**SAMPLING FISH AND MACROINVERTEBRATE**

**RESOURCES IN TIDAL WETLANDS**

**SACRAMENTO-SAN JOAQUIN DELTA**

**Report on**

**PHASE IV PILOT MONITORING IN 2018**

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# Preface

Much of the tidal wetland restoration in the Sacramento-San Joaquin Delta and Suisun Marsh (the Upper SF Estuary) is being constructed under the premise that wetland restoration will increase the resilience of threatened fish. However, extracting patterns is very difficult because of the extreme spatial and temporal variability inherent in estuaries in general, and California estuaries in particular. Given limited funds, we must determine the correct level of replication to answer management questions without cost becoming prohibitive.

The Fish Restoration Program Monitoring Team (FRP) is tasked with developing monitoring plans for tidal wetland sites restored pursuant to requirements in the 2008/2009 Biological Opinions for state and federal water project operations (USFWS 2008, NMFS 2009, CDFW 2009). We led the Interagency Ecological Program (IEP) Tidal Wetlands Monitoring Project Work Team (PWT) in developing the Tidalwetland monitoring framework for the upper San Francisco Estuary (hereafter "Framework"; PWT 2017a). The PWT has developed a set of conceptual models and hypotheses for how wetlands benefit  
fish (Sherman et al. 2017). These were the basis for recommendations for sampling methods to evaluate  
effectiveness of restoration projects (PWT 2017b). However, there are still outstanding questions as to  
the appropriate temporal and spatial sampling strategies to test these hypotheses.

Meso- and macroinvertebrates, including amphipods, mysids, insects, copepods and isopods, are important food resources for tidal wetland fish, but are often patchily distributed and highly variable (Slater and Baxter 2014, Whitley and Bollens 2014, Baxter et al. 2015, David et al. 2016). The spatial and temporal variability inherent in these taxa make them difficult to monitor. While we have already tested several monitoring methods for these groups of invertebrates, monitoring change over time requires understanding the level of spatial and temporal replication necessary for statistical validity. Information on meso- and macroinvertebrates is necessary to address framework hypotheses F2-F5, which were derived from the PWT’s Food Web Conceptual Model (Secondary Production tier, Hartman et al., In Press) and Chinook Salmon Tidal Wetland Model (Environmental Drivers and Habitat Attributes tiers, Goertler et al. In Press).

Even for established methods, such as zooplankton trawls, more research is needed to determine the spatial and temporal extent of inference that can be made for a given metric. Multiple long-term monitoring surveys sample the pelagic realm for zooplankton using well-established methods (Hennessy et al. 2009). However, it is unclear the extent to which zooplankton communities differ between the deep channel habitat currently sampled and the wetlands that our program will sample (Kimmerer et al. 1998, 2002; Bollens et al. 2014). Understanding differences in communities between channels and wetlands is also necessary to detect exchange between these habitats that is predicted to increase food availability in the channel (Framework hypotheses F8-F10, Hartman et al., in In Press).

Fish are also highly variable across the Upper SF Estuary. Although fishes ranging from larvae to adults are sampled regularly, just a few sampling programs focus on small channel/shallow water/vegetation edge habitats. We have tested several different types of gear for catching fish in these habitats in the North Delta (Cache Slough Region). We need a better understanding of the function of these gears across estuarine gradients and how catches compare to long-term IEP monitoring. Fish community sampling in long-term monitoring is needed to determine the presence of listed species (hypotheses P4 and P14), to provide specimens of listed fish for various potential studies of diet, condition, and growth (hypotheses F4-F7), and to understand the potential for predation on and competition with the listed species (hypothesis S4), aspects of “capacity” in the Habitat Attributes tier of the Chinook Salmon Tidal Wetlands conceptual model (Goertler et al., In Press).

## Pilot Monitoring Phases

We conducted a Phase I “gear exploration” from July to October 2015 (Contreras et al. 2016). Based on results from that effort, successful methods were selected for inclusion in the second phase of pilot work. Phase II occurred from February through July 2016, and provided a more rigorous evaluation of gear feasibility during the time of year listed fish are most likely to be using wetlands (Contreras et al. 2017). Phase II included quantitative comparisons of abundance, size, composition, and diversity which led to recommended gear types. The recommended gear types from Phase II were used in the Phase III study that further refined the spatial and temporal sampling schemes necessary to evaluate monitoring hypotheses, and began testing the extent to which IEP’s channel-based monitoring surveys can be used to make inferences on tidal wetlands.

After each pilot phase, results were reviewed by the PWT before inclusion in development of plans for the next phase. The results of all our research will be considered by the PWT in recommending methods for future long-term monitoring. Note that final decisions on the best approaches for long term sampling will be made based on the results of this pilot effort, as well as many additional factors such as take of listed species, logistics, cost, resource availability, and the availability of comparable data from other sampling programs.

## Project Objectives

* Determine the extent to which long-term IEP sampling reflects conditions in nearby shallow-water and wetland habitats.
* Determine whether gear efficiency evaluations are feasible using new sampling technology (i.e., ARIS sonar).
* Determine the level of spatial and temporal replication necessary to make sampling design recommendations for long-term monitoring.
* Begin developing a baseline of biomass, community composition, and fish condition for fish and invertebrates near planned tidal restoration and comparison sites. This will allow us to make pre-and-post-restoration comparisons for evaluating restoration progress.

## 

# Part 1: Phytoplankton and Invertebrate spatial and temporal variability

Project Component Lead: Rosemary Hartman

## Introduction

Understanding the variability of zooplankton and macroinvertebrates will allow us to evaluate appropriate timing and replication of samples for characterizing macroinvertebrate communities that provide fish food in restored tidal wetlands. Macroinvertebrates associated with vegetation and shallow water habitat, such as amphipods and insect larvae, have been historically under-studied in this system; however, they provide the majority of salmonid diet composition in these areas (Sommer et al. 2001, Maier and Simenstad 2009, Bottom et al. 2011, David et al. 2016), and are a component of Delta Smelt diets when smelt occur in areas of high macrophyte production (Whitley and Bollens 2014). Because these groups are understudied, it is unclear how variable biomass and community composition are within a site, between sites and between seasons. Because epifaunal invertebrates are a smaller percentage of smelt diets (Slater and Baxter 2014) and are less mobile than zooplankton, it may only be necessary to sample these macroinvertebrates once or twice per year. If sampling is limited, we want to determine what time of year has greatest overlap between listed fish species and their food supply.

SAY SOMETHING ABOUT THE IMPORTANCE OF FALL FOOD AVAILABILITY!!

Phytoplankton are considered to be one of the key carbon sources in the aquatic food web. However, not all phytoplankton are created equal. Diatoms and green algae are preferred over cyanobacteria and flagellates in most Cladocera and copepod species studied in this estuary (Orsi 1995), though more recent studies suggest cyanobacteria make up a large part of copepod diets when they occur in high abundance (Stephanie Owens, SFSU, pers. Comm.). Furthermore, preferred food differs by species of zooplankton and species of phytoplankton (Bouley and Kimmerer 2006, Jassby 2008) (Cloern and Dufford 2005). In the past, most evidence pointed to pelagic phytoplankton as the key driver of the Bay-Delta food web (Canuel et al. 1995, Sobczak et al. 2002, Sobczak et al. 2005), however, more recent work suggests benthic, epiphytic, and wetland-derived carbon may plan a more important role than previously realized (Schroeter et al. 2015).

Given the importance of species identity when assessing phytoplankton’s role in the food web, we need some phytoplankton community monitoring on FRP sites. Once we start regular monitoring of restoration sites, we will take phytoplankton samples concurrently with monthly zooplankton samples. However, we should first establish a baseline of phytoplankton variability within the wetlands. Therefore, this year I want to collect phytoplankton concurrently with the “spring blitz” sampling of macroinvertebrates.

Study questions:

1. How do invertebrate and phytoplankton communities change from year to year?
   1. How does water year type affect community composition?
   2. How do invertebrate communities change post-restoration? (not assessed this year)
2. Are there significant differences between channel habitat, managed wetlands (pre-restoration), and tidal wetlands (remnant and/or post-restoration)?
3. What food is available for listed fish species throughout the year?
   1. How do fall food resources compare to spring food resources?
   2. When during the spring is most important to sample?

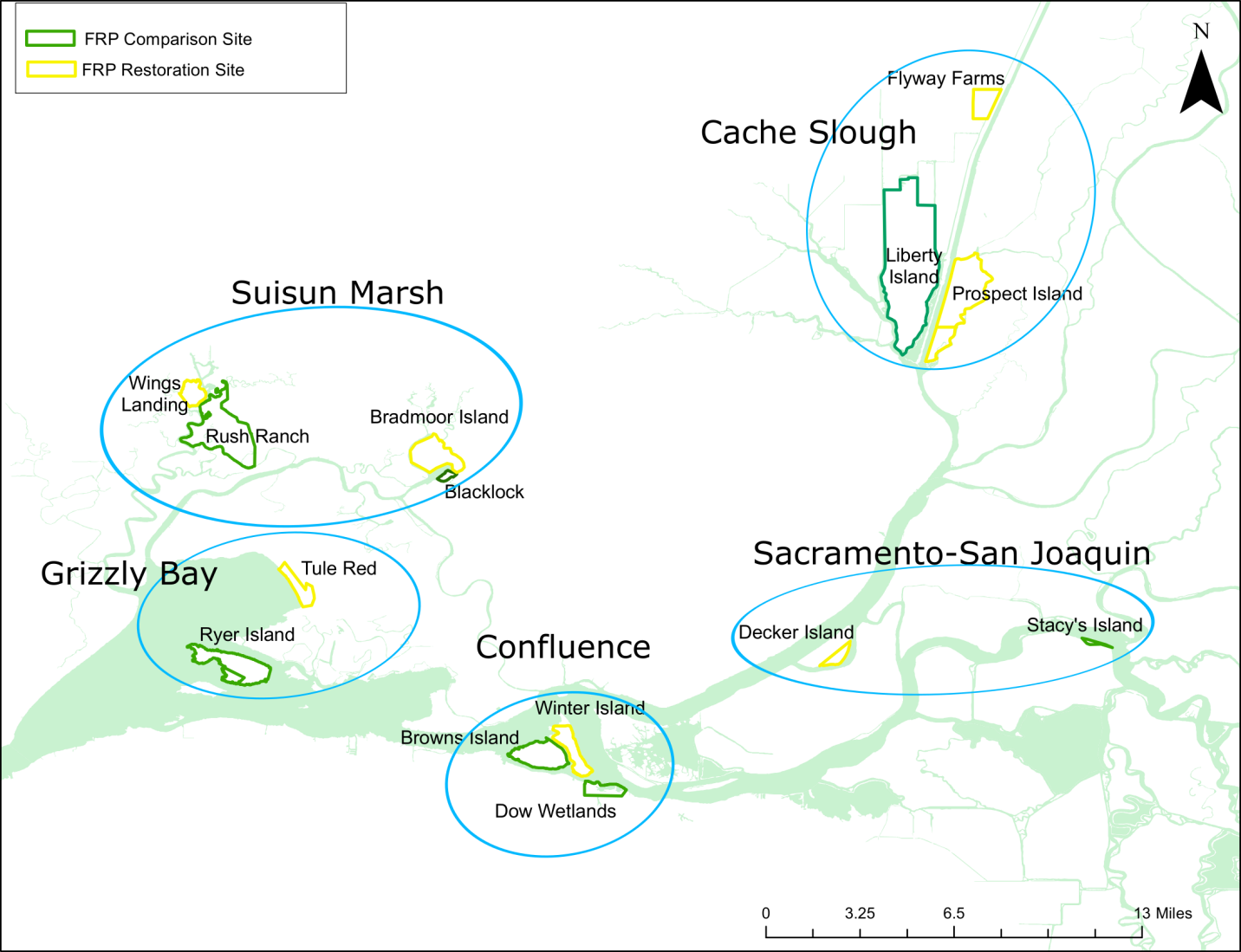
## Methods

### Sampling Sites

To answer questions 1 and 2, sampled FRP restoration sites and surrounding wetlands distributed across the Delta and Suisun Marsh FRP (Table 1, Figure 1). These sites incorporate varying salinity and surrounding land use (the “ecoclines” identified in the IEP TWM PWT conceptual models, see Hartman and Sherman, in press). Sites for 2018 were an expanded set of sites sampled in 2017, and repeating many of these sites will allow for year-to-year comparisons. Coupling pre-restoration sampling with comparison sites will allow for a Before-After, Control-Impact design, once restoration is completed on some of these sites. While we do not expect our restoration sites to develop exactly like the comparison sites, the network of comparison sites provide a background, or “ambient” condition for the estuary that will allow us to better see the effectiveness of the restoration actions.

