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SAN FRANCISCO ESTUARY INVASIVE SPARTINA PROJECT

MONITORING PROGRAM QUALITY ASSURANCE DOCUMENT



JANUARY 31, 2008

San Francisco Estuary Invasive Spartina Project

Monitoring Program Quality Assurance Document

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For the

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January 31, 2008

Updated October 2009

This report was prepared for the California Coastal Conservancy's San Francisco Estuary Invasive *Spartina* Project with support and funding from the following contributors:

California Coastal Conservancy

CALFED Bay-Delta Program, Ecosystem Restoration Program (California Department of Fish and Game Interagency Agreement #PO685608)

California Wildlife Conservation Board (MOU #99-054-01 and subsequent)

National Oceanic and Atmospheric Administration under the American Recovery and Reinvestment Act of 2009 (Award #NA09NMF4630318)

EPA Title and Approval Page

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Section 1. Introduction

This document presents the management structure and protocols for data collection, handling, storage, analysis, and dissemination for the San Francisco Estuary Invasive *Spartina* Project's (ISP) Monitoring Program, and shows how these protocols ensure data of the appropriate type and quality for its intended uses.

The ISP was created in 2000 by the State Coastal Conservancy to eradicate four introduced and highly invasive *Spartina* (cordgrass) species and their hybrids in the San Francisco Estuary (Estuary). The ISP is comprised of two major programs, the Control Program, which plans and conducts coordinated regional *Spartina* treatment, and the Monitoring Program, which collects and interprets environmental data needed to conduct treatment and evaluate progress and effects. The ISP also conducts public outreach and research, coordinates and streamlines permitting processes, and advises the Conservancy and others regarding long-term strategies to protect wetland restoration projects from future non-native *Spartina* invasions.

<u>The primary goal of the Monitoring Program</u> is to provide the Control Program timely, high quality data regarding the location and extent of non-native *Spartina*, so that the Control Program staff may plan and rapidly implement cost-effective weed control measures and determine when site-specific and regional control objectives have been met. In addition, the Monitoring Program provides accurate data on the status of endangered California clapper rails at the *Spartina* treatment sites, to allow *Spartina* control to be implemented while avoiding significant long-term adverse effects and minimizing short term adverse effects on rails, in compliance with State and Federal regulatory requirements.

The Monitoring Program has three subcomponents described pursuant to California Department of Fish and Game/Ecological Restoration Program contract agreement number PO685608 (which provides partial funding for the program). The Monitoring Program subcomponents include inventory monitoring, efficacy monitoring, and clapper rail monitoring. The objective of each of these is as follows:

- **Inventory Monitoring** To assess and map the Estuary-wide distribution and abundance of non-native *Spartina* species (including *S. alterniflora* and hybrids, *S. densiflora* and hybrids, *S. anglica*, and *S. patens*), to identify any new populations and track the change in distribution and abundance over time, as the *Spartina* Control Program is implemented. A specific hypothesis to be tested is whether the Control Program is achieving its objective of reducing the extent of non-native *Spartina* by at least 50% per year at all existing sites and preventing establishment of new infestations.
- Efficacy Monitoring To assess the efficacy and non-target effects of *Spartina* control measures implemented at specific sites to support planning for follow-up treatment and to determine the most effective (and cost-effective) treatment methods. A specific hypothesis to be tested is whether treatment methods implemented by the ISP are effective for controlling the non-native *Spartina* at existing infestation sites.
- **Clapper Rail Monitoring** To assess and map the distribution, abundance, and habitat association of endangered California clapper rail at *Spartina* treatment sites to determine whether clapper rail populations are adversely affected by *Spartina* treatment activities, to estimate the magnitude of any such affect, and to provide critical information so that *Spartina* control strategies can be developed to minimize short-term adverse effects on clapper rail populations. The primary hypothesis to be tested is

whether control of non-native *Spartina* is being achieved with minimal adverse shortterm effects (within limits permitted by U.S. Fish and Wildlife Service under Intraservice ESA §7 Consultation, see <u>www.spartina.org/project_documents/2008-</u> <u>10 Spartina_BO_Amend.pdf</u>) and no adverse long-term impacts to California clapper rail populations.

Quality assurance (QA) within the ISP Monitoring Program is the overall integrated system of management activities involving planning, implementation, documentation, assessment, reporting, and quality improvement to ensure that the data collected for the above three purposes are useful and reliable. Quality assurance guides the entire program from training field staff to analyzing the data.

Quality control (QC) is part of quality assurance. It is the activities that are performed during data collection which ensure the data collected are of the highest quality. Quality control is also the process by which we document the separate components of our quality assurance plan. For instance, the data dictionary a field biologist fills in to record the field data and the photographs taken to document the location and morphology of plants sampled for DNA analysis are both examples of quality control.

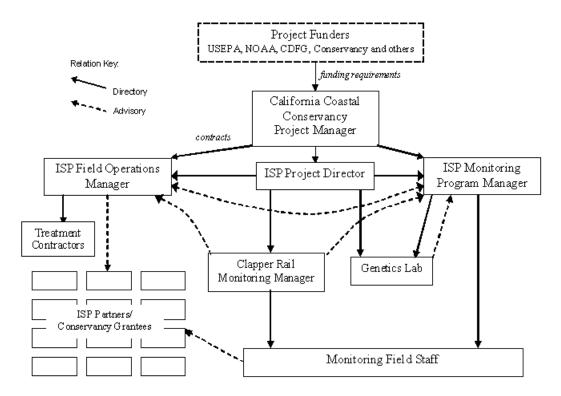
Section 2. Monitoring Program Management

2.1. Involved Parties and Roles

The ISP is a "project" of the California State Coastal Conservancy (Conservancy). The ISP consists of a Project Manager at the Conservancy, the "ISP Management Team," comprised of the ISP Director, the Field Operations Manager and Assistant Manager, and the Monitoring Program Manager (all consultants contracted to the Conservancy), various field staff for monitoring work, and grantees and contractors that implement *Spartina* treatment. Laboratory analysis of plant samples is provided by a licensed commercial laboratory. An organizational structure for the ISP and the Monitoring Program is provided in **Figure 1**. The responsibility of each position is described below, and individuals who fill these positions are listed in **Table 1**.

The Conservancy <u>Project Manager</u> creates and manages contracts with ISP consultants, agreements with grantees responsible for treatment activities, and the Interagency Agreement for lab analysis of *Spartina* samples, and administers state and federal grants awarded to the Conservancy for purposes of management and implementation of the ISP Control and Monitoring Programs. The Project Manager also provides oversight of and coordination with, ISP consultants, and agency and nonprofit partners.

The <u>ISP Director</u> provides day-to-day coordination, oversight and management, assures compliance with all regulatory requirements, and sets the direction for ISP goals, objectives, research, and strategies. The Director provides daily supervision for the other ISP consultants, including the managers of the monitoring and control programs.



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Figure 1. ISP Monitoring Program Organizational Chart (Revised November 2009)

Table 1. Project Organization Roles and Responsibilities (Revised November 2009. Table format from Wetlands Quality Assurance Project Plan Guidance, Ver. 1.0, USEPA Region 9, 2004)

0			1
Title/Responsibility Name/Qualifications		Organiza- tional Affilia- tion	Phone/email
EPA Project Officer Advises on project compo- nents funded by USEPA (wa- ter quality monitoring and <i>Spartina</i> treatment), assists development of QAQC pro- cedures and documents.	Luisa Valiela USEPA Region 9	USEPA	(415) 972- 3400 Valiela.Luisa@ epamail.epa. gov
NOAA Project Officer Advises on project compo- nents funded by NOAA (in- cluding, but not limited to, ISP management, <i>Spartina</i> & clapper rail monitoring, <i>Spartina</i> treatment, public outreach).	Summer Morlock NOAA Restoration Center, Community-based Restoration Program	NOAA	Summer. Morlock@ noaa.gov
Project Manager Administers project and manages primary contracts and grants.	Maxene Spellman Conservancy Project Manager for ISP since it's inception in 2000. Familiar with all aspects of the project, adept at managing multiple funding sources.	Conservancy employee	(510) 286- 0332 mspellman@ scc.ca.gov
Project Director Oversees, directs, and is responsible for all aspects of ISP activities and direction.	Peggy Olofson , Olofson Environmental, Inc. Professional Engineer, business owner, 15 yrs experience as project manager in San Francisco tidal marshes, including manager of San Fran- cisco Estuary Baylands Ecosystem Habitat Goals Project, 10 yrs experience as staff engineer for San Francisco Regional Water Quality Control Board, 8 yrs as ISP Director. B.S. (Magna Cum Laude) in Environmental Resources Engineering, Humboldt State University, California.	Contractor to Conservancy & OEI Princi- pal	(510) 548- 2461 x201 prolofson@ spartina.org
Monitoring Program Manager, Quality Assur- ance Manager Oversees all aspects of the Monitoring Program, designs monitoring and data man- agement protocols, interprets and reports results. QA offi- cer for all <i>Spartina</i> -related data collection.	Ingrid Hogle A botanist with extensive experience developing weed mapping systems, expert in ArcGIS and work flow development. 4 yrs as Monitoring Pro- gram Manager for ISP. M.S. in Ecology from Uni- versity of California, Davis, and B.S. in Natural Resources & Environment from University of Michigan.	Contractor to Conservancy	(510) 548- 2461 x205 ibhogle@ spartina.org
Assistant Monitoring Program Manager Plans and schedules all Spartina surveys, assures data files are checked in and out in accordance with QA procedure. Collects all photo- point data.	Tripp McCandlish A fisheries and wildlife biologist with 6 yrs experi- ence monitoring and assisting control of non- native <i>Spartina</i> in the San Francisco Estuary, 2 yrs as Assistant Monitoring Program Manager. B.S. in Limnology and Marine Biology, San Fran- cisco State University, California.	OEI Employee	(510) 548- 2461 cmccandlish@ spartina.org
Clapper Rail Monitoring Manager, Quality Assurance Officer Plans and schedules all clapper rail surveys, obtains permits, trains staff, prepares reports to funders and regu- latory agencies. QA officer for all clapper rail-related data collection.	Jen McBroom A wildlife biologist with 10 yrs experience study- ing avian ecology in the San Francisco Estuary, has extensive experience with endangered spe- cies, including California clapper rails, and the permitting and reporting processes. 5 yrs as ISP Clapper Rail Monitoring Program Manager for ISP. B.S. (Cum Laude) in Wildlife, Fisheries, and Conservation Biology from University of Califor- nia, Davis.	OEI Employee	(510) 548- 2461 x206 jtmcbroom@ spartina.org

		Organiza-	
Title/Responsibility Name/Qualifications		tional Affilia- tion	Phone/email
		lion	
Field Operations Manager Primary user of monitoring data; develops and revises regional treatment strategies, writes site specific control plans for partners, oversees control work in field.	Erik Grijalva A noxious weed expert for 10 yrs, skilled in man- ual and chemical control methods as well as all aspects of plant identification and mapping. ISP Field Operations Manager for 6 yrs. B.S. degrees in Zoology and in Botany from University of Washington.	Contractor to Conservancy	(510) 548- 2461 x202 ekgrijalva@ spartina.org
Field Operations Assistant Manager, Water Quality Monitoring Manager, Qual- ity Assurance Officer Assists and advises Field Ops Manager in all areas; plans, implements, reports water quality monitoring for NPDES compliance; QA offi- cer for <i>Spartina</i> treatment and water quality monitoring.	Drew Kerr A noxious weed expert for 10 yrs, former coordi- nator of the Wetland & Aquatic Weed Program in King County, WA, ISP Field Ops Assistant Man- ager for 4 yrs. Herbicide chemistry expert, skilled in all aspects of data management and quality control. B.S. degrees in Environmental Policy and in Economics from University of Michigan, and a certificate in Wetland Science and Man- agement form University of Washington. State of California Certified Qualified Applicator, with spe- cialization in aquatic pesticide application.	Contractor to Conservancy	(510) 548- 2461 x203 dwkerr@ spartina.org
Field Biologists Conduct Spartina monitoring using GPS data loggers, col- lect plant samples for genetic analysis, input and manage data.	Field Biologists All staff have background in botany, ecology, wetlands biology, and/or avian ecology. All staff are extensively trained in tidal marsh plant, bird, and endangered species identification, plant sampling, marsh and boat operations safety, and other skills, as needed.	OEI Employ- ees and/or sub- contractors	(510) 548- 2461 x201 prolofson@ spartina.org
Administrative Assistant Receive plant samples from field staff, package and ship samples to lab, prepare chain of custody	Stephanie Ericson Day-to-day office manager for ISP and OEI. B.A. in Political Science from State University of New York, and M.A. in Economics, University of Maryland.	OEI Employee	(510) 548- 2461 x200 sericson@ spartina.org
Graduate Student- Researcher Conduct research on <i>Spartina</i> hybrids and labora- tory methods, and advise ISP	- Laura Feinstein CalFED SeaGrant Fellow 2008-2010, studying the frequency, distribution, and ecological impact of cryptic hybrid <i>Spartina</i> , to help us develop		lfeinstein@ ucdavis.edu
Professor Advisor of Grad Student re- searcher	Dr. Don Strong Professor, Department of Evolution and Ecology, University of California, Davis.	University of California, Davis	drstrong@ ucdavis.edu
Genetics Laboratory Manager Oversee analysis, assure quality control.	Dr. Gene Hookstra General Manager of Genetic Services, Eurofins STA Laboratories. Ph.D. in Plant Breeding and Quantitative Genetics from the University of Ne- braska.	Eurofins STA Laboratories, Colorado	Genehook- stra@ eurofin- sus.com

The <u>Monitoring Program Manager</u> oversees all aspects of the Monitoring Program's *Spartina* inventory and efficacy monitoring, and is responsible for the design and implementation of monitoring protocols, data editing, quality control and assessment, and annual reporting of results. The Monitoring Program Manager is the QA Officer for all *Spartina*-related data collected by the Monitoring Program.

The <u>Assistant Monitoring Program Manager</u>, working under the Monitoring Program Manager, is responsible for scheduling and coordinating the day-to-day logistics associated with inventory and efficacy monitoring.

The <u>Clapper Rail Monitoring Manager</u> is responsible for planning, implementing, and interpreting annual surveys of the distribution and abundance of endangered California clapper rails within selected sites. The Clapper Rail Monitoring Manager is the QA Officer for all clapper rail-related data collected by the Monitoring Program.

The Monitoring Program relies on extensive field work by specially trained <u>field biologists</u>, most of whom are employed by Olofson Environmental, Inc. specifically for work on the ISP. The activities of the monitoring program include mapping and evaluating the distribution of non-native *Spartina* throughout approximately 25,000 acres of marsh and mudflat within the San Francisco Estuary and vicinity, as well as monitoring and analyzing the efficacy of treatment efforts at selected sites. Additional field biologists with training in avian ecology and special permits from U.S. Fish and Wildlife Service and California Department of Fish and Game assist with the annual clapper rail surveys, data entry and GIS data creation and management. Field biologists collect and edit data using Global Positioning System (GPS) in the field and Geographic Information System (GIS) in the office.

The <u>Field Operations Manager</u> and the <u>Field Operations Assistant Manager</u> oversee the Control Program and are responsible for short and long-term planning and coordination of *Spartina* treatment activities. The Field Operations Manager relies on the Monitoring Program for *Spartina* location, treatment efficacy (i.e., annual reduction in infested area), and clapper rail data to assess the effectiveness of previous control activities and develop and revise annual site-specific *Spartina* control plans. The Field Operations Manager oversees implementation of the *Spartina* control plans by subcontractors and staff of public agencies and nonprofit organizations (that usually receive grant funding for this purpose from the Conservancy) to assure proper implementation of all treatment actions and mitigation measures required by the ISP *Spartina* Control Program Programmatic EIS/R and U.S. Fish and Wildlife Service Biological Opinions. The Field Operations Assistant Manager designed and tested the photo point monitoring protocols implemented as part of treatment efficacy monitoring, and provides assistance as needed in acquiring and managing the photo point images.

<u>A commercial genetics lab</u> is contracted through the Coastal Conservancy or Olofson Environmental, Inc. to conduct genetic (DNA) testing of plant tissue samples to determine species (including hybridization) of selected plants. Currently (2009), the contracted lab is Eurofins STA Laboratories in Longmont, Colorado. The laboratory manager is Dr. Gene Hookstra, and the Laboratory Quality Assurance Plan for *Spartina* analysis is provided in **Appendix 6**.

<u>The UC Davis Spartina Laboratory</u>, under the direction of Dr. Donald Strong, Department of Evolution and Ecology, has been an important contributor to the ISP since its inception in 2000. The lab conducted much of the early research in invasive *Spartina* on the Pacific Coast, and developed and published the laboratory procedures for genetic analysis by RAPD and microsatellite methods. The laboratory is currently conducting research on the frequency, distribution, and ecological impact of cryptic hybrid *Spartina*, to help the ISP develop improved management tools for eradication. The lab researchers also continue to advise the ISP staff and management on matters related to hybrid sampling and analysis.

2.2. Special Training Requirements/Certification

All field staff conducting *Spartina* inventory and treatment monitoring are required to undergo team and personalized training as described under Data Quality Objectives for Measurement Data – Comparability (Section 3.3).

All field staff receive marsh safety training. Marsh safety protocols include awareness of dangers of walking in the soft mud/marsh, among channels, rock, water and other potential physical hazards. They also include appropriate behavior in sensitive species habitat. **Appendix 1** contains the ISP's *Safety Guidelines for Working in Marshes, Mudflats and other Intertidal Terrain*.

All persons involved in clapper rail monitoring are required to undergo training before qualifying to conduct independent clapper rail surveys. The training provided by ISP enables new surveyors to receive the necessary permits from the U.S. Fish and Wildlife Service and the California Department of Fish and Game. Training includes review of standardized U.S. Fish and Wildlife Service-approved survey protocols, familiarization with recordings containing calls of common and threatened and/or endangered marsh bird species, at least ten site visits with an experienced observer during which rails are detected, and review of data entry protocols. Staff are tested in all skills before being recommended to USFWS and CDFG for permits. Copies of current permits for monitoring are provided in **Appendix 7**.

2.3. Persons Responsible for Quality Assurance

The Monitoring Program Manager is the QA Officer for Spartina monitoring data, and the Clapper Rail Monitoring Program Manager is the QA Officer for clapper rail monitoring data. However, managers and field biologists alike take responsibility for both quality control of data collection and quality assurance via the quality assurance protocols outlined in this document. The Field Operations Assistant Manager is the QA Officer for *Spartina* treatment and water quality monitoring.

Independent data review is sought on Monitoring Program data collection and analysis methods through periodic program reviews, such as the Monitoring Program Review held in April 2006 (see **Appendix 2**).

The ISP Management Team may recommend changes and updates to this Quality Assurance Document as needed. The relevant QA officer is responsible for making the changes and assuring that they get appropriately integrated into the various programs.

Section 3. Monitoring Program Quality Objectives

Data and measurement quality objectives are usually established for representativeness, completeness, comparability, accuracy, and precision (Stanley and Vener 1985). Data and measurement quality objectives for the Monitoring Program were established based on the needs of the Control Program and partners. Quality assurance measurement requires the establishment of procedures to ensure quality objectives are met. The sections below summarize ISP data and measurement quality objectives, and what quality assurance measures are being taken to ensure that these quality objectives are met. Greater detail is provided in **Appendix 3**.

3.1. Representativeness

The concept of representativeness within the Monitoring Program refers to the sufficient sampling, temporally and spatially within the project area, to effectively and efficiently provide useful data to the Control Program. The ISP has specific representativeness objectives for each component monitoring activity.

Inventory monitoring of non-native *Spartina* has a goal of surveying 100% of all potential *Spartina* habitat within the Estuary and adjacent outlying areas identified as high-risk habitat for invasion by non-native *Spartina* as frequently as necessary to advise the Control Program of the location and extent of plants requiring treatment. Potential *Spartina* habitat is determined by expert opinion and includes those areas monitored in past years plus any new habitat discovered by site visits, aerial photo interpretation or communication with partners or others. The ISP works with a variety of project partners and conducts outreach to the extent possible under current funding to ensure that new populations of *Spartina* are reported to the ISP. Potential habitat boundaries are expanded as new habitat is discovered or created, and are surveyed by qualified staff or partners in all subsequent field seasons.

The Monitoring Program works with the Control Program and the California Coastal Conservancy to determine the appropriate frequency of inventory surveys at all sites. Full inventory surveys were initially conducted throughout the Estuary over a two-year span from 2000 to 2001. A partial inventory in 2003, when only a sample of sites were surveyed for non-native *Spartina*, was considered inadequate for the needs of the ISP. Full inventory surveys have been conducted on an annual basis from 2004 to present (2009), with most monitoring sites surveyed once each year. Beginning in 2008, some sites were surveyed twice per year, while sites where no non-native *Spartina* has been found following three consecutive years of surveys were slated for surveying every other year. The frequency of surveys is reviewed annually by the ISP to ensure that surveys are taking place with the appropriate frequency to best meet Control Program needs, within project budget restrictions.

The objective for representativeness of genetic sampling is to confirm known and identify unknown *Spartina* species through a combination of (1) genetic verification sampling (verification of field identification of *Spartina* species, including native and non-native) and (2) sampling of all unknown *Spartina* plants at each monitoring site. Constraints on 100% sampling of unknown *Spartina* species include access to plants, density and degree of infestation as well as the time and money involved in genetic sampling.

The objective for representativeness in photo monitoring site selection is to select a sample of sites that represents the broadest possible variety of marsh types, treatment methods, infestation size and stage, and marsh locations (north, south, east and west) throughout the Estuary with replication of these characteristics among as many sites as possible. Within each site, the

goal of actual photo point selection is to maximize the amount of qualitative data captured by each photo, and to graphically portray the effects of treatment in a manner that is representative of the entire site. Sites with a higher degree of complexity require multiple images (i.e., photo points) to detail the progression of treatment at the site over time.

The objective for representativeness in treatment efficacy monitoring applies to site selection and plot location within sites. Sites are selected to represent a variety of marsh types, treatment methods, and marsh locations (north, south, east and west) throughout the Estuary with replication of these characteristics among as many sites as possible. The objective for representativeness in transect and plot location is to maximize representation of *Spartina* control effects within the area of each site.

Annual monitoring for clapper rail presence or absence is conducted at treatment sites where control will occur before the breeding season is over. The site's survey area is determined by creating 200m buffer around each listening station. The survey results are extrapolated to entire site to estimate CLRA population at site.

3.2. Completeness

Completeness is defined as "a measure of the amount of data collected from a measurement process compared to the amount that was expected to be obtained under the conditions of measurement" (Stanley and Vener 1985).

The ISP has established a completeness goal of 100% for the recording of location, size, and percent cover of non-native *Spartina* patches discovered during *Spartina* inventory monitoring. Data are checked and edited in a GIS by both the field staff who collected the data and by the Monitoring Program Manager throughout the field season and in the process of data synthesis at the end of the season. When errors are found and corrected, the individual performing the correction records his or her name as well as the date and description of the data modification in the attributes of the corrected feature. Data are checked for completeness by the Monitoring Program Manager and the rest of the ISP Management Team throughout the season and once again at the end of the season in preparation for data analysis.

The ISP has established a goal of recording every detectable non-native *Spartina* occurrence within a site, as well as every occurrence throughout the Estuary (i.e., the discovery and documentation of new pioneering sites). Due to the extensive area surveyed by the ISP, we cannot be certain that we will find every stem of *Spartina*. Rather, we base our completeness objective on mapping all *Spartina* patches which have reached a "detection threshold". This threshold varies based on plant height, patch extent, surrounding vegetation, topography, tidal conditions, and site accessibility. Given the physical constraints inherent in the field work performed by the ISP, and the understanding that neither visual surveys nor photo interpretation of aerial imagery can achieve 100% detection of all *Spartina* locations, or the analysis of associated data. Since 2006, we document the completeness of site surveys by recording, in GIS, the extent of area surveyed each year and the method used to complete the survey.

To ensure completeness within inventory sites, several changes to inventory methods were tried in 2006 and 2007, and were fully implemented in 2008. Prior to 2006, field staff were not provided field maps with past years' data, as it was assumed that knowledge of past non-native *Spartina* locations would bias a complete inventory. Due to the extremely successful treatment of non-native *Spartina* at many sites, however, we are finding weakened, less visi-

ble non-native *Spartina* patches in many areas. Without maps of the previous year's data, these patches can be difficult to locate and identify. In 2006 and 2007, inventory field maps and/or background GPS files were prepared for select sites. Field staff found this information to be very useful. Since 2006, all inventory data from all past years has been made available as both printed map and GPS data files during inventory monitoring efforts. Field staff are trained to conduct a full inventory throughout a site and to navigate to former patches to check for regrowth. By requiring both re-inventory and re-visit to treated patches, we help to ensure that remnant populations of treated *Spartina* are not overlooked.

As a second level of quality control, the Control Program reviews Monitoring Program data for accuracy and completeness during and at the end of the field season. The Control Program uses current and past year *Spartina* location and attribute data to prepare for upcoming field work, and uses this data to prepare field maps prior to supervising control work. When questions arise, the Control Program staff communicate directly with the field biologist(s) or Monitoring Program Manager to resolve potential data errors. During control work or reconnaissance site visits, Control Program staff note any non-native *Spartina* patches found in the field which were not present on the maps. Control Program staff notify the Monitoring Program Manager of patches missed by field staff, and may record the location and extent of these patches in GIS.

As *Spartina* mapping becomes more difficult (as patches become smaller and less obvious across the landscape), the Monitoring Program and Control Program have begun meeting every two weeks to review *Spartina* inventory data collected since the last meeting. Data are checked for completeness, accuracy of species identification, and spatial accuracy. Potential data problems are thus noted and resolved throughout the season. Such meetings (termed "Tour de *Spartina*") took place at the end of the 2007 field season, the end of the 2008 field season, and approximately every two weeks during the 2009 field season.

For spring efficacy monitoring, the goal for completeness in terms of location and timing of photos is determined by the annual planning and documentation needs of the Control Program. All photos are taken to maximize marsh extent and to minimize the horizon within the view frame, thus optimizing the completeness of data recorded. Number of photos per site, and direction of photo (which determines amount of treated area in photo view), are reviewed by the Control Program at the end of each treatment season. Photo points are added or removed from the following year's schedule as determined by the Control Program at this annual review. Timing of spring efficacy monitoring is scheduled to allow the most complete observation of treatment efficacy at the earliest date possible. Early site visits and recording of observed efficacy prior to the start of the control season, in late May, provides valuable information for the planning of current year control efforts.

The minimum goal for summer efficacy monitoring data completeness is 90%. A power analysis of 2004-2005 treatment monitoring data conducted in 2006 (**Appendix 4**) indicated that a 10% change in non-native *Spartina* cover could be detected with 90% confidence with data from a minimum of twenty-seven (27) 0.25 x 0.25-meter plots ("quadrats") per site. Most sites contain 30 such plots, which allows for a 90% level of completeness to suffice for the necessary level of sampling to detect a significant change in non-native *Spartina* cover. While every effort is made to collect data at all plots at each site, due to the many physical and legal constraints regarding entrance of field biologists into the marsh to conduct these surveys, all plots may not be able to be surveyed during a single site visit. When necessary, a return visit will be scheduled to ensure that at least 90% of the plots are sampled at each site.

The completeness objective of genetic testing is to sample each site at a sufficient level of detail to ensure that we are identifying and subsequently treating all non-native *Spartina*. The level of genetic testing is currently based on recommendations from the 2006 Monitoring Program Review (**Appendix 2**), confidence of observer field identification skills, site history, and the needs of the Control Program. Three levels of sampling may be used: haphazard and subjective sampling of samples during inventory monitoring, seedling sampling, and transect sampling. Data collection protocols for these three types of sampling are described in section 4.1.2 below.

For clapper rail monitoring, completeness can be described for both population survey measurement and protocol completeness. The US Fish and Wildlife Service California clap-per rail protocol is designed to "detect presence or absence of clapper rail breeding activity". ISPmodified clapper rail monitoring surveys are designed to estimate the number of clapper rails in a marsh during breeding season. Because there is no means by which true population numbers can be measured, we cannot assess the level of completeness of our detection of individual clapper rails at a site. Survey completeness assesses the degree to which monitoring efforts comply to protocol. The ISP has established a protocol completeness goal of 100% for clapper rail monitoring. Given the physical constraints inherent in the field work performed by the ISP, and the understanding that 100% detection of exact numbers of clapper rail at each location is not possible using even the best available technologies, failure to achieve this goal will not preclude the analysis and reporting of clapper rail data. All reports will indicate the degree of completeness of protocol surveys performed.

3.3. Comparability

Comparability is defined as "the confidence with which one data set can be compared to another" (Stanley and Vener 1985).

All components of ISP monitoring data collection, management processes and reporting mechanisms are designed for maximum comparability with similar organizations. Standardization, where practical, and the use of data collection and reporting formats which make data useful to cooperating organizations is accomplished through continuing education and training to maintain an understanding and application of best practices. Review of scientific literature, attendance of professional meetings, participation in technical trainings and workshops, etc. provide a forum for ISP staff to communicate with peers in the fields of conservation, restoration and related sciences in order to stay abreast of cur-rent technologies and data standards.

All Monitoring Program field staff are provided extensive training prior to conducting monitoring, and every effort is made to retain our experienced staff to improve data reliability and efficiency. Each year, both experienced and new field monitoring staff receive group and personalized training in *Spartina* field identification, non-target marsh plant species identification, distance and diameter estimation, cover class estimation, and any new monitoring methods or GPS/GIS technology. Field staff work in pairs throughout the season for safety, efficiency and continued calibration of distance, diameter and cover class estimates.

To measure comparability of field identification of *Spartina* species among staff, the Monitoring Program Manager reviews the results of samples field-identified and collected for testing by staff person. Field staff genetically sample all accessible populations of morphologically ambiguous *Spartina*, and sample *Spartina* species in which they have high confidence in their field identification of species. The observer's confidence (low, medium, high) of species field identification is recorded for each sample. Genetic results are used to confirm staff field identifications, and to document each staff member's skill at field identification.

Comparability of genetic data is ensured by adherence to published methods for genetic analysis. Analytical methods and quality assurance protocols of the UC Davis Lab, which performs genetic testing for the ISP, are described in Exhibit A of the interagency agreement between the Coastal Conservancy and the Regents of the University of California (**Appendix 5**), and/or any contracted lab (in 2009, this was Eurofins STA Laboratories, and their Quality Assurance Plan is provided in **Appendix 6**).

To ensure and document comparability of non-*Spartina* plant identification, field staff train together, work in pairs, and prepare annual voucher specimens of non-target plants to confirm field identification of species recorded in treatment efficacy monitoring plots.

Inventory and summer treatment efficacy monitoring data are collected, processed, and reported to allow comparability between years. Inventory design and data collection methods are generally consistent from year to year, with changes fully documented. Any changes in methodologies are accounted for during inventory data analysis and reporting to ensure comparability of data between years, with a full explanation of changes and any resulting caveats to the data being fully disclosed in all data transmittals and reports.

Standardization and accurate relocation of spring efficacy monitoring data collection ensures comparability between years. Point positions and bearings are recorded and relocated using a GPS unit, and marsh and patch characteristics are recorded using a specially-designed data dictionary to ensure comparability of data between observers and between years.

The Control Program began conducting annual herbicide applicator calibration training in 2006 in an effort to standardize herbicide application rates at a level shown to achieve the highest efficacy and lowest waste of herbicide and time. Standardized calibration adds to the comparability of all efficacy monitoring data, improving confidence in observed correlations between cause and effects of treatment and vegetation response.

3.4. Accuracy and Precision

The term "accuracy" is defined as the difference between a measured value and the true or expected value. Accuracy values are not possible for measured parameters for which "true" or expected values do not exist. "Precision" is a measure of repeatability, and refers to the degree of mutual agreement among individual measurements. Accuracy represents an estimate of systematic error, whereas precision represents an estimate of random error. Together, accuracy and precision provide an estimate of the total error or uncertainty associated with an individual measured value.

Accuracy and precision objectives are defined as appropriate for each individual component of the ISP based on program requirements and practical considerations of the constraints of field data collection.

The accuracy and precision requirements of *Spartina* inventory monitoring data have been determined based on the needs of the Control Program. The ISP Control Program requires that inventory monitoring of *Spartina* populations be adequate to inform field operations, which include various methods of herbicide or solarization treatments. The degree of spatial accuracy and precision required by the Control Program and the realities of field conditions determine mapped "patch size". Patch size refers to the spatial extent of contiguous *Spartina* plants which are grouped together in a GPS and/or GIS feature. For all practical purposes,

patch size is determined by the degree of precision reasonably captured in the field. In sites with very few non-native *Spartina* plants, highly precise data is recorded (individual plants or clusters are mapped with a patch differentiation threshold of 5+ meters). In sites with heavy infestations of non-native *Spartina* and/or in inaccessible areas, a patch differentiation threshold of up to 30+ meters may be applied. The Control Program has stated that a maximum patch differentiation threshold of up to 0.5 acre is acceptable in highly infested areas.

