once.

Estimated time:

1. Allow prep time for grinding and sieving samples
2. Day 1 approx. 1 hour
3. Day 2 approx. 3 hours

Materials needed:

1. 2 mm sieve
2. 600 ml beakers (one for each sample)
3. sodium metaphosphate (5g per sample + 5g extra)
4. 1 L graduated cylinder
5. 1 L cylinders (1 per sample + 1 extra)
6. stir bar

7. DI water
8. Shaker table
9. Thermometer
10. Hydrometer
11. Parafilm
12. Timer
13. Squirt bottle

Day 1:

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

Day 2:

1. After stirring, transfer contents of beaker to a 1 L cylinder with same number as beaker, using DI water to wash the remaining soil and residue into the cylinder. Add DI water to bring the volume of the cylinder to the 1 L mark.
2. Make a blank by adding 5 g of sodium metaphosphate to 1 L of DI water. Mix thoroughly on a stir plate.
3. Samples must be between 15.6 and 24.4 C.
4. Seal the top of the cylinder with parafilm, and invert the cylinder several times until all the soil is suspended.
5. Immediately after completing mixing, start time and lower the hydrometer gently into the cylinder. Take the hydrometer reading and temperature after 40 seconds.
6. Rinse the hydrometer with DI water and continue to next sample. Do not forget to record the temperature and hydrometer reading for the blank also.
7. Record the temperature and hydrometer reading again 2 hours after the start time. (Do NOT shake the samples again!). Record the temperature and hydrometer reading for the blank also.
8. Enter data into datasheets for calculation of %silt, %clay, and % sand. See figure 10 in *TBF. 2021. Soil Grain Size and Organic Content Standard Operating Procedures.*

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

To wash cylinders:

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

Laser Grain Size Analysis

Materials:

1. 50-cc syringe with bottom cut off and edges sharpened
2. Pre-labeled whirlpaks/Ziplocs with location (subtidal, intertidal, marsh plain), sediment grain size, site ID, and date

Field Methods:

8. Three sediment samples will be taken at each sampling zone.
    a. Two cores will be adjacent to benthic faunal cores
        i. One core at or near the bottom of the channel, depending on the size of the channel (adjacent to subtidal benthic core, SOP 6)
            1. Using the high tide mark as a reference point, collect cores ~0.5m below mean low water at the edge of the channel
            2. For lagoonal or BBE, samples should be taken in areas of the lagoon that are inundated most of the time.
                a. Areas that are predominantly sandy/rocky should be avoided
                b. If these areas are unknown, users can focus sampling at ~0.5m below the water surface at a point proximate to the shoreline.
        ii. One core at the channel edge (adjacent to intertidal benthic core, SOP 6), at the edge of the marsh plain, where the vegetation starts.
    b. One core on the marsh plain
9. At each collection spot, identify a ~5 x 5 cm area without vegetation near the benthic core
10. Insert a 50-cc syringe all the way into the sediment, pulling the plunger up while simultaneously pushing the tube into the sediment
11. Extract the syringe filled with sediment
12. Extrude the sediment into labeled whirlpak/ Ziploc
13. Clean syringe by rinsing with estuary water and then reuse the syringe for subsequent samples
14. All samples brought back to lab on ice for analysis and storage until shipped to designated lab for processing.

SOP 6: Sediment cores – benthic infauna

Protocol:

Bight '18 Field Sampling & Logistics Committee. 2018. Sediment Quality Assessment Field Operations Manual. Appendix L - Bight'18 Brackish Estuary core and Extension Pole Construction SOP Plus Sampling guide. SCCWRP, Costa Mesa, CA *https://ftp.sccwrp.org/pub/download/BIGHT18/Bight18SedQualityFieldManual_Appendices.pdf*

TBF. 2021. Benthic Invertebrates Standard Operating Procedures. Unpublished protocols. The Bay Foundation, Los Angeles, CA. https://cms.santamonicabay.org/wp-content/uploads/2021/03/L3-Estuarine-Wetland-Manual-V.2_FINAL_web.pdf

Objective: This SOP provides a standard approach to collecting sediment cores in order to assess the density and composition of benthic infauna.

We outline two methods for collecting benthic infauna –

1. Small benthic infauna
2. Large benthic macrofauna

Described here are the field protocols for both methods and the preservation of cores for small benthic infauna. For specific laboratory sorting and taxonomic classification, see TBF. 2021.

Small benthic infauna

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). 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). We provide a sampling method for collecting and assessing the density and composition of benthic infauna. However, our method provides the minimum sampling one should take when assessing the benthic community. Because our method only extracts two cores per sampling zone (six cores per estuary), our data won't allow us to characterize individual estuaries, rather we will only be able to make gross comparisons among estuaries.

If users want to be able to characterize estuarine condition within an estuary, then we recommend adjusting the following parameters to meet your needs:

1. Sample replication – Users should increase replication to include samples along the channel from the mouth to the head of the estuary. Additionally, users should include a diversity of habitats within their samples – SAV beds, the marsh plain, the subtidal channel, and the intertidal channel. Along a channel, users could increase replication to include the channel edge and the bottom of the channel.
2. Depth –Depth can influence invertebrate biomass and can have less of an impact on abundance (Valenca and Lo Santos 2013). Therefore, the top layer of sediment to 2 cm has to be included in order to have a majority of infauna invertebrates (0-2 cm – Levin et

al. 1998). Without at least 2 cm of sediment, the core would not be representative of any sediment invertebrate community, and it would be recommended to ignore those cores for data comparison.

3. Area of core – The core diameter or grab size (and thus area) should be chosen carefully as gear size targets particular size classes of organisms. For example, smaller core sizes will target macrofauna typically in the smaller size (e.g., 1- 2 mm) range and are likely to exclude megafauna, such as large clams or crabs. Core size chosen should be consistent with existing studies or published literature on macrobenthos from the site and/or nearby marshes.
4. Sampling frequency - The reproductive biology, abundances, and feeding habits for many estuarine species vary greatly with the seasons and correlated environmental factors. In some cases, a species may be classified as carnivorous during the winter, herbivorous during the spring, and omnivorous during the summer and fall (Light's manual 4th edition). Therefore, users should consider sampling in all seasons.

Parameters: benthic infauna abundance, diversity, and biomass

Materials:

1. 1 subtidal sediment core – 10cm diameter x 10cm depth with *optional* water tight, pressure fit lids (purchase or construct as below)
2. 1 intertidal sediment core – 10cm diameter x 10 cm depth
3. 1-gallon, sealable bags or Nalgene jars
4. Whirlpacks
5. Waterproof paper tags
6. 3-4, 5-gallon buckets
7. Cooler

Subtidal Core Construction: Adapted from Bight 2018

1. *Building Materials*:
    a. 18", 2" diameter – Schedule 40 PVC pipe –
        i. https://www.homedepot.com/p/Charlotte-Pipe-4-in-x-2-ft-PVC-DWV-Sch-40-Pipe-PVC-07400-0200/100566597
        ii. https://www.amazon.com/gp/product/B01F2UDNLK/ref=ppx_yo_dt_b_search_asin_title?ie=UTF8&psc=1&pldnSite=1
    b. 4" – galvanized steel riser clamps - https://www.homedepot.com/p/The-Plumber-s-Choice-4-in-Riser-Clamp-in-Galvanized-Steel-04CLRSG/309742682
    c. 4" – rubber pipe cap w/ stainless tightening clamp - https://www.homedepot.com/p/The-Plumber-s-Choice-4-in-Pvc-Flexible-Pipe-Cap-with-Stainless-Steel-Clamps-E04714/305860719
    d. Saw w/ PVC blade
    e. Rasp file, Dremel tool, etc.
    f. Duct tape, some manner of waterproof tape, or heavy rubber band
2. *Construction*:
    a. If the pipe is longer than 18" trim to appropriate length. Eighteen inches a recommendation, but a few inches shorter or longer is fine if ergonomically more comfortable.

b. Using the file or Dremel tool or other preferred device, cut a bevel at one end of the tube, this will become the bottom (Figure 5.6.1). This will aid in penetrating the sediment.
c. Measure 10 cm up from the bottom of the core and place a wrap of tape or rubber band (Figure 5.6.2). This will be the minimum depth guide for inserting the core into the sediment.
d. Place the riser clamp approximately 12 – 15 inches from the bottom of the core (Figure 5.6.2). This will be the handle for inserting and removing the core from the sediment. Replace the hex nuts used to tighten the clamp with wing nuts for ease of adjustment by hand.
e. Consider wrapping the ends of the riser in waterproof electrical tape to make the handle more comfortable to push and pull on.
f. Ensure rubber cap fits the end of the corer.

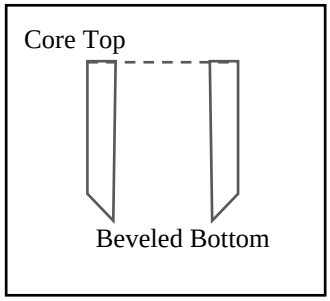


Figure 5.6.1 Cut a bevel at one end of the tube.

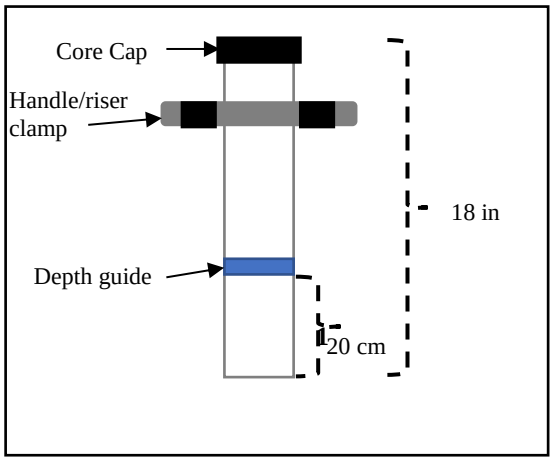


Figure 5.6.2 Constructed core.

Optional Core Pole for non-wading deployment: Adapted from Bight 2018

1. *Building Materials*
    a. 10ft length of 2” diameter PVC pipe

b. 4”x 2” rubber reducing coupler - https://www.homedepot.com/p/The-Plumber-s-Choice-4-in-x-2-in-PVC-Flexible-Reducing-Coupling-with-Stainless-Steel-Clamps-E53844X2/305473560
c. 2” diameter spring-load check valve - https://www.homedepot.com/p/EZ-FLO-2-in-PVC-Solvent-In-Line-Check-Valve-20457/205858374?MERCH=REC-_-pip_alternatives-_-204237842-_-205858374-_-N&

2. *Construction*:
   a. Attach check valve to one end of the pole (compression or cement fitting). Make sure direction of the valve is flow up the pipe from the bottom. (Figure 5.6.3)
   b. Attach 2” side of couple to the check vale. Tighten the gasket.
   c. Mark 2-cm increments along the length of the pole. This will be used to estimate core penetration into the sediment
   d. Drill drainage holes every 5-6 cm along length of the pole

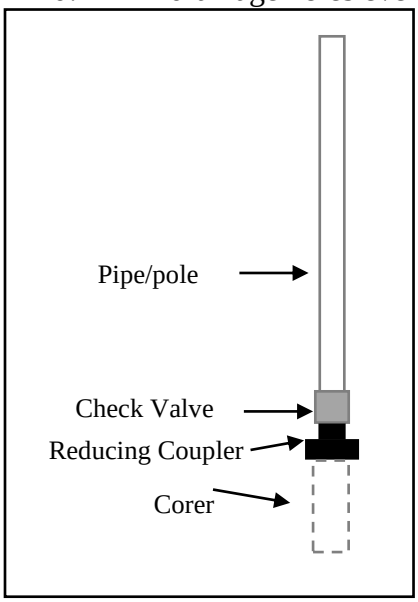


Figure 5.6.3 Core pole for non-wading deployment.

