

# Seedling Germination and Salinity Tolerance in cordgrass: *Spartina foliosa* and *Spartina alterniflora* x *foliosa* Hybrids

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**Abstract** An exotic cordgrass species, *Spartina alterniflora*, hybridized with the native cordgrass, *S. foliosa*, after its 1970s introduction into the San Francisco (CA) estuary. Hybrids are spreading rapidly throughout the Bay, potentially altering the estuary ecosystem, which supports more than a million shorebirds including the endangered California Clapper Rail. Previous studies were conducted on the salinity tolerance of the plants in both individual species and hybrids between the two, however; the salinity tolerance of the seeds had not been previously studied. The goal of this study was to determine the salinity tolerance of the seeds in both the native and hybrid species. Twelve hybrid cordgrass plants were selected based on their experimentally determined salinity tolerance (assessed using end-of-experiment biomass, conducted at UC Davis), their ability to produce seed, and their genetic background (ranging from 0% to 93% *S. alterniflora*). Two *S. foliosa* individuals were collected from a restored tidal marsh in San Francisco Bay, genetic analysis indicated was uninvaded by hybrids. No genetic analysis was performed on the seeds. There were 2 pre-germination treatments (low and high salinity) and 3 germination treatments (low, medium, high salinity) (= 6 treatments). Results show that the pre-germination environments do not have an effect on the seeds. The germination environments do, however, affect the seeds and an increase in salinity leads to a decrease in germination. Parent clones also affect the salinity tolerance of the seeds. These results could have enormous implications for the future of native *S. foliosa* and the species that it supports.

## Introduction

It has been widely recognized that introduced species can pose threats to native ecosystems and alter them dramatically. This has been documented many times throughout history. For example, *Lantana depressa*, an endemic plant to Dade County, Florida is hybridizing with the introduced species common in southern gardens, *L. camara*, the hybrids combine the vigor of the alien with the local adaptations of the native, resulting in a loss of native species (Levin 2002). The most numerous exotic, spreading rapidly throughout the San Francisco estuary, is hybrids between *Spartina alterniflora* (Smooth cordgrass) and *Spartina foliosa* (California cordgrass) (Ayres *et al.* 2004). These hybrids have the potential to permanently alter the estuary ecosystem (Callaway and Josselyn 1992). The salt marshes in the San Francisco Bay supports a variety of species including endangered species such as the California clapper rail (*Rallus longirostris obsoletus*) and the salt marsh harvest mouse (*Rerthrodontomys raviventris*). Over 1 million migratory shorebirds, on the Pacific Flyway Route, pass through the San Francisco estuary annually feeding on the invertebrate fauna of unvegetated mudflats during low tide. “Hybridization is perhaps an equal or greater threat to *S. foliosa* than is ecological competition with *S. alterniflora*” (Ayres *et al.* 1999). *S. foliosa* is a native perennial salt marsh grass found from Bodega Bay, CA to Baja, Mexico (San Francisco Estuary Invasive Spartina Project Page 2003, elect. comm.). It is anticipated that if the hybrid population is left unchecked *S. foliosa* will become the first naturally dominant plant species to go extinct in its own ecosystem since the passage of the Endangered Species Act in 1973 (San Francisco Estuary Invasive Spartina Project Page 2003, elect. comm.).

*Spartina alterniflora*, also a perennial cordgrass, is native to the eastern and gulf coasts of the USA and was introduced in the San Francisco Bay in the mid-1970s (Ayres *et al.* 1999). Previous studies have shown that *S. alterniflora* can grow in elevational zones both higher and lower (9-20cm) than the native *S. foliosa* (Callaway and Josselyn 1992). However, *S. alterniflora* x *foliosa* hybrids pose a greater threat to the San Francisco Bay than *S. alterniflora* because they have greater morphological and reproductive vigor than either parental species (Ayres *et al.* 2003). *Spartina alterniflora* x *foliosa* hybrid plants coalesce while also accreting and stabilizing sediment around them,

converting tidal mudflats to cordgrass meadows (Daehler and Strong 1996). This process increases the elevation of the mudflat for further colonization (San Francisco Estuary Invasive *Spartina* Project Page 2003, elect. comm.). Colonization alters marsh hydrology and channel habitat dramatically while increasing the risk of upland flooding and decreasing the area of mudflats used by foraging birds (Callaway and Josselyn 1992).