Empirical accuracy and precision is also determined to meet the needs of the ISP. Due to the extensive area surveyed (approximately 45,000 acres of marsh and mudflats), empirical parameters are designed to maximize efficiency while collecting data with adequate accuracy and precision to inform monitoring and control efforts. Data collected include field identification of *Spartina* species, cover class estimates for non-native *Spartina* within patches, and patch diameter or width estimates for point and line features, respectively. Accuracy of field identification is assessed by sampling of *Spartina* plants for genetic testing, as described above.

Summer efficacy monitoring plot design was altered in 2006 to account for issues with precision and accuracy by standardizing plot size to improve comparability between years. Prior to 2006, treatment monitoring plots were centered on non-native Spartina plants and were variable in diameter, with plot diameter determined by Spartina patch size in the initial year of plot set-up. Some plots were less than one meter away from others, and the accuracy of the GPS units did not allow for precise plot location under one meter. Also, once Spartina plants were eradicated and thatch was been cleared, there was no visible sign of plot centers, adding to the confusion at sites with a number of small, adjacent plots (as at former S. densiflora clones at Point Pinole). In 2006, plot size was standardized to a 3-meter radius circular plot centered around a 0.25 x 0.25-meter quadrat. Quadrat locations are determined by GPS with an accuracy of +/- 1 meter based on GPS unit specifications. Quadrat boundaries are determined in the field using a 0.25 x 0.25-meter frame to ensure precision of collected density data. Larger, circular plot boundaries are estimated by trained field staff. Cover class data for all species within both plots and quadrats are estimated with the assistance of cover class diagrams. The use of cover classes reduces the precision of data, thus improving the comparability of data collection among field biologist teams. Similarly, the use of large, 3-meter radius plots reduces the precision of cover data, thus improving the comparability of data collection among years.

Summer efficacy monitoring requires greater spatial accuracy and precision of GPS data than inventory monitoring activities because the sampling design requires re-locating permanent plots to monitor changes in vegetation following treatment activities. Field staff record and re-locate treatment monitoring plots in the field using GPS units capable of sub-meter accuracy (Trimble GeoXT 2003 models). Point coordinates are recorded by averaging at least 10 positions at the center of the plot. (Since 2006, new locations have been recorded by averaging at least 30 positions, with positions collected every 1 second.) While the ideal goal for spatial accuracy and precision would be the exact re-location of permanent plots, we cannot achieve this level of accuracy without the use of survey-grade GPS and/or permanent markers in the field. Although GPS unit specifications indicate accuracies within +/- 1 meter, in actuality, re-location of positions may be accurate to only within up to three meters, due to inaccuracies in GPS positioning from standard error sources (number of visible satellites, atmospheric interference, etc.), which cannot be corrected through post-processing of data.

Sampling design, field procedures, and analysis of summer efficacy monitoring data take into account this difficulty in accurate and precise plot relocation. The potential for GPS inaccu-

racy up to three meters results in 39-100% overlap in area between the original and relocated 3-meter radius plots. Because quadrats are located in the center of the 3-meter plots, they fall within the boundaries of the original and/or relocated plots, despite GPS inaccuracies. Where plot locations were obviously inaccurate, falling outside of past or present non-native *Spartina* patches, GPS coordinates were re-logged within the closest patch of non-native *Spartina*, using the average of at least 30 positions to improve accuracy over previous data collection. (Note: re-logging of coordinates only takes place when it is certain that the lack of non-native *Spartina* at the plot is due to GPS error rather than to treatment and subsequent removal of non-native *Spartina* from that location.) Semi-permanent markers (bamboo stakes) were placed in the field in 2007 in an attempt to improve the accuracy and precision of plot relocation in subsequent years. Analysis of summer efficacy monitoring includes a thorough review of data for outliers and a comparison of results to field observations. Outliers and data which do not fit with field observations are reviewed for accuracy. If they are deemed accurate, these data are included. If they are deemed in the reporting of results.

Accuracy and precision of spring efficacy monitoring photo point location and field of view is ensured by using GPS coordinates, compass bearings, and printed photos from previous monitoring events. Photo monitoring sites are revisited using high-accuracy GPS units to relocate coordinates. A compass is used to direct the observer to the same bearing used in previous years, to ensure that the photo will capture the same view and accurately portray the visual time series at that infestation site. Beginning in 2008, printed copies of digital photographs will be taken into the field, to assist the observer in documenting the exact same scene from year to year. The removal of significant proportions of the *Spartina* infestation makes the spatial identification of the previous "scene" more challenging, necessitating this extra measure to improve accuracy and precision.

Accuracy and precision of genetic sampling results are determined by blind, haphazard duplicate sample testing. Field biologists collect duplicate leaf samples from the same stem at a rate of approximately one duplicate per twenty samples. To avoid bias, the lab is not notified that these samples come from the same plant. Results are compared at the end of the field season to assess degree of accuracy (final species determination) and precision (final count of genetic markers for each species within each sample).

Section 4. Sampling and Assessment

The Monitoring Program conducts annual *Spartina* inventory monitoring, treatment efficacy monitoring, and clapper rail monitoring. Monitoring design, data collection, handling, storage, analysis and dissemination are described separately for each type of monitoring below. The approximate timeline for this work is shown in **Table 2**.

4.1. Inventory Monitoring

4.1.1. Inventory Monitoring Design

The inventory monitoring process is designed to survey all known potential non-native *Spartina* habitat in the Estuary, and to record the location, extent, and percent cover of all non-native *Spartina* detected. Components of this design include: identification of potential non-native *Spartina* habitat, definition of monitoring site names and boundaries, obtaining proper permissions to access these sites, and selecting the proper method, timing, mode of access and tidal conditions to enable thorough inventory of non-native *Spartina* at each site.

The ISP identified most potential *Spartina* habitat in the Estuary by 2004. Beginning in 2007, possible new areas of potential habitat were identified by checking existing monitoring sites against tidally influenced sites delineated in WetlandTracker (SFEI), EcoAtlas (SFEI 2005) and the National Wetlands Inventory (USFWS) in a GIS, by discussions with project partners, and by visual analysis of high resolution aerial imagery including imagery available from the National Agricultural Inventory Program ("NAIP"; USDA 2005), the US Geological Survey ("USGS"; USGS 2003), and online sources including Microsoft Live Maps (Microsoft), Google Maps (Google) and Google Earth (Google). These efforts continue, and as new potential non-native *Spartina* habitat is created and/or discovered, these new areas are added to the list of existing monitoring sites.

The Monitoring Program Manager and Assistant Monitoring Program Manager maintain a list and GIS shapefile of monitoring sites covering all known *Spartina* habitat. Site names and boundaries are developed by the Monitoring Program to fit the program's needs, and may or may not conform to the site boundaries or names of the source data (e.g. WetlandTracker). Sites boundaries are determined in part by mode of access, with sites sometimes being lumped or split depending on number of field staff days necessary and the mode of access used to survey the site.

To maximize the ability of field staff to detect populations of non-native *Spartina*, the inventory monitoring process is designed to take place during the height of the *Spartina* growing season (May through November), at the lowest possible tides, using the mode(s) of access with the least possible impact on the marsh habitat while permitting effective and efficient data collection.

Inventory may be conducted using field surveys or aerial imagery interpretation. Aerial imagery interpretation is used to inventory large and/or inaccessible *Spartina* infestations that are visible in high resolution color infrared (CIR) imagery and would be difficult or impossible to map at the required levels of accuracy, precision and completeness using field surveys alone. Land, water, or air-based field surveys are conducted in all other areas. The ISP uses the same field staff for both field surveys and, when used, aerial imagery interpretation work to ensure the consistency and quality of inventory data.

Field Survey Data Acquisition Design

The timing and mode of access for acquiring field survey data is designed to maximize the likelihood and efficiency of detecting all non-native *Spartina* during an inventory survey. Acquisition of field survey data requires site access at appropriate tide levels and weather conditions for maximum visibility of non-native *Spartina* habitat. Monitoring is conducted primarily during the summer and fall, the peak of the growing season, when the phenology of non-native *Spartina* is best for field identification. Because *S. densiflora* and *S. patens* flower and senesce earlier in summer than *S. alterniflora*/hybrids, monitoring of sites known to contain these two species is initiated first, where allowed. In marshes where endangered California

		Anticipated Month of				
Program	Activity	Initiation	Completion	Products	Anticipated Product Completion	
	Field data collection, processing & editing; annual survey boundary delineation	May	November	Shapefiles and maps shared with project part- ners, ERP and others. Data integrated into an- nual inventory report.	Draft shapefiles & maps available by request within 1 week of data collection. Final data due in annual report.	
Inventory Monitoring	Color IR imagery collec- tion & aerotriangulation (AT) *if used	September	January	Imagery & aerotriangula- tion solutions delivered to ISP as specified in sub- contract	As specified in subcon- tract	
iventory	Color IR imagery orthorectification *if used	November	January	Orthorectified color IR imagery	Within 60 days of im- agery & AT solution delivery to ISP	
In	Ground truthing and photo interpretation- based digitizing *if used	December	February	Data integrated into an- nual inventory report	March 15 th of each year	
	Data QC, analysis and presentation	November	March	Data integrated into an- nual inventory report	March 15 th of each year	
cacy	Field data collection	June	October	Data integrated into an- nual inventory report	March 15 th of each year	
Treatment Efficacy	Photo point acquisition	May	June	Digital photos and GIS data organized and avail- able to Control Program via ISP Server	Early July of each year	
Tre	Data QC, analysis and presentation	December	February	Data integrated into an- nual inventory report	March 15 th of each year	
Clapper Rail Monitoring	Field data collection and data entry	January	April	Data integrated into an- nual inventory report	July 15 th of each year	
	Data analysis	April	June	Graphs and comparative analysis	July 15 th of each year	
	Data reporting	April	July	Annual Clapper Rail Re- port; Natural Diversity Database Entry	July 15 th of each year	

Table 2. Monitoring Program Annual Timeline

clapper rail (*Rallus longirostris obsoletus*) are determined to be present, all monitoring takes place as allowed by the project's Biological Opinion from the U.S. Fish and Wildlife Service Endangered Species Division. Modes of access are selected to allow efficient inventory monitoring at the lowest feasible tide. While low tide levels allow the greatest visibility of *Spartina* habitat, access to some sites requires access by boat, thus necessitating that surveys be conducted at medium or high tides. Mode of access and associated tide levels necessary to allow access vary by site. Modes of access for field surveys (including ground truthing of aerial photos) include foot, bicycle, airboat, kayak, motorboat (Zodiac, Klamath, Boston whaler), and helicopter. Details regarding the date(s) surveyed, surveyor names, modes of access and associated tide levels used for inventory at each monitoring site are recorded annually.

Aerial Imagery Acquisition Design

The timing, extent and technical specifications for aerial imagery acquisition are designed to maximize the ability to detect non-native *Spartina* in the imagery. The Monitoring Program Manager works with the Control Program and field staff to determine, on an annual basis, which areas, if any, should be inventoried using aerial imagery interpretation. This decision is based on confidence of species' identification, extent and density of infestation at the site as well as issues of site accessibility. Sites with extensive infestations and/or extremely difficult access were prioritized for imagery acquisition prior to 2008. In 2007, quality assurance audits of the digitized data confirmed what had been noted by photo interpreters: the reduction in *Spartina* density and extent due to control measures had made it difficult to impossible to use photo interpretation as an accurate method of mapping non-native *Spartina*. The decision on whether to use aerial imagery acquisition for *Spartina* inventory mapping, and at what sites, is reviewed annually. Aerial imagery has not been acquired or used by the Monitoring Program for inventory monitoring purposes since 2007.

4.1.2. Inventory Data Collection Methods

Field Survey Data Collection

Inventory field survey data includes mapping of non-native *Spartina*, location of *Spartina* genetic sampling, and mapping of the endangered marsh bird's beak, *Cordylanthus mollis* ssp. *mollis*.

At each site, trained staff map the location and areal extent of non-native *Spartina* species using a Global Positioning System (GPS) handheld sensor and data entry unit. All GPS units are loaded with a custom *Spartina* monitoring data dictionary created using ArcPad Studio or Pathfinder Office software. ArcPad or TerraSync software is used by staff in the field to record data using these custom data dictionaries. When using ArcPad, past years' data and informative background layers are loaded onto the units as well using a standard protocol for data checkout (**Appendix 11**, Section A).

Plant location data are entered into the unit as points, lines, or polygons, depending on the extent of the invasion. A linear array of clones, for example, along a creek bank, is typically mapped as a line. A cluster or meadow of clones is mapped as a polygon if possible. In many cases, for efficiency or in sites where direct marsh access is restricted, a cluster of individual clones may have been mapped as a single point, with the number of clones per cluster noted. In these cases, the point feature's diameter and percent cover attributes are recorded to reflect a circular plot encompassing all non-native *Spartina* and the percent cover of non-native *Spartina* within that plot. Only one species is recorded per feature. At sites where more than

one non-native *Spartina* species grow together (e.g. Creekside Park), field staff record separate GPS features for all non-native *Spartina* species.

The GPS units automatically collect data on date, time, location, area and perimeter (if polygon data), and length (if line data). Field staff manually enter additional data including species field identification, species identification confidence, diameter (if point data), width (if line data), cover class of recorded species, and survey conditions. Since 2008, field staff record two kinds of cover class attributes for each *Spartina* feature. The first is visual estimation of absolute aerial cover class. The second is a visual estimation of the coverage of herbicide required to treat the non-native *Spartina* within the feature. Any other relevant information is noted and entered in a "comments" field.

The ISP is also required to map all locations endangered plant species found in non-native *Spartina* sites. At the single site (Southampton Marsh, Benicia State Recreation Area) where both non-native *Spartina* and endangered marsh bird's beak grow, ISP field staff map the extent and percent cover of this plant in GIS using *Cordylanthus* point, line and polygon features in the inventory data dictionary. Field staff are trained to look for endangered species at all monitoring sites, and will map and notify U.S. Fish and Wildlife Service if new populations of endangered species are discovered.

Spartina species identification in the field is confirmed by genetic verification of fieldidentified native and non-native *Spartina* samples collected by field staff for genetic testing. The inventory data dictionary includes a point feature to record the precise location of the *Spartina* samples collected. DNA point features are linked by sample code to the associated point, line or polygon feature containing the *Spartina* patch from which the sample was taken.

Species Identification and DNA testing

Genetic testing of *Spartina* samples is performed to identify unknowns and confirm field identification of *Spartina* species. *Spartina* species are identified based on plant morphology, density, and marsh position in the field (as described in the ISP Field Identification Guide, Zaremba 2001), as well as genetic testing. Identification of *Spartina* species is complicated by (1) the variation of morphological characteristics due to phenotypic variation from site to site and year to year given variable environmental conditions (as expressed in native *S. foliosa* and hybrids), and (2) morphological ambiguity between hybrids and their parent species, especially in highly backcrossed hybrid populations. Morphologically ambiguous plants or patches are mapped as "Possible *S. alterniflora* x *foliosa* hybrids", "Possible *S. anglica*", or "Possible *S. densiflora* x *foliosa*", as applicable. Where possible, samples of these morphologically ambiguous plants are collected for genetic testing. Samples of plants identified by field staff as *S. alterniflora*/hybrids and *S. foliosa* with high confidence are also sampled at monitoring sites throughout the season to confirm species identification.

In addition to morphologically ambiguous plants, there are also morphologically cryptic *S*. *alterniflora* hybrids (i.e., plants with *S*. *alterniflora* genes which are not readily distinguishable in the field). These plants may be discovered through genetic testing of point samples of "known" species and through seedling or transect sampling methods.

At the leading edge of the hybrid invasion, within sites recently colonized by *Spartina*, or in the outlying areas of the Estuary where marshes are still dominated by native *S. foliosa*, mature *Spartina* plants may be sampled by transect or seedlings may be collected for genetic analysis to assess the extent of the invasion or to confirm native species identification. Prior to 2006, when sampling mature plants, transects were run the length of the marshes and *Spartina* leaves collected every 10 meters. Transect-collected leaves were randomly sub-sampled for

genetic analysis. Data on the exact locations of each sample were not collected. Beginning in 2006, the exact location of each sample was recorded for all genetic sampling (transect and individual plant).

For each genetic sample, a collection location is recorded using GPS, and a single undamaged leaf 10-20 cm in length is individually labeled. Samples are kept refrigerated until mailed overnight to the lab where genetic testing is performed using RAPD (Random Amplified Polymorphic DNA) nuclear DNA markers according to the methodology described in Daehler *et al.* (1999). Once genetic testing is complete, the lab results are then incorporated into the GIS data layer's attribute table.

Some plants may have backcrossed to the extent that, while they contain some *S. alterniflora* genes, these cannot be detected through RAPD testing. These are referred to as genetically cryptic hybrids. The ISP and the UC Davis Lab, which discovered hybrid *Spartina* in the Estuary and which continues to advise and perform RAPD analysis for the ISP, are currently involved in research and discussion of how to quantify the probability of non-detection of hybrids using current methods of genetic testing, and how to assess and address the potential risks of these genetically cryptic hybrids in the Estuary.

Starting in 2009, based on the research of Christina Sloop and Laura Feinstein, the ISP requested the use of additional RAPD markers as well as microsatellite markers to increase the level of testing and thus the ability to detect highly backcrossed hybrids.

Aerial Imagery & Associated Data Collection

Imagery Processing

In years when the project determines that inventory monitoring would be most effectively done via aerial imagery interpretation at select sites, color infrared (CIR) photography is obtained via contract with a firm specializing in such imagery acquisition and processing. Imagery is orthorectified and mosaiced by either the contractor or by the ISP in-house using imagery processing software. Specifications for color infrared imagery acquisition and processing are outlined in **Appendix 9**.

Following orthorectification and mosaicing, current-year CIR images are visually checked to ensure less than two meters difference in overlap with NAIP and USGS "orthophotos" (geometrically corrected aerial photographs). This check is performed by loading CIR images into an ArcGIS 9.2 (ESRI) map document along with NAIP 2005 (USDA 2005) and USGS 2003 high resolution orthophotos (USGS 2003), then using the swipe tool in ArcMap to visually compare feature locations. If errors greater than two meters are detected, the ISP and the contractor who provided the CIR imagery work together to resolve the errors.

Ground Truthing and Field Reconnaissance

Field staff use a combination of ground truthing and field reconnaissance to inform photo interpretation.

Ground truthing involves recording percent cover of all vegetation in 3m radius circle around each of 30 random points within the site to be digitized. This information is used to help interpret the CIR imagery by identifying the vegetation corresponding to patterns in the imagery. While the imagery changes from year to year, ground truthing points are permanent points once initially selected. This allows for comparison of changes in vegetation patterns and/or imagery quality from year to year.

Random points for ground truthing are selected from within the boundary of the potential *Spartina* habitat using the Hawth's Tools (Beyer 2004) "Generate Random Points" in ArcMap. Tool parameters are set to create 100 points spaced at a minimum distance of 10 meters apart using an unstratified sampling design. Random points falling in inaccessible areas, such as water or deep mudflat, are deleted. Remaining points are prioritized 1-50. (Although only 30 points will be ground truthed, extra points are provided in case points 1-30 prove to be inaccessible in the field.) Points are imported from shapefile format into Pathfinder Office, then loaded onto a Trimble GeoXT GPS unit for field location and data recording using TerraSync software. Data are recorded using the Monitoring Program data dictionary's ground truthing feature to allow efficient and standardized data recording in the field and transfer of field-recorded data into GIS on return.

Field reconnaissance involves subjective ground truthing as well as marking up printed, large scale images of CIR imagery with notes in the field. The mark-up of printed maps directly trains the eyes of the staff person to recognize visual patterns in the imagery which correspond to non-native *Spartina* patches. Subjective ground truthing and partial inventory insure correct location of field notes by linking GPS points to surveyor observations at useful locations. In subjective ground truthing, the surveyor records up to 10 subjectively located *Spartina* points of 3-meter radius (with variable cover classes) and up to 10 non-*Spartina* points (with various vegetation types) of 3-meter radius – selected in the field to assist with photo interpretation efforts in the office. These data are also recorded using the data dictionary's ground truthing feature, for consistency. Partial inventory is performed using the data dictionary's inventory features (see Field Survey Collection methods) to record Pt_Spartina, Ln_Spartina, and Poly_Spartina features as deemed appropriate to assist with photo interpretation in the office.

To facilitate use of 8 $\frac{1}{2}$ " x 11" printed images for field reconnaissance, a map grid is created using Hawth's Tools to delineate blocks of views to be printed at a scale between 1:1000 and 1:2000. This grid and it's associated larger scale maps are printed and carried in the field along with a GPS, sharpie and clipboard. To provide "landmarks" to help identify surveyor position in the field, and to aide in location of ground truthing points, ground truthing points are included on these maps, identified by their priority numbers. Examples are given in **Appendix 10**.

Digitizing

Following ground truthing and field reconnaissance, staff identify and delineate polygons identifying non-native *Spartina* patches visible in orthorectified CIR imagery within a GIS using a process called heads-up digitizing. All digitizing edits are done in a map document containing the following raster data layers: current-year CIR "orthoimagery" (geometrically corrected digital imagery), past-year CIR orthoimagery, NAIP and USGS high resolution true color imagery, and vector layers: current-year and past-year ground truthing data, field reconnaissance data, and *Spartina* inventory data. Although patches may be recorded as points, lines or polygons, polygon features are generally preferred; digitization of polygons is efficient and provides the greatest accuracy and precision. The digitizer attributes each feature with his or her name, date of digitization, *Spartina* species, and percent cover of non-native *Spartina*.

4.1.3. Inventory Data Handling

Prior to collection of field data, data dictionaries are created on a desktop computer using Pathfinder Office (Trimble) or ArcPad Application Builder (ESRI). Data dictionaries are saved on the ISP Server and uploaded onto all ISP GPS units.

Following collection of field data in a GPS unit, data are downloaded, processed, viewed and edited using GIS software and following ISP protocols (**Appendix 11**). Field data are collected using TerraSync (Trimble) and/or ArcPad software loaded onto GPS units. Data are downloaded from the GPS units to the ISP Server via Pathfinder Office or ActiveSync, using desktop computers at the ISP office which are networked to the Server. Data collected using TerraSync are differentially corrected in Pathfinder Office using the nearest base station. New and updated features from these differentially corrected data files are exported to GIS shape-file format in Pathfinder Office, retaining all TerraSync-generated attributes and projecting files to the UTM Zone 10N coordinate system, NAD 1983 datum. Data collected in ArcPad are checked in using standard protocols (**Appendix 11**, Section B).

Field-collected are loaded into an annual geodatabase for editing by staff members, and are visualized and edited in ArcGIS map documents using customized symbology styles. Data editing by field staff includes checking attribute and location data for accuracy. As a quality control measure, staff date and describe all data modifications, and date their final QC of their data.

The contracted genetics lab provides genetic results to the Monitoring Program Manager in Excel (Microsoft) format. The Monitoring Program Manager updates and maintains the annual ISP genetic results table, also in Excel format. The annual genetic results table is linked in ArcGIS to the DNA sample location layers in the current-year geodatabase using a join on the sample identification code used by both ISP staff and lab staff. This link allows for the automatic update of all genetic results and associated symbologies in all ArcGIS maps containing DNA sample layers when the ISP genetics table is updated. Symbology in ArcMap indicates both the genetic results and the field identification of the *Spartina* species. Staff review genetic results as they come in, update their final species determination for related inventory features in a Final Species attribute column, and date their final determination.

4.1.4. Inventory Data Storage

Inventory data is stored on the ISP Server, which is backed up weekly. Current-year data is backed up daily. Back-ups are stored for 6-7 months.

Field-collected data are stored in both their original and edited formats, allowing review and/or reprocessing of original data when necessary. Data storage includes:

- Backups of original GPS files.
- Storage of potentially edited GPS files.
- Storage of zipped base files for differential correction to allow reprocessing of differential correction if necessary.
- Storage of exported shapefiles.
- Backup of current-year geodatabase prior to loading of new data files.
- Offsite storage of data backups.

4.1.5. Inventory Data Analysis

Analysis of inventory data includes summarization of annual bay-wide non-native *Spartina* abundance and distribution, and calculation of the change in distribution and abundance from previous surveys. Data are analyzed using ArcGIS software, with additional data analysis taking place as needed using statistical software such as JMP (SAS).

Summary statistics of *Spartina* inventory monitoring data are calculated based on the summation of gross area, net area and treatment area within spatial boundaries (subsite, region, and Estuary). For graphic and geographic representation of changes in *Spartina* cover over time, cover classes may be grouped into low (<10%), medium (10-50%) and high (>50%).

Gross area is calculated as the area of non-native *Spartina* features in GIS, with calculation of (point area) = πr^2 and (line area) = (length) * (width). Note that this calculation of gross area is highly influenced by the method used to record *Spartina* locations in the field or in aerial photo interpretation (see Collection Methods), as the "patch size" differs by survey method.

Net area is calculated on a per feature basis as (gross area) * (percent cover), using the mean of the percent cover class recorded in the field as the percent cover multiplier. This is the same calculation used to determine "total area of solid cover" reported by Ayres *et al.* (2004), and is a measure of estimated aerial cover of non-native *Spartina*.

Treatment area is calculated on a per feature basis as (gross area) * (treatment cover), using the mean of the percent cover class recorded in the field as the treatment cover. As described earlier, treatment cover is the estimated absolute aerial percent cover of the feature that should be sprayed with herbicide in order to effectively treat the mapped non-native *Spartina*.

Changes in gross, net, and treatment area between years are reported as both difference and percent difference.

4.1.6. Inventory Data Dissemination

Internal

Inventory data are shared internally through use of personal and shared GIS map documents which contains up-to-date inventory data layers as well as background imagery and ancillary data layers. The Monitoring Program Manager is responsible for the development and maintenance of GIS layers and shared map documents. Shared map documents include spatial and attribute table data for all GIS monitoring data recorded by the Monitoring Program from 2000 to present. Staff are trained in the use of GIS to visualize and query data, and consult with the Monitoring Program Manager when additional assistance is needed.

<u>External</u>

Inventory data are shared with project partners on a per request basis and through the annual monitoring report. Data are shared in the form of maps, shapefiles, and data summaries, as requested.

4.2. Efficacy Monitoring

4.2.1. Efficacy Monitoring Design

The ISP conducts two types of treatment efficacy monitoring. The first, "Spring Efficacy Monitoring," consists of photo monitoring at select treatment sites. Photo monitoring is conducted in the spring and fall to estimate the effectiveness of the previous season's control ef-

forts and to enable planning for the current control season. The second, "Summer Efficacy Monitoring," is a quantitative survey conducted at a pre-selected subset of sites (stratified across all treatment sites by *Spartina* species, treatment method, and habitat or marsh type) during the peak of the growing season to measure the efficacy of *Spartina* treatment methods

Spring Efficacy Monitoring Design

Spring efficacy monitoring is designed to inform current-year control efforts through observation and subjective analysis of the efficacy of the previous year's treatment. Spring efficacy monitoring sites, and photo point locations at each site, are determined by the Control Program's Field Operations Manager and Assistant Manager using the guidelines in the ISP Photo Point Establishment Protocols (**Appendix 12**). Sites are selected in the office, using a GIS to determine the optimal location(s) for photo points. Exact location and compass direction of photo points is determined during the initial site visit, and is recorded using a GPS data logger.

The spring efficacy monitoring schedule is designed to take place at low tide, which allows the greatest visibility of *Spartina*, beginning in late May each year. A second round of photos is taken between September and October each year.

Summer Efficacy Monitoring Design

Summer efficacy monitoring is designed to quantify treatment efficacy at a sampling of sites, and thus follows a rigorous experimental design. The study design uses permanent monitoring plots established in the first year of measurement, prior to treatment, which are re-sampled one season following treatment, and each subsequent season. This approach for treatment efficacy monitoring is consistent with the approach suggested in the guidelines developed by Collins *et al.* (2001).

Summer efficacy monitoring sites are selected by the ISP to encompass a range of marsh types and treatment methods. Sites selected for treatment efficacy monitoring are reported in annual monitoring reports. A full description of each site and the treatments implemented can be found in the *Spartina* Control Program Site-Specific Plans (available at www.*Spartina*.org/control/sites.php).

For small infestations where individual clones are mapped as points, all mapped plants (up to a maximum of 30 plants per site) are monitored. If more than 30 plants are mapped, a sample of 30 permanent monitoring plots are selected along transect(s) within the site.

Experimental design for those summer efficacy monitoring sites initiated in 2006 was established using GIS and ground truthing in the field to locate 30 random points within non-native *Spartina* populations along three restricted-random transects at each site. The restricted random sampling procedure divided each site into three equal-length segments along the linear axis, then randomly positioned three sampling transects perpendicular to the segment, with 10-meter minimum spacing between each transect. This design was employed to create good interspersion of transects throughout the marsh, and to create more than one transect for purposes of statistical analysis of mean transect values. Along transects, points with 10-meter minimum spacing and containing non-native *Spartina* were selected as potential sampling points. A final randomization of potential points was used to determine which 30 points would be sampled in the field. Based on the observed spread and clonal growth patterns of non-native *Spartina*, 10-meter spacing was determined by ISP staff to be sufficient to provide independence among sampling points, thus allowing use of parametric statistical tests to infer results from these plots to the entire marsh. Transects established prior to 2006 were designed so that transect length and location would intersect the longest possible extent of the infestation within the constraints of site accessibility. (Site access is often highly constrained by tides, channels, and deep mud that restrict the ability to physically enter portions of many sites.) Every 10 meters along the transect, a coin toss determined whether or not to sample at that point. At selected points, the exact location of the permanent monitoring plot was determined by a toss of the .25 x .25-meter quadrat. Although this sampling design did not employ strict randomization, testing of spatial autocorrelation between plots indicated that stem density reduction values were neither clustered nor dispersed, but were randomly distributed among sites (San Francisco Estuary Invasive Spartina Project 2007).

Scheduling and strategy of summer efficacy monitoring is designed to comply with the U.S. Fish and Wildlife Service Biological Opinion (USFWS 2003; USFWS 2004; USFWS 2005), to allow completion of monitoring at all selected sites during the growing season (June through October), and to create the least possible disturbance to marsh biota. To enable completion of monitoring at all sites, field work may begin as early as June, once plants have reached identifiable phenological states. At sites with California clapper rail present, site entry dates are restricted within the limits set by the U.S. Fish and Wildlife Service under the ISP Biological Opinion. Sites are entered at low tide, following the ISP Marsh Safety protocol (**Appendix 1**). Site maps, which display labeled plot locations and (where possible) channel delineations overlaid onto high resolution aerial photos, are prepared ahead of time and carried in the field to assist in navigating the plots with the least impact to the marsh and clapper rails, as mandated in the Biological Opinion.

4.2.2. Efficacy Monitoring Data Collection

Spring Efficacy Monitoring Data Collection

At each Spring efficacy monitoring point, the biologist shoots a single photo. Photos are taken using a digital camera as specified in the ISP Photo Point Establishment Protocols (**Appendix 12**).

Location and bearing of spring efficacy monitoring photo points are recorded during the initial year of monitoring using a Trimble GeoXT GPS unit to record a point feature, averaging at least 10 GPS positions, with an associated monitoring code. A compass bearing is determined and recorded to document the angle at which the photo was taken. In subsequent years, positions and bearings are relocated using a Trimble GeoXT GPS unit and a compass. Beginning in 2008, printed copies of digital photographs will be taken into the field, to assist the observer in documenting the exact same scene from year to year.

Summer Efficacy Monitoring Data Collection

Summer efficacy monitoring data collection involves relocation of permanent plots and the standardized recording of vegetation data at each plot. Permanent plots are re-located using a Trimble GeoXT GPS unit, which records these locations at up to sub-meter accuracy. Printed maps are brought into the field to assist field staff in minimizing their footprint while entering and exiting the marsh to access plots.

Treatment efficacy data is collected at two scales: within 3-meter radius plots, and within .25 x .25-meter quadrats. The data collected from the 3-meter radius plots includes the following:

- Cover class for all live plant species
- Cover class for all recognizable dead plant species

- Sediment type (mud, sand, cobble, other)
- Cover class for bare ground
- Cover class for wrack
- Non-native *Spartina* vigor (high/medium/low)
- *Spartina* life history (vegetative growth/flowering/senescent)
- Treatment efficacy (estimate of percent non-native *Spartina* dead or weakened by treatment within plot)

Data collected from the .25 x .25-meter quadrats includes the following:

- Stem height of live and dead stems of non-native *Spartina*. A single stem is selected to be indicative of the median height within the quadrat; height is measured from the mud surface to the base of the highest leaf of the selected stem
- Count of live and dead stems of non-native Spartina
- Cover class for each plant species
- Cover class for bare ground
- Cover class of wrack

4.2.3. Efficacy Monitoring Data Handling

All GPS-collected efficacy monitoring data are downloaded from the GPS units and exported into GIS shapefiles as described under Inventory Data Handling.