Field Methods: Adapted from TBF 2021.

1. Points for benthic cores will be taken within each sampling zone.
2. At each zone, two cores will be taken – a subtidal core and an intertidal core.
3. Using the high tide mark as a reference point, collect subtidal cores ~0.5m below mean low water at the edge of the channel with the subtidal corer (10cm diameter x 10cm depth)
   a. For lagoonal or BBE, samples should be taken in areas of the lagoon that are inundated most of the time.
      i. Areas that are predominantly sandy/rocky should be avoided
      ii. If these areas are unknown, users can focus sampling at ~0.5m below the water surface at a point proximate to the shoreline.
4. First, push the 10 cm diameter corer into the sediment to a depth of 10 cm, then affix the lid (if present)
5. Extract the sample from the sediment and place core into either labeled 1gall, sealable bags with an internal label identifying the sample or a labeled jar.

6. Pre-label jars or bags with location (subtidal, intertidal), benthic invert core, siteID and date
7. Next, take an intertidal sediment corer (10 cm diameter x 10 cm depth) at the edge of the marsh plain, where the vegetation starts.
    a. An additional intertidal core will be taken in order to homogenize the sample and extract a subset of samples in 1.5ml Eppendorf tubes for sediment eDNA (SOP 4)
8. First, push the 10 cm diameter corer into the sediment to a depth of 10 cm, then affix the lid (if present)
9. Extract the sample from the sediment and place core into either labeled 1gall, sealable bags with an internal label identifying the sample or labeled jar.
10. All samples can be brought back to the lab in containers and in a cooler on ice (if the field day is long and if the day is hot).

Optional Non-Wading Collection for subtidal core*:* Adapted from Bight 18' Field Manual Appendix

1. NOTE: this approach is suggested if one were sampling from a boat. It works well for muddy and sandy mud sediments. It does not work well for coarse sand and gravel
2. Assemble the coupler to the pole.
3. Attach the core to the wide side of the coupler. Make sure that the core is seated all the way into the coupler and that the gaskets are tight.
4. Place the pole+corer over the side of the boat, gently resting at the sediment surface.
5. Note the approximate cm mark of the water along the pole, this is the starting point.
6. Gently push the core into the sediment at least 10cm and no more than 15cm, approximating the distance with the 2cm markings on the pole.
    a. Make sure the core goes into the sediment vertically and the pole+core doesn't flex out at the point of the coupler
7. Gently pull core from the sediment. It may require some jostling and twisting of the core to break the seal with the sediment.
8. Keep the core vertical as you pull it out of the water. As the bottom of the core breaks the surface of the water, use your hand or a wide flat object to prevent any slippage of the sediment out of the tube
9. Place the corer in or over the sample bin or sieving device
10. Remove the core from the coupler, allowing the sediment to slide out. Gently rinse the insides of the core into the bin/sieve.
11. If the sample looks less than the minimum required depth, then collect a new core.
12. Process as normal.

Laboratory Methods:

1. Both of the intact benthic cores should be preserved using formalin (8% formaldehyde with Rose Bengal stain).
2. The jars should be filled with sample material to 50-70% capacity, leaving at least 30% uncovered space for further processing.
3. The jar should then be filled with saltwater leaving 10% available open space.
4. 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 zone ID, sample location within the channel (i.e. subtidal vs intertidal), and date

    a. 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.
5. In the laboratory, jars should be initially preserved with a 10% formalin saltwater solution.
6. Make sure to use a spoon (or similar object) to mix up the sample without cutting up the mud in order to ensure that the formalin gets mixed throughout the core.
7. NOTE: If preservation in formalin is not possible, samples can be placed in ethanol temporarily for transport or until formalin is available, then the ethanol (or alternative preservative) should be drained over designated sieve size and replaced by formalin (as above).
8. NOTE: The TBF 2021 laboratory method recommends a standard fixing of unsorted fauna and detritus in 10% seawater-buffered formalin for 2 to 5 days, followed by a transfer to 70% ethanol for preservation and storage. If one is uncomfortable using formalin or if the fauna may need to have their DNA extracted, use the following alternate method although the trade-off of not using formalin is that ethanol-preserved samples are not well-preserved and would need to be put in formalin at some point or at least sorted on a short-time frame
    a. Prepare a solution of 95 parts 95% non-denatured ethanol to 5 parts glycerin.
    b. Place sieved, but unsorted material in a clean, glass or plastic screwtop container. Use a large enough container such that the material occupies no more than 66% of the volume of the container (i.e., 1:2 ratio of ethanol to material).
        i. Allow room for more ethanol if the sample contains a large amount of organic matter (e.g., rhizomes, leaf litter, wood, etc)
    c. Fill the container with the ethanol-glycerin solution at minimum of 1:2 ratio of ethanol to material
    d. 24hrs after the initial addition of ethanol, decant the overlying ethanol using a 250-um sieve. Return any material retained on the sieve to sample container
    e. Add a fresh amount of the ethanol-glycerin solution, maintaining the same ethanol-material ratio as before
    f. 48hrs after the initial addition of ethanol, decant the overlying ethanol using a 250-um sieve. Return any material retained on the sieve to sample container.
    g. Add a fresh amount of only 95% ethanol (omit the glycerin), maintaining the same ethanol-material ratio.

References:

Carlton, J.T. (editor). 2007. The Light and Smith Manual: Intertidal Invertebrates from Central California to Oregon Fourth Edition

Hilty, J. and Merenlender, A. 2000. Faunal Indicator Taxa Selection for Monitoring Ecosystem Health. Biological Conservation, 92, 185-197.

Hodkinson, I.D., and Jackson, J.K. 2005. Terrestrial and aquatic invertebrates as bioindicators for environmental monitoring, with particular reference to mountain ecosystems. Environmental Management 35:649–666.

Levin, L., T. Talley and Hewitt, J.. 1998. Macrobenthos of Spartina foliosa (Pacific Cordgrass) saltmarshes in southern California: Community structure and comparison to a Pacific mudflat and a Spartina alterniflora (Atlantic Smooth Cordgrass) marsh. Estuaries 21:129-144.

Valenca, A. P.M.C, Dos Santos, P. 2013. Macrobenthic community structure in tropical estuaries: the effect of sieve mesh-size and sampling depth on estimated abundance, biomass and composition. Journal of the Marine Biological Association of the United Kingdom 93(6): 1441-1456.

Large benthic macrofauna

Parameters: benthic macrofauna abundance and diversity

Materials:

1. 1 large sediment core – 10cm diameter x 30cm depth with *optional* water tight, pressure fit lids
2. 1 large sieve – 3mm mesh screen
3. 3-4, 5-gallon buckets

A minimum of 6 sediment cores should be taken at each station (3 on the marsh plain, 3 near either side of the channel – intertidal, and 3 in the middle of the channel - subtidal) to characterize the macroinfaunal invertebrate assemblage.

1. Run a minimum of 2 transect lines from the subtidal (bottom of the channel) across a elevational gradient to the marshplain
2. Push the 10 cm diameter corer into the sediment to a depth of at least 30 cm
3. Extract the sample from the sediment and place core onto a large sieve with a 3-mm screen
4. Identify, count, and release each organism
    a. If an organism cannot be identified in the field, it can be preserved in formalin and ethanol for later identification to the lowest possible taxonomic level.
5. Record data on datasheet, making sure to include
    a. species name,
    b. species count by dead and live,
    c. length or weight measures,
    d. Note other non-target organisms as occurring (e.g. polychaetes).
    e. GPS point for core
    f. Substrate type (e.g. mud, sand)

Figure 5.6.3 Example of corer constructed for sampling, and 3 mm sieve being used in Huntington Beach Wetlands to sieve a core.

SOP 7: SAV and macroalgal surveys

Protocols:

Seagrass Cover and Density. 2020. Tennenbaum Marine Observatories Network, MarineGEO, Smithsonian Institution. https://marinegeo.github.io/assets/modules/seagrass-density/marinegeo_protocol_seagrass_density.pdf

Methods and Guidance on Assessing the Ecological Functioning of Submerged Aquatic Vegetation in Southern California Estuaries and Embayments. 2020. Southern California Coastal Water Research Project. https://ftp.sccwrp.org/pub/download/DOCUMENTS/TechnicalReports/1136_SeagrassAssessmentFramework.pdf

California Eelgrass Mitigation Policy and Implementing Guidelines. 2014. NOAA Fisheries: West Coast Region. https://media.fisheries.noaa.gov/2020-08/cemp_oct_2014_final.pdf

Short, F.T., Coles, R.G., Short, C.A. 2015. SeagrassNet Manual for Scientific Monitoring of Seagrass Habitat, Worldwide edition. University of New Hampshire Publication. 73 pp. http://www.seagrassnet.org/assets/about/SeagrassNet%20Worldwide%20Manual%202015.pdf

McLaughlin K., M. Sutula, M. Molina. 2019. Standard Operating Procedure (SOP) for Macroalgal Collection in Estuarine Environments. Technical Report #872. Southern California Coastal Water Research Project. Costa Mesa, CA

Objective: This SOP provides a standard approach to assess seagrass or Submerged Aquatic Vegetation (SAV) distribution (areal extent, percent cover), species composition, and shoot density and to evaluate macroalgal abundance, a key indicator for eutrophication in California estuaries.

For the purpose of monitoring SAV, we focus on SAV that is traditionally found in seagrass beds (i.e., *Zostera* spp., *Ruppia maritima*). We ignore kelp beds.

If sites do have kelp beds (e.g., Drakes Estero), users may want to monitor their beds to achieve a more comprehensive sampling of condition. We recommend partnering with the MPA subtidal group to develop surveys or alternatively use Reef Check, PISCO protocols, and/or annual drone surveys to map out kelp canopy during peak biomass months (September to October).

Additionally, if sampling of SAV can only occur one time per year, we recommend sampling during periods that capture the maximum growth of SAV. For example, in southern California, maximum growth of eelgrass occurs in the fall (September-November), while peak growth occurs earlier (August-October) for central and northern California. Sampling should therefore occur between September and October.

For the purpose of monitoring macroalgae, this SOP provides protocols to sample two major habitat types: 1) intertidal (mud or sand) flats and 2) subtidal surface habitat. Intertidal flats are

the unvegetated band of habitat found in the lower intertidal zone. The subtidal surface habitat represents the algae in areas where algae is prolific on the surface of the water.