To answer question 3, we conducted increased sampling at one site (Decker Island) four times throughout the spring, and sampled in the fall at a subset of the locations where Delta Smelt are found most often. We chose spring for our spatially intensive sampling, because this is when there is the highest density of juvenile salmonids rearing in the estuary (Williams 2012), Delta Smelt are spawning in the freshwater Delta (Sommer and Meija 2013; Baxter et al. 2015), and Longfin Smelt are present and spawning in the marshes surrounding Suisun Bay (Grimaldo et al., in press). However, the importance of food supply during the fall, while juvenile smelt area rearing, is considered one of the major factors in population resilience (Brown et al. 2014; Baxter et al. 2015). Therefore, chose to characterize potential food supply in areas important to smelt in the fall (October or November).

Due to limited resources, we will targeted fall sampling only in areas likely to have high smelt abundance. In most years, a majority of the population migrates to the Low Salinity Zone (LSZ), to rear (Baxter et al. 2015). This area generally stretches from Suisun Bay to the Confluence, though exact location depends on water year type. An analysis of data from the Fall Midwater Trawl Survey from September and October 2010-2016 showed the majority of the smelt caught where in this area (Figure 2. CDFW data available: https://www.wildlife.ca.gov/Conservation/Delta/Fall-Midwater-Trawl). There is an additional, resident population in the Cache Slough Complex (Baxter et al. 2015), so we sampled the subset of wetland sites near these “smelt- hot- spots” during the fall, at a lower intensity than spring sampling. Before conducting the fall sampling, we used data from the FMWT and the USFWS’s Enhanced Delta Smelt Monitoring survey to asses the most recent data on Delta Smelt distribution.

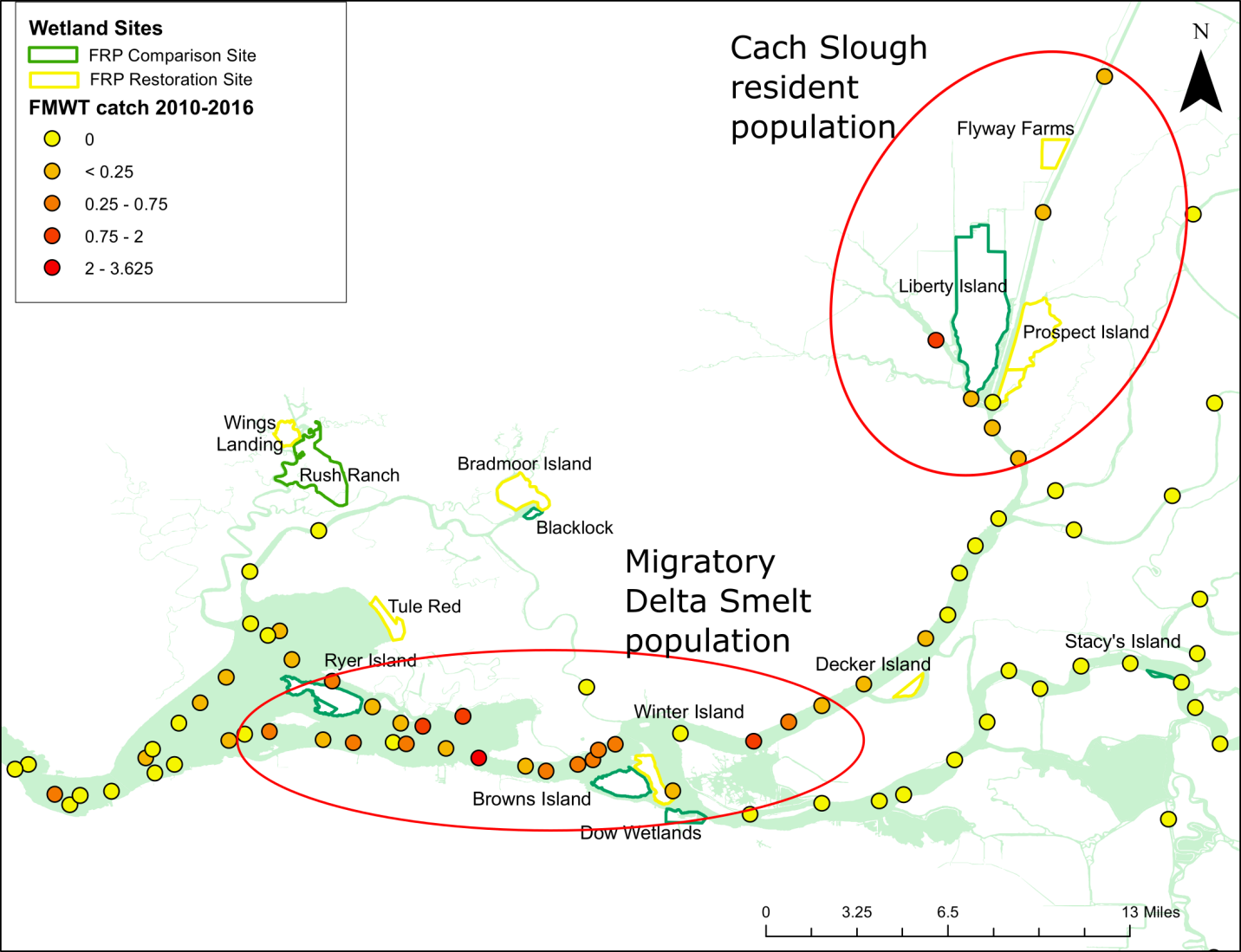


**Figure 1.** Sites that were sampled during the spring of 2018. Within each region (outlined in blue), we compared pre-project data at planned restoration sites (where relevant) and from currently diked or muted tidal wetlands, with data from associated channel habitat and post-restoration data (where available).

**Table 1.** Sample sizes for spring sampling in Phase IV. Sample numbers differ based on site size and habitat availability. Where possible, we will coordinate with existing studies to avoid double-sampling. Decker Island will be sampled more frequently than the other sites.

\* sampling types with the potential to take listed fish.

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Samples near | region | site type | Vegetation (maximum) | Benthic core | Mysid trawl\* | Neuston \* | Phytoplankton | Zoop trawl\* | **Total** |
| Winter Island | Confluence | diked wetland | 12 | 6 | 6 | 6 | 6 | 6 | **36** |
| Browns Island | Confluence | tidal wetland | 10 | 6 | 6 | 6 | 6 | 6 | **34** |
| IEP station 801 | Confluence | channel | 4 | 4 | 4 | 4 | 6 | 4 | **20** |
| Tule Red | Grizzly Bay | diked wetland | 12 | 6 | 6 | 6 | 6 | 6 | **36** |
| Ryer Island | Grizzly Bay | tidal wetland | 12 | 6 | 6 | 6 | 6 | 6 | **36** |
| Grizzly Bay | Grizzly Bay | shallow open water | 0 | 6 | 6 | 6 | 6 | 6 | **24** |
| Decker Island | Sac River | diked wetland | 16 | 12 | 12 | 12 | 15 | 12 | **64** |
| Stacy’s Island | Sac River | tidal wetland | 4 | 4 | 4 | 4 | 6 | 4 | **20** |
| IEP station 705 | Sac River | channel | 6 | 4 | 4 | 4 | 6 | 4 | **22** |
| IEP station 724 | Cache Slough | channel | 4 | 4 | 4 | 4 | 6 | 4 | **20** |
| Flyway Farms | Cache Slough | tidal wetland | 6 | 4 | 4 | 4 | 6 | 4 | **22** |
| Prospect Island | Cache Slough | tidal wetland | 12 | 6 | 6 | 6 | 6 | 6 | **36** |
| Liberty Island | Cache Slough | tidal wetland | 10 | 6 | 0 | 6 | 24 | 0 | **22** |
| Blacklock | Suisun | tidal wetland | 6 | 4 | 4 | 4 | 6 | 4 | **22** |
| Bradmoor | Suisun | diked wetland | 10 | 6 | 6 | 6 | 6 | 6 | **34** |
| Little Honker Bay | Suisun | shallow open water | 10 | 6 | 6 | 6 | 6 | 6 | **34** |
|  |  | **Total** | **144** | **96** | **90** | **96** | **129** | **90** | **516** |



**Figure 2.** Sites that were sampled in fall of 2018. Colored dots represent long-term Fall Midwater Trawl sampling location, with color indicating average size of Delta Smelt catch during September and October, 2010-2016. Largest Delta Smelt catches occurred in the Low Salinity Zone near the Confluence and entrance to Suisun Bay, with an additional “hot-spot” in the Cache Slough complex. We concentrated fall sampling on these regions.

**Table 2.** Sample sizes for fall sampling in Phase IV. \* sampling types with the potential to take listed fish.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Samples near | region | site type | Vegetation (maximum) | Mysid trawl\* | Zoop trawl\* | **Total** |
| Winter Island | Confluence | diked wetland | 6 | 6 | 6 | **18** |
| Browns Island | Confluence | tidal wetland | 6 | 6 | 6 | **18** |
| Ryer Island | Grizzly Bay | tidal wetland | 6 | 6 | 6 | **18** |
| Prospect Island | Cache Slough | managed | 6 | 6 | 6 | **18** |
|  |  | **Total** | **30** | **30** | **30** | **90** |

### Habitat Types and Sampling gears

#### Vegetation

Previous studies showed very high replication was necessary to differentiate between vegetation types (Contreras et al. 2016, 2017). Therefore, we randomly distributed our sweep net samples through all vegetation types present on the site in proportion to their abundance, rather than specifically targeting samples from each vegetation type. We haphazardly choose 6-12 sampling locations per site and used sweep nets to sample vegetation.

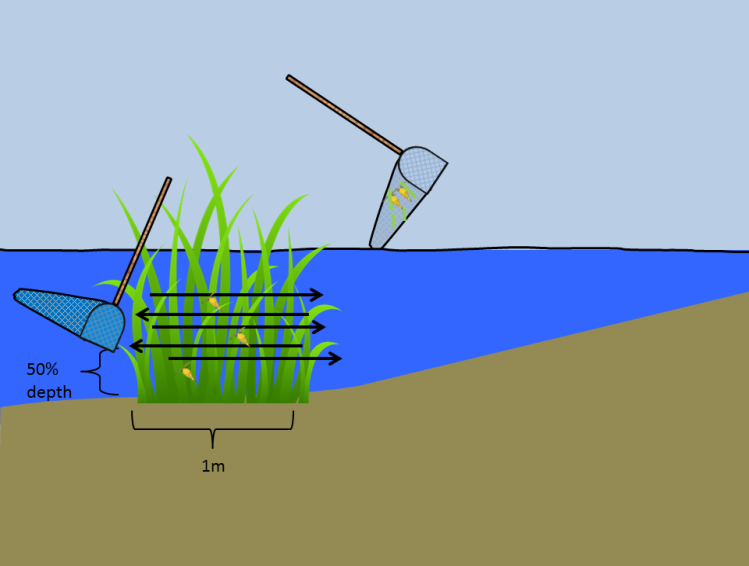
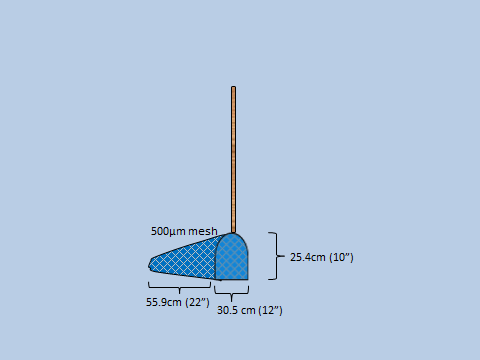
**Sweep nets:** Sweep nets are a simple but effective way to sample the invertebrate community. Sweep nets may capture higher species diversity than many passive methods, though with higher variability in biomass (Turner and Trexler 1997).We will use a 25cm x 30cm D-frame net with 500 micron mesh for all sweep net samples (Figure 3A). The sweep net technique wase adapted slightly in different vegetation types.

**EAV:** Emergent vegetation (EAV) samples occurred in the dominant emergent vegetation species, usually either Schaenoplectus spp., Typha spp. , or Phragmites astralis. All samples will be taken within 1m of the edge, since vegetation will be less dense and easier to sample. This is also the area where salmonids have been shown to forage most effectively (Simenstad and Cordell 2000). We took five, one-meter sweeps through the edge of the vegetation, scraping the vegetation as much as possible to knock invertebrates off the stems (Figure 3B). We then rinse down the net and preserved all invertebrates in ethanol for later identification.

**SAV:** Submerged vegetation may have adverse effects on native fish due to the high occurrence of invasive predatory fish (Ferrari et al. 2014), but it may also provide high invertebrate production (Rozas and Odum 1988). To sample in SAV, we took the same five, 1-meter sweeps as for EAV and collected any vegetation within the border of the net after the sweep is completed (Figure 3D). The sample will be placed on ice for processing in the lab. In the lab, we will rinse the vegetation, remove all invertebrates, and preserve the invertebrates for later identification. Any vegetation captured in the sweep net will be dried to a constant weight to standardize the sample.