Spring Efficacy Monitoring

Digital photos are downloaded to the ISP server and to a portable hard drive. Digital photos are named using a standard naming convention ("ABCDPP##_YYRX", where ABCD = Treatment subsite code, PP = photo point, ##= 2-digit photo point number, YY = 2-digit year, R = round and X = round number), and saved to a single folder. This allows easy and rapid viewing of all monitoring photos using photo viewing software, as well as efficient preparation of PowerPoint (Microsoft) presentations.

Point location and characteristic data are saved within the exported GIS shapefiles. Shapefiles are combined into a single shapefile for each round of photos.

Summer Efficacy Monitoring

Exported GIS shapefiles are loaded into a personal Access geodatabase designed to contain the same domains (drop-down menus) as in the data dictionary used to collect the data. Data are checked for accuracy by the Monitoring Program Manager using attribute and location queries in ArcGIS. Field staff are consulted when potential errors are found. Any corrections or modifications to data are noted in the attribute table of the feature modified.

4.2.4. Efficacy Monitoring Data Storage

Data is stored on the ISP Server, which is backed up daily. Back-ups are stored for 6-7 months.

GPS and GIS data are stored in both their original and edited formats, allowing review and/or reprocessing of original data when necessary. Data storage includes the following:

• Backup of original GPS TerraSync rover files

- Storage of potentially edited GPS TerraSync rover files and differentially corrected TerraSync files
- Storage of zipped base files for differential correction to allow reprocessing of differential correction if necessary
- Storage of exported shapefiles
- Backup of efficacy monitoring shapefiles and geodatabases prior to and during editing
- Offsite storage of data backups

4.2.5. Efficacy Monitoring Data Analysis

Treatment efficacy monitoring data are analyzed by using parametric and non-parametric statistics to calculate the effects of treatment methods on treatment efficacy and on all other vegetation. Efficacy is measured by plot and quadrat percent cover reduction of non-native *Spartina*, and by change in live non-native *Spartina* stem height and quadrat stem density percent reduction one year following treatment as the measure of efficacy. Stem density percent reduction is calculated as [(stems(Year 1)-stems(Year X))/stems(Year X)]. Negative stem density percent reduction values are coded as zeros (as an increase in stem density indicates zero efficacy). Effects on all other vegetation are measured based on changes in species presence/absence and changes in percent cover of other species over time.

Treatment methods are combined into six classes: dig, cover, mow, herbicide glyphosate, herbicide imazapyr, and no treatment. Supplemental environmental and treatment data is evaluated for its ability to detect differences in efficacy based on elevation, surfactant, application method, and contractor implementing control.

Data are analyzed in two different ways: assuming independence between all plots and all sites, and assuming independence between sites only. Due to unequal sample sizes among sites, data can not be blocked by site for analysis. Independence among all plots is tested in ArcGIS using the Spatial Autocorrelation Moran's I (Spatial Statistics) Tool prior to regression analysis to avoid violating assumptions of data independence. This tool uses feature locations and feature values to evaluate whether patterns in feature similarity are clustered, dispersed or random. The tool calculates both the Moran's I Index value and a Z score evaluating the significance of the index value (ESRI Developers Network 2007). We also test for spatial autocorrelation of stem density percent reduction at all plots. Results are also analyzed without assuming independence among plots and subsites by summarizing mean log efficacy data by treatment method and subsite.

Treatment efficacy is analyzed as a function of treatment method on measures of treatment efficacy (e.g. stem density percent reduction) using JMP statistical software (SAS) to perform matched pair analysis and comparison of means using one-way analysis of variance (ANOVA) and the non-parametric Wilcoxon/Kruskal-Wallis Test.

4.2.6. Efficacy Monitoring Data Dissemination

Spring Efficacy Monitoring

Internal

Qualitative analysis of treatment efficacy based on observation during spring efficacy monitoring data collection are shared with the ISP Management Team through discussions and email communication throughout the field season. Digital photos are linked to a GIS photo point layer and available to all ISP staff via the networked server immediately following download and processing.

External

Photo monitoring results are presented to the public via PowerPoint presentations during meetings and educational outreach sessions hosted by the ISP on an ongoing basis. All photos and GIS shapefiles are available to interested parties on request.

Summer Efficacy Monitoring

Internal

Qualitative analysis of treatment efficacy based on observation during summer efficacy monitoring data collection are shared with the ISP Management Team through discussions and email communication throughout the field season. The results of quantitative analysis of summer efficacy monitoring data are disseminated to the Control Program in the form of graphs and discussions as soon as results are analyzed.

External

The results of quantitative analysis of summer efficacy monitoring data are disseminated to the public in the annual monitoring report following quality review by the ISP Management Team. Data are presented in the form of graphs, tables, and a discussion section within the report.

4.3. Clapper Rail Monitoring

4.3.1. Clapper Rail Monitoring Design

Clapper rail monitoring is designed to inform the Control Program of current breeding-season clapper rail distribution and to detect changes in regional clapper rail abundance over time as *Spartina* treatment progresses. Sites to be surveyed are selected by the Clapper Rail Monitoring Manager before the breeding season begins based upon: planned *Spartina* treatment at the site; previously documented clapper rail presence at the site; and previously documented presence of clapper rail habitat associations at the site. Within each survey site, listening stations are created at 200-meter intervals, using a GIS in the office to calculate the distance between stations. To minimize disturbance to habitat and for observer safety, stations are established along peripheral footpaths, levees, and boardwalks where possible. The number of listening stations at each site varies due to site area, configuration, and accessibility. For consistency and repeatability, all efforts are made to use existing survey stations from previous years and/or observers.

4.3.2. Clapper Rail Survey Data Collection

California clapper rail breeding-season call-count surveys are conducted between January 15 and April 15 using standardized survey protocols approved by the U.S. Fish and Wildlife Service (**Appendix 14**). At sites where *Spartina* treatment is planned and where clapper rail are known or suspected to occur, Protocol A surveys are used to measure clapper rail abundance at the site. At sites where *Spartina* treatment is planned and where clapper rail abundance ously been documented and are expected to be absent, Protocol C surveys are used to determine clapper rail presence or absence and to map the distribution of rails in the region. Planned *Spartina* treatment sites that do not have suitable clapper rail habitat are not surveyed using either of the call-count methods. Instead, clapper rail habitat associations at the site are evaluated and documented using the Protocol F survey.

4.3.3. Clapper Rail Survey Data Handling

The minimum and maximum numbers of rails breeding at each site are estimated on the basis of all surveys completed during the season. Usually this is the maximum of both minimum and maximum birds detected across three surveys. Locations of birds at higher density sites, especially where birds may have been detected from multiple points or by multiple observers, are plotted using triangulation in ArcGIS. Triangulation at these sites improves the accuracy and precision of the estimates of number of clapper rails detected during each round.

4.3.4. Clapper Rail Survey Data Storage

Clapper rail survey data are stored in an Access database, originally designed by Point Reyes Bird Observatory (PRBO). Estimates of rail locations are entered into a GIS feature class and stored in a geodatabase. Data associated with geographic location are linked to data stored in tabular format in Access.

4.3.5. Clapper Rail Survey Data Analysis

The mean of the final clapper rail minimum and maximum estimates are calculated for each site every year. This value is divided by the area surveyed to produce an estimate of density generally referred to in wildlife ecology as an abundance index, in terms of birds per hectare (Bart *et al.* 2004; birds/ha). The abundance index can be used to estimate the clapper rail population at the entire site by multiplying by site area as calculated in GIS. Sites are grouped into regions based upon U.S. Fish and Wildlife Service regional boundary designations. Annual population estimates are summed by region and graphed by year. A regression line is calculated in order to assess population trends by region over time as *Spartina* control is achieved.

For the sites with the highest predicted potential impacts to rail populations, the ISP has phased treatment across multiple years and has implemented mitigation recommendations, primarily involving enhancing vegetation by planting gumplant and channel creation.

4.3.6. Clapper Rail Survey Data Dissemination

<u>Internal</u>

Clapper rail breeding season survey results are shared with the Control Program to aid in nonnative *Spartina* control planning. Additionally, the mapped distribution of clapper rails within each treatment site helps the Control Program in refining strategies to minimize affects *Spartina* treatment on California clapper rails while meeting *Spartina* control objectives.

External

The ISP clapper rail call-count survey database is shared with PRBO for Estuary-wide analysis of clapper rail population trends. Additionally, the California Natural Diversity Database is updated annually with the ISP clapper rail program survey results, as required by our California Department of Fish and Game permits. The results of the regional population trend analysis are shared with the U.S. Fish and Wildlife Service.

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Appendix 1:

Safety Guidelines for Working in Marshes, Mudflats and other Intertidal Terrain

Safety Guidelines for Working in Marshes, Mudflats and other Intertidal Terrain

San Francisco Estuary Invasive Spartina Project

Intertidal marshes and mudflats present many unique hazards to workers and researchers who must access intertidal areas during the performance of their activity. The following is a summary of some of the hazards one may encounter when accessing these areas, and suggested precautions. This list is not comprehensive of all hazards that could possibly be encountered. Caution and common sense should be exercised at all times while in these areas to minimize risk and avoid injury.

Teams

Always travel with a partner when entering or working in tidal areas. There are many hidden hazards associated with tidal marshland travel and work that may not be readily discernable at first glance. A team of at least two individuals adds a necessary level of safety for any work or activity taking place in the marsh.

Supervisors should provide daily worker safety briefings prior to commencing work on site. Briefings should include any new information about the work conditions on the site, weather conditions, team assignments, equipment condition, or other pertinent issues.

Channels

Watch for hidden channels and holes in the marsh plain as you traverse the marsh. Often smaller channels in the marsh are obscured by vegetation. These channels can be quite deep, and may result in a sprain or pulled muscle, or possible fractures. Use a probe, like a stick or staff to check ahead of your path for unseen channels. Keep alert for vegetation changes, like Grindelia (Gumplant), which grows along channel edges, and may indicate a hidden channel.

Channel banks can sometimes be quite soft, and the mud that lines the channel can often be unstable. It is not unusual to sink deeply into these muds. This could be dangerous during an incoming tide. Always probe the mud within channels to test its ability to support your weight before stepping forward.

Channels often block direct routes through the marsh. These channels can be quite small or very large. Great caution should be observed when considering crossing these channels. It may seem worthwhile in most cases to leap across the channel to get to the other side. This should

only be done on the smaller channels, when your partner is able to follow, when you have surveyed the route for alternate paths around the channel, and at low tide. Large channels should be avoided entirely, and placing driftwood bridges over these channels is not advised. Workers may fall from unstable bridges into larger channels and risk injury, drowning, hypothermia, or equipment destruction. It is best in these situations to find a way around the channel.

Mudflats

Wear mudders. Mudflats at low tide can be dangerous to the unprepared. Often these flats are extremely soft, making travel over them slow and messy. Without proper footwear made for travel over mudflats (called "mudders"), workers may sink up to their thighs in mud.

If feet sink into a soft mudflat or channel bottom, do not make violent movements in an attempt to get free. Pull gently and steadily, holding the boot top with your had if practicable. Try twisting your ankles within your boots or waders. If you can extract one foot (with or without the boot), rest the leg on the surface and gradually free the other leg. Leave the shoes or boots behind if you need to!

If stuck deeply in mud or quicksand, you can often extract yourself by lying down and distributing your weight over the mud surface. Move to firm ground using a "leopard crawl" (spread eagled, face down, keeping the maximum area of the body in contact with the ground at all times).

Bring a rope and a partner (of course). If you are working in an area with very soft mud (especially if you don't have mudders) bring a rope to help pull a stuck partner out. Make sure that the person doing the pulling is standing on as firm a surface as possible. The person being pulled should lie on the mud surface and distribute their weight broadly to prevent additional sinking.

Chemical or Physical Hazards

Many marshland areas have been historic sites of dumping or disposal, and many more have accumulated recent debris or wrack that contains all manner of refuse. As a result, some areas have large amounts of this waste material, and in some cases, toxic waste or hazardous chemicals. Crew leaders should make themselves aware of known chemical or toxic waste issues associated with a site, notify workers, and plan appropriate precautions, including (but not limited to) the following.

Wear suitable protective footwear. At a minimum, wear footwear able to prevent puncture by sharp objects. Nails, glass, chunks of concrete, rusty metal and other debris can cause severe injury workers without appropriate footwear. Ideally, wear hip or chest waders with reinforced soles, that are resistant to puncture, tearing or chemicals.

Develop a site-specific safety plan. In areas where there are known concentrations of toxic or hazardous substances, a site-specific safety plan should be prepared and an appropriately trained hazardous materials expert should be retained to supervise work. At a minimum on such sites, wear protective gloves and eyewear and long-sleeve shirts, and thoroughly wash all clothing after work in the marsh. Workers should also thoroughly wash themselves with soap and water following work activities.

Mark and report any found toxic or hazardous materials. If potentially toxic or hazardous materials are discovered, mark the area and report it to the appropriate authorities (the County hazardous Materials Office and/or the Regional Water Quality Control Board). The area should be avoided until the material has been assessed and mitigated or removed from the site.

Tides

Always be aware of the tidal schedule prior to work in the marsh. When possible, time work to commence on an ebb tide and cease on the incoming tide or earlier. Allow ample time to return to non-tidal areas before the incoming tide starts to advance across the work site. This general rule may be modified in higher marsh habitats where tidal action is lessened, but workers should always be alert and mindful of working in situations where the incoming tides may trap them, and allow ample time for exiting the marsh prior to an incoming tide.

If in doubt, get out. Tides can rise extremely quickly in some areas, and it is possible that rising tides may outpace the ability of workers to outrun the increasing water levels, especially in soft muds or heavily vegetated marsh plains. If you are not certain of the tide schedule, leave early.

Be prepared to swim. If you are caught by an incoming tide, be prepared to lie down and "ride it in", using the

help of a rope held by your partner, if you have both of these. Keep momentum moving shoreward and toward the shallower areas. Don't try to stand up in mud unless you are sure it is stable. Follow the suggestions for working in mudflats, above, if you find yourself in soft mud.

Weather

Wind, rain, fog or other inclement weather can mean the difference between a safe work site and an extremely dangerous one. Winds usually occur in the early afternoon or late morning during the summer months, though dangerous weather patterns can occur at any time of the year. Rainfall may subject workers to hypothermia if unprepared, or may result in potentially dangerous floodwaters. Winds can increase wave action, and whip up salt spray or dust. Fog can decrease workers ability to communicate or discern potential hazards in the marsh.

Check weather conditions prior to and during work activities within the marsh. Weather is reported on a number of websites, such as <u>www.weatherunderground.org</u> and <u>http://weather.yahoo.com</u>. Check the forecast before you go out, and pay attention to the conditions when you get to the site.

If in doubt, get out. It is ill advised to go into marshland terrain in bad weather or poor visibility. Pay attention, and modify work plans if needed to insure the safety of all personnel.

Communications

Maintain lines of communication between workers in the marsh. When more than one team will be working in the marsh at any one time, it is advisable to have a communication link (via walkie-talkie or cell phone, if coverage permits) to a land base and between teams. In the case of injury, discovery of hazardous materials, endangered species, or cultural artifacts, or for other reasons, land-based assistance can be contacted from the field for immediate help or first aid. On-the-ground coordination via phone or walkie-talkie when crews are spread out over the marsh can help to avoid dangerous situations.

Appendix 2:

Meeting Summary, San Francisco Estuary Invasive Spartina Project Monitoring Program Review, April 27, 2006

Meeting Summary San Francisco Estuary Invasive *Spartina* Project Monitoring Program Review

Prepared By

Gina Bartlett, Facilitator Center for Collaborative Policy, CSUS

Meeting Held: April 27, 2006

Introduction

On April 27, 2006, the San Francisco Estuary Invasive Spartina Project (ISP) Monitoring Program hosted a scientific review committee to solicit feedback on the Monitoring Program. The meeting goals, which the committee achieved, were to:

- Review and improve current field-based monitoring protocols,
- Evaluate the value of and make recommendations on remote sensing and geodatabases for the program,
- Recommend how to improve accuracy of existing field monitoring and GIS components, and
- Develop a consensus recommendation on an adequate sampling plan to confirm "eradication" of a hybrid.

The purpose of this document is to summarize the recommendations and outcomes of the scientific review committee's discussions on those monitoring protocols. Generally, the scientists place the program in high regard.

Presentations

For each topic under discussion, an expert presented the issue and typically made some recommendations. The scientists then discussed and formulated recommendations for the program.

Current ISP Monitoring Program Overview | Katy Zaremba

The Trouble with Hybrids | Debra Ayres

- Geodatabases The Potential Value and Recommendations for ISP Monitoring Program | Barry Nickel
- **Remote Sensing Potential Value and Recommendations for ISP Monitoring Program** | Trish Foshci

Case Study – Remote Sensing of *Spartina* in Willapa Bay, Washington | Janie Civille

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Invited, Unable to Attend

Steve Schoenig, CA Dept of Fish & Game, Integrated Pest Control Stuart Seigel, Wetlands and Water Resources

Monitoring Program Overview (K. Zaremba)

The *Spartina* Inventory Monitoring Program maps the distribution and abundance of non-native *Spartina* throughout the San Francisco Estuary and local outer-coast marshes. The 2004-5 monitoring methods were modified from the 2001 methods described in the "Guidelines to Monitor the Distribution, Abundance, and Treatment of Non-indigenous Species of Cordgrass in the San Francisco Estuary" (Collins *et al.* 2001) and published in "Spread of exotic cordgrasses and hybrids (*Spartina* sp.) in the tidal marshes of San Francisco Bay, California, USA" by Ayres *et al.* 2004. Monitoring methods have been modified since the 2001 and 2003 surveys to improve data quality and efficiency of the data collection. In 2004 and 2005 the ISP conducted a full estuary-wide inventory to map all occurrences of non-native *Spartina* to assure detailed information for use by the estuary-wide Spartina Control Program. In a single site visit, both inventory and at a sub-set of sites, treatment efficacy data are collected.

Staff Training

All field monitoring staff are provided extensive training prior to conducting monitoring, and every effort is made to retain experienced staff to assure data quality. All monitoring staff receive personalized training each year. Each new field staff spends at least 3-5 initial monitoring days chaperoned by the lead field biologist. New field staff genetically sample all suspicious *Spartina* until genetic results confirm their field identification and predictions. Once the lead field biologist determines that the field staff is adequately trained, the new field staff is paired with other more experienced field staff. All field monitoring staff receive marsh safety training. Marsh safety protocols include awareness of dangers of walking in the soft mud/marsh, among channels, rock, water and other potential physical hazards. Marsh safety protocols include appropriate behavior in sensitive species habitat. Finally, all monitoring staff are required to work in pairs whenever in the field.

Field Methods

All field monitoring takes place during the summer/fall, the peak of the growing season, when field identification of marsh vegetation is at its best. Where endangered California clapper rail (Rallus longi*rostris*) are present, monitoring takes place after September 1, outside the rail-breeding season. At each sampling site, monitoring staff (monitors) map the location and extent of non-native *Spartina* species using a Global Positioning System (GPS) handheld sensor and data entry unit (Trimble GeoXT). Monitors enter plant data into the data dictionary as points, lines, or polygons, depending on the extent of the invasion. The GPS units automatically collect data on date, time, location, area and perimeter (if polygon data), and length (if line data). Field staff manually enter additional data including site name, species identification, species identification confidence, clone identification (if applicable), clone diameter (if point data), clone number (multiple for cluster points), average clone size, width (if line data), sample name (if applicable), *Spartina foliosa* and other native vegetation species percent cover class (if line of polygon data), marsh elevation (high, medium, low), sediment type (sand, mud, cobble, or other), mud or bare ground percent cover class and survey conditions. Any other relevant information is entered in a "comments" field. As noted above, in 2004 the percent cover classes were modified from the 2001 and 2003 methods. The cover classed for lines and polygons included: <1% seedlings, <1% mature, 1-10%, 10-20%, 20-30%, 30-40%, 40-50%, 50-60%, 60-70%, 70-80%, 80-90%, and 90-100%. Species identification is confirmed by sampling at least 3-5 plants per monitoring site for genetic analyses. A single plant leaf is collected, labeled with indelible ink and placed in a labeled zip lock bag. Plant samples are kept refrigerated until mailed overnight to Dr. Debra Ayres at the UC Davis Spartina Laboratory. Once genetic testing is complete, the results are incorporated into the GIS database. Ambiguous plants are also sampled to confirm species identification. At the leading edge of the hybrid invasion, or in the outlying areas of the Bay where marshes are still dominated by native *S. foliosa, Spartina* plants are sampled by transect to confirm native species identification.

Aerial Photo Interpretation Methods

Aerial photo interpretation is used for large, less accessible *Spartina* infestations. Given the challenge to distinguish between native and non-native *Spartina* species using aerial photo interpretation, the photo interpretation mapping methods are used only for sites previously confirmed by genetic

analysis to be non-native *Spartina*. Color infrared photos are taken at 1:6000 feet scale at low tide during the peak of the growing season at the same time each year to allow yearly comparison. Photos are scanned at 1200 dpi and rectified by subcontractors. Once rectified, the photos are imported into ArcView 3.3, and ArcGIS 9.0 for review and analysis. Rather than using marsh area boundaries, polygons are digitized at 1:500 scale, specifically targeting the *Spartina* meadows and patches. Photo interpreters examine the photos and distinguish the *Spartina* from mud/sand, channel and other marsh vegetation. The digitized polygons are given a cover class. Field data points are used to ground truth the photo interpretation.

Treatment Efficacy Monitoring

A variety of treatment methods have been used to control non-native *Spartina*. During 2001-2005, three types of treatment were applied: manual (digging and covering), mechanical (mowing), and chemical (herbicide). The treatment sites are monitored pre- and post-treatment, with the treatment efficacy results measured in the next growing season. All sites will continue to be monitored (given adequate funding) until all known non-native *Spartina* is "eradicated".

The methods for collecting Treatment Efficacy data are modified from the "Guidelines to Monitor the Distribution, Abundance and Treatment of Non-indigenous Species of Cordgrass in the San Francisco Estuary" (Collins *et al.* 2001). Before treatment, the extent of non-native *Spartina* is mapped to determine the total area of non-native *Spartina*. Treatment efficacy data is collected at 0.25 meter² and 10 meter² plots. The data collected from each includes measurements of overall plant vigor, patch size, patch species cover, patch mud/bare ground percent cover, patch percent cover wrack, plant/clone vigor, marsh elevation, *Spartina* life history, site (marsh) type. The 0.25 meter² plot data includes plant stem height, native and non-native *Spartina* stem density, plant species percent, percent flower *Spartina*, sediment type, percent stems in flower, percent cover wrack, percent cover mud/bare ground. In addition to the efficacy monitoring plots, the entire treatment site is mapped again according to the Inventory Monitoring Methods to determine the area of non-native *Spartina* cover after treatment. Each year following treatment, the initial pre-treatment monitoring points will be updated with new post-treatment data. These data are exported to both Excel and then Systat for review and examination for outliers and errors.

Data Management and Statistical Methods

Data on species, location, and area covered are entered into the *Spartina* Monitoring Data dictionary on handheld GPS units (Trimble GeoXTs) in the field. Supplemental notes are added as needed. Once in the office, data are downloaded to Pathfinder software, differentially corrected, and reviewed by the data collectors/field monitoring staff and the lead field biologist. After review, the data files are exported to ArcView 3.3. All files are backed up regularly to CD and to the server. Once all data files are collected for the season, the site files are merged into a single data set, sorted by data collector, exported to Excel for another round of review by the data collectors. Files are edited as necessary and then combined and imported into ArcView/ArcGIS for preliminary GIS analysis, and then into Excel and Systat for further statistical analyses.

The first statistical analysis is a cross-tabulation of categorical names to check for typographical errors and duplications. Summary statistics are then calculated for quantitative variables to check for unreasonable ranges and outliers. The program tests the hypothesis that there is an increase in area covered by non-native *Spartina* using an ANOVA with annual inventory data. The hypothesis that a particular species, site type, bay region (latitude), elevation or sediment type has an influence on the change in area covered is tested with an Analysis of Variance (ANOVA). Treatment efficacy is determined using an ANOVA on the difference between 2004 and 2005 vegetation data (i.e. the dif-

ference between species cover, density, height, etc.). We also test the influence of species, elevation, site type or sediment type on overall treatment efficacy using an ANOVA.

Data quality is assured by having all data collected using the same method, by direct entry of data in the field and by using trained field biologists who have all been instructed and mentored by the project manager. Handwritten notes are made if needed to supplement the machine-entered data. Quality of data is further assured by multiple editing checks of the data and frequent back-ups to CD of intermediate and final data files.

Recommendations on the Monitoring Program

Inventory and Treatment Monitoring Sampling Parameters

- The value of measuring plant height to measure treatment efficacy was questioned, but it was determined that the Program should continue measuring height. (Height of the plant can prove valuable to birds and other wildlife. Height is also an indicator of chemical efficacy. Ground-level photo monitoring / photo-point visual tracking can document change over time.)
- LiDAR data might be a more accurate tool to collect elevation data rather than the field observation of low, mid or high marsh using plant vegetation. LIDAR has been acquired for the south end of the Bay And the ISP staff may be able to acquire some free data to use as a pilot to test the accuracy and usefulness of the data. Alternatively, the program staff could work with USGS, NOAA or Pt. Reyes National Seashore to develop proposals to acquire some of their data for a pilot study.
- Standardize genetic sampling number by site size. *(See section below "Recommendations on Hybrid Sampling Approach")*.
- Given that assigned cover class varies by observer, consider setting standards with a class cover card or other tool to standardize observation of variable foliage and stem density.
- Share tools and protocols throughout the area among agencies and mappers in an invasive plant manual.

Statistical Analysis Options

- Consider Bayesian approaches instead of parametric for the statistical analyses. Bayesian approaches provide the probability that the event happened. Parametric approaches test whether an event was due to random chance, test hypotheses, and analyzes experimental design, but are not necessarily the right approach to landscape ecology. Within 10 years, some think Bayesian will be the standard in statistics.
 - CART is effective for clarification and regression. Good for multivariate analysis, not great for small samples. Maggi Kelly volunteered to help with this through her lab at UCB.

Feedback on Analyses Presented

- Consider incorporate South Bay elevation data into the treatment efficacy analyses, data may be available from other partners/stakeholders.
- Efficacy might better be explained as a percentage related to control work rather than growth rate. (Presenting the treatment effects as "Negative Efficacy" seems un-

clear/unintuitive. Stating that the treatment isn't effective is more strait forward. Positive value seems difficult to understand.)

Recommendations on Hybrid Sampling Approach

Sampling

- Identifying cryptic cordgrass in existing sampling may be possible. Cryptic cordgrass is likely to show up in areas of high density and diversity. Marsh plains, not mud flats, should be a targeted sampling area for cryptic cordgrass.
- Currently, field staff take 3-5 genetic samples of *Spartin* per "site". Run power analysis to determine number of samples required per "area".
- To complete an accuracy assessment of sampling, increasing the sample number of "known" *S. foliosa* was recommended.
- Consider taking seven samples (instead of five currently) and deliberately select two foliosa of small size to potentially identify hybrids.
- Have land managers augment the monitoring program. Develop protocols so land mangers can use them and seek funding to support sampling. Also, land managers should be able to send samples to ISP labs. Seek needed funding.

Outreach

- Develop further outreach on the ongoing threat of the *Spartina* hybridization invasion and the potential for the public spreading *Spartina* unintentionally. (Web site underway already.)
- Develop survey/monitoring protocols for land managers, stakeholders and general public (Further develop current field identification brochures and website.)
- Educate people working in the marsh, including restoration workers, biologists and surveyors and others to prevent accidental spread of seed.
- Circulate *Walking in the Marsh (Marsh safety and sensitive species guidelines)* to all field staff. (Giselle Block, USFWS SPBNWR, to provide.)
- Continue working with Save the Bay and other outreach organizations.

Other

- Consider using airboats to rapidly survey the Bay mudflats (quicker than kayak or by foot).
- Seek funding for hybrid sampling long-term, in perpetuity if needed. Perhaps establish an endowment. The appropriate agency for maintaining or housing this task was unknown. Both BCDC and Coastal Conservancy were recommended.
- Given the alarming spread of *Spartina*, consider instituting a genetic sanctuary pilot project for pure native *S. foliosa*. Elk Horn Slough National Estuarine Preserve was a recommended location as they are contemplating new marsh restoration (There is currently no native or non-native *Spartina* in the Preserve). (Mike Vasey offered himself as contact).

• However, a risk assessment on the effect of *S. foliosa* on the ecosystem would be needed since it is absent at this point.

Recommendation on Data Management

Criteria for Database Management— Rectify staff problems & meet their needs.

- Improve data management protocols (20,000 records).
- Establish topology rules, which will improve data quality, reduced redundancy of data, i.e. points and lines that are adjacent.
- Maintain data accessibility. There are large amounts of data and the ability to integrate all the types of data including past data sets is crucial.
 - Establish transferability to monitors who are not ISP staff and to other ISP staff within the project. Ensure that all users are using the most recent data.

Skill Base: 3 GIS Users on Staff

8 of 10 Staff have Access

Options for Database Management Generated by the Group

- ESRI products such as the Geodatabase are already developed, understood, and support is available. However, licenses are expensive, and costs are associated with gaining expertise.
- WIMS coupled with ArcGIS Geodatabase for mapping
- Open source, such as MySQL, that integrates with ArcGIS products.
- Integrate web-mapping capabilities such as ArcIMS.

The group recommended that ISP run a pilot study on one marsh with new data (as opposed to importing old historical data) using a few alternative data management systems. Once ISP decides on a data management system, ISP can – complete the migration and transfer of current data, Previous years data can be transferred at some later point. The recommendation was to run a pilot study on one marsh using:

- 1) Geodatabase
- 2) WIMS
- 3) Open source, such as MySQL

Background on alternative data management systems

1) Geodatabase (The following are taken from "Object Modeling and Geodatabases" and "Modeling our World" book chapters provided by Barry Nickle, SFSU)

The Geodatabase is a new object oriented database model. Object oriented data modeling lets you characterize features more naturally by letting you define your own types of objects by defining topological, spatial, and general relationships and by capturing how the objects interact with each other. It allows one to make features in GIS data sets "smarter" by endowing them with natural behaviors, allowing different sorts of relationships to be defined among the features. Unlike with shapefiles, the Geodatabase model lets you implement the majority of custom behaviors without writing code.

Benefits of the Geodatabase model include:

- 1) A uniform repository of geographic data
- 2) Data entry and editing is more accurate
- 3) Users work with more intuitive data objects
- 4) Features have a richer context
- 5) Better maps can be made
- 6) Features on a map display are dynamic
- 7) Shares of features are better defines
- 8) Sets of features are continuous
- 9) Many users can edit a geographic data simultaneously

2) WIMS=Weed Information Management System

WIMS is an *Access* database that has been tailored to serve weed managers' needs. Although thorough and well thought out, it is strictly a database that can be queried to provide information. WIMS is not a geodatabase or a map. WIMS is valuable for land managers; managers can query volunteer information, wind speed, treatment parameters, and other useful reports. *Access* is the same database software that ESRI uses for their personal geodatabases in ArcGIS so WIMS would be compatible with the GIS data. WIMS has no raster and no topology, but it does have spatial data: simplified polygons with up to 15 points. Developed by BLM and The Nature Conservancy in partnership with USGS and National Wildlife Refuge Association, the Sonoma Ecology Center is further developing WIMS. All these programs are available for free download from The Nature Conservancy web site. San Francisco Bay data could be imported into it. All land managers would have the same desktop interface, and it does not require GIS. Using the same program across the Bay would be advantageous.

There are two software applications used in WIMS: Arc PAD and Access. ArcPAD is an ESRI application that runs on PDA-type units. Anyone can load aerial photographs into the display. This bypasses the creation of shapefiles (maps) and imports the monitoring data directly into Access. Minimizing the number of applications used to process data is preferable.

Recommended Next Steps on WIMS

- Talk to Sonoma Ecology Center about WIMS.
- Talk to Humboldt who's transferring data to WIMS the summer of 2006.
- Consider integrating data dictionary into WIMS. Staff would be able to use same handheld devices.
- Consider crosswalk to WIMs from another database such as a ISP Personal Geodatabase

3) Open source, such as MySQL

Open source applications are free alternative to the more expensive ESRI product line.

However, they would likely require a fully committed database manager.

Other Feedback and Discussion

- The **Data Dictionary** is comprehensive and an effective tool.
- ISP should strive to minimize the number of applications necessary to process data. i.e. Shapefiles exported to or viewed in Excel, then to Access, to Systat, to ...etc.
- Access Pros-Cons:
 - o Limited to single-user editing.
 - o Allows behavior-attribute domains, relationships and topology.
 - o Has size limitations.
 - Shapefile structure can be hard to manage in Access.
 - Problems: Use topology for cleaner data and cumbersome data management.