For perennially tidal or for intermittently tidal estuaries that are open to tidal exchange for most of the year, the intertidal and surface protocol is recommended because of logistical issues and costs associated with deep subtidal sampling. However, if an estuary regularly features a seasonally restricted or closed inlet condition, particularly during the growing season (April-November), sampling the estuary utilizing a benthic subtidal protocol is strongly recommended.

Below we provide a minimalist approach to assessing the abundance of SAV and macroalgae. If funding and time allows, we recommend and outline more intensive sampling.

Parameters: SAV diversity and distribution, shoot density, macroinvertebrate abundance; Macroalgae abundance

Materials:

1. 50 cm x 50 cm quadrat
2. 25 x 25 cm quadrat
3. 50-m metric transect tape
4. 25-m metric transect tape
5. 2 PVC marker poles
6. GPS
7. Digital camera
8. Short ruler
9. Percent cover quadrats ($1m^2$ quadrat divided into 16 smaller squares using string, PVC pipes, and elbow joints) for macroalgae
10. Pole with attached scoop (the pole should be able to reach the depth of the SAV; various items could be used for the scoop; spaghetti ladle, rake, etc.)

Field Methods:

Minimalist approach

At the bare minimum, we recommend measuring the presence and absence of SAV and macroalgae while doing water quality surveys (SOP 2).

1. At each water quality point, users will use a pole and a scooper to scoop up the bottom sediment.
2. SAV and macroalgae will be identified by species
    a. Species should be noted on the SOP 2 datasheet

Intensive approach

SAV

Field methods for seagrass surveys adapted from MarineGEO 2020 and SCCWRP 2020

1. Locate SAV or seagrass beds on sampling map

a. Determine number of beds and overall size of beds on sampling map
    i. SAV imagery may help delineate bed number and size
    ii. West coast Eelgrass imagery can be found on PMEP website and can also be readily visible via Google Earth imagery depending on site and quality of imagery
    iii. If no maps are available, then users can rely on site recon prior to sampling.
b. Based on bed size, determine number of transects (2-3)
    i. If beds are too narrow to achieve more than 1 transect, then sample a different bed if available
c. If possible (the bed is large enough), run 3 transect tapes (the interior transect should be prioritized)
    i. Inshore (shallow)
    ii. Interior
    iii. Offshore (deep)
    iv. If beds are too short (< 50 m), but with adequate width, then transects can be run in a zig-zag formation
d. Sampling maps and site-recon will help determine if SAV can be surveyed via
    i. Waders/wetsuit at low tide
    ii. Snorkel at low tide

2. Setup transects parallel to shoreline
3. Along the transect tape, lay down a quadrat immediately adjacent to the transect line
    a. Every 8m for percent cover; n=6; 50 cm x 50 cm quadrat
    b. Every 16m for shoot density; n=3; 25 cm x 25 cm quadrat
4. Record the GPS coordinates of the endpoints of the transect.
5. If transect is less than 50m, deploy quadrats at 6 (percent cover) and 3 (shoot density) equidistant points along the transect
    a. If transect is less than 10m, reduce number of percent cover quadrats to 3 and do concurrently with the shoot density measures
6. Use and record visual estimates of percent cover, benchmarked to standardized percent cover images*
    a. Standardize personnel using the SeagrassNet manual - http://www.seagrassnet.org/assets/about/SeagrassNet%20Worldwide%20Manual%202015.pdf
    b. See Figure 5.7.1
    c. If possible, percent cover should be estimated for each species. Species could include -
        i. *Zostera marina*
        ii. *Zostera pacifica*
        iii. *Zostera japonica*
        iv. *Ruppia maritima*
        v. Other SAV species
7. Record the presence and approximate size of large mobile benthic macroinvertebrates (>10cm), including crabs, snails, etc.
8. In every *other* replicate (unless transect is <10 m), obtain a measurement of shoot density by counting and recording the number of seagrass shoots within a 25 x 25 cm quadrat

    a. In some estuaries, shoot density (as well as percent cover) may not be possible with waders or on snorkel due to water quality hazards, reduced visibility, and/or channel depth
    b. If taking shoot density or percent cover in the field is not possible, we recommend using regional historic monitoring data, if available.
        i. If this is not available, then users should either attempt to collect shoot density and percent cover with scuba or side-scan sonar technology or omit SAV monitoring.
        ii. If funding is available, we recommend using scuba over using regional historic monitoring data.
9. Repeat steps for every replicate and pre-determined number of transect lines
10. The extent of the bed should be estimated by using GPS.
    a. If the GPS has the capability, users can draw polygons with the GPS and walk (or kayak/boat) around the extent of the bed.
    b. If users cannot walk polygon boundaries, then five GPS points should be taken.
        i. The four corners
        ii. The middle of the bed
11. On the field datasheet record the following: sampling zone, transect #, location (inshore, interior, offshore), depth/tidal height, substrate, quadrat #, vegetation species, percent cover, shoot density, bed area, macroinvertebrate species, macroinvertebrate abundance, and whether a photo was taken.

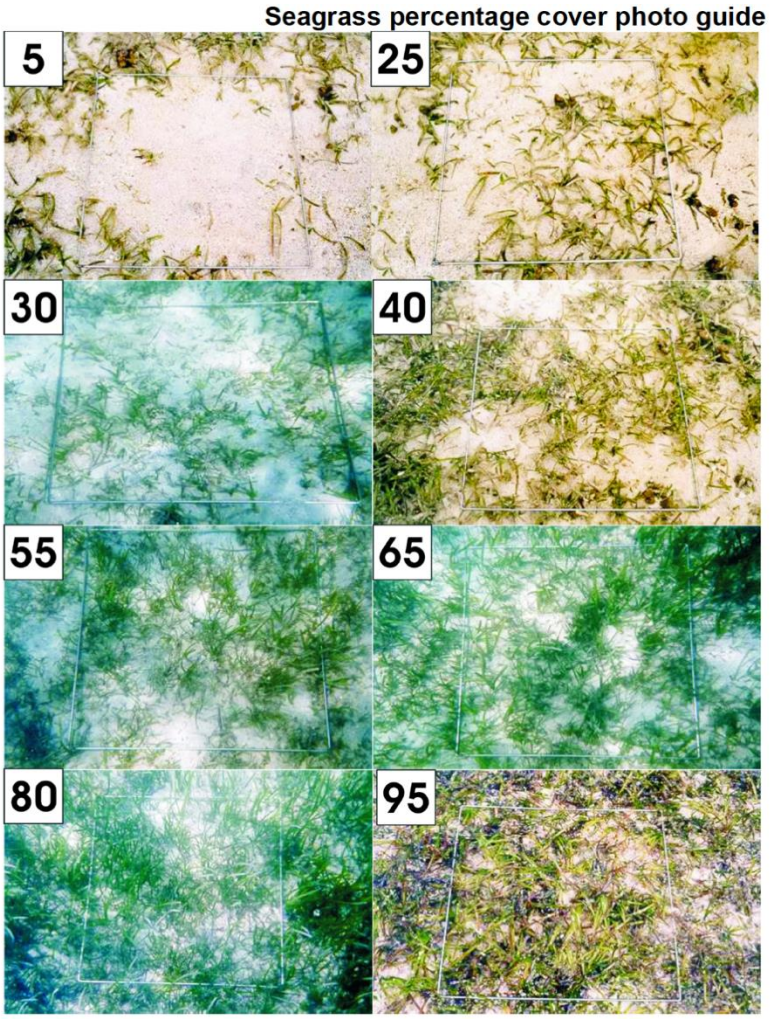


Figure 5.7.1. Standardized percent cover photo guide from SeagrassNet manual. Personnel should standardize percent cover estimates using this guide and among themselves.

*If scuba is not an option and snorkel is not feasible, cameras mounted on PVC could be used to take standardized photos of SAV. These photos may help determine visual cover estimates.

Macroalgae

Intertidal habitat

The intertidal habitat survey for macroalgae should be prioritized. This survey can be completed in conjunction with the vegetation surveys (SOP 11)

Field methods for macroalgae surveys adapted from McLaughlin et al. 2019

1. Locations for macroalga surveys are critical in assessing overall macroalga abundance.
    a. In general, transects for each sampling zone will be located on the mid- to upper-mudflats and below the mean lower low water level (MLLW). See Figures 5.7.2 and 5.7.3 for layout of transects and sampling locations.
    b. Transects should be adjacent or near to sampling zones

    c. Transects can be parallel to the vegetation transects in SOP 11
    d. At each station, a minimum of one transect should be laid out (ideally three).
        i. For intensive site monitoring, additional transects should be added to represent approximately 15-30% of the channel length.
        ii. Each transect should be 25 meters long
        iii. Within a channel, sampling of the intertidal flat should always occur on the depositional bank of the channel.
2. When approaching a station minimize footprints within the designated sampling areas.
3. At the edge of the emergent vegetation stake in a small piece of grey PVC pipe or stake to mark the start of the transect and record the GPS coordinates on the data sheet.
4. Carefully lay the transect tape out to 25 m near the edge of the emergent vegetation parallel to the shore.
5. Record the GPS coordinates of the endpoints of the transect. Record the distance in meters from the PVC pipe.
6. Carefully lay out the second transect tape out to the same distance as the first (either 60 m or 30 m) between the MLLW and the emergent vegetation parallel to the shore. Stake in the landward end of the transect.
7. Record the GPS coordinates of the endpoints of the transect. Record the distance in meters from the PVC pipe to the transect.
8. Carefully lay the third transect tape out to 60 m or 30 m below the MLLW line and parallel to the shore.
9. Record the GPS coordinates of the endpoints of the transect. Record the distance in meters from the PVC pipe to the transect.
10. The oceanward location of each transect is designated as distance 0 m along the transect.
11. For each transect –
    a. Every 5m along the 25-m transect, lay the $1m^2$ quadrat next to the transect
        i. Randomly select the side of the transect to lay the quadrat
    b. Data to be collected are:
        i. Cover class (open, wrack, dead plant material, trash, and algae)
        ii. Percent cover by species of macroalgae (for all species in each subplot)
            1. If plants are present in the plots, include them in the percent cover counts
        iii. Photo
    c. Collect percent cover
        i. 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%
        ii. Each species (and whether it is alive or dead) should be recorded as one cover class.
        iii. Visual estimates of cover are made using a modified Daubenmire cover class system using a 7-point scale. Because canopies may overlap, cover estimates may total more than 100% (Figure 5.11.3)
    d. Take a photo of each quadrat
        i. Lay the small ruler along the edge of the quadrat
        ii. Take a photo of each plot by holding the camera directly above the plot, ensuring that the whole quadrat and ruler is in the frame of the shot

