Pulling Together: Managing Invasives 2010 - Submit Final Programmatic Report (Activities)
Grantee Organization: Humboldt Bay Harbor, Recreation and Conservation District
Project Title: Humboldt Bay Regional Cordgrass Eradication (CA)

Project Period	06/30/2009 - 06/30/2012
Award Amount	$59,925.00
Matching Contributions	$65,950.00
Project Location Description (from Proposal)	South Humboldt Bay, California, within the Humboldt Bay National Wildife Refuge and Bureau of Land Management lands.
Project Summary (from Proposal)	Eradicate dense-flowered cordgrass on over 100 acres of salt marsh and develop a regional eradication plan through associated mapping, outreach, and research activities. Project will result in the restoration of native salt marsh communities in the Humboldt Bay region through the eradication of dense-flowered cordgrass and increased public awareness of the value of salt marsh and the threat from invasive species.
Summary of Accomplishments	Spartina eradication efforts have been implemented throughout the Salmon Creek, Hookton Slough, and White Slough Units of the Humboldt Bay National Wildlife Refuge in South Humboldt Bay. 125 acres has achieved a 'maintenance' level status with minimal annual monitoring and treatment needed. Signficant research on the longevity of the Spartina seedbank was completed, and a Spartina symposium was held presenting research on spartina in Humboldt Bay. Comprehensive mapping of Spartina in Humboldt Bay was completed, and utilized heavily in the production of the Regional Spartina Eradication Plan, and the associated Environmental Impact Report. The Spartina eradication effort in Humboldt Bay has expanded dramatically in recent years, thanks in part to the support of the National Fish and Wildlife Foundation
Lessons Learned	Spartina eradication is a long term effort, and both the research and the eradication efforts supported by NFWF helped refine the eradication methods currently in use, and the planning and budgeting for full eradication in Humboldt Bay. We have found that retreatment at key seasonal points is critical to avoid substantial regrowth after the initial treatment. Similarly new seedlings emerge each spring, and rapid treatment of these seedling 'flushes' is much more efficient if performed quickly after emergence. While not a new lesson, the importance of widespread community engagement remains critical for this and similar projects. Spartina densiflora has been present in large amounts for over a hundred years in Humboldt Bay, so many residents and stakeholders respond negatively to its removal without significant educational efforts. Until the native marsh species return, eradication efforts leave what appears a barren surface, which triggers a negative response that the project has to be prepared and sympathetic to, even as we educate the community about the benefits of the project.

Conservation Activities	Acres of Dense spartina treated to maintenance level
Progress Measures	Other (Acres)
Value at Grant Completion	125
Conservation Activities	Public Outreach (Spartina Symposium, Public Volunteer Days)
Progress Measures	Other (Number of Events)
Value at Grant Completion	4

Conservation Activities	Regional Mapping of Spartina to support Eradication Planning
Progress Measures	Other (Regional Mapping Completed)
Value at Grant Completion	Complete

U.S. Fish and Wildlife Service
Humboldt Bay National Wildlife Refuge
Humboldt County, California

Spartina Densiflora Invasion Ecology and the Restoration of Native Salt Marshes at Humboldt Bay National Wildlife Refuge

Prepared by: Andrea J. Pickart, Ecologist
Humboldt Bay National Wildlife Refuge
Arcata, CA 95521

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EXECUTIVE SUMMARY

This report documents activities undertaken through a series of grants awarded to Humboldt Bay National Wildlife Refuge between 2006 and 2011. The common goal of these grants was to contribute to a protocol for controlling the invasive cordgrass *Spartina densiflora*, which has proliferated through the salt marshes of Humboldt Bay and adjacent estuaries. The individual grants focused on different components of *Spartina* control and salt marsh restoration, including research on invasion ecology/impacts that could inform the development of new control techniques and regional control strategies. Following are highlights of the results:

- 31 previously untreated acres of salt marsh on the Mad River Slough were restored through the removal of invasive *Spartina densiflora*.
- Mature plants were killed in 1-2 years through the application of a subsurface "grind" technique using a tri-blade brushcutter applied directly on the shallow rhizomes. Resprouts were treated at approximately 6-month intervals.
- Above-ground parts of plants were mowed, raked and burned or removed, however a new method of "mulching" the above-ground material with the brushcutter now eliminates this step.
- Dense *Spartina* seedling flushes emerged after the first treatment (mean 240/m^2), and were flamed or removed with brushcutters. Subsequent seedling emergence was much lower. Seedling density in the first year was positively correlated with cover of *Spartina* prior to treatment. This initial flush of seedlings may be emerging from the seed bank, and a "deep" grind (4-6 in) has subsequently been shown to minimize seedling emergence and eliminate much of the seed bank.
- A successional trend during restoration was documented, in which bare areas resulting from treatment became colonized first by filamentous algae (first winter following treatment). Native vascular plants, especially pickleweed, began colonizing the first summer after treatment and significantly increased in cover during the second summer. Canopy closure was achieved between 2 and 4 years after initial treatment.
- *Spartin*a seedling emergence following treatment was positively correlated with algal mats, which may have reduced desiccation in the spring.
- Application of a first treatment in summer (vs. winter) resulted in fewer *Spartina* resprouts but more *Spartina* seedlings. Pragmatically, timing of treatment is more likely to be a function of crew availability and site accessibility. Sites are far more accessible in summer due to tides, and weather is more suitable.
- Revegetation can be accomplished using "plugs" of native salt marsh dominants (pickleweed or salt grass) planted at any time between December and April. Both pickleweed and salt grass exhibited extremely high survivorship. Earlier transplants resulted in more rapid canopy closure, and canopy composition shifted to predominance by pickleweed by the end of the first summer after planting.
- Canopy closure occurred in all areas, included those not planted, by year 4, suggesting that revegetation is not a required step in marsh recovery.
- Arrowgrass, a brackish and high marsh plant, appeared resistant to the mechanical treatment, resprouting from rhizomes vigorously in treated areas in the first spring

after treatment, and accelerating vegetation recovery. However, this species was largely confined to areas with freshwater input.

- In areas without freshwater input, pickleweed was the dominant colonizer, emerging from seed in the first or second summer after treatment. Salt grass was observed to recruit only vegetatively from established stands bordering controlled areas, but even in these areas pickleweed was often the first colonizer.
- The rare salt marsh annual Humboldt Bay owl's clover responded dramatically and positively to restoration, with the population in the restored area increasing from approximately 3,000 individuals pre-restoration to over 99,000 five years post-restoration.
- Continued maintenance to remove newly established plants will be required until regional eradication is completed.
- *Spartina densiflora* has extremely high fecundity (35-47 million seeds/ac), and a persistent seed bank lasting at least two years. Viable seed in the seed bank was reduced at most sites after two years (when replenishment was prevented) but remained the same in the site characterized by the densest seed bank. Seed bank studies will continue in order to determine longevity.
- Seed bank density, ranging from an average of 100 to 3,805 seeds/m^2 of surface area in the first year, was strongly correlated to above ground abundance of *Spartina*, suggesting that seeds may primarily enter the bank at the site of seed production.
- Top mowing of dense *Spartina* can be used to suppress seed production when complete control isn't feasible. Mowing in July completely suppressed seed production, mowing in April reduced it by 90%. Top mowing resulted in increased native cover, but also increased seed production in the second year. These results suggest that annual mowing would be needed for this method to be effective.
- *Spartina* continued to increase in density in the control plots at one experimental restoration site over a two year period, indicating that the invasion was still in progress, even in moderate to dense areas. There are many areas around Humboldt Bay where *Spartina* can be observed spreading to new areas, but this study suggests that it is continuing to increase in density even in areas where it appears to be fully established.

ACKNOWLEDGMENTS

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U.S. Fish and Wildlife Service Coastal Program
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Humboldt Bay Harbor, Recreation and Conservation District

The work documented in this report is the product of contributions by many individuals and a few organizations, whose dedication and perseverance are worthy of high praise. Following is an alphabetical listing of those who contributed. My apologies for any oversight/omission I may have made.

Armando Aispuro, Simona Augyte, Ben Bridegroom, Bethany Baibak, California Conservation Corps, California Department of Forestry and Fire Protection (High Rock Camp), Adam Canter, Ana Canter, Ryan Cecil, Patti Clifford, Andrea Craig, Desiree Davenport, Ashley Dickinson, Annie Eicher, Michelle Fuller, Friends of the Dunes, David Garza, Joel Gerwein, Laurel Goldmsith, Paula Golightly, Trevor Goodman, Jessica Graff, Zack Grazul, Kira Hawk, Erik Jules, Rebecca Langhans, Heather Lagarde, Luc Lagarde, Gillian Levy, Steve Lewis, Sara March, Matt Mitchell, Eric Nelson, Britney Newby, Scott Owen, Sheldon Owen, Rose Patenaude, Kate Proctor, Kristina Prosser, Heather Rickard, Peter Rowland, Conor Shea, Mike Swank, Ellen Tatum, Mark Thom, Adam Wagschal, Emily, Walter, Derek Weatherbee, and Jason Zito

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INTRODUCTION

Spartina densiflora is a South American cordgrass that has aggressively invaded salt marshes at Humboldt Bay and Eel River estuary. The species was introduced to Humboldt Bay in the late 1800s (Kittelson and Boyd 1997), and has since been transported or dispersed to a number of salt marshes between San Francisco Bay, California and the Strait of Georgia, British Columbia. Because of the severe ecological impacts posed by invasive members of the genus *Spartina*, political leaders from California, Oregon, Washington and British Columbia have jointly committed to eradicate all non-native populations of the genus *Spartina* along their coasts by 2018 (WCGA 2008, Pacific Coast Collaborative 2010).

Between 2002 and 2004 staff at Humboldt Bay National Wildlife Refuge carried out the first experimental control project targeting *S. densiflora* on a 4-ac (1.6-ha) salt marsh island in the Lanphere Dunes Unit of HBNWR (Pickart 2005). Based on the success of this effort, a larger, pilot restoration project was initiated in 2006 and eventually encompassed 12.5 ha (31 ac) on the Lanphere and Ma-le'l Dunes Units (Pickart 2008). In 2008 a research component was added to the restoration program, funded by the State Coastal Conservancy. Research goals included the refinement of control techniques, documentation of restoration impacts (both positive and negative) and a further understanding of *Spartina densiflora* invasion ecology. Based on the success of these pilot projects, in 2010 the USFWS funded the control of *S. densiflora* within the entire refuge for a cost of $1 million (USFWS 2011a). Monitoring and experimental elements of the larger project will yield even more insights needed to accomplish regional eradication. This report is divided into two sections, the first (Pilot Restoration) detailing the result of restoration efforts that began in 2006. The second part (Research Results) documents the results of research projects carried out from 2008 to 2012.

I. PILOT RESTORATION

LOCATION

Restoration was carried out on a total of 12.5 ha (31 ac) of salt marsh in the Mad River Slough within the Lanphere and Ma-le'l Dunes Units, Humboldt Bay National Wildlife Refuge, California (Fig. 1). Restoration consisted primarily of control of *Spartina densiflora*, however, revegetation techniques were also tested in several areas.

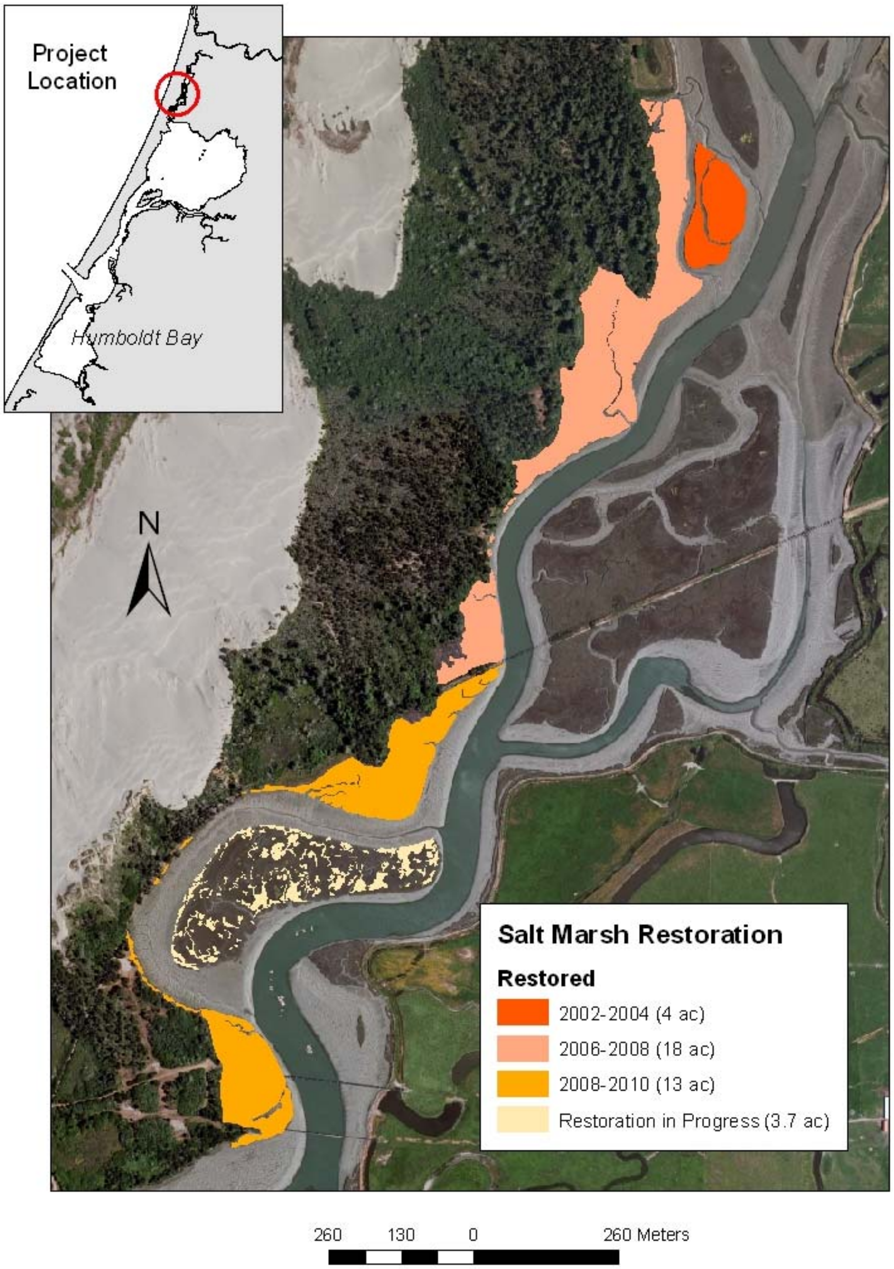


Figure 1. Location of *Spartina densiflora* control, restoration are covered by this report was carried out from 2006-2010 and covers 12.5 ha (31 acres).

METHODS

Mapping Methods

Baseline mapping of *Spartina* on the Lanphere and Ma-le'l Dunes Units was carried out from June through August of 2006 prior to the start of restoration. Occurrences of *Spartina* were mapped on the ground by streaming data with a Trimble GeoXT. Mapping relied on a combination of polygons for continuous occurrences, lines for linear occurrences, and points for isolated occurrences of a single or a few tightly spaced individuals. A minimum mapping unit was not defined due to the logistics of working in areas bisected by many tidal creeks. All polygon boundaries and linear occurrences were walked. All mapped features were attributed with cover/abundance data consisting of two cover classes: less than 60% cover, and greater than or equal to 60% cover. After completion, spatial data were checked for quality, and topology was cleaned to eliminate any incomplete or overlapping polygons.

Monitoring Methods

Several different types of monitoring were utilized for different aspects of the project. In addition to baseline mapping of *Spartina* and quantitative, plot-based sampling, photo-points were used to document the site prior to and throughout the project. Photo-monitoring is an effective means of assessing the overall quality of the site, and is particularly effective for outreach, as the change depicted can be dramatic.

All quantitative, plot-based monitoring methods are detailed in the Monitoring Plan (USFWS 2009a). Within the plan are stated management (restoration) objectives as well as the monitoring objectives tied to them. Monitoring covered response to *Spartina* removal as well as to revegetation efforts. The monitoring plan was revised over time to accommodate changes in management applied through the process of adaptive management. Following is a summary of the different components of monitoring.

Overall response to *Spartina* control

To follow overall salt marsh recovery following the removal of *Spartina*, a series of permanent baselines were established along the east and west edges of the northern Lanphere salt marsh (Fig. 2). A .5-m x .5-m gridded quadrat was placed at intervals of 10 m along transects spaced at 10 m, for a total sample size of n≈180 encompassing 4.2 ha (10.3 ac) of salt marsh (total plots varied slightly by time interval due to the random start). The plots were monitored twice per year, in spring and fall, between spring 2007 and 2010, with a final sample carried out in summer 2011. The variables recorded depended on monitoring interval, since the interest shifted over time, but two variables were consistently collected: percent cover by species, and density of *Spartina* culms. Cover was measured by visual estimation in six cover classes over the entire plot, using the grid to assist w estimation. Density of *Spartina* (culms) was measured in a subplot consisting of four of the gridded subdivisions, or .04 m^2. At the beginning of the project, density of resprouts was recorded separately from that of seedlings for both spring and fall intervals, but after the first year it became difficult to discern older juveniles from resprouts in the fall, and only total *Spartina* density was recorded for fall monitoring intervals. During spring monitoring intervals *Spartina* seedlings could be distinguished from resprouts or juveniles, and were recorded for all years through 2011. In the earlier time periods, cover of bare mud, filamentous algae, and wrack were also recorded. Filamentous algae became too difficult to measure as the vascular plant canopy closed, and this measurement was later omitted.

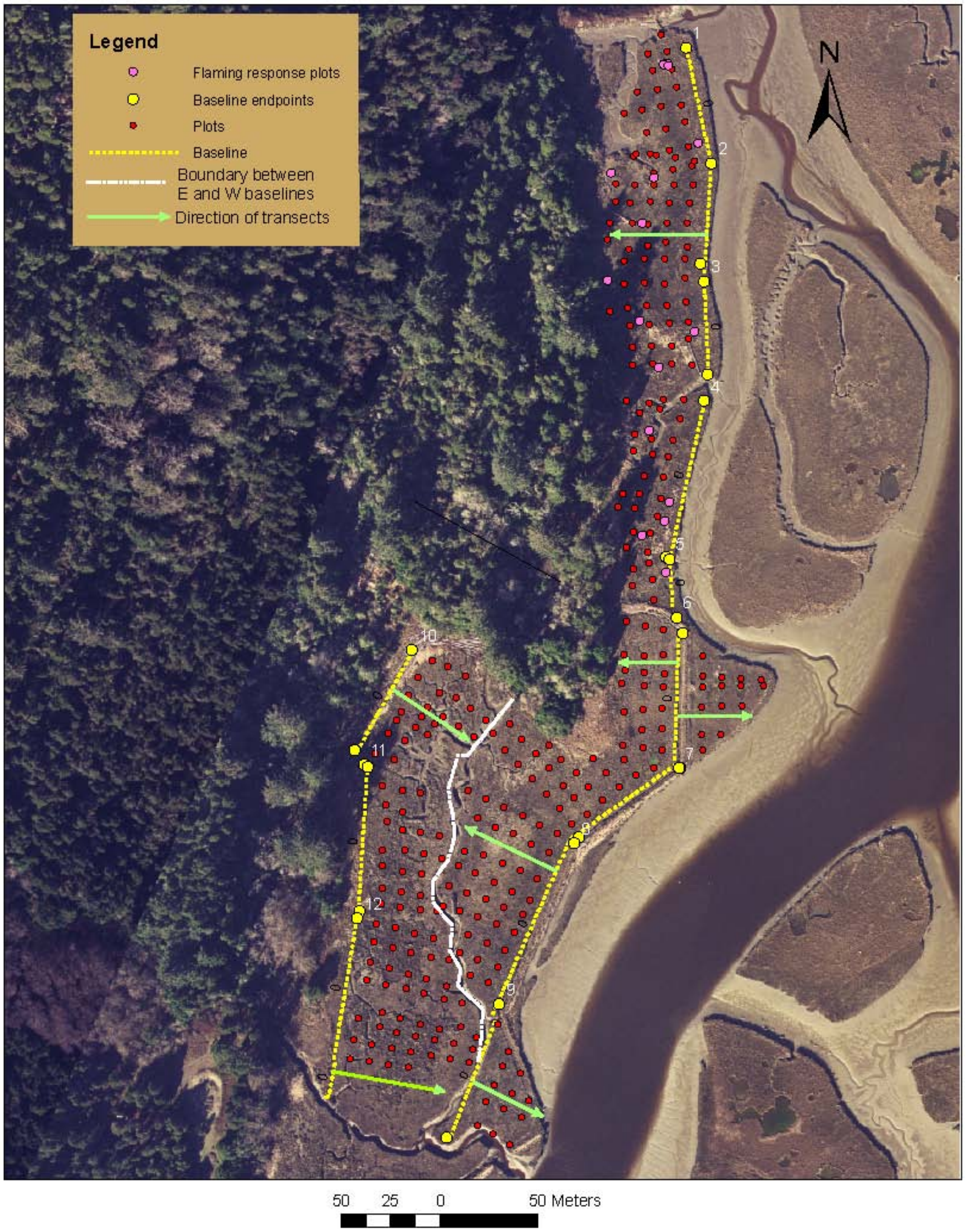


Figure 2. Layout of monitoring transects and seedling plots.

A separate sample (n=15) was established to follow the fate of flamed *Spartina* seedlings, and to measure the response of native plants to flaming. The same size plots were used, but were placed subjectively in order to capture seedling presence (seedling and native species response to flaming, n=10) and absence (to compare change in native cover, no treatment, n=5). These permanent plots were sampled monthly from the end of April through the end of October, 2007 (Fig. 2). Variables measured included percent cover by species (by size class), percent cover of mud, filamentous algae, and wrack, and cover and density of *Spartina* seedlings and resprouts (culms).

Response of Rare Plants

One of the concerns identified for *Spartina* control was the potential for detrimental impacts to the two rare salt marsh annuals, Humboldt Bay owl's clover (*Castilleja ambigua* ssp. *humboldtiensis*) and Point Reyes bird's beak (*Cordylanthus maritimus* ssp. *palustris*). These plants are characteristic of the high elevation marsh plain, and are also abundant at the margins of salt marsh habitat, either along the edges of abandoned dikes or at the ecotone between salt, brackish and freshwater marsh. Both of these niches are also colonized by *Spartina*. The refuge already had a monitoring program in place for these taxa that allowed indirect assessment of impacts of *Spartina* removal (USFWS 2009b). The *Castilleja* monitoring program had a component that included mapping and censusing all *Castilleja* occurrences on the mainland salt marsh (possible even when *Spartina* was abundant due to the very visible coloration of the plant). This sampling program was continued throughout the restoration project. Mainland occurrences were mapped with a Trimble Geo-XT, or drawn on printed air photos and later heads-up digitized. Individuals were counted, or for very large occurrences, the total was visually estimated incremementally and associated with the spatial record. Monitoring was conducted during peak flowering, usually between mid-May and mid-June.