Recommendations on Remote Sensing

- Continue to collect new imagery each year to correct for inaccuracies.
- Use reliable data and techniques
 - Continue to use airborne aerial color infrared photography for areas with limited access. Classify color infrared photos in later summer and classify into thematic rasters.
 - Continue to use computer-assisted visual/manual interpretation
- Improve orthorectification and radiometric adjustments
 - If the budget can support it, three permanent ground control disks at each marsh were recommended. These would provide known locations and could be used for geometric and radiometric corrections. Costs would be associated with initial installation and ongoing maintenance.
 - If the budget can support it, set up adjacent flight lines with large overlap (60%) rather than small groups of individual photos. This would simplify and improve orthorectification and could include mudflats thereby providing greater potential for early detection. Full stereo coverage is best.
- Use in-house orthorectification of imagery (or shapefiles if really needed). This would provide more control and more information on fly.
 - Hire someone or assign the orthorectification task to current staff.
 - Note: raster-to-vector conversion will always introduce error. Vector data are easier to reposition and re-project with less error than raster.
 - Consider GIS capabilities and use of existing databases.
- Types of accuracy testing recommended: 1) Is it new? Can you see it? Find it in the field? Are you producing a good map? 2) Can you confirm your data in the field year-to-year, i.e. in the long term?

- Foster student interns for algorithm development.
 - Trish Foschi, SFSU or Maggi Kelly, UCB student?
- Study problem areas, multi-temporal approaches, etc.
 - Five-inch pixels might be finer than program needs. Recommend 0.5 to 1 meter spatial resolution. This is fine enough for the subject and coarse enough to reduce "noise" and data volume.
 - There is too much variability at 0.5 m pixels resolution.
 - In addition it is costly, given the needed data storage space and manipulation by software, which is also expensive. Plus, this scale does not solve the variability problem with the photos.
 - If scanning is to be done in house, the ISP will need a high quality scanner to avoid introducing scanner distortions. 8-bit (256 gray tones) scan of the image is sufficient and more than photos can actually resolve, therefore, higher levels are non-applicable. It doesn't make sense to scan film with more than 256 gray tones, so why go to 16-bit scans and introduce thousands of colors that aren't even there. (Note: Scanning can be provided by the photographer.)
- Consider using QuickBird or Iconos hyperspectral imagery and adding it to the pilot. QuickBird may be able donate time and resources. When using QuickBird, consider timing related to tidal flow so it serves as a good supplemental source.
 - John Bourgeois at HT Harvey offered sharing some their hyperspectral imagery for a Pilot.

Appendix 3:

Quality Assurance Objectives for the San Francisco Estuary Invasive Spartina Project Monitoring Program

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NVENTOR	Y MONITORING – GPS fie	eld data collection, pr	rocessing & editing; /	Inventory Monitoring – GPS field data collection, processing & editing; Annual survey boundary delineation	lineation
Quality Attribute	Representativeness	Completeness	Comparability	Accuracy	Precision
Data Generated for Measure- ment Quality Definition	Summary of percent and location of potential inva- sive Spartina habitat sur- veyed, and any gaps in sur- vey area not covered by photo interpretation, in an- nual report.	 Calculation and reporting of number and location of features missed during inven- tory and subsequently added by other staff. Calculation and reporting of number and location of features with missing data, and description of measures taken to remedy these errors. 	Summary of differences in location, extent, per- cent cover, and net cover collected by each field staff at the same site during training and mid-season, as meas- ured in a GIS.	 Storage of pre- and post- processed data files and process- ing documentation files available for troubleshooting if necessary. Summary of GIS feature modification in annual report. Summary and discussion of species confirmations and changes following evaluation of genetic test results in annual report. 	 Summary of min, max, average and standard deviation of number of GPS positions recorded per feature type. Summary of number of features recorded as low, medium or high cover classes as an indication of patch delineation precision.

Invento F boundary	InvenтоrY Moniтoring – Grounc boundary delineation	truthing and photo i	nterpretation-based o	digitizing; Orthorectification	INVENTORY MONITORING – Ground truthing and photo interpretation-based digitizing; Orthorectification; Annual photo interpretation boundary delineation
Quality Attribute	Representativeness	Completeness	Comparability	Accuracy	Precision
Quality Objective	 Visit and record ground truthing data at approxi- mately 30 points per site to be digitized Complete survey of all invasive Spartina habitat within color IR imagery not surveyed during GPS field data collection 	Document and report the species, location and extent of all inva- sive Spartina discov- ered each year	Utilization of ground truthing and standardi- zation of photo inter- pretation (PI) tech- niques to limit differ- ences in PI feature digi- tizing among staff and differences between PI and GPS field collected data to no more than +/- 15%	 Ortho-rectification of color infrared images to an accuracy of 5m or less. Feature location accurate to visibility in GIS at 1:500 scale with features displayed over orthorectified color infrared im- agery taken at low tide. Accuracy of species identifi- cation as determined by genetic testing 	 (1) Precision of feature location as possible by digitizing in GIS at 1:500 scale using available color infrared imagery. (2) Precision of Spartina patch de- lineation as required by Control Pro- gram and as possible at 1:500 scale using available imagery. NOTE: During PI, multiple, individ- ual patches within 30+ m are often recorded as a single polygon feature with a lower cover class. This lower precision may not be sufficient for control purposes at some sites.
QA Sample Type or Measurement Proce- dure	Annual GIS delineation of photo-interpreted areas digi- tized by ISP field biologists. Gap analysis in GIS to lo- cate any potential Spartina habitat not surveyed in the field or using photo inter- pretation.	 Visual assessment of GIS maps with 1m true color aerial photo background by all Monitoring Program and Control Program staff to ensure com- pleteness of populations documentation Run queries in GIS and/or Access to find and fix missing attrib- ute data associated with Spartina features. 	 Photo interpretation staff train together, ini- tially ground truthing and digitizing data at the same site and re- viewing results. Staff periodically review each other's work, shar- ing and refining species identification and per- cent cover estimate interpretation tech- niques. Comparison of re- sults of GPS field sur- vey versus PI digitizing at a sample site. 	 Assessment of accuracy of color IR ortho-rectification by comparison of color IR to NAIP 2005 imagery in select locations. Staff assess the accuracy of their PI by (a) symbolizing fea- tures by cover class to allow ease of data checking and (b) returning to the site following PI digitizing to check the species and cover class of selected and accessible digitized features. Genetic testing is performed when species determination is questionable and when sample collection is possible. 	Review by Control Program staff to assess usefulness of patch size de- lineation.

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Invento F boundary	Invenтоry Moniтоring – Ground boundary delineation	truthing and photo i	nterpretation-based	digitizing; Orthorectification	Inventory Monitoring – Ground truthing and photo interpretation-based digitizing; Orthorectification; Annual photo interpretation boundary delineation
Quality Attribute	Representativeness	Completeness	Comparability	Accuracy	Precision
Fre- duency of Use	Ongoing during and at com- pletion of PI efforts	Ongoing during and at completion of PI efforts	Ongoing during and at completion of PI efforts	Ongoing during and at comple- tion of PI efforts	Ongoing during and at completion of PI efforts
Data Generated for Measurement Quality Definition	Summary of percent and location of potential inva- sive Spartina habitat sur- veyed using aerial photo interpretation, and any gaps in survey areas, in annual report.	 Calculation and reporting of number and location of features missed during photo interpretation and sub- sequently added by other staff. Calculation and reporting of number and location of features with missing data, and description of measures taken to remedy these errors. 	 Description of any discrepancies in PI me- thods or results between staff. Summary of differ- ences in location, ex- tent, percent cover, and net cover of invasive Spartina as collected using GPS in the field versus using PI at a sample site. 	Description of ortho- rectification, photo interpreta- tion, and/or species identifica- tion difficulties and their resolu- tion in annual report.	Summary of number of features re- corded as low, medium or high cover classes as an indication of patch de- lineation precision, and discussion of Control Program comments, if any.

	Precision	Results of data summary and analysis will be presented at a scale appropriate for the degree of error inherent in each data type	ISP staff will review draft monitoring report to ensure all data are presented using the appropriate precision		ISP staff will respond directly to the author of the draft with suggestions for final revision
		Results of analysis w scale appr of error in type	ISP staff v monitorin data are pi appropriat	Annual	ISP staff v to the auth suggestion
	Accuracy	All analyses and presenta- tion of results will be com- pletely accurate to the best of our knowledge.	ISP staff will review draft monitoring report to ensure all analytical results were performed and presented accurately	Annual	ISP staff will respond di- rectly to the author of the draft with suggestions for final revision
sentation	Comparability	Data will be analyzed and presented following standard scientific and statistical protocols and formats	 Scientists including ISP & California Coastal Conservancy staff will review proposed analytical techniques. A periodic formal expert review of the methods and results of the Monitoring Program will be conducted 	Annual	Reviewer comments will be docu- mented and considered by the moni- toring report author
C, analysis and pres	Completeness	All summary data deemed useful and nec- essary for reporting will be included in the an- nual report	ISP staff will review draft monitoring report to ensure completeness	Annual	ISP staff will respond directly to the author of the draft with sugges- tions for final revision
INVENTORY MONITORING – Data QC, analysis and presentation	Representativeness	Analysis and presentation of all inventory data re- cords by site, subsite, region and ISP area.	ISP staff will review draft monitoring report to en- sure all levels of data analysis are satisfactorily analyzed and presented	Annual	ISP staff will respond directly to the author of the draft with suggestions for final revision
INVENTORY	Quality Attribute	Quality Ob- jective	QA Sample Type or Measurement Procedure	Frequency of Use	Data Generated for Measurement Quality Definition

TREATME	UC I	Experimental Design, Data Collection, Analysis & Presentation	Collection, Analysis & Pre	esentation	
Quality Attribute	Representativeness	Completeness	Comparability	Accuracy	Precision
Quality Objective	Sites for treatment efficacy moni- toring were selected by the ISP to encompass a range of wetland and treatment types throughout the Bay's regions with site type repli- cation where possible. Number of plots per site selected to achieve statistical power to detect mean- ingful change in species cover over time.	Data collection: Survey cover class of each plant species in each plot for each year of sam- pling - as possible given field restrictions, with a goal of 90- 100% completeness. Data analysis & reporting: All usable data will be used in data analysis, and all meaningful results will be reported.	Data collection will be stan- dardized within and between years and individual data col- lectors to allow comparability of data. Data analyses will use stan- dard statistical techniques to allow comparability with other scientific studies.	All permanent plots are to be accurately relocated to within <1 meter annually. All cover class data will be accurate to within +/-15%.	Precision of data collection is determined by cover class categories, which are de- signed to meet but not ex- ceed the ability of staff to visually assess percent cover. Precision of reported data analysis results will not exceed precision of data collection.
QA Sample Type or Measurement Proce- dure	Describe wetland type, treatment type and region for each site. Con- duct power analysis with data to determine power to detect differ- ences in species cover over time.	Check for missing data in all feature attribute fields in GIS. ISP staff will review draft monitoring report to ensure completeness in analysis and reporting.	Field staff will informally cali- brate their percent cover esti- mates while working in pairs on a daily basis. Final data will be assessed by the Moni- toring Program Manager for comparability between years. Experts will be consulted re- garding the appropriate use of analytical techniques.	Check for outlier values and topology/ location errors	Monitoring Program Man- ager and ISP staff will re- view cover class categories and reported analysis results to ensure precision values meet quality objectives.
of Use Fre-	Annual	Annual	Annual	Ongoing throughout field season	Annual
Data Generated for Measurement Quality Definition	Summarize number and location of site/treatment/ region replica- tion. Report results of power analysis.	If possible, return to collect data. If not possible, report and discount missing data (as op- posed to species absence) when conducting data analysis. ISP staff will respond directly to the author of the draft monitor- ing report with suggestions for final revision	Report on any difficulties in percent cover estimation by field staff, and report any data found to be not usable due to incompatibility between years. Documentation of consultation with experts regarding appro- priate use of analytical tech- niques.	Report on outlier values and other po- tential errors found during data analysis. Fully describe data limitations in annual monitoring report.	ISP staff will respond di- rectly to the author of the draft monitoring report with suggestions for final revi- sion

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	Precision	Precision of clapper rail loca- tions by comparison between field mapped locations and GIS calculated locations	Visual comparison between clapper rail locations plotted on field map to those offset in GIS using estimated distance and direction recorded on da- tasheet	Ongoing throughout field sea- son	Distribution map of precise clapper rail locations at ISP treatment sites
	Ι	Precision of tions by cor field mappe GIS calcula	Visual com clapper rail on field ma GIS using e and directic tasheet	Ongoing th son	Distribution m clapper rail loc treatment sites
	Accuracy	Accuracy of clapper rail locations using GIS to trian- gulation of individuals de- tected from multiple stations	Visual assessment in GIS of intersecting vectors created in GIS by offsetting esti- mated distance and direction to detected rails as recorded on datasheet	Ongoing throughout field season	Distribution map of accurate clapper rail locations at ISP treatment sites
	Comparability	Standardization of clapper rail survey protocols for training, data collection, and data storage	Use standardized clapper rail survey protocols which are approved by the USFWS. Use stan- dardized clapper rail survey database which was created by PRBO.	Annual meeting of California clapper rail biologists to dis- cuss any protocol modifications	Annually updated clapper rail database is shared with PRBO for final storage and Estuary-wide popula- tion analysis
	Completeness	Summary of clapper rail abundance at ISP treat- ment sites and clapper rail population estimates on a regional level	Calculate clapper rail density within area sur- veyed to extrapolate to entire site and sum by region	Annual	Summary of annual clap- per rail abundance and distribution
CALIFORNIA CLAPPER RAIL MONITORING	Representativeness	Annually assess the abundance of California clapper rail at ISP treatment sites which support clapper rail habitat associations, as determined by the program manager using survey protocol F.	Conduct annual breeding season surveys for California clapper rail at selected ISP sites using survey protocols A & C	Between January 15 – April 15 annually	Summary of annual clapper rail regional abundance and distribution map
CALIFORN	Quality Attribute	Quality Ob- jective	QA Sample Type or Measurement Procedure	Frequency of Use	Data Generated for Measurement Quality Definition

Appendix 4:

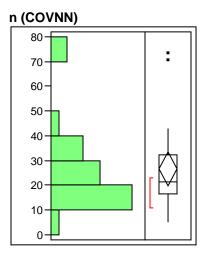
Determination of Treatment Efficacy Monitoring Sample Size for the San Francisco Estuary Invasive Spartina Project Monitoring Program

Determination of Treatment Efficacy Monitoring Sample Size for the San Francisco Estuary Invasive *Spartina* Project Monitoring Program

Calculated estimates of sample sizes, below, are based on 04-05 "treatment monitoring" data using data from $\frac{1}{4} \times \frac{1}{4}$ m quadrats. Full plot data from variable-sized plots was not analyzed for this purpose.

Non-Native Spartina COVER change detection

Number of independent $\frac{1}{4} \times \frac{1}{4}$ m quadrat samples per site required to detect a 10% change in live non-native Spartina cover within that site with a 90% certainty of detecting that difference, if it occurs, and a 10% chance of making a false-change error (concluding that a difference occurred when it actually did not).



Quantiles

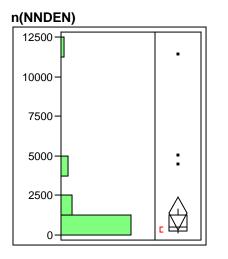
100.0%	maximum	73.123
99.5%		73.123
97.5%		73.123
90.0%		56.451
75.0%	quartile	32.254
50.0%	median	21.612
25.0%	quartile	16.384
10.0%		11.831
2.5%		5.333
0.5%		5.333
0.0%	minimum	5.333

Moments

Mean	26.669609
Std Dev	16.511751
Std Err Mean	3.370447
upper 95% Mean	33.64191
lower 95% Mean	19.697308
Ν	24

Non-Native Spartina DENSITY change detection

Number of independent ¹/₄ x ¹/₄ m quadrat samples per site required to detect a 10% change in live tiller density within that site with a 90% certainty of detecting that difference, if it occurs, and a 10% chance of making a false-change error (concluding that a difference occurred when it actually did not).



Quantiles

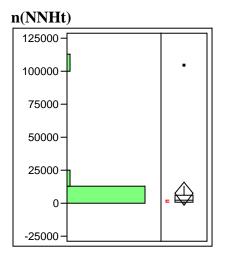
100.0%	maximum	11336
99.5%		11336
97.5%		11336
90.0%		4693
75.0%	quartile	1247
50.0%	median	503
25.0%	quartile	279
10.0%		184
2.5%		134
0.5%		134
0.0%	minimum	134

Moments

Mean	1366.6082
Std Dev	2454.6833
Std Err Mean	501.06014
upper 95% Mean	2403.13
lower 95% Mean	330.08631
Ν	24

Non-Native Spartina HEIGHT change detection

Number of independent $\frac{1}{4} \times \frac{1}{4}$ m quadrat samples per site required to detect a 10% change in live stem height within that site with a 90% certainty of detecting that difference, if it occurs, and a 10% chance of making a false-change error (concluding that a difference occurred when it actually did not).



Quantiles

100.0%	maximum	103924
99.5%		103924
97.5%		103924
90.0%		11022
75.0%	quartile	5878
50.0%	median	2001
25.0%	quartile	1047
10.0%		669
2.5%		207
0.5%		207
0.0%	minimum	207

Moments

Mean	7366.8847
Std Dev	20816.693
Std Err Mean	4249.1897
upper 95% Mean	16157.003
lower 95% Mean	-1423.234
N	24

Appendix 5:

Exhibit A Interagency Agreement to Regents of the University of California Agreement No. 06-050

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The Regents of the University of California Agreement No. 06-050 Page 3 of 3

EXHIBIT A (Interagency Agreement)

The Regents of the University of California (UC Regents), by and through the University of California, Davis (UC Davis), and its Strong Laboratory (Laboratory) will provide services to the State Coastal Conservancy (Conservancy), in connection with the Conservancy's San Francisco Estuary Invasive *Spartina* Project (ISP), as follows:

Task 1. Genetic Analysis

Perform genetic analyses of cordgrass (*Spartina* sp.) samples submitted by the Conservancy or its ISP contractors (referred to collectively in this agreement as "ISP staff") to determine whether the plants are native *S. foliosa*, exotic *S. densiflora* or *S. alterniflora*, or hybrids. Samples will be collected, stored, and analyzed following protocols shown in **Attachment A**, or other protocols recommended by the Laboratory and approved by the Conservancy.

The ISP staff will submit samples taken from two categories of sites; inventory sites where results are not needed until 3-6 weeks after receipt of the samples, and treatment sites where results are needed within 2 weeks of receipt of the sample. The sampling season will begin in June and run until December, with peak sampling occurring in September and October. The number of samples will range from 50 to 150 samples per week for a total of approximately 1,000 samples. The Laboratory will provide analyzes as requested where possible. However, the Laboratory has determined that a maximum of 72 samples can be processed and analyzed in any one week period. The Laboratory will implement a priority assignment method that will allow processing of high priority ("treatment") samples first. Samples that cannot be analyzed within three days of receipt will be extracted and stored at -20°C until analyses can be run.

Task 1 Deliverables: Results of analyses in electronic (excel) format emailed to <u>ibhogle@spartina.org</u>. Chain of custody documentation and data records with gel photographs are to be maintained at UC Davis, and are to remain available to the Conservancy for inspection for 5 years.

Task 2. Scientific Consultation and Support

On request, provide ongoing scientific support to the ISP staff, including advising on the monitoring program design, assisting with analysis of data, and reviewing and advising on scientific materials prepared by and for the ISP staff. Review relevant *Spartina* and invasion biology literature and advise ISP staff regarding short and long-term monitoring and control strategies. Keep the ISP staff apprised of *Spartina* research or data analyses being conducted by students or faculty within the Department using Conservancy-funded data, and provide copies of interim and final results as they become available.

Task 2 Deliverables: Informal reports and consultation for the purpose of providing advice/analyses to ISP staff as requested. Copies of interim and final results of research/analyses conducted using data developed with funding from Conservancy.

APPENDIX 5 - Page 2 of 5

The Regents of the University of California Agreement No. 06-050 Page 3 of 3

EXHIBIT A (Interagency Agreement)

Attachment 1 to Exhibit A

Protocols for Spartina Sampling and Analysis

1. SAMPLING PROTOCOLS

Samples are to be collected by the ISP staff and shipped to the Laboratory following the protocols below. These sampling protocols have been developed in consultation with the Laboratory; however, neither the Laboratory nor UC Davis are responsible for sample collection or shipment.

- a. <u>Established Plant Samples</u> A sample from an established plant consists of a fresh leaf cutting approximately 25 cm (10 inches) long. A leaf cutting can be either individually labeled using a "Sharpie" pen writing directly on the leaf, or placed in a labeled zip lock bag. Labeled leaf cuttings can be bagged singly or together. See Item 1.c. for transect sampling.
- b. <u>Seedling Samples</u> A seedling sample consists of the entire seedling. A seedling sample can be either individually labeled using a "Sharpie" pen writing directly on a leaf, or placed in a labeled zip lock bag. Groups of seedlings can be bagged singly or together. See Item 1.c. for transect sampling.
- c. <u>Transect Samples</u> Transect sampling is used to estimate the general genetic background of plants in a larger marsh. Transect samples may consist of leaf samples, seedling samples, or a combination of these. Transect samples should be taken by walking parallel to the longest axis of a marsh and taking a sample every 10 paces (about 10 meters apart). Wide marshes may require 2 or more transects. It is not necessary to label each leaf or seedling in a transect sample. All samples should be placed in a single bag and delivered to the Laboratory as indicated in Item 1.d. If the submitted number of leaves/seedlings exceeds 18, the transmittal letter should indicate the desired number of sub-samples to be evaluated (see Item 3).
- d. <u>Sample Storage and Shipment</u> The quality of the sample deteriorates as soon as it is collected. Samples should be refrigerated as soon as possible after leaving the field up until the time they are sent via Federal Express to the Laboratory at the following address:

Dr. Debra Ayres (530-752-6852) Department of Evolution and Ecology 1 Shields Avenue University of California, Davis Davis, CA 95616

Only Federal Express is to be used as delays have occurred at the UC Davis end when sent by USPS Priority mail. Samples must be sent no later than Thursday to assure arrival by Friday (to avoid week-end storage by Federal Express). If necessary, ISP staff will keep samples collected late in the week under refrigeration over the weekend, and ship on Monday. The envelope shall be clearly labeled "REFRIGERATE UPON ARRIVAL". ISP staff will notify the Laboratory by email (<u>drayres@ucdavis</u> and <u>aflee@ucdavis.edu</u> or other lab technician specified by D.R. Ayres) when a package is shipped, and will provide Laboratory advance notice of planned shipments whenever possible.

- 2. **SAMPLE RECEIPT AND TRACKING** Laboratory staff will place and keep the envelope under refrigeration upon its arrival at the Department of Evolution and Ecology and after pick-up by the Laboratory. Samples will be logged into a lab notebook dedicated to Conservancy analyses, and they will be given code labels as designated in the transmittal letter. ISP Monitoring Program Manager, Ingrid Hogle, will be notified by e-mail (<u>ibhogle@spartina.org</u>) of their arrival. A notation with date will be made in the log as the sample progresses through extraction, PCR amplification, scoring, data entry and analysis, and sending the results to Ms. Hogle. This, and the spreadsheets where the data is entered, will document the custody chain of the samples.
- 3. **SUB-SAMPLING PROTOCOL** –Sub-samples will be drawn at random by the laboratory staff when requested for seedling or transect sampling (see Items 1.b-c). If the sub-sample size has not been specified in the transmittal letter, the sub-sample size will be as specified in Item 1.c.. Variation from this protocol must be approved by Ms. Hogle prior to lab analysis.

APPENDIX 5 - Page 3 of 5

The Regents of the University of California Agreement No. 06-050 Page 3 of 3

EXHIBIT A (Interagency Agreement)

Attachment 1 to Exhibit A

- 4. EXTRACTION METHODS Samples will be extracted using Qiagen DNAeasy extraction kits. Extraction methods are detailed in a protocol handbook that accompanies the Qiagen kits. Laboratory staff is proficient and experienced with the kits, and has experienced no extraction failures with them. However, to guard against potential sample loss as a result of extraction failure or other causes, laboratory staff shall store extra plant material at -20°C until all analyses have been successfully run.
- 5. **ANALYTICAL METHODS** There are currently no "U.S. EPA approved" or other "standard" analytical methods for determining the genetic background of cordgrass samples. The analytical methods that will be used under this contract were developed by the Laboratory, as described below. These methods may be revised or updated by the Laboratory over time. The revision and update of methodology may occur without amendment of this agreement, provided that the Conservancy is notified and provided with written documentation of the revisions or updated methodology and has approved it in writing.
 - a. <u>Polymerase Chain Reaction</u> The PCR methodology is detailed in Daehler *et al.* 1999 (Daehler, C.C., C.K. Antilla, D.R. Ayres, and D.R. STRONG. 1999. Evolution of a new ecotype of *Spartina alterniflora* (Poaceae) in San Francisco Bay, California, USA. *American Journal of Botany* 86: 543-546). Each sample will be run with 3-6 primers, and 6 primers will be used where finer determinations or uncertain results require more genetic resolution.
 - b. <u>DNA fragment scoring</u> The scoring method is generally described in Ayres et al 1999 (Ayres, D.R., D. Garcia-Rossi, H.G. Davis, and D.R. Strong. 1999. Extent and degree of hybridization between exotic (*Spartina alterniflora*) and native (*S. foliosa*) cordgrass (Poaceae) in California, USA determined by random amplified polymorphic DNA (RAPDs). *Molecular Ecology* 8: 1179-1186). The species specific bands are generated from Operon primers; the primer code, diagnostic band molecular weight (in base pairs of DNA), and the species (*A or F*) that the band is specific to are as follows: A2 575 bp (*F*); A17 725 bp (*F*); B7 550bp (*A*), 650bp (*A*), 800 bp (*F*); C10 470 bp (*A*); D5 600 bp (*A*), 1100 bp (*F*); D11 575 bp (*A*).
- 6. **QUALITY CONTROL** All laboratory analyses will be performed under the direction and supervision of Dr. Debra Ayres. Any reaction that produces ambiguous results will be repeated. The Laboratory does not routinely perform laboratory duplicates. However, the Conservancy may submit field duplicates or request laboratory duplicates as needed for the ISP QA/QC program.
- 7. REPORTING OF RESULTS The results for each sample shall include the sample ID number, genetic determination (native *S. foliosa*, pure *S. alterniflora*, or hybrid), number of primers run, number of markers used to make the genetic determination, and any comments from the laboratory technician regarding confidence in the banding results. Data and comments will be entered in excel spreadsheets, and spreadsheets will be e-mailed to the ISP staff, Ms. Hogle (<u>ibhogle@spartina.org</u>), as results are completed.
- 8. **MAINTENANCE OF RECORDS** Laboratory will maintain chain of custody documentation and data records with gel photographs, and these will be available for inspection for 5 years.

Proposed Amendment #1 to Exhibit A, Agreement 06-050

Proposed by UC Davis, October 31, 2007

Revise existing three sentences of Item 6, Quality Control, to be listed as subitems a, b, and c. Add new text i, ii, and iii, to read as follows:

6. Quality Control

- a. All laboratory analyses will be performed under the direction and supervision of Dr. Debra Ayres.
 - i. Gel Scoring the laboratory technician who scores each electrophoretic gel will date and initial the data sheet in the laboratory notebook; Dr. Ayres will review the scoring, correcting if necessary, and initial and date the data sheet.
 - ii. Data will be entered into an Excel spreadsheet only after the data sheet has been reviewed and initialed by Dr. Ayres. The laboratory technician who enters the data will date and initial the data sheet in the laboratory notebook; correct data entry must be verified by a second person before the data is transmitted to the ISP. This person can be another lab technician, or Dr. Ayres.
 - iii. Exception to the above will occur during those periods when Dr. Ayres is the only person working in the genetics lab, in which case her sole approval of the accuracy of gel scoring and data entry will be sufficient.
- b. Any reaction that produces ambiguous results will be repeated.
- c. The Laboratory does not routinely perform laboratory duplicates, however, Conservancy may submit field duplicates or request laboratory duplicates as needed for the ISP QA/QC program

Proposed Amendment #2 to Exhibit A, Agreement 06-050

Proposed by UC Davis, January 22, 2008 (method has reportedly been in use since Spring 2006)

Append the following to Item 4. Extraction Methods:

The lab may also use the following DNA extraction protocol described by Daehler *et al.* (1999):

Genomic DNA was extracted from 100 mg of fresh leaf tissue by grinding in liquid nitrogen and incubating the homogenate at 50 C in 1000 mL of Guidet's (1994) extraction buffer. After 1–2 h, the proteinase K was denatured by heating, and samples were centrifuged at 8000 3 g for 10 min; 500 mL of supernatant was withdrawn and combined with 50 mL of 2 mol/L sodium acetate and 500 mL isopropanol. The solution was placed at 0 C for at least 1 h and then centrifuged as above. The pellet was recovered and suspended in 100 mL Tris-EDTA buffer; 5 mg of RNAase were added and the sample was incubated at 37C for 20 min. The DNA was precipitated by adding 10 mL of 2 mol/L sodium acetate and 250 mL 100% ethanol and chilling at 0C for at least 1 h. The pellet was recovered and resuspended as above. Centrifugation was repeated as above; 90 mL of supernatant were withdrawn and mixed with 200 mL Tris-EDTA buffer.

Appendix 6:

Quality Assurance Project Plan for the Genotypic Evaluation of Spartina Leaf Samples

Quality Assurance Project Plan for the

Genotypic Evaluation of Spartina Leaf Samples

contracted by

San Francisco Estuary Invasive Spartina Project Peggy Olofson Olofson Environmental, Inc. 2612-A 8th St. Berkeley, CA. 94710 Tel: (510) 548-2461 ext 201 Email: prolofson@spartina.org

prepared by

Gene Hookstra, Ph.D. Division Manager – Genetic Services Eurofins STA Laboratories 1821 Vista View Drive Longmont, Colorado 80504 Tel: (303) 651-6417 Email: <u>genehookstra@eurofinsus.com</u>

September 25, 2009

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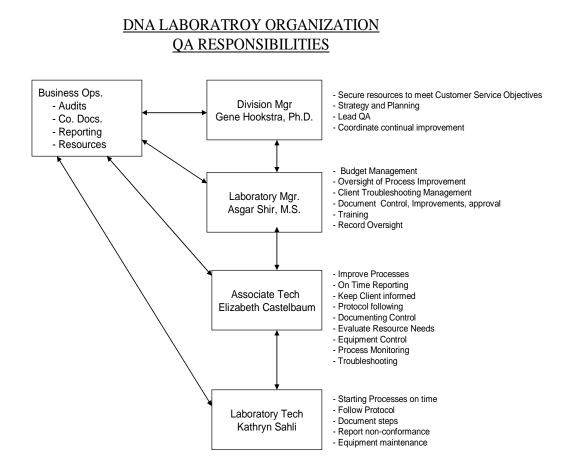
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Appendix B: Quality Assurance Manual	

Mission Statement: Eurofins STA Laboratories (ESTA) is dedicated to help clients succeed by providing expert, knowledgeable, confidential accurate and timely agricultural product testing and diagnostic services using innovative and reliable methods that are scientifically validated.

Quality Statement: Eurofins STA is committed to excellence in agricultural testing services and superior customer service through defined, measured and accountable quality assurance programs.

Laboratory and Quality Assurance Organization: Flow chart defining positions, individuals and responsibilities.



