

2004 & 2005

SAN FRANCISCO ESTUARY INVASIVE SPARTINA PROJECT MONITORING REPORT FOR 2004 & 2005





San Francisco Estuary

Invasive Spartina Project

Monitoring Report for 2004 & 2005

Prepared by the

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1.0 INTRODUCTION

The San Francisco Estuary (Estuary) covers more than 750 square miles, including nearly 70,000 acres of tidal marsh and mudflats and 50,000-100,000 acres of salt production ponds and agricultural areas that could potentially be restored to tidal marsh by removal of levees. The Estuary's tidal marshes are populated by a variety of native plants, such as *Spartina foliosa* (Pacific cordgrass), *Grindelia stricta* (gumplant), *Salicornia virginica* (pickleweed), and *Distichlis spicata* (saltgrass), which supply habitat for a rich and diverse array of native and migratory marsh species (Goals Project 1999). In the last several decades, four non-native cordgrasses, including *Spartina alterniflora* (Atlantic cordgrass), *S. densiflora* (Chilean cordgrass), *S. anglica* (English cordgrass), and *S. patens* (saltmeadow cordgrass), were introduced to the Estuary. Each of these species is known to be an aggressive invader outside of its native range, and each has demonstrated varying degrees of invasiveness since establishing in the Estuary. Of particular concern, S. *alterniflora* hybridized with native *S. foliosa* (and their offspring backcrossed with the parent species and with one another), producing an extremely robust and fertile "hybrid swarm," which threatens the ecological integrity of the Estuary's existing and potential future restored tidal wetlands and mudflats¹ (Daehler and Strong, 1996; Goals Project 1999; Ayres *et al.* 2003; California Coastal Conservancy 2003; Ayres *et al.* 2004).

The State Coastal Conservancy responded to the threat of this invasion in 2000, by establishing the San Francisco Estuary Invasive *Spartina* Project (ISP). The purpose of the ISP is to implement a coordinated, regionwide program to control and eventually eradicate non-native *Spartina* species from the Estuary and outer California coast marshes. As part of its regional program, the ISP conducts annual baywide and outer coast monitoring to track and map the extent and rate of spread of nonnative *Spartina*, and to monitor the efficacy of the ISP's *Spartina* Control Program's treatment efforts. This report presents the results of regionwide monitoring conducted by the ISP in 2004 and 2005.

1.1 INVENTORY MONITORING

The ISP began regionwide inventory monitoring in 2000 to track and map the extent and rate of spread of non-native *Spartina*. The results of the 2000-2001 monitoring were published by Ayres *et al.* (2004). At the

time of the 2000-2001 survey, there were 483 net acres (total acres of solid cover) of non-native *Spartina* in the Estuary, with 470 net acres being *S. alterniflora* hybrids. It was estimated that the marsh area invaded by exotic *Spartina*, termed "**gross area**,"* was 5,300 acres. The distribution of nonnative *Spartina* was shown in maps (**Appendix 1**).

Monitoring was again conducted in 2003, and the results were reported by Zaremba and McGowan (2004). Monitoring in 2003 used a sampling of sites, rather than a full inventory, and an effort was made to extrapolate the results to subregional and baywide estimates of rate of spread and area covered. Based on the 2003 results, the average increase in **net area** between 2000 and 2003 for all species of non-native *Spartina* was 244%, with *S. al*-

* Reporting Spartina Area

Two methods are used to measure and report area of nonnative *Spartina*, "**net cover**" and "**gross area**." "Net cover" refers to the actual amount of the *Spartina*, and is calculated using GIS to represent the coverage as if all non-native *Spartina* plants were contiguous (i.e., compacted onto one discrete area). Net cover is not very useful for planning and management purposes, as it does not give an accurate picture of the marsh area that will need to be treated or monitored. For this purpose, "gross area" is used, which is defined as the area invaded to some extent by non-native *Spartina*. Gross area is calculated using GIS and a point/line-buffering strategy. Cover class categories (low, moderate, high) are used to define *Spartina* impact within gross areas for planning and management purposes.

terniflora/hybrids expanding at the greatest rate, estimated at 177% to 390%, depending on the Bay region. Based on this rate of spread, the baywide non-native *Spartina* net acreage in 2003 was estimated at 900-1,200

¹ Unless otherwise noted, this document will refer to *S. alterniflora* and the hybrids formed between *S. alterniflora* and *S. foliosa* (and all subsequent generations of hybrids resulting from backcrossing, etc.) collectively as "*S. alterniflora*/hybrids".

acres. No real effort was made in 2003 to estimate gross acreages based on the limited data. In 2004 and 2005, the ISP resumed the regionwide inventory, using a combination of field monitoring and aerial photograph interpretation techniques.

1.2 SPARTINA TREATMENT

In 2002 and 2003, ISP partners conducted small-scale *Spartina* control efforts at a half dozen treatment sites using manual treatment methods (digging, trampling and covering, and mowing). In 2004, with the completion of programattic and site-specific environmental reviews under the California Environmental Quality Act (CEQA), National Environmental Protection Act (NEPA), Section 7 of the Endangered Species act, and state and federal water quality laws, treatment was expanded to encompass portions of 19 treatment sites (39 subsites)², and glyphosate herbicide was added to the treatment techniques. Also in 2004, the ISP initiated pilot testing of imazapyr herbicide, which was to be registered for use in California the following year. In 2005, imazapyr herbicide was approved, and the ISP treated portions of 22 treatment sites, including treatment within 77 of 141 known subsites. **Figure 1** shows the location of the 2004 and 2005 treatment site complexes. **Appendix 2** provides a list of treatment sites and a summary of treatment methods used during 2002-2005. Treatment methods are generally described in the ISP's Programmatic EIS/R (Conservancy 2003). Specific treatment approaches are described in site-specific control plan prepared for each site



Figure 1. Location of 2004 and 2005 Treatment Sites Within the San Francisco Estuary and Adjacent Outer Coast Marshes. Each treatment site may be comprised of two or more "subsites", which are identified by letters (a through z). Appendix 2 contains a complete list of treatment sites and subsites.

² The ISP's *Spartina* Control Program uses a combination of marsh system and ownership/management boundaries to delineate treatment "sites", which are typically complexes of multiple subsites. For convenience, this report sometimes uses the term "site" to refer to subsites as defined by the ISP Control Program.

(ISP 2004, 2005). *Spartina* treatment operations are reported annually by the ISP Control Program (Grijalva 2004, Grijalva and Kerr 2006)

1.3 TREATMENT EFFICACY MONITORING

Treatment efficacy monitoring was initiated at the sites treated in 2003 (Zaremba and McGowan 2004). The 2003 efficacy monitoring determined that digging or trampling and covering had variable efficacy up to 95%, depending on how carefully the treatment was conducted, and that mowing resulted in no noticeable treatment effect. Also in 2003, pre-treatment monitoring was conducted at sites slated for treatment in 2004, to provide baseline information for the evaluation of the 2004. In 2004 and 2005, efficacy monitoring was conducted at 22 and 44 subsites, respectively. **Appendix 2** shows the sites at which treatment efficacy monitoring was conducted during 2002-2005.

The remainder of this report presents methods, results, and analyses of inventory and efficacy monitoring conducted in 2004 and 2005.

2.0 METHODS

2.1 INVENTORY MONITORING METHODS

In 2004 and 2005, the ISP conducted extensive inventories of non-native Spartina within the San Francisco Estuary and local outer coast marshes. Unlike the 2003 inventory, which was limited to only a subset of sites, the 2004 and 2005 efforts attempted to map all occurrences of non-native Spartina in sufficient detail to inform the ISP's treatment plans. The monitoring methods used in 2004 and 2005 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" (Ayres et al. 2004). In 2004, new Trimble GeoXT GPS units were purchased, improving GPS location data from ± 3 meter accuracy to ± 1 meter accuracy, and additional cover classes were added to provide more detailed estimations of cover. Complete drop-down menus of site names, site codes, native and non-native species lists, sediment type and marsh type were added to the data dictionary. Where needed, more color infrared aerial photos were taken. Improved methods of aerial photo interpretation were incorporated into the protocols, including digitizing Spartina specifically at 1:500 scale rather than digitizing marsh area boundaries with estimations of Spartina cover. Treatment efficacy monitoring was conducted in a subset of treatment sites to provide detailed information on effects of treatment methods on vegetation cover and Spartina density. Details of the treatment efficacy monitoring data parameters are provided in Section 2.2.