Spartina Control Methods

Initial Treatment Methods

When on-the-ground restoration started in July 2006, the treatment method was one that had been developed during a previous experimental restoration on the 4-ha (10-ac) salt marsh island of the Lanphere Dunes Unit (Pickart 2005). The island consisted primarily of high elevation marsh, with dense *Spartina* confined to the island margins and edges of tidal creeks. Stands of dense *Spartina* were much smaller than on the mainland marsh that was the focus of this larger restoration project. For the small island occurrences, a corded weedeater was used successfully over a two-year period to control *Spartina*. Plants were repeatedly mowed to the marsh surface and eventually died. In the newer, larger project, the corded weedeater was quickly found to be inadequate for mowing the dense stands of *Spartina* found in this lower elevation marsh, and was replaced with a three-pronged ("triblade") metal-bladed brushcutter. The brushcutter is heavier and requires a harness and safety equipment. First, restoration crew members top-cut the *Spartina* using a side to side motion (referred to as "sweeping"), a process that generates a large amount of wrack which must later be raked and burned or removed. With a metal blade, the restoration crew found that they could apply the blades slightly below the surface, slicing into the rhizome repeatedly at increasing depth. This method was referred to as "progressive cut." Over time, a new method evolved that became known as the "grind" method. After cutting above-ground stems and leaves the blade on the brushcutter is rotated and applied such that the plane of the blade is tilted as it comes in contact with rhizomes, and the rhizomes are ground into small fragments (Fig. 3). This method results in a large amount of debris (mud, plant fragments) that is flung into the air Most of the Lanphere Dunes Unit salt marsh was treated initially with the progressive cut method, but resprouts were later treated with the grind method. The Ma-le'l salt marsh was treated primarily with the grind method from the beginning. Since the project was completed, crewmembers have further refined the treatment, using smaller "mulching" motions on above-ground biomass to reduce accumulation of wrack and eliminate the need for its disposal. Experiments are currently underway to determine optimal grinding depth to increase efficiency (fewer visits to treat resprouts) while minimizing impacts due to disturbance and elevation loss (USFWS 2011b).

Figure 3. Orientation of three-pronged metal brushcutter blade. The area shown is relatively low in *Spartina* cover, so there is not as much debris being ejected as when this treatment is used in dense *Spartina*.

Labor for restoration was from a variety of sources. Throughout the project, a crew of 2-5 contractors was employed. The number of the crew varied with season, availability of labor, and funding. New crewmembers were trained by experienced members to run brushcutters, refuel, and make repairs. At times, when labor was crucial and/or funding or contributed labor was available, the experienced crew supervised California Conservation Corps (CCC) or California Department of Forestry and Fire Protection (CDFFP) crews. Safety training was provided for all crewmembers. Crews had to walk to the worksite carrying equipment. Restoration on the Lanphere marsh required a walk of 1.2 km (0.8 mi) from the refuge office to the marsh access point, and then up to 0.5 km (.3 mi) to reach the targeted area. Crewmembers returned once per day to refuel. Parts of Ma-le'l marsh were closer to its parking area, but again, to reach a given restoration site workers walked up to 1.2 km (.75 mi). The walking time was therefore a significant portion of overall labor. Portable toilets were placed at the office (Lanphere) and parking area (Ma-le'l).

The timing of the initial treatment in the majority of the area was dictated strictly by funding and logistics. The Lanphere (northern) salt marsh was begun in summer 2006, while the Ma-le'l (southern) marsh was begun in fall 2008. However, by 2008 additional funding had been obtained to study the effect of treatment start date, so some experimental areas in Ma-le'l had a delayed start date (see Part II). The time it took to complete the initial treatment was also a function of funding and labor availability. During the period 2006-2011 the number of mowers varied from 2 to 5. During most of the restoration period there were two mowers working at a time.

Wrack Disposal Methods

Wrack created during the first months of treatment in summer through fall 2006 was initially left in place. Wrack occurs in all *Spartina*-invaded marshes, and tends to accumulate at the upper edges of the marsh during seasonal high tides. After monitoring the extent of wrack in spring 2007, and finding a negative relationship between wrack and native species recovery (discussed later under Results), this approach was modified. Wrack was raked in April 2007 (primarily by CDFFP crews), piled, and burned during summer months when tides were lower. *Spartina* occurring in the Ma-le'l marsh, first treated in fall 2007, was raked and piled in winter 2008. At this season, the wrack was too wet to burn. Piles were hauled on tarps to the adjacent upland railroad berm and the Humboldt Bay Municipal Water District pipeline landing, and piled. A refuge equipment operator was assisted by a CCC crew in moving the large piles into a dumptruck using a front end loader (Fig. 4). The wrack was transported to a composting facility. This method of disposal was costly, and the use of the "mulch" method on above-ground biomass has since eliminated the need for disposal.

Figure 4. Front end loader moving *Spartina* wrack on to dump truck.

Seedling Treatment Methods

Following the initial treatment in summer and fall 2006, a large amount of bare mud was exposed. Based on this and subsequent *Spartina* treatment, it is apparent that a flush of *Spartina* seedlings typically occurs in the first spring (approximately March) following treatment. Seedlings are particularly dense in areas where freshwater input is high. Studies are underway to determine what proportion of this seedling flush derives from the seed bank (see Research section) as opposed to seed rain/dispersal from the preceding fall and winter. For this project, a large seedling flush was observed in the spring of 2007 in the Lanphere marsh, with extremely high densities occurring locally. The abundance of seedlings was estimated during the first interval of the monitoring design described above, and a smaller sample (see above) was monitored in order to measure response to flaming treatment. The flaming treatment consisted of backpack propane torches used to burn seedlings (Fig. 4). When seedlings occurred intermixed with returning or relict native vegetation the flame was applied over the entire area. In subsequent years, after native vegetation recovery had progressed, seedlings were sparser and more localized. Treatment of seedlings during this point in the restoration shifted to use of the brushcutter, but using the sweeping rather than the grinding technique. In most cases seedlings can be "nicked" out with the blade, minimizing disturbance.

Figure 4. CDFPP crews use backpack propane torches to flame dense seedling areas.

Resprout Treatment Methods

After the first summer or fall mow, each marsh (Lanphere and Ma-le'l) was treated twice per year to remove resprouts. The timing of, and interval between, treatments varied somewhat due to uneven funding or labor availability, and localized portions of the marsh probably received more or less frequent treatments. Resprouts were removed with brushcutters, but it was much easier to target individual plants once the dense above-ground biomass was gone, and no wrack removal was needed. In a few areas that proved resistant to the brushcutter treatment, (particularly upland margins with a sandy substrate), shovels were used to remove rhizomes. In general, the first (spring) resprout treatment was much more time consuming, and by the third year, the fall treatment was done with trowels, with plants were bagged and transported off site. Since 2011, only one treatment has been needed per year.

Revegetation Methods

When the project began, there was little literature regarding the need for revegetation in salt marshes in our region. Past restoration projects involved restoring tidal influence without considering vegetation, with the result that *Spartina densiflora* dominated all sites soon after restoration (Kittelson and Boyd 1997). Experiences with salt marsh restoration in other parts of California and the Pacific Northwest indicated that *Salicornia* could readily colonize restored marshes, but that other species might need to be intentionally introduced (Sullivan 2001, Thom et al. 2002). Since this project involved denuding existing salt marshes, erosion was a potential concern. When it was observed that extensive, bare areas persisted after the first summer, revegetation experiments were initiated in the following winter. Revegetation also offered an appropriate vehicle for volunteer involvement. The brushcutter technique was too dangerous for volunteers, but it was still a goal of the project to engage the local volunteer force both as an outreach tool and to garner support for the project.

Revegetation experiments were carried out in the winter and spring of 2007-2008, from December to April. Most of the literature reviewed recommended early winter for revegetation, but given the long rainy season in coastal northern California, it was hypothesized that later plantings might be successful. At the time of this project, a site in the southern refuge that supported salt marsh vegetation behind dikes as a result of leaky tidegates was planned for increased connection to the a river channel. The increased freshwater influence was expected to cause halophytic vegetation to convert to brackish/freshwater species. A portion of this salt marsh vegetation was salvaged for use in revegetation on the north refuge.

Material was harvested two ways. At first, blocks of salt marsh vegetation were removed manually with shovels and placed in plastic buckets. Later, a refuge equipment operator removed larger blocks using a backhoe and placing them on the dike, after which they were loaded by hand into a 4-wheel-drive pickup. In both cases, harvest areas were selected that appeared to be a single species monoculture of either *Salicornia* or *Distichlis*. The excavated blocks were sawed into smaller chunks, and material was then transported to the Ma-le’l parking lot, where it was loaded into an ATV and taken via trail to the upland disturbed meadow adjacent to the Lanphere salt marsh. This was accomplished in the week prior to a given revegetation volunteer day. Blocks were covered with tarps to prevent desiccation. The day before the volunteer day, the crew would cut the larger chunks into blocks approximately 13 cm (5 in) square, filling buckets with one of the two species and labeling it accordingly (Fig. 5).

A total of five revegetation experimental sites were subjectively chosen on the Lanphere marsh in areas of persisting bare mud in December 2007 (Fig. 6). The first site was considered a test site to work out logistics. By the second date the crew had developed a protocol. They would measure and pin-flag a planting grid on the marsh, labeling each flag by species. Species were arranged systematically through the planting sites, without considering elevation. Elevation data were not yet available, although the pre-project, high density of *Spartina* was an indication of low elevation for all bare areas. This approach would allow for sorting out of species by individual tolerances. Plugs were grouped by species into sets of four (Fig. 7). Corner points of each planting area were marked with rebar. When volunteers arrived for the planting day, they were first given an introduction, and then they placed the pre-cut plugs at flags (for some events the crew did this step). Volunteers opened a hole with a shovel, placed the plug, then closed and tamped the hole. When there was excess mud from the hole it was placed nearby on bare mud. Planting days were held on December 12, 2007 (dry run), January 19, March 18, April 13, and April 26, 2008. After planting, the crew would quality check the site, recording exactly which species were planted where to facilitate follow up monitoring. Most of the volunteer days were sunny and/or dry, one was cancelled and rescheduled. During the workday, planks were placed over especially mucky areas to make it easier for volunteers to negotiate.

Figure 5. Crewmembers place plugs in plastic buckets for volunteers to carry to marsh.

Monitoring consisted of tracking survival of plugs and recovery of vegetation over the entire plot. Four additional bare, low-elevation areas were designated as control areas so that the role of the propagules could be discerned in the vegetation recovery process (Fig. 6). To determine survival of propagules, each site was visited monthly for

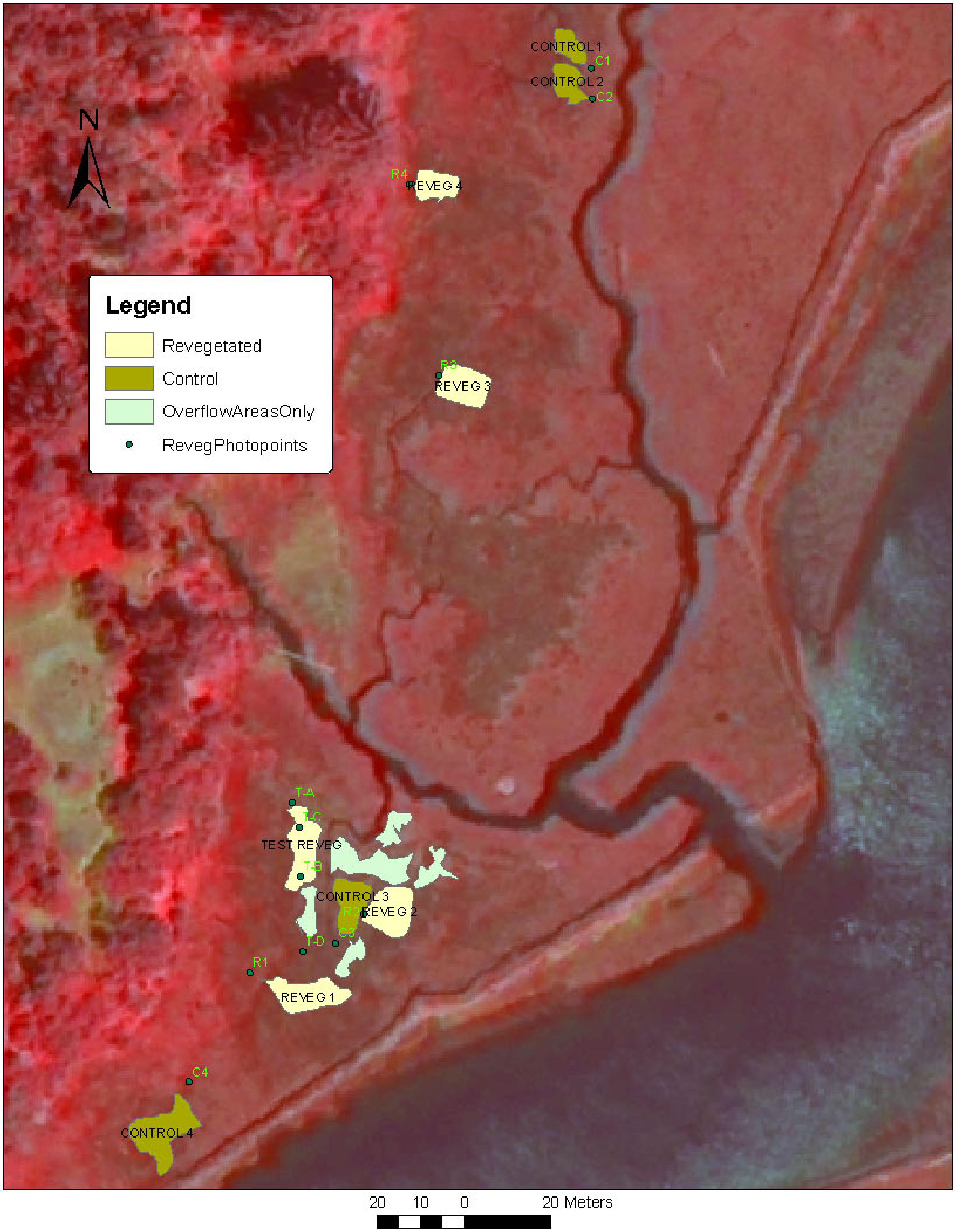
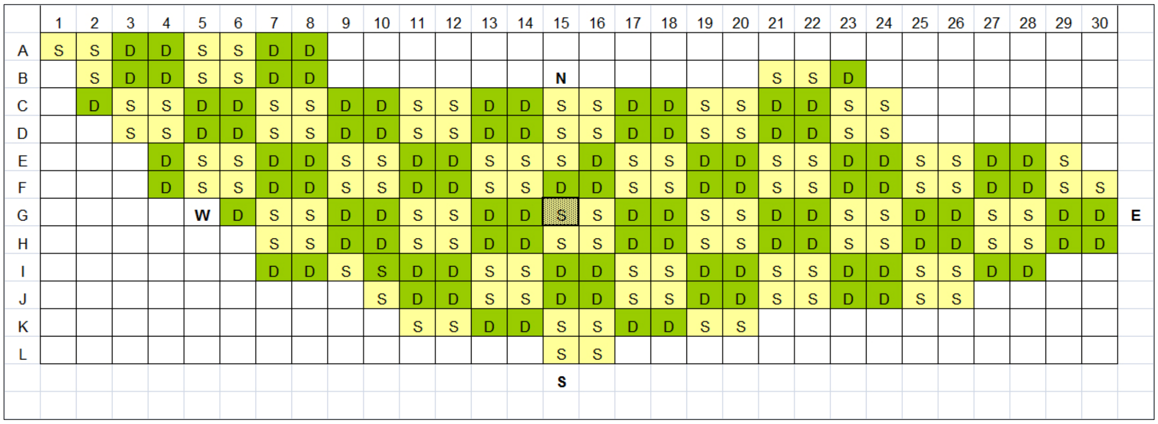


Figure 6. Location of experimental revegetation areas.

the first growing season after plugs emerged from dormancy (the date of emergence from dormancy differed among planting dates). A printout of the planting grid was used by the sampler to facilitate location of original plugs in relation to rebar (Fig. 7). The plugs were easy to identify because even by the end of the season the original shape of the plug was discernable at the surface. After the first year no attempt was made to distinguish plugs using quantitative monitoring. Recovery of salt marsh vegetation in revegetated and control areas was monitored using .5m x .5 m quadrats placed systematically in relation to the axes defined by corner rebar markers. Spacing was varied to result in a sample size of 30 for each planting/control area. Total cover and cover by species were recorded using visual estimation and cover classes. Vegetation sampling was first carried out in May 2008 and repeated in September 2008. In October 2008 abiotic sampling was conducted. A total of 12 abiotic samples were placed systematically in each revegetation area. Total Station elevations were recorded for each sample. Soil cores were also collected of the top 20 cm (8 in) of soil. Cores were bagged, labeled and sent to A&L Western Laboratories (Modesto, California) for analysis of organic matter, salinity, and pH.



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H							S	S	D	D	S	S	D	D	S	S	D	D	S	S	D	D	S	S	D	D	S	S	D	D	
I							D	D	S	S	D	D	S	S	D	D	S	S	D	D	S	S	D	D	S	S	D	D			
J										S	D	D	S	S	D	D	S	S	D	D	S	S	D	D	S	S					
K											S	S	D	D	S	S	D	D	S	S											
L															S	S															
															S																

Figure 7. Layout of revegetation Plot 1. S=*Salicornia pacifica*, D = *Distichlis spicata*. The shape of the plot is determined by available bare area that not dissected by tidal creeks.

RESULTS AND DISCUSSION

Spartina Mapping Results

A total of 10 ha (25 ac) of *Spartina* was mapped as continuous occurrences in the project area (Fig. 8). An additional 339 m^2 (0.1 ac) was mapped as linear occurrences, assuming a mean width of 1 m. Of the total area, 1.2 ha (3.0) ac was characterized by cover values over 60%. Although mapping was carried out over the islands within the refuge boundary, these results aren't displayed.

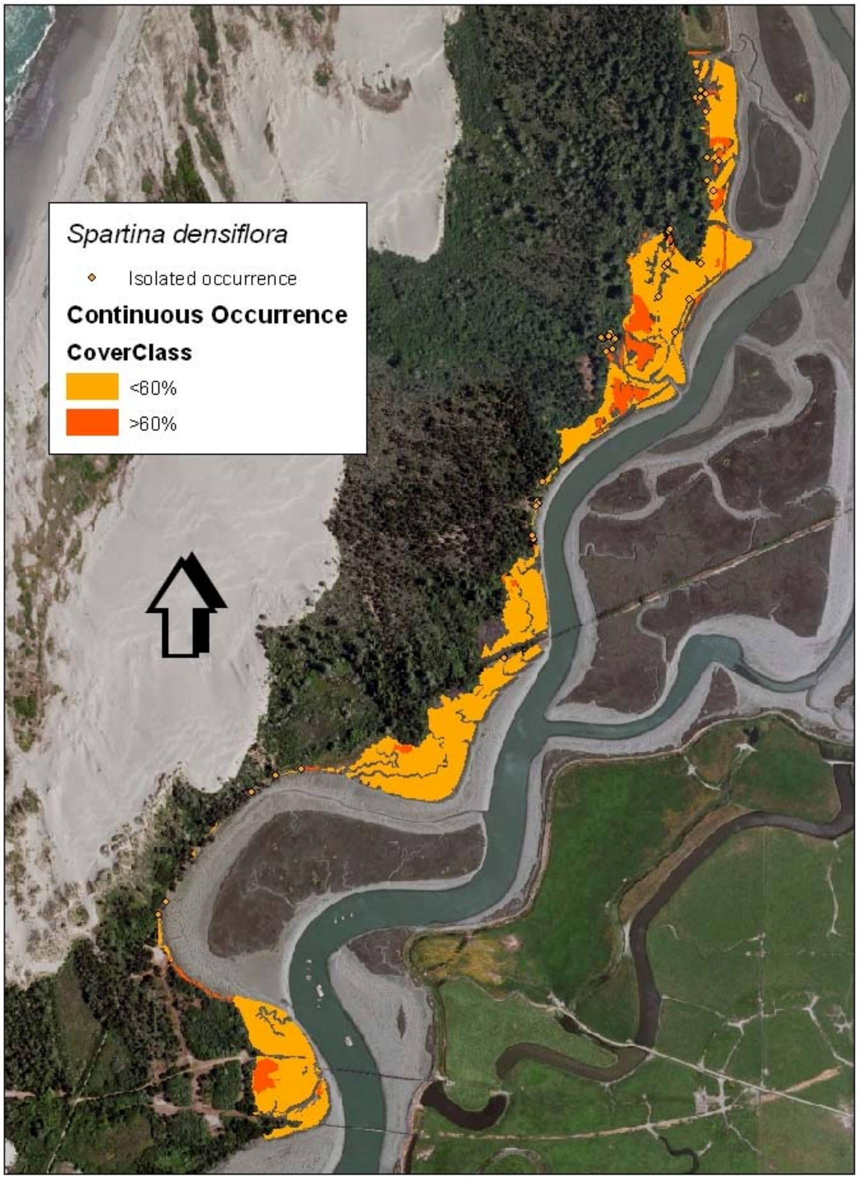

Figure 8. Distribution of *Spartina densiflora* prior to restoration.

Spartina Control and Native Species Recovery Results

The success of *Spartina* control over the entire project area was dramatic (Figs. 9-10). Quantitative sampling of *Spartina* density was not carried out prior to the first treatment; however, the subsequent four years of monitoring illustrate the recovery of the marsh vegetation.

Figure 9. Dense *Spartina densiflora* prior to treatment, April 2007.

Figure 10. Same area as above two years later (June 2009).

Figure 11 depicts the change in *Spartina* density (all seedlings and resprouts combined) as well as changes in percent cover of dominant species, filamentous algal mat, and wrack between 2007 (post-treatment) and 2010. The area sampled (see Fig.2) was treated initially between July 2006 and Feb. 2007. At the time of the first post-treatment monitoring (April 2007), the high density of *Spartina* (mean 62.2/.25m^2, SE 9.3) was highly influenced by seedlings that emerged in March 2007. Treatment of seedlings and resprouts during the summer of 2007 resulted in a dramatic decline in *Spartina* total density (seedlings plus resprouts) by spring 2008 (from 62.2 to 3.1/.25m^2). It is not possible to pinpoint when all original (pre-restoration) *Spartina* plants were killed because of the difficulty discerning older juveniles from resprouts. However, based on observations that included examining root and rhizomes of remaining plants, it appears that the vast majority of *Spartina* in the area first treated in summer 2006 through spring 2007 was dead by the end of 2008. This conclusion is borne out by monitoring. The graph in Figure 11 shows there was very little reduction in *Spartina* density after 2008; recruitment from seedlings resulted in the flattening of the mortality curve.