12. Once the tapes are laid out, take a digital photograph of the station from the oceanward end.

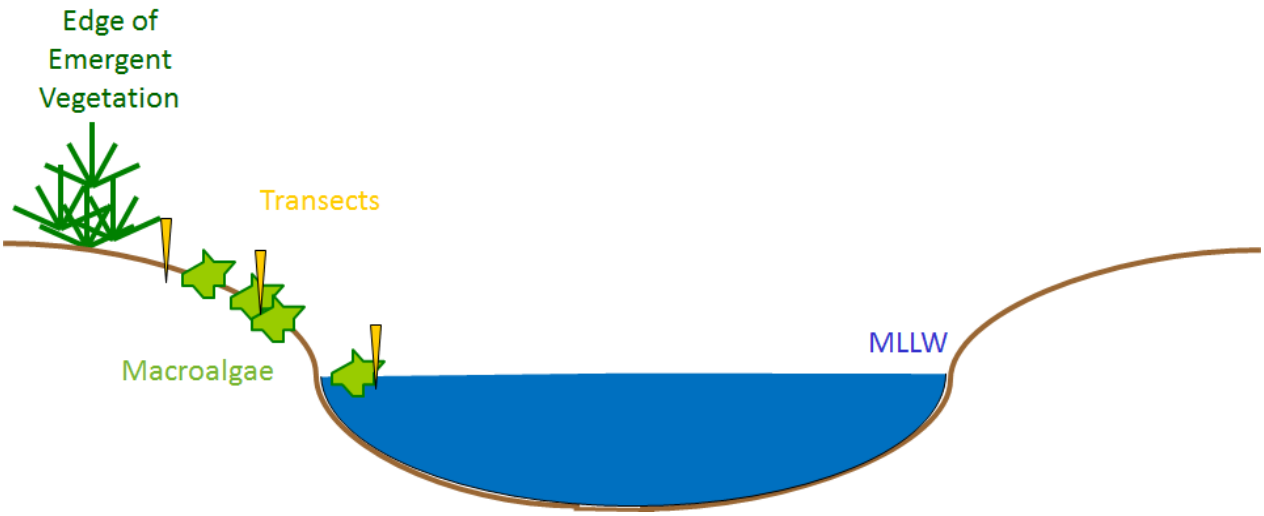


Figure 5.7.2. Cross sectional view of one station with three transects. One transect is laid out near the emergent vegetation, another between the vegetation and MLLW, and a third within the shallow subtidal (McLaughlin et al. 2019).

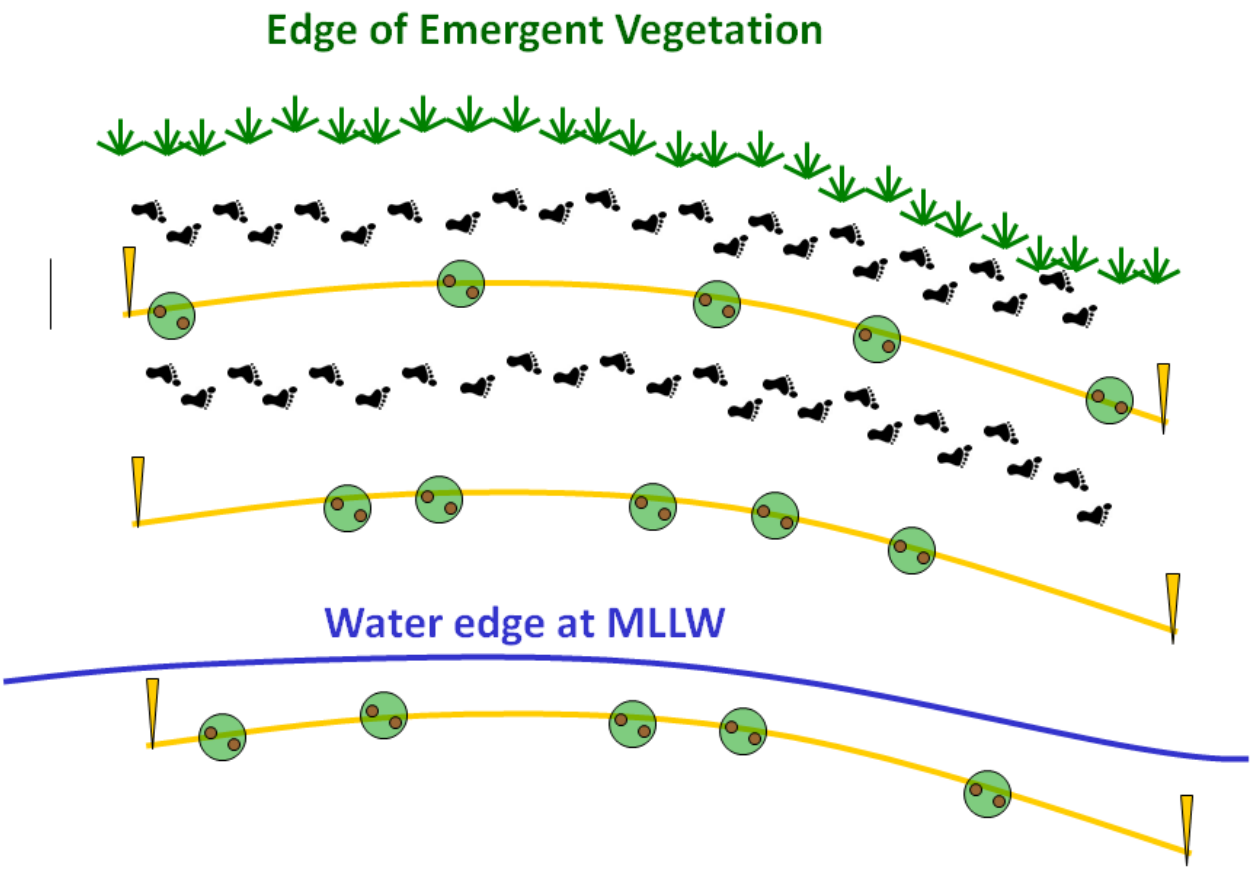


Figure 5.7.3. Top-down view of transect layout at one station in the intertidal flats and shallow subtidal. Three transects per station are set along the shore with one transect near the emergent vegetation, another transect between the vegetation and MLLW, and below the MLLW. Green circles represent macroalgal quadrats. Note that it is not recommended to step on the transect path, but to walk in between transects (McLaughlin et al. 2019). **For the EMPA protocols, we recommend spacing quadrats every 5m (contradictory to the diagram).

Floating subtidal habitat

To survey the free-floating algae on the water surface, we recommend estimating the abundance of algae across the 25 m intertidal transect and channel width.

1. Measure the channel width from the water edge intertidal 25-m transect to the opposite channel edge (Figure 5.7.4).
    a. Estimate the area of the sampling zone by multiplying 25 m by the channel width
2. Estimate the total percent cover of the free-floating algae within the transect area
    a. Estimate percent cover using 25% bins
        i. 0%, 25%, 50%, 75%, 100%
    b. Note any species if able to identify
3. Record the estimated percent cover on the SOP 7 macroalgae datasheet on the line corresponding to the water edge intertidal transect

To get a more comprehensive assessment of the macroalgae, algae could be collected in order to estimate macroalgae biomass at each station.

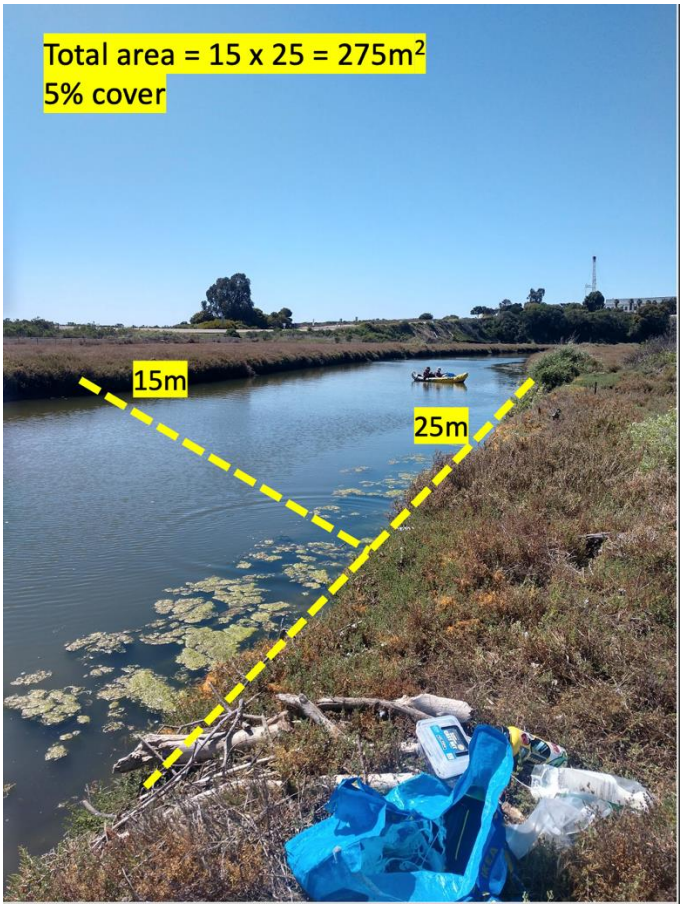


Figure 5.7.4. Example of estimated channel width perpendicular to the 25m transect tape for an estimated sampling area of 275m^2. Within the sampling area, the percent macroalgae was estimated to be 5% cover.

SOP 8: Fish – BRUV (Baited Remote Underwater Video)

Protocol:

CSULB and TBF. 2021. Fish Cameras Standard Operating Procedures. Unpublished protocols. The Bay Foundation, Los Angeles, CA. https://cms.santamonicabay.org/wp-content/uploads/2021/03/L3-Estuarine-Wetland-Manual-V.2_FINAL_web.pdf

Objective: This SOP provides a standard approach to fish species abundance estimates using an underwater baited video setup. This approach focuses on benthic and water column fish abundance.

Parameters: Fish species abundance

Materials:

1. PVC pipe, 6ft, white
2. PVC pipe, 0.5m, clear
    a. https://www.amazon.com/gp/product/B01F2UDNLK/ref=ppx_yo_dt_b_search_asin_title?ie=UTF8&psc=1&pldnSite=1
3. PVC primer and cement, with T-fitting
4. Nylon
5. Bait: chopped shrimp and squid
6. Knife and cutting board, for bait
7. Micro SD card, 64-GB and adapter
8. GoPro camera
    a. https://www.amazon.com/gp/product/B08JHFLL4Z/ref=ppx_yo_dt_b_search_asin_title?ie=UTF8&psc=1&pldnSite=1
9. Battery extension packs
10. Secci disk for visibility estimate (optional but recommended)
11. Waterproof GoPro cases
    a. https://www.amazon.com/gp/product/B08JVF6S49/ref=ppx_yo_dt_b_search_asin_title?ie=UTF8&psc=1&pldnSite=1
12. GoPro attachments (Figure 5.8.1)
    a. https://www.amazon.com/gp/product/B01LCLVBU8/ref=ppx_yo_dt_b_search_asin_title?ie=UTF8&psc=1&pldnSite=1
13. External hard drive (approx. 1TB per season needed)

Figure 5.8.1. Go-pro attachments needed for BRUV.