2.1.1 Staff Training

All monitoring staff were provided extensive training prior to conducting surveys, and every effort was made to retain experienced staff from year to year to improve data reliability and efficiency. Each year, both experienced and new staff received personalized training in *Spartina* field identification and any new monitoring methods or GPS/GIS technology. Each new staff spent at least 3-5 days chaperoned by the lead field biologist for personalized training. Because the primary plants being mapped are in fact hybrids between two *Spartina* species (*S. alterniflora* x *foliosa*) and their backcrosses with both parents and other hybrids, there are frequently challenging hybrids that are diffcult to identify in the field. New staff were encouraged to collect samples of all "suspicious" *Spartina* for genetic analysis until laboratory results consistently confirmed their field identifications and the lead field biologist determined that their identification skills were sufficient³. Once initial training was complete, the new staff was paired with other more experienced field staff for the remainder of the first season.

All field staff received a minimum of 15 hours of marsh and boating safety training. Marsh safety training addressed such things as the of dangers of working in a tidal environment, walking in the soft mud/marsh among channels, rocks, and other potential physical hazards, and marsh work in poor weather conditions. Staff were also provided field and classroom training in appropriate protocols for working in sensitive species habitat.

2.1.2 Field Methods

All field monitoring was conducted during the summer and fall, the peak of the growing season, when the phenology of marsh vegetation and in particular, *Spartina alterniflora x foliosa* hybrids (*S. alterniflora*/hybrids, see footnote on page 1), is best for field identification. In marshes where endangered California clapper rail

³ Even with extensive training and experience, there are expected to be hybrids that appear so similar to *S. foliosa* as to defy accurate field identification based on morphology. These are termed to be "morphologically cryptic hybrids," and they will become an important focus of the monitoring program in future years, as the population of visible hybrids is reduced.

2.0 Methods

(Rallus longirostris obsoletus) were determined to be present, all monitoring took place after September 1, after the rail breeding season. At each site, trained staff mapped the location and areal extent of non-native Spartina species using a Global Positioning System (GPS) handheld sensor and data entry unit (Trimble GeoXT). A custom Spartina monitoring data dictionary was created using Pathfinder Office software and uploaded onto the GeoXT's (Appendix 3). Plant location data were 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, was typically mapped as a line. A cluster or meadow of clones was 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 attribute was recorded to reflect the total diameter of all clones within the cluster *as if* clones were adjacent and coalesced into 90-100% aboveground cover.

The GPS units automatically collected data on date, time, location, area and perimeter (if polygon data), and length (if line data). Field staff manually entered 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 (for genetic sam-



ISP Field Biolgist entering inventory data into a Trimble GeoXT GPS and data entry device

pling, if applicable), percent cover data for Spartina foliosa, Cordylanthus spp., and "other high marsh vegetation" (if line or 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, including presence of other nonnative invasive species (non-Spartina genera), was noted and entered in a "comments" field. As decribed above, diameter of non-native Spartina within point features was adjusted in the field to reflect 90-100% percent cover of non-native Spartina plants within the feature. If cover in the field was less than 90%, the recorded diameter of the point feature was reduced to reflect the total diameter of all

clones within the cluster *as if* clones were adjacent and coalesced into 90-100% aboveground cover. As previously noted, in 2004 the percent cover classes were modified from the 2001 and 2003 methods. The cover classes 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%. As explained above, cover class for all point features was 90-100%. (See **Appendix 3** for more details.)

Spartina species were identified based on genetic testing, plant morphology, density, and marsh position (as described in the ISP Field Identification Guide, Zaremba 2001). Identification of *S. alterniflora*/hybrids is complicated by the variation of morphological characteristics expressed in some hybrids. Plants displaying one or more *S. alterniflora* hybrid characteristics (i.e. taller or thicker culms than the surrounding *S. foliosa*; pink to red culm color) were mapped as *S. alterniflora*/hybrids, or "unconfirmed hybrids". Species identification was confirmed by sampling at least 3-5 plants per monitoring site for genetic analyses using published methods (Ayres *et al.* 2004). Plant samples were kept refrigerated until mailed overnight to Dr. Debra Ayres at the UC Davis *Spartina* Laboratory. Plant samples were tested for species using RAPD (Randomly Amplified Polymorphic DNA) nuclear markers (Daehler and Strong 1997; Ayres *et al.* 1999). Once genetic testing was complete, the *Spartina* Laboratory results were then incorporated into the GIS data layer's attribute table. In addition to a sample of field-identified non-native hybrids, any ambiguous plants were also sampled for genetic analysis to confirm their 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 were sampled by transect for genetic analysis to confirm native species identification. Transect and point sampling were also employed at selected marsh restoration sites to determine the percent invasion. Transects

were run the length of the marshes sampling every 10 meters (m) for genetic analysis and to find any hidden *S. alterniflora*/hybrids (i.e., plants with *alterniflora* genetics which are not readily distinguishable in the field).

2.1.3 Aerial Photo Interpretation Methods

Aerial photo interpretation was used to inventory larger *Spartina* infestations that would be extremely timeconsuming to map using the field based monitoring methods. In 2004 and 2005, 112 and 128 photos were taken, respectively. 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 dominated by non-native *Spartina*. All *Spartina* mapped in these sites was assumed to be *S. alterniflora* hybrid or *S. densiflora*, depending on the site.

Color infrared photos were taken at 1:6000 feet scale at low tide during the peak of the growing season (between August-October). The photos were taken at the same time each year, when the plants were at peak of growth and still green, to allow accurate yearly comparison. Photos were scanned at 1200 dpi and rectified by subcontractors. In 2004, USGS Digital Ortho Quarter Quads (DOQQs) were used, and in 2005 USGS true color orthorectified photos were used as the baseline reference layers for rectification. The 2005 rectified images had positional errors (RMSE) of less than 1.0 m; much improved



Example of color infrared aerial photograph digitized to show non-native *Spartina* (shown in red)

from the 2004 photos rectified using dated USGS DOQQs. Once rectified, the color infrared photos were imported into ArcView 3.3 (or ArcGIS 9.1) for review and analysis. Rather than using marsh area boundaries and levees to guide the digitization of *Spartina* invaded marsh (as in 2001), we digitized polygons delineating the boundaries of *Spartina* meadows and patches within the marshes at 1:500 scale. Trained photo interpreters, familiar with the sites to be digitized, mapped the *Spartina* by using a mouse or tablet pen to manually trace the outline of visible *Spartina* patches over color infrared imagery displayed on a computer monitor (a method called heads-up digitization). Photo interpreters examined the photos and distinguished the *Spartina* from mud/sand, channel and other marsh vegetation using color, texture, and growth form. Mud/sand flats and channels typically exhibit dark grey-blue color hues, whereas other marsh vegetation, such as *Salicornia virginica* and *Jaumea carnosa*, is light pink, and *Spartina* is bright orange-red to dark red. The relatively tall and erect *Spartina* typically exhibits a unique velvety texture relative to the shorter, even texture exhibited by *Salicornia virginica* or the pimply, branching appearance of *Grindelia stricta*. An additional distinguishable feature for *Spartina* is its circular growth pattern of individual clones, which eventually coalesce into meadows with scalloped edges.

The digitized polygons were then given a cover class (modified as in the field methods, including: 1-10%, 10-20%, 20-30%, 30-40%, 40-50%, 50-60%, 60-70%, 70-80%, 80-90%, and 90-100%.). *Spartina* identification on the aerial photos was aided by *Spartina* points, lines, and polygons mapped during the summer-fall field monitoring efforts (when the aerial photos were taken). Areas of the polygons were calculated using the ArcView Xtools extension. The digitized non-native *Spartina* areas were then merged with the field-based data into final annual data sets.

2.1.4 Genetic Analysis Methods

Genetic testing of *Spartina* samples was performed in both 2004 and 2005 to identify unknowns and confirm field identification of *Spartina* species. A haphazard sample of three leaves per invaded marsh was collected to confirm species identification. In addition, leaf samples were collected from all populations of questionable genetics that could be accessed in the field. For each genetic sample, a collection location was recorded using GPS, and a single undamaged leaf 10-20 cm in length was individually labeled and sent to the *Spartina* Lab at the University of California, Davis. Genetic testing was performed using RAPD (Random Amplified Polymorphic DNA) nuclear DNA markers according to the methodology described in Daehler et al. (1999).