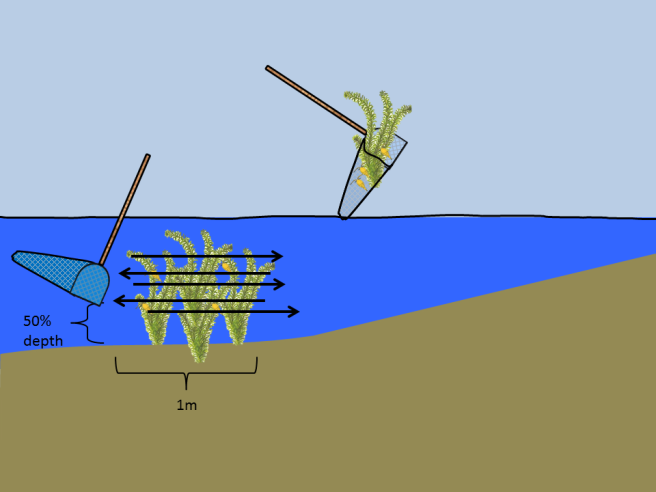
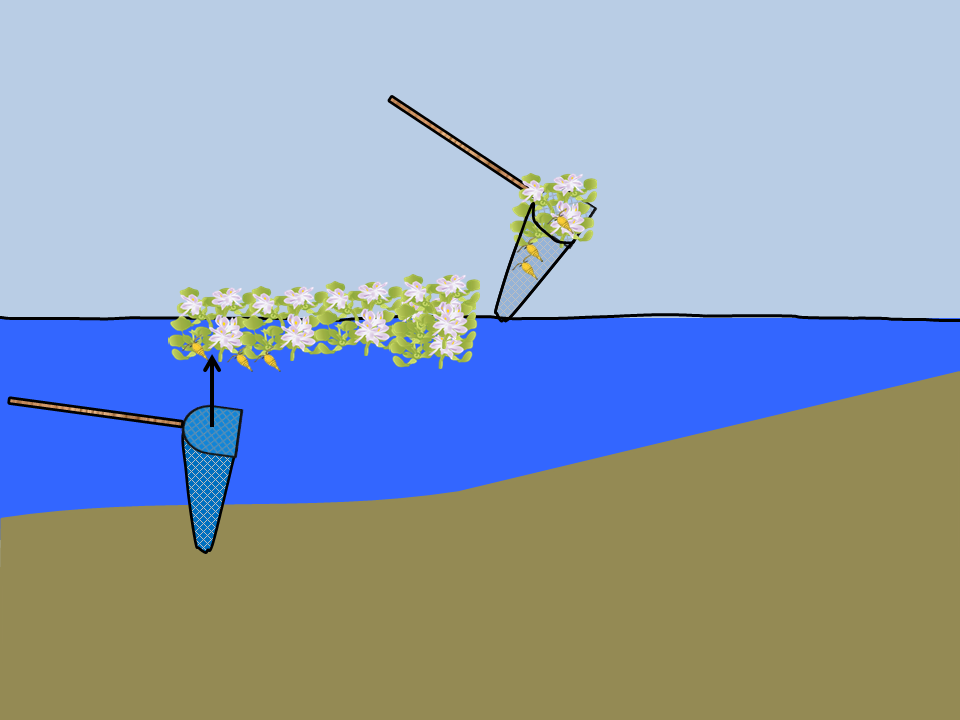
To allow us to make inferences for broad-scale invertebrate-vegetation relationships, we will take an SAV rake sample immediately after collecting a sweep net sample at each SAV site (see Chapter 5).

**FAV:**  Sampling techniques in FAV will depend on FAV species. For *Eichhornia crassipes*, *Hydrocotyle,* and *Azolla*, we harvested a 25cm x 30cm sample from below using the same d-frame net and severing the connection to surrounding plant material with shears (Figure 3C; methods currently being used by USDA aquatic weeds lab, E. Donely Marineau, pers. comm). We placed the roots of the plant material and associated invertebrates on ice. Upon return from the field, we will separate the invertebrates from the vegetation and dry the plants to a constant weight. *Ludwigia* spp. is a creeping emergent, and does not form discrete, easy-to-harvest clumps. Therefore, it will be sampled with five 1-meter sweeps, as for EAV.



B

A



C

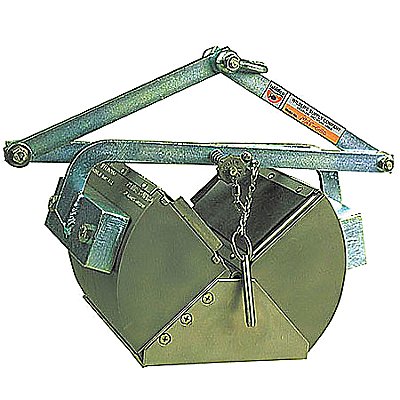
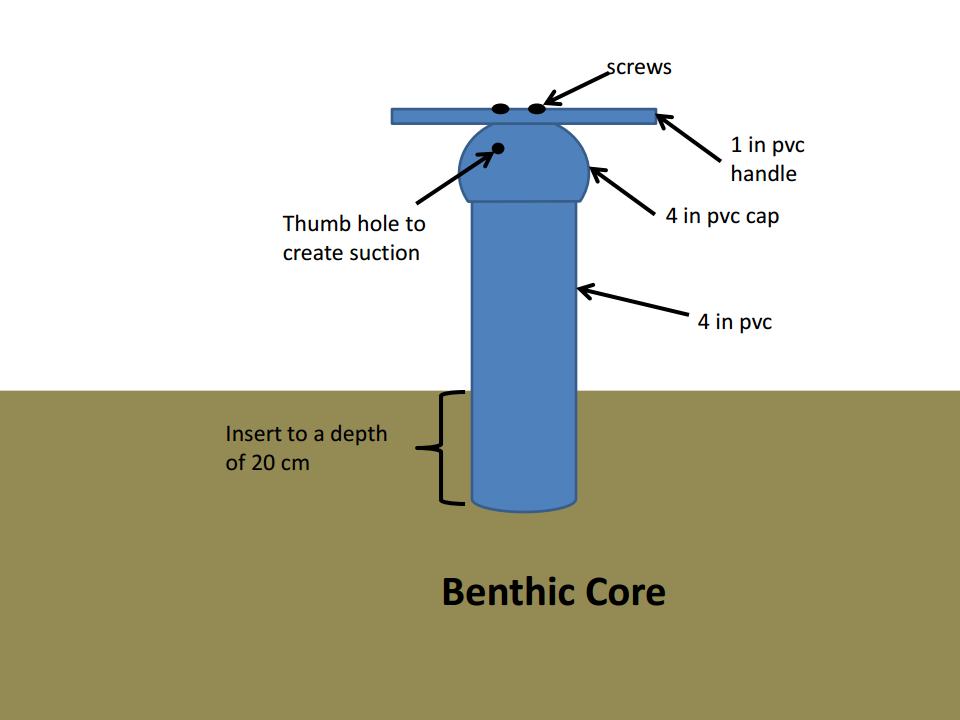
D

**Figure 3:** **A)** Specifications of the sweep net. **B)** Use of sweep net in emergent vegetation. **C)** Use of sweep net in submerged vegetation. **D)** Use of sweep net in floating vegetation.

#### Open water and channel

Our open water sampling patches were haphazardly distributed across all unvegetated open water and channels > 1.5m across. Methods used in open-water have a long history of use in monitoring in the Delta, and will allow us to compare our measurements in vegetated wetlands to conditions in channels and make comparisons to long-term data sets. Methods included: zooplankton and mysid trawls, PVC cores and ponar grabs.

**Benthic core:** Benthic cores have been used extensively to quantify chironomid and amphipod populations, as well as bivalves and other infauna in tidal wetlands (Wells 2015, Howe et al. 2014, CDFW unpublished data). While many chironomids and amphipod life stages present in fish diets are pelagic (S. Slater, pers. comm), they also have benthic life stages. In shallow water (<1.5m), we took a 4in (20cm) diameter benthic core (Figure 5A), hand-deployed to a depth of 20 cm. In deep water >1.5m, we will use a 9x9 in ponar grab modified for use in hard substrates (as per USFWS Liberty Island Monitoring, L. Smith pers. comm, figure 5B), with three samples at each site. This may be in conjunction with substrate analysis during fish sampling to minimize disturbance and maximize efficiency. The core will be washed and sieved on board the boat to remove the sand/mud and preserve any organic detritus and invertebrates. Two crew members will estimate % silt, sand, and gravel in the field, and average the values. We will calculate effort as catch per surface area of substrate sampled.



**Figure 5**: A) Benthic core made of 4” PVC pipe for use in shallow water (<1.5 meters). B) Ponar grab for use in water greater than 1.5 meters.

**Mysid and zooplankton nets:** Mysid nets have been used extensively to characterize macrozooplankton in the water column. Macrozooplankton includes amphipods and mysids that are large components of fish diets (Feyrer et al. 2003, Slater and Baxter 2014). We sampled macrozooplankton in the water column using a 50 cm mouth diameter (0.500mm mesh size) mysid net and sampled mesozooplankton with a 14.6 cm diameter (0.150 mm mesh size) zooplankton net attached or held alongside (similar to EMP methods, Hennessy 2009). These nets were held approximately 1 m to the site of the bout, 10 cm below the surface of the water and trawled at 1-2 mph for five minutes. A flowmeter mounted in the net measured sample volume, and effort was standardized by catch per liter of water sampled.

Where channels are less than 1.5m in width, and no open water is present, we placed the net in the channel on an ebb tide, and allow the tidal current to flow through the net for five minutes instead of trawling the net. After retrieval, the nets were be rinsed from the outside to wash down the sample into the cod end. All content collected in a cod end was preserved in 70% ethanol for identification in the lab.



**Figure 6**. Set up of oblique sled for mounting mysid and zooplankton nets.

**Neuston tow:** Emerging insects and collembola found at the surface of the water are an important feature in salmonid diets, and are commonly sampled using neuston tows and drift nets (Sommer et al. 2001, Howe et al. 2014). The neuston net is a 45cm x 30 cm rectangular net, 1m long with 0.500mm mesh towed half-way out of the water to sample invertebrates on the surface of the water (Figure 7A, B). We will tow the neuston net at the surface of the water from the side of the boat via a davit or boat-hook. In narrow channels, we may pull the net along the edge of emergent vegetation by hand (Figure 7C; as in Howe et al. 2014). We will standardize effort by the distance of the tow calculated by GPS track multiplied by width of net to calculate surface area of water sampled. After retrieval, all content collected in a cod end will be preserved in 70% ethanol for later ID. Crew will remove and release any fish that are visible in the sample before preservation.

**Phytoplankton:** At each zooplankton trawl site, a single, 150 mL sample of water will be collected from the surface and preserved with Lugol’s iodine for identification of phytoplankton community composition.