Figure 5.8.2 Picture of constructed BRUV with shrimp bait in pantyhose.

We recommend deploying the BRUVs right when you arrive at the field site. BRUVs need to be deployed at each sampling zone for about 2 hrs. We recommend deploying cameras so they are visible to shore crew.

The mouth dynamics of lagoonal systems will influence video quality. For example, closed conditions will affect the effectiveness of BRUVs due to poor visibility. If visibility is poor and no fish are seen on the camera footage, this should be marked as a failed sampling event. We recommend returning to the estuary when the mouth is open or visibility improves. A BRUV with zero captured fish does not mean the estuary does not have fish.

Field Methods

1. 2 BRUVs (Figure 5.8.2) should be deployed at each sampling zone.

2. Secure GoPro camera to PVC T-frame as pictured in Figure 5.8.2.
    a. Fasten GoPro to attachments (base + 1 larger attachment + 1 smaller attachment), and secure camera to the arm of the T-frame using zip ties (5.8.3).
3. Cover zip ties with electrical tape (Figure 5.8.4) if using zipties.
4. Chop shrimp and squid (or other available bait) and place in a bag made of nylon.
5. Place bait approximately 0.3m from GoPro, ensuring that its position is visible on camera.
6. Samples should be collected at low-to-mid tide, when oyster beds are minimally submerged (if oyster beds are present). Ideally, this would be done consistently on an ebbing or flooding tide.
7. At each site, place the base of the T-frame into the sediment 1m into the subtidal (as noted on site map), at a randomly selected position along the shoreline facing away from shore (towards deeper water).
8. Set up Go Pro with standard settings. These will vary with model number.
    a. For Go Pro 9's, setting should be 1080p resolution and 60 frames per second
    b. For ho Pro 10's, setting can be 4000 resolution and 60 frames per second
9. Record for two hours, the maximum recording time for Go Pro HERO9 or older models with battery extension pack.
10. Recollect camera. Transfer files to an external hard drive using an SD card converter. Ensure all cameras are cleared and that both cameras and battery packs are charged before the next sampling date.
    a. When transferring the videos, it is useful to double check the visibility and camera angle to ensure that the video is usable. If the bait is not visible, data from this video will not be counted. the video should be repeated if possible.
11. Record the time in, time out, and depth of each BRUV on the Estuary Checklist.

Figure 5.8.3 Black tape covering zip ties on BRUV, as well as 3D printed attachment options.

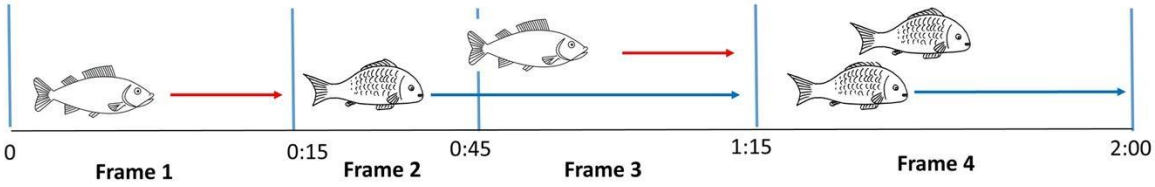
Figure 5.8.4 More tape additions to secure GoPro attachments.

Video Analysis

$MaxN_{species}$ is an abundance estimate often used in video analysis to account for bias associated with fish entering and leaving the camera's field of view (Cappo et al., 2003; Wakefield et al., 2013; Mallet & Pelletier, 2014). It is considered a conservative measure of abundance, as it may underestimate the size of larger schools of fish, but it prevents individual fish from being counted multiple times and gives a reliable minimum abundance within the sampling area for many species (Schobernd et al., 2013).

1. The first 5 minutes of each video should not be counted as this is the time it typically takes for the sediment to settle. Fish in this timeframe can be noted, but any fish in this time period should not be counted as data (Figure 5.8.5).
2. To be processed, the bait should be visible in the video. If it is not visible due to water quality or if the camera angle is incorrect, this should be noted in the metadata, but the data from this video will not be counted. If possible, the video should be repeated in the same season.
3. Analyze each 2-hour video sample using VLC media player at up to 3x speed.
4. For each fish passing through the frame of view, record ID to the lowest taxonomic level possible, as well as the time it enters and leaves the field of view.
    a. Watching and then rewatching the video is a useful way to determine if the fish enter and leave the video as a solo group or if another fish or school of fish enter while the first fish or school of fish is still in the frame.
5. Abundance should be estimated overall for the 2-hour session and by species for the two-hour session as MaxNspecies, the maximum number of individuals present in the field of view at one time.
    a. Schooling fish (when numbers are in the hundred) can be recorded as a school instead of trying to count all fish.
6. Total time on camera for each species as well as species richness, overall video period MaxN, and MaxN by each species should be reported for each sampling period video (Figure 5.8.5).

a. MaxN: The maximum number of fish in a given frame regardless of species (e.g.if there were 3 bat rays in the frame, then n=3) (e.g. if there were 3 bat rays and 2 mullets in the frame, then MaxN = 5).
b. MaxNs: The maximum number of fish in a given frame by species (e.g. if there were bat rays and 2 mullets in the frame, then MaxNs = 3 for rays and MaxNs =2 for mullet; every species in a row)



Frame #	Time	Species	MaxN	MaxNs	Notes
1	0 – 0:15	Red fish	1	1	
2	0:15 – 0:45	Blue fish	1	1	
3	0:45 – 1:15	Red fish	2	1	
3	0:45 – 1:15	Blue fish	2	1	
4	1:15 - 2	Blue fish	2	2	

Figure 5.8.5 Example of 4 data frames with associated scoring of MaxN and MaxNs.

References

Wakefield, C. B., Lewis, P. D., Coutts, T. B., Fairclough, D. V., & Langlois, T. J. 2013. Fish assemblages associated with natural and anthropogenically-modified habitats in a marine embayment: comparison of baited videos and opera-house traps. PloS one, 8(3), e59959.

Mallet, D., & Pelletier, D. 2014. Underwater video techniques for observing coastal marine biodiversity: a review of sixty years of publications (1952–2012). Fisheries Research, 154, 44-62.

Cappo, M., Harvey, E., Malcolm, H., & Speare, P. 2003. Potential of video techniques to monitor diversity, abundance and size of fish in studies of marine protected areas. Aquatic Protected Areas-what works best and how do we know, 455-464.

Schobernd, Z. H., Bacheler, N. M., & Conn, P. B. 2013. Examining the utility of alternative video monitoring metrics for indexing reef fish abundance. Canadian Journal of Fisheries and Aquatic Sciences, 71(3), 464-471.

SOP 9: Fish – Seine

Protocol:

TBF. 2021. Fish Beach Seine Standard Operating Procedures. Unpublished protocols. The Bay Foundation, Los Angeles, CA.
https://cms.santamonicabay.org/wp-content/uploads/2021/03/L3-Estuarine-Wetland-Manual-V.2_FINAL_web.pdf

Beach Seines. 2020. Tennenbaum Marine Observatories Network, Marine- GEO, Smithsonian Institution.
https://marinegeo.github.io/assets/modules/fish-seines/marinegeo_protocol_beach_seines.pdf

Objective: This SOP provides a standard approach to quantitatively assess the distribution, relative abundances, species richness, and diversity of fish.

Understanding estuarine fish communities relies on quantification of density and/or species richness of fish. Seines are one of the most widely used gear types for sampling estuarine fishes (e.g., Allen 1982; Allen et al. 1992) because they capture a wide variety of species and are relatively easy to use. However, seines themselves are biased towards smaller, mid-water and sometimes slower fish than other methods such as beam trawls or hook and line fishing. As explored thoroughly in Steele et al. 2006a and 2006b, various factors about seines including mesh size, length, skill of fishers, and block netting can influence the density and species richness estimates. The choice of seine may vary with the project goals and should be carefully considered by the practitioner.

Parameters: fish species abundance, species richness, diversity, age class/biomass

Materials:

1. 30ft l, 6ft h, 1/8in mesh beach seine (no bag)
2. 25m transect tape
3. 3-4, 5 gal buckets or large plastic pool
4. Calipers
5. Rulers or meter sticks

Field Methods: Field methods for fish seines incorporate protocols from both TBF 2021 and MarineGEO 2020.

1. As determined through species accumulation curves (see data document), four seine pulls will be performed at each sampling zone on an ebb tide. The end of the first seine is the start of the second seine, and so on.
    a. Lay out 25 m transect which will mark the seine pull
    b. Stretch out seine, walking along the shoreline
    c. Mark the orientation of how you will pull the seine on the data sheet
        i. Perpendicular to shore –the seine is perpendicular to shore and users are walking along the shore (Figure 5.9.1)

        ii. Parallel to shore – users start offshore and walk the seine towards the shore moving offshore to inshore
    d. Walk the seine 25 m
        i. Close ends at end of transect by forming a C and
        ii. Pull onto land
    e. Transfer fish immediately from the nets into buckets filled with estuary water to be measured and identified to species
2. Measure and count the catch
    a. If there are fewer than 10 individuals of a species, all fish standard lengths (most anterior part of the upper or lower jaw to caudal peduncle) should be measured to the nearest millimeter (Figure 5.9.2)
    b. If more than 10 individuals of a given species are collected in a given seine, only the first 10 randomly selected individuals of each species should be measured. The remaining fish of that species (> 10) should be counted and held for release in the buckets
    c. Fish that are too small (typically those ≤ 10 mm) to accurately identify in the field should be labeled as juveniles
    d. Record macroinvertebrate or other by-catch data
    e. Retain fish until all 4 seines are complete at a sampling zone
3. Once a location has been fully counted and measured, the fish may be released outside of the immediate zone area (to avoid recapture).

Figure 5.9.1 Perpendicular seine pull - the seine is perpendicular to shore and users are walking along the shore.

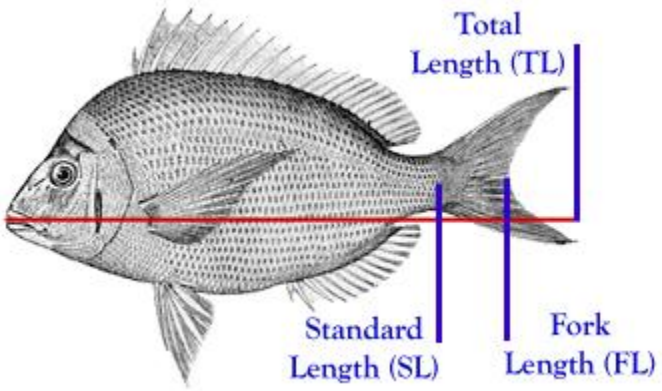


Figure 5.9.2 Standard length on an example fish (source: https://fishionary.fisheries.org/tag/standard-length/)

References:

Allen, D. M., S. K. Service, and Ogburn-Matthews, M.V. 1992. Factors influencing the collection efficiency of estuarine fishes. Transactions of the American Fisheries Society 121:234–244.

Allen, L. G. 1982. Seasonal abundance, composition, and productivity of the littoral fish assemblage in upper Newport Bay, California. Fishery Bulletin 80:769–790.