2.2 TREATMENT EFFICACY MONITORING METHODS

A subset of treatment subsites were monitored pre- and post-treatment, with post-treatment efficacy results being measured one growing season after treatment. In 2004 and 2005, efficacy monitoring was conducted at 22 and 44 subsites, respectively (**Appendix 2**). Subsites were selected to encompass a range of marsh types and treatment methods. A summary of site characteristics and treatment information for each 2004 efficacy monitoring site is provided in **Appendix 4**. 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).

The methods for collecting treatment efficacy data were consistent with the approach suggested in the guidelines developed by Collins *et al.* (2001). The study design used permanent monitoring plots established in the first year of measurement, prior to treatment, which were re-sampled one season following treatment. The location of these permanent plots was recorded using a Trimble GeoXT GPS unit, which records these locations with sub-meter accuracy. For small infestations where individual clones are mapped as points, all mapped plants (up to a maximum of 30 plants per site) were monitored. If more than 30 plants were mapped, a sample of 30 permanent monitoring plots were selected along a single transect within the site. Transect length and location were established to intersect the longest possible extent of the infestation within the contraints of site accessibility. (As noted above, 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 m along the transect, a coin toss determined whether or not to sample at that point. Permanent monitoring plot locations were haphazardly determined by a toss of the .25 x .25 m quadrat at these selected points.

Treatment efficacy data was collected at two scales: within .25 m x .25 m plots and within the clone/plant or patch. The clone/plant or patch was defined as the size of the clone (with the boundary of the genetic clone determined by the field biologist), or as a 10 m^2 default patch size if the sample point fell within a *Spartina* meadow. The data collected from each of these sampling locations included the following:

- Patch size (diameter, 10 m maximum)
- Spartina cover within the patch (cover class, live and dead)
- Mud/bare ground cover class
- Wrack cover class
- Plant/clone vigor (high/medium/low)
- Marsh elevation (high/medium/low)
- Spartina life history (vegetative growth/flowering/senescent)
- Site type⁴

The .25 m x .25 m plot data included:

- Stem height of live and dead stems of native and non-native *Spartina* (a single stem was selected to be indicative of the median height within the quadrat; height was measured from the mud surface to the base of the highest leaf of the selected stem)
- Stem density of live and dead native and non-native *Spartina* (number of stems/quadrat)

⁴ The ISP has defined four general "site types", which it uses in planning and evaluating *Spartina* treatement, and particularly the effects of herbicide applications on water quality. The site types are as follows: Type I: Tidal and microtidal marsh, former diked baylands, and back barrier marsh; Type II: Fringing tidal marsh, mudflat, and estuarine beach; Type III: Midsized or major tidal slough, creek, or flood control channel; Type IV: Urbanized riprap, docks, boat ramps, and marinas. Sometimes sites contain multiple types, and so a predominant type may be assigned, or the site may be further broken down and evaluated in smaller units.

- Plant species percent cover (cover class of each plant species found within the quadrat)
- Sediment type (mud, sand, cobble, other)
- Percentage of *Spartina* stems in flower
- Mud/bare ground cover class
- Wrack cover class

2.3 DATA MANAGEMENT AND STATISTICAL METHODS

2.3.1 Data Management & Quality Assurance

Data on species, location, and area covered were entered in the field into the *Spartina* monitoring data dictionary in Trimble GeoXT GPS units, as described above. Supplemental notes were added as needed to clarify entries. Following field data collection, GPS data were downloaded, differentially corrected, and reviewed by the data collectors and the lead field biologist using Trimble Pathfinder Office software. After review, the data files were exported to ArcView 3.3 shapefiles. All original and intermediate files were backed up regularly to CDs. Files were sorted by data collector and exported to Excel for another round of review by the data collectors. Once all data files were collected for the season, the individual site files were merged into a single shapefile and viewed in ArcView for preliminary GIS analysis. The attribute table of the final shapefile was then exported into Excel and SYSTAT 10 (SPSS 2000) for statistical analysis. Categorical names were cross-tabulated to check for typographical errors and duplications. Summary statistics were calculated for quantitative variables to check for unreasonable ranges and outliers. Any errors detected in the SYSTAT screening tests were corrected in the GIS shapefile and the Excel spreadsheets.

Using GIS to manage the treatment monitoring data sets, all pre- and post- treatment data collected in 2005 was separated out of the inventory data set and joined to the previously collected 2004 treatment monitoring data, using the monitoring point's "Clone ID name" as the common field. The initial pre-treatment monitoring points from 2004 were updated with the 2005 post-treatment efficacy data. This data was exported to both Excel and then SYSTAT for review and examination for outliers and errors. The final summary pre/post treatment data was analyzed to determine a quantitative assessment of treatment efficacy.

A second round of data editing was completed along with transformation of GIS shapefiles into a Spartina inventory personal Access geodatabase in February 2007 using ArcGIS 9.0. At this time, all features collected in the field as points and lines between 2004 and 2005 were converted to polygon features using the point diameter and line width data associated with the feature and recorded at the time of data collection. Percent cover of all point features was calculated as 95%, based on the field methods described above. Area was calculated as line length x line width rather than relying on area calculation of buffered lines in GIS, which can cause odd buffer shapes due to slight curvatures in the line, and which result in discrepancies between the area recorded in the field and the area of the buffered polygon feature. Where point diameter or line width values were missing (see Table 1), a diameter of 1 m was assumed for point data and a width of 1 m was assumed for line data. This value was chosen to be a conservative estimate, as populations under 1m² are uncommon. Point features collected by the Santa Clara Valley Water District (SCVWD) in the southern South Bay in 2005 were given a diameter of 0 if the comments field indicated "merged with [another patch]" or "could not locate patch in 2005". Final species names for features collected by SCVWD were updated to reflect the results of genetic testing. (Note this change of many features from "unknown" to known species identification is not reflected in the maps provided in this report. This change *is* reflected in all data tables and data analyses in this report.)

Cover classes were grouped into low (<10%), medium (10-50%) and high (>50%). Species names were combined for consistency (S. alterniflora and S_alterniflora were combined, for example).

2.3.2 Statistical Methods

Inventory Monitoring Data

Summary statistics of *Spartina* inventory monitoring data were calculated based on the summation of gross area and net cover within spatial boundaries (subsite, region, and Estuary). **Gross area** was calculated as the GIS feature area described above. Note that this calculation of gross area is not standardized, and is dependent on the method used to record *Spartina* locations in the field or in aerial photo interpretation (see Introduction). The resulting gross area calculation is thus markedly different from the calculation of "total gross acreage" reported by Ayres et al. (2004), which standardized gross area per feature by buffering and dissolving features, then establishing a 1 acre minimum affected area threshold. Changes in gross area and net cover between 2004 and 2005 are reported as both difference (2005 values minus 2004 values) and percent difference [(2005 values – 2004 values)/2004 values]. **Net cover** was calculated on a per feature basis as gross area x 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 in Ayres et al. (2004).

Treatment Efficacy Monitoring Data

Treatment efficacy monitoring data were analyzed by using parametric and non-parametric statistics to calculate the effect of 2004 treatment method on treatment efficacy. Efficacy was measured by plot and quadrat percent cover reduction, change in live *Spartina* stem height and quadrat stem density percent reduction one year following treatment as the measure of efficacy. Stem density percent reduction was calculated as [(stems(2004)-stems(2005))/stems(2004)]. Negative stem density percent reduction values were coded as zeros (as an increase in stem density indicates zero efficacy).

Treatment methods were combined into 6 classes: dig, cover, mow, herbicide glyphosate, herbicide imazapyr, and no treatment. Supplemental environmental and treatment data was evaluated for its ability to detect differences in efficacy based on elevation, surfactant, application method, and contractor employed.

Data were 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 could not be blocked by site for analysis. Independence among all plots was tested in ArcGIS 9.1 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 tested for spatial autocorrelation of stem density percent reduction at all plots. Results were also analyzed without assuming independence among plots and subsites by summarizing mean log efficacy data by treatment method and subsite.

Treatment efficacy was analyzed as a function of 2004 treatment method on measures of treatment efficacy (e.g. stem density percent reduction) using JMP statistical software (SAS 2005) to perform matched pair analysis and comparison of means using one-way ANOVAs and the non-parametric Wilcoxon/Kruskal-Wallis Test.

Year	Number of point features missing diameter	Number of line features missing width
2001	32 of 1698 total points	0
2003	7 of 1434	10 of 201
2004	117 of 3496	12 of 637
2005	117 of 3076	0 of 495

Table 1. Summary of Annual Inventory Monitoring Data with Missing Data Parameters

3.0 RESULTS

3.1 INVENTORY MONITORING RESULTS

The results of the 2004 and 2005 inventory monitoring, categorized by general cover class of impacted areas, are summarized in Tables 2 and 3 and illustrated in Figures 2 and 3. In Table 2, gross area indicates the spatial extent of area impacted, and cover class category indicates the degree of infestation within this extent. (Gross area, infested area, and area impacted are used synonymously throughout this document. Where used, net cover is referenced as such.)