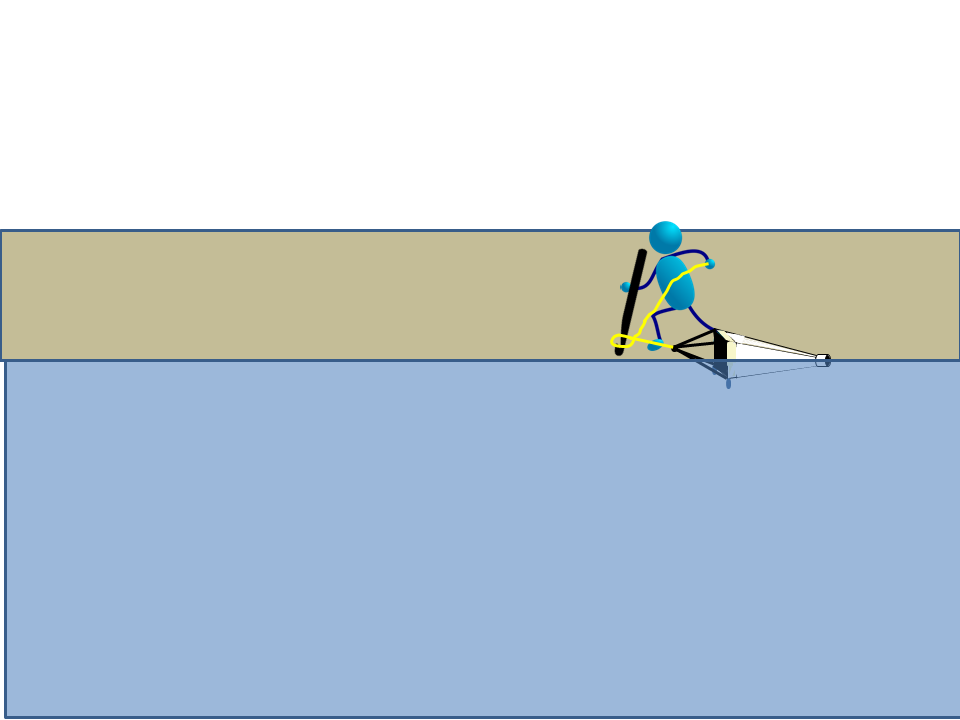
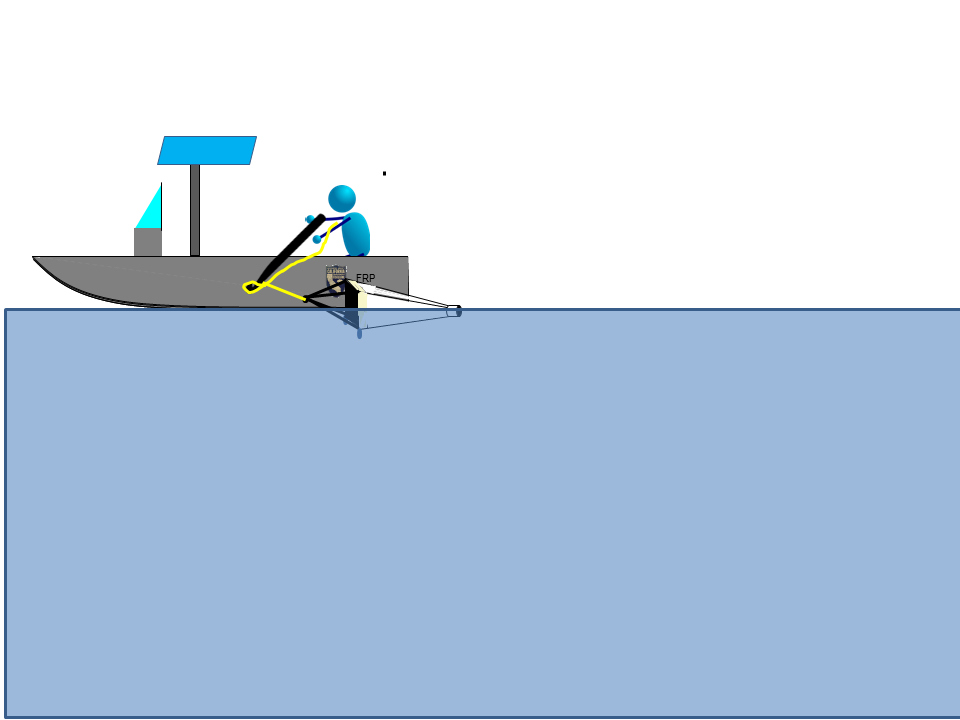
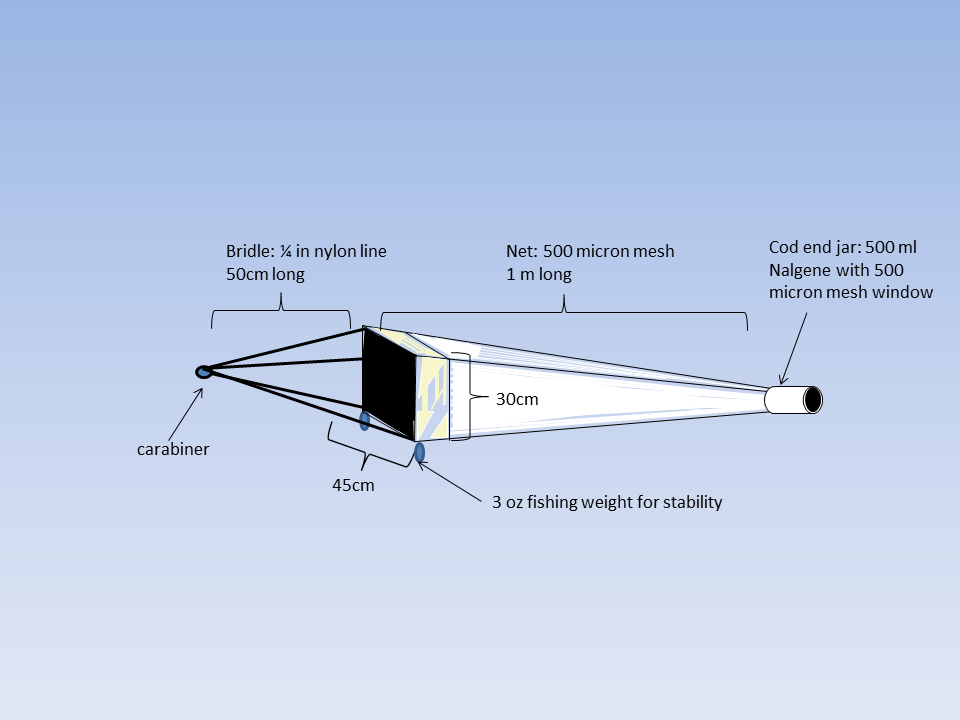
#### Phytoplankton spatial variability

To better differentiate between the sources of phytoplankton in the water column, we will intensively sample a wider variety of habitats at one site (Liberty Island). At Liberty, will take phytoplankton from four microhabitats within the wetland:

1. 6 Submerged vegetation samples – algae will be scraped from a 10-cm section of Egeria densa, or dominant form of submerged vegetation
2. 6 emergent vegetation samples – algae will be scraped from a 10-cm section of *Schoenoplectus acutus*, or dominant form of emergent vegetation.
3. 6 Benthic samples (Epipelic or Episammic) – algae will be rinsed from the mud/sand collected by a 10-cm PVC core, or petite ponar grab
4. 6 Pelagic – phytoplankton collected as per “Water Quality Grab Samples SOP”

To answer question 3, we will conduct increased sampling at one site (Decker Island) throughout the spring, approximately every two months (January, March, May, June, August). Additional phytoplankton community composition, chlorophyll, and nutrient samples will be taken during phytoplankton blooms, if large-scale blooms are observed. “Blooms” will be categorized as any event with chlorophyll concentrations >=10 ug/L, or when phytoplankton is visibly observable in the water column. We will contact all long-term monitoring programs that regularly sample *chl-a* or zooplankton during the spring and summer and ask to be informed if a bloom may be occurring.

Cell counts and community composition will be processed by EcoAnalysts.



**Figure 7:** A) Specifications for the neuston net. B) Deploying the neuston net alongside a boat. C) Deploying the neuston net from shore.

### Laboratory Methods

**Taxonomic effort:** Invertebrates will be sorted to taxonomic level according to their importance in fish diets (see Table 3). Mysids, isopods and amphipods will be sorted to Genus; insects will be sorted to Family. Zooplankton caught incidentally in macroinvertebrate samples may be identified to a higher level of identification (Family or Order) but will be identified to Genus or Species in zooplankton samples. The first twelve individuals of each taxonomic group per sample will be measured to the nearest 0.1mm using an ocular micrometer. Benthic infauna (most importantly invasive bivalve grazers *Corbicula* and *Potamocorbula*), are not commonly found in salmon or smelt diets; however, their indirect influence on the food web makes them important to predicting availability of production to the pelagic food web (Lucas and Thompson 2012, Kimmerer and Lougee 2015). Therefore, all bivalves will be identified to genus and measured along the longest axis to the nearest mm.

#### Macroinvertebrates

All samples will be sorted to extract invertebrates from plant material and detritus, and invertebrates will be identified by a Senior Laboratory Assistant (SLA) or Scientific Aide. A subset of samples will have identifications checked by an Environmental Scientist for quality assurance. Another subset of samples will be checked by an outside lab (Wayne Fields of Hydrobiology), for external quality assurance.

**Subsampling:** Approximately 400 invertebrates from each sample will be identified. If more than 400 invertebrates are present in a sample, or more than four hours are required for processing, they will be quantitatively sub-sampled using a grid tray.

**Table 3.** Levels of taxonomic resolution recommended for each group of taxa commonly found in invertebrate samples.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Phylum | Subphylum | Class | Order | Level of ID |
| Annelida |  | all | all | Class |
| Arthropoda | Chelicerata | Arachnida | all | Class |
| Arthropoda | Crustacea | Maxillopoda: Copepoda | all | Genus |
| Arthropoda | Crustacea | Malacostraca | Amphipoda | Genus |
| Arthropoda | Crustacea | Malacostraca | Cumacea | Class |
| Arthropoda | Crustacea | Malacostraca | Decapoda | Species |
| Arthropoda | Crustacea | Malacostraca | Isopoda | Genus |
| Arthropoda | Crustacea | Malacostraca | Mysidea | Species |
| Arthropoda | Crustacea | Branchiopoda | Cladocera | Genus |
| Arthropoda | Crustacea | Ostracoda | Podocopida | Order |
| Anthropoda | Hexapoda | Collembola | All | Class |
| Anthropoda | Hexapoda | Insecta | All | Family |
| Mollusca |  | Bivalvia | All | Genus |
| Mollusca |  | Gastropoda | All | Family |
| Nematoda |  | All | All | Phylum |
| Platyhelminthes |  | All | All | Phylum |

#### Zooplankton

All samples will be filtered and washed in a 150 μm mesh sieve. Filtered zooplankton will be diluted to a set volume depending on the concentration of zooplankton and/or detritus. 1mL subsamples will be placed on a Sedgewick-Rafter cell (S/R cell) glass slide. All organisms will be identified to the taxonomic resolution identified in Table 2. At least 5 slides, but no more than 20 slides will be processed for each sample, targeting 6% of the total sample. This subsample will then be extrapolated to calculate the total number of organisms in the sample in individuals per m2.

All samples will be processed by a trained Senior Laboratory Assistant (SLA). A subset of samples will have identifications checked by a second SLA for quality assurance.

#### Phytoplankton

### Analysis

We will calculate catch-per-unit-effort (CPUE) and biomass-per-unit-effort (BPUE) for each sample using formulas listed above. We will use published and ongoing diet studies of juvenile Chinook Salmon and Delta Smelt (CDFW unpublished data, Slater and Baxter 2014) to look for degree of overlap in taxa present in fish diets and in our samples, and calculate separate measures of CPUE and BPUE with only taxa important to salmon or smelt diets.

To answer Question 1 on the inter-annual variation in macroinvertebrates, we will compare samples from 2018 to samples from 2017 and 2016 for sites that were sampled in multiple years. We will use mean catch, CPUE and BPUE to compare biomass and density across sites within years and between years using generalized linear models (GLMs). We will test the fit of all possible models and their first-order interactions using Akaike’s Information Criterion corrected for small sample sizes (AICc) (Anderson 2008, Gotelli and Ellison 2012). Environmental variables may be used as co-variates to explain potential differences in catch between areas, and final models will be presented graphically with standard errors. Since we only have two to three years of data per site, we will not be able to differentiate between variance due to water year type and variance due to other inter-annual factors, however the same analyses can be conducted in future years when we have more data per water year type. This data will also provide the “Before” of our Before-After Control-Impact design.

To answer Questions 2 on the differences between wetland sites, we will compare CPUE, BPUE, and community composition of samples from our spatially extensive spring sampling event of 2018. These different wetland sites will provide the “Control-Impact” blocks for our BACI design in future analyses. We will analyze the data from the subset of stations with both Spring and Fall sampling by analyzing CPUE of organism found in Delta Smelt diets for the life stage at observed in proximity to the sites.

We will use mean catch, CPUE and BPUE to compare biomass and density across habitat types within sites, among sites, and among regions using generalized linear models (GLMs), with the predictor variables listed in Table 4. We will test the fit of all possible models and their first-order interactions using Akaike’s Information Criterion corrected for small sample sizes (AICc) (Anderson 2008, Gotelli and Ellison 2012). Environmental variables may be used as co-variates to explain potential differences in catch between areas, and final models will be presented graphically with standard errors.

To detect differences in community composition, we will permutational multivariate analysis of variance, non-metric multidimensional scaling, and/or canonical correspondence analysis using the same set of predictor variables.

**Table 4.** Potential predictor variables

|  |  |  |
| --- | --- | --- |
| Variable | Variable type | Description |
| Region | Categorical | Region of the estuary as shown on Figure 1 |
| Site type | Categorical | Depth and water management regime (diked wetland, tidal wetland, shallow open water, or channel) |
| Habitat type | Categorical | Depth of water and presence of vegetation (Emergent wetland, SAV, FAV, open-water, or benthic) |
| Distance from GG | Continuous | Distance from the Golden Gate, in Km. |

To answer Question 3, we will analyze the four sampling events from Decker Island to see when CPUE and BPUE of fish food invertebrates are maximal. We will test the fit of linear and quadratic equations to see when biomass peaks. Hydrologic variables, such as river stage, degree days, or Delta outflow may be used as covariates, where necessary. We will compare any trends in invertebrate catch to trends in salmon and smelt catch at nearby long-term monitoring stations using Man-Kendall tests or Granger tests to see whether highest fish food availability occurs at the same time as fish presence. This may be repeated for specific fish life stages and size classes of invertebrates as necessary (i.e., Smelt Larvae Survey catch compared to copepod nauplii, DJFMP juvenile Chinook salmon catch compared to amphipods and chironomids). We will use these analyses to make recommendations of timing of macroinvertebrate sampling for long-term monitoring programs. We will also use our comparisons between the spring and fall sampling events to recommend where and to what extent fall sampling should occur.