Genetically tested hybrid plants showed a wide range (10ppt-40ppt) of tolerance to salinity in a previously conducted common greenhouse experiment (Pekenham-Walsh, 2003). The purpose of this experiment is to determine if hybrid seeds also show a wide range of salinity tolerances, however; no genetic analysis was conducted on the seeds. Seeds are carried by tides and these tides have the potential to spread the invasion throughout the San Francisco Bay and outside of the estuary (Ayres *et al.* 2003). Seeds that can germinate in higher salinities will have an advantage over those less tolerant to salinity because they can establish at higher elevations (D.A. 2003, elect. comm.).

I hypothesize that seeds with a low tolerance to salinity will be those of *S. foliosa* due to its restriction in tidal marshes to lower salinity sites, and its poor growth in high salinity in the greenhouse relative to several hybrids. I also hypothesis that a subset of hybrid plants will produce seeds with higher salinity tolerance (D.A. 2003, elect. comm.). This experiment tests whether the tolerance of salinity, ranging from 10ppt to 40ppt, in established plants is correlated with the salinity tolerance of their progeny and whether there is interaction between the pre- and germination salinity environments (D.A. 2003, elect. comm.).

## Methods

**Data collection** Seeds were collected yielding varying seed sets (Table 1) in November 2003 from twelve in situ *Spartina alterniflora* x *foliosa* hybrid plants located at Cogswell Marsh in Hayward, CA and two in situ *S. foliosa* plants at Mt. View tidal marsh, which is uninvaded by hybrids, at Shoreline Park, Mt. View, CA.

Clone ID	*A	*B	*C
C1-5	146	98	126
C2-11	66	60	64
C2-13	53	77	47
C2-5	46	96	64
C2-6	146	86	183
C2-8	88	92	116
C3-1	118	40	124
C3-13	58	30	30
C3-4	38	42	58
C3-5	84	76	96
C3-7	92	153	81
C3-8	68	56	62
SF-1	228	184	228
SF-2	162	220	164

Table 1

Seed Set Data: C=*S. alterniflora* x *foliosa* hybrid clones and SF=*S. foliosa* clones

Entries denote the number of seeds per replicate (each replicate is the total of 2 inflorescences)

\*3 Replicates: A, B, C

The salinity tolerance of the parent plants was experimentally determined in 2002 by vegetatively propagating them and subjecting them to 3 salinity levels (10ppt, 25ppt, and 40ppt) in a greenhouse experiment conducted at the University of California Davis (UCD) (Pakenham-Walsh 2003). Those plants which had reliable seed production over the years were selected for this experiment (D.A. 2003, pers. comm.).

Viable seeds were determined by removing the florets from the inflorescence and gently finger pressing the seeds. Previous studies have shown that “filled” seeds determined using this method contain fertile embryos. Eighty-four small-perforated ziplock bags were used to store the seeds once they were separated from the unfilled florets. Six bags were necessary for each clone (14 total clones) because each replicate was separated into either a high or a low salinity and there were 3 replicates per clone ( $2 \times 3 = 6$ ) (‘A’, ‘B’, ‘C’). Each replicate consisted of 2 inflorescences. Each replicate was divided in half and one perforated bag was immersed in high salinity (undiluted seawater~30ppt) while the other was immersed in a low salinity solution (25-30% seawater~8ppt) at 4°C for 8 weeks (total) at UCD. These treatments were chosen to simulate the natural overwintering environment of floating on tidal waters, and a more

benign low salinity environment to determine the effect of seawater on germination. The perforations were large enough to allow the solutions to flow into the bags, while small enough to prevent the seeds from escaping. For clones with seed numbers too small (<140 seeds total) to receive all 6 treatments (2 pre-germination treatments and 3 germination treatments), the seeds received the full strength seawater pre-treatment only. Pre-germination treatment is necessary for optimum germination in *Spartina* (Seneca 1974). After the pre-germination treatment, seeds were thoroughly rinsed in de-ionized (DI) water and arbitrarily and equally divided into 3 germination environments (low: 10ppt, med: 25ppt, and high: 40ppt). These germination environments simulate the various salinity zones occurring at varying elevations found throughout salt marshes (brackish, estuarine waters, and hyper-saline respectively) and were consistent with those used in the common greenhouse experiment. The solutions were created using Instant Ocean, manufactured by Aquarium Systems USA & France, and added to autoclaved DI water to prevent pathogen contamination using a refractometer to determine salinity. To allow accurate replacement of evaporated water with DI water during the course of the germination period of 10 weeks, a line 8mm from the bottom of the petri-plates was drawn on the side of the plates. The seeds were then placed in small sterile petri-plates (diameter=7cm) and 77mm<sup>3</sup> of the appropriate solution was added to each petri plate. The plates, were numbered from 1 to 222 (total number of samples) and the assignment of any replicate/individual/treatment to each plate was randomly determined. The petri-plates were arranged numerically on a lab shelf so each plate was well lit during the germination phase, this ensured that the placement of any replicate/individual/treatment was randomly determined. The room was kept at room temperature, ranging from 60<sup>0</sup> F to 80<sup>0</sup> F.