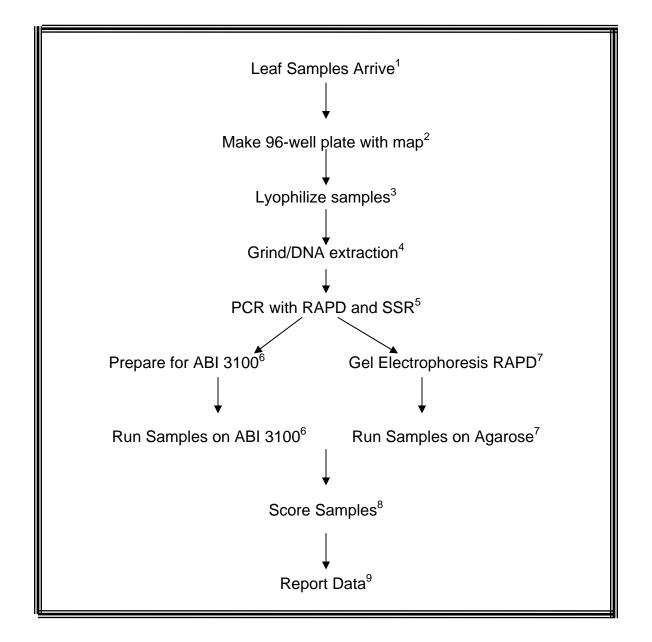
Name	Position	Qualifications
Gene Hookstra	Division Manager	Ph.D in quantitative genetics, 25
		years experience in managing
		plant related projects
Asgar	DNA Laboratory Manager	M. S. in Genetics and Plant
Shirmohamadali		breeding, 12 years experience in
		managing DNA laboratories
Elizabeth	Associate Technician II	M. S. in Plant Breeding and
Castelbaum		Genetics, 1 year experience and
		2 years TA in laboratory during
		graduate school
Kathryn Sahli	Laboratory Technician II	Eight years experience in plant
		pathology and genetics
		laboratories

Key Project Personnel/Qualifications:

Facilities Description: Eurofins STA Laboratories operates two laboratories, one in Gilroy, California, and the company headquarters in Longmont, Colorado where subject project is being conducted. The DNA laboratory operates in approximately 2500 square feet with approximately 125 linear feet of bench space. This laboratory is fully furnished with all equipment needed to operate a modern DNA laboratory including but not limited to the following:

Refrigerators Fume Hoods Lyophilizer Thermocyclers Sequencing equipment Gel electrophoresis equipment Computers Secure chemical and sample storage areas

Project Objective: Eurofins Sta Laboratories will genotype and report results of *Spartina sp.* leaf samples collected by San Francisco Estuary Invasive Spartina Project following protocols detailed in <u>Laboratory Protocols for Genotyping</u> <u>Spartina</u> prepared by Laura Feinstein, Ph.D. Candidate, U.C. Davis for the Invasive Spartina Project, June 13, 2009. (Appendix A).



Laboratory Sample Flow Procedures:

1. Leaf Samples: Leaf sample collection to be performed by Olofson Environmental, Inc. prior to sending to ESTA. Leaf samples are to be individually packaged in plastic baggies properly labelled and sent as fresh leaf on blue ice or frozen for next day delivery and will include a hard copy inventory with an electronic copy sent via email. Upon arrival samples will be inventoried and secured in a dedicated refrigerator prior to processing.

- 2. 96-well Plate format with Plate Map: Initial processing will begin with development of an electronic and hard copy map assigning specific entry to a specific plate and plate coordinate.
- 3. Lyophilize Samples: Standard Operating Procedure (SOP) per manufactures recommendations.
- 4. Grind/DNA Extraction: Qiagen DNAeasy Plant Mini Kit as detailed in included instruction pamphlet with the kit and following protocols detailed in Appendix A, pg 10
- 5. PCR with RAPD and SSR: Protocols detailed in Appendix A, pg 12
- 6. Prepare and run samples for ABI3100 sequencer: Protocols detailed in Appendix A and SOP provided by Applied Biosystems.
- 7. Prepare and run samples for RAPD Gel Electrophoresis: Protocols detailed in Appendix A, pg 13
- 8. Score Samples: All entries will be scored by two individuals to ensure accuracy in an electronic format following defined allele sizes as detailed in Appendix A, pg 14 (RAPD), pg 18 (SSR)
- 9. Report Data: Data will be reported electronically in format defined by Olofson Environmental, Inc.

Quality Control: Eurofins STA Laboratories Quality Assurance Manual is not included but the Index page is (Appendix B) and if more information is required it will be provided. It should be noted we are in the process of QA revision as STA was purchased by Eurofins in September of 2008. Below are specific exerts (Level 1) from subject manual which cover specific areas pertinent to proper QA monitoring

Audits

Internal Audit: An internal audit of the QMS must be performed at least once during every 12 months by the Quality Team. Audits will be performed in accordance with the elements of the ISO 9001:2000 standard.

Quality audits of selected areas shall be conducted at the discretion of the Quality Team, Senior Management or at any time a problem is discovered. An Audit Team will be appointed by the QA Coordinator and will consist of an audit leader and 1-2 Quality Team individuals that have no direct responsibility in the area being inspected.

The Audit Leader will give sufficient notice to the manager before the audit takes place. The audit team will review all documentation relevant to the audit and an audit checklist will be prepared to ensure a systematic examination of the area. Data gathered from the audit will be reviewed by the Quality Team and presented to the BU manager in a written report within one week of the audit. The audit report shall include:

Purpose and area: Describe the initiating factors for the audit, limitation of the audit, and the area being audited.

Observations and deficiencies: Give a detailed account of the current practices and list the deficiencies in protocols, documentation, etc., along with the relevant ISO standard.

Follow-up: Define plans for a follow-up review to establish individual responsibilities and completion dates.

Based on the Audit Report, the Manager and QA Team shall agree on a corrective action plan within 7 days of notification. The corrective action plan shall be included in the audit report 18 and will be reported to Senior Management their quarterly report. In cases of disagreement on the corrective action plan, the President will determine the final plan for corrective action. A list of all audits listing deficiencies, individuals with assigned responsibilities, and dates for completion shall be maintained by the QA Coordinator in accordance to the procedures in section

Corrective Action Contingencies:

8.3 Control of Nonconforming Product. Critical nonconformance of a product is determined by managers or designated AT process control positions after adequate training. If during processing, any component of the process (media, buffers, chemicals, equipment, etc.) is nonconforming, the AT or DM must be notified to determine if the nonconformance is critical to the outcome of the test. If the nonconformance is critical, the component will be removed from use and/or an equivalent component will be substituted. Any substitutions used in the process or interruption of processing should be noted on the accompanying lab sheet(s).

If before or during processing, any customer sample is found to be nonconforming, the AT or DM must be notified to determine if the nonconformance is critical to the outcome of the test. What is considered to be nonconforming is listed in level 3 documents specific to each department in the company. If it is determined to be critical, the customer sample will be removed from processing and the customer contacted to determine a resolution. If errors in processing are discovered before a certificate of analysis is issued to the customer, the AT or DM will determine a course of action. Errors in processing that are discovered after a certificate of analysis has been issued to the customer require that the customer be contacted by the AT or DM, and/or by management. Any customer sample or processing component that is determined to be unusable will be removed from use unless the problem can be resolved. **Record Keeping Procedures:** Electronic media is used to store all documents and all documents are maintained in the "QA folder" on the shared directory of the company server. The QA folder is maintained by the QA Coordinator and all STA personnel have access to these documents and are trained in their proper use. The computer system is backed up daily.

Appendix A

Laboratory Protocols for Genotyping Spartina

Prepared by Laura Feinstein, Ph.D. Candidate, U.C. Davis, for the Invasive Spartina Project

Jun 1, 2009

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C. 10X Loading Dye	
D. 100 BP DNA ladder	
E. 10X TBE buffer	
F. DNTPs	

I. Introduction

The protocols described here are those used by the U.C. Davis *Spartina* lab in the laboratory of Professor Donald R. Strong for the purpose of genotyping *Spartina*. Most of the work is targeted at differentiating native *S. foliosa* from exotic *S. alterniflora* and *S. alterniflora x foliosa* hybrids. There also are RAPD genetic markers that are useful for identifying two other species and hybrids that occur in San Francisco Bay. *S. densiflora* is originally from South America and produces sterile hybrids with *S. foliosa*. *S. anglica* is an allopolyploid hybrid of *S. alterniflora x maritima* that arose in England and was introduced to San Francisco Bay.

Both RAPD and microsatellite markers are useful for diagnosing hybrid *S. alterniflora x foliosa*. RAPDs have the advantage of being relatively fast and inexpensive because they can be scored with gel electrophoresis. The small

differences in base pair length of the microsatellites means they are genotyped on a capillary sequencer, a more costly technology than gel electrophoresis. A two-tiered approach can combine the two methods. First, all samples can be tested with RAPD markers. Next, samples yielding inconclusive or questionable results can be tested with microsatellite markers.

II. DNA Extraction

The U.C. Davis *Spartina* lab has chiefly used two methods for extracting *Spartina* DNA. The first, the kit method, produces DNA that is usable both when analyzing with gel electrophoresis (as with RAPD markers) and for genotyping (for analyzing microsatellite markers). The second DNA extraction method, Proteinase K, produces acceptable results for testing RAPDs with gel electrophoresis, but it may leave behind impurities that interfere with genotyping. Therefore using the kit method is recommended if there is the possibility that a sample may be tested with microsatellites.

A. Kit DNA Extraction Protocol

Use the Qiagen DNAeasy Plant Mini Kit. A detailed instruction pamphlet is included with the kit.

Web page:

http://www1.qiagen.com/Products/GenomicDnaStabilizationPurification/DNeasyPlantSystem/DNeasyPlantMiniKit.aspx

B. Proteinase K DNA Extraction Protocol

Supply list:

- 1.7 mL eppendorf tubes, labeled with sample number
- 0.5 L liquid nitrogen
- one mortar and pestle per sample
- Kimwipes
- 1 mL extraction buffer stock per sample (see stock solutions)
- 1 mg proteinase K per sample
- dry bath
- hot plate
- 1000 mL beaker
- centrifuge
- 1 mL and 200 µL pipettes and tips
- 2N sodium acetate
- 70% isopropanol
- 100% ethanol
- TE buffer (see stock solutions)

Procedure:

- Start with fresh, green leaf tissue. Samples can be stored in the refrigerator for a few days prior to processing. They should be free of other living organisms, such as mildew and scale insects (e.g., *Haliaspis spartina*, which look like white dots on the leaves, about ¼" long). Ideally the tissue should be young, such as a green leaf near the top of the tiller; older plant tissue tends to accumulate structural tissues that can interfere with the DNA extraction.
- 2. Wash plant samples in DI water and dry with Kimwipes.
- 3. Weigh out 100-120 mg fresh plant tissue. Cut across the leaf into small, 1/8" wide strips. Place in labeled, 1.7 mL eppendorf tube. Note: At this stage, the tissue can be frozen at -20°C for a few weeks before continuing with the extraction. Upon removing from the freezer, place the tubes on ice until the sample is ground with liquid N in step 5.
- 4. Dissolve 1 mg of proteinase K per 1 mL of extraction buffer. Make enough for 1 mL of extraction buffer per sample to be extracted.
- 5. Turn on dry bath and set to 50°C (make sure temp. remains under 56°C)
- 6. Place plant tissue in mortar; carefully pour small volume liquid N into mortar (enough to fully cover plant sample) and grind plant material into a fine powder.
- 7. Once liquid N is fully evaporated, add 1 mL of extraction buffer/proteinase K mixture and grind until mixture is a fine slurry.
- 8. Return sample to labeled tube and place in dry bath for 1 hour to incubate.
- 9. While waiting for samples in dry bath, boil 800 mL of water in a 1000 mL beaker on a hot plate.
- After incubation, proteinase K must be denatured by heating. Remove samples from dry bath and turn off hot plate. Place samples in a floating waterbath rack and open the tubes. Float rack in beaker for 5 minutes. When time elapses, remove from water bath, close caps and allow to briefly cool.
- 11. Centrifuge samples at 7800 rpm for 10 minutes. The DNA will be suspended in solution.
- 12. Pipette 500 μL of the liquid supernatant into fresh, labeled tubes, being careful not to take up any solid debris. Discard pellet and remainder of liquid.
- 13. Add 10% by volume 2N sodium acetate (50 μL)
- Add 1 volume 70% isopropanol (500 μL). Invert several times and/or vortex to mix. Place samples in -20°C freezer for at least 1 hour, or overnight. Steps 12 and 13 will precipitate the DNA.
- 15. Harvest pellet by cold centrifuging at 7800 rpm for 10 minutes.
- 16. Pour off supernatant and dispose of it. Drain tube upside down on Kimwipe for ~ 1 minute.
- 17. Dissolve pellet in 100 μL TE buffer. Mix with pipette tip. Change tips between samples.

- 18. Reprecipitate DNA by adding 10 μL 2N sodium acetate and 250 μL 100% ethanol, in that order. Mix by inversion and/or vortex, then place in -20°C freezer for at least 1 hour or overnight.
- 19. Harvest pellet as in steps 14 and 15, and dissolve as in step 16.
- 20. Cold centrifuge at 7800 rpm for 10 minutes.
- 21. Decant 75 μL of supernatant, being careful not to disturb pellet, into fresh, labeled tubes. Toss pellet and remainder of supernatant.
- 22. Reprecipitate DNA by adding 7.5 μL 2N sodium acetate and 75 μL 100% ethanol, in that order. Mix by inversion and/or vortex, then place in -20°C freezer for at least 1 hour.
- 23. Harvest pellet as in steps 14 and 15, and dissolve as in step 16. Centrifuge as in step 19 and decant as in step 20.
- 24. Find DNA concentration on Nanodrop or spectrophotometer.
- Dilute DNA sample to 20ng/µL.
 Note: For RAPD analysis, U.C. Davis always diluted DNA using TE buffer. However, excess TE buffer can interfere with genotyping. If samples will be tested with microsatellite primers, diluting with molecular grade water is preferable.
- 26. Store samples in refrigerator if they will be used within a few weeks. For longer storage, place in freezer at -20°C or below.
- III. Analysis with RAPD Markers

A. PCR

Supply list:

- Purified DNA (20ng/µL), up to 14 samples
- TE buffer
- 1.7 mL tubes
- 0.6 mL tubes
- Taq: U.C. Davis lab uses Promega GoTaq (5 $U/\mu L$)
- Buffer: U.C. Davis uses Promega clear 5x buffer
- MgCl₂: U.C. Davis lab uses the MgCl₂ that comes with Promega GoTaq (25mM)
- dNTPs (10µM each)
- Molecular grade double-distilled H₂O
- RAPD primers (see Appendix 1A)
- mineral oil
- 96 well PCR plates
- 96 well cooling plate

Note: Prepare a volume of master mix 5-10% greater than required for the total number of PCR assays to be performed, as there will be some pipetting loss. Procedure:

1. In a 1.7 mL tube, mix reagents to the following proportions

- $8.154 \mu L \text{ of } H_2 O \text{ per reaction}$
- 2.7 µL of 5x buffer per reaction
- $1.62 \mu L \text{ of MgCl}_2 (25 \text{ mM}) \text{ per reaction}$
- 0.27 µL of dNTPs (10mM each) per reaction
- .108 µL of TAQ (5u/uL)
- 2. Vortex to mix
- Divide mixture into six 0.6 mL tubes in equal proportion. To each submaster mix, add 0.72 μL per reaction of the appropriate RAPD primer. Note: A17 requires twice as much primer concentration: add 1.44 ul).
- 4. Place PCR plate into cooling plate.
- 5. To each well of the PCR plate, add 13.5 µL of submaster with primer. Group RAPD primers in horizontal rows.
- 6. Add a drop of mineral oil to each filled well.
- 7. To each well containing, add 1 μ L of DNA, being careful to eject the DNA below the mineral oil. Group each DNA sample in vertical columns.
- 8. Cover PCR plate with its plastic cover.
- 9. PCR program:
 - 1. Denature at 94°C for 1:30
 - 2. Followed by 40 cycles of:

94°C for 15 seconds

42°C for 30 seconds

- 72°C for 2 minutes
- 3. Hold at 4.0°C.

10. Store in refrigerator until loaded in gel. PCR product can safely be stored in refrigerator for up to a week.

B. Gel Electrophoresis

Supply list:

- Molecular biology-grade agarose (U.C. Davis lab uses GenePure LE)
- 0.5X TBE buffer
- DI H₂O
- PCR product
- 100 BP DNA ladder (see stock solutions)
- 10X Loading dye (see stock solutions)
- Ethidium bromide in solution

Procedure:

- 1. Prepare a 1.75% gel (i.e. 1.75 grams of agarose per 100 mL TBE buffer).
- 2. Add .3 µL of loading dye to each PCR well.
- 3. Load the correct volume of DNA ladder (per the instructions that come with the ladder) to two wells per tier in the gel.
- 4. Load 13 µL of PCR product to each well in the gel.
- 5. Run at 120 volts until the loading dye has nearly reached the next tier.

- 6. Place in ethidium bromide stain for 3 minutes, then move to the DI water bath for 7 15 minutes.
- 7. Photograph under UV light.
- 8. Score the gel.

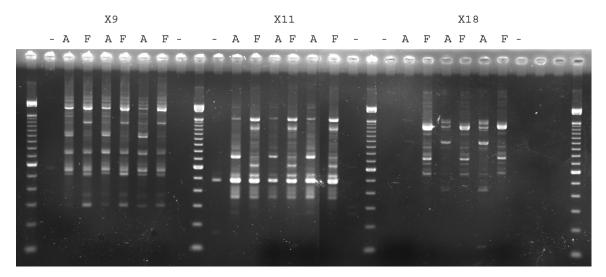
The species-specific bands scored at each primer are noted below. The number indicates the base pairs, the name in parentheses indicates in which species the marker occurs.

Primer	Base Pair (species)
A2	575 (foliosa)
A17	725 (foliosa)
D5	600 (alterniflora), 1100 (alterniflora), 550 (densiflora), 800
	(densiflora)
D11	575 (alterniflora)
B7 *	550 (alterniflora), 650 (alterniflora), 800 (foliosa/densiflora), 700
	(densiflora), 1200 (densiflora)
C10	470 (alterniflora)
X9	1000 (alterniflora)
X11	750 (alterniflora)
X18	950 (alterniflora), 750 (foliosa)**
B10	750 (anglica)
C12	575 (anglica)
F10	900 (anglica)
G2	1050 (anglica)
H7	650 (anglica) 750 (anglica) 1500 (anglica)

- * Note: B7 is the trickiest primer to score because S. foliosa has a double band at 575 and 625. The alterniflora bands are wide and bright and can occur either at 550, 650, or both. An individual S. alterniflora plant could have either band or both (but always has at least one).
- ** X18 also has a species-specific band at 450 for *S. alterniflora*, but Laura Feinstein of U.C. Davis had difficulty getting this band to show up clearly in her gels.

Image of the X9, X11, and X18 tests:

"-" indicates the negative controls, "A" indicates alterniflora, and "F" indicates foliosa. The rest of the lanes are ladder or are empty.



IV. Analysis with Microsatellite Markers

A. PCR

Supply list:

For each reaction, the following amounts are added to yield a total volume of 14 uL:

- 5 μ L DNA template at 20 ng/ μ L
- $2 \mu L 10 x$ buffer
- 0.5 µL dNTPs (10 uM)
- 1.5 μ L forward labeled primer (10 μ M)
- 1.5 μ L reverse primer (10 μ M)
- 0.03 µL TAQ DNA polymerase (5 U/µL)
- MgCl, volume depending on optimal concentration for primer (see Appendix 1B)

• Add molecular grade double distilled water to make a total volume of 14 μ L Procedure:

1. To make master mix for a 96 well reaction, multiply all reactants by 100 and add to a 1.7 mL Eppendorf tube stored on ice.

2. Vortex master mix for a few seconds, then centrifuge at ~14000 rpm for 3-5 seconds.

- 3. Aliquot 9 µL of Master Mix into each tube of a 96 well plate stored on a cold block.
- 3. Add 5 µL of DNA (20ng/µL) to each tube.
- 4. Cover each plate with a sealing mat.
- 5. Run the PCR program appropriate for the primer.

PCR program:

- 1. Denature at 94°C for 2 minutes
- 2. 29 cycles of:

94°C for 0:45 seconds

T_a for 45 seconds (see Appendix 1B)

- 72°C for 1 minute
- 3. Extension phase, 72°C for 6 minutes
- 4. Hold at 4°C
- 6. Store PCR plate at -20°C.

B. Genotyping

- Quantify the DNA concentration in the PCR product. This can be done by running 2.5 µL of the PCR product on a 2% agarose gel with a marker of known concentration, or by using a 96-well plate reader.
- Dilute the product to the concentration required by the sequencing instrument using double-distilled water. Note: For the Genomics Facility at UC Davis, PCR products ranging from 100-200 base pairs in length should be at a concentration of 0.2-0.6 ng/µL. PCR products from 200-500 base pairs in length should be 0.6-2.0 ng/µL.

Primers SPAR. 20, 8, 2, and 18 yield products from 100-200 base pairs long, while all other primers give products from 200-500 base pairs long.

- Transfer 5 µL of product from each well of the PCR plate to the corresponding well in the multiplex plate. Up to 5 sets of products labeled with different fluorescent dyes and/or of differing base pair lengths can be added to a plate. Recommended multiplex groups are given in Appendix 1.
- 4. After all products have been added to the multiplexed plate, seal with USA scientific sealing film and label. Store at -20°C.
- 5. Genotype the products. Note: The facility at UC Davis uses an ABI 3730 XL capillary sequencer from Applied Biosystems.
- 6. Alleles characterizing *S. foliosa* and hybrids are listed in Appendix 1B. The presence of any one allele that is not listed in column four, "*S. foliosa* alleles," is evidence that the sample is a hybrid plant.

Appendix 1 - Primers

A. RAPD Primers

A single RAPD primer serves as both the forward and reverse primer. We use standard, salt-free primers ordered from Invitrogen and dilute with TE buffer to a concentration of .004 nM/ μ L.

Standard set for diagnosing *S. alterniflora, S. foliosa, S. alterniflora x foliosa, and S. densiflora* (these six primers were used for San Francisco Estuary *Spartina* assessments between 2001 and 2008):

B. Microsatellite Primers

table, MgCl₂ (µL) gives the volume to add of MgCl₂ when using 2 µL/reaction of Qiagen 10x PCR buffer, which already successfully amplify the target product and the population frequency of hybrid-specific alleles. The last column in the The primers are listed in approximate order of their usefulness in diagnosing hybrids as a function of how often they

!			:		T_a^*	MgCl ₂	MgCl ₂
Primer ID	Forward sequence	Reverse sequence	S. foliosa alleles	Alleles occurring only in hybrids	(°C)	(MM)	(nL)
Spar.15	ATTTGCTGCTTTTGGTAGAC	GTAGAACAATGGAAGAATGC	266	269, 271, 273, 275, 279, 285, 296	51	2.83	0.39
Spar.26	TTCAACTGGCGTAGTGATTCC	AACATTTCCGACTGGTAGAGC	263	275, 277, 281, 283, 285, 286, 291	58	3.17	0.58
Spar.20	ACCGTGCCTCAGCTACTG	GGTGTTTCCTCGCATAGATC	171	173, 175, 177, 179, 181	52	2.17	0.02
Spar.23	GGGAAGTGAAATCTGGTTGC	GCTTGCTTGTCTCAGTCC	262, 264, 266, 268	248, 250, 274	55	2.17	0.02
Spar.25	CGGTAGAGGGGGAGTTGTGG	GCTTGGGAGATGAGACTGGAC	245, 249	253	69	3.00	0.48
Spar.08	CTAAGGTCCCAAACGACGAC	GCGACGAGCGAGGATTTAC	193	180, 188	58	2.50	0.20
Spar.28	CACCGTTCAATCACAGTT	GGAAGCAGGAGGGGGTTGG	416	419,476	59	2.00	0.00
Spar.02	GAAGGACGAGTCTCATTTGG	GGCTGCCCCTGTTTCACG	193	201, 213	56	2.50	0.20
Spar.27	CATCAAAGCAAGAGGA	GACACCAACGGAACTG	314	304, 309, 321, 323, 328, 331	50	2.17	0.02
Spar.09	GTGGCCTAGCCTATCGACCT	TGAATGGAAAGGGGAAATGA	279, 285, 294	273, 277, 292, 296	58	2.50	0.20
* T	* T _ Appropriate tomocrotiuro						

T_a = Annealing temperature contains 15mM MgCl₂.

Groups suitable for multiplexing together (no more than two overlapping size ranges):

Plate 1.	Plate 2.
Spar.15	Spar.23
Spar.26	Spar.28
Spar.20	Spar.02
Spar.25	Spar.27
Spar.08	Spar.09

B. Microsatellite Primers

The primers are listed in approximate order of their usefulness in diagnosing hybrids as a function of how often they successfully amplify the target product and the population frequency of hybrid-specific alleles. The last column in the table, $MgCl_2$ (µL) gives the volume to add of $MgCl_2$ when using 2 µL/reaction of Qiagen 10x PCR buffer, which already

					T _a *	MgCl₂	MgCl ₂
Primer ID	Forward sequence	Reverse sequence	S. foliosa alleles	Alleles occurring only in hybrids	(°C)	(mM)	(µL)
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Spar.09	GTGGCCTAGCCTATCGACCT	TGAATGGAAAGGGGAAATGA	279, 285, 294	273, 277, 292, 296	58	2.50	0.20

* T_a = Annealing temperature

contains 15mM MgCl₂.

Groups suitable for multiplexing together (no more than two overlapping size ranges):

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Plate 1.	Plate 2.
Spar.15	Spar.23
Spar.26	Spar.28
Spar.20	Spar.02
Spar.25	Spar.27
Spar.08	Spar.09

2. Divide into five aliquots of 160 μ L in .7 mL tubes. Use one of these tubes at a time (it can remain on the workbench) and freeze the rest until needed.

E. 10X TBE buffer

- 1. Place in 1 L fleaker
 - 108 g Tris
 - 55 g boric acid
 - 8.3 g EDTA
- 2. Add DI H₂O to bring up to 1 L. Add stir bar and place on stir plate until clear (should take 20 minutes or more)
- 3. Cap with aluminum foil.

F. DNTPs

The directions below are for making a dNTP mixture of 10 mM per dNTP. The volumes given are based on Promega dNTPs which come at 100 mM concentration.

- 1. Combine in a 1.5 µL tube
 - 50 µL Promega dATP
 - 50 µL Promega dTTP
 - 50 µL Promega dGTP
 - 50 µL Promega dCTP
 - $300 \ \mu L$ molecular grade ddH₂O
 - 2. Mix by vortexing, split into 5 100 µL batches in .7 mL tubes, and freeze.

Appendix B

Quality Assurance Manual

Longmont, CO

Gilroy, CA 00-1-001-rev 1

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All printed copies of this document are considered to be uncontrolled

Appendix 7:

Monitoring Program Permits

Permits for Spartina Project Monitoring Program

October 2009

Permits from US Fish and Wildlife Service and California Department of Fish and Game specify terms and conditions for conducting *Spartina* treatment, and *Spartina* and California clapper rail monitoring.

All three activities are addressed under the 2003 U.S. Fish and Wildlife Service's Programmatic Formal Intra-service Endangered Species Consultation (1-1-03-F-02156) and amendments (most recently, May 20, 2009; 81420-2008-F-1546-2). The consultation documents (including the "biological opinions" can be viewed and downloaded from the ISP "Project Documents" webpage (<u>http://www.spartina.org/project.htm</u>), under the section entitled "Section 7".

Clapper rail monitoring requires additional terms, conditions, and authorizations from the U.S. Fish and Wildlife Service under Section 10(A)(1)(a) of the Endangered Species Act, and from the California Department of Fish and Game, through a Memorandum of Understanding and via Scientific Collection Permits for individual biologists. Current copies of these permits are provided in this section.



United States Department of the Interior

FISH AND WILDLIFE SERVICE Pacific Southwest Region 2800 Cottage Way, Suite W-2606 Sacramento, California 95825-1846



IN REPLY REFER TO: PSR/Recovery

EFn 24 2009

Olofson Environmental, Inc. RECEIVED

MAY 0 1 2009

Signed

Dear Permittee:

Enclosed is your U.S. Fish and Wildlife Service recovery permit issued under section 10(a)(1)(A)of the Endangered Species Act (ESA), 16 U.S.C. 1531 et seq., and its implementing regulations.

Please refer to the permit number in all correspondence and reports concerning permit activities. Engagement in any activity pursuant to this permit constitutes understanding and acceptance of the Special Terms and Conditions attached to your permit.

By accepting this permit and conducting activities authorized by it, you are agreeing to adhere to the attached terms and conditions. Failure to meet permit terms and conditions could result in ESA section 9 take violations, or suspension/revocation of this permit.

Please be aware that some species named in your recovery permit may also be listed under various State Endangered Species Acts or otherwise be of special concern to the States. As such, activities affecting those species may not be conducted without first obtaining the appropriate State permits. Federal permits do not supersede State authorizations.

If you have any questions regarding this matter, please contact Daniel Marquez at 760-431-9440. Thank you.

Sincerely,

langered Species Division

Enclosures



DEPARTMENT OF THE INTERIOR U.S. FISH AND WILDLIFE SERVICE			3-201 (1/97)
FEDERAL FISH AND WILDLIFE PE	RMIT	2. AUTHORITY-STATUTES 16 USC 1539(a) 16 USC 703-712	
1. PERMITTEE OLOFSON ENVIRONMENTAL, INC. 2612-A 8TH STREET		REGULATIONS (Attached 50 CFR 17.22 50 CFR 21.23 50 CFR 21.27 50 CFR 13	0
BERKELEY, CA 94710 U.S.A.		3. NUMBER TE118356-1	AMENDMENT
		4. RENEWABLE YES NO 6. EFFECTIVE 04/24/2009	5. MAY COPY YES NO 7. EXPIRES 04/23/2013
8. NAME AND TITLE OF PRINCIPAL OFFICER (If #1 is a business) PEGGY R. OLOFSON PRESIDENT	9. TYPE OF PERMIT ENDANGERED SPECIES		I
10. LOCATION WHERE AUTHORIZED ACTIVITY MAY BE CONDUCTED ON LANDS SPECIFIED WITHIN THE ATTACHED SPECIAL TERM	S AND CONDITIONS		
 CONDITIONS AND AUTHORIZATIONS: A. GENERAL CONDITIONS SET OUT IN SUBPART D OF 50 CFR 13, AND SPECIFIC CONDITIONS MADE A PART OF THIS PERMIT. ALL ACTIVITIES AUTHORIZED HEREIN MUST BE CARRIED SUBMITTED. CONTINUED VALIDITY, OR RENEWAL, OF THIS PERMIT IS SUBJECT TO COMPL FILING OF ALL REQUIRED INFORMATION AND REPORTS. B. THE VALIDITY OF THIS PERMIT IS ALSO CONDITIONED UPON STRICT OBSERVANCE OF A C. VALID FOR USE BY PERMITTEE NAMED ABOVE. D. Further conditions of authorization are contained in the attached Special 	DUT IN ACCORD WITH AND FOR THE P ETE AND TIMELY COMPLIANCE WITH A LL APPLICABLE FOREIGN, STATE, LOC	URPOSES DESCRIBED IN TH LL APPLICABLE CONDITIONS,	E APPLICATION NCLUDING THE
ADDITIONAL CONDITIONS AND AUTHORIZATIONS ALSO APPLY 12. REPORTING REQUIREMENTS ANNUAL REPORTS DUE: 1/31 See permit conditions for further reporting requirements.			
ISSUED BY ACTING TITLE ENDANGERED SPECIES	S DIVISION CHIEF		DATE 04/24/2009



United States Department of the Interior

FISH AND WILDLIFE SERVICE Sacramento Fish and Wildlife Office 2800 Cottage Way, Room W-2605 Sacramento, California 95825-1846



Olofson Environmental, Inc. RECEIVED

OCT 2 6 2009

Signed _

In reply refer to: 81420-2010-TA-0036

Peggy Olofson Olofson Environmental, Inc. San Francisco Estuary Invasive Spartina Project 2612-A 8th St. Berkeley, California 94710

Subject:

Change to List of Authorized Individuals for Permit TE-118356

Dear Ms. Olofson,

This is in response to your electronic mails dated October 13, 2009 and October 19, 2009, requesting changes to the list of individuals authorized to conduct activities pursuant to section 10(a)(1)(A) permit TE-118356. Specifically, you have requested Stephanie Chen be added to the list of authorized individuals to independently conduct activities with respect to the California clapper rail (*Rallus longirostris obsoletus*). You have also requested that April Robinson and Ode Bernstein be removed from the list of authorized individuals.

We have reviewed the material transmitted with your letter and authorize the addition of Stephanie Chen to the permit under the conditions stipulated therein. We have also removed Ms. Robinson and Mr. Bernstein.

Please contact staff biologist, David Kelly at (916) 414-6492, if you have questions regarding this response.

Sincerely,

cell

Eric Tattersall Deputy Assistant Field Supervisor





United States Department of the Interior

FISH AND WILDLIFE SERVICE Sacramento Fish and Wildlife Office 2800 Cottage Way, Room W-2605 Sacramento, California 95825-1846



LIST OF AUTHORIZED INDIVIDUALS TE-118356-0.5

1. Individuals authorized to independently survey for rails, pursuant to this permit:

Jennifer McBroom, Allison Nelson, Julia Stalker, Leonard Liu, Jeffrey Lewis, Jeanne Hammond, Whitney Thornton, Vicki Trabold, and Stephanie Chen.

Supervised individuals may conduct activities pursuant to this permit only under the direct, on-site supervision of the above named individuals. On-site supervision for rails is defined as a supervised individual conducting activities within view and control of an independently authorized individual.

Basic education, training, and experience in the general needs for observation, minimizing harassment, and handling of all classes of wildlife species that may be encountered (such as in traps or while moving through habitat) is needed for those conducting work under supervision of authorized individuals. A list of all individuals participating in supervised work, an account of the times worked, names of those authorized individuals supervising the work, a statement about whether the work of the supervised individuals was satisfactory, and resumes showing relevant experience should be included in the reports required by this permit.