Data used to answer our questions on timing and replication of samples will also provide pre-project data for evaluating the food web benefits of restoration sites.

1. To answer our first question on the major differences between the phytoplankton communities in the wetland channel versus the exterior channel, we will first categorize the water-column phytoplankton based on their most likely habitat. This will be done be comparing relative abundance of various taxa in the water column at Liberty Island with the abundance of tax on each microhabitat (SAV, EAV, benthic, pelagic). Data from Liberty will be supplemented by literature review on algal growth forms and expert opinion (Tiffany Brown, DWR”s EMP study). We will then compare community composition of phytoplankton in the larger set of wetlands to samples collected by FRP in the exterior channels, or data collected by EMP’s phytoplankton survey. These data will be compared using PerMANOVA or other multivariate methods, as appropriate.
   1. We expect to see significantly different communities exiting the wetlands than in the exterior channel, and differences will be driven by higher abundances of epibenthic and epiphytic taxa in the wetland channels.
   2. Samples from the surface of vegetation and benthic substrates within the wetland will have greater overlap in community composition when compared to tidal channels than with exterior channels.
2. To answer our second question on differences between wetlands, we will compare community composition from channels within diked wetlands and tidal wetlands across the salinity regime. These data will be compared between management regimes using PerMANOVA, and across the salinity gradient using CCA, or other multivariate methods, as appropriate.
3. To answer our third question on seasonal variability, we will compare community composition at Decker Island over time.
   1. We expect to see an increase in the proportion of cyanobacteria in samples of phytoplankton from tidal channels later in the spring/summer, as assessed by a beta regression or permutational MANOVA. This will likely require multiple years of data before it can be shown statistically.

## Results

## Discussion

# Chapter 2: Channel-Shoal Gear Comparison

## Introduction

### Nutrients

### Zooplankton

Mesozooplankton are recognized as the largest component of Delta Smelt diets (Slater and Baxter 2014) and a significant component of salmon diets (Sommer et al. 2001). Our conceptual models postulate that tidal wetland restoration sites will have higher production and availability of zooplankton when compared with existing channel habitat and pre-project conditions (Hartman et al., in press). In order to support this hypothesis, we must compare zooplankton we collect within the wetland to zooplankton collected from the channels. We will leverage existing datasets from long-term monitoring programs currently sampling pelagic and channel habitat whenever possible, but we need a better understanding of how these samples compare to samples taken concurrently from adjacent wetlands. Water depth, substrate, presence of vegetation, presence of benthic grazers (clams), and differences in fish community may alter the zooplankton community (Bollens et al. 2014, Kimmerer et al. 2014). Furthermore, changes to the physical environment will affect the efficiency of our sampling gear.

By sampling wetlands concurrently with existing channel sampling, we can characterize some of these sources of variation. During Phase III sampling we conducted sampling of wetland habitat adjacent to eight of the long-term stations sampled by 20mm, however the extremely high water year of 2017 meant that results from that study may not be applicable to all years. Therefore, we will repeat our spring sampling alongside some 20mm sites, and add fall sampling alongside the Fall Midwater Trawl (FMWT) survey’s zooplankton sampling stations. We will test for differences in zooplankton catch between channel habitats in which IEP samples and the shallow littoral habitats in which we sample. This will also give us a better understanding of the spatial variability in zooplankton in wetlands across the estuary.

In the original study plan, we proposed sampling zooplankton concurrently with CDFW’s 20 mm and Fall Mid-Water Trawl surveys. Instead, we would like to replace some of our concurrent sampling with 20mm and FMWT with concurrent sampling during CDFW/DWR’s Environmental Monitoring Program (EMP) sampling at some of our FRP stations (i.e. sample concurrently at EMP site 64/D22 instead of 20mm & FMWT site 706, Figure 8). This change will allow us to compare both mesozooplankton and macrozooplankton (mysids), and will allow us to add an analysis of wetland nutrients. The FRP sampling locations, timing, and effort will not change, and listed species take will not increase.

Mesozooplankton questions:

1. How do mesozooplankton and macrozooplankton communities in the littoral and wetland habitat compare to open water habitat?
   * How do these communities change over the course of the year?
   * How do these communities change along the salinity gradient?

Proposed Nutrient questions:

1. Are there differences in nutrients, chlorophyll, and organic carbon concentrations between the wetland and the exterior channel?
2. Are nutrients limiting phytoplankton production?
3. Are excess nutrients a causal factor for harmful algal blooms on our sites?

### Fish

The extent to which at-risk fish species will benefit from tidal wetland restoration in the San Francisco Estuary is unknown (Brown 2003, Herbold et al. 2014). However, restored wetlands in other areas have shown to be productive food sources and provide refuge from predation (Gray et al. 2002, Shreffler et al. 1992, Simenstad et al. 1982). The Fish Restoration Program Monitoring Team was established to monitor the benefits of tidal wetland restoration to at-risk fish species in the San Francisco Estuary. Comparing fish communities and their condition pre- and post-construction can inform how at-risk fish benefit from tidal wetland restoration.

Littoral habitat provides benefits to at-risk fish species, such as salmon, which rear in littoral areas, and Delta Smelt, which inhabit the littoral zone to maintain their position during ebb tides when migrating (McLain and Castillo 2009, Bennett and Burau 2015). However, many of CDFW’s long term monitoring studies sample open water habitat due to gear size, boat size, and absence of vegetation. Sampling littoral and open water habitat simultaneously can provide insights into how fish species utilize different habitats. Similar to the 2017 work plan, the Fish Restoration Program will sample littoral habitat near planned tidal wetlands concurrently with mid-channel sampling by the IEP Summer Townet and Fall Midwater Trawl surveys. For 2018, work will be expanded to tidal wetland reference sites near Ryer and Browns Island.

## Methods

### IEP Surveys

The EMP survey monitors water quality, phytoplankton, meso-/marco-zooplankton, and benthic invertebrates in the upper estuary throughout the year. Zooplankton is collected using a steel sled with paired mesozooplankton (160 μm mesh) and macrozooplankton (500 μm mesh) nets. Phytoplankton is sampled using water collected from submersible pump.

The 20mm Survey monitors Delta Smelt distribution throughout their historical spring range in the Sacramento-San Joaquin Delta and San Francisco Estuary during the spring. The 20mm survey targets Delta Smelt in the post-larval and juvenile life stage, at lengths >20mm. The net is a cone shaped plankton net 5.1 meters in length with an opening circumference of 4.9 meters (1.5 cubic meters). Zooplankton is collected concurrently with a 160 μm mesh modified Clarke-Bumpus net mounted on the frame with its own flowmeter. The survey samples at 40 stations throughout the estuary and completes three 10-minute tows at each station. Zooplankton are only sampled at the first of these tows (Damon, 2015).

The FMWT survey was designed to study Striped Bass distribution throughout the upper estuary, but has since become an integral part of monitoring Delta Smelt and Longfin Smelt distribution and abundance during the fall. Beginning in 2010, meso- and macro-zooplankton sampling was added at 32 of the 122 regular fish sampling sites. Zooplankton is collected after fish trawling is complete, using a same sized EMP steel sled with paired mesozooplankton (160 μm mesh) and macrozooplankton (500 μm mesh) nets. We will sample near five EMP sites, five 20mm sites, two FMWT sites, in adjacent tidal channels or fringing marsh (Table 5, Figure 8), using a paired mysid and zooplankton net as described in Chapter I (Figure 6). These sampling sites were chosen based on their proximity to future FRP restoration sites or comparison wetlands. Thus, we will be able to use these stations to establish a pre-project baseline for zooplankton production, and determine to what extent the effect of restoration is detectable in nearby long-term survey monitoring. Note that while we will discontinue the 2017 macroinvertebrate sampling in Lindsey Slough, we will continue to survey zooplankton alongside 20mm to increase our power to compare data between years.

### FRP Sampling

#### Zooplankton

FRP gear and methods are easily comparable to IEP’s methods. The most important difference is our gear will be trawled obliquely through the water for five minutes instead of ten minutes, to reduce potential for take of listed fishes. If the tidal channels or marsh habitat is too short to take a full five-minute tow, the tow time will be reduced. Alternatively, gear may be held in the mouth of a tidal channel to sample water flowing out of the channel on an ebb tide instead of being trawled.

EMP collects samples monthly, but our sampling will be limited to March-June and September-December. The 20mm survey samples every two weeks, usually March-June. We will sample monthly (every other 20mm survey), in nearby wetlands as close to the same time as possible. If it is not possible to sample at the same time as the 20mm survey, we will sample the following day at the same point in the tidal cycle. The FMWT survey samples once per month, September-December. We will sample concurrently in nearby wetlands as close to the same time as possible. If it is not possible to sample at the same time as the FMWT survey, we will sample the following day at the same point in the tidal cycle.

#### Nutrients

Nutrient sampling methods will follow methods used by EMP as closely as possible. At each FRP sampling site, we will take two to three nutrient samples. Three samples will be taken at wetland sites that have tidal influence. We propose to take one sample:

1. Deep within the wetland as possible, where the water will have the greatest influence from the wetland and least influence from the channel;
2. At breach/outlet of the site where water is actively moving in or out of the site;
3. Approximately 100 m outside the site, where we expect some influence of the wetland on water quality in the surrounding channel.

At pre-restoration sites that have no tidal action, we propose to collect a sample:

1. Within the managed wetland, if there is water on the site and access is feasible;
2. Approximately 100 m outside the planned breach location.

We will collect 2 L of water using a bucket. For dissolved nutrient samples, we will filter 100 mL water and save the filtrate on ice; for total nutrients, we will fill the sample bottle with unfiltered water, place the sample on ice, and freeze as soon as possible. We will measure chlorophyll florescence from 10 cm below the water’s surface using a YSI 6600 sonde. The sonde will be calibrated once per week using grab samples of environmental water analyzed for chlorophyll and pheophytin at Bryte labs.

#### Fish

During routine open water monitoring of the Summer Townet and Fall Midwater Trawl surveys, a beach seine will be used to concurrently sample the littoral habitat adjacent to existing wetlands or future restoration sites. During instances a beach seine cannot be used to sample, a lampara net will be used to sample the site.

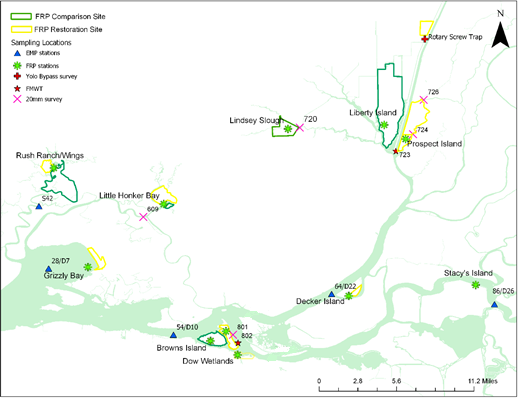
The beach seine measures 15m long x 1.2m high (3mm delta square mesh) net and is deployed one of two ways.

Primary Beach Seine Deployment Method (Standard Method): One crew member will walk out into the water (up to 1.2m in depth) holding one end of the net to measure the width and depth of the seine site. The second crew member will walk to the first crew member and place their seine pole where depth was recorded. The first crew member will walk parallel to the length of the shore and note seine length and site depth. Both crew members will haul the beach seine up on the shore, leaving the cod end bag in the water. The crew will fill a tub with water and place the cod end bag in the tub along with any fish caught in the “wings” of the seine.

Secondary Beach Seine Deployment Method (“Drag and Drop” Method): There are instances where the sampling substrate is composed of ankle high soft mud and difficult/unsafe to walk on. During these instances, a boat will help deploy the net. The boat will drive to one side of the sampling beach area. Both crew members and the beach seine will get off the boat and onto shore. A line will be wrapped around the middle of the beach seine and tied to the bow of the boat. Both Crew members will each grab one side of the beach seine close to where the net is tied. The boat will slowly back up and the crewmembers will keep the net taught to prevent the net from touching the surface of the water. Once crewmembers reach the end of their wing, the boat will remove the tie around the middle of the beach seine and drop it into the water. Both crew members will haul the beach seine up on the shore, leaving the cod end bag in the water. The crew will fill a tub with water and place the cod end bag in the tub along with any fish caught in the “wings” of the seine.

Lampara net sampling will occur if no beach is present in shallow habitat (< 3m). The vessel will deploy one side of the gear that is attached to an empty anchor bag (the bag fills with water) and buoy. The net will be deployed in a circular fashion around the site (much like a purse seine). The anchor bag will be brought back onboard and both sides of the net will be retrieved back onboard. The crew will fill a tub with water and place the cod end bag in the tub along with any fish caught in the “wings” of the lampara net.

All ESA-listed fish will be measured to nearest fork length millimeter (mm). For all other fish species, 30 fish will be measured to the nearest fork length mm and all remaining fish will be counted.