Total area impacted by all invasive Spartina species and their hybrids (total gross area) throughout the Estuary in 2005 was 1,395 acres, with 99% of this area (1,386 acres) infested with the aggressive S. alterniflora/ hybrids (including 0.05 acre gross area of genetically-confirmed pure S. alterniflora at 13 points around the Bay). The second most abundant species, S. densiflora, covered 7.57 gross acres (concentrated mainly in the Corte Madera Creek watershed in Marin County), including 35 field-identified populations of the hybrid between S. densiflora and the native S. foliosa (total gross area 0.10 acre). The other two non-native Spartina species continue to infest just one marsh each, with S. anglica impacting 0.08 acre and S. patens impacting 0.73 acre.

Distribution of cover classes among species is indicative of both the degree of the infestation and the methods used to conduct inventory monitoring. Inventoried populations of the two non-native Spartina species of greatest extent and highest control priority, S. alterniflora/hybrid and S. densiflora, included populations at all three general cover class categories (low, moderate, and high percent cover). Populations of S. anglica, S. patens, S. densiflora hybrid, and pure S. alterniflora were inventoried using only the high cover class, indicating smaller, more concentrated populations and/or greater use of point features to record locations of these populations. Areas of low cover class may illustrate low density patches resulting from annual spread within sites or dispersal to new sites, may indicate the effects of treatment and a reduction in density from previously higher cover classes, and/or may be a result of changes in inventory method from year to year.

		Count of	Features	Gross Ar	ea (acres)
Spartina spp.	Count of Features Gross Area (a) Cover Class 2004 2005 2004 a high 57 13 1.07 a low 18 457 38 a moderate 277 904 317 a high $7,246$ $8,717$ 742 a high 16 17 0.08 a low 0 14 0 a moderate 60 58 1.64 a high $1,200$ $1,023$ 3.65 a moderate 1 0 0.001 a high 51 133 0.59 a moderate 8 1 0.48 a high 50 24 3.84 a	2005			
S. alterniflora	high	57	13	1.07	0.05
	low	18	457	38	321
S. alterniflora x foliosa	moderate	277	904	317	185
	high	7,246	8,717	742	881
S. anglica	high	16	17	0.08	0.08
	low	0	14	0	0.25
S. densiflora	moderate	60	58	1.64	2.35
	high	Pr Class 2004 2005 2004 2005 nigh 57 13 1.07 0.05 low 18 457 38 321 derate 277 904 317 185 nigh 7,246 8,717 742 881 nigh 16 17 0.08 0.08 ow 0 14 0 0.25 derate 60 58 1.64 2.35 nigh 1,200 1,023 3.65 4.87 derate 1 0 0.001 0 nigh 51 133 0.59 0.73 derate 8 1 0.48 0.05 nigh 50 24 3.84 0.98 nigh 0 35 0 0.10			
S. natons	moderate	1	0	0.001	0
S. pateris	high	2004 2005 2004 2005 57 13 1.07 0.05 18 457 38 321 277 904 317 185 7,246 8,717 742 881 16 17 0.08 0.08 0 14 0 0.25 600 58 1.64 2.35 1,200 1,023 3.65 4.87 1 0 0.001 0 51 133 0.59 0.73 8 1 0.48 0.98 0 35 0 0.10			
Unknown: S. foliosa or	moderate	8	1	0.48	0.05
x alterniflora hybrid	high	7,246 $8,717$ 742 881 16 17 0.08 0.08 0 14 0 0.25 60 58 1.64 2.35 $1,200$ $1,023$ 3.65 4.87 1 0 0.001 0 51 133 0.59 0.73 8 1 0.48 0.05 50 24 3.84 0.98 0 35 0 0.10			
S. densiflora x foliosa	high	0	35	0	0.10
* 0	Link 50%			10 50%	1

Table 2	Croos	Aroo	of Non	nativo	Snortina	Spanian	by Covor	Close	2004	and '	2005
	01055	Alea	0111011-	nauve	Spartina	Species	Dy Cover	Ulass,	2004	anu 4	2005

Cover Classes: High = > 50% cover moderate = 10-50% cover

low = < 10% cover



Figure 3. Distribution of Invasive Spartina in the San Francisco Estuary 2004



Figure 4. Distribution of Invasive Spartina in the San Francisco Estuary 2005

		Gross A	rea (acres)		Net Cover (acres)					
Spartina Species	2004	2005	% Change 04-05	Difference 04-05	2004	2005	% Change 04-05	Difference 04-05		
S. alterniflora/hybrid	1,098	1,386	26%	288	704	770	9%	67		
S. anglica	0.083	0.081	-3%	-0.002	0.081	0.064	-18%	-0.014		
S. densiflora	5.29	7.47	41%	2.18	3.02	4.22	40%	1.20		
S. densiflora x foliosa	0	0.10	-	0.10	0	0.09	-	0.09		
S. patens	0.59	0.73	25%	0.15	0.55	0.69	26%	0.14		
TOTAL (not including unknowns)	1104	1,395	26%	291	707	776	10%	68		
Unknown S. foliosa or x alterniflora hybrid	4.32	1.04	-76%	-3.28	3.58	0.94	-74%	-2.64		

Table 3. Gross Area and Net Cover of Non-native Spartina Species Estuary-wide, 2004 and 2005

As described above, all field-collected GPS point features are recorded as high cover, with patch diameter being estimated to indicate the diameter of the patch if all plants were at 95% density. Cover class of all field-collected GPS line and polygon features indicate the actual percent cover observed in the field, and cover class of digitized polygons indicates the actual percent cover observed on the color infrared photographs being interpreted.

As in previous years, the amount of non-native *Spartina* varied markedly by subregion. ⁵ **Figure 4** shows the distribution within the Estuary of the two priority species, S. *alterniflora*/hybrids and *S. densiflora*/hybrids. **Table 4** shows the distribution of all non-native *Spartina* species by subregion and cover class category. The greatest overall *Spartina* acreage is concentrated in the Central and South San Francisco Bay regions. Of the total *S. alterniflora*/hybrid-impacted area, 74% (1,022 acres) is located in the South Bay and 17% (231 acres) in the Central Bay. The Southern South Bay is still relatively lightly infested over its many marsh acres, containing only 9% (131 acres) of the total Estuary-wide area of *S. alterniflora*/hybrid; the North Bay has only 0.1% (less than 2 acres). *S. densiflora*/hybrid is less-widely distributed, with 99% (7.6 acres) located in the Central Bay, just a few small patches in the North Bay, and a few small outlier patches in Tomales Bay. The single *S. anglica* infestation is in the Central Bay, and the *S. patens* site is in Suisun Bay.

3.1.1 Estuary-wide Population Change 2004-2005

As shown in **Table 3**, Estuary-wide populations of non-native *Spartina* expanded significantly between 2004 and 2005. Total gross acreage of all non-native *Spartina* species expanded by 26% (291 acres) from 1,104 to 1,395 acres, with both priority species showing a similar annual rate of increase. Net cover of all non-native *Spartina* increased by 10%, indicating that the increase in gross acres impacted is a result of a true increase in *Spartina* cover throughout the Estuary, not simply a consequence of a change in method of impacted area estimation.

<u>Spartina alterniflora/hybrids</u>

As discussed in Section 2 (Methods), starting in 2005, all plants with *S. alterniflora* characteristics were recorded as *S. alterniflora* x *foliosa* hybrids unless genetically determined to be pure *S. alterniflora*. Thus we calcu-

⁵ For reporting purposes, the ISP generally uses the subregional boundaries defined by the San Francisco Estuary Institute's EcoAtlas, with one additional division of the South Bay region, as shown in Figure 2. The North Bay consists of the area north of the San Rafael Bridge, the Central Bay is between the San Rafael and San Mateo Bridges, the South Bay is between the San Mateo and Dumbarton Bridges, and the Southern South Bay is south of the Dumbarton Bridge.



Figure 4. Distribution by Subregion of S. *alterniflora*/hybrids (left) and *S. densiflora*/hybrids (right) in the San Francisco Estuary 2005. All areas shown are gross acres (explained on page 1), and percentages are percent of the total gross area.

lated a 26% rate of increase of area impacted by *S. alterniflora* plus *S. alterniflora* hybrids ("S. *alterniflora*/hybrid"), up 288 acres from 1,098 to 1,386 acres, the greatest area increase of all non-native *Spartina* species.