Steele, M.A., Schroeter, S.C, and Page, H.M. 2006a. Sampling Characteristics and Biases of Enclosure Traps for Sampling Fishes in Estuaries. Estuaries and Coasts 29(4): 630-638.

Steele, M.A., Schroeter, S.C, and Page, H.M. 2006b. Experimental Evaluation of Biases Associated with Sampling Estuarine Fishes with Seines. Estuaries and Coasts 29(6B): 1172-1184.

SOP 10: Crab traps

Protocol:

Hughes BB, Wasson K, Tinker MT, Williams SL, Carswell LP, Boyer KE, Beck MW, Eby R, Scoles R, Staedler M, Espinosa S, Hessing-Lewis M, Foster EU, Beheshti KM, Grimes TM, Becker BH, Needles L, Tomoleoni JA, Rudebusch J, Hines E, Silliman BR. 2019. Species recovery and recolonization of past habitats: lessons for science and conservation from sea otters in estuaries. PeerJ 7:e810.

TBF. 2021. Fish Minnow Trap and Enclosure Trap Standard Operating Procedures. Unpublished protocols. The Bay Foundation, Los Angeles, CA.

Objective: This SOP provides a standard approach to assess the distribution, relative abundances, species richness, and diversity of crab and small fish species. Multiple trapping methods will be used based on the landscape, as well as to capture a broader species richness.

Parameters: crab species abundance, diversity, age class/biomass

Materials:

1. Willapa shrimp pots [https://www.westmarine.com/buy/willapa-marine--shrimp-pot—5555727, *modified according to Hughes et al. 2019]
2. Minnow traps [https://smile.amazon.com/South-Bend-Wire-Minnow-Trap/dp/B00GI9WS84/ref=sr_1_4?crid=32TNWXYFX41ZH&keywords=Minnow+traps&qid=1658203236&sprefix=minnow+traps%2Caps%2C349&sr=8-4]
3. Tennis ball cannisters with small holes drilled in bottom to hold bait in shrimp pots
4. Plastic toothbrush holders for minnow trap bait
5. Floats
6. Weights or anchors
7. Bait
8. 3-4, 5 gal buckets
9. Rulers or meter sticks
10. Calipers
11. Scale
12. Zip ties
13. Garden trawl

Description of the traps:

Baited (anchovy) shrimp pots (Figure 5.10.1 left) will be used with modified openings that allow access to large crabs, yet still prevent access or entrapment of any larger predators, such as sea otters, birds and harbor seals. The dimensions of the shrimp pots are 24”L x 24”W x 9”H, with four openings that are rigidly supported and ~10 cm in diameter, which allows for access of the larger cancrid estuarine crabs, yet still retaining the smaller crab species. There are no doors on the openings. Traps will be deployed on the substrate.

Baited minnow traps (Figure 5.10.1 right) will be used to catch smaller fish and invertebrates. One trap will be deployed 1-2m above the shrimp pot in the water column and one trap will be deployed attached to the shrimp pot on the substrate. Note: trap arrays need to be deployed subtidally if using the floating minnow trap.

Figure. 5.10.1 Shrimp pots (left) and minnow traps (right) used for crab traps.

Field Methods – shrimp pots + minnow traps:

1. Before the field –
    a. Modify shrimp pots according to above description
    b. Drill small holes in the bottom of 1 empty tennis ball cannister and fill halfway with either anchovies or sardines (used for bait)
    c. Fill two plastic toothbrush holders with either anchovies or sardines
    d. Let bait-filled holders sit overnight for 24 hours in order to enhance the bait aroma and attractiveness.
2. Locations for traps will be pre-determined on sampling maps and correspond with sampling zones
    a. Each trap will be deployed at or adjacent to each sampling zone.
    b. Locations will be chosen based on landscape features
        i. SAV
        ii. Main channel
        iii. Subtidal (sand or mud)
3. Secure shrimp pots to the bottom of a float line that can extend from the substrate to the surface (Figure 5.10.2)
4. Attach the subsurface minnow trap 1-2 m above the shrimp pot, along the line, with a mini float to keep it buoyant in the mid-water at varying tides
    a. In a shallow system if the water is < 1 m, then no top minnow trap should be deployed.
5. Attach a second “benthic” minnow trap directly to the shrimp pot with zipties.
6. Add the tennis ball cannister of bait in the shrimp pot and zip tie (or any other locking mechanism) the top door shut
7. Add 1 toothbrush holder of bait in the minnow traps and zip tie shut
8. Drop the trap array at the sampling location and attach the top of the line to a float
    a. Depending on water conditions,
        i. secure the shrimp pot to the substrate using a small (5 lb anchor) or
        ii. add weights (5-10 lbs) to the shrimp pot
        iii. in high current areas it’s best to use both anchor and weights.
9. If the system is shallow, then deploy trap array from the channel edge.

a. Tie two lines onto a PVC pole (Figure 5.10.3)
b. Push the PVC pole into the mud, or if sediment size is too large (sand) then use either a sand anchor or 10 lb anchor, and deploy either:
    i. Along the channel edge
    ii. 1-2m off of the channel edge (Figure 5.10.4)
c. Drop the minnow traps into the water, ensuring that they remain submerged during low tide

10. Retrieve all traps ~24hrs later
11. Record the time in, time out, and depth of each trap on the field data sheet and Estuary Checklist.

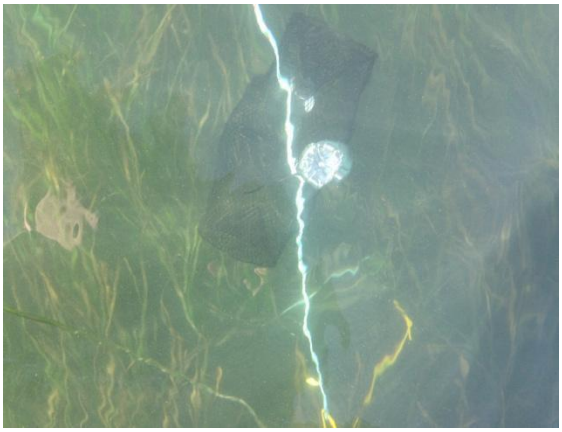



Figure. 5.10.2 The photo on the left shows the deployment of our trapping unit with the minnow trap and the shrimp pot below it on the substrate for a mudflat site. The photo on the right shows a deployment of our trapping unit in an established eelgrass plot. The tidal range that we are operating in is 0-2m relative to NAVD88, which is within centimeters of MLLW. Traps are always deployed in positions along the main channel where they will be fully submerged for the full 24 hours.

Figure. 5.10.3 In shallow systems, both minnow traps can be attached to a single PVC pole and deployed off the channel edge.

Figure. 5.10.4 In shallow systems, minnow traps can be attached to a single PVC pole and deployed off the channel edge with the PVC anchored into the vegetation [photo: TBF 2021].

Field Methods – Retrieval of traps

1. Retrieve shrimp pots and minnow traps by loosening any anchors and gently pulling to the surface
2. Transfer crabs from the traps into buckets to be measured and identified to species
3. Identify crab species, weigh on scale, and measure carapace width to the nearest millimeter.
4. For crab, note the sex of the animal. In most crab the width of the abdomen will reveal sex, with females have a notabally wider abdomen than males.
5. After being counted and measured, crabs should be transferred to a release bucket.
6. Record by-catch (fish, macroinvertebrates, etc.)
    a. If fish are caught, record species and measure fish standard length (mm).
7. Once a trap has been fully counted and measured, the crabs may be released outside of the immediate zone area (to avoid recapture) or when all trapping is completed.

SOP 11: Marsh plain vegetation and epifauna surveys

Protocol:

TBF. 2021. Vegetation Cover Surveys Standard Operating Procedures. Unpublished protocols. The Bay Foundation, Los Angeles, CA. https://cms.santamonicabay.org/wp-content/uploads/2021/03/L3-Estuarine-Wetland-Manual-V.2_FINAL_web.pdf

EMAP 2002. West coast pilot. Intertidal assessment: California intensification. Environmental Monitoring and Assessment Program.
https://www.sfei.org/sites/default/files/biblio_files/3_EMAP_Quality_Assuranc~000.pdf

Objective: This SOP provides a standard approach to evaluate three important plant community parameters in tidal marshes: 1) plant species diversity, 2) community physical structure, and 3) the invasion of non-native species. This protocol is to be used for estuarine wetland habitats including the backshore (i.e., upland transition zone), marsh plains, channel margins, tidal pannes and ponds, and foreshore (i.e., channel-ward transition zones from the marsh plain to open tidal mud flats).

Parameters: Marsh plain vegetation diversity and structure, invasive species

Materials:

1. 25-m transect tape
2. Meter stick or measuring tape (for vegetation canopy heights)
3. Percent cover quadrats ($1m^2$ quadrat divided into 16 smaller squares using string, PVC pipes, and elbow joints)

Field Methods:

1. Locations for vegetation surveys are critical in assessing overall plant community parameters
    a. Survey areas will be pre-selected on sampling maps and marked with lines
    b. Transects should be adjacent or near to sampling zones
    c. Transects should include multiple habitat zones in the marsh platform -
        i. the backshore (i.e., upland transition zone)
        ii. marsh plains
        iii. channel margins
        iv. tidal pannes and ponds
        v. foreshore
2. The number and direction of transect lines will follow the EMAP 2002 protocols (Figure 5.11.1). Each transect is placed parallel to the channel at the same elevation.
    a. 1 transect – 25m transect, parallel to the mainstem channel along the foreshore (low marsh), but not in the water or on a mudflat.
    b. 1 transect – 25m transect, parallel to the mainstem channel, 20m back from the foreshore (mid marsh), 10 m from any other channel
    c. 1 transect – 25m transect, along the backshore in the high marsh.
    d. *Additional transects can be added based on the number of marsh platforms

3. For each transect –
    a. Every 5m along the 25-m transect, lay the $1m^2$ quadrat next to the transect
        i. Alternate sides of the transect when laying the quadrat
    b. Data to be collected are:
        i. Cover class (living vegetation, dead plant material, open/barren ground, wrack/unattached flotsam, trash)
        ii. Percent cover by species for all species in each plot
            1. Distinguish between live and dead species as separate percent cover categories
        iii. Height of the tallest plant of each species in each plot (both living and dead)
        iv. Epifauna species and abundance
    c. Collect percent cover
        i. Surveys should be conducted using 1 m^2 PVC quadrats
            1. Total percent cover and cover by individual species should be recorded for the whole $1m^2$ quadrat area.
            2. To increase the accuracy of the $1m^2$ estimate, use a quadrat subdivided into 16 squares (Figure 5.11.2)
        ii. 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%
        iii. Each species (and whether it is alive or dead) should be recorded as one cover class.
        iv. Visual estimates of cover are made to exact cover estimates. Multiple data recorders should calibrate their cover estimates before making measurements in the field.
            1. These more precise cover estimates can be scored back in the laboratory using a modified Daubenmire cover class system with 7-point scale.
            2. Because canopies may overlap, cover estimates may total more than 100% (Figure 5.11.3)
    d. The species and height of the tallest plant (to the nearest cm) is recorded for each species in each quadrat.
        i. From these data, the height of the tallest plant in each plot can be extracted
        ii. In addition, the number of plant layers (estimate of complexity) can also be determined.
    e. Identify epifauna species and abundance
        i. For the purposes of this protocol, epifauna species are defined as the bottom-dwelling and mud-dwelling species, such as snails and crabs. We do not include terrestrial insects (bees, leafhoppers, scales) in the epifauna counts.
        ii. For each quadrat, count and record the total abundance for each epifauna species
        iii. Crab burrows should also be counted to record evidence of crabs
        iv. If $1m^2$ quadrats are too large to count epifauna, then $0.25m^2$ quadrats can be used to count a subset of individuals