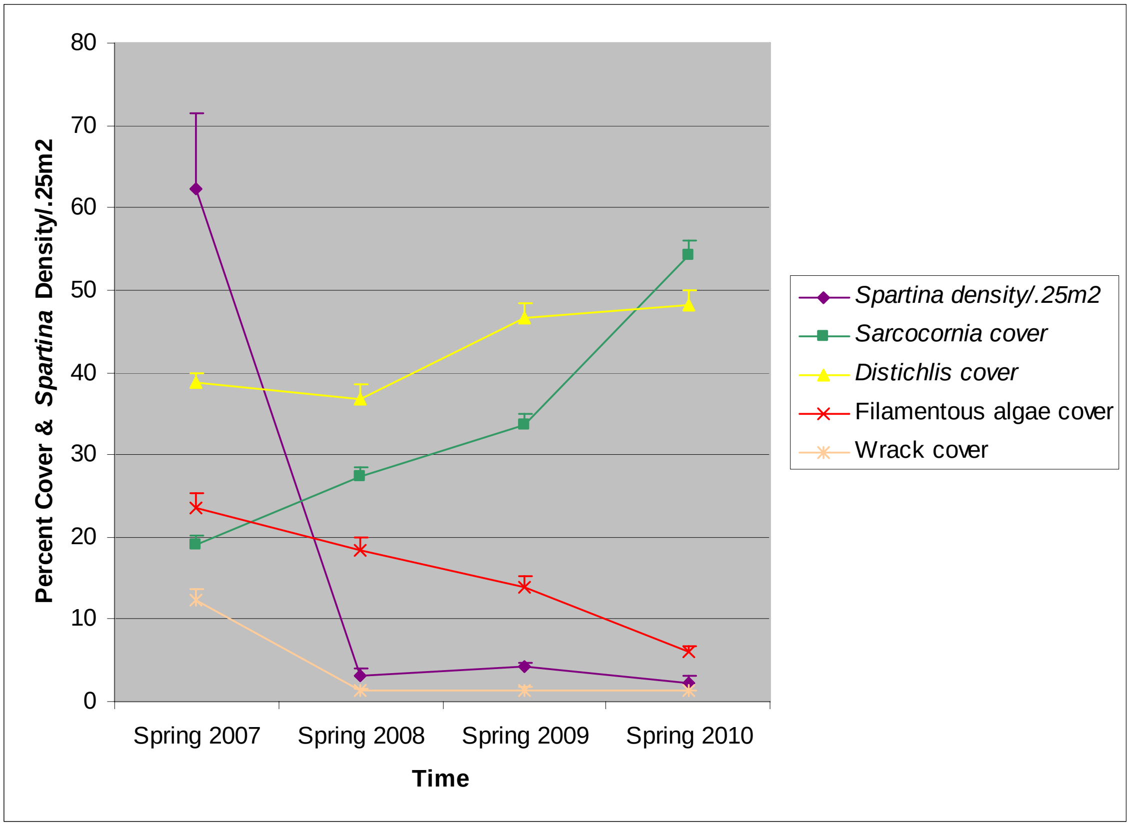

Figure 11. Changes in total density of *Spartina* culms (seedlings plus resprouts), and total cover of native dominants, filamentous algae, and wrack from 2007-2010 (pre-treatment density was not measured; the first measurement in spring is post-treatment, note that density is per quarter square meter).

Because seedlings can be missed by mowers and become adults before being detected and removed, the resprouts detected after 2008 represent new plants established after the initial treatment. By 2011, all *Spartina* seen in the spring sample were seedlings (Fig. 12). Exceptions observed outside the sampled area were a small area of sandy substrate where the *Spartina* had moved from the salt marsh to an adjacent dune, and a few margins of tidal creeks where *Spartina* still persisted. The latter phenomenon could have been due to greater vigor along tidal creeks, difficulty mowing *Spartina* adequately along steep unstable creek banks, or a combination.

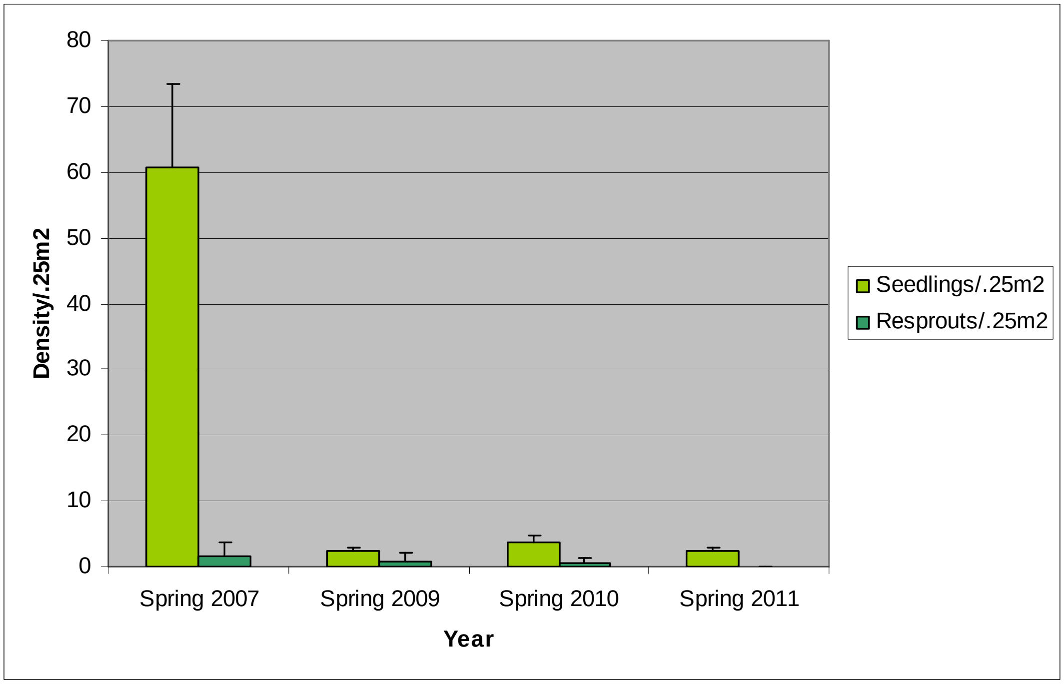

Figure 12. Changes in *Spartina* resprouts and seedlings/.25 m^2 from 2007-2011.

One conclusion of the initial sampling in April 2007 was the potential role of wrack in suppressing native plant recovery. A significant negative correlation was found between native species cover and wrack (r=-0.41, p <.05). Monitoring also shed light on successional trends during native species recovery (further discussed in the Research Results below). During initial stages of recovery, bare areas are colonized by thick mats of green filamentous algae, commonly *Rhizoclonium riparium* and *Chaetomorpha linum* (Fig. 13). As vascular plants recover, filamentous algal mats on the mud are displaced, although datioms and cyanobacteria become more prevalent later (Augyte and Pickart 2012). In the first spring following removal, in areas of high freshwater input (at the dune/salt marsh ecotone) *Triglochin maritima* responded vigorously to *Spartina* removal, exhibiting dense regrowth and expansion (Figs. 14 and 15). *Triglochin* was present prior to restoration but increased in the absence of *Spartina* through expansion by rhizomes. By the third year *Triglochin* cover had decreased in the sampled area as other salt marsh species increased but remained highest in freshwater-influenced areas. *Salicornia* began rapidly colonizing in the first spring after treatment, and increased significantly in cover every year through 2010, becoming the dominant species. Cover of *Distichlis* was much

Figure 13. Green filamentous algal mat covering mud in a treated area.

less dynamic, remaining static at first and then increasing slowly. The steeper increase in *Salicornia* can be attributed to two factors. First, *Salicornia* tends to occur at higher cover than *Distichlis* in the low marshes where *Spartina* is most abundant, thus it is disproportionately affected by control activities. In addition, *Salicornia* is known to be a prolific colonizer in salt marshes, and has been observed elsewhere to be the dominant species in recovering, restored California marshes (Sullivan 2001). Dense seedlings of *Salicornia* were observed in treated areas, even in areas of higher elevation marsh with surrounding vegetation dominated by *Distichlis* (Fig. 16). Conversely, few *Distichlis* seedlings were seen even in areas surrounded by *Distichlis*. The dominance of *Distichlis* observed during the monitoring in the spring following initial treatment (Fig. 11) is likely due to disproportionate removal of *Salicornia* (as opposed to *Distichlis*) habitat, but the subsequent dominance of *Salicornia* by 2010 is the combined result of the recovery of low marsh vegetation as well as *Salicornia*'s superior colonizing ability. Areas of bare mud persisted in the most hypoxic/anoxic areas through 2009, however by 2011 the canopy had closed over all but a few small areas (Fig. 17).

Over time, succession may result in a decline in *Salicornia*, with a concomitant increase in other salt marsh species. By 2010, *Jaumea carnosa* and *Limonium californica* cover significantly increased in cover (Fig. 15), although they are still a minor constituent of the marsh in the sampled area. *Spergularia marina* showed an initial increase from 2007 to 2009 followed by a decline in 2010, in keeping with the positive response to disturbance often shown by this species. The nature of the brushcutter treatment increases topographic heterogeneity. Beginning in 2009, retreatment of new juveniles was done using the grind method, which penetrates more deeply than the progressive cut method. As a result, the marsh becomes punctuated with localized low spots. Topographic heterogeneity in salt marshes has been shown elsewhere to increase species richness (Zedler et al. 1999), and some authors recommend planting multi-species assemblages in salt marsh restoration projects (Morzaria-Luna et al. 2004). Additional research and monitoring is needed to determine whether, over time, the topographic heterogeneity introduced by this restoration method results in increased species diversity.

Figure 14. Dense *Triglochin maritima* emerging in an areas of high freshwater input in April after first treatment (*Spartina* wrack had not yet been raked).

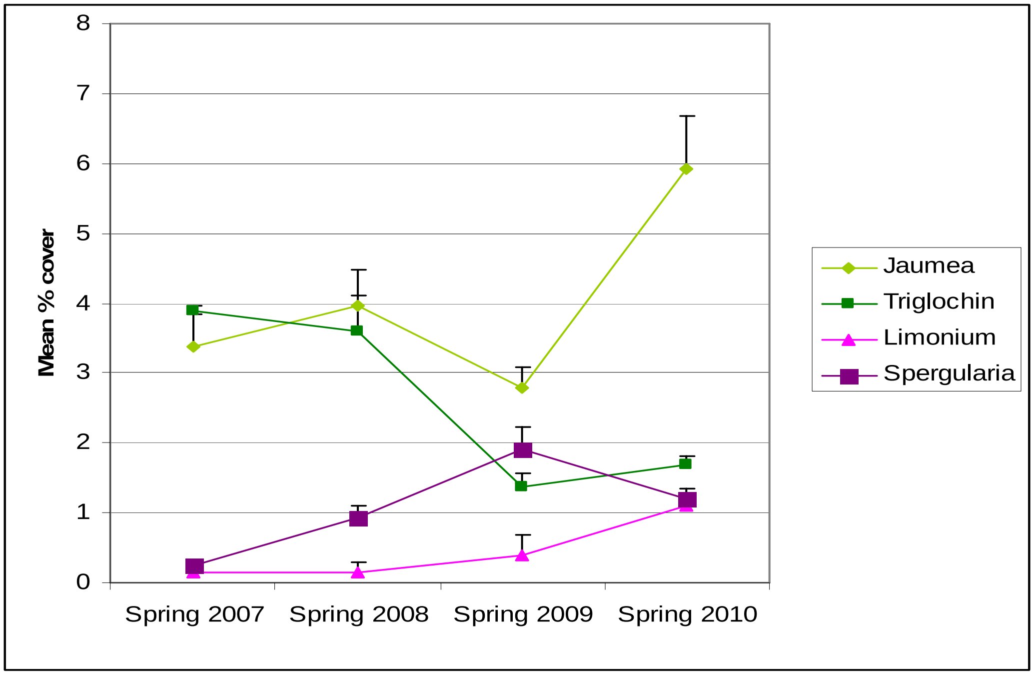

Figure 15. Change in mean cover (SE) of non-dominant constituents of the restored marsh.

Figure 16. Dense *Salicornia* seedlings emerging in an area where *Distichlis* was the dominant in surrounding vegetation (Larger *Distichlis* plants can be seen emerging from rhizomes).

Figure 17. Photographs of the same area show a hypoxic area that resisted recovery gradually being colonized by *Salicornia.*

Spartina Seedling Recruitment and Control Results

As seen in Fig.12, *Spartina* seedling density was greatest in the spring following initial treatment (mean = 240/m^2, SE 37.0). Although numbers declined steeply after the first year, density of seedlings in the spring monitoring exceeded density of resprouts for all years. Seedling density in the first spring was related to *Spartina* cover prior to treatment. Over all areas pre-mapped as the dense *Spartina* cover class ($\geq$60%), mean density of seedlings was 923/m^2 (SE 199), compared with the mean of 140/m^2 (SE 26) in the lower cover class (<60%). The difference was statistically significant ($p<.001$). This sampling design could not discern whether the seedling response in areas of previous dense *Spartina* was the result of a seed bank in those areas, favorable environmental conditions for both seedlings and mature *Spartina*, or a combination. A Pearson bivariate correlation analysis on the first spring monitoring interval data, when seedlings were most abundant, revealed that seedling density was positively correlated with filamentous algal cover ($r=.37$, $n=315$, $p<.01$). This relationship was maintained over subsequent years when far fewer seedlings emerged ($r=.34$, $n=1,267$, $p<.01$). It is possible that the filamentous algal mats retained moisture, facilitating germination and seedling survival. Seedlings showed no other relationships other than a negative correlation with *Distichlis* cover ($r=-.362$, $n=315$, $p<.05$). High marshes of Humboldt Bay are dominated by *Distichlis*, whereas *Spartina* is dominant in low to medium elevation marshes (Eicher 1987), suggesting the possibility of invasion resistance in *Distichlis*-dominated marshes. Falenski (2007) found that marshes least invaded by *Spartina* in Humboldt Bay were characterized by high elevation, less reduced soils, abundant shallow drainage channels and low available phosphorus in soils.

By the third spring after treatment (2009), *Spartina* seedling density had dropped to 9.5 seedlings/m^2 (n = 386, SE 2.3). Seedling density was not quantitatively monitored in the second year (2008), but qualitative observations showed that it was similarly low in that year. Density remained low for the two additional years the project was monitored (2010-2011) (Fig. 12). An ANOVA followed by Least Significant Difference post-hoc tests demonstrated that the reduction in density was significant between the first spring and all other springs ($p<.001$), but the lower densities in all years following the first were not significantly different from each other. Thus, new *Spartina* recruitment into the restored marsh is maintained at an equal level as mortality through eradication efforts. Recruitment may be enhanced by repeated disturbances caused by seedling and juvenile removal each year. Seedlings were treated in the spring soon after emergence. By the fall there was usually some additional recruitment (see below) but in addition, missed seedlings had become juveniles. That some juveniles are also missed is evidenced by the fact that there are some isolated flowering plants observed each summer. Since plants don’t flower until the second year (Castillo and Figueroa 2009), these plants represent seedlings from two years prior that were missed both as seedlings and juveniles. Seedlings (as well as all *Spartina*) are most visible in the late winter when native species have died back. *Salicornia* is completely deciduous, while *Distichlis* goes dormant and acquires a brown color. Against this backdrop the green *Spartina*, which doesn’t go completely dormant, is very apparent. However, the second treatment of seedlings and juveniles needs to occur at the end of summer, in order to find any missed flowering plants and to prevent seed set. Unfortunately, at this season non-flowering plants are very difficult to distinguish and so are unlikely to be detected until the following spring. These seedlings will likely have developed rhizomes by the time they are treated in the spring, requiring greater disturbance to remove them. Obviously, this feedback loop of seedling recruitment, disturbance from control, and recruitment into disturbed areas cannot be broken until 1) the seed bank is exhausted and 2) regional eradication removes new seed rain and dispersal.

In the first year of the project seedling recruitment and response to treatment was monitored more intensively in order to test the effect of using propane torches to flame seedlings, as well as the response of the native plants to this treatment. Although the sample size was small, results were statistically significant. The single flaming treatment resulted in a mortality rate of 80%. Following these plots monthly revealed that seedlings continued to be recruited on the marsh throughout the summer. Both the flamed and control plots (the latter had no seedlings at the start of the monitoring) continued to show seedlings present, although the control area peaked in June, while the flamed area had seedlings emerging until August (Fig 18). The difference in late seedling emergence could not be attributed to treatment due to the substantially different seedling presence at the start. The flaming treatment suppressed native plant recovery in the first summer (Fig. 19), with control plots increasing in cover 700% (from a mean of 12 to 88%), and flamed plots increasing only 350% (from 9% to 33%). However, this advantage was clearly erased by the end of the project, as evidenced by photographs showing virtually 100% cover in areas that had been covered in dense seedlings and then denuded by flaming (Fig. 20). After the first seedling flux, and in areas where sparse seedlings occurred among dense native vegetation, the brushcutter was used for removing seedlings. When seedlings are young, the blade can be used to nick out the seedlings without going as deeply as when using the grind technique. The efficiency of using flaming as opposed to brushcutting for large areas of dense seedlings is dependent on timing and labor availability. There are tradeoffs of efficiency and impacts, and the age of the seedlings is critical. After only a few weeks, seedlings require much more sustained flaming to kill.

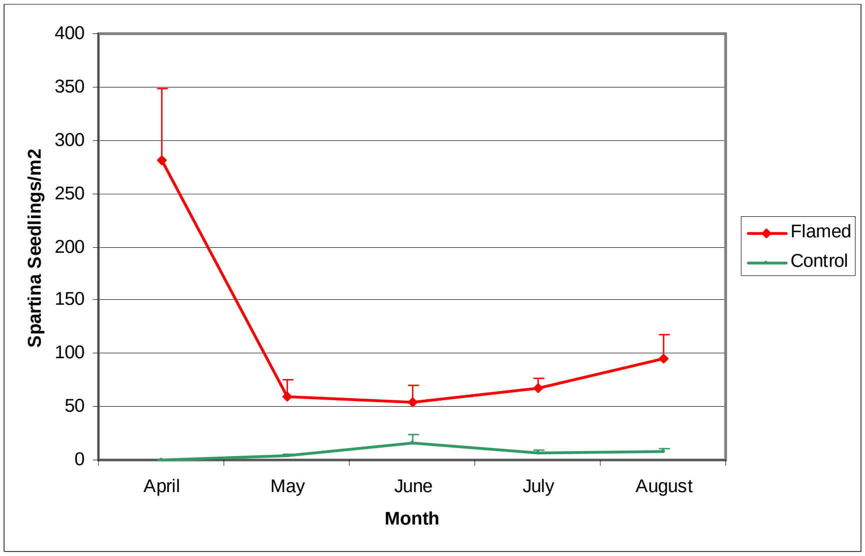

Figure 18. Response of *Spartina* seedlings to a single flaming treatment administered in April (red). The green curve represents recruitment of seedlings into non-flamed areas that had no seedlings present at the time of the first seedling flush in March-April.

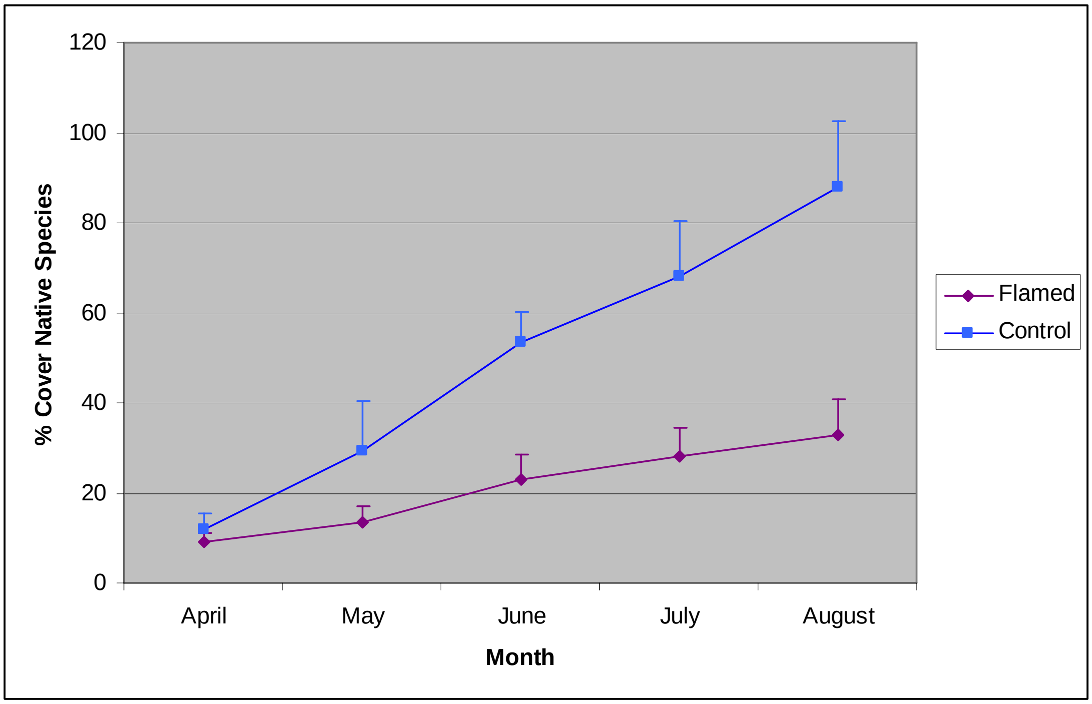

Figure 19. Colonization and/or recovery of native species in flamed areas compared with nonflamed areas that had no *Spartina* seedlings present

Figure 20. The area shown is the same as that in Fig. 4 (note large driftwood in background) and received extensive flaming in Spring 2007. In this photograph (taken June 2011) the area is characterized by 100% cover.

Rare Plant Response Results

The two rare plant species, Humboldt Bay owl's clover (*Castilleja ambigua* ssp. *humboldtiensis*) and Point Reyes bird's beak (*Cordylanthus maritimus* ssp. *maritimus*) are both hemiparasitic annuals generally found on high elevation salt marshes (Eicher 1987, Bivin et al. 1991). *Castilleja* populations on the Lanphere mainland marsh have been censused and mapped periodically since 1988, allowing an assessment of their response to *Spartina* removal. *Spartina* eradication had a dramatic, positive effect on *Castilleja* population size (Figs. 21-22). Population size has been documented to vary over time as it does for many populations of annuals (USFWS 2009c), thus it can be difficult to separate a treatment effect from background variation. In this case, the magnitude of change, as well as the lack of a similar increase on a nearby salt marsh island, indicate a strong treatment effect. Before restoration in the years 1988-2004 the mainland population size fluctuated between 1,000 and 3,800. In the first spring following restoration (2007), when vascular plant cover was still relatively low, the *Castilleja* population increased to 6,213. In 2008 the island *Castilleja* population peaked at its highest level since monitoring began in 1989, but by 2009 densities had dropped substantially (USFWS 2009c). On the mainland, the positive conditions of 2008 were apparently amplified by restoration, as the population increased to 38,490. Since 2008 *Castilleja* density on the island has continued to drop, while the mainland, restored population increased to 79,661 in 2009 and 99,485 in 2011.