<u>Spartina anglica</u>

S. anglica showed little change, actually decreasing 3% from 0.083 to 0.081 impacted acre (in the absence of treatment).

Spartina densiflora and hybrids

Hybrid *S. densiflora* x *foliosa* plants were detected in 2005 for the first time at three locations in the Central Bay.Gross *S. densiflora-* and *S. densiflora* x *foliosa-*impacted area expanded by 43% (2.3 acres) from 5.3 to 7.6 impacted acres.

<u>Spartina patens</u>

Although the population of *S. patens* is relatively small, it showed a similar expansion rate of 25% from 2004 to 2005, increasing 0.14 acre from 0.59 to 0.73 impacted acre.

Undetermined Spartina sp.

The area of plants of "unknown" species dropped significantly, from 4.32 acres in 2004 to just over 1.0 acre in 2005, a decrease of 3.3 impacted acres (77%).

				Gross are	ea (acres)			Net area	a (acres)	
Region	Spartina species	Cover Class	2004	2005	% Change 04-05	Change Area 04-05	2004	2005	% Change 04-05	Change Area 04-05
		low	0.0	7.6		7.6	0.0	0.4		0.4
	Solution Spartina Species S. alterniflora or hybrid S. anglica S. densiflora S. densiflora S. densiflora x foliosa Unknown S. alterniflora or hybrid S. densiflora Unknown S. densiflora Unknown S. densiflora	moderate	59	28	-54%	-32	18	7	-59%	-11
	hybrid	high	179	196	9%	17	152	159	5%	7
		Total	239	231	-3%	-7	170	167	-2%	-3
>	S. anglica	high	0.083	0.081	-3%	-0.002	0.077	0.064	-18%	-0.014
Ba		low	0.00	0.25		0.25	0.00	0.01		0.01
al	S. densiflora	moderate	1.6	2.4	44%	0.7	0.4	0.7	103%	0.4
htr	o. actioniora	high	3.6	4.9	33%	1.2	2.7	3.5	31%	0.8
ပဳ	S. densiflora S. densiflora x foliosa	Total	5.3	7.5	41%	2.2	3.0	4.2	40%	1.2
	S. densiflora x foliosa	high	0.00	0.10		0.10	0.00	0.09		0.09
	S. densiflora S. densiflora x foliosa Unknown (S. foliosa or alterniflor bybrid)	moderate	0.0	0.0	-100%	0.0	0.0	0.0	-100%	0.0
	(S. foliosa or alterniflora	high	3.2	0.1	-97%	-3.1	2.9	0.1	-97%	-2.8
	hybrid)	Total	3.3	0.1	-97%	-3.2	2.9	0.1	-97%	-2.8
		high	0.0	1.0		1.0	0.0	0.2		0.2
	5. alterninora ol	moderate	0.2	0.6	228%	0.4	0.2	0.6	212%	0.4
3ay	<i>S. alterniflora</i> or hybrid	Total	0.2	1.6	755%	1.4	0.2	0.8	350%	0.6
orth E	S. densiflora	high	0.005	0.001	-69%	-0.003	0.004	0.001	-69%	-0.003
Ň	Unknown (S. foliosa or alterniflora hybrid)	high	0.04	0.04	14%	0.01	0.02	0.04	67%	0.02

Table 4. Inventory of Non-native Spartina by Subregion and Cover Class, 2004 and 2005

3.1.2 Regional Population Change 2004-2005

Regional expansion of non-native *Spartina* generally followed Estuary-wide patterns, with a few notable exceptions (**Table 4**). The only species with significant change in gross coverage in more than one Bay region was *S. alterniflora*/hybrid. This result is expected, since 99% of all *S. densiflora* is in the Central Bay and all other species and hybrids are in just one location and/or region.

<u>Central Bay</u>

From 2004 to 2005, ISP monitoring efforts recorded a 3% decrease (7.4 acres) in gross acreage of *S. al-terniflora*/hybrid-impacted areas in the Central Bay, down from 239 to 231 gross acres. A corresponding three acre decrease in net cover of *S. alterniflora*/hybrids (from 170 to 167 net acres) in the Central Bay verifies that this decrease in gross acreage is indeed due to a reduction in non-native *Spartina* biomass, not simply a difference in inventory methodology.

The relatively small, single Central Bay population of *S. anglica*, at Marin's Creekside Park, decreased 3% from 0.083 acre to 0.081 acre (gross), with a corresponding decrease of 18% (0.014 acre) net cover. This high ratio of net:gross decrease of *S. anglica* may indicate a lack of spread and a nearly 20% decrease in cover of *S. anglica* at Creekside Park, or it may be an artifact of the change from field-based inventory in 2004 to aerial photo interpretation-based inventory in 2005.

Central Bay populations of *S. densiflora* increased 2.2 acres in gross area and 1.2 acres in net cover, with increases in net cover and gross area expansion of *S. densiflora* occurring at all levels of infestation (low, moderate and high). No new *S. densiflora* sites were located in 2005, and one population (a single clone on Marin Island) was extirpated in 2005.

4	SoboSpartina speciesNeg unocS. alterniflora or hybridNeg unocS. alterniflora or hybridS. foliosa or alterniflor hybridNeg unocS. alterniflora or hybridNeg unocS. alterniflora or hybridNeg unocS. alterniflora or hybridNeg unocS. alterniflora or hybridNeg unocS. alterniflora or hybridNeg unocS. patens or hybrid			Gross are	a (acres)		Net area (acres)				
Regio	Spartina species	Cover Class	2004	2005	% Change 04-05	Change Area 04-05	2004	2005	% Change 04-05	Change Area 04-05	
		low	38	194	410%	156	2	10	439%	8	
	SolutionSo	moderate	229	156	-32%	-73	51	41	-19%	-10	
Bay	hybrid	high	552	673	22%	121	465	534	15%	69	
th		Total	819	1022	25%	203	518	586	13%	68	
no	YeaSpartina speciesYeaS. alterniflora or hybridYeaS. alterniflora or hybridUnknown (S. foliosa or alterniflora hybrid)YeaS. alterniflora or hybridYeaS. alterniflora or hybridYeaS. alterniflora or hybridYeaS. alterniflora or hybridYeaS. alterniflora or hybridYeaS. alterniflora or hybridYeaS. alterniflora or hybrid	moderate	0.5	0.1	-89%	-0.4	0.17	0.01	-93%	-0.16	
S		high	0.34	0.36	6%	0.02	0.32	0.33	4%	0.0	
	hybrid)	Total	0.81	0.41	-49%	-0.40	0.49	0.35	-30%	-0.15	
		low	0	119		119	0.0	6.0		6.0	
_ ≥	S. alterniflora or hybrid	moderate	28.8	0.4	-99%	-28.4	4.5	0.2	-96%	-4.4	
Ba	hybrid	high	11.8	11.5	-2%	-0.3	10.9	10.7	-2%	-0.2	
th th		Total	41	131	224%	91	15	17	9%	1	
So Sol	Unknown (<i>S. foliosa</i> or <i>alterniflora</i> hybrid)	high	0	0	125%	0	0	0	125%	0	
ц,		moderate	0.001	0.000	-100%	-0.001	0.0003	0.0000	-100%	-0.0003	
uist 3ay	S. patens	high	0.59	0.73	25%	0.15	0.55	0.69	26%	0.14	
Sc		Total	0.8	1.2	52%	0	0.76	1.16	53%	0.40	
iter ast	SpeciesSpeciesS. alterniflora or hybridUnknown (S. foliosa or alterniflora hybrid)S. alterniflora or hybridS. batensS. alterniflora or hybridS. alterniflora or hybridS. alterniflora or hybridS. alterniflora or hybrid	high	0.003	0.000	-100%	-0.003	0.003	0.000	-100%	-0.003	
ပိုပ်		high	0.0003	0.0001	-69%	-0.0002	0.0003	0.0001	-69%	-0.0002	

Field-identified *S. densiflora x foliosa* plants were discovered in the Central Bay for the first time in 2005. Possible *S. densiflora* hybrids were identified at Piper Park (two clones), Corte Madera Marsh Reserve (six clones), and Creekside Park (27 clones).