21 Oct 2009

Alecka Eric Tattersall

Deputy Assistant Field Supervisor

This List is only valid if it is dated on or after the permit issuance date.



State of California - The Resources Agency DEPARTMENT OF FISH AND GAME ARNOLD SCHWARZENEGGER, Governor

Olofson Environmental, Inc. RECEIVED MAY - 6 2008

Signed



April 2, 2008

Ms. Peggy Olofson, P.E., President Olofson Environmental, Inc. 2560 9th Street, Suite 216 Berkeley, California 94710

Dear Ms. Olofson:

Wildlife Branch

1416 Ninth Street, 12th Floor

Sacramento, California 95814

At your written request, the Department of Fish and Game (Department) is formally authorizing Olofson Environmental, Inc. (Olofson) to take the California clapper rail (Rallus longirostris obsoletus) (rail) during field studies. The rail is a State-listed endangered species.

Our authorization is in the form of a Memorandum of Understanding (MOU) between Olofson and the Department. The MOU is a special permit prepared pursuant to the California Endangered Species Act. For the purpose of this agreement, we consider you to be the executive signatory and Ms. Jennifer T. McBroom of Olofson to be the principal investigator. The MOU supersedes the e-mail authorization for taking the rail that I provided to you on March 30, 2006.

We enclose two identical originals of the MOU. If you and Ms. McBroom concur with the terms of the agreement, I ask that each of you sign and date both documents in the provided spaces on pages 5 and 6. Retain one fully-signed MOU for Olofson's records and as a master for making required copies for certain field personnel. Please return the other signed MOU to Dr. John R. Gustafson, Nongame Unit, at the letterhead address. He is the Department's representative for the agreement until April 30, 2008.

Through April 30, please address all inquiries regarding the MOU to Dr. Gustafson by e-mail at JGustafs@dfg.ca.gov or by telephone at (916) 327-8847. On or after May 1, 2008, please send inquiries about the MOU and requests for further action on the agreement by surface mail to Ms. Randi Logsdon, Nongame Unit, at the letterhead address. We include in this mailing a List of Authorized Individuals for the MOU.

Sincerely,

John R. Due tapon Dale T. Steele, Manager

Wildlife Program

Enclosures

cc: See page two.

Conserving California's Wildlife Since 1870





California Natural Resources Agency DEPARTMENT OF FISH AND GAME http://www.dfg.ca.gov Wildlife Branch Nongame Wildlife Program 1812 9th Street

Sacramento, CA 95811

April 6, 2009

LIST OF AUTHORIZED INDIVIDUALS

for the

Memorandum of Understanding (MOU) (became effective on April 2, 2008; expires on October 31, 2012) with

Olofson Environmental, Inc.

(2560 9th Street, Suite 216; Berkeley, California 94710) allowing take of the following species during field studies: *California clapper rail*

- 1. We, the Department of Fish and Game, permit the persons named herein to conduct only those studies of the California clapper rail (*Rallus longirostris obsoletus*) that Olofson Environmental, Inc. (Olofson), has agreed verbally or in writing to undertake as the primary contracting or research entity.
- 2. For studies authorized by the MOU, we concur with Olofson's designation of Ms. **Jennifer T. McBroom** as the *principal investigator*, a role described in paragraph 14 of the MOU.
- 3. We authorize each of the following persons to work as a *field investigator*, which is a designation described in paragraph 14 of the MOU, only if listed as an 'independent' worker in the list of authorized persons for federal recovery permit TE-118356: Mr. Ode M. Bernstein, Mr. Jeffrey T. Lewis, Mr. Leonard Y. Liu, Ms. Allison R. Nelson, Ms. April H. Robinson, Ms. Jeanne Hammond, Ms. Whitney Thornton, Ms. Julia K. Stalker, and Ms. Vicki Trabold.
- 4. This List of Authorized Individuals (LAI) supersedes the LAI dated February 27, 2009.



FOR: Lyann Comrack, Staff Environmental Scientist and Department Representative for MOU

Conserving California's Wildlife Since 1870

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State of California—The Resources Agency DEPARTMENT OF FISH AND GAME 2008 SCIENTIFIC COLLECTING PERMIT JUSTIFICATION

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ADDITIONAL CONDITIONS, AUTHORIZATIONS, AND APPROVALS ARE AS FOLLOWS:

You may salvage dead mammals, except bats, cougars, marine mammals, fully protected or federally-listed species unless additionally authorized. Salvaged specimens of the Mohave ground squirrel shall be deposited only in the mammalian collection of the Natural History Museum of Los Angeles County, Los Angeles, California. See also Mandatory Conditions/Number Authorizations.

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- NUMBER AUTHORIZATIONS REQUESTED BY PERMITTEE / NUMBER AUTHORIZATIONS ISSUED BY DFG -

- You may take or possess marine fishes (excluding salmon, striped bass, and sturgeon) not to exceed sport fishing bag limits; sport fishing seasons and size limits shall apply. Cowcod, giant sea bass, garibaldi, gulf grouper, broomtail grouper, and white shark may not be taken. Authorized methods of take: hand, hook and line, seine (except beach seine), dip nets, spear, dredge, hand tools, and trap (when marked with Scientific Collecting Identification Number (SCIN)).
- 2. You may take or possess marine aquatic plants not to exceed 10 pounds wet weight in the aggregate. Eel grass, surf grass, and sea palm may not be taken. Authorized method of take: hand and hand tools.
- 3. You may take or possess marine invertebrates not to exceed sport fishing bag limits; sport fishing seasons and size limits shall apply. Lobster, abalone, and live rocks may not be taken. Authorized method of take: hand, hook and line, seine (except beach seine), dip nets, spear, dredge, hand tools, and trap (when marked with your SCIN).
- 4a. You may take <u>terrestrial</u> invertebrates and <u>vernal pool</u> invertebrates excluding Standard Exceptions (see Mandatory Condition A). Threatened or Endangered Species inadvertently collected shall be immediately released and reported to the Department of Fish and Game (DFG), Wildlife Branch, Scientific Collecting Permit Biologist, 1812 9th St., Sacramento, CA 95811 on copies of California Native Species Survey (CNSFS) forms (see Mandatory Condition H) within three business days. Authorized methods of take: hand and net.
- 4b. You may take <u>aquatic</u> invertebrates from streams and lakes excluding Standard Exceptions (see Mandatory Condition A). Threatened or Endangered Species inadvertently collected shall be immediately released and reported to the DFG at (916) 327-8335 within two business days. Authorized methods of take: hand, net, dip net, Surber sampler, drift net, dredge, and plankton net.
- 5a. You may capture and release an unlimited number of freshwater fishes, including Special Concern Species but excluding all other Standard Exceptions, only for the purposes of identification and documentation. Seasons and size limits shall not apply. Threatened or Endangered Species inadvertently collected shall be immediately released and reported to the DFG at (916) 327-8335 within two business days. Authorized methods of take: hand, hook and line, seine, dip net, and minnow trap (when marked with SCIN). Electrofishing and gill netting are not authorized without express written approval from the DFG office responsible for the area where collections are proposed.
- 5b. You may sacrifice, as voucher specimens (for the purposes of identification and documentation), up to 3 individuals per species of nongame freshwater fish species that are Special Concern Species as requested in your SCP application but excluding all other Standard Exceptions. Seasons and size limits shall not apply. Threatened or Endangered Species inadvertently collected shall be immediately released and reported to the DFG at (916) 327-8335 within two business days. Authorized methods of take: hand, hook and line, seine, dip net, and minnow trap (when marked with your SCIN). Electrofishing and gill netting are not authorized without express written approval from the DFG office responsible for the area where collections are proposed.
- 6a. You may capture and release an unlimited number of amphibians and reptiles, including Special Concern Species, but excluding all other standard exceptions, only for the purposes of identification and documentation. All animals captured shall be released alive and unharmed at the capture site and may not otherwise be removed from the wild unless authorized by the DFG. Specimens of Threatened or Endangered Species inadvertently collected shall be immediately released and reported to the DFG, Wildlife Branch, Scientific Collecting Permit Biologist, 1812 9th St., Sacramento, CA 95811 on copies of CNSFS forms (see Mandatory Condition H). Authorized methods of trapping are hand, dip net, minnow seine, lizard noose, snake tongs, snake hook, and live-trap (when marked with your SCIN). You shall report to the DFG's Wildlife Branch at (916) 445-0879 within three days any incidental injury or mortality of individuals of Standard Exception Species.
- 6b. You may purposefully capture (see Mandatory Condition M) and release an unlimited number of small mammals, excluding all Standard Exceptions, but only for the purposes of identification and documentation. You shall immediately release all captured individuals alive, unharmed, and unmarked at the site of capture and shall not otherwise handle these animals without additional authorization from the DFG. You shall immediately release any inadvertently-captured individuals of Standard Exception species and report their capture to the DFG, Wildlife Branch, Scientific Collecting Permit Biologist, 1812 9th St., Sacramento, CA 95811 on copies of CNSFS forms (see Mandatory Condition H). You shall also contact the DFG's Wildlife Branch at (916) 445-0879 within three days to report any incidental injury or mortality of individuals of Standard Exception Species. Authorized methods of trapping are live-traps, Sherman- or Tomahawk-brand, of appropriate size to avoid capture of medium-sized mammals or carnivores.
- 7a. You may salvage dead amphibians, reptiles, and mammals. You shall leave specimens that are unsuitable for donation at the site or shall completely destroy them through burial or incineration. You shall obtain additional permission to salvage on private or public property from the landowner or custodian of that property. You shall tag each salvaged specimen with the following information:(a) date of salvage and location of the specimen; (b) name of the person who salvaged the specimen; and (c) the permit number under which the specimen was salvaged. You shall deposit all salvaged specimens in a public scientific or educational institution within one month of acquisition.
- 7b. You may salvage dead freshwater fishes. Standard Exceptions (see Mandatory Condition A) salvaged shall be reported to the DFG at (916) 327-8335 within two business days for disposition instructions. All specimens must be donated to a public scientific or educational institution.
- You may take birds in accordance with your federal permit(s). The DFG may require additional or modified conditions that will be noted on your SCP. You shall immediately release any inadvertently-taken Threatened or Endangered Species and report their capture to the DFG, Wildlife Branch, Scientific Collecting Permit Biologist, 1812 9th St., Sacramento, CA 95811 on copies of CNSFS forms (see Mandatory Condition H).
- 9. You may take species that are federally-listed or are both State- and federally-listed in accordance with your existing federal permit(s). The DFG may require additional or modified conditions that will be noted on your SCP. If you are using traps to take mammals, you must follow Mandatory Condition M. You shall immediately release any Threatened or Endangered Species not authorized in your federal permit and report their capture to the DFG, Wildlife Branch, Scientific Collecting Permit Biologist, 1812 9th St., Sacramento, CA 95811 on copies of CNSFS forms (see Mandatory Condition H).

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State of California-The Resources Agency DEPARTMENT OF FISH AND GAME 2008 SCIENTIFIC COLLECTING PERMIT JUSTIFICATION

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I certify that I have read, understand, and agree to abide by, all conditions of this permit, the applicable provisions of the FGC, and the regulations promulgated thereto. I certify that I am not currently under any Fish and Game license or permit revocation or suspension, and that there are no other legal or administrative proceedings pending that would disqualify me from obtaining this permit. I agree that if I make any false statement as to any fact required as a prerequisite to the issuance of this permit, the permit is void and will be surrendered where purchased, and I understand that I may be subject to prosecution pursuant to FGC Section 1054 or to other administrative actions pursuant to Section 746, Title 14, of the CCR.

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State of California—The Resources Agency DEPARTMENT OF FISH AND GAME 2008 SCIENTIFIC COLLECTING PERMIT JUSTIFICATION

DFG USE ONLY

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SECTION 4 - PERMIT JUSTIFICATION - Required for ALL activities, including permit renewals.

FIRST NAME	M.I.	LAST NAME	CHECK HERE IF PROPOSING
Leonard	Y	Liu	TO TAKE STANDARD EXCEPTIONS

Use the space below to summarize your research (purpose, methods/techniques, species and numbers to be collected, collection locations, and disposition of specimens). If requesting standard exceptions, marking/tagging, captivity, or sacrifice, specific details as described above must be included for each species and activity requested. Attach additional pages if necessary. Attach copies of appropriate federal permits and additional State permits (e.g., Memorandum of Understanding) to avoid delay of processing. You may be asked to send in a *detailed* study proposal for standard exception species during the review process.

Call count surveys for California Clapper Rail (Rallus longirostris obsoletus) in San Francisco Bay region following standardized, federally-approved protocols. Current Sect. 10 permit with Olofson Environmental, Inc. TE118356-0. Between 10 and 50 sites will be surveyed per year with passive and active (tape-response) methods.

Nest monitoring for California Clapper Rail for Olofson Environmental Inc., California Coastal Conservancy's Invasive Spartina Project, and/or PRBO Conservation Science. Section 10 permits in process.

Banding of California Clapper Ralls for PRBO Conservation Science. Section 10 permit in process.

Salvaging unhatched eggs and carcasses of California Clapper Rails, Ca. Black Rails, and other tidal salt marsh species for San Francisco Estuary Institute food web studies and contaminant studies, with final disposition with the Museum of Vertebrate Zoology and/or per the PRBO Conservation Science's MOU with DFG (attached).

As part of a long-term bird population study on tidal marshes, birds of the species listed below will be captured (mist-netting of adults and juveniles) and banded (color and USGS-numbered) to assist in identifying individuals, map their densities, assess parentage, and assess survivorship. Nestlings will be banded to allow estimation of dispersal distance and survivorship.

Birds to be captured, banded, and released:

Samuels Song Sparrow (Melospiza melodia samuelis), Alameda Song Sparrow (M. m. pusillula, Suisun Song Sparrow (M. m. maxillaris), Common Yellowthroat (Geothlypis trichas sinuosa), Marsh Wren (Cistothorus palustris); all less than 100 per year. Birds will be collected in San Francisco Bay tidal marshes, including the counties of Marin, Sonoma, Napa, Alameda, Solano, Contra Costa, San Mateo, and Santa Clara.

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the issuance of this permit, the permit is void and v	vill be surrendered where purch	ased, and I understand th	iat I may be subject	to prosecution pursuant to FG
Section 1054 or to other administrative actions pu	rsuant to Section 746, Title 14	of the CCR.	<u>e 6907</u>	
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Clear Form



SCIENTIFIC COLLECTING PERMIT AMENDMENT FORM

State of California-The Resources Agency

DEPARTMENT OF FISH AND GAME

You are required to complete and submit an Amendment Form when requesting a change to an existing Scientific Collecting Permit or when your affiliation changes.

DFG USE ONLY							
	THIS	PERMIT IS VALID:				ANENT ID NUMBER	
FROM	_//_	THROUGH	_!_	_/	SC-	7204	

BEFORE COMPLETING AMENDMENT: Read instructions, permit descriptions, mandatory conditions, and authorization requested or issued on reverse page of this amendment form and current Scientific Collecting Permit (SCP) Application www.dfg.ca.gov/licensing/pdffiles/fg1379.pdf. <u>Complete all portions of the attachment</u> If the DFG returns this amendment, it becomes part of and must be attached to your valid, existing SCP. Type or print dearly.

FIRSTNAME	M.t.	LASTNAME			CHECKHERE IF PROPOSING TO	
Leonard	Y.	Liu				
AFFILIATION		Check here if you want	future	TITLE		
Olofson Environmental Inc.; PRBO C	onservation Scier	ce correspondence mailed	to your affiliation.	biologist		
PERMITTEE'S MAILINGADDRESS		ł	DAY TELEPHK	ÓNE	FAXNUMBER	
165 Oddstad Dr. #77			()510-	409-5105	()	
CITY	STATE	ZIP CODE +4	E-MAIL ADDF	RESS		
Vallejo	CA	94589	lenliu1@ya	hoo.com		
AFFILIATION'S MAILING ADDRESS	•	CITY		STATE	ZIP CODE + 4	
3820 Cypress Dr. #11		Petaluma		CA	94954	
AMENDMENT JUSTIFICATION	- Required for	ALL activities		· · · ·		

Use the space below to summarize your research (purpose, methods/techniques, species and numbers to be collected, collection locations, and disposition of specimens). If requesting standard exceptions, marking/tagging, captivity, or sacrifice, specific details as described above must be included for each species and activity requested. Attach additional pages if necessary. Attach copies of appropriate federal permits and additional State permits (e.g., Memorandum of Understanding) to avoid delay of processing. You may be asked to send in a detailed study proposal for standard exception species during the review process. You may not conduct new collection activity until you have received an approved validated amendment.

Call count surveys for California Clapper Rail (Rallus longirostris obsoletus) in San Francisco Bay region following standardized, federally-approved protocols. Current Sect. 10 permit with Olofson Environmental, Inc. TE118356-0. Between 10 and 50 sites will be surveyed per year with passive and active (tape-response) methods.

Nest monitoring for California Clapper Rail for Olofson Environmental Inc., California Coastal Conservancy's Invasive Spartina Project, and/or PRBO Conservation Science. Section 10 permits in process.

Banding of California Clapper Rails for PRBO Conservation Science. Section 10 permit in process. Salvaging unhatched eggs and carcasses of California Clapper Rails, Ca. Black Rails, and other tidal salt marsh species for San Francisco Estuary Institute food web studies and contaminant studies, with final disposition with the Museum of Vertebrate Zoology and/or per the PRBO Conservation Science's MOU with DFG (attached).

As part of a long-term bird population study on tidal marshes, birds of the species listed below will be captured (mist-netting of adults and juveniles) and banded (color and USGS-numbered) to assist in identifying individuals, map their densities, assess parentage, and assess survivorship. Nestlings will be banded to allow estimation of dispersal distance and survivorship. Birds to be captured, banded, and released:

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I certify that I have read, understand, and agree to abide by, all conditions of this permit, the applicable provisions of the FGC, and the regulations promulgated thereto. I certify that I am not currently under any Fish and Game license or permit revocation or suspension, and that there are no other legal or administrative proceedings pending that would disqualify me from obtaining this permit. I agree that if I make any false statement as to any fact required as a prerequisite to the issuance of this permit, the permit is void and will be surrendered where purchased, and I understand that I may be subject to prosecution pursuant to FGC Section 1054 or to other administrative actions pursuant to Section 746, Title 14, of the CCR.

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DF FISH AND GAME USE ONLY			
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FOR DEPARTMENT (All pages of attachments in AUTHORIZATIONS: 8



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SECTION 5						
FIRST NAME	M.I.	LASTNAME				
Leonard	Y.	Liu				
ADDITIONAL CONDITIONS, AUTHORIZATIONS, AND APPROVALS ARE AS FOLLOWS:						

You may not band clapper rails without being so authorized on a federal recovery permit, a federal bird banding permit, and a current State MOU on which you are specifically named. You may not broadcast survey for, nest monitor, pursue, intentionally handle and band, nor otherwise take Samuel's song sparrow, Alameda song sparrow, San Francisco common yellowthroat, or Clark's marsh wren without being so authorized on a current MOU or letter permit on which you are specifically named. Contact John Gustafson, jgustafs@dfg.ca.gov, 916-327-8847. Passive surveys are authorized without an MOU (if neither approaching birds or nests nor conducting species-specific broadcast calls). You may band the above bird species of special concern (BSSC) if incidentally captured in your mist nets. Intentional pursuit and capture of BSSC in areas where they are known to occur requires an MOU.

Except as authorized by your federal bird banding permit for banding mortalities or by your federal recovery permit, you may not salvage birds without being so authorized by a valid federal Migratory Bird permit (Special Purpose - Salvage) from the U.S. Fish and Wildlife Service (http://www.fws.gov/permits/ mbpermits/birdbasics.html) on which you are specifically named.

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FOR DEPARTMENT OF FISH AND GAME USE ONLY		. al Hai
ALL PAGES OF ATTACHMENTS NOTED IN THIS PERMIT SHALL REMAIN WITH THIS PERMIT	AT ALL TIMES.	19 11
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State of California—The Resources Agency DEPARTMENT OF FISHAND GAME SCIENTIFIC COLLECTING PERMIT APPLICATION	2007 SCIENTIFIC COLLECTING PERMITSTAMP	2007 DUPLICATE
PERMIT MUST BE IN IMMEDIATE POSSESSION WHILE COLLECTING	No <mark>801103-02</mark> Date <mark>4/4/2008</mark>	No. 000
W RENEWAL - Copy of previous permit and report of specimens captured/salva		plication will be return

NEW
 Z_ RENEWAL - Copy of previous permit and report of specimens captured/salvaged must be attached or application will be returned.
CHECKONE: RESIDENT_\$55.50 NONRESIDENT_\$185.00 STUDENT_\$18.50_RESIDENT_AND NONRESIDENT
 DFG USE ONLY

THIS PERMIT IS VALID:	PERMANENT ID NUMBER
FROM_10/15/2008 through 10/15/2010	SC- 8629

BEFORE COMPLETING APPLICATION: Read instructions (last page), permit descriptions, mandatory conditions, and authorization requested or issued on reverse page of application. Complete all portions of the application (sponsor's section may be required). Type or print clearly.

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State of California—The Resources Agency DEPARTMENT OF FISHAND GAME 2008 SCIENTIFIC COLLECTING PERMIT JUSTIFICATION

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2006 MOU and 2006 Fublic Interest Notice (PIN). Any new activities proposed will require additional State authorization through an amendment bud post another 30-dep WIN before new advitties with these species can begin. Such though field work may be further delayed, depending on any comments the Department may receive during the notice period. INVOU Reporting section shall be modified as follows: Paragraph 18 and 19b are valid, please mail to Lyann Comrack. Exclude Paragraph 19a and Paragraph 20, mail CNDDB forms to the BDB address listed on the top of the CNDDB form. Do NOT send CNDDB forms to Lyann Comrack. Interpret address listed on the top of the CNDDB form. Do NOT send CNDDB forms to Lyann Comrack. Interpret address listed on the top of the CNDDB form. Do NOT send CNDDB forms to Lyann Comrack. Interpret address listed on the top of the CNDDB form. Do NOT send CNDDB forms to Lyann Comrack. Interpret address listed on the top of the CNDDB form. Do NOT send CNDDB forms to Lyann Comrack. Interpret address listed on the top of the CNDDB form. Do NOT send CNDDB forms to Lyann Comrack. Interpret address listed on the top of the CNDDB form and the top of the CNDDB forms to Lyann Comrack. Interpret address listed on the top of the CNDDB form and the top of the CNDDB forms to Lyann Comrack. Interpret address listed and the top of the CNDDB form and the top of the CNDDB forms to Lyann Comrack. Interpret address listed and the top of the complexity integration and the top of the top of the CND. Interpret address listed and the top of the complexity integration and the top of the regulations promine. Interpret address listed address listed and the top of the top	ollowing standardi nvironmental Inc. ctive (tape-respor	zed, feder TE11835 (se) metho (lapper rail sha 2th St. Sacran	ally-appro 6-0. Betwo ods.	veen 10 and 50	Current s) sites will contact shall	section 10 permit be surveyed per be changed from John C	with Olofs year with Sustafson to Ly ails that is curr	on passive and ann Comrack, rently permitted under
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State of California—The Resources Agency DEPARTMENT OF FISHAND GAME 2008 SCIENTIFIC COLLECTING PERMIT ATTACHMENT

CHECK APPLICABLE BOX(ES):

(Special status species in "A",		_	IEND EXISTING PERMIT lust attach copy of current permit			S BUT USE OF MARKING, FROM WILD, ETC.
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	FROM		RMIT IS VALID: _ THROUGH// //	SC-	IANENT ID NUMBE ダイマン	R
BEFORE COMPLETING ATTAC	L				10.20	issued on reverse page of application.
Complete all portions of the att	achment. If rec	quired, use this fo	rm to describe the purpose of your w	/ork <u>or</u> to request an a	mendment to an exis	ting SCP. Return the attachment with
FIRST NAME 1 //	uthorizations an	M.I.	If the DFG returns this attachment, LAST NAME	it becomes part of and	d must be attached to	
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FOR DEPARTMENT O	F FISH AND	GAME USI	ONLY			
AUTHORIZATIONS: (See re		SIGNED AND	DATED	SH	ALL REMAIN WIT	H THIS PERMIT AT ALL TIMES.
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DFG USE ONLY						
THIS PERMIT IS VALID:	PERMANENT ID NUMBER					
FROM/ THROUGH/	SC- 95(3	5				

INSTRUCTIONS: <u>Complete the top portion of this form only</u>. Return the authorizations and approvals form with your completed application and attachment form. If the DFG returns this form, it becomes part of and must be attached to your SCP. Type or print clearly.

FIRST NAME) effer	MI. T	LAST NAME Lewis		DATE OF BIRTH 3/2/1978
AFFILIATION Olofson Enviro	mental, I.	Check here if you want fu correspondence mailed to		Techan
2436 10 Standards	Śł.		DAYTELEPHONE (503)830-0248	FAX NUMBER
Berkeliy	ŠTATE	ZIP CODE+4 94710	E-MAIL ADDRESS	wis@ yahoo.com
AFFILIATION'S MAILING ADDRESS S	+, S., +, 216	CITY Berkeley	J STATE CA	ZIP CODE+494710

You may salvage dead mammals, except bats, cougars, marine mammals, fully protected or federally-listed species unless additionally authorized. Salvaged specimens of the Mohave ground squirrel shall be deposited only in the mammalian collection of the Natural History Museum of Los Angeles County, Los Angeles, California. See also Mandatory Conditions/Number Authorizations.

FOR DEPARTMENT OF FISH AND GAME USE ONLY	
PAGE(S) OF ATTACHMENT SIGNED AND DATED	SHALL REMAIN WITH THIS PERMIT AT ALL TIMES.
AUTHORIZATIONS: (See reverse for explanation)	

Randi Logsdon	C CN = Randi Logsdon C = US, O = Department sh and Game, OU = Wildlife Programs Branch ste: 2008.02.11 14:09:10 -06'00'	t of 2 .		DATE 3.	DATE
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State of California—The Resources Agency DEPARTMENT OF FISHAND GAME 2007 SCIENTIFIC COLLECTING PERMIT AUTHORIZATIONS AND APPROVAL
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DFG USE ONLY						
THIS PERMIT IS VALID:	PERMANENT ID NUMBER					
FROM// THROUGH//	SC- 9799					

INSTRUCTIONS: <u>Complete the top portion of this form only.</u> Return the authorizations and approvals form with your completed application and attachment form. If the DFG returns this form, it becomes part of and must be attached to your SCP. Type or print clearly.

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You may salvage dead mammals, except bats, cougars, marine mammals, fully protected or federally-listed species unless additionally authorized. Salvaged specimens of the Mohave ground squirrel shall be deposited only in the mammalian collection of the Natural History Museum of Los Angeles County, Los Angeles, California. See also Mandatory Conditions/Number Authorizations.

FOR DEPARTMENT	OF FISH AND	GAME	USE ONLY
PAGE(S) OF	ATTACHMENT	SIGNED	AND DATED

SHALL REMAIN WITH THIS PERMIT AT ALL TIMES.

AUTHORIZATIONS: (See reverse for explanation)

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Appendix 9:

Specifications for Aerial Imagery Acquisition

San Francisco Estuary Invasive *Spartina* Project Specifications for Aerial Imagery Acquisition

I. Objectives of Aerial Imagery Acquisition and Processing

- (1) Acquisition of color infrared stereo imagery following the technical specifications listed below (Project Details).
- (2) Scheduling of imagery acquisition to ensure imagery is:
 - i. Free of atmospheric interference (no fog or clouds).
 - ii. Free of glare (taken at maximum sun angle).
 - iii. Taken at low tide (to ensure visibility of mudflat vegetation).
 - iv. Taken during flowering of non-native Spartina.
- (3) Communication between Subcontractor and ISP Monitoring Program Manager to confirm suitable dates and times for aerial photography capture to meet these requirements.
- (4) Scanning of imagery following technical specifications detailed below.
- (5) Collection of airborne GPS (ABGPS) at the time of imagery acquisition.
- (6) Aerotriangulation (AT) of imagery following technical specifications detailed below.
- (7) Delivery of raw imagery and AT solution to the ISP in a digital format compatible with Leica Photogrammetry Suite (LPS) 8.7 software OR delivery of orthorectified imagery with <2m RMS (root mean square) error.

II. Required Deliverables

- (1) Images meeting specifications below, delivered in tiff or geotiff format, as appropriate, on external hard drive.
- (2) If ISP will orthorectify imagery, contractor will also deliver:
 - i. Camera calibration report (LPS-compatible).
 - ii. ABGPS data (LPS-compatible).
 - iii. Camera-integrated IMU data (LPS-compatible) if available.
 - iv. Triangulation solution (LPS-compatible).

III. Specifications

- (1) Photography will be flown for standard stereo-coverage with 60% endlap and 30% sidelap.
- (2) If CIR film is used:
 - i. Acquire images at 1:10,000 scale.
 - ii. Scan color negatives at 25 microns (3 bands, 8 bit resolution) for a pixel resolution of approximately 0.25m.
- (3) If a digital camera is used:
 - i. Capture 4-band, 16-bit per band, 25 cm ground sample distance (GSD) digital imagery. (Note: 25 cm GSD applies to imagery prior to pan-sharpening.)
 - ii. If possible, capture an additional panchromatic band, 16-bit per band, at GSD higher than 4-band multi-spectral data during the acquisition of 4-band data
 - iii. Apply radiometric and atmospheric corrections to imagery.
- (4) Capture of flight timing, trajectory and airborne GPS data.
- (5) A current camera calibration report will be provided with an LPS-compatible camera file.
- (6) *If available*, a camera with an integral IMU will be used and IMU data will be collected during photo acquisition.
- (7) ABGPS data (and IMU data if collected) will be reduced with appropriate CORS data and the acquired photography will fully triangulated using soft-copy methods.
- (8) Triangulation solution will be provided electronically for easy input to LPS.
- (9) Camera station data resulting from the ABGPS solution will be provided as a separate file.
- (10) Where available, ground points will be selected and used to close the Airborne GPS solution.
- (11) Mapping datums will be NAD83 and NAVD88; projection will be UTM, Zone 10.
- (12) Orthorectify imagery using existing DEM and collected IMU and airborne GPS data.
- (13) Mosaic and tile imagery.

IV. Schedule Constraints & Deadlines

(1) Schedule Constraints

- i. Photography will be flown at low tide (preferably <3') and maximum sun angle (preferably 20-40 degrees) at dates and times determined in consultation with the ISP Monitoring Program Manager.
- ii. Photography will be flown under cloud and fog-free conditions.
- iii. Photography will be flown at non-native *Spartina* flowering stage as determined by ISP Monitoring Program Manager (typically August/September).

- (2) Deadlines
 - i. Potential dates for flights will be determined by July 15, 2007.
 - ii. Products will be delivered within 75 calendar days of the date of photo acquisition.

V. Resources Provided:

The ISP Monitoring Program Manager will provide:

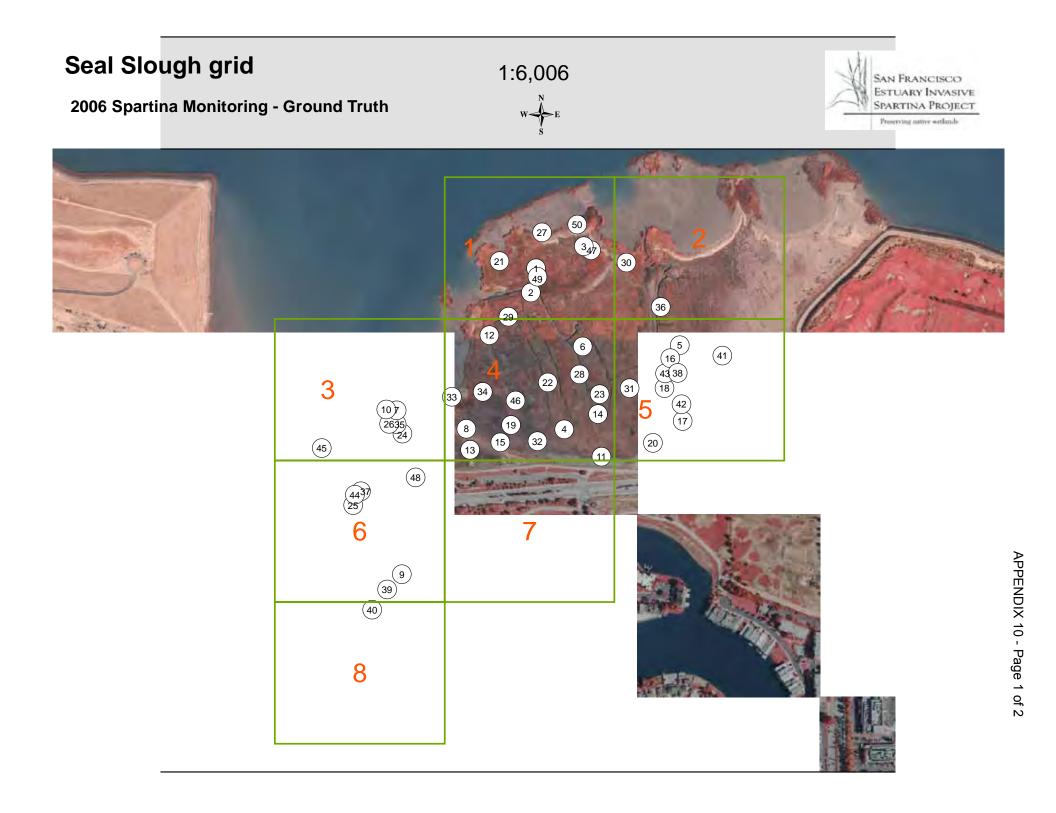
- (1) Shapefiles and maps of aerial extent of project areas.
- (2) Schedule of appropriate dates and times for photo acquisition to meet plant phenology, tide and sun angle requirements.