A more direct study of rare plant impacts was carried out by Eicher and Pickart (2011) at the Jacoby Creek salt marsh. During the one year period covered by the study, (documenting changes over 2 generations of annual plants) restoration treatment did not have any negative impacts on *Castilleja*.

Cordylanthus maritimus ssp. *palustris* has historically not been censused on the mainland like *Castilleja*, primarily because its green color makes it much more cryptic. Particularly before *Spartina* was removed it was impractical to spot *Cordylanthus* from any distance. *Cordylanthus* overlaps with *Castilleja* in terms of habitat and they are frequently found together (Bivin et al.1991). Although the response of *Cordylanthus* to *Spartina* removal wasn't quantitatively monitored, observations suggest that restoration had a similar positive effect on this species. In many of the areas that *Castilleja* was mapped, *Cordylanthus* also occurred. It was seen additionally in many areas where *Castilleja* was less common or absent (Fig. 23).

Castilleja and *Cordylanthus* have been previously documented to be positively affected by disturbance, especially compaction (Newton 1986). The mechanical treatment certainly caused both disturbance and compaction, but also resulted in a significant decrease in shading by *Spartina*. *Castilleja* fecundity probably didn't increase proportionately with its numbers, as many dense individuals of small stature occurred in some places. However, the population clearly benefited from restoration for as long as four years following initial disturbance.

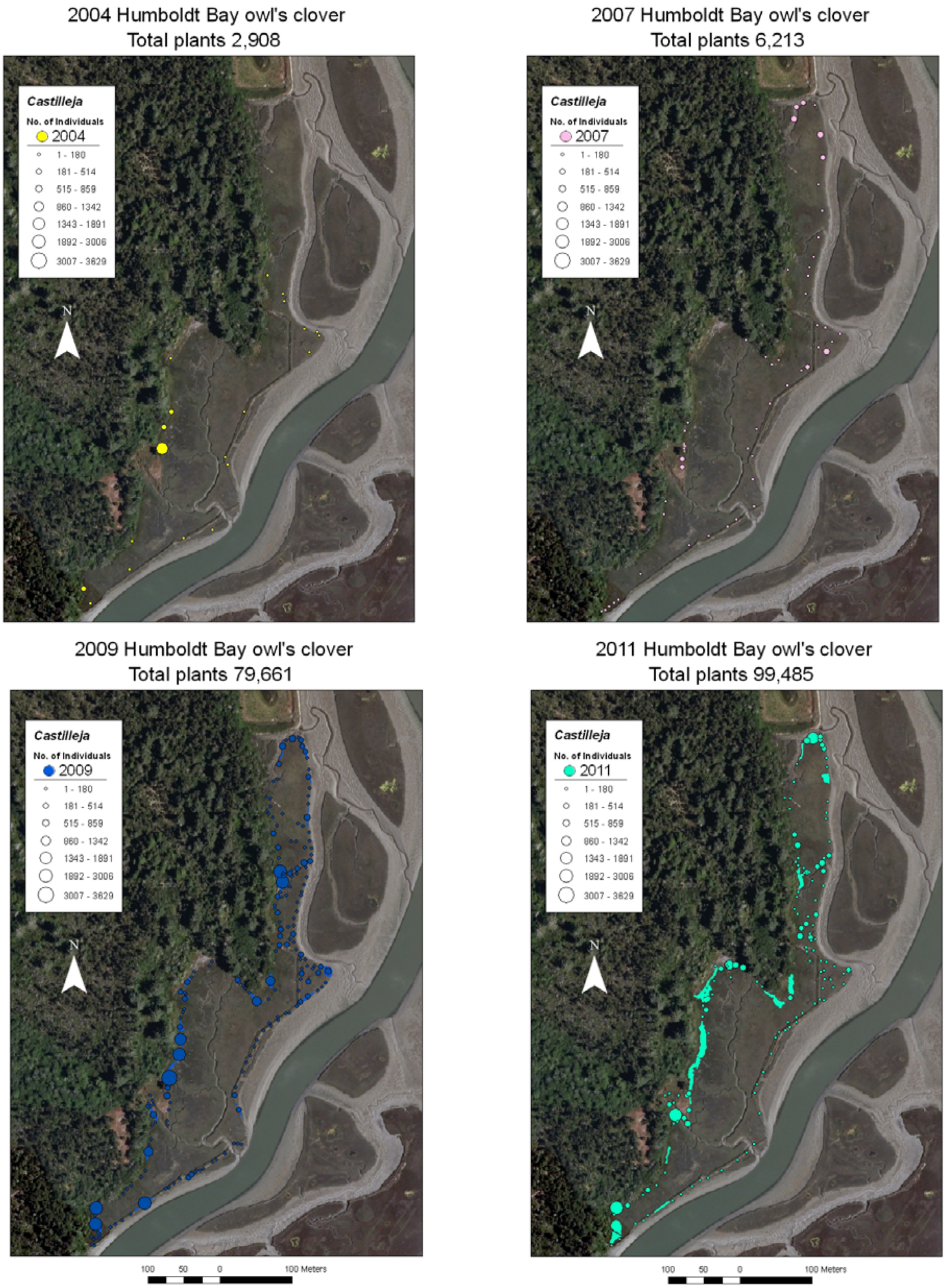

Figure 21. Maps showing the change in abundance and distribution of *Castilleja ambigua* ssp. *humboldtiensis* two years before restoration (top left), the spring immediately following restoration (2007 top right), and two and four years after initial *Spartina* removal (bottom).

Figure 22. *Castilleja* and *Cordylanthus* three years after treatment in a brackish area previously dominated by *Spartina* (Also note *Limonium californica*).

Figure 23. *Cordylanthus* seedlings emerging in an area of low cover where *Spartina* was removed. (presence of *Limonium* in photo indicates high marsh).

Revegetation Results

Survivorship of plugs was high for both species. Plugs of *Salicornia* had a mean survival rate of 99%, and *Distichlis* 98% by the end of the first summer. These results confirm that a late planting date (end of April) did not hamper survival. However, earlier plantings emerged from dormancy sooner, and recovery of vegetation occurred most quickly in plots planted earlier, thus an early planting date can accelerate recovery. After plugs broke dormancy in the first summer, *Salicornia* plugs quickly sent runners into bare areas (Fig. 24), whereas *Distichlis* plugs were static and eventually overwhelmed by *Salicornia*. However, recovery of the canopy in the control areas, as well as observed colonization from remnant *Salicornia* within or at the edges of revegetated areas, demonstrated that revegetation wasn't necessary for closure of the canopy. An example illustrating the recovery of one of the revegetation areas is shown in Figs. 25-27.

Figure 24. *Salicornia* expanding from plugs, 7 months after December planting.

Because of the superior colonizing ability of *Salicornia*, the species composition of the planted areas shifted over the course of the summer over planting. At the time of planting, all areas were bare and plantings resulted in a ratio of 1:1 *Salicornia:Distichlis*. By September this ratio had shifted to 80:1 in terms of cover.

By the end of the growing season in 2009, both revegetated and control plots were approaching 100% cover. One exception was Revegetation Area 4, which was located at the edge of the brackish/salt marsh interface. A small portion of this revegetation area

Figure 25. Revegetation Area 1 (center third of photograph), March 2008 prior to planting. Note dormant *Salicornia* at edges.

Figure 26. Same area as above, July 2008. *Salicornia* spreading from plugs, but most vigorously from remnant vegetation at edges.

Figure 27. Same area as above, June 2009. Canopy cover has closed, and is dominated by *Salicornia.*

closest to the freshwater source did not become colonized by vascular plants until the end of 2010. This was the latest area to be planted, and a thick algal mat developed prior to revegetation and persisted in this area until 2010. By 2011 the area supported a high diversity of brackish marsh species, with little *Salicornia* (Figs. 28-30).

Figure 28. Revegetation Area 4 prior to planting, late April 2007 (planting "holes" flagged). Filamentous green algal mat in foreground.

Figure 29. Same area in June 2009 with persisting bare areas covered with dried algal crust.

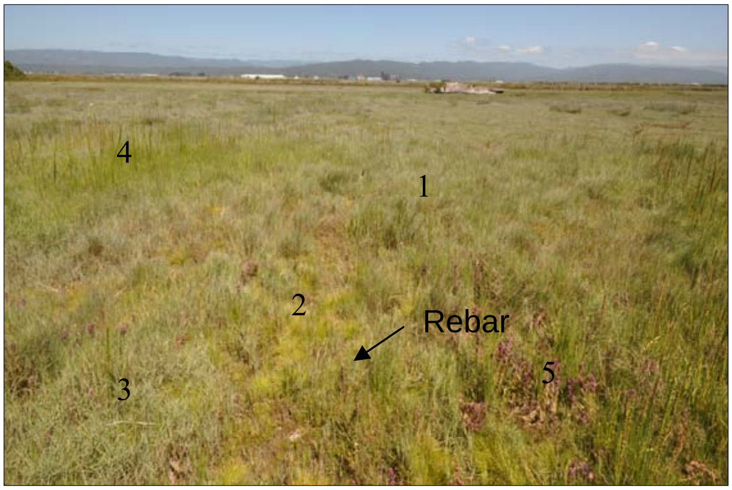

Figure 30. Same area in June 2011. Some of the species present include *Schoenoplectus maritimus* (1), *Scirpus cernuus* (2), *Distichlis* (3), *Triglochin maritima*, already emerged at time of planting (4), and *Castilleja* (5).

Abiotic sampling shed light on the performance of the two species. The superior colonization and expansion of *Salicornia* was related to elevation and salinity. Due to the sampling design, it was only possible to assess correlations between the mean of each variable (elevation, salinity, mean percent cover by species) rather than individual samples. This greatly reduced the sample size (n=4). Even so the high marsh species *Distichlis* was highly negatively correlated with salinity (r=-.99, p<.01), and *Salicornia* was negatively correlated elevation (r=-.91 p<.10).

In other parts of California, restorationists have suggested that dominance by *Salicornia* is undesirable in a salt marsh restoration due to its competitive ability and tendency to form monopsecific stands (Sullivan 2001). However, in Humboldt Bay *Salicornia* is the sole or dominant constituent of many low marsh areas where freshwater input is low or lacking,and low marsh as a vegetation type has been most impacted by the spread of *Spartina densiflora*. For this reason, *Salicornia* -dominated vegetation is an appropriate goal for low marshes that have been invaded by *Spartina*. Our revegetation trials indicate that abiotic conditions, rather than competition, drive species composition at this early stage of colonization. Abiotic forcing factors have been documented to structure vegetation of the low marsh in other parts of California (Grewell et al. 2007). Ultimately, as vegetation modifies environmental conditions, and with sedimentation, greater diversity may be achieved.

This project demonstrates that revegetation is not a required restoration step after *Spartina* removal in our region, which is important in light of the labor intensity and high cost of mechanical *Spartina* eradication. However, there are compelling reasons to include revegetation when possible. Our trials demonstrated that revegetation accelerates native plant recovery of the marsh, which can be important in the early stages of restoration when exposed bare mud or algal mats increase the likelihood of *Spartina* recruitment from seed. In addition, revegetation is one of only a few tasks that can be carried out by volunteers. Engaging volunteers in restoration is an important step in gaining community backing, and increases awareness and support needed to maintain restored areas. The volunteer days for this project did not attract a large number of participants, but those who volunteered displayed a high level of interest and commitment, with several returning for additional events.

SUMMARY AND CONCLUSIONS

The project clearly demonstrated the feasibility of using mechanical methods (specifically brushcutters) to control *Spartina densiflora* in north coast salt marshes. Until seed production and seedling recruitment are eliminated, it will not be possible to eradicate this species. However, the results of this study show that numbers of individuals can be reduced to a level that requires a relatively low amount of annual labor. In 2010 the spring treatment consisted of a total of 335 ph (omitting the walk to and from the marsh) for 12. 5 ha (31 ac), or approximately 27 ph/ha (10 ph/ac). While this level of maintenance is not unreasonable for a restoration project, it is clearly desirable to achieve eradication through regional restoration efforts, as is currently planned.

Vegetation recovery can occur without revegetation efforts, although revegetation accelerates recovery and could potentially increase diversity in areas of suitable salinity.

In Humboldt Bay *Spartina* occurs most densely in low marsh areas, which were most likely dominated by *Salicornia* prior to invasion. Areas with high freshwater input recovered with higher species diversity both with and without revegetation.

Marsh vegetation had recovered substantially, reaching nearly complete canopy closure between two and three years after restoration was initiated. Isolated, low and/or hypoxic areas continued to gain cover four years after completion. Disturbance to the marsh canopy continues as the result of ongoing removal of newly recruited *Spartina* seedlings. These repeated treatments result in a heterogeneous microtopography that could be beneficial to ecological functioning of the salt marsh. The predominance of *Salicornia* in restored areas may simply reflect the pre-invasion conditions; unfortunately no extensive reference areas of low marsh survive in Humboldt Bay. Alternatively, restoration activities may have contributed to the reversal of succession by incrementally lowering elevation, increasing inundation, salinity and anoxia. Over time increased species diversity may occur, although ongoing maintenance will continue to create low elevation niches. Ecological characteristics of restored marsh other than vegetation are discussed in Research Results below.

II. RESEARCH

OBJECTIVES

The research component of *Spartina* eradication was initiated in 2008, with funding from the Coastal Conservancy (07-196), USFWS's Coastal Program and USFWS Volunteers Working With Invasives Program. Additional funding was provided through an agreement with Humboldt Bay Harbor, Recreation and Conservation District (number) and through the 2010 large *Spartina* eradication allocation by the U.S. Fish and Wildlife Service. The goals of this project were 1) to provide an expanded understanding of the invasion ecology of *Spartina densiflora* in Humboldt County, and 2) to further refine mechanical treatments. These two components, and their interaction, can assist in planning for regional eradication. The Coastal Conservancy has funded the development of a Regional Eradication Plan and a supporting Environmental Impact Report. In support of this effort, the following objectives were met and are documented herein or in separate documents:

- Documentation of the extent and severity of the regional *Spartina densiflora* invasion through a regional mapping effort (this component of the project is presented in a separate report (Grazul and Rowland 2011).
- Determination of the nature of the *Spartina densiflora* persistent seed bank over two years.
- Refinement of the mechanical brushcutter eradication techniques and exploration of additional mechanical treatments.
- Impacts of *Spartina densiflora* and its eradication on invertebrate communities, presented in a completed Humboldt State University Master's thesis (Mitchell 2012).
- Impacts of *Spartina densiflora* on rare salt marsh plants; results presented in a separate report (Eicher and Pickart 2011).
- Impacts of *Spartina densiflora* mechanical removal on tidal creek geometry. Results are still pending for this objective, with final measurements scheduled for summer 2012.
- Impacts of *Spartina densiflora* on nonvascular salt marsh plants; results presented in a separate report (Augyte and Pickart 2012).

SEED BANK RESEARCH

Purpose

The objectives of the study were to determine whether *S. densiflora* has a persistent seed bank, document the size of the seed bank, and the longevity as allowed by the duration of the study. Although the genus *Spartina* has been characterized as generally lacking a seed bank (Ungar 2001, Wolters and Bakker 2002), the great abundance and high density of seedlings of *S. densiflora* observed in areas following *Spartina* removal presented the possibility of a seed. The existence of a seed bank would strongly affect strategies for regional eradication.

Methods

Five locations around Humboldt Bay were selected to investigate the presence of a persistent *Spartina densiflora* seed bank: Mad River Slough, Jacoby Creek mouth, Elk River mouth, Woodley Island, and South Spit (Fig. 31). The sites were selected with the intent of encompassing both island and mainland salt marshes in South and North Bays. Previous observations suggested that seed bank characteristics might differ between island and mainland marshes, and between low and high elevation marshes. Since little is known about dispersal of *S. densiflora* by tidal currents and how that might affect a seed bank, both North Bay and South Bay marshes were tested. Each site contained both high and low marsh.

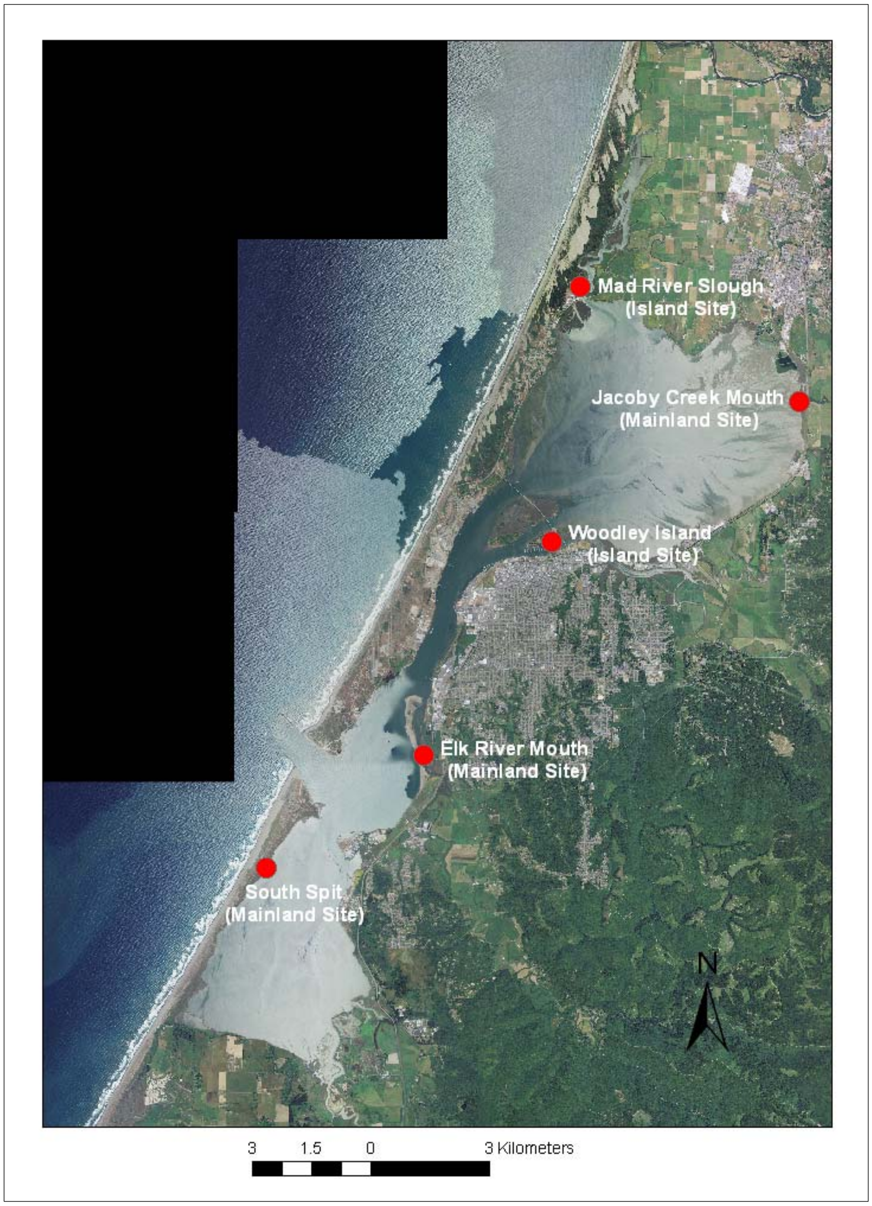

Figure 31. Location of seed bank study sites in Humboldt Bay.

At each marsh location, ArcMap 9.3 was used to delineate low and high marsh using 2009 aerial photographs. Areas of high *Spartina cover* (>60%) were used to indicate low marsh, and areas of low cover (<30%) were used to indicate high marsh (along with other physical attributes). A grid with a randomly generated start point was superimposed on the air photos in ArcMap to allow for systematic placement of 20 samples in high marsh and 20 in low marsh at each site. When locations were unsuitable in the photograph (e.g. they fell within a tidal creek) the nearest location was used. A Trimble GeoXT was used to navigate to each potential sample. All sites were visited prior to the onset of seed maturation in August 2010. An estimation of cover by species (to the nearest 5%) was made for the 1m x 1m quadrat. Any *Spartina* present within the center 30 cm x 30 cm of the plot (and a buffer) was clipped to ground level. A mesh screen (<1-mm) was used to cover the subplot. Screens were cut in advance, with additional length along each dimension. A saw was used to cut a shallow slit on each side of the subplot, so that the edges of the screen could be inserted. Landscape staples were then used to anchor all sides of the screen, thus ensuring that no new seed could reach the surface (Fig. 32). Based on this premise, all *Spartina* seeds present below the mesh originated in the seed set of 2009 or earlier. Once these barriers were in place, collection of soil cores could proceed without the risk of contamination by subsequent seed rain.

Figure 32. A seed bank subplot covered with mesh, after clipping stems and leaves of *S. densiflora*.

All seed bank plots were visited one time between October 2010 and April 2011 At this time, the screen was removed, and a 4-cm-deep soil core with a surface area of 10 cm x 10 cm was cut with a pull saw, removed with a spade, and placed in a labeled plastic bag. The 4-cm depth was chosen based on a pilot study carried out the previous year. The screen and staples were then replaced. Towards the end of the sampling period, *Spartina* had begun to regrow so was reclipped. After sampling completion all sites were revisited in summer 2011 and checked, with nets replaced as needed.

Once all samples were collected from a given site, cores were refrigerated while awaiting processing. Each sample was then placed in a plastic bucket, and agitated in water using a hose with nozzle. This resulted in dispersion of the clay, with additional hand manipulation as needed. The sample was then poured through a graduated series of sieves (4.75 mm, 2 mm and 1mm), and rinsed again. Starting with the top sieve, technicians placed a small portion of the sample on a plate and added water. Tweezers or dissecting probes were used to separate material and locate seeds. The process was repeated until the entire core was examined. Although the majority of seeds were found in the top (4.5 mm) sieve, some were also located in the lower, finer sieves. Damaged seeds or empty enclosing bracts were discarded, and all seeds were placed on a damp paper towel and enclosed in a 2.5-cm^2 sealed plastic bag. The bags were placed back into the refrigerator until all samples for that location were completed, and then shipped to Ransom Seed Laboratories in Carpenteria California. This seed lab had previously worked with *Spartina densiflora* and was familiar with interpreting tetrazolium staining results. The lab tested seeds for viability using a tetrazolium stain.

These methods were repeated in the second year, but only low marsh samples were collected due to funding restrictions. Samples were collected from inside the original plots, avoiding the areas affected by the first year samples. Nets were replaced as needed and *Spartina* clipped to allow for a third year of testing in 2012-13.