<u>North Bay</u>

North Bay populations of *S. alterniflora*/hybrids increased at the highest rate within the Estuary (755% increase in gross acres, 350% increase in net cover). What began as a small infestation in 2004 covering 0.2 gross acres at Point Pinole and the Richmond shoreline, expanded to 1.6 gross acres by 2005. In addition, two individual *S. alterniflora*/hybrid clones were identified and lab-confirmed along the Novato Bayfront in 2005.

The gross area and net cover of *S. densiflora* both decreased by approximately 70% in the North Bay due to the successful control efforts at Point Pinole, home to the single North Bay population of *S. densiflora*.

<u>South Bay</u>

The South Bay, the subregion with the greatest concentration of *S. alterniflora*/hybrids, saw a 25% (203 acre) increase in impacted area, increasing from 819 to 1,022 acres. Increases in both gross area and net cover of *S. alterniflora*/hybrids in the South Bay were noticeable in both high and low cover classes. Populations with high cover classes accounted for the greatest increase in net cover in the South Bay, while low cover populations accounted for the most acres of newly impacted land in the region. This apparent increase in gross area and net cover is likely due in part to the enhanced monitoring ability enabled by the increased use of photo interpretation to monitor many of the South Bay sites in 2005. The change from field-based monitoring to aerial photo interpretation predictably increases the amount of low coverage acreage inventoried as a result of the different methodologies used (see Section 2, Methods, for a complete description).

Table 5. *S. alterniflora*/hybrid Population Change in the Southern South Bay Subregion 2004 & 2005. The substantial increase in gross area shown at Cooley Landing is an artifact of a change in monitoring method between the two years.

		Site Name	N& Calever	5 Marshes Daton & Aud Daton & Aud	ubon Marshes	Le Creek Upst	Allo Baylands	n Bay Marshe	s Intainester	Warshes av Landing Salt	Ponds
	Site Number	05a	05b	05c	05f	8	15a	15b	16	TOTAL	
No. of	2004	36	55	19	0	42	116	2	53	323	
Features	2005	170	55	7	2	57	146	6	2	445	
	2004	1.42	5.75	0.78	0	0.43	5.44	0.02	26.71	40.55	
C	2005	5.14	1.72	0.18	0.02	0.65	4.12	0.05	119.39	131.27	
(acres)	Difference 2004-2005	3.72	(4.04)	(0.60)	0.02	0.22	(1.32)	0.04	92.69	90.72	
	% Change 2004-2005	262%	-70%	-77%	all new	51%	-24%	224%	347%	224%	
	2004	1.35	3.77	0.74	0	0.41	5.17	0.02	4.01	15.46	
Net Cover	2005	4.5	1.63	0.17	0.02	0.62	3.91	0.05	5.98	16.87	
Net Cover (acres)	Difference 2004-2005	3.15	(2.14)	(0.57)	0.02	0.21	(1.26)	0.03	1.98	1.42	
	% Change 2004-2005	234%	-57%	-77%	all new	51%	-24%	224%	49%	9%	

<u>Southern South Bay</u>

The Southern South Bay subregion, the location of the majority of the South Bay Salt Ponds Restoration Project area, experienced a 9% increase of S. alterniflora/hybrid net cover, resulting in a net cover of nearly 17 acres impacting a total of over 131 acres (Table 5). At first glance, monitoring results seem to indicate that the number of acres impacted by S. alterniflora/hybrids in the Southern South Bay increased by 224%, spreading from 41 acres in 2004 to 131 acres in 2005. In actual fact, a large part of this apparent increase is an artifact of a change in monitoring method at Cooley Landing, which resulted in an apparent increase of nearly 93 gross acres. This site was digitized on infrared aerial photographs in very fine detail in 2004, and the entire infestation given a corresponding moderate cover class. However, in 2005, aerial photographs were not available, so the entire marsh was digitized at a very coarse level and given a low cover class to adjust for the lack of detail. The overall acreage of *Spartina* for this site was actually similar between years, but a shift of 119 acres from moderate to low occurred as a result. Actual change in the extent of S.alterniflora/hybrids was apparent at the other Southern South Bay sites, however, as indicated in Table 5. Nearly four acres of new S. alterniflora/hybrid patches were located in the Mowry and Calaveras marsh areas in 2005, while just over four acres of S. alterniflora/hybrid was successfully controlled in the Dumbarton and Audubon marshes between 2004 and 2005. Also of note, two new populations of S. alterniflora/hybrid were located in the upstream reaches of Coyote Creek in 2005.

<u>Outer Coast</u>

A single, small (135 ft²) *S. alterniflora*/hybrid clone was found at Limantour Estero in Drake's Bay at Point Reyes National Seashore and was successfully removed (100% eradicated) by digging and covering following detection in 2004.

Eight *S. densiflora* clones found at Tom's Point in Tomales Bay in 2004 were treated by digging and covering in 2004. Nine *S. densiflora* clones found at Tom's Point in Tomales Bay in 2005 were in new locations (up to 541 feet away). Treatment was effective in 2004, and the new clones detected and treated in 2005 were small, resulting in an approximate 70% reduction in extent and cover of *S. densiflora* at Tom's Point from 2004 to 2005.

3.1.3 Population Change by Subsite 2004-2005

Subsite-specific 2004 and 2005 inventory data, in the form of stacked bar graphs, are presented by site in **Appendix 5**. The graphs show the gross area and net cover of non-native *Spartina* for each year. Gross area changes between years can indicate changes in extent as well as changes in monitoring methodologies. Corresponding change (or lack of change) in net cover can aid in interpreting gross area change. Change in gross area may be due to population expansion (indicated by increases in both gross area and net cover), densification of existing stands (increase in net cover with no change in gross area), population contraction (indicated by a decrease in net cover, with or without a decrease in gross area), or change in monitoring methodology (dramatic change in gross area with little or no change in net cover).

3.1.4 Genetic Results 2004 and 2005

In 2004, a total of 208 samples from 125 sites were genetically analyzed at the UC Davis *Spartina* Laboratory. Of these, lab results from 151 samples (73% of all samples) from 26 sites confirmed *Spartina* species field identifications. These included confirmation of 79 native *S. foliosa* from six sites, and confirmation of 72 *S. alterniflora* x *foliosa* hybrids from 20 sites. Seventeen samples (8% of all samples) from seven sites were misidentified in the field as *S. foliosa*, and determined in the lab to be *S. alterniflora* x *foliosa* hybrids. Only three samples from three sites were determined to be misidentified natives (plants that were field identified as *S. alterniflora* hybrids, but were genetically determined to be *S. foliosa*). A total of 37 samples from 23 sites were field-identified as hybrids but determined in the lab to be genetically pure *S. alterniflora*.

In 2005, 168 samples from 129 sites were successfully analyzed. Laboratory results confirmed field identification for 86 samples (51% of all samples) from 38 sites. Confirmed identifications included 42 *S. foliosa* samples from 14 sites and 44 *S. alterniflora x foliosa* hybrid samples from 24 sites. Only three samples (2% of all samples) from three sites were misidentified in the field as *S. foliosa*, and determined in the lab to be *S. alterniflora x foliosa* hybrids (all sites were different from those in which *S. foliosa* was mistakenly identified in 2004). Natives misidentified in the field as hybrids accounted for 45 samples from nine sites (28% of all samples), the majority of these samples (26) being taken from plants in the Southern South Bay, where native *Spartina* morphology is much more robust and resembles *S. alterniflora* hybrid morphology in more northern reaches of the Estuary. Unknowns determined by genetic testing accounted for 24 samples from 10 sites, and 10 samples from nine sites were identified as *S. alterniflora* hybrid but determined to be pure *S. alterniflora*. This last result is not surprising, as field identification of pure *S. alterniflora* versus hybrids was abandoned in 2005, at which point all field identification assumed hybridity of plants exhibiting *S. alterniflora* characteristics. (See Methods for more information.)

The results of successful RAPD genetic analyses of *Spartina* samples taken during 2004 and 2005 inventory monitoring efforts are presented in **Appendix 6**. (Samples for which lab reactions were unsuccessful are not listed.)

3.2 TREATMENT EFFICACY MONITORING RESULTS

Varying treatment methods and levels of treatment took place at non-native *Spartina* sites in 2004, resulting in variable influence on extent and cover by site. Treatment took place at 39 subsites in 2004 (**Appendix2**). Treatment included digging at eight subsites, covering at five subsites, aerial application of an aquatic formulation of the herbicide imazapyr at three subsites, and treatment with an aquatic formulation of the herbicide glyphosate using a variety of application methods (helicopter, truck, Argo, backpack and boat) at 25 subsites.