**Figure 8**. Zooplankton sampling stations. We will take zooplankton macroinvertebrates, and nutrients samples from shallow wetland habitats (green stars) in proximity to established 20mm sampling sites (pink Xs), established FMWT zooplankton sites (orange shrimp), and EMP sites (blue triangles).

**Table 5.** Sample numbers for meso- and macro-zooplankton trawls and nutrient samples taken concurrently with other monitoring programs.

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Survey | Months | | FRP Site | Long Term Site | Mysid Trawl | Zoop Trawl | Nutrient Water Sample | Total |
| 20mm | March-June | | Little Honker/Blacklock | 609 | 4 | 4 | 24 | 32 |
| 20mm | March-June | | Lindsey Slough | 720 | 4 | 4 | 12 | 20 |
| 20mm | March-June | | Prospect Island | 724 | 4 | 4 | 12 | 20 |
| 20mm | March-June | | Liberty Island | 723 | 4 | 4 | 12 | 20 |
| 20mm | March-June | | Winter, Dow | 801 | 8 | 8 | 24 | 40 |
| FMWT | Sept-Dec | | Prospect, Liberty | 723 | 8 | 8 | 24 | 40 |
| FMWT | Sept-Dec | | Winter, Dow | 802 | 8 | 8 | 24 | 40 |
| EMP | March-Dec | | Grizzly Bay | 28/D7 | 8 | 8 | 16 | 32 |
| EMP | March-Dec | | Rush Ranch/Wings | S42 | 8 | 8 | 24 | 40 |
| EMP | March-Dec | | Decker Island | 64/D22 | 8 | 8 | 24 | 40 |
| EMP | March-Dec | | Stacy’s Island | 86/D26 | 8 | 8 | 24 | 40 |
| EMP | Sept-Dec | | Browns | 54/D10 | 4 | 4 | 12 | 20 |
|  |  |  | |  | 76 | 76 | 232 | 384 |

### Lab methods

Zooplankton will be processed using the same methods as IEP’s 20mm Survey. In brief:

All samples will be filtered and washed in a 150 μm mesh sieve. Filtered zooplankton will be diluted to a set volume depending on the concentration of zooplankton and/or detritus. 1mL subsamples will be placed on a Sedgewick-Rafter cell (S/R cell) glass slide. All organisms will be identified to the taxonomic resolution identified in Table 2. At least 5 slides, but no more than 20 slides will be processed for each sample, targeting 6% of the total sample. This subsample will then be extrapolated to calculate the total number of organisms in the sample in individuals per m2.

All samples will be processed by a trained Senior Laboratory Assistant (SLA). A subset of samples will have identifications checked by a second SLA or Environmental Scientist for quality assurance.

Macrozooplankton will be processed using the same methods as described in Chapter 1. Because the FMWT survey only enumerates mysids and amphipods in their samples, we will only compare catches of these taxa, and use catch of other taxa for separate analyses of spatial variability.

Nutrient samples are processed at DWR’s Bryte laboratory using standard methods (http://www.water.ca.gov/bdma/meta/discrete.cfm).Timing

Processing of zooplankton samples is expected to take 2-8 hours per sample, depending on density of the sample and amount of algae. Processing mysid samples is expected to take 1-4 hours per sample. We expect all samples to be processed within 6-8 months. If processing time is prohibitively long, we will re-evaluate subsampling procedures, and zooplankton samples will be processed preferentially over the remaining mysid samples.

### Analysis

To compute biomass per unit effort (BPUE), we will use the average biomass by life stage established for common zooplankton taxa collected by the 20 mm Survey and FMWT survey (Hennessy unpublished data).

To answer question 1 on spatial variability, we will compare CPUE and BPUE from samples collected across habitat types and over the salinity gradient using generalized linear models (GLMs), with predictor variables listed in Table 6. We will test the fit of all possible models and their first-order interactions using AICc (Anderson et al. 2000, Gotelli and Ellison 2012), though we may not be able to incorporate both Collection Group and Habitat type in the same model due to co-linearity. Environmental variables may be used as covariates to explain potential differences in catch between areas.

We will also use analysis of similarities, non-metric multidimensional scaling, and/or canonical correspondence analysis to test differences in community composition between habitat types and across the salinity gradient.

To test differences in nutrients and carbon between the wetland and the channel, we will graph chlorophyll, nutrients, and organic carbon versus sampling location (inside, breach, outside, far outside), over time at each site. A generalized linear model will be attempt compare these values statistically, but it is unlikely any detection will occur with one year of data. To determine whether nutrients are limiting phytoplankton production, we will compare nutrient and chlorophyll concentrations in the wetland to published literature values for nutrient concentrations and ratios in the Delta. A GLM of all the chlorophyll and nitrogen data will be best modeled by a parabolic curve or show a threshold of chlorophyll above which nitrogen decreases. To answer causal factors for algal blooms, we will compare visual reports of *Microcystis* and other cyanobacteria with nitrogen concentrations.

**Table 6.** Potential predictor variables for models of spatial and intra-annual variation in zooplankton catch and biomass.

|  |  |  |
| --- | --- | --- |
| Variable | Variable type | Description |
| Julian day | Continuous | Day of year sample was collected |
| Collection group | Categorical | EMP, 20mm survey, FMWT survey, or FRP survey |
| Habitat type | Categorical | Deep channel, tidal channel, or shoals. |
| Distance from GG | Continuous | Distance from the Golden Gate, in Km. |

This work will help us determine the future level of replication necessary to support assessment of restoration project goals. Data used to answer our questions on timing and replication of samples will also provide pre-project data for evaluating the food web benefits of restoration sites.

## Results

## Discussion

# CHAPTER 3: Methods Development

Project Component Lead: Dave Contreras

## Introduction

### ARIS Sonar

Due to procurement issues obtaining an ARIS sonar device this year, we would like to reinitiate a component from the 2017 work plan. Last year, we briefly worked with the USGS observing how fish behave within wetland breach sites using Dual frequency IDentification SONar (DIDSON) and Adaptive Resolution Imaging Sonar (ARIS) devices. We used gill nets and boat electrofishing to confirm species identity, but because we fished within the sonar field-of-view, we were able to observe fish response to the gears (e.g., a few fish appeared swim towards the gill net and then do a u-turn away from the net). We would like to see if an ARIS sonar device can be used to determine how effective electrofishing and gill net sampling techniques are, and whether particular biases of the methods could be identified. Full gear efficiency studies are labor intensive, requiring planning and the recognition of the limitations of the evaluation technique. We propose to explore gear evaluation techniques in 2018 and plan a comprehensive gear efficiency study for gill netting and boat electrofishing for 2019. We will also explore the extent to which an ARIS would be useful in monitoring fish communities in wetland habitats that are difficult to sample with other gears (e.g., adjacent to emergent vegetation).

Fish study questions:

* Can the ARIS sonar be used as a tool to determine gill net and electrofishing efficiency?
* Can ARIS sonar be used to monitor fish use of wetlands with decreased take of listed species?

### SAV methods devlopment

## Methods

### Location and Timing

Sampling will occur from Suisun Bay to the North Delta near existing managed or tidal wetlands, some of which are slated for restoration: Ryer Island, Tule Red, Bradmoor Island, Winter Island, Decker Island, and Prospect Island (Figure 9). Sampling sites were chosen based on the ability to sample effectively and in proximity to established long-term monitoring sites (Figure 9).



- Summer Townet

- Fall Midwater Trawl

- Beach Seine or Lampara Haul

- ARIS & Electrofishing or Gill Net

**Figure 9.** Map of the proposed sampling sites. Four ARIS locations will be selected at each transect.

Sampling will occur from January through December of 2018 (Table 7). All beach seine or lampara sampling will begin in June and end by December to coincide with Summer Townet and Fall Midwater Trawl surveys. ARIS evaluations of the boat electrofisher and gill net will occur from January to June.

**Table 7.** Maximum sample numbers for gear types and sampling period. \* denotes long term monitoring gear types.

|  |  |  |
| --- | --- | --- |
| **Gear Types** | **N** | **Sampling Period** |
| Beach Seine (or lampara) | 156 | Jun-Aug |
| Townet\* | 42 |
| Beach Seine (or lampara) Midwater Trawl\* | 88  36 | Sep-Dec |
| Boat Electrofisher with | 30 | Jan-May |
| ARIS |  |
| Gill Net with | 30 | Apr-Jun |
| ARIS |  |

### Description of Sampling Methods

Data collected with all samples include: date, location, weather, tide stage/water depth, water velocity, surface and bottom specific conductance, surface and bottom water temperature, DO,

### ARIS Evaluation of Boat Electrofisher and Gill Net

An Adaptive Resolution Imaging Sonar (ARIS) will be used to evaluate the efficiency of boat electrofishing and gill net sampling in the vicinity of Decker and Prospect Islands. Four sites will be sampled in both Horseshoe Bend and Miner Slough using the following methods. An ARIS unit will be mounted on the hull of a kayak to record the presence and behavior of fish in a defined sampling area. The kayak will make a slow pass of the sampling site approximately 15m away and record fish presence within the sampling area. Once the site has been recorded with the ARIS, the site will be electrofished. A Smith-Root electrofishing vessel with a 5.0 GPP electrofisher will be used to sample the site following the ARIS recording. Crew members will stand on the bow of the vessel operating a foot pedal, using eight to ten second bursts of electricity along one shoreline. All fish will be collected with a 5mm mesh dip net and placed in a live well, measured, and counted.

To estimate efficiency of gill net sampling, an ARIS unit will be mounted on the gill net vessel. The gill net will measure 30.5m long x 1.8m high and is composed of various mesh panels, where the largest mesh panel is 15.2cm. Gill nets will be set at four sites in both Horseshoe Bend and Miner Slough. Each net will be deployed by a vessel parallel to the shore, anchored by two 8lb weights. As the net is sampling, a slow pass will be made with the ARIS unit along the net face to record fish behavior near the gear. After 60 minutes of sampling, the ARIS unit will be shut off and the gill net retrieved. All fish will be placed in a large bin with water. All ESA-listed fish will be measured. Only 30 fish of other species will be measured; all remaining fish will be counted. In the office, fish counts and length estimates will be made from images captured by the ARIS unit.

### SAV survey techniques

Rake collections

Sonar w/Biobase

## Analysis

The littoral and open water habitat catch-per-unit-effort (CPUE), species composition, and fork lengths will be summarized for each species in a table or graph. Size-frequency distributions will be plotted for common and listed fishes by habitat type. Environmental data will also be summarized by habitat type in a table. The CPUE of fish captured and size frequency distributions between the lampara net, townet, and midwater trawl will be analyzed using an ANOVA or non-parametric equivalent, and Kolmogorov-Smirnov two sample tests. MANOVA, ANOSIM, or other multivariate analyses will be used to test for differences in community composition.

Gear efficiency trials using the block net, sonar, and electrofishing will be qualitatively analyzed. Selection of possible sampling locations, set-up time, and learning how to effectively sample will be the focus of these trials. Data gathered from successful gear efficiency trials can be used during a formal 2019 gear efficiency test.

## Results

## Discussion

Chapter 4: Submerged Aquatic Vegetation

Contact: Daniel Ellis

1 Feb 2018

Adjustments:

SAV mapping using a sonar is limited by the boat’s ability to drive safely above the SAV. In channels, SAV is often found along the banks, where water is shallower and flow slower. This is also an area that is covered by floating vegetation. That vegetation, along with the narrow sonar beam in shallow depths, makes sonar mapping of those areas nearly impossible (for example: interior channels of Browns and Winter). For these reasons: sonar mapping at sites with such channels will not be conducted near the banks. The sonar will be utilized while travelling up the main channels primarily to capture the presumed absence of SAV in those channels. Assessments of SAV at sites across the delta will be compared using the methods laid out in #1. SAV mapping will be an auxiliary tool but not the primary tool.

**Notes:**

1. Many more hypotheses can be proposed for the long-term. The following hypotheses focus on SAV and invertebrates. Fish have not been addressed yet here ☹ .
2. Questions 1 thru 4 utilize the same methodology. Questions 5 and 6 have unique methods.

**Major SAV questions:**

1. **What is the natural variability in SAV cover, composition, and turnover at intertidal wetland sites around the delta (site-level)?**

**Rationale:** In order to contextualize transformations of habitats at wetland restoration sites it will be imperative to understand the landscape-level factors that control large-scale trends in SAV cover and composition. This question will require multiple years of study.

**Hypotheses:**

1. Natural patterns of SAV cover, composition, and turnover will vary with local and landscape-level factors. After years of data collection, sufficient data should allow for the uncovering of the most important parameters that control SAV.

**Methods (SAV Mapping):**

1. **Pre-field work**

**Site selection:**

1. **Liberty Island & Prospect Island**

Rationale: This pair of reference and restoration sites has varying densities of SAV cover. The sites can be used to compare SAV turnover and invertebrate associations in the Cache Slough area.