Results and Discussion

1. Seed Bank Size

The first-year results of the seed bank study are summarized in Fig. 33. Results are expressed as the density of viable seeds/m^2 surface area (to a depth of 4 cm). A persistent seed bank was confirmed for all sites, primarily in the low marsh. Density of the seed bank differed considerably among sites, but over all sites, the mean density was 1,540 seeds/m^2 (SE 3.0) for low marsh sites and 15 seeds/m^2 (SE 5.2) for high marsh sites. For low marsh, this translates to over 6 million seeds/acre.

An ANOVA indicated that seed bank density differed significantly by site ($p < .001$) and marsh elevation ($p<.001$), with a significant interaction between the two factors site and elevation. Over both elevation strata, Jacoby Creek was the only site to be significantly higher in density than all other sites based on post-hoc Student-Newman-Keuls and Tukey HSD tests.

The significant interaction between site and stratum resulted from the fact that the low marsh stratum contributed to the significant difference among sites while the high marsh stratum did not. This difference may be at least partially explained by the positive correlation shown between *Spartina densiflora* cover and density of viable seeds ($r=.51$, $p<.001$, $n=199$). The correlation between *Spartina* cover and viable seeds differed by

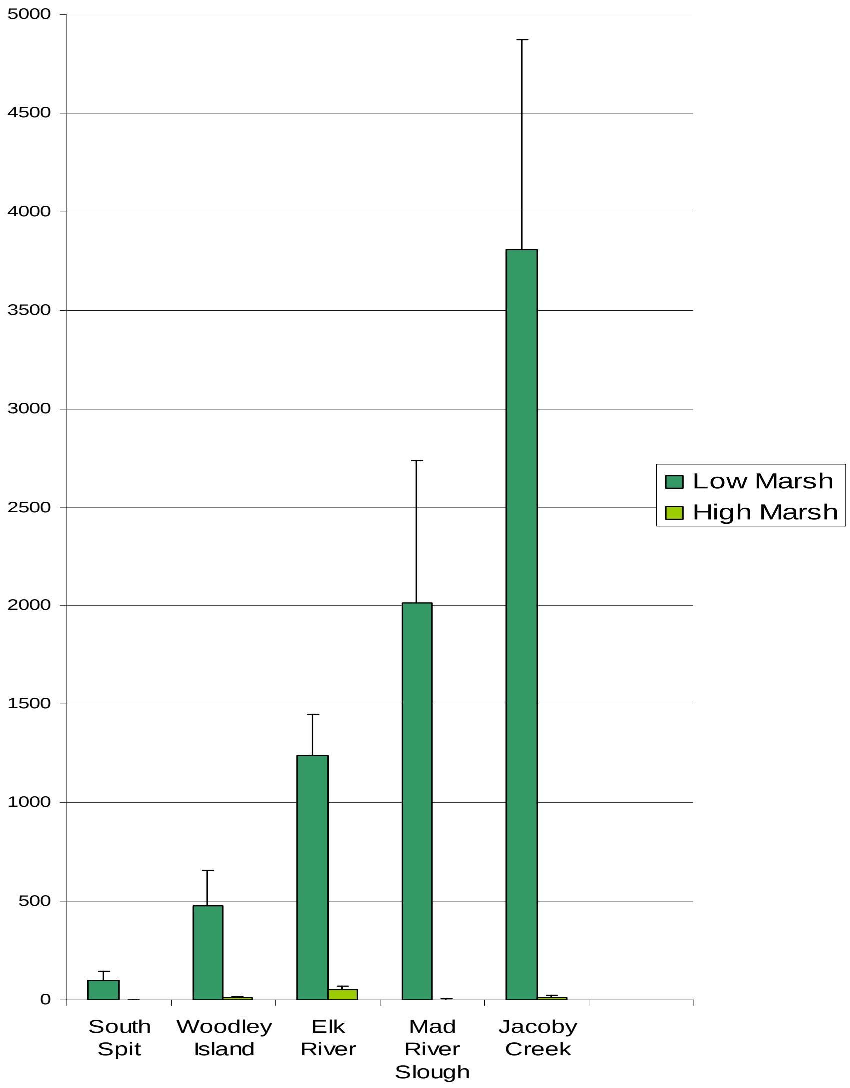

Figure 33. Density (viable seeds/m^2 surface area) by site and stratum (low marsh vs. high marsh)

site, with the strongest correlation at the Elk River (r=.83, p<.001, n = 39), and the lowest correlation at Woodley Island (r=.42, p < .001, n = 40).

This study did not measure all of the variables that could influence the differences in seed bank density among sites. The only independent variable measured was the percent cover (by species) of standing vegetation at seed set. *Spartina* cover in the low marsh, although always dominant, varied in its abundance among sites, with Jacoby Creek having the highest cover, significantly higher than all other sites (mean = 90.5 %, p<.05), and South Spit lower than all other sites (mean = 28%, p<05). These two sites also represent the low and high of the seed bank abundance range for low marsh. The dense area of *Spartina* at Jacoby Creek developed between 1970 and sometime in the 1990s, a period of rapid sedimentation resulting from timber activities in the watershed

(Fig. 34). This origin explains the robustness of the Jacoby Creek *Spartina* stand; unlike most low marsh areas around the bay the salt marsh formed after *Spartina* was well distributed in the Bay. The influence of freshwater from Jacoby Creek probably gave *Spartina* an additional competitive edge in quickly colonizing the site, more rapidly than native species could establish. High deposition rates would facilitate incorporation of *Spartina* seed into the seed bank. Overall, *Spartina* seed bank abundance appears to be driven by the abundance of standing *Spartina*.

This study did not analyze the composition of the non-*Spartina* seed bank, but observations suggest that *Distichlis spicata* seeds were the most abundant species represented in the high marsh seed bank. This observation suggests a similar correlation with above ground dominants for the high marsh, although the density of *Distichlis* seeds in the high marsh was far lower than that of *Spartina* seeds in the low marsh.

Explaining these patterns will require additional studies. The fact that *Spartina* seed bank abundance is correlated with above ground *Spartina* cover suggests that input to the seed bank may be primarily via seed rain that is trapped in the immediate vicinity of plants, as opposed to seeds that arrive from other geographic areas by tide. In addition, *Spartina* may be better able to trap seeds originating from other areas at the surface (and/or sediment deposition rates are higher than in native vegetation), resulting in more incorporation of seed into the soil. This seems plausible, since *Spartina* plants are denser and taller than native species, and create more drag and surface heterogeneity. A new study is now in place to distinguish the relative contributions of seed rain compared with dispersal from other parts of the marsh to the *Spartina* seed bank. Those results will help to clarify the picture.

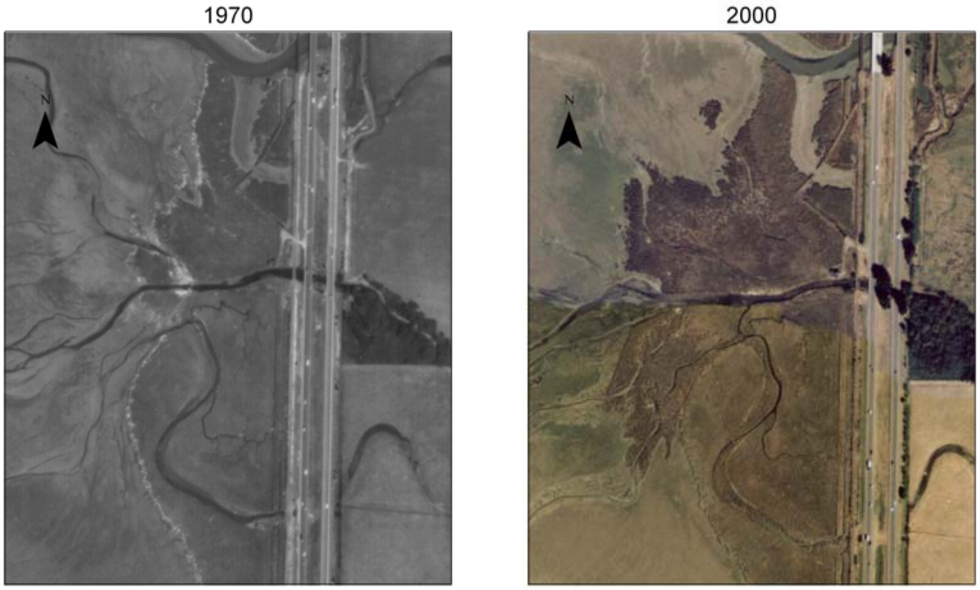

Figure 34. Aerial photographs showing the expansion of *Spartina*-dominated salt marsh between 1970 and 2000 at the mouth of Jacoby Creek.

2. Seed Bank Longevity

In the second year only low marsh plots were sampled due to funding constraints. A few plots were discarded because of tears in the screen. The mean number of viable seeds/m^2 of surface area decreased from 1,540 to 924 seeds/m^2 (SE 3.2). Seed bank densities in the three middle-range sites declined (Fig. 35), while those at the highest and lowest density did not. Jacoby Creek continued to significantly differ from all other (p=.001).

In the second year Jacoby Creek continued to stand out as an anomalous site characterized by a very high density of viable seeds. Given the reduction seen in mid-range sites, the conditions that support such a large seed bank at this site appear to also foster longevity. Sampling of additional independent site variable is recommended for the third year of seed bank sampling to determine whether environmental conditions may be contributing to persistence. These results have relevance for regional eradication planning, suggesting that where conditions are similar to Jacoby Creek, an eradication treatment that reduces or eliminates the seed bank (such as the deep grind method) would be preferable to one that does not (shallow grind or herbicides). Until the full longevity of the seed bank is known, it is difficult to predict how significant a factor it will be in regional eradication.

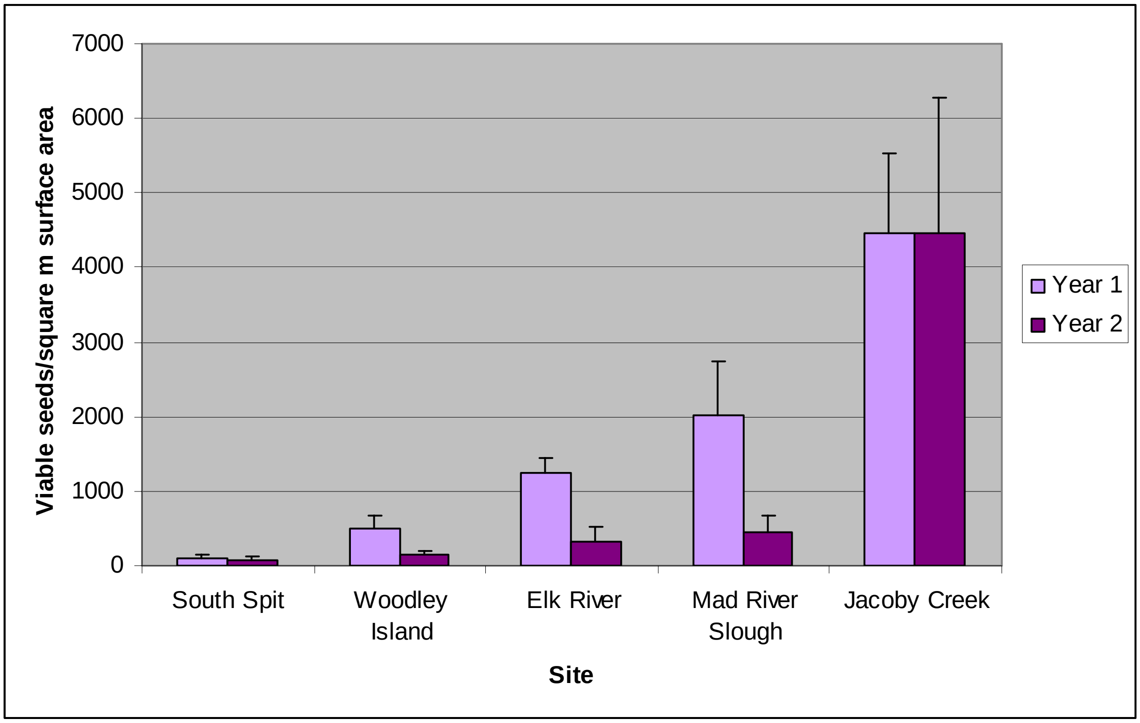

Figure 35. Changes in the persistent seed bank over a two year period by site.

REFINEMENT OF BRUSHCUTTER ERADICATION TECHNIQUES

Purpose

This component of the research was carried out to look for potential efficiencies in the brushcutter treatment based on seasonality of application, to examine the viability of using a top mow technique to prevent seed set and allow for delays in treatment while avoiding new seed production, and to document changes in algal functional groups through the treatment and recovery process (Augyte and Pickart 2012). It was anticipated that the season in which treatment was first applied would influence the rate of both seedling emergence and resprouting frequency. Although it would have been ideal to test four different seasonal starting dates, funding dictated that only a summer and winter date could be tested. These two seasons provided the greatest contrast. In summer, both *Spartina* and native plants are out of dormancy, and there are longer periods of daylight without inundation during which plants can photosynthesize (both due to longer days and due to differences in tidal periodicity). In contrast, native plants are dormant during winter and it is believed that this phenomenon provides a competitive edge to *Spartina* (Kittelson and Boyd 1997). It was hypothesized that winter treatment of *Spartina* might reduce this competitive advantage, and that seasonality might affect Spartina seedling response and recovery of both vascular and nonvascular native plants..

Methods

The investigation was carried out on a salt marsh at the Ma-le'l Dunes Unit, HBNWR. The site consists of a mainland salt marsh adjacent to the North Spit on the Mad River Slough. A pipeline of the Humboldt Bay Municipal Water District crosses the site, but experimental plots were placed away from the footprint of the pipeline. The area had previously been mapped into two *Spartina* cover strata, low to moderate (<60%) and high ($\geq$60%). Four treatment areas and one control area were placed in each of the two strata (Fig. 36). Each treatment/control area was 15-m square, encompassing 225 m^2. The following treatments/control were randomly assigned such that one replicate of each treatment/control was located in medium and one in high cover of *Spartina.*

1. Late seed suppression mow (at anthesis):
   At anthesis (July 2008) plants were mowed to a height sufficient to remove inflorescences.
2. Early suppression mow (at flowering shoot onset):
   At onset of flowering (May 2009) plants were mowed to a height of 20 cm.
3. Eradication Mow, summer start (Aug. 2008). Retreated at 6 month intervals.
4. Eradication Mow, winter start (Jan 2009). Retreated at 6 month intervals.
5. Control (no treatment)

Figure 36. Experimental design for the brushcutter treatment refinement experiment (note that base map is from 2009 after restoration so abundance of *Spartina* is not apparent.

In July and early August 2008, prior to the treatment, baseline monitoring was conducted. Five 15-m-long transects were placed systematically 2.5-m apart, and six plots were located such that they fell within *Spartina* patches along each transect, resulting in a sample size of 30. A .5-m x .5-m quadrat was carefully placed along a stretched tape, to allow for precise relocation of plots. Different measurements were collected depending on the treatment, as described below:

Late Seed Suppression (treated August 2008): The density of flowering culms was measured in each plot. Inflorescences were collected in all sample plots and the long axis measured. Outside the plot, 30 randomly chosen inflorescences were marked in areas of high *Spartina* density and 30 in areas of low-medium density. After seed maturation but prior to dispersal, the inflorescences were clipped and the number of mature seeds counted. A regression equation was developed to predict potential seed set (mature seeds) by inflorescence length. This equation was then used to estimate potential seed set over the entire treatment area.

Early Seed Suppression (treated May 2009): Density of culms was recorded using the same sampling design as above.

Summer and Winter Mow (treated August 2008 and January 2009): Density of culms was recorded. Percent cover (total and by species) was estimated using a gridded quadrat and six cover classes.

Control: Density of culms was recorded. Percent cover (total and by species) was estimated using a gridded quadrat and six cover classes. Seed set was estimated by collecting and measuring the lengths of inflorescences in sample plots following the methodology used in the Early Seed Suppression treatment areas.

Treatments were applied to the entire plot as well as a buffer area of 1 m (Fig. 37). At the time this experiment was initiated, the standard mowing treatment consisted of a top-mow (sweep-cut rather than mulched) followed by a light grind, with wrack later raked and removed from the site. Treatment areas were revisited every six months. In the mowing treatment areas, any resprouts or seedlings were retreated using the light grind method.

Figure 37. Summer mow (left) and winter mow (right) brushcutter treatments being applied.

Results and Discussion

A. Fecundity and Seed Suppression Treatment

For plants monitored outside treatment areas, length of inflorescence ranged from 6.8 cm to 22.0 cm, with a mean of 12.8 cm (SE 0.25). Seeds per inflorescence ranged from 30 to 333 with a mean of 11.6 (SE 5.5). The variability was not related to the stratum; i.e. there was no significant difference between both inflorescence length and seeds/inflorescence between low-medium density and high density *Spartina* areas (p=.753 and .788 for inflorescence length and seeds/inflorescence respectively).
A linear regression showed that inflorescence length is a fair predictor of potential seed set (R^2= .60), using the equation below. For all sampled inflorescences, the mean seeds produced per cm of inflorescence length was 8.42. Because seeds were not examined for viability, this equation can only predict the potential seed set.

Number of Seeds = (-99.9) +16.8(length of inflorescence in cm)

Using the regression formula, the number of inflorescences in sampled plots in treatment and control areas was used to estimate total potential seed production for the baseline (pre-treatment) late seed suppression treatment areas as well as control treatment areas in late summer 2008. Since the treatment had not yet been applied, samples within a stratum were pooled (over control and treated) to form a more robust estimate of seed production. In the low-medium density *Spartina*, mean inflorescence number was 84.6/m^2 with predicted potential seed production of 8,693 seeds/m^2. In high density *Spartina* mean inflorescences/m^2 was 109.8, with potential estimated seed production of 11,632 seeds/m^2. This represents a range of seed production between and 35 and 47 million seeds/acre.

The late seed suppression treatment administered in August 2008 resulted in virtually no seed set that year. Mowed seed heads were examined at intervals after mowing to ensure that seeds were not developing on inflorescences on the ground. One year later in August 2009, density of flowering stems in the late seed suppression mowed plots was significantly higher ($p<.001$) than both the pre-treatment density (same plot one year earlier) and the density found in control plots that same year (Fig. 38). This result is significant because it suggests that top-mowing can be effective for one year, but may actually stimulate flowering when the plants grow back.

The early seed suppression treatment carried out in May 2009 didn't entirely eliminate flowering the same year, but reduced it to a very low level, offering the possibility that there is a wider window of opportunity to carry out a suppression treatment (Fig. 39).

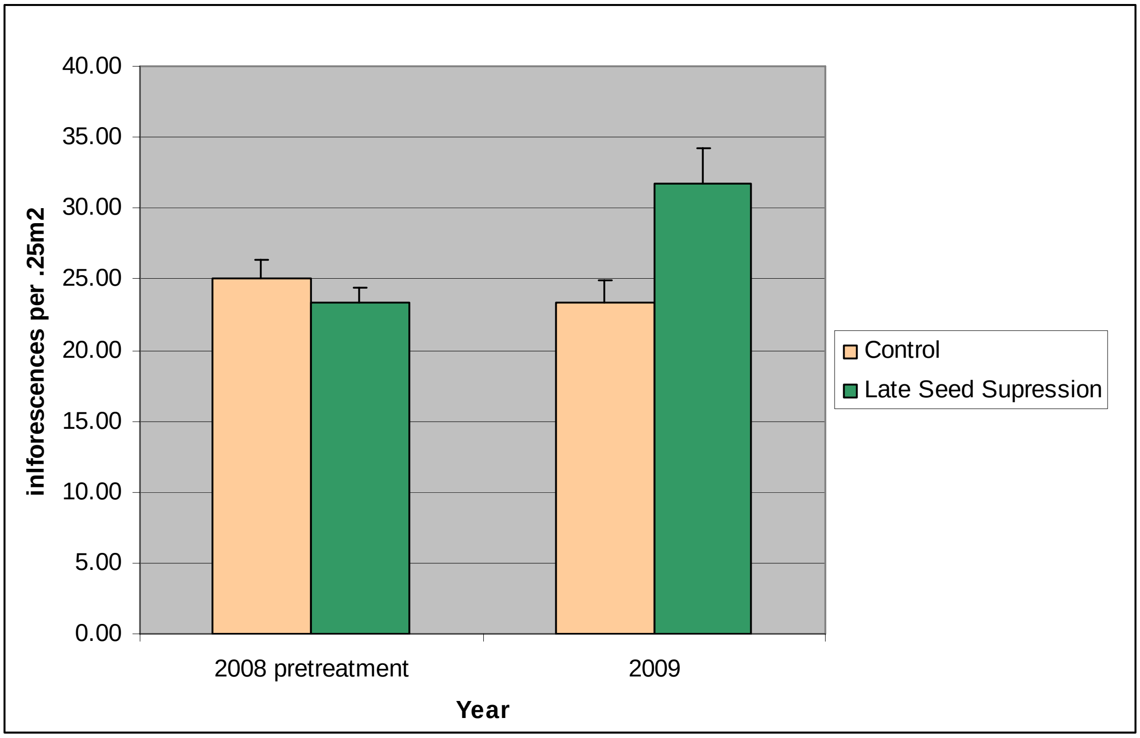

Figure 38. Fecundity prior to treatment for late seed suppression mow, and one year after treatment, expressed as the number of inflorescences per .25 m^2, compared with controls.

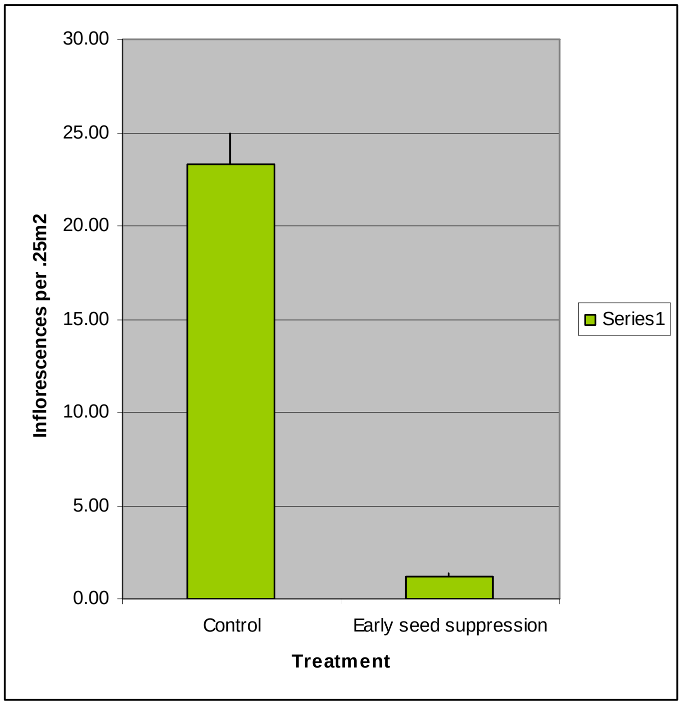

Figure 39. Results of early suppression mow 3 months after application, expressed as the number of inflorescences per .25 m^2, compared with control samples.