The germination observations in the petri-plates initially took place 3-4 times per week for 2 weeks and then 1-2 times per week for 8 weeks at the University of California Berkeley (UCB). The germinated seeds were counted/documentated and removed from each plate. Observations of the petri-plate solutions took place during the germination monitoring and if the solution appeared cloudy it was drained off and replaced with fresh solution of the same salinity. These methods are consistent with others used when germinating and monitoring *Spartina* (Seneca 1974, Seneca and Blum 1984). Some seeds

did not germinate during the 10-week observation period, however; this could be due to seed immaturity or infertility and not necessarily due to salinity tolerances.

These methods address the question of whether the seeds of hybrid *S. alterniflora* x *S. foliosa* can tolerate high (40ppt) or low salinities (10ppt), successfully, regardless of the parent plants. UCD has conducted experiments suggesting that the hybrid plants grow better in both higher and lower salinities than the parental species. These salinities ranged from 10ppt to 40ppt, however; it is unknown whether the seeds can also withstand these extremes. The maximum salinity threshold for *S. alterniflora* germination ranges from 60ppt to 80ppt (Mooring *et al.* 1971) and *S. foliosa* occurs in the lowest salinity zone found in native tidal marshes due to consistent flushing by the tides (Ustin *et al.* 1982). I expect hybrid seeds will have the largest range of salinity tolerance resulting from both parent species. *Spartina* spreads by belowground rhizomes but also by seeds that are carried by the tides so if they are deposited in areas either higher or lower in salinity relative to the parent plants, they could potentially inhabit these areas. The objective of this experiment was to discover if this is true and to what extent (their salinity range tolerations).

**Data Analysis** A One-way Anova (Analysis of Variance) analysis was performed to test overall effects of pre-germination salinity on germination, effects of germination environment, effects of plant genotype, and any interactions that may occur between these factors. All alpha levels, or threshold P values, were set at 0.05 to ensure statistically significant results. The relative (Low/High) salinity tolerance in both the parent plants and the seeds were assessed. A Tukey test, also known as a “honestly significant difference test” or “wholly significant difference test, was employed because it is the most suitable and most widely used multiple comparison procedure in comparing means with large sample sizes (n=222) (Zar 1999). A linear regression was also performed correlating salinity and germination to predict the effects that salinity changes would have on the proportion of *Spartina* germinated. JMP Start Statistics software was used to conduct all statistical analyses (Sall *et al.* 2001).

## Results

The pre-germination environments were not statistically significant (prob>f, 0.9628) and no interaction was found between the pre- and germination environments (prob>f,

0.9403) so the pre-germination treatment was consequently dropped from the further analysis (Fig. 1).

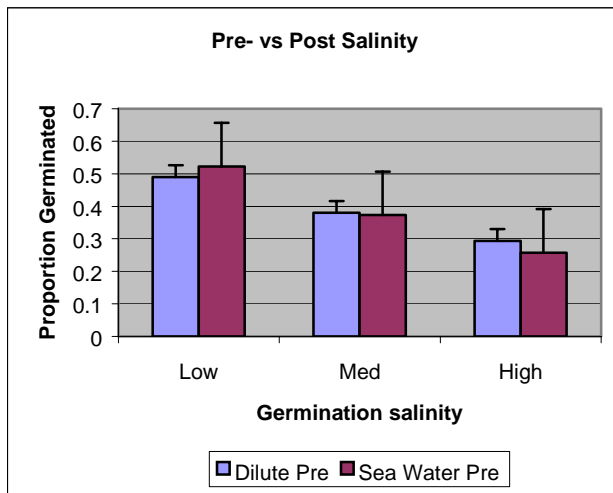


Figure 1  
Pre-germination environments are Dilute Seawater=25-30%~9ppt and Undiluted Seawater=100%~33ppt. Germination salinities are Low=10ppt, Medium=25ppt, and High=40ppt.