1. Epifauna abundance can be scaled on data sheets multiplying the abundance by 4

4. On the field datasheet record the following: sampling zone, transect #, habitat (low, mid, high/upland), quadrat #, cover class, species, live or dead, % cover, max height, epifauna species, epifauna abundance, and epifauna quadrat size

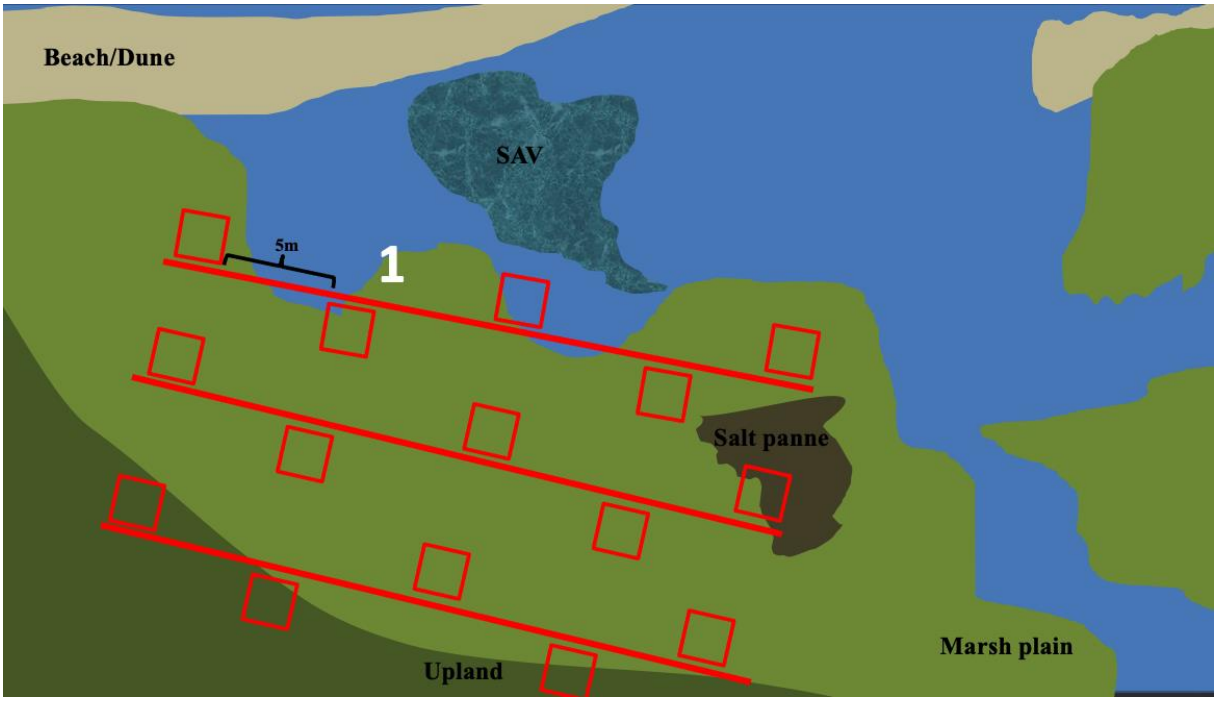
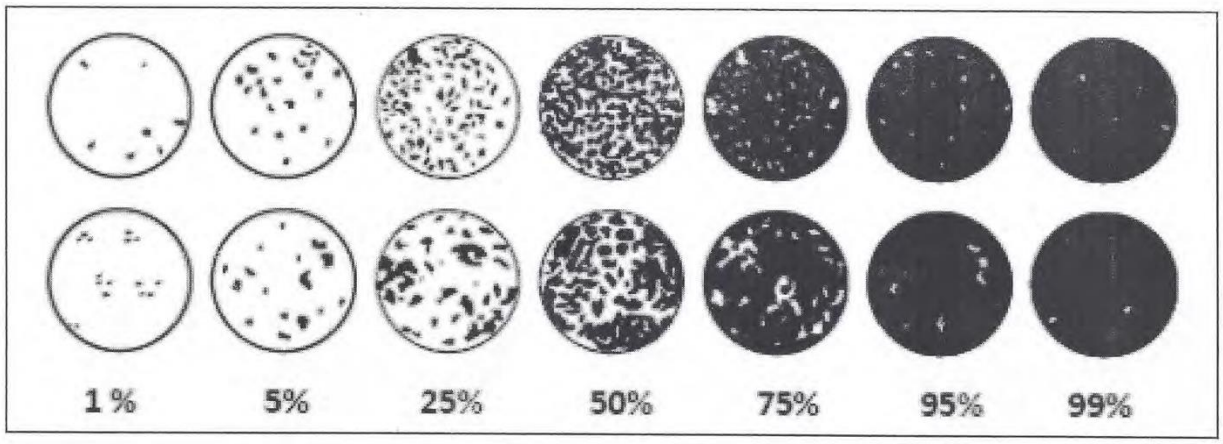


Figure 5.11.1 Example lagoon with three vegetation transects (foreshore, mid, and backshore) and five quadrats every 5m along each 25m transect line. This configuration would be focused at 1 sampling zone. Similar transect lines could be set up at the other zones.

Figure 5.11.2 1m^2 PVC quadrat, divided into 16 squares (TBF 2015).



COVER CLASSES

1	>0-1%
2	>1-5%
3	>5-25%
4	>25-50%
5	>50-75%
6	>75-95%
7	>95-100%

Figure 5.11.3 Modified Daubenmire cover class system using a 7-point scale from TBF 2015.

SOP 12: Topographic surveys

Objective: This SOP provides a standard, repeatable approach to characterizing estuarine topography and morphology and assessing change over time. This approach emphasizes major landscape features (inlets, marsh plains, ponds, channels, levees, etc.) as well as artifacts of the primary fluvial and marine processes that influence overall estuarine topography and geomorphology (e.g., floodplain deltas, overwash fans, backbeach runnels, etc.). This approach utilizes abundant transects to capture gradients in elevation as well as inundation depth, duration, and frequency. Technicians may decide to co-locate vegetative sampling plots along these transects to capture the influence of hydrologic and geomorphic change on vegetation community composition (see gradsects in Parker et al. 2011).

Parameters: Elevations (vertical), planform morphology (horizontal)

Materials: For most smaller/supratidal estuary systems, ground-based survey methods utilizing RTK GPS equipment and/or a differential GPS with post-correction equipment should be suitable to characterize estuarine topography. Larger/subtidal systems, especially those with deep subtidal ocean inlets and channels, may require the use of boat-based surveys to capture estuarine bathymetry. It may not be safe and/or practicable in some cases to capture subtidal bathymetry, especially where strong currents/waves are present. Technicians shall use their best professional judgment when developing work plans to ensure the safety and efficiency of topographic surveys.

Field Methods:

Estuaries often contain unstable features, such as unconsolidated mudflats, that may be unsafe to access on foot. Prior to initiating any topographic surveys, technicians shall review aerial photographs of any subject estuary, consult local experts, and ideally visit in-person to assess any potential access and safety challenges. Technicians should pay special attention to the stability and safety of recent, unvegetated sediment deposits, such as overwash fans and floodplain deposits, as well as lagoon inlet channels and associated unvegetated mudflats that have been recently dewatered.

Surveys must be timed to avoid impacts to rare, sensitive, or special-status species, such as endemic plants and nesting birds. Technicians shall consult with local resource agencies and obtain any necessary access training and/or permits. In fully tidal estuaries, surveys should be timed during a low tide, to capture intertidal features. In BBEs, surveys should be timed when the inlet is open and the system is drained; they may not need to be at low tide depending on the system.

1. **Site reconnaissance.** Prior to initiating topographic surveys, technicians shall review current and historic aerial photographs, USGS topo quads, and related materials describing the estuary's environmental setting, and identify the following features:

    a. Ocean inlet
    b. Main fluvial channel (in BBEs, the channel that serves as the primary connection between the watershed outlet and the ocean inlet) and associated floodplain/delta

c. Any significant distributory channels that branch off the main fluvial channel
d. Backbarrier and/or tidal marsh plain
e. Any backwater/hydraulically isolated features such as oxbow ponds, unvegetated flats, and related features that may only be hydraulically connected to the main fluvial channel and/or ocean inlet during high water events
f. Estuarine-terrestrial transition zones
g. Beach foreshore and backshore and any associated dune systems
h. Overwash fans, relict inlet locations, and other shoreline features
i. Any artificial features such as fill pads, embankments, bridges, culverts, or other infrastructure that could influence estuarine hydrology and/or morphology

Technicians should also use available materials and their best professional judgment to anticipate likely locations and mechanisms of future estuarine geomorphic change, such as floodplain deposition, channel evulsion, inlet movement, etc., and include these locations in the topographic transects and profiles listed below where practicable. Survey metadata should include the rationale for including these locations in the topographic surveys.