Transect-based treatment efficacy monitoring was completed at 22 treatment sites in 2004, with follow-up monitoring in 2005. Measurement of non-native *Spartina* stems/quadrat was found to be both less subjective and more effective than estimation of % cover/quadrat in detecting annual change in *Spartina* abundance within monitoring plots, and thus was used for analysis of treatment efficacy from 2004-2005. Number of plots per site surveyed both years varied from four to 38. As described in Section 2 (Methods), those





sites with fewer than 30 plots were generally sites where less than 30 plants were treated. Of the 22 sites sampled, non-native *Spartina* removal methods in 2004 included covering with tarp (2 sites, 4 plots total), digging (2 sites, 38 plots total), glyphosate herbicide (13 sites, 307 plots total), imazapyr herbicide (3 sites, 78 plots total), and mowing (1 site, 3 plots total). Four sampling sites, with a total of 82 plots, received no treatment (note that one untreated site had only one plot). Charts of the change in stem densities at individual sites within each treatment type are presented in **Figure 5**. Lack of sufficient replication within the sites monitored in 2004 and 2005 prevented analysis of the potential impact of factors such as surfactant, application method, and contractor employed on treatment efficacy.

Analysis of treatment efficacy monitoring data summarized by subsite and treatment method indicates no sig Figure 4. Efficacy of 2004 Treatment Method. Efficacy of 2004 treatment method as measured by mean percent reduction in stems per quadrat along treatment efficacy monitoring transects (2004-2005). Bar height indicates the average of calculated subsite mean percent reduction in stems per quadrat. Error pars indicate standard error among subsite mean values. There is no standard error for mowing treatment, as mowing was used at only one monitored subsite in 2004.nificant difference in percent stem density reduc-



Bars represent mean number of stems reduced per quadrat. "X" represent numbers of plots (equal to number of quatrats) surveyed per method per site.

tion between treatment methods. Percent stem density reduction values were log-transformed to improve normal fit (Shapiro-Wilk W Test p = 0.0131). Neither parametric nor nonparametric tests indicated significant differences between treatment methods (One-way ANOVA p = 0.0663; Wilcoxon/Kruskal-Wallis Test P = 0.1433). These results are likely due to the low subsite-based sample sizes of all 2004 treatment methods other than glyphosate herbicide.

Testing of spatial autocorrelation between plots indicated that stem density reduction values were neither clustered nor dispersed, but were randomly distributed among sites (Moran's Index = 0.281, Z Score = 0.014 standard deviations). These results allowed for the analysis of unblocked data by indicating independence between sampling points.

Descriptive and non-parametric statistical results on unblocked data indicate that of the treatment methods attempted in 2004, the most effective methods were digging (79% efficacy), covering (71% efficacy) and imazapyr herbicide (36% efficacy). Neither glyphosate treatment (26% efficacy) nor mowing (5% efficacy) was more effective than no treatment at all (**Figure 6** and **Appendix 7**). Interestingly, no treatment resulted



Figure 6. Efficacy of 2004 Treatment Method. Efficacy of 2004 treatment method as measured by mean percent reduction in stems per quadrat along treatment efficacy monitoring transects (2004-2005). Bar height indicates the average of calculated subsite mean percent reduction in stems per quadrat. Error pars indicate standard error among subsite mean values. There is no standard error for mowing treatment, as mowing was used at only one monitored subsite in 2004.

in a 27% average reduction in stem counts/quadrat at the four sites sampled. This can be explained by the small but natural reduction of density within the center of a stand as a clone expands, and/or by the fact that quadrat placement is approximate within the limitations of GPS technologies and may vary by up to $1m^2$ from year to year.

Non-parametric statistics must be applied to these results, since reduction in stem numbers are not normally distributed and transformations to normality were unsuccessful. The non-parametric Wilcoxon/Kruskal-Wallis Test (**Appendix 7, Table 2**) applied to the unblocked data finds that efficacy differs significantly by treatment (p<0.0001), and indicates that digging, covering, and to a lesser degree treatment with imazapyr, has a positive impact on reducing stem numbers. Parametric statistics are also given (**Appendix 7, Table 1**) for comparison purposes, but the reader is cautioned to view parametric analysis with caution due to the non-normal distribution of input data.

4.0 DISCUSSION

While the full-scale control efforts of the ISP are just beginning and have not yet achieved the anticipated level of *Spartina* reduction, it appears that these efforts have slowed the expansion. In addition, expansion within some sites may have slowed as individual clones come into contact and simply coalesce into a meadow rather than colonize a new area. While there are still thousands of vulnerable acres in the Estuary, the rate of expansion in some areas with large infestations may be leveling off due to lack of available space or the less favorable conditions of remaining, uninvaded sites. Expansion rates of *S. alterniflora*/hybrids from 2004 to 2005 were significantly lower than found by previous years of ISP monitoring, which had indicated a greater than exponential (> 100%) rate of spread. Net cover of non-native *Spartina* increased by 9%, for a total net coverage of 770 acres of *Spartina* infesting over 1,386 gross acres throughout the Estuary, indicating an overall reduction in rate of spread compared to previous years. Only one Bay region, the North Bay, continued to show a greater than exponential increase in net cover of 350% from 2004 to 2005 for *S. alterniflora*/hybrid, and this only relates to a very small area of *Spartina* (less than two acres). The South Bay and Southern South Bay showed much more moderate increases in net cover.

Total gross area impacted by *S. alterniflora*/hybrids increased by 26% from 2004 to 2005, resulting in 288 newly infested acres. This large increase in impacted acres can be attributed to the continued aggressive expansion from the large 2004 starting acreage as well as the widespread distribution of this invader. Differences in inventory methods between years account for at least 92 of these additional acres, as noted in the discussion of the Southern South Bay results in Section 3, above.

There was also a significant reduction (77%) in the area of "unknown" samples from 2004 to 2005, a probable result of the regular training that the field crew receives, as well as the benefit of several years of experience. However, several issues guarantee that there will always be some specimens that can't be identified in a given year. On occasion, the genetic analysis of *Spartina* samples fails to provide enough of the nine markers to determine species or hybrid origin. In addition, with the enormous task of performing an inventory of the entire Estuary each year, and the complex tidal marsh environment that can be impossible to access on a given day, suspect plants occasionally cannot be analyzed and must remain unknown for one additional season.

Although *S. alterniflora*/hybrids are by far the most abundant and widespread species of non-native *Spartina* in the San Francisco Estuary, *S. densiflora* is locally very abundant in the Corte Madera Creek watershed in Marin County, and poses a serious threat to these wetlands. The infestation of 7.5 acres, expanded by 40% since 2004, illustrates the aggressive nature of this species. If it had been introduced more widely around the Estuary, it is probable that the infestation would be much larger. However, since *S. alterniflora*/hybrids have a much greater tolerance for tidal inundation, they can thrive at much lower marsh elevations, while the *S. densiflora* prefers higher marsh and creek banks. This has probably hampered the dispersal of *S. densiflora* outside of Corte Madera Creek because it cannot establish new clones on open mudflats or fringe marsh where its seeds or vegetative propagules are deposited. As seen from its expansion within Marin, however, once it gets a foothold in an area it is capable of spreading as rapidly as *S. alterniflora*/hybrids.

The majority of the changes observed in the total gross area within cover class categories for *S. al-terniflora*/hybrids between 2004 and 2005 are attributable to the change in inventory method from ground-based to aerial photo interpretation in large areas of the South and Southern South Bay regions. This change in methodology resulted in the Estuary-wide low cover class (< 10%) increasing by a dramatic 744% (283 acres) from an area of 38 acres in 2004 to 321 acres in 2005, while the moderate cover category (10-50%) decreased 42% (132 acres) from 317 to 185 acres. The high cover class (> 50%) still describes the majority (64%) of the infestation, however, a reflection of the continued reliance on field-based inventory methods throughout the majority of the Estuary.

Although the changes observed in the South and Southern South Bay regions are largely explained by the change in monitoring methodology, there still exists concern over any increase in low cover areas due to expansion of non-native *Spartina* into new areas, such as was seen in other regions of the Bay. Low cover

4.0 Discussion

areas (in the absence of effective treatment) can result from the dispersal of seed or vegetative propagules to mudflats or other marsh systems on the edge of high or moderate density areas, distant enough from the existing clones to be considered a new patch. Observed decreases in moderate cover, and simultaneous increases in high cover, could be related to within-marsh expansion with clones coalescing into denser meadows and continuous stands.