The seed suppression treatment, in addition to reducing seedset, was hypothesized to increase native cover by reducing the competitiveness of *Spartina densiflora*. Top mowing greatly reduces shading, a factor that likely contributes to *Spartina*'s competitive advantage over the low native vegetation. A GLM followed by Tukey HSD and SNK post-hoc tests demonstrated that both stratum and treatment significantly affected native cover in August 2009 (one year after the late seed suppression treatment and 3 months after the early seed suppression treatment). For this analysis, native cover for the three dominant species (*Salicornia, Distichlis* and *Jaumea carnosa*) was translated from cover class to proportion using the midpoint of the cover class, and an arcsine transformation was used. The GLM was significant both for stratum (p<.001), and treatment (p=.003), with native cover higher in the low-density *Spartina* treatment areas. To remove the obvious effect of stratum, An ANOVA was then performed on the high density stratum only. Using arcsine transformations, the ANOVA was significant at p<.01. Post hoc tests indicated that both seed suppression treatments resulted in significantly greater native cover than the control, but that the two treatments did not differ from each other. The actual differences in percent cover are shown in Fig. 40. The early successional species *Salicornia* contributed most strongly to the native species response. Based on this analysis, the use of top-mowing to reduce seed production has the added benefit of causing a small but significant increase in native cover. This benefit is realized in as little as 3 months for the early seed suppression treatment, so repeated top mowing would be expected to slowly shift towards higher native cover.

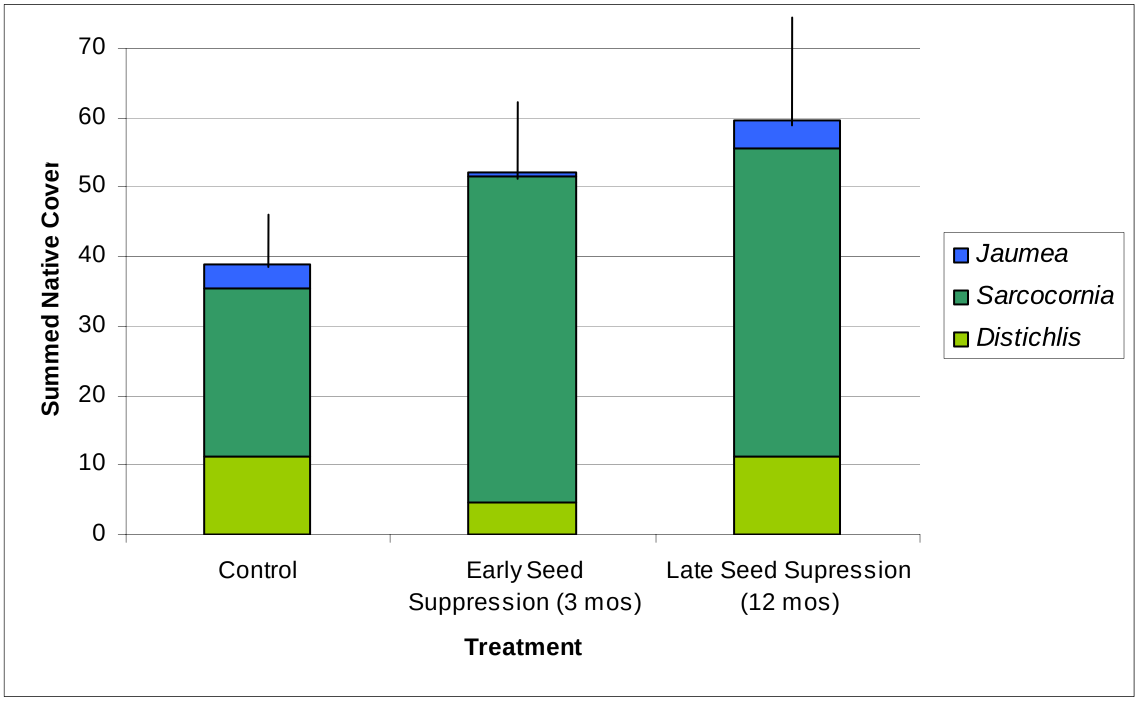

Figure 40. Response of native cover to two seed suppression treatments. Note that the bars represent summed cover for the three species, which is not necessarily total cover since species can overlap in the canopy. The error bar represents the standard error for the summed cover.

B. Eradication Treatments.

The final sample of *Spartina* density was conducted in August 2010, two years after the summer mow and 19 months after the winter mow was implemented. At this time, mortality (over both strata) was 99% for the summer mow (SE = 0.3%) and 94% for the winter mow (SE = 1.3%). Although mortality was only slighter higher in the summer mow plots (Fig. 41), the difference was statistically significant (p<.001).

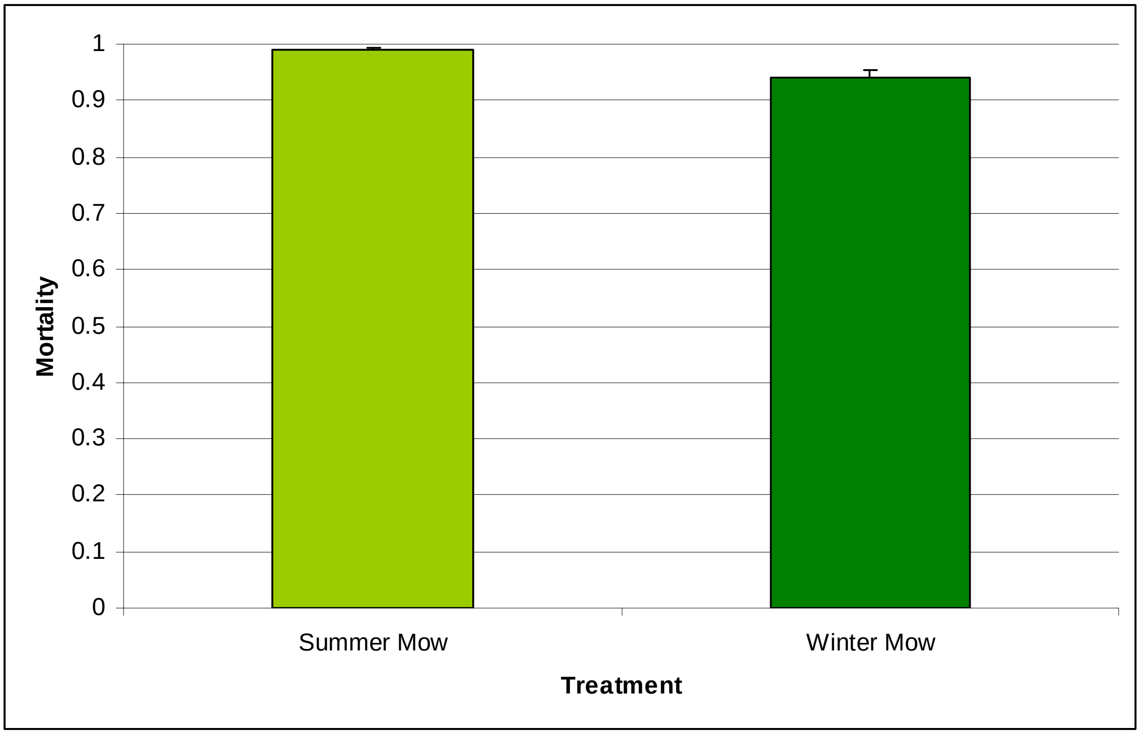

Figure 41. Differences in *Spartina* mortality by treatment (summer vs. winter-implemented first mow).

The summer mow treatment had a 6-month advantage in terms of start time. The experiment was terminated after two years (when all remaining *Spartin*a was treated), so it is possible that the winter mow would have achieved the same effect if that experiment had been left to run for 24 instead of 17 months. However, the summer mow treatment also had a steeper mortality curve than the winter treatment (Fig. 42).

The most dramatic and unexpected result of the experiment was the change in culm density in control plots over the two year period. Averaged over both strata, the culm density almost doubled after the first year, and nearly doubled again after the second year. The change in density was examined for each of the two strata (Fig. 43), and revealed that the increase was even steeper in the high density stratum. These patterns suggest that the invasion at the site was still progressing, which has implications for prioritization of regional eradication work.

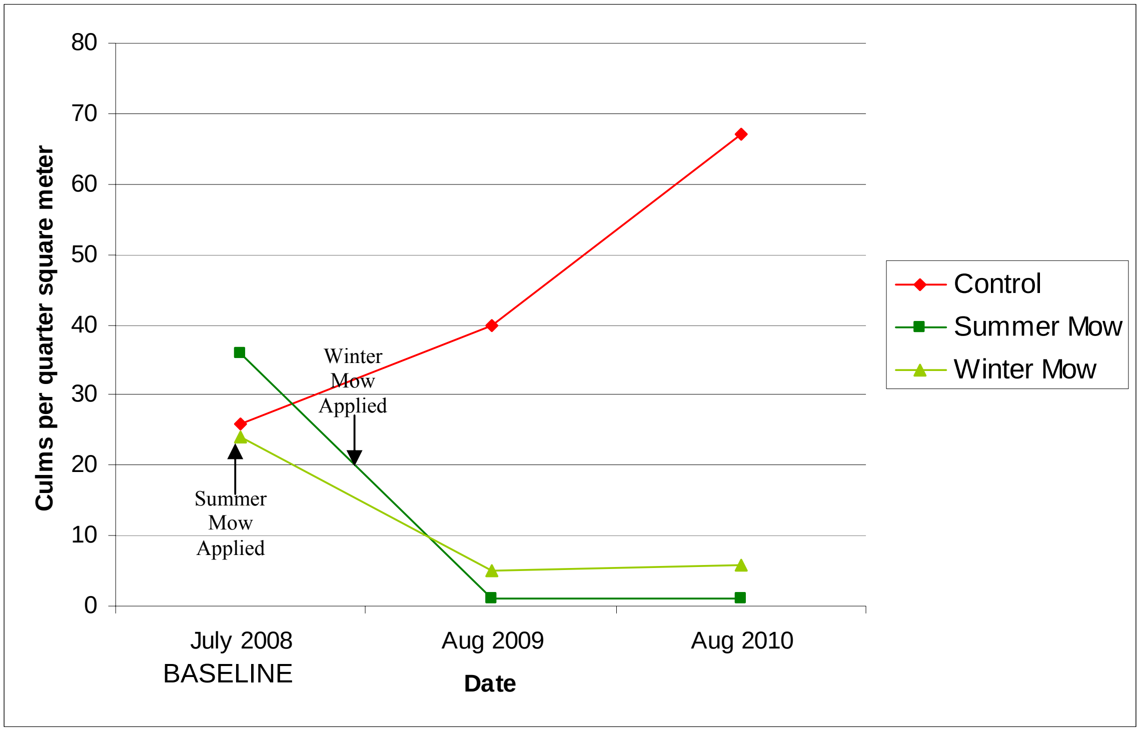

Figure 42. Changes in the density of *Spartina* (culms/.25m^2) over two years by treatment.

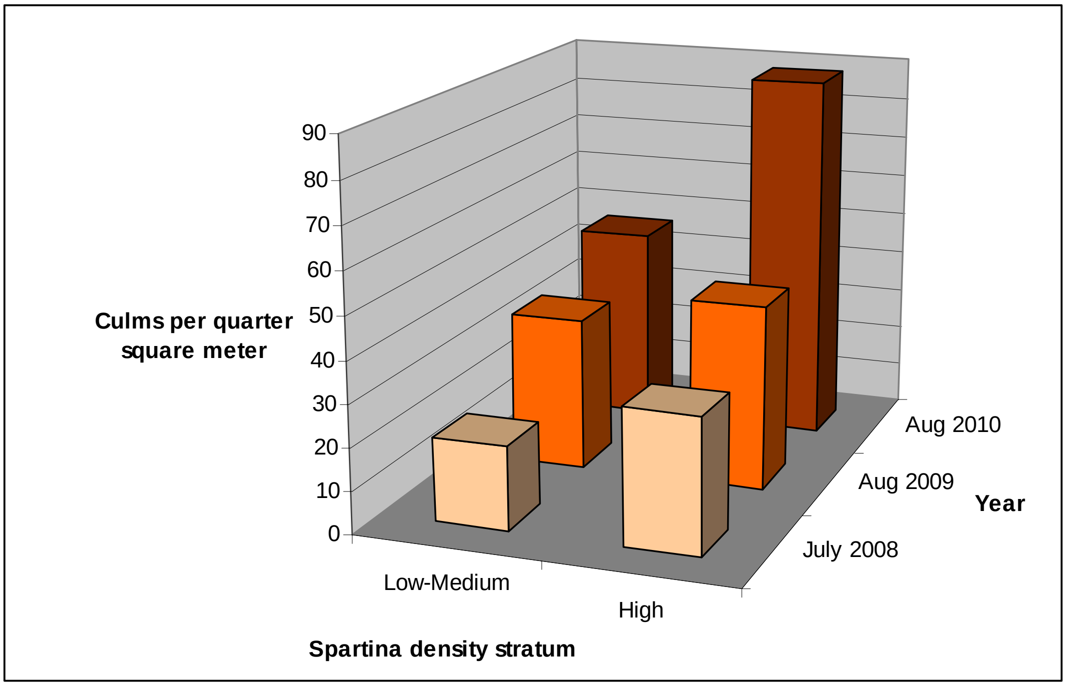

Figure 43. Increases in *Spartina* density (culms/.25m^2) in two cover/density strata for untreated control plots over two years.

To assess relative efficiency of the two treatments (summer vs. winter) it is also important to consider emergence of *Spartina* seedlings following treatment. Over both strata, the density of seedlings was far higher in the summer mow plots (Fig. 44). The results should be tested again in different years to be certain that the effect is consistent, but certainly this experiment suggests a seedling response that is highly affected by seasonality of treatment. A possible explanation lies in the greater amount of time that the summer plots were exposed before the season of seedling emergence (usually March). Monitoring of the restoration area discussed in the first section of this report found a positive correlation between seedling emergence and algal cover. The longer period in which the plots were exposed would allow increased colonization and succession by nonvascular species, as discussed in the section on algal response below. Treatment of seedlings is not nearly as time consuming as that of resprouts, but it can be argued that the efficiencies in labor delivered by the summer mow are at least partially offset by the increase in labor needed to remove seedlings. Monitoring of seedlings in this study confirms that *Spartina* seedling emergence is very low in areas that are not disturbed by treatment.

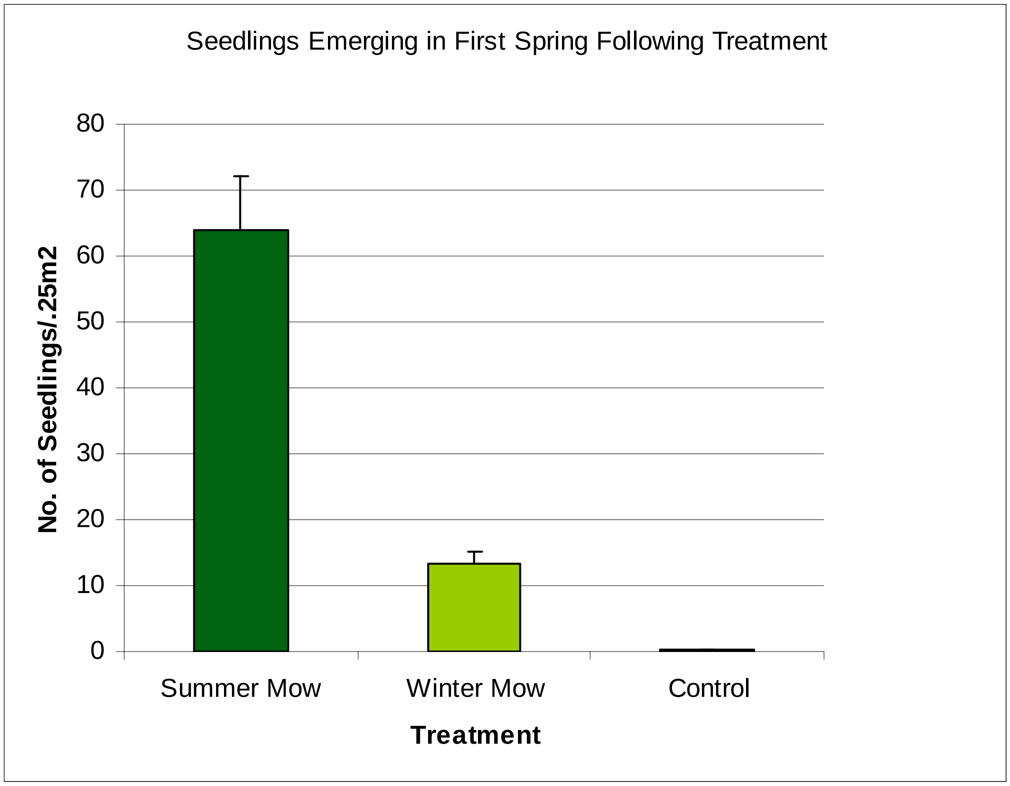

Figure 44. Differences in spring *Spartina* seedling emergence (March 2009) in areas first mowed in summer (August 2008) compared with those first mowed in winter (January 2009).

SUMMARY AND CONCLUSIONS

The research component of this project provided a better understanding of *Spartina* densiflora invasion ecology as well as implications for its control. The documentation of a variable but potentially large seed bank that persists for at least two years has strong relevance for treatment strategies. Experiments conducted subsequent to those documented in this report have shown that a grind treatment to a depth of 4-6 inches almost eliminates this seed bank, significantly reducing seedling emergence. Further studies are in progress to determine whether the depth needed to eliminate the seed bank slows recovery of native plants. Until the full longevity of the seed bank is known, there is an argument for treating dense *Spartina* with a deep grind for the combined benefits of reducing seedling emergence, minimizing the number of required number of resprouts treatments, and permanently eliminating the seed bank. New experiments begun since the conclusion of this study suggest that use of a mini-tiller may be more efficient than a brushcutter in some cases, however, it will be important to determine the effect of this new treatment on the seed bank.

The high fecundity of *Spartina* has led to the concern that any reproducing plants near a restored areas will significantly increase recruitment. However, results of this study seem to point to the seed bank as being a much greater contributor to seedling recruitment. This question will be answered definitively by two studies that are proposed or currently in progress. One study will quantify the proportion of seedlings emerging on restored salt marsh that originate in the seed bank as opposed to seed rain and dispersal. The second will investigate the number and viability of seeds that are dispersed by tide. Until these questions are answered it is difficult to determine the most efficient strategies for regional eradication.

The most important conclusions arising from the projects documented in this report (and related studies cited herein) are that *Spartina densiflora*-dominated salt marsh can be restored to native salt marsh with improved ecosystem function (as measured by invertebrate diversity, vascular and non-vascular plant diversity) within a period of 4-5 years. Restoration using the methods documented here is labor intensive, but has the potential to eliminate persistent *Spartina* seed banks. Revegetation is not required, but long term monitoring will be needed to determine whether initial dominance by the native colonizer *Salicornia pacifica* yields to greater species diversity over time, particularly given the microheterogeneity introduced by the brushcutter treatment. The success of the restoration efforts documented here provide strong incentive to pursue regional eradication of *Spartina densiflora* in the Humboldt Bay Region.

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U.S. Fish and Wildlife Service
Humboldt Bay National Wildlife Refuge
Humboldt County, California

Spartina densiflora Eradication Project

Project Update July 28, 2011

Project Goals

To eradicate an invasive cordgrass species, *Spartina densiflora*, from all lands within the Humboldt Bay National Wildlife Refuge (Refuge)

To serve as a model and first stage for the proposed regional eradication of *S. densiflora* from the rest of Humboldt Bay and two adjacent estuaries (Eel River and Mad River)

To demonstrate the feasibility and success of using mechanical methods of eradication for *S. densiflora* over a large area

Project Area

- The Refuge is comprised of 3,379 ac (1,367 ha) of freshwater and estuarine wetlands and coastal dunes distributed within 10 management units around Humboldt Bay, including 257 ac (104 ha) salt marsh and 141 ac (57 ha) brackish marsh

Work Accomplished To Date

Primary treatment of *S. densiflora* (primarily using brushcutters) is close to completion at all units within the Refuge except the Eureka Slough Unit and the Table Bluff Unit. Due to the high density of *S. densiflora* throughout this site, it is being considered for treatment with mechanized equipment. Use of heavy equipment at this site (such as MarshMaster 2 with mowing attachment) would both greatly increase efficiency and serve as an experimental and demonstration site for the upcoming regional eradication. Work is underway to complete permitting and environmental compliance to test this method in fall 2011.

In locations or conditions where use of brushcutters was infeasible (e.g., the sides of rock levees), plants were removed using shovels and/or picks, stockpiled, and then either ground on site using brushcutters or hauled off-site to compost

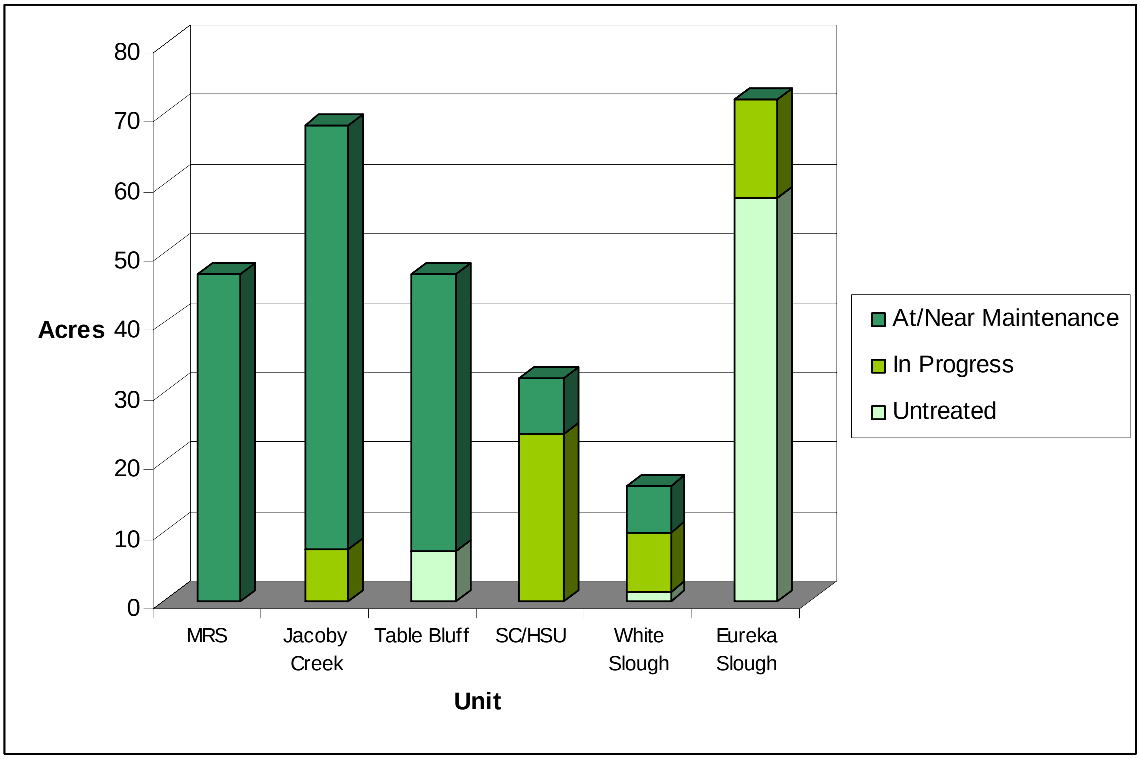
Some sites of *S. densiflora* are located on marsh islands, and we accessed these by the placement of floating docks purchased for this purpose.