**Note:** There is no expectation that Liberty and Prospect Island are directly comparable sites this year, since Prospect is currently muted tidal, and has very different habitat. Post-restoration, their habitats should increase in similarity, and post-restoration changes can be accessed.

1. **Browns Island & Winter Island**

Rational: This pair of sites has varying SAV cover as well. These sites can be used to test the same questions in the higher-salinity region near the confluence.

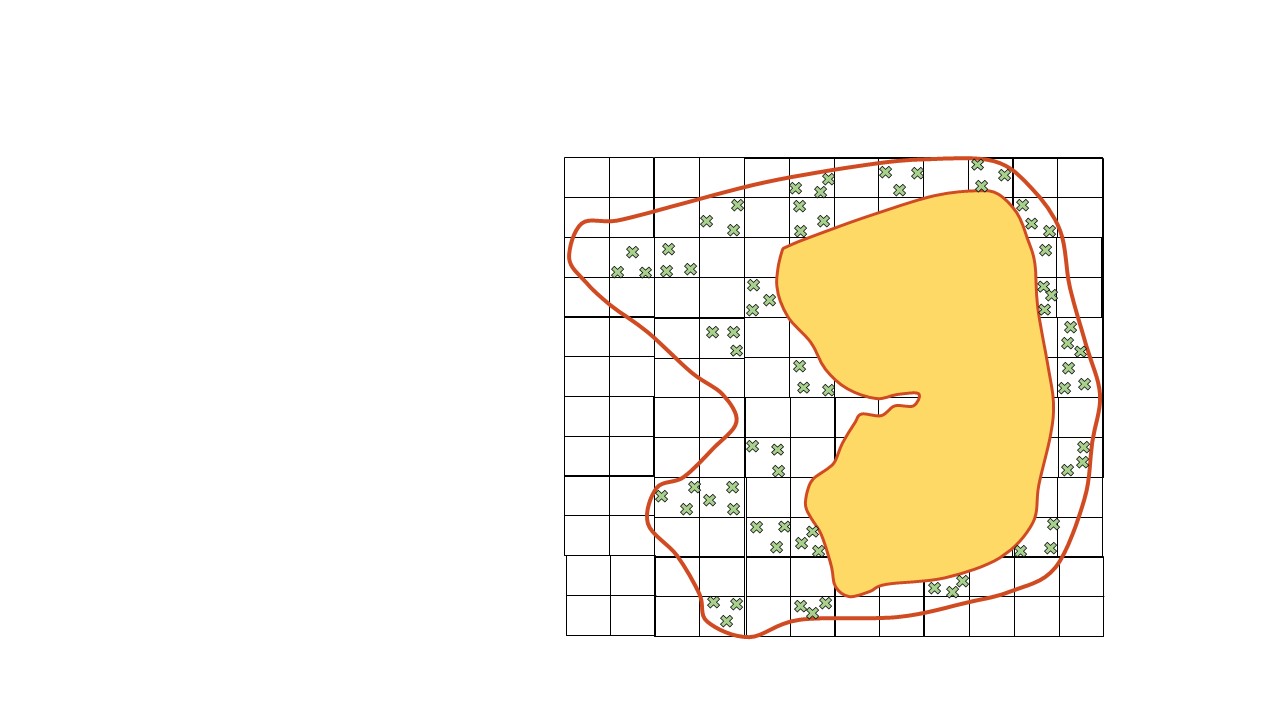
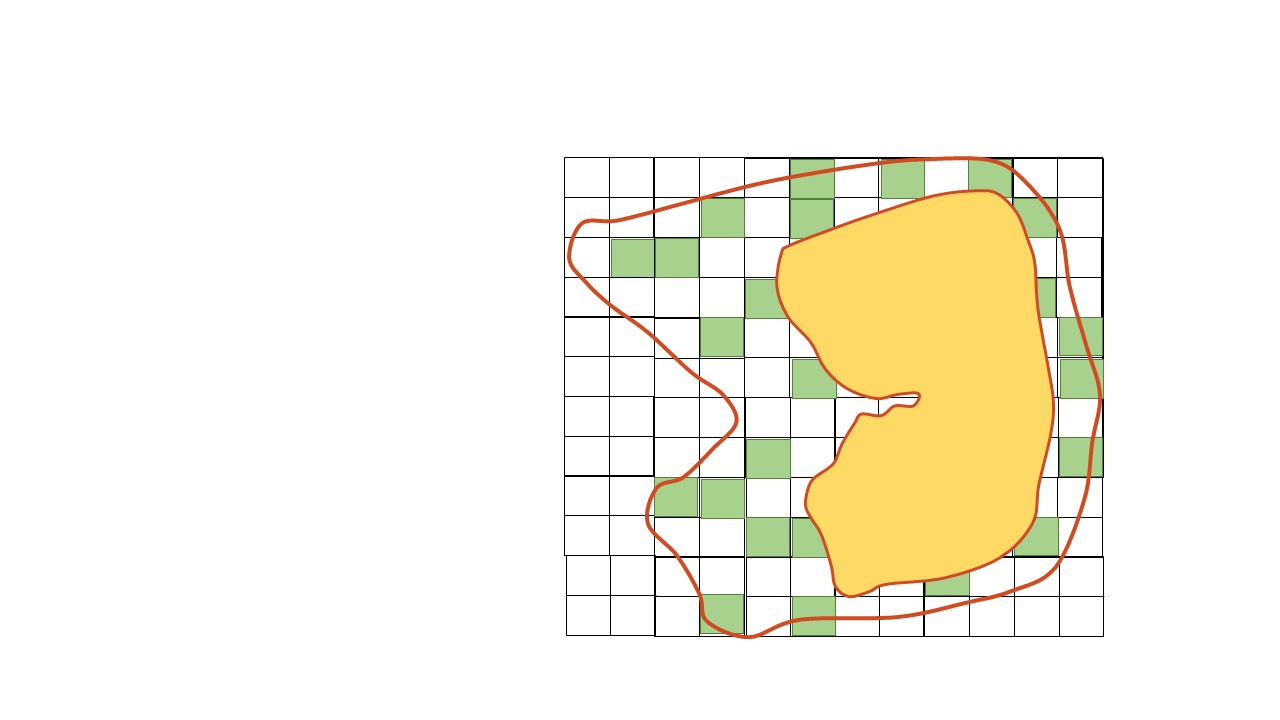
**Sample design (timing):**

1. **January/February, March, June, August, & October**

Rationale: Expect to find lower SAV density in winter. We want to observe the change in biomass and species composition through an annual cycle. This will help to observe SAV turnover on an annual basis. In addition, this special study will aid in determining the necessary intensity of future sampling efforts. Ideally, we would repeat this sub study to obtain an estimate of turnover in wet and dry years.

**Sample design (spatial):**

**~10 x 10 m quadrats–**



In general, sampling points will be selected in a random-stratified grid pattern. A grid of 10 m x 10 m squares will be spread over a site map. Grid cells which fall within SAV depth ranges will be counted. Of those grid cells, ~20–30% will be selected for sampling with a minimum of 30 and a maximum of 100. Three replicate samples will be collected haphazardly using a SAV rake from the grid cell to ensure that samples do not overlap. If logistical constraints preclude the use of some grid cells in the field, the closest replacement can be selected from the remaining grid cells after random selection. Sampling designs will be modified as necessary to sites with different characteristics.

**Note:** In order to compare sites from across the delta (where the majority of sites have SAV in shallow depths and fewer sites have SAV deeper) a lower limit to the bottom depth will be selected to exclude some deeper SAV patches.

**Rationale:** In order to scale estimates of invertebrates from within a patch of SAV to the scale of an entire site (March, October), assessments of SAV across the site will be made (March, October). A 10 x 10 m quadrat is a realistic grid size to navigate to quickly and also provides a small scale from which SAV data can be collected accurately. Turnover and spatial cover will be assessed by sampling (January, March, June, August, and October). Sampling the same way across the year will allow for seasonal patterns to be uncovered.

1. **Field work**
2. Relevant field notes will be taken upon reaching a site.
3. A list of pre-selected quadrats will be navigated to.
4. Upon reaching a quadrat, the SAV rake will be lowered into the water and three samples will be collected using standard SOPs for SAV rakes.
5. SAV composition will be taken from % tines covered by SAV.
6. SAV will be weighed using a salad spinner.
7. SAV need not be kept.
8. Should a quadrat be unsafe to navigate to, or conditions challenging for another reason, the closest navigable quadrat can be selected using the list of quadrats and an associated map.
9. **Laboratory**
10. SAV Mapping and assessment should require no laboratory work on a regular basis.
11. If the salad spinner is not available, or if relationships between wet and dry SAV are not suitable for converting between one another, SAV will be labeled clearly, bagged, and returned to lab for weighing.
12. **Analysis:**

**Hypotheses:**

**H1:** Natural patterns of SAV cover, composition, and turnover will vary with local and landscape-level factors. After years of data collection, sufficient data should allow for the uncovering of the most important parameters that control SAV.

**Analysis:** SAV biomass, biovolume, annual turnover, and indices of diversity can be analyzed as the response factor of a suite of environmental and biological data. A GAM or GLM can be used to test for the most significant parameters controlling SAV metrics. SAV composition can be visualized along with the above-listed parameters using CCA and nmds. GAMs or multivariate regression analysis can be used to determine the significant explanatory factors of such relationships. Significance can be set to 0.05. These analyses can be conducted for each site individually to uncover site-specific factors of importance. A single delta-wide model will incorporate all sites and will uncover landscape-level patterns.

1. **Do the dynamics of SAV cover, composition, and seasonal turnover differ by wetland location within the Delta (Landscape-level)?**

**Rationale:** Many factors which can control SAV occur at the landscape scale. Collecting monitoring data across the delta will inform management more accurately. Normal SAV cover, composition, and turnover may look very different at sites farther from or closer to the golden gate bridge. This would affect the determination of success at restoration sites. This question will require multiple years of study for a full understanding but preliminary findings will be available after the first complete year of sampling.

**Hypotheses:**

1. The magnitude SAV turnover at each of four wetlands will differ.
2. The rates of change in SAV biomass will differ for the different species among the four sites.

**Methods (SAV Mapping):** Methods will be the same as in question 1.

**Analysis:**

**Hypotheses:**

**H1:** The magnitude SAV turnover at each of four wetlands will differ.

**Analysis:** The rate of SAV turnover can be calculated between pairs of time points (January to March, January to October, March to October, and January to January). Metrics of turnover will be aerial coverage, biomass, and biovolume. Differences can be tested using t-tests. Significance can be set to 0.05.

**H2:** The rates of change in SAV aerial cover, biomass, and biovolume will differ for the different species of SAV among the four sites.

**Analysis:** The rate of SAV turnover can be calculated between pairs of time points for species of SAV (January to March, January to October, March to October, and January to January). Metrics of turnover will be aerial coverage, biomass, and biovolume. Differences can be tested using t-tests. Significance can be set to 0.05.

1. **Are SAV cover, composition, and turnover related to delta outflows?**

**Rationale:** Delta outflow has a major impact on the vegetation and aquatic biota of the delta ecosystem. Salinity, sedimentation, and a suite of other factors change as the distance from the golden gate bridge increases. Monitoring how SAV interacts with these factors will allow for a better understanding of what should be expected for SAV at sites within the delta. This question will require multiple years of study.

**Hypotheses:**

1. Patterns of SAV cover, composition, and turnover will largely be related to flow and salinity. Therefore, gradients of those two factors will be the largest explanatory factors of SAV because they are associated with location within the delta.

**Methods (SAV Mapping):** Methods will be the same as in question 1.

**Analysis:**

**Hypotheses:**

**H1:** Patterns of SAV cover, composition, and turnover will largely be related to flow and salinity. Gradients of those two factors will be the largest explanatory factors of SAV, these are tightly tied to location within the delta.

**Analysis:** SAV metrics of cover can be analyzed using multiple parameters, including flow and salinity, in a GLM or GAM. If flow and salinity are indeed strong predictors they will explain a larger portion of the variance in the model. SAV composition relationships to predictor variables can be visually assessed using CCA and nmds plots. SAV metrics of composition can be analyzed using ANOSIM. In addition, CCA can produce visualizations. If location within the delta is a significant factor, groupings of SAV may cluster by regions in the delta. Metrics of turnover will be aerial coverage, biomass, and biovolume. Differences can be tested using t-tests. Significance can be set to 0.05.

1. **How do SAV cover, composition, and turnover respond to restoration of intertidal wetland sites?**

**Rationale:** The alteration of habitats as is planned with the restoration sites in the delta is anticipated to have a multitude of interacting effects. In order to adaptively manage restoration sites, it will be important to identify successful and unsuccessful restoration components that can be employed in future restoration plans or incorporated into ongoing restorations. This question will require multiple years of study prior to and after restoration actions occur.

**Hypotheses:**

1. Restoration sites will evolve after levee breaching proceeds. This evolution will consist of a change in the composition and cover of SAV. (SAV–invertebrate associations will likely follow changes in SAV at the site.)