Treatment efficacy data suggest that digging, covering, or imazapyr application are the most effective methods of control. Although no statistically significant difference between treatment methods was detected when data were summarized by subsite and treatment method, differences were observable in the field, and the inability to detect these differences is likely due to low subsite sample size for all treatment methods except glyphosate treatment. As reported, analysis of data with the assumption of independence among plots and among subsites supports field observations of differences in efficacy among treatments.

Digging and covering are most effective on small populations. The biology of *S. densiflora* lends itself well to digging because it is a cespitose grass, growing in discrete, compact bunches as opposed to the clonal growth habit and extensive below-ground rhizome system of *S. alterniflora*/hybrids. Digging and/or covering will continue to be used by the Friends of Corte Madera Creek to eradicate *S. densiflora* from their Marin watershed. These methods will also be utilized in the later stages of eradication for all of the introduced *Spartina* species, after larger infestations have been reduced to scattered patches. In addition, these methods are appropriate for volunteer events and will be incorporated into the long-term monitoring strategy for maintaining invasive *Spartina* eradication around the Bay.

While digging or covering are only suitable in small treatment areas, imazapyr application is appropriate in a wide range of situations. If applied to large areas of marsh, digging or covering are highly disruptive to the fragile marsh ecosystem and substrates, causing unacceptable environmental impacts. While effective on a small scale, these methods are labor and waste-disposal intensive, and thus are impractical on a large scale.

Imazapyr can be applied over large areas of contiguous *Spartina* by helicopter, eliminating the disruption to the marsh environment caused by manual methods. Aerial application allows treatment to occur while plants are still actively growing and have not set seed. In clapper rail marshes, where marsh entry is not allowed until September 1, the end of breeding season, aerial application is permitted as early as July 15 according to the ISP's USFWS Biological Opinion (USFWS 2003, 2004, 2005). Translocated herbicides such as imazapyr rely on a healthy target plant to circulate the herbicide down into the roots for effective control. Consequently, treatment that is implemented after *Spartina* begins to senesce may result in lower efficacy, as well as the release of viable seed into the Estuary to spread the infestation. More experience with imazapyr is needed to understand how to improve the efficacy, if possible, and to reduce cost, time, and the amount of chemical entering the environment. Since the 2004 imazapyr treatments did not include any ground application methods, the effectiveness of this approach could not be analyzed.

The treatment efficacy data regarding glyphosate showed very poor results, actually on the same level as no treatment at all. Although the cause of the poor efficacy is unknown, it is suspected that because glyphosate adsorbs tightly to sediments, it is binding to the silt and salt deposited on the *Spartina* by the tides. This would inactivate the herbicide before it can penetrate the leaf cuticle and enter the cordgrass to begin its mode of action. In addition, glyphosate cannot be applied at low volume from helicopter, and the importance of this method to the ISP was discussed above. The full registration of the aquatic formulation of imazapyr (Habitat®) in California in August 2005 allowed the *Spartina* Control Program to begin to transition away from glyphosate in the 2005 treatment season.

Treatment efficacy monitoring data shows that mechanical mowing cannot be used to control *Spartina*. Perennial grasses have evolved with regular herbivory pressure, with many species "overcompensating" by growing faster, taller, stouter, etc. with each successive "mowing". Even repeated mowing each growing season would cause limited mortality to invasive *Spartina*, and mowing is impractical in hazardous marsh systems and soft Bay mud. Mowing is a useful seed suppression tool if full treatment cannot be implemented immediately, and may be helpful for preparing a site for covering, but it will not work on its own to control *Spartina*. This method was used at only one site in 2004, Blackie's Creek mouth, by volunteers of Tiburon Audubon in an attempt to stop seed production and dispersal until a better treatment strategy could be developed and approved.

5.0 CONCLUSIONS

It is the intention of the Invasive *Spartina* Project to use the results of the analyses contained in this and future monitoring reports to guide our work to arrest and reverse the spread of, and eventually eradicate, non-native *Spartina* from the San Francisco Estuary. We believe that this goal is achievable based on the current extent and rate of spread of *Spartina* in the Estuary. We estimate that, as of 2005, invasive *Spartina* impacts 2% and covers just over 1% of the approximately 69,432 acres of mudflat and marsh in the Estuary (Goals Project 1999). The current net coverage of 776 acres of *Spartina* covering an extent of 1,395 gross acres represents a noticeable increase since initial monitoring efforts in 2001 recorded a total of 482 acres of net cover within the Estuary (Ayres et al. 2004). The rate of expansion has dramatically slowed between 2004 and 2005, likely due to both control efforts and saturation of available habitat in older populations. The wide-scale initiation of treatment and improvement of methods justifies optimism that extent, net cover, and rate of spread will decline substantially in subsequent years.

The 2004-2005 ISP monitoring efforts have shown that all species of invasive *Spartina* in the San Francisco Estuary are continuing to expand, with the exception of the single infestation of *S. anglica* that showed a slight decrease in area. *S. densiflora* had the greatest rate of gross area expansion (54%) and net cover increase (43%), illustrating how problematic this species could be if it was more widely introduced around the Estuary. Since *S. densiflora* has mainly only expanded within the Corte Madera Creek watershed where it was introduced, this species appears to have difficulty establishing new infestations around the Bay, probably because of its preference for higher marsh and creek banks as opposed to open mudflat. The dominant invaders, *S. alterniflora*/hybrids, showed a gross expansion rate of 30%, a net cover increase of 10%, and represent 99% of the acreage of invasive *Spartina* species around the Estuary.

The rate of expansion has slowed considerably from the greater-than-exponential spread seen just a few years ago. The 2004 treatment season was the first year of large-scale *Spartina* control efforts throughout the Estuary, which helped to reduce the expansion in some of the main dispersal areas, probably in combination with some spatial constraints of late-stage infestation sites as discussed above. Future control efforts incorporating the best available science and treatment technologies should now move beyond the ability to simply slow expansion and actually begin to yield large reductions in invasive *Spartina* acreage in the coming treatment seasons.

Monitoring program capabilities have significantly improved since 2001, enabling a more extensive and more precise recording of *Spartina* location data in 2004 and 2005. The ISP monitoring program will continue to update monitoring methods to adapt to the best available technology and practices to ensure accurate monitoring of *Spartina* population change over time. Staff training in plant identification will continue to ensure correct identification of *Spartina* and other, non-target marsh species for both inventory and treatment efficacy monitoring. New and existing partnerships with be nurtured to enhance the ability to detect new invasive *Spartina* populations, especially in new and remote areas of the Estuary.

The addition of a treatment efficacy monitoring component in 2004 and 2005 has enabled quantification of treatment method success at control sites around the Estuary. Expansion of treatment efficacy monitoring from 22 to 44 sites in 2005, with expected follow-up in 2006, will greatly increase our ability to detect differences in efficacy attributable to treatment method. With this expanded treatment monitoring we hope to have sufficient statistical power to also detect changes in non-target plant species within monitoring plots.

Based on the analyses and findings presented in this report, the ISP concludes the following:

Monitoring Recommendations

- Add treatment efficacy monitoring sites to include greater replication of treatment methods among sites.
- Improve data collection methods to prevent collection of spatial data with incomplete collection of attribute values.

- Expand data quality control protocols to include checking of spatial (position) data by data collectors in addition to continued checking of attribute data.
- Calculate gross acreage following the protocol of Ayres et al. (2004), and include these results in future reports to allow comparisons with 2001 monitoring data.
- If possible, implement minimum patch threshold standards in the field and in digitizing efforts to improve comparability of total gross areas between years.
- Record and report absence data in future inventory monitoring efforts. (Absence data documents the location and extent of those areas searched where no invasive *Spartina* was found.)
- Continue and expand genetic testing of *Spartina* plants for species identification and confirmation.

Control Recommendations

- Continue an aggressive control approach for all four species of non-native *Spartina* in the Estuary to reverse their spread and start to reduce net *Spartina* acreage.
- Encourage ISP partners to incorporate imazapyr into the Control Program in areas where the Site-Specific Plans call for use of herbicide in the initial control efforts.
- Evaluate efficacy of ground-based imazapyr application.
- Discourage use of glyphosate to control *Spartina* due to the low efficacy consistently documented around the Bay.
- Utilize the highly effective manual control methods (digging and covering) where appropriate to reduce dependence on herbicide while still ensuring a high rate of efficacy.
- Consider mowing only as a seed suppression tool if full treatment cannot be implemented immediately. Mowing may be helpful for preparing a site for covering, but it will not work on its own to control Spartina.

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