2. **Point collection (Minimalist Approach).** At a minimum, technicians should collect elevation data at the following locations (Figure 15.12.1) -
    a. Unique plant communities representing variability in inundation across the vegetated marsh plain
    b. Different channel and marsh plain elevations and physical features (examples listed above)
    c. Estuarine-terrestrial transition zones
    d. Water elevation loggers, to tie the recorded water elevation to the marsh plain measurements
    e. In the vicinity of the sampling stations
    f. At the beginning and end of each vegetation transect

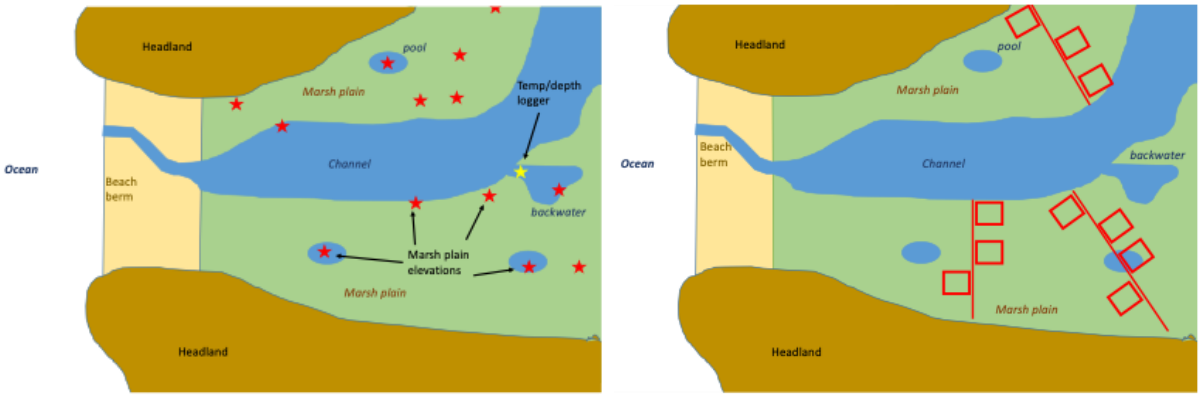


Figure 5.12.1 Example lagoon with suggested locations for topographic surveys

3. **Point collection (Intensive Approach).** If more in-depth topographic data is needed, technicians should establish the following topographic profiles and transects where safe and practicable (Figure 5.12.2) –

a. Main fluvial channel thalweg profile from the ocean inlet (starting at the foreshore) upstream to the watershed outlet
b. Thalweg profiles perpendicular from the main fluvial channel upstream into any distributory channels (ending at the marsh plain/floodplain)
c. Cross-sections of the main fluvial channel:
    i. At the ocean inlet
    ii. Immediately upstream of any beach/dune system
    iii. In between confluences with major (typically 2 nd order or above, or with a width greater than or equal to roughly one third the width of the main channel) distributory channels
    iv. At the watershed outlet/floodplain delta
    v. At /near any artificial hydraulic constraints such as bridges, embankments, or other infrastructure, on the upstream and downstream sides of those constraints
d. At least three representative profiles of the backbarrier/tidal marsh plain extending perpendicular from the main fluvial channel thalweg upslope through the estuarine-terrestrial transition zone if possible
e. At least two representative cross-sections of the beach from the foreshore, landward through the backshore, extending over any dune systems into the backbarrier/tidal marsh (tying into the profiles in (c) if practicable)
f. One shore-parallel profile of the backshore in the vicinity of the ocean inlet, especially if a backshore runnel is present
g. One shore-parallel profile of the dune crest in the vicinity of the ocean inlet

Points shall be collected along these profiles and cross-sections roughly every 2 m (or 1 m in smaller systems) and at major features/breaks in slope. Technicians shall also capture points at:

a. Water elevation loggers, to tie the recorded water elevation to the marsh plain measurements
b. In the vicinity of the sampling stations
c. At each vegetation quadrat

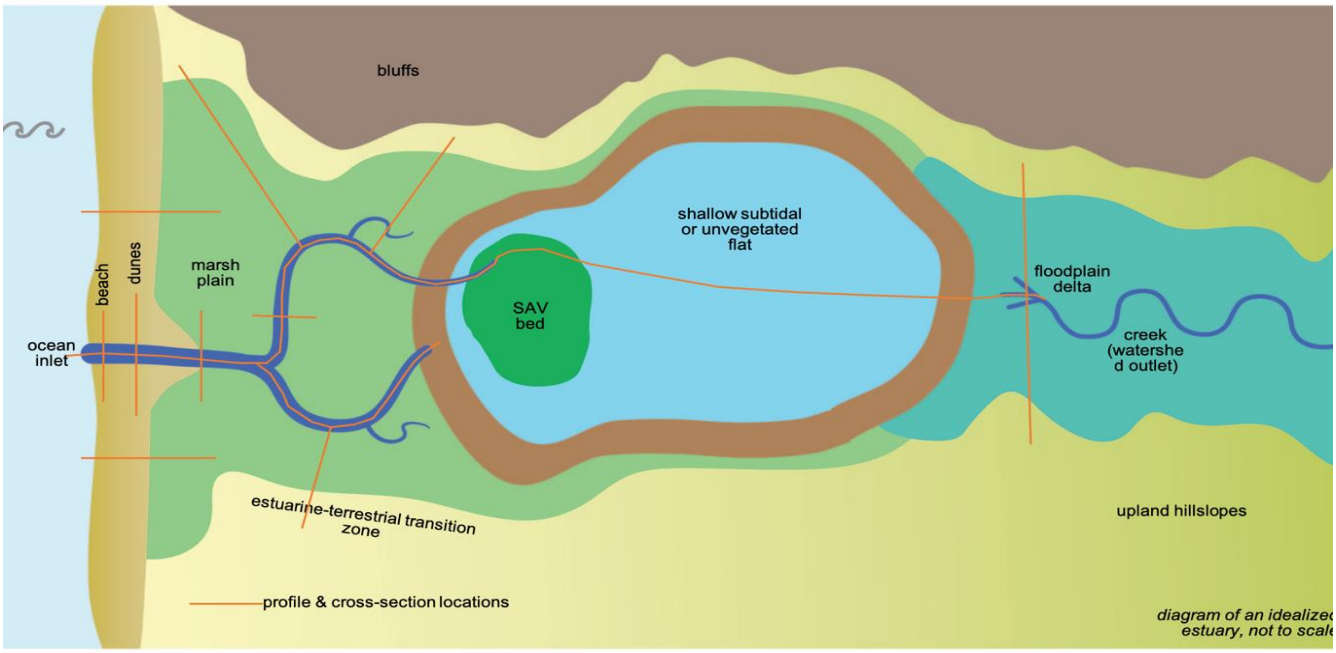


Figure 5.12.2 Example lagoon with suggested profile and cross-section locations (brown lines) for topographic surveys. RTK points will be taken every 1-2m along each cross-section depending on the size of the estuary. Not shown – RTK points should be taken at all data instrument locations.

4. Data Management

All elevation data will be uploaded to the Estuary MPA online data management system utilizing the SOP 14 data template. *Under development.*

SOP 13: Sediment accretion rates

Protocol:

Freeman, C.M, K.M. Thorne, and G. Guntenspergen. 2015. Surface elevation tables: standard operating procedure. US Geological Survey, Western Ecological Research center, San Francisco, CA.
https://www.usgs.gov/states/maryland/science/surface-elevation-table?qt-science_center_objects=0#qt-science_center_objects

Objective: This SOP provides a standard approach to quantify relative surface deposition and erosion on the marsh surface or pond bottom using feldspar.

Parameters: sediment accretion

Materials:

1. Feldspar clay
    a. There are many materials which you can use to quantify surface accretion.
    b. We have settled on using feldspar clay, following USGS protocols.
    c. Feldspar typically comes in 50 LB bags - From a single bag you can expect to get about 6 feldspar plots (50cm x 50 cm) established
2. Respirator mask for feldspar
3. 1⁄2" pvc or fiberglass poles - for marking the boundaries of the sampling area for the feldspar
4. Putty knife
5. Digital calipers or metric ruler

Field Methods:

Installation

1. Locations for feldspar will be marked on sampling maps prior to entering the field
    a. Feldspar areas should be paired with vegetation transects
    b. Locations should be on the marsh platform in the low elevation zone
    c. Depending on the estuary, 1 to 3 plots should be deployed
        i. If the system is dynamic with many marsh plains, higher replication should be used to capture potential variation
2. Mark the boundaries of the plot containing the feldspar using PVC poles (0.5m x 0.5m)
    a. Using a GPS, record the PVC pole locations
    b. If possible, the feldspar plot elevation should be recorded using an RTK
3. Using a small cup, sprinkle the feldspar on the marsh surface (Figure 5.13.1)
    a. There is enough material for about 6 plots (50 cm x 50 cm) per 50 LB bag.
    b. If the plot is vegetated, use an extra stake to knock the feldspar off the plants.

Figure 5.13.1 Feldspar plot. Photo from USGS 2015.

Collection

1. Feldspar samples should be collected one time per year (one year following installation)
2. Sediment plugs or brownies will be cut to extract sediment samples and measure sediment deposition over the feldspar.
    a. If you can still see the feldspar on the ground surface, do not take a measurement. You can estimate accretion to be <1 mm.
3. If you can no longer see feldspar on the ground surface, take a measurement
    a. Systematically select a location within the feldspar plot
        i. Sampling location = n*10cm from north west corner
        ii. n=number of years since creation, n>10 move to next 10cm "row")
        iii. See sampling grid, Figure 5.13.2.
    b. Start by cutting a small four-sided "brownie" with a putty knife, (about 3 cm x 3 cm) from within the feldspar marker plot (Figure 5.13.3).
        i. Use a putty knife, angled downwards, simply push in on all 4 sides to create an upside-down pyramid
    c. If there are extensive roots, it helps to have a sharp knife. A long-serrated kitchen knife works well.
4. Next pry the brownie out of the ground from one of the sides. The brownie will be about 3 cm x 3 cm x 6 cm in size
    a. The core needs to be deep enough to include the feldspar marker
    b. It's a bit more challenging to keep the brownie intact if the sediment is coarse-grained and sandy (like Carmel River Lagoon)
    c. Avoid touching the top of the brownie because you will be measuring the accretion of sediment on the top
5. Measure the depth of the feldspar marker on each side of the brownie (4 readings) from the surface to the feldspar, and calculate the mean (Figure 5.13.4).
6. Put the brownie back into the ground to minimize impacts to the marker area

1	2	3	4	5	6	7	8	9	10
11	12	13	14	15	16	17	18	19	20
21	22	23	24	25	26	27	28	29	30
31	32	33	34	35	36	37	38	39	40
41	42	43	44	45	46	47	48	49	50
51	52	53	54	55	56	57	58	59	60
61	62	63	64	65	66	67	68	69	70
71	72	73	74	75	76	77	78	79	80
81	82	83	84	85	86	87	88	89	90
91	92	93	94	95	96	97	98	99	100

Figure. 5.13.2. Sample extraction location grid by years since feldspar placement

Figure. 5.13.3. Example of a Feldspar “brownie.”

Figure. 5.13.4. Measuring the feldspar “brownie”. Photo from USGS 2015.

SOP 14: Trail cameras

Protocol:

SCCWRP. 2015. Hydrologic and Geomorphic Changes to Southern California Estuaries and Lagoons During Episodic Events Associated with the 2015-2016 El Niño: Insight to Potential Future Response to Sea Level Rise. Project Field Sampling Protocols.

TBF. 2021. Photo Point Standard Operating Procedures. Unpublished protocols. The Bay Foundation, Los Angeles, CA.

Objective: This SOP is to document temporal changes in berm/mouth condition, inundation, and berm topography.

Parameters: mouth dynamics

Materials:

1. GoPro or Reconyx cameras
2. Zip ties
3. Stake

One or more fixed photo-station(s) will be established at each estuary. At these camera locations, GPS coordinates, including height, and the direction(s) of the photo station will be documented. These stations will be used to document temporal changes in berm/mouth condition, inundation, and berm topography. If possible, semi-automated camera stations (e.g., GoPro or Reconyx cameras) will be deployed in order to capture morphological changes to the lagoon over the course of a few months. If a fixed photo station cannot be installed digital photos should be taken at several locations around the beach berm and surrounding nearshore area to capture any migration to the entrance channel as soon as possible and safe following storm events.