Secondary treatment and treatment of spring emerging seedlings is currently underway for all sites that have received primary treatment; this phase involves treating resprouts and seedlings as needed using brushcutters.

A quantitative expriment designed to test the efficacy and efficiency of different grinding depths was implemented at the Jacoby Creek Unit. Large plots were delineated and subjected to one of four treatments: Deep grind, Light grind, 3-step Deep grind, and Control. The three types of grinding have all been used on the project but it is difficult to compare their relative costs and benefits without the quantitative experiment. Each treatment is replicated 2-3 times, and within each replicate we have quantitative samples in which we measure Pre and post-treatment Spartina density, new seedling emergence, and recovery of native species, as well as abiotic variables of elevation and oxidation-reduction potential. We also have a contractor collecting samples of invertebrate species. To date, we have been able to show that the deep grind method actually results in less elevation loss initially than light grind (presumably due to the aeration association with the sidecast material). We have also shown that the light grind treatment results in about twice the number of spring seedlings. We are currently analyzing the labor requirements for treatments and will be monitoring resprout rates. Initially we had planned to test manual digging as a method since it had been used over relatively large areas on another project. However, it quickly became evident that in the dense low elevation Spartina, digging is not a viable method for removal as it essentially becomes a trenching process due to the density of plants.

Progress Update, *Spartina densiflora* Refuge Eradication Program
Humboldt Bay National Wildlife Refuge
February 19, 2013

TREATMENT PROGRESS



- Progress to date is shown in above chart. Over the fall and winter, retreatment was carried out at Jacoby Creek, Salmon Creek/Hookton Slough and White Slough Units. A 3-person crew has been employed by the Harbor District to carry out resprout treatments. Approximately $30,000 is remaining in our contract to the District, which will go towards continued crew work and equipment purchase and maintenance.

Restored salt marsh island on Hookton Slough, 2 years after project begun.

Restored high marsh at Jacoby Creek Unit, with rare plants, 2 years after project begun.

TESTS OF NEW EQUIPMENT FOR REMAINING AREAS

- Experimental marshmaster/rototiller treatments were carried out at the Eureka Slough Units, funded by the State Coastal Conservancy. The rototiller attachment was used successfully and efficiently following a top mow with a mowing attachment. Response is being quantitatively monitored.

- Based on the marshmaster experiments, we have arranged to have loan of a marshmaster from Klamath, and will be using it beginning in April-May on the dense areas of Eureka Slough Unit, which is the largest remaining area of *Spartina*. We have also procured an airboat, needed to transport the marshmaster across open water. Our refuge equipment operator has received training for both.

- Early tests of a new power-tool, the minitiller, are promising. Unlike brushcutters, these are designed for subsoil use. They are lighter and less likely to cause strain or injuries with sustained use. They were tested at Ma-le'l island and shown to be faster than brushcutters, but will require more time at the follow-up treatments stage (but may also reduce time-to-recovery since they don't treat as deeply as brushcutters). *Spartina* must be top-mowed first (similar to brushcutters). When the marshmaster is used at Eureka slough, we will be having crews do larger test plots using the mintiller to get a better sense of its efficiency.

- Monitoring was carried out prior to the marshmaster trials, including the areas to be tested with minitillers. Additional measurements of elevation will be collected in order to document whether the marshmaster has an affect on bank erosion.

Above, marshmaster "swimming" to site (left), and with rototiller attachment following a top mow (right). Left, operator using a minitiller following top mow.

PROGRESS TOWARDS REGIONAL ERADICATION

- The Programmatic EIR and Draft Regional Eradication Plan were released by the State Coastal Conservancy. The comment period has recently closed, and a final plan is expected soon.

- The Coastal Conservancy is expected to award $500K to the Humboldt Bay Harbor District around May to begin control work off-refuge, including permitting. Funding to continue work on-refuge while regional control is occurring is expected to come from this grant or from a pending NAWCA grant.

- The third year of seed bank sampling was completed. All but the last site has been analyzed, and shows a significant decrease in the persistent seed bank. Some sites are approaching seed bank depletion. The site with the highest seed bank density saw a reduction for the first time. The Coastal Conservancy will be funding an additional year of testing.

- Monitoring of quantitative plots continued at Jacoby Creek Unit. Established in 2011, these areas are being closely monitored for changes in elevation, oxidation-reduction potential, and vegetation. A graduate student has been studying changes in benthic nvertebrate species composition. Our measurements have documented that brushcutter treatments result in an initial elevation loss, which is recovered within 2 years.

- A volunteer intern from Bangladesh on an ambassadorship program at Humboldt State carried out a study of Spartina seed dispersal via rafting on wrack.

Seed bank research, including collection of samples by a volunteer (left) and sieving by contractors and volunteers (right).

A volunteer collects wrack samples to analyze seed viability.

- A graduate student completed a Master's thesis comparing the terrestrial invertebrates in our restored Lanphere/Ma-le'l marshes and an invaded *Spartina* marsh. A shift in community structure was found, indicating that *Spartina* invasion lowers the richness of low canopy and epibenthic invertebrates, and increases relative population size of an invasive snail.

- A new Pathways student began a thesis looking at the buoyancy of *Spartina* seed bank, and the dispersal of seeds on tides.

- A graduate thesis was completed documenting that, despite the much higher above-ground biomass of *Spartina*-dominated marshes, the net ecosystem productivity is actually higher in native marshes, likely due to the contribution of micro- and macroalgae.

- The two-year study following changes in micro- and macroalgae in the restored marsh at Ma-le'l documented that removal of *Spartina* increased both the abundance and diversity of algal communities.

- The State Coastal Conservancy carried out an experiment using herbicides to treat *Spartina* at the Harbor District's property. Although the 2-year study is still in progress, it indicates that herbicides results in uneven mortality and require secondary mechanical treatment.

OUTREACH

- A People for Pickleweed volunteer event was held at the Salmon Creek Unit in September. The event was coordinated by HBNWR staff, Friends of HBNWR, and Friends of the Dunes. About 75 volunteers removed *Spartina* clumps manually using shovels, with the plants composted off-site. Food was served by our partner groups, who also created a memorial t-shirt.

Final Programmatic Report Narrative

Instructions: Save this document on your computer and complete the narrative in the format provided. The final narrative should not exceed ten (10) pages; do not delete the text provided below. Once complete, upload this document into the on-line final programmatic report task as instructed.

1. Summary of Accomplishments

- Detailed mapping of *Spartina densiflora* has been completed throughout the project area in a GIS format and has been used as a key part of the completed Regional Eradication Strategy for Humboldt Bay and the associated Environmental Impact Report.

- Eradication efforts are ongoing on the USFWS Refuge properties. Over 200 acres have received primary treatment to date, and approximately 125 acres are at 'Maintenance' level, having received multiple treatments and now requiring annual monitoring and minor control efforts to eliminate new seedlings.

- Multiple experiments have been established examining the characteristics of the Spartina densiflora seedbank; comparing different treatment methods, examining revegetation of native species, and comparing the ecology of treated, untreated, and native salt marsh.

- A Spartina Symposium was held in December 2011 to present research results and ongoing progress in the eradication effort.

- Annual volunteer eradication days have been held from 2010 onward with 50 to 75 volunteers working on manual removal in areas where motorized equipment is not appropriate.

2. Project Activities & Outcomes

Activities

- Describe and quantify (using the approved metrics referenced in your grant agreement) the primary activities conducted during this grant.

The primary activity conducted under this grant was the mechanical treatment of Spartina densiflora on salt marsh in Southern Humboldt Bay. The main method used was hand crews with metal bladed brush cutters grinding 1-3" into the soil surface to destroy the root mass of the plants. Approximately 125 acres dominated by Spartina was treated multiple times to achieve a maintenance level control with almost no Spartina remaining. Regrowth of native vegetation is occurring without planting efforts.
Another key activity supported by this grant was a seed bank research program, which gathered key data on the longevity of the Spartina seeds in Humboldt Bay.

- Briefly explain discrepancies between the activities conducted during the grant and the activities agreed upon in your grant agreement.

Mapping Spartina throughout the Humboldt Bay Area was originally proposed, but was subsequently funded through the State Coastal Conservancy, and the NFWF funds were utilized, with approval, for the seedbank research project and the mechanical treatment methods.

Outcomes

- Describe and quantify progress towards achieving the project outcomes described in your grant agreement. (Quantify using the approved metrics referenced in your grant agreement or by using more relevant metrics not included in the application.)

As shown in the attached progress update sheets, over 200 acres of Spartina dominated salt marsh have received treatment and approximately 125 acres have been treated multiple times to achieve a 'maintenance level' descriptor. This indicates that minimal annual monitoring and spot treatment of resprouts or new seedlings is conducted as the native marsh species grow in.

3. Lessons Learned

Describe the key lessons learned from this project, such as the least and most effective conservation practices or notable aspects of the project's methods, monitoring, or results. How could other conservation organizations adapt their projects to build upon some of these key lessons about what worked best and what did not?

The large scale of the Spartina infestation in Humboldt Bay has provided ample opportunity for adaptive management in terms of comparing the effectiveness of different treatment methods. Detailed tracking of crew time and expenses across similar sized plots was critical to make this data useful. As with most invasive plant control projects, timing of follow up treatments is critical and missing a key spring seedling flush results in secondary treatment requiring more effort than it otherwise would.

Public support for the project has been challenging at times because a) Spartina has been a dominant feature of the salt marsh for many decades, so most people see it as effectively 'native' vegetation, and b) eradication of densely infested areas leaves the marsh surface appearing largely barren for the first six to 12 months until native vegetation grows in. Concern about the potential for increased erosion from treated areas, and increased vulnerability to sea level rise with the lower native vegetation have and sea level rise have been consistent themes from the community. The Regional Eradication Plan and EIR have addressed these issues, although not to everyone's satisfaction.

4. Dissemination

Briefly identify any dissemination of lessons learned or other project results to external audiences, such as the public or other conservation organizations.

Research results and progress to date were presented at a Spartina Symposium held in December 2011. The current status of the mapping and treatment efforts were summarized in the 2013 Spartina Eradication Plan for Humboldt Bay, and the associated Environmental Impact Report, both available at www.humboldtbay.org

Presentations have been made to the Wiyot Indian Tribe, the Eureka City Council, and the California Department of Fish and Wildlife as part of gathering landowner and community support for the Regional Eradication Plan and EIR.

5. Project Documents

Include in your final programmatic report, via the Uploads section of this task, the following:

- 2-10 representative photos from the project. Photos need to have a minimum resolution of 300 dpi and must be accompanied with a legend or caption describing the file name and content of the photos;
- report publications, GIS data, brochures, videos, outreach tools, press releases, media coverage;
- any project deliverables per the terms of your grant agreement.

POSTING OF FINAL REPORT: *This report and attached project documents may be shared by the Foundation and any Funding Source for the Project via their respective websites. In the event that the Recipient intends to claim that its final report or project documents contains material that does not have to be posted on such websites because it is protected from disclosure by statutory or regulatory provisions, the Recipient shall clearly mark all such potentially protected materials as "PROTECTED" and provide an explanation and complete citation to the statutory or regulatory source for such protection.*

U.S. Fish and Wildlife Service
Humboldt Bay National Wildlife Refuge
Humboldt County, California

The Distribution of *Spartina densiflora* in the Humboldt Bay Region: Baseline Mapping

Prepared By:
Zackary I. Grazul and Peter D. Rowland
Humboldt Bay National Wildlife Refuge
6800 Lanphere Rd.
Arcata CA 9552185

August 2011

INTRODUCTION

Spartina densiflora, an invasive salt marsh cordgrass native to the coasts of Argentina and Brazil (Bortolus 2006), was introduced to Humboldt Bay in the late 1800s and has since invaded over 90% of the bay's salt marshes (Pickart 2001). Restoration of *Spartina*-dominated salt marsh has been the focus of research and management at the Lanphere and Ma-le'l Units of Humboldt Bay National Wildlife Refuge (HBNWR) since 2004 (Pickart 2005, 2008). Based on the success of early research and experimental trials, the California State Coastal Conservancy and USFWS jointly funded a 35-ac (14 -ha) *Spartina* eradication/salt marsh restoration project on the Lanphere and Ma-le'l Dunes Units of the refuge. At the onset of this pilot project in 2006 all *Spartina* within the Lanphere Dunes, Ma-le'l Dunes, Salmon Creek, and Hookton Slough and White Slough Units was mapped using a two-cover-class system to serve as a baseline data set (Pickart 2008). As the project progressed successfully, the Conservancy awarded additional funds to the Service to carry out research designed to refine control methods as well as to map the regional infestation of *Spartina* in the Mad River and Eel River estuaries and Humboldt Bay. This regional mapping effort was begun in 2009. In 2010 the Refuge received $1 million in USFWS funding to complete the removal of *Spartina* within the entire Refuge boundary. The decision was made at this time to complete Refuge *Spartina* mapping as a first phase of regional mapping. This report addresses both of these mapping efforts while concentrating on documenting the regional project. The refuge mapping is detailed in Grazul and Rowland (2010). The baseline data in this report represent conditions prior to the initiation of the large-scale refuge eradication project, much of which has already been implemented as of the publication of this report. A separate inventory of eradication efforts in progress or completed was conducted to allow for an up to date assessment of the distribution of *Spartina.*

PROJECT AREA

The *Humboldt Bay regio*n is defined as the area captured by the Mad River to the north, and the Eel River delta to the south, including the river mouths, Humboldt Bay, and all associated sloughs, estuaries, and tidal channels. Most notably these include: Mad River Slough, McDaniel Slough, Jacoby Creek, Eureka Slough, Elk River, White Slough, Salmon Creek, Hookton Slough, and the Salt River. Also included in the regional area were islands and structures such as levees and tide gates, including the Eureka waterfront and Arcata wastewater treatment facility. (Fig. 1)

Within the region, a search area was defined by identifying known coastal marsh habitat including salt marsh and adjacent brackish marshes. Photo interpretation and site visits were used to identify other areas of interest outside known *Spartina* habitat including agricultural fields behind tide gates, upland marshes and relict salt marsh features.

METHODS

Mapping of *Spartina* took place in two phases. The first phase was within the Humboldt Bay National Wildlife Refuge. Refuge mapping was more detailed and included collection of ecological data (Grazul and Rowland, 2010). The regional mapping focused exclusively on cover and distribution of *Spartina*. Regional mapping methods were developed so that the two data sets were easily integrated.

Whereas the first phase of the project was field-based to the highest degree possible, the larger regional effort relied heavily on photointerpretation. At this point in the project, the mappers had developed a high degree of skill in photointerpretation. Photo-interpretation was carried out using several sets of imagery, including NAIP 2005 and 2009 true-color imagery, and Humboldt Bay 2009 true-color imagery. The 2009 Humboldt Bay imagery proved to be most useful in detecting *Spartina* remotely. Heads-up digitizing was aided by ground truthing.

Mapping Features

Mapping utilized a combination of line and polygon features. Attributes recorded differed for each of these and were associated with features within a geodatabase.

Lines

Spartina plants distinct from the surrounding vegetation and found to be growing in linear occurrences (as is common along tidal creeks, roads and levees) were mapped as lines. Length and width of the lines were recorded allowing for the calculation of an area for the occurrence.

Polygons

Polygons were placed into three different classes based on percent cover of *Spartina*. The following cover classes were designed based on the ease of estimation as well as the labor needed for mechanical treatment:

0% <Cover Class 1 <25%
26% <Cover Class 2 <60%
61% ≤Cover Class 3 <100%

In addition to these cover classes, a separate layer was created to identify restoration efforts. These areas are placed within three categories: “Maintenance Level”, “In Progress”, and “Planned”. Maintenance Level areas are currently characterized by no more than 1% cover of *Spartina* as the result of new recruitment, and control efforts are ongoing to remove new juvenile plants and seedlings until such time as regional eradication is complete. Areas identified as In Progress, are currently undergoing some phase of treatment. Planned areas have been identified as targets for restoration by land managers, although these efforts may still require funding or additional organization. Data was collected in the form of paper and digital maps from restoration project managers in the region.

RESULTS

A total of 1,671.49 acres (117.00 ha) of salt marsh on the Humboldt Bay Region were infested with *Spartina* as represented by polygon or line features (Table 1, Fig. 2). Of this total, approximately 191 acres are currently undergoing restoration (treatment for *Spartina*), or have restoration efforts planned by a managing agency. In addition, more than 56 acres of salt marsh was identified as being restored to maintenance level in the Mad River Slough within the Lanphere and Ma-le’l dunes units of the Humboldt Bay National Wildlife Refuge (Table 2, Figs. 3-4). Larger scale maps depicting the distribution of Spartina in the four subareas (Mad River, North Humboldt Bay, South Humboldt Bay and Eel River) is shown in Fig. 5-8.

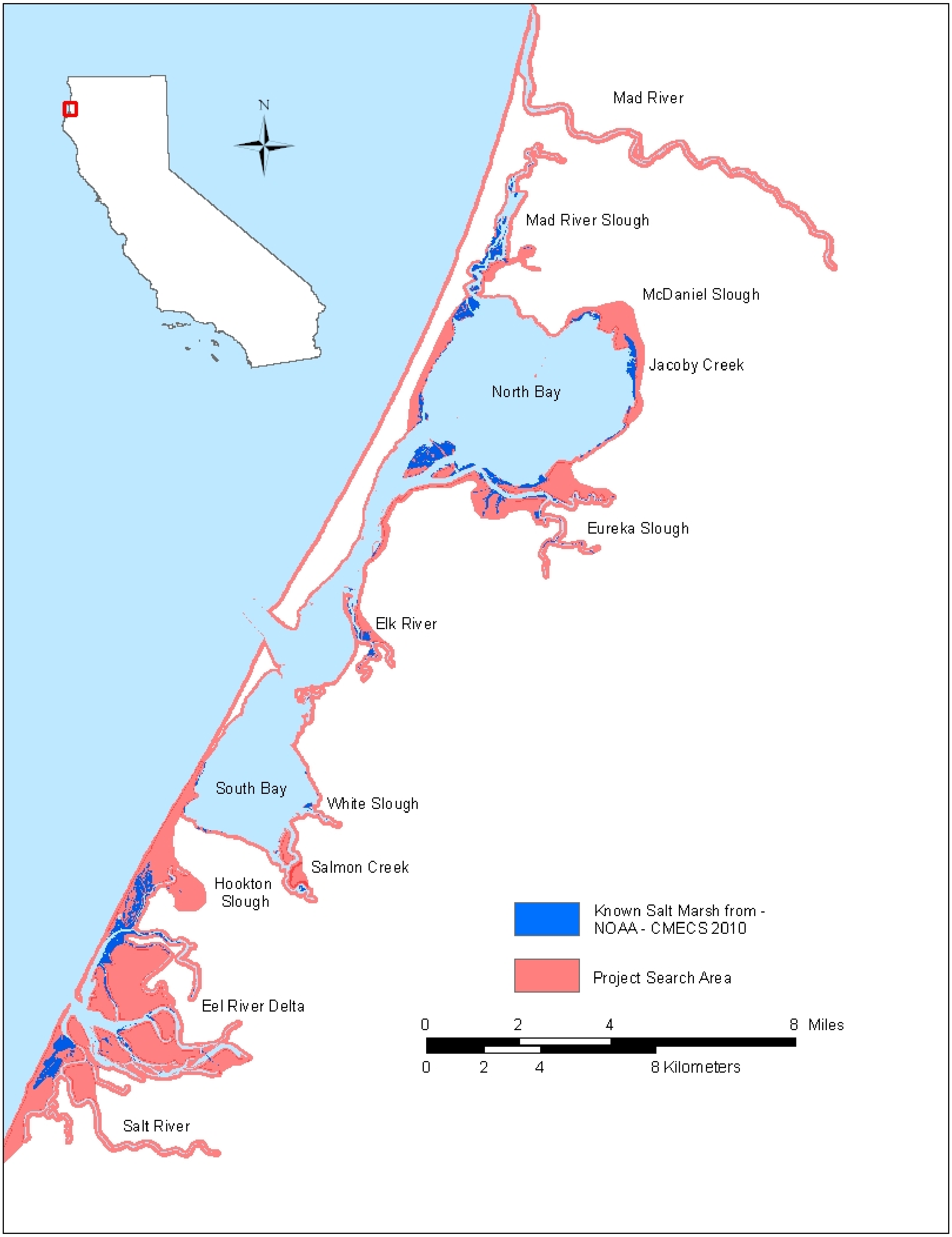


Figure 1. Major rivers, sloughs, bays, and estuaries in the Humboldt Bay Region. A search area was defined for the regional *Spartina* mapping effort using NOAA's Coastal and Marine Ecological Classification Standard for the area from 2010, Photo Interpretation (PI), and a series of field visits.

Table 1. Total acres infested by *Spartina densiflora* mapped as linear and polygon features distributed by cover class within the Humboldt Bay Region.