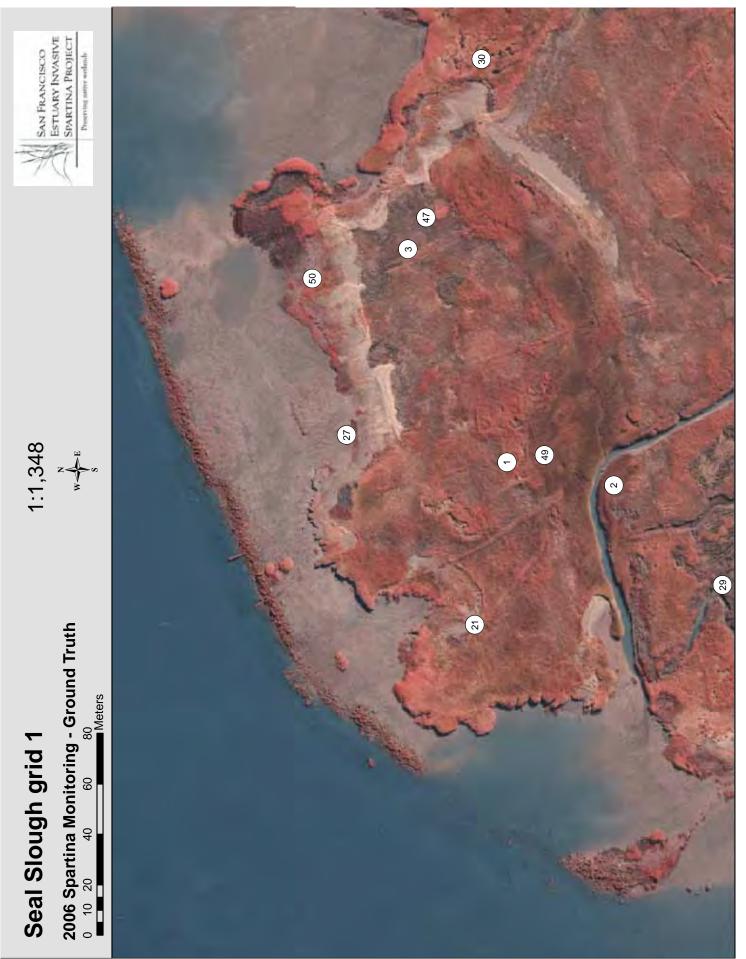
VI. Criteria for Selecting a Contractor

- (1) Resources and ability to perform all tasks and provide all deliverables to the specifications detailed in this RFP.
- (2) Proven experience in acquisition of aerial imagery of marshes and mudflats of the San Francisco Estuary for purposes of photo interpretation of biological features. (Provided via examples of past work and references.)
- (3) Proven experience in scanning of color infrared negatives to meet specifications of no glare or other interferences which would hinder photo interpretation of biological features in marshes or on mudflats. (Provided via examples of past work and references.)
- (4) Proven track record for providing on-time delivery of satisfactory materials as specified in contracts. (Provided via examples of past work and references.)

Appendix 10:

Seal Slough Grid – Example of maps used for ground truthing and field reconnaissance of aerial imagery





Appendix 11: Monitoring Program Data Handling Protocol

Spartina Monitoring Program Data Handling Protocols

Revised July 2009

In 2008 and 2009, the ISP transitioned from using Terra Sync and Pathfinder Office for managing GPS data, to using ArcPad, and refined protocols to improve the speed and efficiency of work flow. This protocol describes the methods used to transfer data between the GIS computer system, and the GPS units used to collect data in the field.

A. Data Check Out Protocol

This seven part process is for checking data out of ArcGIS for use in the field with ArcPad.

Part 1: Selecting Data for Copy Out

This step selects what data will be available for viewing but not editing in ArcPad.

1. Open ArcPad_for_Inv_2009.mxd within Y: \2009 ArcPad\ArcPad MXDs.

BEWARE: No other map document (mxd) may be open on your computer, or this checkout will not be logged and thus will not be able to be checked in!

- 2. If the ArcPad toolbar is not visible, click on the grey space within the toolbars and select the ArcPad toolbar. (If it is already checked on but is not visible, uncheck it then recheck it, and it should appear.)
- 3. Turn on relevant layers within the "Copy out" group layer. This is the data you will view but not edit. These layers should normally include the following:
 - a. To Do 2009* (Expand group to check that all layers within this group are checked on.)
 - b. Past Year's Data
 - i. ArcPad Past Years DNA results (Expand and check as above)
 - ii. ArcPad Past Years Inventory data (Expand and check as above)
 - iii. Today's Inventory Boundary
 - iv. Use layer Properties>Symbology tab to symbolize today's inventory boundary.
 - v. Apply ISP Spartina style for Today's Inventory Boundary.
 - vi. You may need to symbolize by Scheduled and/or Method to narrow it down to today's inventory boundary for this particular checkout.
 - c. CLRA polygons

Appropriate "Copy out" layers may sometimes include other layers including but not limited to the following:

- d. Grid backgrounds
- e. Channels

- f. Control Program subareas
- 4. Be sure that layers are in the appropriate order (top to bottom) for viewing in ArcPad. (Note: ArcPad does not support grouping of layers, but does follow the order (top to bottom) in which they are exported from ArcMap.)
- 5. Turn on all layers within the "Checkout (for editing)" group layer. (CTRL-expand to check to be sure all layers within this are checked on. Cordylanthus layers should be turned off except at Southampton Marsh.)
- 6. Using Selection>Interactive Selection Method, select all features relevant to this site's inventory. (Note: You may want DNA samples from nearby sites, etc. if you think this would be helpful to the field staff doing inventory.)
- 7. Continue adding and removing from current selection until you have selected exactly those features you want to export to ArcPad.
- 8. Zoom to Selected Features. Ensure that only those features you want to export are selected.

Note: If ANY features selected are only partially in the view frame at time of checkout, those layers will have errors and will at best not be visible and at worst will cause ArcPad to crash. So it is <u>very</u> important to Zoom to Selected Features, and to return to this exact view extent when you check out the data.

Part 2: Selecting Data for Check Out (for editing)

This step selects what features will be available for editing in ArcPad. If the same features are also selected in the previous step, for copy out, that is okay.

- Using View>Bookmarks, go to "Check Me Out". (You may notice that I have Cordylanthus turned off most of the time...we'll only add this when we are checking out data for inventory at Southampton.. By the way, this bookmark takes you to "fake" data located in Annabel State Park...just a random location outside our project area.)
- 2. Using Selection>Interactive Selection Method>Add to Current Selection, select all of the features visible (smiley face and silly hat).



3. Using the Go Back to Previous Extent button (big blue back arrow) to return to your previous extent. (See note above about how important it is that you do not alter your extent at this point.)

Part 3: Get Data for ArcPad Wizard

- 1. Click on the Get Data for ArcPad icon within the ArcPad toolbar to start the Get Data for ArcPad wizard.
- 2. First screen:
 - a. Select All
 - b. Next>

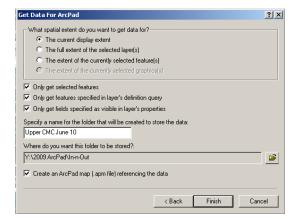
- 3. Second screen:
 - a. Ensure that the Database for checkout is Y:\2009 Edits\inventory_2009.mdb
 - b. Leave unchecked the "Only check out schema..." box.
 - c. Select All
 - d. Next>

*** Note: Once you hit Next> on this second screen you are committed to this checkout. Even if you decide to delete it later, please follow through and Finish this checkout. <u>Hitting <Back on the third screen will result in ArcMap crashing</u> and you will have to do all of the above steps all over again!***

- 4. Third screen:
 - a. Choose the first option under "What spatial extent do you want to get data for?": The current display extent
 - b. Check all three boxes below:
 - ✓ Only get selected features
 - ✓ Only get features specified in layer's definition query
 - ✓ Only get fields specified as visible in layer's properties
 - c. Specify a name that indicates the site <u>and</u> which survey this checkout is intended for. Recall that we may be conducting multiple surveys throughout the year, both for inventory and treatment surveys.
 - i. Example: "Blackies inv survey 1" or "Blackies May 11"
 - ii. Spaces in the name are OK

iii. Do not use any symbols other than an underscore.

- d. Click the Folder icon next to "Where do you want this folder to be stored?:" and navigate to Y:\2009 ArcPad\In-n-Out
 - ✓ Check the box to Create an ArcPad map (.apm file) referencing the data.
- e. Finish
- 5. Check the Operation Successful box to ensure that the Total Feature Layers all succeeded. If all looks good, click OK.



6. Repeat steps 1-5 above for each GPS unit that will be used in the field. Add the GPS unit letter to the end of the checkout name. (These checkouts with letters on the ends will be used later to check data IN, and will not be actually loaded onto the GPS units. There is no harm in checking out for all units that might possibly be used...the unused checkouts can just be deleted later.)

Example: Upper CMC June 10 H

7. Save map document in the ArcPad MXDs\Checkouts folder with all of the features selected for checkout. This will enable someone else to see what was checked out so they can make a field map...or will enable you to re-do checkout easily if anything went wrong!

Part 4: Preparing Files for GPS units

- 1. Shrink (don't close) ArcMap.
- 2. Open File Explorer and navigate to Y:\2009 ArcPad.
- 3. Click on the Folders view to make your life easier.
- 4. Expand the APLs folder within Y:\2009 ArcPad, then use CTRL-a to select all files in the APL folder.
- 5. Copy these selected .apl files, then Paste them into the checkout folder you made in Y:\2009 ArcPad\In-n-Out (use the first checkout you made...the one *without* a counting number at the end of the folder name).
- 6. When prompted with the Confirm File Replace message box, click on Yes to All.
- 7. Scroll to the bottom of the list of pasted files to find the "leftover" .apl files without any matching shapefiles.
- 8. Delete these "leftovers".
- 9. Open the ArcPad.apm file.
- 10. Check to make sure that the correct layers are visible.
- 11. Open the layers tab (the golden waffles) and click on the blue identify (i) button to select all layers to be identifiable in ArcPad.
- 12. Save and close ArcPad.

Part 5: Housekeeping

- 1. Navigate back to Y:\2009 ArcPad\In-n-Out. Select the checkout folder you just worked with (the one *without* the GPS unit letters).
- 2. Copy and paste this folder into two locations:
 - Y:\2009 ArcPad\Checkouts\Backup Copies and
 - Y:\2009 ArcPad\Checkouts\Ready for GPS
- 3. Once you have copied this folder into both of the above locations, delete it from the In-n-Out location.
- 4. Select all of the checkout folders in the In-n-Out folder from this site that have GPS unit letters at the end of the names.
- 5. *Cut* (CTRL-x) then paste these into Y:\2009 ArcPad\Checkouts\Multiple Checkouts.

6. In-n-Out should now be empty. There should be one copy of the checkout in the Ready for GPS folder, and one in the Backup Copies folder. All of the extra checkouts should be in the Multiple Checkouts folder.

Part 6: Preparing Inventory Field Maps

Typically, you will want to prepare and print a map for use in the field when conducting your surveys.

- 1. Create a map in layout view with the following in mind:
 - a. DNA results from all years should be visible!!!
 - b. Inventory results (at least unique locations*) from all years should be visible!!!
 - c. Today's inventory boundary should be a hollow polygon. (I have found that turquoise or bright yellow are the most visible border colors.)
 - d. NAIP imagery can be made 40% transparent to make the inventory layers more visible without loosing the benefit of the imagery.
 - e. Create separate two maps, one with NAIP and one with World Street Map** as the background layer if a lot of driving/navigating is necessary.
 - f. Include callout graphics to indicate starting and stopping points, and any advice you think might be useful.
 - g. Convert 2009 To Do* point, line, and/or polygon labels to annotation (In The Map, Current Extent only, please!) and alter/drag these as necessary to make them easy to read & not obstructing other features.
- 2. Export to JPEG (96 dpi) and save in Y:/2009 Field Maps/Inventory.

Part 7: Print Field Maps

- 1. If printing on the Dell Color Laser printer, don't forget to select Printer Preferences every time, and select Letter_Portrait or Letter_Landscape from the Print Setup tab for each printing.
- 2. To save ink, use Toner Saving Mode (in the Advanced tab).
- 3. Use waterproof paper if necessary for the day's field work!

🔹 Dell Color Laser 3010cn Properties 🧧 🦹				
Print Setup Paper Tray Graphics Watermark Advanced				
Job Type:		Favorites:		
Normal Print	▼ Setting	🚊 Letter_Portrait	Save Edit	
a		Papar Siza	- Image Orientationa	

B. Data Check In Protocol

This process is for checking in data from ArcPad into ArcGIS. There are five parts, including a section on troubleshooting.

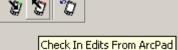
Part 1: Backup

- 1. Open ArcCatalog, and navigate to Y:\2009 Edits.
- 2. Right click on the inventory_2009 geodatabase and select Compact Database. This will take a minute, and the file size should be much reduced afterwards. This will only work if no one is editing or even viewing the geodatabase. If it does not work, at least you can compact the copy you are about to make.
- 3. Right click again and Copy the inventory_2009 geodatabase.
- 4. Paste the copy into Y:\2009 Edits\BKP.
- 5. Rename the backup copy bkp_inventory_2009MMDD where MMDD are today's month and date. If there is an existing backup from today, you can add the time (military style).
- 6. If you were unable to compact the original file, compact your backup file now.

Part 2: Check In

(Note troubleshooting ideas below if you run into problems!)

- 1. *Cut* and paste the folder you wish to check in from Y:\2009 ArcPad\CheckIns\Needs CheckIn to the Y:\2009 ArcPad\ In-n-Out folder. (Multiple folders can be cut and paste into In-n-Out, they don't have to be checked in one by one)
- Rename the folder(s) you just copied into In-n-Out to match the name(s) they were given during checkout. To do this, you will remove the _FLoff part of the folder name(s) so it's just the _X (unit) extension at the end...though it might just be (space)X rather than _X. (You'll see the name in step 5 below, or look in the Y:\2009 ArcPad\Checkouts\Multiple Checkouts folder to see checkout names by unit.)
- 3. Open ArcMap. When prompted, choose to start the program with the map Y:\2009 ArcPad\ArcPad MXDs\ArcPad_for_Inv_2009.
- 4. If the ArcPad toolbar is not visible, make it so by adding it to your toolbars. This may require un-checking and re-checking it from the list of toolbars.
- 5. Right click on Pt_Spartina_09 and click the 'Start Editing' pencil icon from the drop-down menu.



- 6. Click the 'Check in Edits From ArcPad' icon, the one depicting a GPS unit with an arrow pointing out of it.
- 7. A 'Check in Edits From ArcPad' window will appear. Using the **Check Out Name** column to guide you, put check marks in the boxes next to <u>all</u> of the feature classes associated with the Check Out Name you wish to check in. (Note: you can click on

the column heading to sort by Check Out Name if it is not already sorted this way.) Then click 'Check In'.

Feature Class	Check Out Name	Target Database	Folder 🔺
Bird_09	CMER Larkspur June 9_J	Y:\2009 Edits\inv	Y:\2009 A
🗹 DNA_09	CMER Larkspur June 9_J	Y:\2009 Edits\inv	Y:\2009 A
FieldPhotos	CMER Larkspur June 9_J	Y:\2009 Edits\inv	Y:\2009 A
🗹 Generic_Ln_09	CMER Larkspur June 9_J	Y:\2009 Edits\inv	Y:\2009 A
Generic_Poly_09	CMER Larkspur June 9_J	Y:\2009 Edits\inv	Y:\2009 A
🗹 Generic_Pt_09	CMER Larkspur June 9_J	Y:\2009 Edits\inv	Y:\2009 A
🗹 Ln_Spartina_09	CMER Larkspur June 9_J	Y:\2009 Edits\inv	Y:\2009 A
Poly_Spartina_09	CMER Larkspur June 9_J	Y:\2009 Edits\inv	Y:\2009 A
Pt_Spartina_09	CMER Larkspur June 9_J	Y:\2009 Edits\inv	Y:\2009 A
🗹 tracking_polys	CMER Larkspur June 9_J	Y:\2009 Edits\inv	Y:\2009 A 🖕
	over i pv	V10000 F 0.11	VI SOSSA

- 8. A message will ask you if you are sure you want to do this. Respond 'Yes'. A series of messages will most likely then appear with exclamation marks, warnings that various files have no feature updates, and the question, 'Do you still want to check this in?' Respond 'Yes' to all of these.
- 9. Next, click on the Editor icon and in drop-down menu select 'Stop Editing'. A message will appear asking whether you would like to save your edits. Respond 'Yes' to save the data you just checked in.

Part 3: Housekeeping

within the CheckIns folder...

1. Once you have checked in a folder in the In-n-Out folder, add the following to the end of the folder name(s), " cid FL YYYYMMDD" where cid means 'check-in done', FL are the initials of the person who just did the check in, and YYYYMMDD is the date that check in was completed.

If you had to rename the folder to do the checkin (like from S to B or something), please rename it BACK to the original unit letter that needed checkin during this housekeeping step.

2. Cut and paste these checked-in folders with the super long names from In-n-Out to Checkins\Checkin Done.

Part 4: Final Steps

- 1. Close ArcMap if it is not already closed.
- 2. Open ArcCatalog.
- **ISP** Monitoring Program

Edito	•	÷,	87	•0	
- 🖅 :	Star <u>t</u> B	Editin	g		
- Ø s	🍠 Stop Editing				
B 7 :	<u>5</u> ave B	Edits			
[<u>d</u> ove,				

- 3. Repeat the Backup steps above.
- 4. Open ArcToolbox within ArcCatalog.
- 5. Expand 2009 ISP Tools. If this toolbox is not present (at the top of the list of toolboxes), add it from S:\GIS\Toolboxes.
- 6. Run the "Repair and Calculate" tool.

Part 5: Troubleshooting Check Ins

If something goes wrong and you can't check in the folder...

1. Use ArcCatalog to preview the 2009 data to check if any data was actually collected. The shapefiles which may have been edited are those with the same name as the feature classes within the inventory_2009 geodatabase, as listed below.

E- inventory_2009.mdb 💽 axf_100x100_09 • axf_25x25_09 axf_50x50_09 - ⊡ Bird_09 -🖽 Cordy_Ln_09 - 🖾 Cordy_Poly_09 Cordy Pt 09 ⊡ dens_10x10_09 ⊡ DNA 09 FieldPhotos_09 🗄 Generic_Ln_09 🖾 Generic_Poly_09 Generic Pt 09 🗄 Ln_Spartina_09 🖾 Poly_Spartina_09 💽 Pt_Spartina_09 ⊡ ToDo_09 ⊡ tracking_centroids_09 🖾 tracking_polys_09

- 2. Use the ArcCatalog Preview tab to view the table of the 2009 shapefiles waiting for check in and corresponding to the above list.
- 3. Scroll to the far right of the table to see the _CHGCODE field. If no data was collected or updated, all of the _CHGCODE values will be zero (0). If any of the _CHGCODE values are non-zeros, you will need to check the data in one way or another. FYI:
 - a. _CHGCODE = 1 means this is a new feature added in the field
 - b. _CHGCODE = 2 when a feature's attributes are changed in the field
 - c. _CHGCODE = 3 means a feature's geometry (location) was updated in the field
- 4. If no data was collected or updated, move the folder to CheckIn Done, but replace "cid" with a word or two describing the situation (like "empty" or "corrupted" or something like this) so that others will be able to see what you figured out.

- 5. If data was collected, and there is no checkout available, check if the data was already checked in, but the folder was accidentally not moved out of Needs Checkin.
 - a. To do this, Add Data in a map displaying 2009 data (such as ArcPad_for_Inv_2009.mxd or any map called this with a site at the end of the name).
 - b. Add the shapefiles containing features that were created or updated (_CHGCODE >0)...or just add all of the files if you prefer.
 - c. The file names should be highlighted when they are first added to ArcMap. While they are still highlighted you can right click and select "Group". This will group all these new files together for you, making it easier to keep them organized and to remove them from your map when you are done viewing them.
 - d. Open the attribute table of a layer that was updated or has new features.
 - e. Scroll to the __CHGCODE field and Sort Descending, then highlight one of the updated/new features.
 - f. Right click on the grey box to the left of the selected feature's row, and select Zoom to Selected.
 - g. Use the information button in ArcMap to draw a box around and identify all visible layers at the location of this field.
 - h. If only the feature from the newly added layer (the layer with a field named ___CHGCODE at the bottom of the identify fields lists) is identified, this signifies that the data is not present in the inventory 2009 geodatabase, and thus has not been checked in.
 - i. If two features are identified one from the newly added layer and one from the inventory 2009 geodatabase layer AND the attribute data is the same for both features this indicates that this data was already checked in. (The attributes to check for changes are most likely TS_by, TS_date, and TS_record.)
- 6. If the data truly needs to be checked in, and there is no checkout available, you can load the data (if it is all new data) or (if it has checked out data in it) you need to make a redo checkout by opening the saved MXD that was used to check the data out, and redoing the checkout.
- 7. Open the corresponding MXD in <u>Y:\2009 ArcPad\ArcPad MXDs\Checkouts</u>. (It may be in the Done folder at the end of this file path.)
- 8. If you are not sure which MXD to open, you can try to match the files by checking the date/time of the checkout or by viewing the extent and features checked out.
 - a. Using File Explorer, View>Details, check the Date Modified of the 2009_Inventory_Boundary.shp file (or any .shp file that is not modified in the field, such as past years' data). (Do not rely the date/time of any .apl or .vbs files, as these were not created at the time of checkout). You can then check

the Date Modified of the same file within the suspected folder(s) in Backup Checkouts to see which one matches the folder needing check in.

- b. To view the extent and features checked out, open the file named ArcPad (type: ArcPad map document) within the ArcPad folder needing check in. This should open a map in ArcPad (the same ArcPad map that was on the GPS unit). If the map is not zoomed to full extent, click the globe icon. Now you can see what the checkout looked like, and try to match this to what is selected in the suspected ArcMap MXD document(s).
- c. Open the
- 9. Once you have opened the correct MXD:
 - a. Close any other open map documents open on your desktop. No other map document (mxd) may be open on your computer, or the record of this checkout will not be logged. This is okay if you will do the check in immediately, without closing the mxd from which you did the checkout. But if you have 2+ mxds open during checkout, then close the map from which you just did the checkout, the checkout will be lost.
 - b. Open the ISP <u>Check Out protocol</u> Word document. (CTRL-click the underlined words above to hyperlink there!)
 - c. Check that the protocol's *Part 2: Selecting Data for Checkout* was done (i.e. the smiley face visible via the Check Me Out bookmark should all be selected; if it is not, follow the directions in Part 2 to do so).
 - d. Follow *Part 3: Get Data for ArcPad Wizard*, steps 1-6 (don't bother with the last step, step 7).
 - e. Skip Part 4: Preparing files for GPS units

Instead of following *Part 3: Housekeeping*, just save a copy with the word **redo** added at the end of the checkout name to Y:\2009 ArcPad\Checkouts\Backup Copies (example: Blackies 0629 S redo)

Appendix 12: ISP Photo Point Establishment Protocols

Drew Kerr April 17, 2006

ISP Photo Point Establishment Protocols

The ISP will be incorporating photo points into our monitoring program tracking the eradication of non-native *Spartina* around the Bay. Photos will be taken annually from identical points overlooking the infestation to provide a graphic illustration of the effects of removing the *Spartina* from the system and capture the details of this progression over time. The photos will be combined with extensive quantitative monitoring data to qualitatively track marsh condition as it transitions from a system dominated by non-native *Spartina*. Since many of the infested marshes have large monocultures of *Spartina*, photos taken after successful control work should show dramatic results after several seasons, and will reveal some of the salt marsh habitat features that were recovered by the efforts.

Key Photo Point Elements

Since consistency is one of the most important factors in accurately tracking progress with photo points, these standardized ISP Photo Point Establishment Protocols have been developed to help select the best possible views, and to aid in our ability to replicate views from year to year.

- **1.** *Select photo point and composition.* Select the location and view for the photo point following the "Photo Composition Guidelines" and "Determining the Number of Photo Points", below.
- 2. *Record photo point location.* Mark the photo point using a sub-meter accuracy GPS datalogger to ensure that the point can be found at any time in the future. The monitoring data dictionary on the GPS datalogger has been modified to allow us to record details of the photo point along with data that is specific to the type of monitoring event (i.e. spring efficacy, summer efficacy, or inventory) being conducted. The GPS dataloggers will automatically record the date and time of the photos, so there is no need to add that information in an additional field. No permanent stakes or markers will be used for ISP photo points to avoid adding more "litter" to the marsh or levees. This also guarantees that markers will not be vandalized or removed.
- **3.** *Record photo bearing.* Take a compass bearing to record the direction of the photo from the GPS point. The photographer will compose the photo in the viewfinder or screen, and then take a compass bearing directed at the center of the photo area. The compass should be held level at approximately waist height. This bearing is then entered into the GPS unit.
- 4. *Record lens selection.* Use a wide-angle lens whenever possible to maximize the area captured. If the zoom on the camera was used to restrict the photo area for small sites or for some other reason, that should be recorded as a note in the GPS datalogger, and the degree of zoom or approximate focal length. Generally, using no zoom (camera at the widest possible focus) will be optimal. Digital photos can be easily cropped at a later date.

Photo Composition Guidelines

When composing the photo, keep in mind that you are not just taking a snapshot, but rather, you are trying to select an image that will tell the story of invasive *Spartina* control at the site over time. Look for dramatic site characteristics when possible, such as marsh scarps, tidal channels, etc. If these features are heavily infested in Year 1, effective *Spartina* control should make these features look dramatically different over time and illustrate the habitat values that are being recovered or restored. Another example would be recently infested mudflats that should progress from grassy swards to unvegetated areas over several seasons. When photographing large monocultural meadows, try to include some differentiating feature of the vegetation or marsh structure to add interest to the graphic and add a sense of scale. Photographs should capture the "area of influence" to give context to the treated *Spartina*.

Take the photo from a standing posture (rather than crouching or kneeling) so that we will generally maintain the same height over subsequent years, with a small degree of variation between the heights of the photographers. If standing on a rock or other feature to get a better angle, make sure to record that in the GPS notes. To obtain a well-focused image, take care to stabilize the camera if not using a tripod by standing as still as possible. Verify that the shot is in focus by reviewing it on the LCD screen, and take additional shots from the same point if needed to obtain the desired product. Better to take too many shots than too few; with digital photography extras can always be deleted after downloading.

Some sites are just not "photogenic", so we should be mindful that taking multiple points might not always be necessary if they don't provide good additional graphic descriptions of the consequences of *Spartina* control on the site.

Scale in the Photo. It is customary to include permanent landscape features in the background of the photo for reference and to add scale. However, in the case of the ISP, this is not always possible when photographing a large salt marsh that is remote. Whenever possible, include such permanent landscape features or hardscape to assist with lining up the annual update to that photo point. But in general we will rely on the GPS point, compass bearing, and general guidelines on camera angle to replicate the same view in subsequent visits. During clapper rail breeding season, we cannot be in most of the marshes and therefore cannot easily add a scale element. However during Summer Treatment Monitoring and Inventory Monitoring, we will take the photo points with a person or meter board in the marsh to add scale.

Framing the Photo. Minimize the amount of sky in the photo by placing the horizon close to the top of the frame (unless this is impractical for some reason). This will ensure that the same general angle from the photo point is used year after year, and will limit the glare and underexposure/darkening of the marsh and subsequent loss of detail resulting from a bright sky.

Minimize the amount of unrelated foreground (especially if it is not even salt marsh) by positioning the bottom of the frame as close to the near-edge of the infestation as possible. Try to eliminate road, levee, or upland invasives and other distractions in the foreground. If possible, clear dead vegetation in the foreground such as old radish or *Lepidium* stalks that obscure the view from the levee or upland.

In some instances you may have to choose between too much sky and too much foreground. In general, the bright sky has a better chance of ruining the photo than too much foreground, since it has more to do with light and keeping the details of the marsh visible. As long as the *Spartina* infestation is clearly visible (not a small part of the frame in the background), err on the side of minimizing sky and make a note in the GPS that we should crop the photo to reduce foreground. When photographing a narrow, linear site, take the photo at an angle to the infestation to avoid the issues of balancing foreground and sky.

Determining the Number of Photo Points

We will use two general criteria to determine the number of photo points needed to best represent the story at each site: the complexity of the site and overall size/acreage of the infestation. Small sites with little complexity can be captured in one photo point, whereas large sites and sites with a number of habitat types or other interesting features will require multiple shots.

As a general guideline, we'll test these ranges based on acres of *Spartina*:

- < 1 acre and uniform = 1 photo point</p>
- 1-10 acres and uniform = 1-2 photo points
- 1-10 acres and diverse = 3 + photo points (additional to capture specific photogenic areas of the site that are unique and will illustrate the significance of the *Spartina* control at the site)
- 11-35 acres and uniform = 3 photo points
- 11-35 acres and diverse = 4 photo points (possibly photos from the 4 corners of the site, or a similar division to get good coverage over the entire site)
- 35+ acres and uniform = 4 photo points (photos from the 4 corners or similar)
- 35+ acres and diverse = 5+ photo points.

For *linear infestations*, establish a photo point for approximately every 7-10 acres or evenly spaced every 500-1000 feet; one GPS point can be established for two photos by pivoting approximately 180 degrees to get a shot in both directions from the one point.

For <u>*regular polygons*</u>, establish photo points at the corners and possibly at the midpoint of each side (if unique enough to warrant); for a rectangular polygon that could be up to 8 photo points for a large diverse site.

In subsequent visits to update photo points, color copies of previous photos should be brought to the field to help recreate the shots as closely as possible.