**Methods (SAV Mapping):** Methods will be the same as in question 1.

**Analysis:**

**Hypotheses:**

**H1:** 1. Restoration sites will evolve after levee breaching proceeds. This evolution will consist of a change in the composition and cover of SAV.

**Analysis:** SAV data should be tested for autocorrelation using semivariograms. Assuming no corrections are required, individual metrics of SAV cover, biomass, and biovolume can compared between restoration and reference sites. Differences between SAV at discrete time points can be compared using t-tests or a nonparametric version (Wald-Wolfowitz runs test, Mann-Whitney U test). Significance can be set at 0.05.To track progression over time, a GLMM can be run with restoration status as a factor.

1. **How do SAV cover, composition, and turnover affect invertebrate abundance and composition at tidal wetlands?**

**Rationale:** SAV habitat provides a complex architecture within which many invertebrates thrive. Many of these invertebrates are critical food items for endangered Delta Smelt, Longfin Smelt, and Chinook salmon. Identifying characteristics of SAV that harbor large quantities of ideal fish foods is among the goals of the FRP. Insights into SAV–invertebrate dynamics can inform ongoing and future restorations within the delta. This question will require multiple years of study for a full understanding but preliminary findings will be available after the first complete year of sampling.

**Hypotheses:**

1. Estimates of invertebrate productivity will differ with the density of SAV cover.
2. Estimates of invertebrate productivity will differ with the species composition of SAV.
3. Intermediate density SAV will support the most diverse assemblage of SAV-associated invertebrates.
4. Higher rates of change in site-level SAV biomass (i.e. larger net increases since January) will support higher abundances of SAV-associated invertebrates in March and October.

**Methods (Invert–SAV associations):**

1. **Pre-field work**

**Site selection:**

Samples will be collected at all of our sites across the delta while conducting already-scheduled monitoring activities in 2018.

**Sample design (timing):**

March & October-

**Rationale:** Sampling along with invertebrates in the spring blitz to observe real-time associations between SAV composition and cover.

**Sample design (spatial):**

**~1 x 1 m quadrats-**

Sampling for SAV will occur concurrently using a d-frame sweep net followed by a standard SAV rake in the same location.

**Rationale:** SAV-associated invertebrates are small and are thought to be associated with microhabitats created by the SAV around them. Collection of invertebrates and SAV within a small quadrat should ensure that the invertebrates collected are representative of the SAV they came from.

1. **Field work**

Sites will be selected per the current SOPs for SAV-invertebrate samples.

1. Relevant field notes will be taken upon reaching a site.
2. A sweep net sample will be collected and stored for invertebrates.
3. A SAV rake sample will be collected from the exact location from which the sweep net sampling was conducted.
4. SAV composition will be taken from % tines covered by SAV.
5. SAV will be weighed using a salad spinner.
6. SAV need not be kept.
7. **Laboratory**
8. Sample jars and field data will be entered into the necessary databases and tables.
9. Invertebrates captured in the sweep net will be separated and enumerated to the lowest taxonomic level possible.
10. Dry weight calculations of vegetation caught in the sweep nets will be conducted in the laboratory.
11. **Sample processing**
12. SAV weights from vegetation caught in a sweep net will be added to vegetation from SAV rakes at the same point.
13. Invertebrate data will be assessed using counts, measurements, and as CPUE based on the dry weight and wet weight of the SAV in the sweep net.
14. Invertebrates will be assessed in association with the SAV the SAV rake collected but SAV rake collections will not be incorporated in CPUE’s.
15. **Analysis:**

**Hypotheses:**

**H1:** Estimates of invertebrate productivity will differ with the density of SAV cover.

**Analysis:** SAV data should be tested for autocorrelation using semivariograms. Assuming no corrections are required, individual metrics of invertebrate productivity and composition can compared with SAV biomass using an ANOVA and GLM. Significance can be set at 0.05.

**H2:** Estimates of invertebrate productivity and composition will differ with the species composition of SAV.

**Analysis:** ANOSIM can be utilized to associate distinct SAV compositions with those in invertebrates. ANOSIM can also be used to compare estimates of invertebrate productivity (abundance, biomass, CPUE) with the SAV they came from. Data can be visualized using a CCA or nmds. If the relationships are real, groupings or types of vegetation will be significantly correlated with groupings of invertebrates in the ANOSIM.

**H3:** Intermediate density SAV will support the most diverse assemblage of SAV-associated invertebrates.

**Analysis:** SAV samples can be ranked based upon their density (biomass, biovolume). If the hypothesis is correct, a plot of (x vs. y) SAV density vs. invertebrate productivity will result in a peak at intermediate levels of SAV density. This can be visualized using a CCA or nmds and analyzed using an ANOSIM. Significance can be set at 0.05.

**H4:** Higher rates of change in site-level SAV biomass (i.e. larger net increases since January) will support higher abundances of SAV-associated invertebrates in March and October.

**Analysis:** Site-level SAV biomass can be estimated from measurements taken at different points in the year. Within each time period two metrics will be calculated: 1. Samples will be treated as random quadrats representing a % of the site and 2. SAV biomass will be interpolated using R and GIS mapping to estimate biomass at the site. These metrics will be calculated for each site and time period. Metrics of invertebrate biomass and diversity can be plotted against SAV biomass and biovolume estimates. A GAM can be tested to confirm that rates of turnover are significantly linked to metrics of invertebrate biomass or diversity. If the relationship between turnover and invertebrates is real, regressions of the two datasets will be significant and SAV turnover will be selected as a significant parameter among others used in the GAM. Significance can be set at 0.05.

1. **How do SAV measurements of biomass by wet weight, dry weight, and bio volume compare to one another; are reliable conversions available to expedite monitoring tasks?**

**Rationale:** Measurements of SAV often rely on dry weights to ensure comparability. Unfortunately the quantity of vegetation and the number of samples required by our monitoring efforts preclude the time required to dry weigh each sample of SAV. This sub study would result in reliable conversions between wet and dry weights for SAV biomass estimates; this will allow the FRP monitoring group to use wet weights taken in the field rather than relying on time-intensive efforts. This sub study will also evaluate if estimate of biovolume collected from the boat’s sonar device can be used in lieu of SAV rakes or to reduce time on site to assess SAV. This question and its study can be completed in less than one complete year of sampling.

**Hypotheses:**

1. Regressions between metrics of SAV biomass on a wet weight and dry weight basis will be significantly correlated to one another.
2. Regressions between metrics of biovolume and biomass will be significantly correlated to one another.

**Methods (SAV weight correlations):**

1. **Pre-field work**

For this sub-study the most common SAV species will be sought out and collected per the normal SAV rake methods: Brazilian waterweed (*Egeria densa*), Curlyleaf pondweed (*Potamogeton crispus*), Carolina fanwort (*Cabomba caroliniana*), Eurasian watermilfoil (*Myriophyllum spicatum*), Coontail (*Ceratophyllum demersum*), American pondweed (*Elodea Canadensis*), Sago pondweed (*Stuckenia spp*.), and Longleaf pondweed (*Potomogeton nodosus*), as well as filamentous algae (which is not a rooted aquatic plant but is often found among SAV samples).

**Site selection:**

Samples will be collected at all of our sites across the delta while conducting already-scheduled monitoring activities in 2018.

**Sample design (timing):**

Year round and some methods will occur primarily in summer

**Rationale:** Sampling for SAV samples should occur year round because SAV sampling and SAV mapping efforts may also occur throughout the year.

1. **Field work**

In the process of other field work and projects, SAV specimens will be collected. Steps I – IV can be carried out during standard field work but may require extra days specifically for these tasks.

1. A SAV sample will be collected using SAV rake and standard SOPs.
2. The sample will be bagged according to species.
3. A sample should be at least 200 grams.
4. Sample bags should be labeled and returned to the laboratory.
5. After collection of SAV rakes across a site for SAV mapping, circle the site with the boat’s sonar turned on and set to map SAV.
6. Save data onto removable device to bring back for analysis.
7. **Laboratory**
8. Each sample should be massed after spinning in a salad spinner for wet weight.
9. Remove from scale and dry in a drying oven until mass is constant.
10. Sample may be discarded after records are backed up.
11. **Analysis:**

**Hypotheses:**

**H1:** Regressions between metrics of SAV biomass on a wet weight and dry weight basis will be significantly correlated to one another.

**Analysis:** Species will be treated individually. For a given species, wet weight and dry weights will be plotted on the X and Y axes. Regression lines and curves will be created to find the relationship with the most highly significant result. If the significance of the relationship is less than 0.05 dry weights will no longer be required and wet weights can be normalized to dry weights at the analysis stage.

**H2:** Regressions between metrics of biovolume and biomass will be significantly correlated to one another.

**Analysis:** Data from the boat’s sonar can be sent to BioBase for conversion to biovolume. In order to compare bio-volumes and the SAV cover at the site two maps will be created. Estimates of SAV from rake samples across the site will be compared to bio-volumes in two ways: first pairs of SAV rakes will be compared with biovolume from maps at that specific point. Second, interpolated maps of SAV rake data will be compared point-by-point to maps of biovolume where there is a union of the two maps. Pairwise comparisons will be made using T-tests. Significance will be set at 0.05.

**Topics of concern / discussion**

1. Differentiating between covariates- flow and SAV density
2. Take instantaneous measurements of flow?
3. Maximum tidal velocity as measured by sondes?
4. Analysis of spatial coverage-
   1. The current design iteration allows for the use of interpolation between points and inclusion in spatial models. This may not work because variability is too high and interpolated error would be also. In that case, standard analysis of SAV as a site will follow ‘b’ below.
   2. SAV can be assessed using the 30 – 100 samples from each site. Each 10 x105 m grid cell will be treated as a quadrat. % cover can be calculated for each site.

# Endangered Species Act Take

The Fish Restoration Program is permitted to incidentally take salmonid and sturgeon ESA-listed species managed by NMFS via their 2009 Biological Opinion on the Long term Operations of the Central Valley Project and State Water Project (confirmed with Brycen Swart of NMFS April 22, 2015 via email). The EMP, 20mm, Summer Townet, and Fall Midwater Trawl Studies have existing ESA take permits for their activities. Tables included in this section show estimated maximum incidental take from FRP monitoring activities in 20172018. Take coverage under IEP is being sought for Delta Smelt only. Other ESA species estimates are listed for informational purposes.

**Table 8.** Take of listed fish species in all FRP sampling. Gears not listed had zero take.

|  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  |  | | Listed fish species take | | | | | | | | |
| Gear Type | Total # of samples: | Chinook Salmon | | Steelhead | Delta Smelt (larval) | Delta Smelt (juv) | Delta Smelt (adult) | Longfin Smelt (larval) | Longfin Smelt (juv) | Longfin Smelt (adult) | Green Sturgeon |
| Mysid Trawls |  | 0 | | 0 | 2 | 0 | 0 | 25 | 0 | 0 | 0 |
| Beach Seine |  | 2 (fall-run) | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Total |  | 2 | | 0 | 2 | 0 | 0 | 25 | 0 | 0 | 0 |

# Data Management and Distribution

Most data will be recorded using paper data sheets developed for our study based on IEP long-term monitoring data sheets.

Data will undergo extensive quality checks and quality assurance before analysis. Field data will be checked for completeness before leaving each station, and each day’s field data sheets will be checked before leaving the field. Any obvious mistakes found during review will be corrected immediately (values outside of what is reasonable, incorrect dates, etc.). Data will be entered into a relational database (Microsoft Access) as soon as reasonably possible, not more than one week after collection. Any notations on the field sheet indicating deviations from the SOP or potential problems with the data will be reviewed and translated into data noted in the database. Data will then be printed and compared against the field data sheets line-by-line to ensure no errors in transcription.

After the data have been checked, we will produce summary statistics, histograms, box plots, or scatter plots to check for outliers and ensure data meets the assumptions of relevant statistical tests.

Data will be stored temporarily on the local CDFW server, backed up once per month. Data will be made publically available as soon as reasonably possible after completion of the project, no later than June 2019. The Access database, as well as flat files in .csv or other non-proprietary format, coupled with metadata and a copy of the final report, will be posted on the CDFW FTP site, and the IEP Tidal Wetlands PWT website. A more robust database using Oracle or SQL Server is currently in development by DWR’s Department of Technology Services, which will be stored on DWR’s server.

## Reports

We will provide a full report to the IEP by June 2019 summarizing all results from this study, in tabular or graphical form, as appropriate, with a full analysis and discussion of results. All Delta Smelt take will be reported to IEP via their on-line reporting system, and all anadromous fish take will be reported to NMFS.

## Publications and Conferences

This project will result in at least one IEP newsletter article, on macroinvertebrate variability, comparisons of zooplankton and fish catch between open water and littoral habitats, and fish gear evaluation techniques. One or more members from the FRP program will also present results at the Bay-Delta Science Conference and/or at the Interagency Ecological Program Annual Workshop.

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