Project Area	Infested Acres	61-100%	26-60%	1-25%	Linear Features
Mad River	7.36	1.88	0	5.47	0.16
North Bay	867.5	314.94	243.37	308.18	14.43
South Bay	140.21	26.71	45.17	68.31	8.57
Eel River	656.42	278.96	171.78	205.66	2.61
Total Infested Acres	1,671.49	622.49	460.32	587.62	25.77

Table 2. Distribution of the 247 acres with completed, ongoing, or planned *Spartina densiflora* treatment efforts; together accounting for 14.7 % of the total regional infestation

Project Area	Restored to Maintenance Level	Restoration in Progress	Restoration Efforts Planned
Mad River	0	0	0
North Bay	56.32	58.9	82.61
South Bay	0	50.15	0
Eel River	0	0	0
Total Acres	56.32	108.24	82.61
Percent of Total Infestation	3.4%	6.4%	4.9%

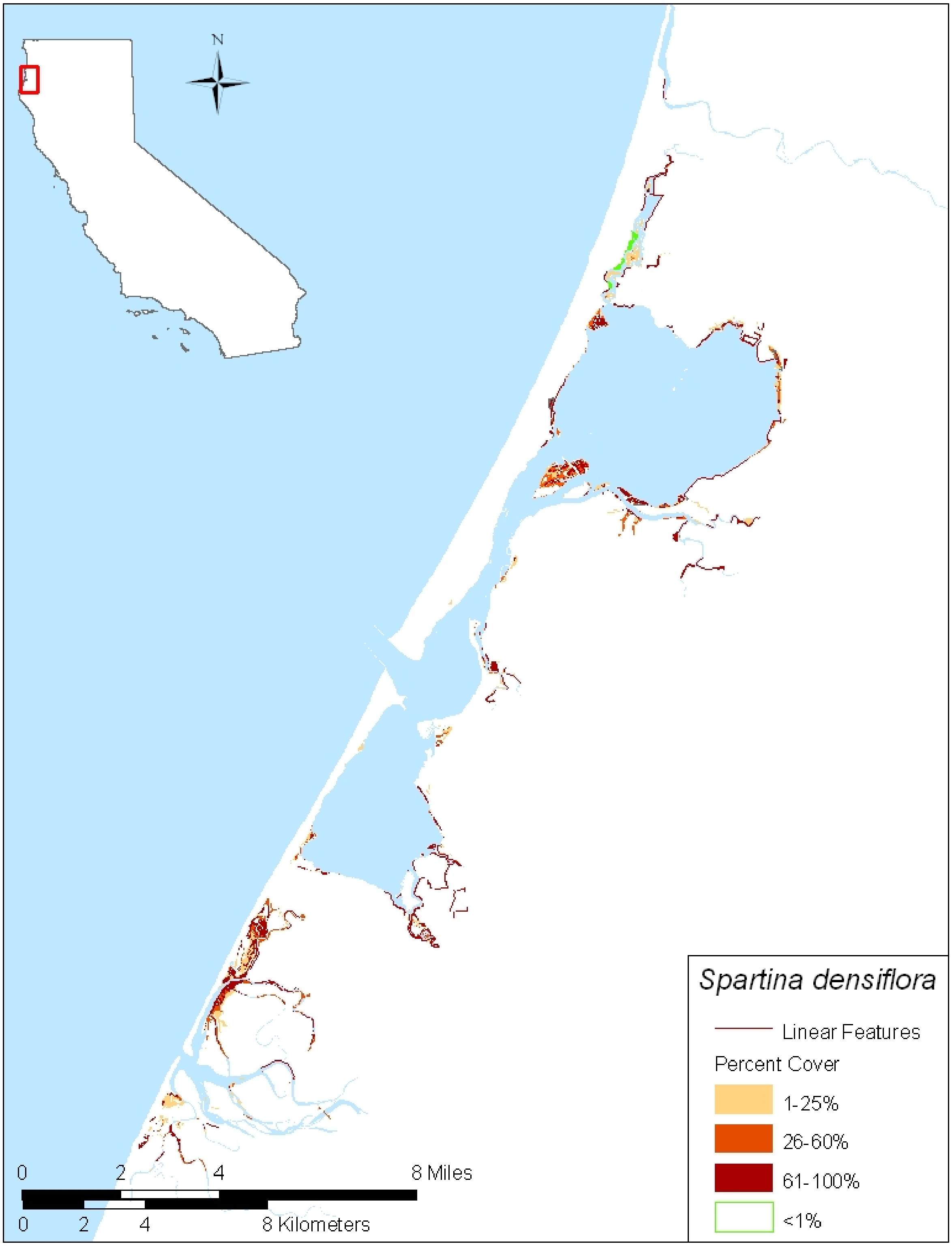


Figure 2. Distribution of *Spartina densiflora* by cover class within the Humboldt Bay Region.

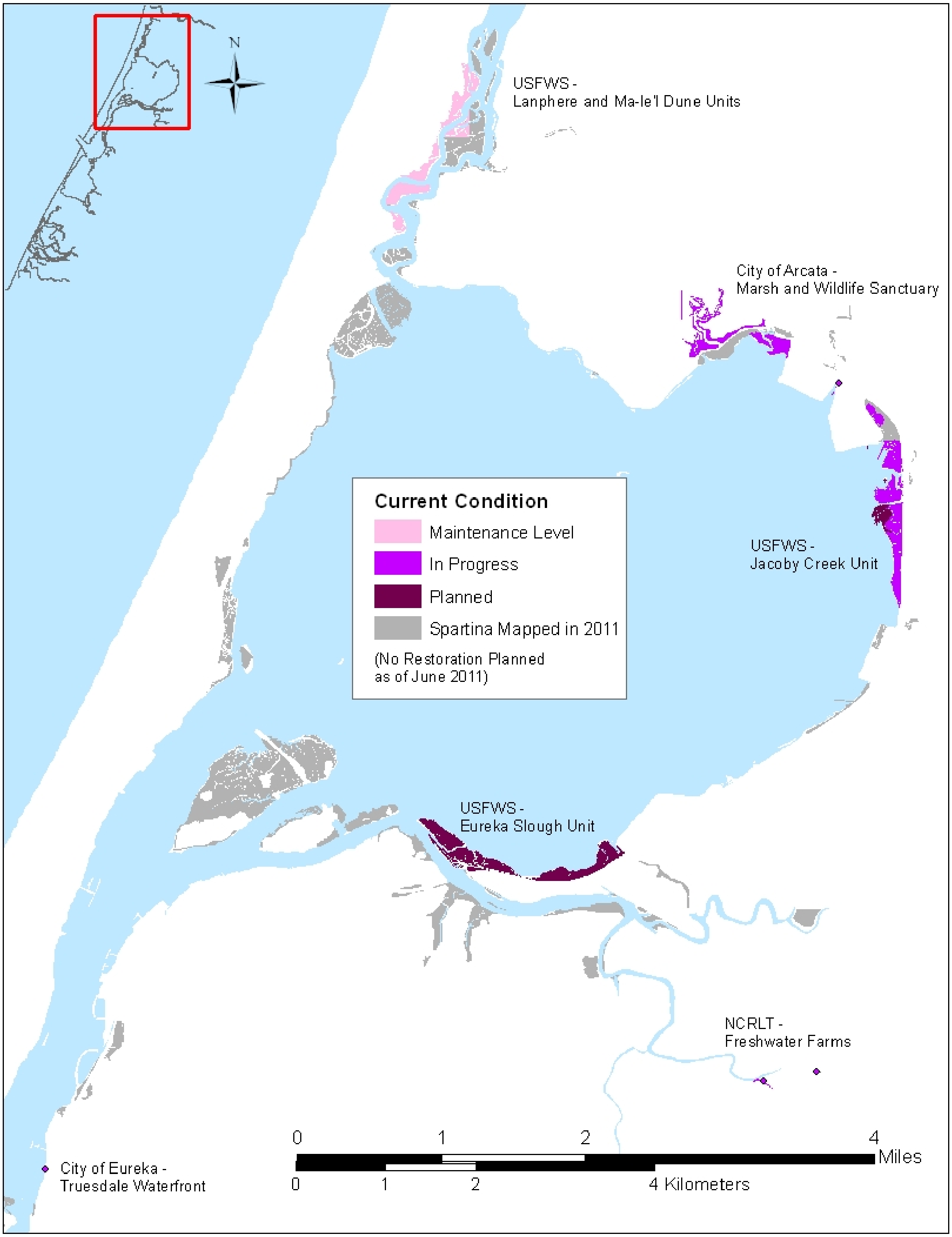


Figure 3. Coastal marsh lands infested with *Spartina densiflora* in the North Bay area of the Humboldt Region that have been restored to maintenance level, are in the process of restoration, or have restoration efforts planned.

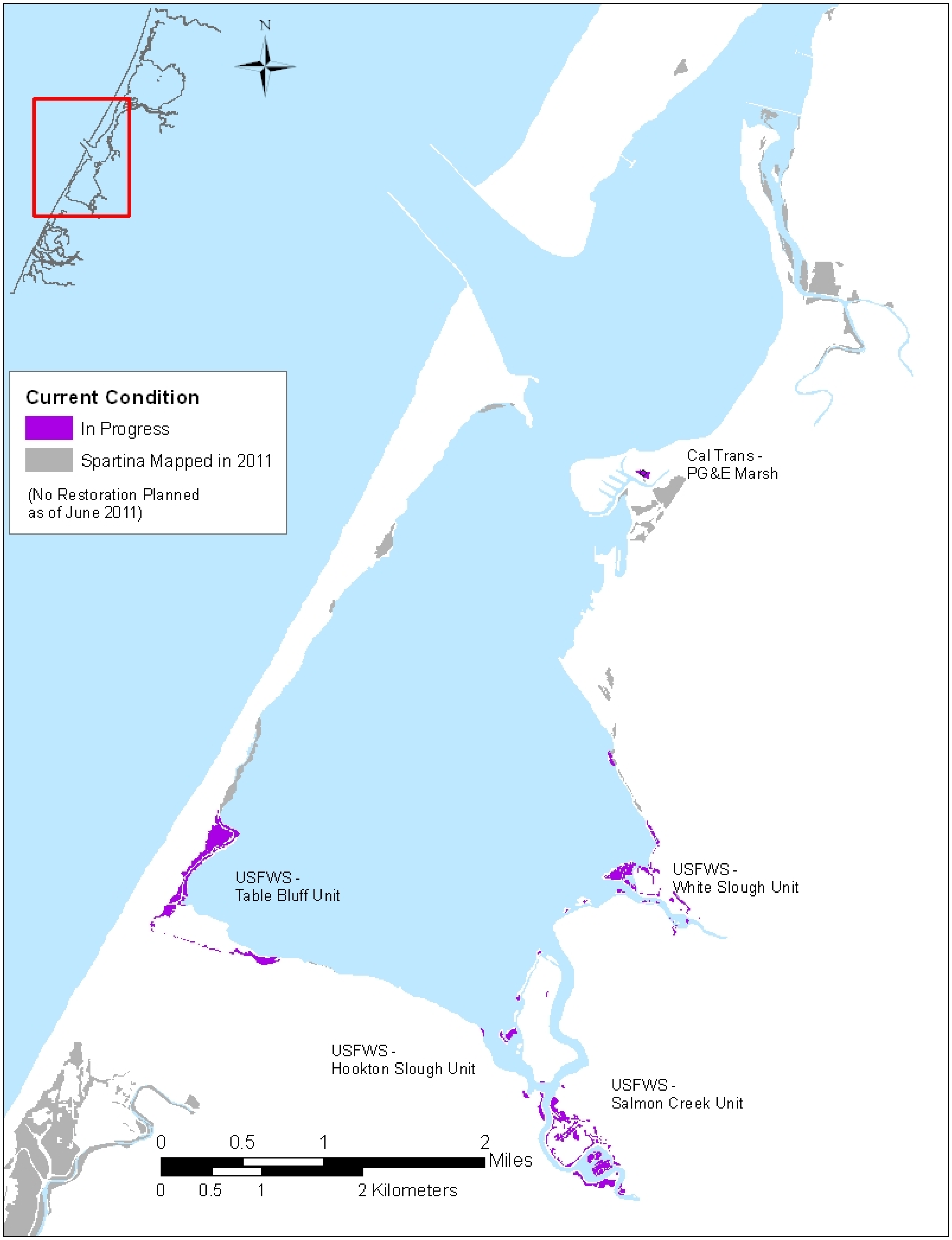


Figure 4. Coastal marsh lands infested with *Spartina densiflora* in the South Bay area of the Humboldt Region that are in the process of restoration, or have restoration efforts planned.

Figure 5. Distribution of *Spartina densiflora* by cover class within the Mad River area of the Humboldt Bay Region.

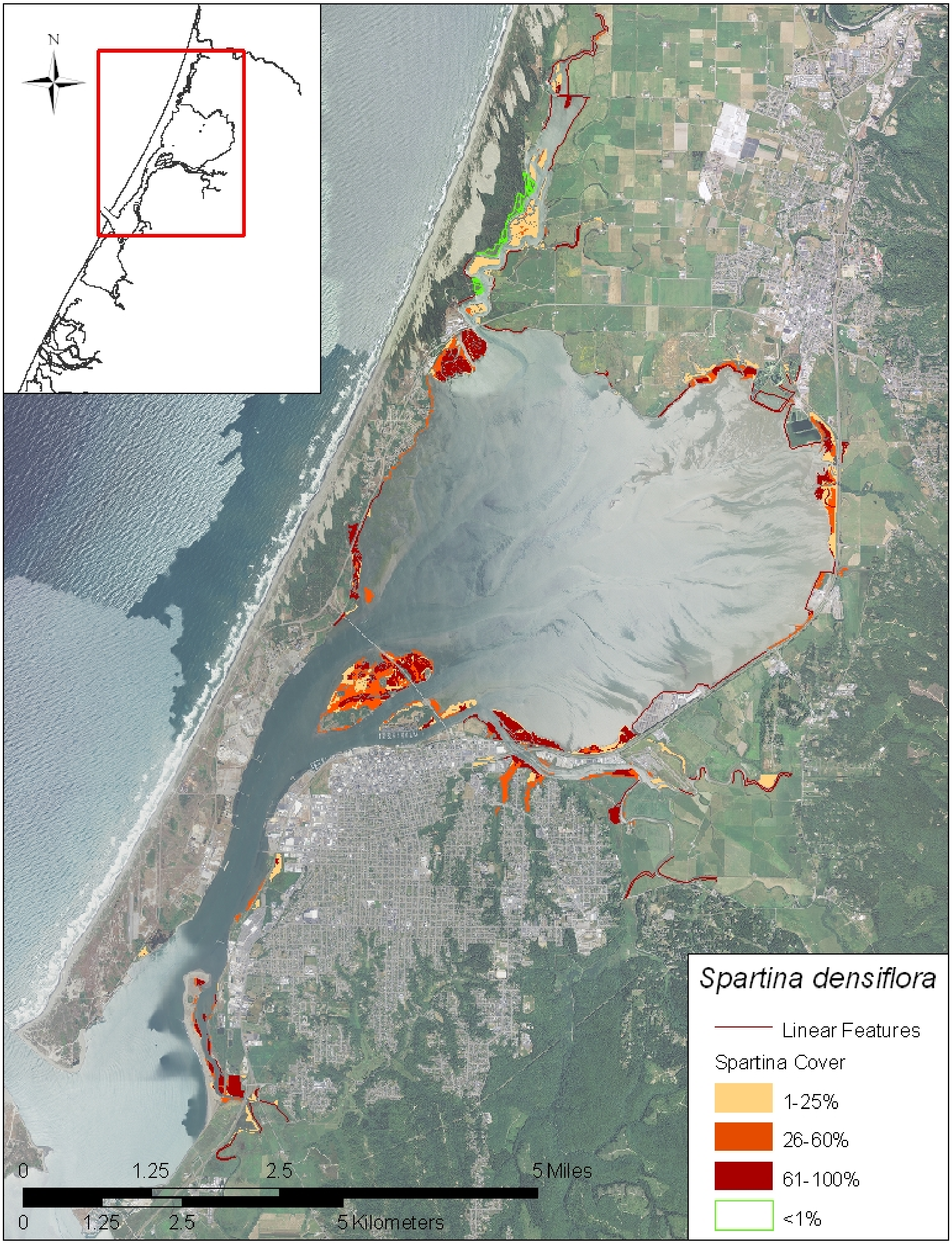


Figure 6. Distribution of *Spartina densiflora* by cover class within the North Bay area of the Humboldt Bay Region.

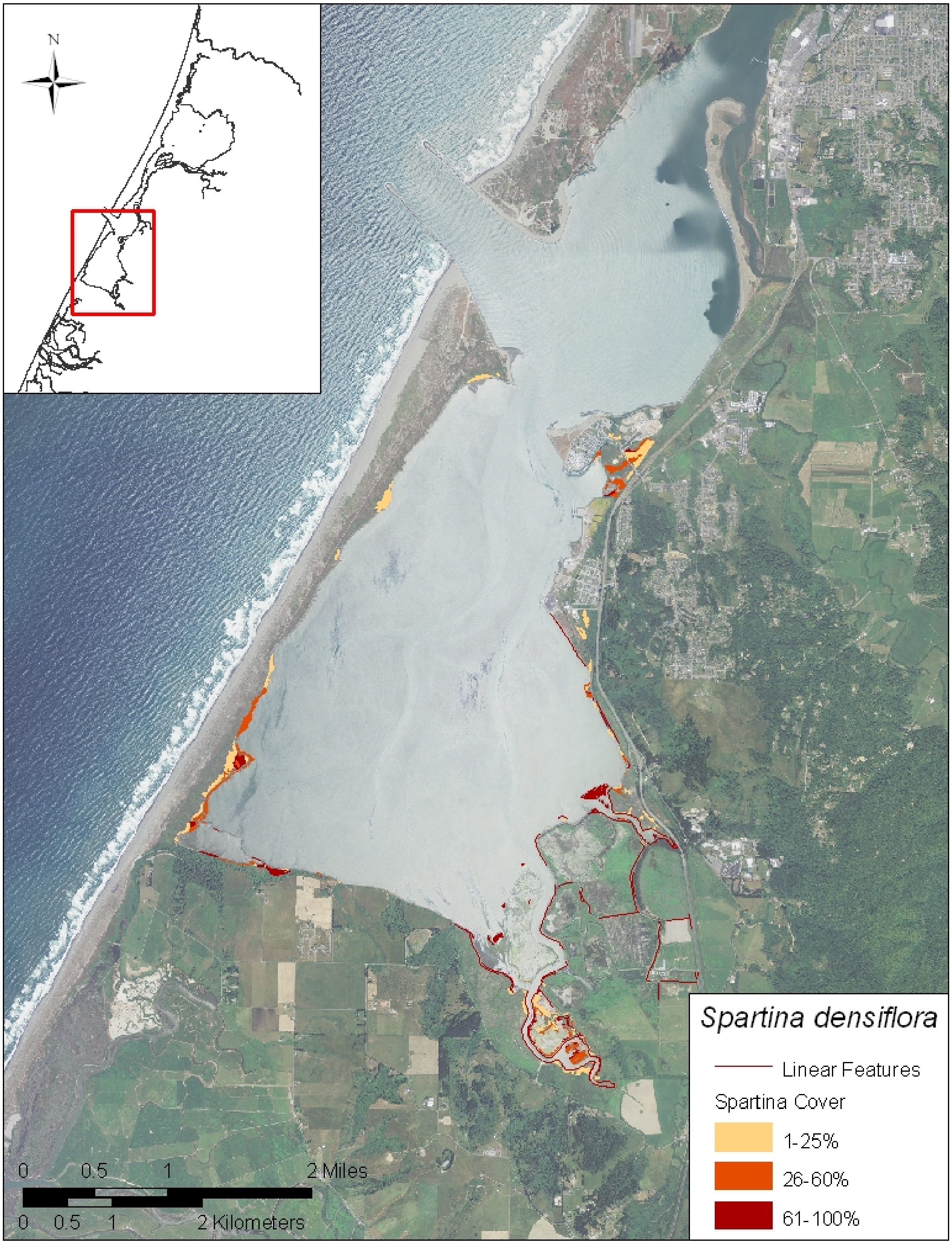


Figure 7. Distribution of *Spartina densiflora* by cover class within the South Bay area of the Humboldt Bay Region.

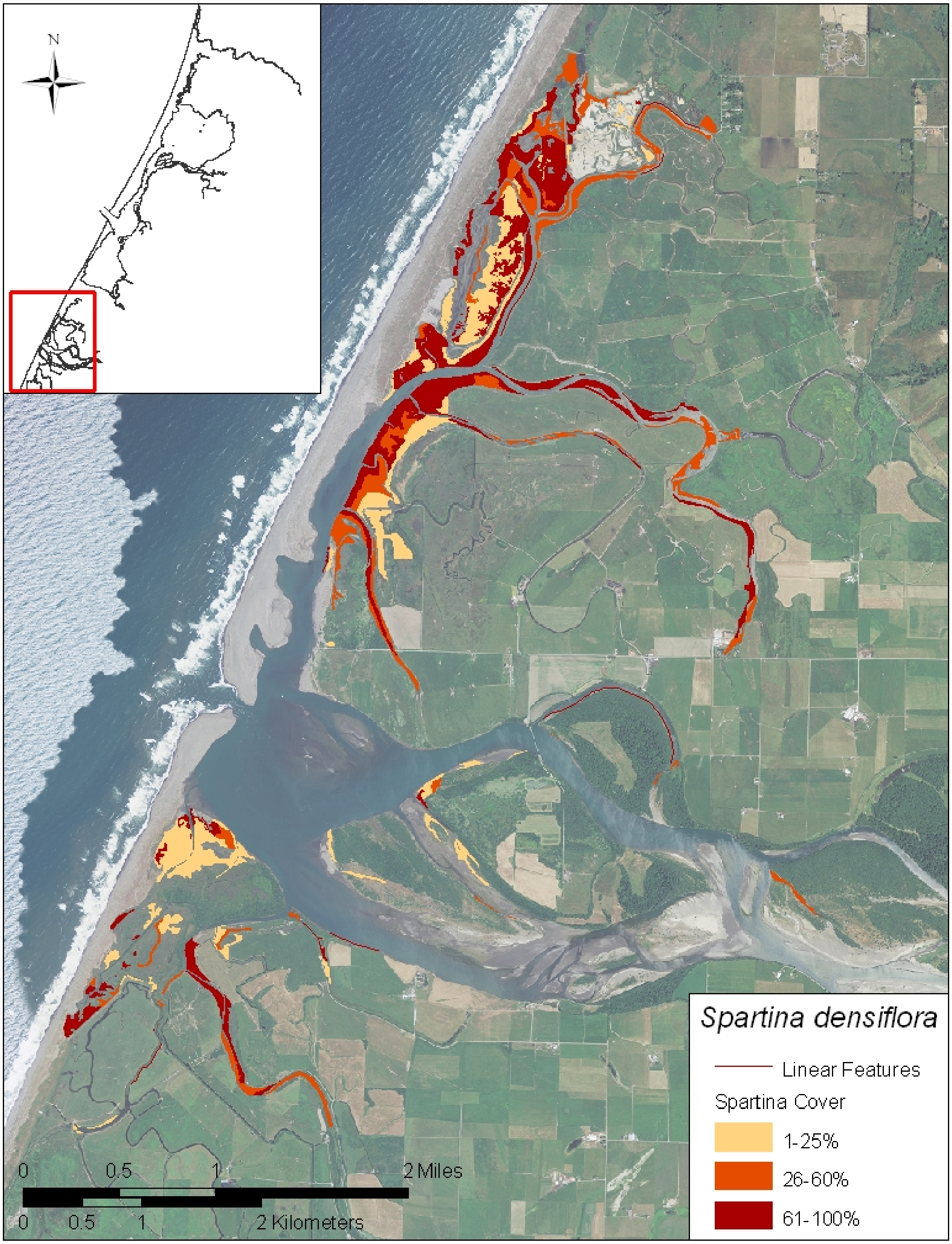


Figure 8. Distribution of *Spartina densiflora* by cover class within the Eel River area of the Humboldt Bay Region.

ACKNOWLEDGEMENTS

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1984

2001


CDC
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Jan 2008
July 2008
July 2009