Appendix 13: Photo Point Data Dictionary

12/17/2007

```
N:\Spring Efficacy Monitoring\2007-MidSeasEff-PhotoPts\2007PPdata\Data Dic\PP2007V1.ddf
Spartina 2007V1
V1
PP2007ALL
                       Point Feature, Label 1 = PhotoPtID, Label 2 = CamBearing
                      Mid-season treatment efficacy monitoring
   Date07
                       Date, Auto generate Create And Update, Year-Month-Day Format
                      Not Permitted, Not Permitted
   Surveyor07
                      Menu, Required, Required
     I_Hogle
     J_Good
     T_ McCandlish
     J_McBroom
     A Good
     H Spautz
     A_Nelson
     A_Robinson
     D Kerr
     E_Grijalva
     K Zaremba
     Other
   PhotoPtID
                      Text, Maximum Length = 30, Photo point ID
                      Normal, Normal
                      Text, Maximum Length = 30
   CamBearing
                       Normal, Normal
   PhotoNumbr
                       Text, Maximum Length = 30, Photp number
                      Normal, Normal
  CamNotes
                      Text, Maximum Length = 50
                      Normal, Normal
   SiteType07
                      Menu, Normal, Normal, depicts habitat type
     Site_Typ_ I:Marsh
     Site_Type_II:Strip
     Site_Typ_ III:Slough
     Site_Type_IV:Urban
   AltLfHst07
                      Menu, Normal, Normal, Avg Site-wide Spartina_Life_History
     N/A Default
     Vegetative growth
     Flowering
     Senescent
     Dead
   AltEffic07
                      Menu, Normal, Normal, overall % kill entire site
     N/A Default
     0%
      .1-.9%
      1-4%
      5-9%
     10-19%
      20-29%
     30-39%
      40-49%
      50-59%
     60-69%
      70-79%
     80-89%
      90-99%
      100%
                      Menu, Normal, Normal, depicts patch vigor with site
   AltVigor07
     N/A Default
     High
     Medium
     Low
     Dead
   AltColor07
                      Menu, Normal, Normal, Color of patch
     N/A Default
     Green
     Pale Green
     Yellow Green
     Brown
   AltRegrw07
                      Menu, Normal, Normal
     N/A Default
     Seedlings
     Mature
     None
     Other
                      Menu, Normal, Normal, %cover live stems within 0.25 meter quad
   AltCov07
     n/a Default
```

0%_identified

0-.9% 1-4% 5-9% 10-19% 20-29% 30-39% 40-49% 50-59% 60-69% 70-79% 80-89% 90-99% 100% DAltCov07 Menu, Normal, Normal, %cover live stems within 0.25 meter quad n/a Default 0%_identified 0-.9% 1-4% 5-9% 10-19% 20-29% 30-39% 40-49% 50-59% 60-69% 70-79% 80-89% 90-99% 100% BareCov07 Menu, Normal, Normal, %cover live stems within 0.25 meter quad n/a Default 0%_identified 0-.9% 1-4% 5-9% 10-19% 20-29% 30-39% 40-49% 50-59% 60-69% 70-79% 80-89% 90-99% 100% DenLfHst07 Menu, Normal, Normal, Avg Site-wide Spartina_Life_History N/A Default Vegetative growth Flowering Senescent SitCvFol07 Menu, Normal, Normal n/a Default 0%_identified 0-.9% 1-4% 5-9% 10-19% 20-29% 30-39% 40-49% 50-59% 60-69% 70-79% 80-89% 90-99% 100% SitCvDFol07 Menu, Normal, Normal, Site cover Dead Fol n/a Default 0%_identified 0-.9% 1-4% 5-9% 10-19% 20-29% 30-39% 40-49%

50-59% 60-69% 70-79% 80-89% 90-99% 100%	
SitCOvSal07 n/a Default 0%_identified 09% 1-4% 5-9% 10-19% 20-29% 30-39% 40-49% 50-59% 60-69% 70-79% 80-89% 90-99% 100%	Menu, Normal, Normal, Site cover Dead Salicornia
SitCvDSal07 n/a Default 0%_identified 09% 1-4% 5-9% 10-19% 20-29% 30-39% 40-49% 50-59% 60-69% 70-79% 80-89% 90-99% 100%	Menu, Normal, Normal, Site cover Dead Salicornia
DenEffic07 N/A Default 0% 09% 1-4% 5-9% 10-19% 20-29% 30-39% 40-49% 50-59% 60-69% 70-79% 80-89% 90-99% 100%	Menu, Normal, Normal, overall % kill entire site
DenVigor07 N/A Default High Medium Low	Menu, Normal, Normal, depicts patch vigor with site
DenColor07 N/A Default Green Pale Green Yellow Green Brown	Menu, Normal, Normal, Color of patch
DenRegrw07 N/A Default Seedlings Mature None Other	Menu, Normal, Normal
DenCov07 n/a Default 0%_identified 09% 1-4%	Menu, Normal, Normal, %cover dead stems within 0.25 meter quad

5-9%	
10-19%	
20-29%	
30-39%	
40-49%	
50-59%	
60-69%	
70-79%	
80-89%	
90-99%	
100%	
DDenCov07	Menu, Normal, Normal, %cover dead stems within 0.25 meter quad
n/a Default	Menu, Mermar, Mermar, Beover dead Stems wrenth 0.25 meter quad
0%_identified	
09%	
1-4%	
5-9%	
10-19%	
20-29%	
30-39%	
40-49%	
50-59%	
60-69%	
70-79%	
80-89%	
90-99%	
100%	
SiteCode	Menu, Required, Required
2PTC	
ALSL	
AFCC	
BAIR	
BLPA	
CMCC	
CCSB	
COCR	
EMCR	
IDMA	
MARO	
NBAY	
OLMA	
OUCO	
PABL	
PCPK	
PTPI	
PO3A	
RABE	
SBMA	
SHAM	
SLHS	
SOSF	
WSFB	
WTCO	
SubSitCode	Menu, Normal, Normal
AHMA	
ALBA	
ALVI	
ANLG	
APCH	
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Text, Maximum Length = 75 Normal, Normal

AbnormalGr	Text, Maximum Length = 30, Abnormal growth Normal, Normal
SiteComm	Text, Maximum Length = 50 Normal, Normal
Landmanger	Text, Maximum Length = 75, Landowner or the manager of property Normal, Normal
TreatDat07	Date, Day-Month-Year Format, Treatment Date Normal, Normal
Treatmnt07 Herbicide Glyphosate Imazapyr Dig Cover Mow Mow & Cover Mow & Cover Mow & Herbicide TreatCom07	<pre>Menu, Normal, Normal, depicts pre vs post treatment monitoring Text, Maximum Length = 30, Contractor/Applicator for the treatment</pre>
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Appendix 14:

ISP Protocols for Surveys of California Clapper Rails

San Francisco Estuary Invasive Spartina Project

Summary of California Clapper Rail Survey Protocols

March 29, 2006

Туре	Common Protocol Name	Description
A	Standard USFWS Transect Survey	As described in Albertson & Downard, 2004 and Spautz 2005. Used for <u>linear</u> sites and for sites with <u>low</u> to <u>medium</u> rail density. One or more observers move from station to station, remaining at each station for 10-minutes. 3 survey rounds, with recording played at end of 3 rd round if no prior detections.
В	Standing or Stationary Survey (not used in 2006)	As described in Albertson & Downard, 2004 and Spautz 2005. Used for non-linear sites with <u>high</u> rail density. Requires one person at each station for $1\frac{1}{2}$ hour. Typically 3 survey rounds, with recording played at end of 3^{rd} round if no prior detections.
С	ISP-Modified Transect Survey	<i>Originally described in Zaremba & Albertson, 2004</i> ; modified in Spautz & Albertson 2006. Used to determine presence or absence of CLRA at sites with low potential for CLRA presence, where <i>Spartina</i> control activities are planned. Same as Type A, except recording is played from first survey round, and surveys are discontinued upon detection.
D	DENWR-Modified Transect Survey (Rough Density)	Used by DENWR biologist in narrow strip marshes with medium to high rail density - Similar to Type C, except densities are extrapolated by Refuge biologist.
E	Winter High Tide Survey	Described by EBRPD pers. comm. CLRA are flushed out of marsh habitat by airboat and counted during winter high tide.
F	Preliminary Habitat Suitability Assessment	Quick assessment by CLRA biologist to determine if suitable CLRA habitat is present; if habitat is suitable, a call count survey protocol C is typically conducted.

San Francisco Estuary Invasive *Spartina* Project California Clapper Rail Survey Protocol A:

"Standard Transect Survey"

March 2006

The ISP's Protocol A <u>is</u> the "walking transect survey" method described in "Draft clapper rail protocol for the San Francisco Estuary large-scale population surveys" (Albertson & Downard 2004) and "Revised draft clapper rail protocol for the San Francisco Estuary large-scale population surveys" (Spautz 2005). The protocol is excerpted verbatim from Spautz 2005, except as otherwise noted. Note: The ISP refers to the "standing survey" method described in Albertson & Downard 2004 and Spautz 2005 as "Protocol B".

General Survey Requirements:

- Permits. Obtain required survey permits: USFWS Endangered Species Permit, ESA Section 10(a)(1)(A); California DFG permit (i.e. Memorandum of Understanding); site-specific permissions (e.g. Special Use Permit from a NWR).
- Training. Observers must be trained to identify clapper rail calls and distinguish CLRA calls from other marsh bird species (see Rail Training document, April 2004). Observers must also be trained to minimize disturbance while conducting surveys (see Walking in the Marsh document, April 2004).
- 3) Tides and moon phase. Conduct surveys when tidal sloughs are less than bank full, <4.5-ft NGVD at the Golden Gate tide station. Tide height at bank full will vary by site. Avoid high (flood) tides. Full moon periods should be avoided during active surveys when tape playback is utilized, as birds may be attracted out of cover or a response may be elicited, and increase the likelihood of predation. There is also evidence of reduced calling rates during full moon periods.
- 4) *Survey Timing*. Morning surveys should be initiated no sooner than one hour before sunrise and extended no more that 1.5 hours after sunrise; evening surveys should begin one hour prior to sunset and extend no more than one hour following sunset. Surveys at a particular location should be spaced at least 1 week apart and should be conducted at both sunrise and sunset.
- 5) *Weather*. Record wind velocities and weather; conduct surveys at winds <10 mph; do not conduct surveys during heavy rainfall.
- 6) Seasonality. Conduct surveys between January 15 and mid-April.
- 7) *Survey Stations*. Stations should be spaced approximately 200-m apart. Stations should be placed on boardwalks or levee tops when possible to minimize disturbance. When surveys are conducted within a marsh, stations should be placed away from slough/channel edges to minimize disturbance to rail species.
- 8) *Data collection*. All rail vocalizations should be recorded, noting the call type, location, and time. Locations where rails are detected should be plotted on a map during the survey with numbered reference codes that correspond to detections on the datasheet. The call types should be coded as follows:

Call Code	Call Description	Number of Birds Indicated*
С	Clapper/clatter by one individual	1-2 birds
D	"Duet"- two individuals clattering simultane- ously	2 or more birds, depending on situa- tion
K	"kek"	1-2 birds
AK	agitated "kek"	1-2 birds
В	"kek-burr"	1-2 birds
V	Visual sighting	1-2 birds per sighting
SK	"squawk"	1-2 birds
SC	"screech"	1-2 birds
СН	"chur"	1-2 birds
Р	"purr"	1-2 birds

* See data interpretation section below for more details about determining number of birds per detection type.

If the bird was definitely or possibly previously detected, e.g. as part of a pair, make this clear on the datasheet. Record when birds were detected simultaneously or nearly so, which will verify they were separate individuals. Calls of other rail species should also be recorded as above, with species clearly marked.

- 9) *Disturbance*. Record all Information on disturbance (e.g., predator sightings or boats) detected during surveys.
- 10) Review the WRMP CLRA protocol (Evens 2002) for other general information (<u>http://www.wrmp.org/docs/protocols/Wetland%20Birds.pdf</u>, p.21 Rails). Defer to the requirements listed above if they are more restrictive than the WRMP protocol.

Survey Specifics

The Protocol A transect survey is suitable for linear sites and for sites with low to medium rail density. Surveys as sites with high clapper rail density should use "Protocol B" – the standing or stationary survey protocol described by Albertson & Downard 2004 and Spautz 2005.

The transect survey may be performed by one or two observers. Listening stations are established at approximately 200 meter intervals along a transect, preferably along the edge of the marsh. The first two of three surveys are passive (listening) for 10-minutes at each station. On the third survey, if a clapper rail was not previously detected within 200 meters of a listening station during the two previous passive surveys or incidentally within the season, recorded calls are played, according to the *"Recorded Call Playback Procedure"* described below. If a clapper rail has been previously detected within 200 meters of a listening station, the third survey should also be passive. There should be a minimum of one week between surveys.

Recorded Call Playback Procedure

A standardized recording of clapper rail calls should be obtained from USFWS. The recording should include a combination of clapper/clatter and duet calls, and there should be at least four complete calls with at least 5-seconds of silence between calls. The recording should be of good quality, and should be played at a volume of 80-90 dB at 1-meter distance from the speaker. A digital sound level meter should be used to calibrate the playback device.

The survey should begin with an initial 5-minute passive listening period, followed by 1-minute of clapper rail calls, and completed with a 4-minute passive listening period (10-minutes/survey). Tape playbacks should be broadcast in all directions over the marsh at a station. Assume rails can hear tapes at distances of \leq 200-m.

Note: Only play recorded clapper rail calls at stations when you are certain rails have not yet been detected. As soon as a clapper rail is detected, stop the recording.

Data Interpretation and Data Analysis

Use the following key to determine how many birds to record for each detection type. Use your "field" judgment to avoid redundancy (overlap) and interpret uncertainty as a range. Keep in mind the part of the breeding season in which your survey occurs.

Detection type	Code	Number of birds	Description	Notes and Exceptions
Clatter	С	1 - 2	Primary territorial call. Rapid series of kek notes, often trailing off at the end.	 Usually clattering individuals are paired. Often it's difficult to determine whether one or two birds are calling, if completely synchronized; thus, the range of 1-2 birds. Example scenario: at the end of a survey session you have 4 distinct duets, 3 single clatters away from duets and away from one another. The estimate for breeding birds would be 11-14 (# duets x 2 = 8 + 3-6 birds represented by clatters).
Duet clatter	D	2	Two bird clattering simultaneously.	 Usually given by a pair, or less often, neighboring territorial males (J. Evens peers. obis. 2005). When chorusing birds are masking one another and you are uncertain whether it was one duet or two, record as 1-2 duets (1-2 pairs) or 2-4 clatters. Again, interpret uncertainty as a range.
Kek	К	1-2	Single sharp "kek" call, given singly or in se- ries, with significant space between calls (as compared to clatter, which is very rapid).	 Given by males, most often when unmated or prior to setting up pair bond, thus is most typically heard early in the season. However, can be given by a mated male throughout the breeding season, thus the range of 1-2 birds. Sometimes paired/breeding birds make random keks or kek-burrs intermingled with clatters, especially at the beginning of the breeding season. If you hear a single kek followed by a duet in the same location, the kekking individual is likely part of the duet pair and would not be counted separately.
Agitated Kek	AK	1-2	As above but higher pitched, rougher, and with what can be inter- preted as an element of alarm. Mid-way be- tween kek and squawk or screech.	• As above, the call may indicate either an unmated or mated male, thus the range of 1-2 birds.

Detection type	Code	Number of birds	Description	Notes and Exceptions	
Kek-burr	В	1-2	One or several rapid "kek" calls followed by a more attenuated, "burrrr". Often re- peated constantly over many minutes, and can be heard about 1 km away, depending on conditions.	 Given by female clapper rails, primarily during pair bond formation or when fertile and soliciting a copulation with her mate, thus, it is most typically heard early in the season. The call is not likely to be given when she is incubating. Later in the season, it may be given when a nest has failed and the female is beginning another nesting attempt. The call may indicate either an unmated or mated female, thus the range of 1-2 birds. A single kek-burr followed by a duet: the individual is likely part of the duet pair and would not be counted separately. 	
Visual	V	1-2		• Clapper rails are most often seen when foraging along tidal channel banks, often near the shelter of overhang- ing vegetation. They are often seen crossing channels, and regularly swim across open water within a channel.	
				 A sighting of one bird may indicate the presence of a pair; thus record as 1 – 2 birds. 	
The following for complete		riptions we	re not included in Albertso	n & Downard 2004 or Spautz 2005, but are provided here	
Squawk	SK	1-2	More highly agitated than an agitated kek,	• Typically given only once as an alarm call. Bird may later make other vocalizations.	
Screech	SC	1-2	More rare than a squawk. Like a squawk but even more high- pitched.	• Typically given only once as an alarm call.	
Churr	СН	1-2	Similar to the last syl- lable in a kek-bur call	• Typically given by a female.	
Purr	Р	1-2	Very soft, like churr or burr.	• Typically given by a female at the nest.	

San Francisco Estuary Invasive *Spartina* Project California Clapper Rail Survey Protocol B

"Stationary Survey"

November 2006

The ISP's Protocol B is the "stationary survey" method described in "Draft clapper rail protocol for the San Francisco Estuary large-scale population surveys" (Albertson & Downard 2004) and "Revised draft clapper rail protocol for the San Francisco Estuary large-scale population surveys" (Spautz 2005). The protocol as written is from Spautz 2005. Note: The ISP refers to the "transect survey" method described in Albertson & Downard 2004 and Spautz 2005 as "Protocol A".

General Survey Requirements:

- Permits. Obtain required survey permits: USFWS Endangered Species Permit, ESA Section 10(a)(1)(A); California DFG permit (i.e. Memorandum of Understanding); site-specific permissions (e.g. Special Use Permit from a NWR).
- Training. Observers must be trained to identify clapper rail calls and distinguish CLRA calls from other marsh bird species (see Rail Training document, April 2004). Observers must also be trained to minimize disturbance while conducting surveys (see Walking in the Marsh document, April 2004).
- 3) Tides and moon phase. Conduct surveys when tidal sloughs are less than bank full, <4.5-ft NGVD at the Golden Gate tide station. Tide height at bank full will vary by site. Avoid high (flood) tides. Full moon periods should be avoided during active surveys when tape playback is utilized, as birds may be attracted out of cover or a response may be elicited, and increase the likelihood of predation. There is also evidence of reduced calling rates during full moon periods.</p>
- 4) *Survey Timing*. Morning surveys should be initiated no sooner than one hour before sunrise and extended no more that 1.5 hours after sunrise; evening surveys should begin one hour prior to sunset and extend no more than one hour following sunset. Surveys at a particular location should be spaced at least 1 week apart and should be conducted at both sunrise and sunset.
- 5) *Weather*. Record wind velocities and weather; conduct surveys at winds <10 mph; do not conduct surveys during heavy rainfall.
- 6) Seasonality. Conduct surveys between January 15 and mid-April.
- 7) *Survey Stations*. Stations should be spaced approximately 200-m apart. Stations should be placed on boardwalks or levee tops when possible to minimize disturbance. When surveys are conducted within a marsh, stations should be placed away from slough/channel edges to minimize disturbance to rail species.
- 8) *Data collection*. All rail vocalizations should be recorded, noting the call type, location, and time. Locations where rails are detected should be plotted on a map during the survey with numbered reference codes that correspond to detections on the datasheet. The call types should be coded as follows:

Call Code	Call Description	Number of Birds Indicated*
С	Clapper/clatter by one individual	1-2 birds
D	"Duet"- two individuals clattering simultane-	2 or more birds, depending on situa-
	ously	tion
K	"kek"	1-2 birds
AK	agitated "kek"	1-2 birds
В	"kek-burr"	1-2 birds
V	Visual sighting	1-2 birds per sighting
SK	"squawk"	1-2 birds
SC	"screech"	1-2 birds
СН	"chur"	1-2 birds
Р	"purr"	1-2 birds

* See data interpretation section below for more details about determining number of birds per detection type.

If the bird was definitely or possibly previously detected, e.g. as part of a pair, make this clear on the datasheet. Record when birds were detected simultaneously or nearly so, which will verify they were separate individuals. Calls of other rail species should also be recorded as above, with species clearly marked.

- 9) *Disturbance*. Record all Information on disturbance (e.g., predator sightings or boats) detected during surveys.
- 10) Review the WRMP CLRA protocol (Evens 2002) for other general information (<u>http://www.wrmp.org/docs/protocols/Wetland%20Birds.pdf</u>, p.21 Rails). Defer to the requirements listed above if they are more restrictive than the WRMP protocol.

Survey Specifics

The Protocol B stationary survey is suitable for larger sites with high clapper rail density, such as Faber-Laumeister, Palo Alto Baylands, Greco Island, Dumbarton Marsh, Colma Creek/San Bruno Marsh, and Arrowhead Marsh. Surveys at linear sites and sites with low to medium rail density should use "Protocol A" – the walking or transect survey protocol described by Albertson & Downard 2004 and Spautz 2005.

The Protocol B stationary survey requires a sufficient number of observers to have one person at each listening station. Listening stations are established along a grid or transect, with stations set apart by approximately 200 meters. Observers are present at each station for an entire 1.5-hour survey period. When calls are recorded, the observer must take care to record the exact time and direction, and best estimate of the distance of the call, so that the data can be reconciled with other observers' data.

Because this procedure is only implemented at sites with a high density of clapper rails, under most circumstances, the survey need only to be repeated twice in a season, with a minimum of one week between surveys. If survey conditions are unsatisfactory for part or all of a survey period, a third survey may be conducted.

The Protocol B stationary survey is a passive listening survey, and does not include playing of recorded calls.

Data Interpretation and Data Analysis

Use the following key to determine how many birds to record for each detection type. Use your "field" judgment to avoid redundancy (overlap) and interpret uncertainty as a range. Keep in mind the part of the breeding season in which your survey occurs.

Detection type	Code	Number of birds	Description	Notes and Exceptions
Clatter	С	1 - 2	Primary territorial call. Rapid series of kek notes, often trailing off at the end.	 Usually clattering individuals are paired. Often it's difficult to determine whether one or two birds are calling, if completely synchronized; thus, the range of 1-2 birds. Example scenario: at the end of a survey session you have 4 distinct duets, 3 single clatters away from duets and away from one another. The estimate for breeding birds would be 11-14 (# duets x 2 = 8 + 3-6 birds represented by clatters).
Duet clatter	D	2	Two bird clattering simultaneously.	 Usually given by a pair, or less often, neighboring territorial males (J. Evens peers. obis. 2005). When chorusing birds are masking one another and you are uncertain whether it was one duet or two, record as 1-2 duets (1-2 pairs) or 2-4 clatters. Again, interpret uncertainty as a range.
Kek	К	1-2	Single sharp "kek" call, given singly or in se- ries, with significant space between calls (as compared to clatter, which is very rapid).	 Given by males, most often when unmated or prior to setting up pair bond, thus is most typically heard early in the season. However, can be given by a mated male throughout the breeding season, thus the range of 1-2 birds. Sometimes paired/breeding birds make random keks or kek-burrs intermingled with clatters, especially at the beginning of the breeding season. If you hear a single kek followed by a duet in the same location, the kekking individual is likely part of the duet pair and would not be counted separately.
Agitated Kek	AK	1-2	As above but higher pitched, rougher, and with what can be inter- preted as an element of alarm. Mid-way be- tween kek and squawk or screech.	 As above, the call may indicate either an unmated or mated male, thus the range of 1-2 birds.
Kek-burr	В	1-2	One or several rapid "kek" calls followed by a more attenuated, "burrrr". Often re- peated constantly over many minutes, and can be heard about 1 km away, depending on conditions.	 Given by female clapper rails, primarily during pair bond formation or when fertile and soliciting a copulation with her mate, thus, it is most typically heard early in the season. The call is not likely to be given when she is incubating. Later in the season, it may be given when a nest has failed and the female is beginning another nesting attempt. The call may indicate either an unmated or mated female, thus the range of 1-2 birds. A single kek-burr followed by a duet: the individual is likely part of the duet pair and would not be counted separately.

Detection type	Code	Number of birds	Description	Notes and Exceptions		
Visual	V	1-2	• Clapper rails are most often seen when foraging along tidal channel banks, often near the shelter of overhang- ing vegetation. They are often seen crossing channels, and regularly swim across open water within a channel.			
				• A sighting of one bird may indicate the presence of a pair; thus record as 1 – 2 birds.		
The following for complete	-	riptions we	re not included in Albertso	n & Downard 2004 or Spautz 2005, but are provided here		
Squawk	SK	1-2	More highly agitated than an agitated kek,	• Typically given only once as an alarm call. Bird may later make other vocalizations.		
Screech	SC	1-2	More rare than a squawk. Like a squawk but even more high- pitched.	• Typically given only once as an alarm call.		
Churr	СН	1-2	Similar to the last syl- lable in a kek-bur call	• Typically given by a female.		
Purr	Р	1-2	Very soft, like churr or burr.	• Typically given by a female at the nest.		

San Francisco Estuary Invasive *Spartina* Project California Clapper Rail Survey Protocol C:

"ISP Modified Transect Survey"

March 2006

2006 Note: This California clapper rail survey protocol was originally developed by Joy Albertson (U. S. Fish & Wildlife Service, Don Edwards San Francisco Bay National Wildlife Refuge) and Katy Zaremba (San Francisco Estuary Invasive Spartina Project) in 2004. The protocol was approved in 2004 by Dan Buford of the Sacramento Office of the Endangered Species Branch of the US Fish and Wildlife Service for use exclusively by the ISP. Hildie Spautz (San Francisco Estuary Invasive Spartina Project) and Joy Albertson updated the protocol in 2006 to add additional clarification and specifications.

Purpose of Protocol C

Protocol C (ISP modified transect survey) was developed to allow the ISP to more efficiently confirm presence or absence of California clapper rails (clapper rails) at certain non-native Spartina-invaded sites, so that Spartina control could be undertaken at sites with no rails during rail nesting season. Protocol C surveys are implemented only at sites where the probability of clapper rail presence is relatively low, i.e., at sites where clapper rails have not previously been detected, but where marginally suitable habitat or other conditions suggest that rails may be present. Protocol C differs from Protocol A (USFWS standard transect survey) in that it allows the broadcasting of pre-recorded clapper rail vocalizations beginning on the initial round of surveys in order to elicit responses from birds in the marsh. If a clapper rail responds, the broadcast is immediately discontinued and not repeated on subsequent survey rounds at that station, and *Spartina* control at that location is postponed until times authorized by the USFWS Section 7 Biological Opinion. In some cases, if clapper rail presence is determined using Protocol C, the ISP may choose to complete the survey using Protocol A to determine the number of birds present at the site.

Determining Protocol Suitability

The suitability of using Protocol C is determined based on whether clapper rails have been previously detected at the site, and whether conditions at the site suggest that clapper rails may be present. The ISP regularly reviews clapper rail records from all known sources, including PRBO Conservation Science, Avocet Research Associates, U.S. Fish and Wildlife Service, San Francisco Bay Bird Observatory, California Department of Fish and Game, East Bay Regional Parks District, and other reliable sources, to identify locations where clapper rails have been detected in the past. Also, the ISP evaluates all planned Spartina treatment sites for potential habitat, and conducts habitat assessment surveys (Protocol F) at any locations that are thought to be potentially, albeit marginally, suitable clapper rail habitat. If the ISP plans to do Spartina control at a location where (1) the collective records do not indicate clapper rails have been previously detected, and (2) the habitat at the site is determined to be at least marginally sufficient for clapper rails, then a Protocol C survey would be performed. If the ISP requires clapper rail data at locations where clapper rail presence was previously confirmed, it would use Protocol A (Standard USFWS transect), rather than Protocol C. Generally speaking, Protocol C surveys are conducted at sites that have a low probability of clapper rail presence.

Protocol Description

All aspects of Protocol C (ISP Modified Transect Survey) are the same as Protocol A (Standard Walking Transect Survey; described in Albertson & Downard, 2004), with the following additions:

Survey Stations: As in Protocol A, transect survey listening stations will be placed 200 meters apart along linear transects. For Protocol C, if the survey occurs on a creek or channel, stations will be placed from the creek or channel mouth, extending upstream for the full length of vegetated marsh sufficient for clapper rails, or to the point where non-native *Spartina* stops, depending on the site.

Broadcasting vocalizations: Recorded clapper rail vocalizations will be broadcast at each station beginning with the first round of surveys. If a clapper rail is detected within 200 m of the survey station, the recording will be immediately turned off, and will not be played again at that station during subsequent rounds. If no clapper rail is detected within 200 m of the survey station, the recording will be played at each of three rounds at that station. Recorded vocalizations will not be played if raptors or other predators are observed nearby, as a response from a clapper rail will increase the rail's likelihood of predation.

Constraints: Surveys will not be conducted when wind, rain, fog, or other conditions unacceptably impair the biologist's ability to detect clapper rails. At sites adjacent to major highways, surveys may need to be conducted on Sunday mornings when traffic noise is at its lowest possible level. Recorded vocalizations will not be played if raptors or other predators are observed nearby, as a response from a clapper rail will increase the rail's likelihood of predation.

Survey determinations: For each survey point, "absence" will be reported only after three rounds of Protocol C surveys are completed with no detection. If less than three rounds are completed without detection, the surveyor may make the determination "absence suspected;" however, this will not be sufficient for the ISP to proceed with control work unless a determination is specifically made by the UFSWS biologist.

San Francisco Estuary Invasive *Spartina* Project California Clapper Rail Survey Protocol F:

"California Clapper Rail Habitat Assessment"

December 10, 2008

Protocol description

The San Francisco Estuary Invasive *Spartina* Project (ISP) is required (under the USFWS Biological Opinion dated September 2005) to conduct surveys for California clapper rails (*Rallus longirostris obsoletus*) to determine clapper rail presence or absence prior to treatment of non-native *Spartina*. Sites that are clearly insufficient to support clapper rails, e.g., stretches of concrete rip-rap with a scattering of small non-native *Spartina* clones, do not require clapper rail surveys. However, sites requiring *Spartina* control exhibit a continuum of habitat characteristics, many of which are documented clapper rail habitat requirements (e.g., extensive channels for foraging and vegetated upper marsh for refuge during high tides). This makes it difficult in some cases to determine whether the habitat at the site is of sufficiently high quality to require a call count survey. In 2005, the ISP developed a standardized method to document the decision whether or not a clapper rail survey was required (Protocol F).

ISP staff consulted with Joy Albertson and Jules Evens to develop a list of required habitat elements for clapper rails based on field knowledge and published sources. This information was used to develop a field checklist to assess the habitat using multiple criteria and to document the decision as to whether the marsh will require a formal clapper rail call count survey. The habitat assessment is typically completed at sites where clapper rails have previously not been documented.

The process of determining whether the site is of sufficient quality to require a call count survey is based on a cumulative score of positive characteristics. Patches with no necessary habitat elements are considered very poor habitat and clapper rail use is "highly unlikely", and require no further clapper rail survey; such sites are determined to be available for early non-native *Spartina* treatment. If the site is poor but is geographically near enough to good habitat or known rail habitat to potentially provide habitat for at least some clapper rail activities (such as foraging or shelter), it will require a call count survey. Potentially good habitat with at least two positive characteristics will also be likely to require a call count survey, but this will be site-dependent. Possibly good habitat or likely good habitat (with at least four or six characteristics, respectively) will require a call count survey.

If call count surveys are required, the biologist will generally recommend using clapper rail call count survey protocol "C", which is conducted at apparently low quality sites where clapper rails are not likely and have not been previously documented. However, it is possible that the site is of sufficiently high quality that clapper rails are at least moderately likely and a standard call count protocol "A" survey will be recommended.

Habitat characteristics documented to be associated with California clapper rails and included on the habitat assessment datasheet include the following:

- 1. Young or mature restoration site (at least 50% vegetated)
- 2. Upper marsh vegetation present
- 3. Vegetated levee slopes
- 4. Marsh patch size > 10 ha
- 5. Closer than 500 m to nearest marsh with documented clapper rail presence
- 6. Fully tidal
- 7. Saline
- 8. High proportion of *Salicornia virginica*, tall hybrid *Spartina* clones, and/or *Grindelia stricta* cover
- 9. At least a few second and third order channels, or highly channelized

Habitat characteristics associated with California clapper rail absence and included on the habitat assessment datasheet as negative characteristics include the following:

- 1. New restoration site < 50% vegetated
- 2. Upper marsh vegetation absent
- 3. Levee slopes unvegetated
- 4. Small marsh patch size (< 1 ha)
- 5. Distance to nearest known marsh with clapper rails > 1000 m
- 6. Sparse vegetation in rip-rap
- 7. Highly muted tidal regime or non-tidal
- 8. Freshwater

CLRA survey protocol "F" - Clapper rail habitat assessment datasheet 2008

Study site _____

Date _____

Obs _____

All these questions can be answered by checking the appropriate box, with the exception of the percent vegetation, which requires a number

Variable	Options	+/- for CLRA	Data
Restoration status	natural		
	mature restoration site	+	
	restoring < 10 years but > 50% vegetated	+	
	new restoration site, less than 50% vegetated	-	
	unknown		
Elevation	upper marsh present (S. virginica, Grindelia)	+	
	upper marsh absent (S. europea & Spartina only)	-	
Levees	levee slopes vegetated	+	
	levee slopes unvegetated	-	
	no levees, natural upland gradient, vegetated > 50%	+	
	no levees, natural upland gradient < 50% vegetated	-	
Marsh size	largest patch < 1 ha (100 x 100 m)	-	
	largest patch 1 to 10 ha		
	largest patch > 10 ha	+	
Marsh width (linear	<20 m		
marsh)	20 to 50 m		
	50 to 200 m		
	>200 m		
Distance to nearest	distance < 500m	+	
CLRA + marsh	distance 500 - 1000 m		
	distance > 1000m	-	
	unknown		
Marsh configuration	surrounded by levees on 4 sides		
	no levee on bayward edge		
	linear marsh with slough		
	sparse vegetation in riprap	-	
Tidal regime	fully tidal	+	
	slightly muted tidal		
	highly muted/managed	-	

	unknown		
Salinity	saline	+	
	moderately brackish	+	
	brackish to fresh (S. acutus/Typha present)	?	
Spartina hybrid	complete (marsh 100% Spartina)		
invasion	moderate		
	sparse		
Vegetation cover	percent Salicornia virginica	+	
(as percent of vegetation)	percent tall Spartina hybrids	+	
	percent short form Spartina hybrids		
	percent <i>S. foliosa</i>		
	percent Grindelia	+	
	percent other		
Channelization	highly channelized	+	
	a few second and third order channels	+	
	no channels > first order	?	
	only one channel with narrow marsh strip either side		
	no channels, marsh at bay edge		
Open water	ponds > 75% of marsh		
	ponds 50 - 75% of marsh		
	ponds 25 - 50%% of marsh		
	ponds < 25% of marsh		
	ponds absent		
Overall likelihood of	highly unlikely		
CLRA use	poor but close enough to good habitat that may be in home range & used for foraging		
	potentially marginal habitat; at least 2 positive characteristics		
	possibly good habitat; at least 4 positive characteristics		
	likely to be good habitat; at least 6-8 positive characteristics		
	other notes		
Recommendation	"A" regular CLRA survey 3 rounds		
for CLRA surveys	"C" call response survey 3 rounds		
	no further surveys necessary		