Germination for the three germination environments peaked towards the beginning of the experiment, a spike in the middle, and trailed off towards the end. As time increased, generally, germination decreased (Fig. 2).

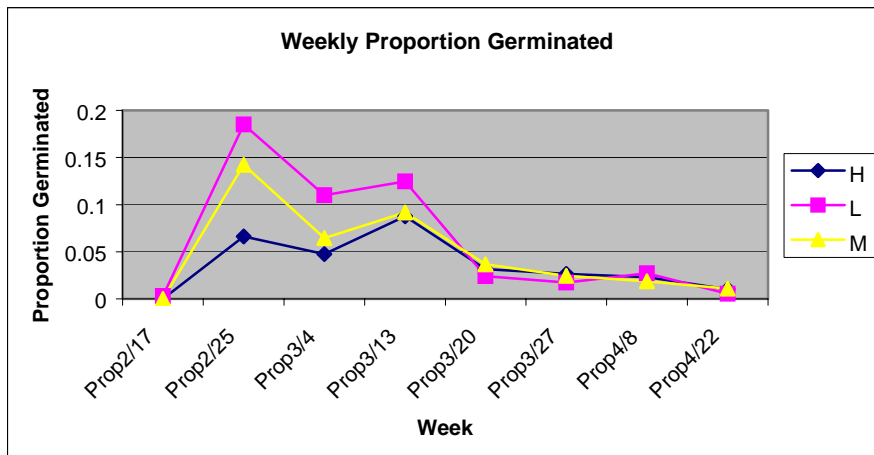


Figure 2  
H=40ppt, M=25ppt, L=10ppt

Significant ( $p < 0.05$ ) results were found for both the germination environments and the effects of plant genotype on the proportion germinated and the interaction occurring between these factors. The germination environment had an effect on the proportion

germinated (prob>f, <0.0001) indicating that increases in salinity lead to decreases in proportion germinated (Fig. 3).

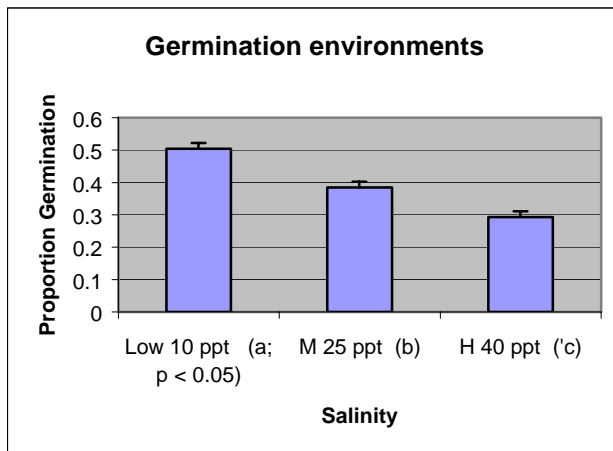


Figure 3

A linear regression, or line of “best fit,” using least square means of 10ppt, 25ppt, and 40ppt was calculated to predict how changes in salinity would affect germination within the observed range (Fig. 4).

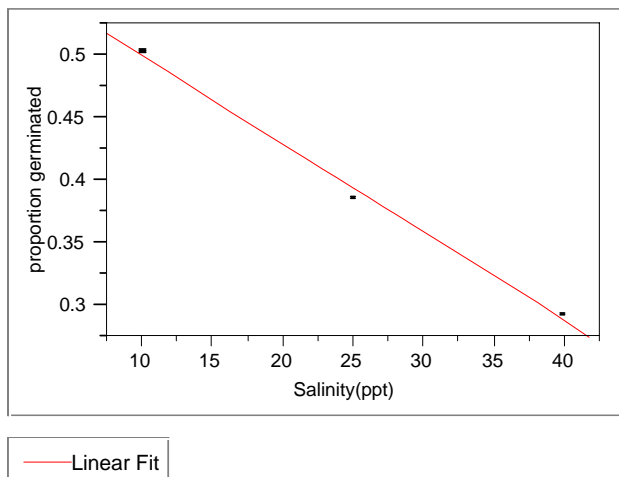


Figure 4

$$\text{Proportion Germinated} = 0.569943 - 0.0070397 * \text{Salinity(ppt)}$$

$$R^2 = 0.99463$$

Plant genotype also significantly affects proportion germinated (prob>f, <0.0001). The interaction between these two variables also yields a statistically significant result (prob>f, 0.01) indicating that both the germination and the plant genotype have an effect



on the proportion germinated and these results would not occur simply by chance alone (Fig. 5).

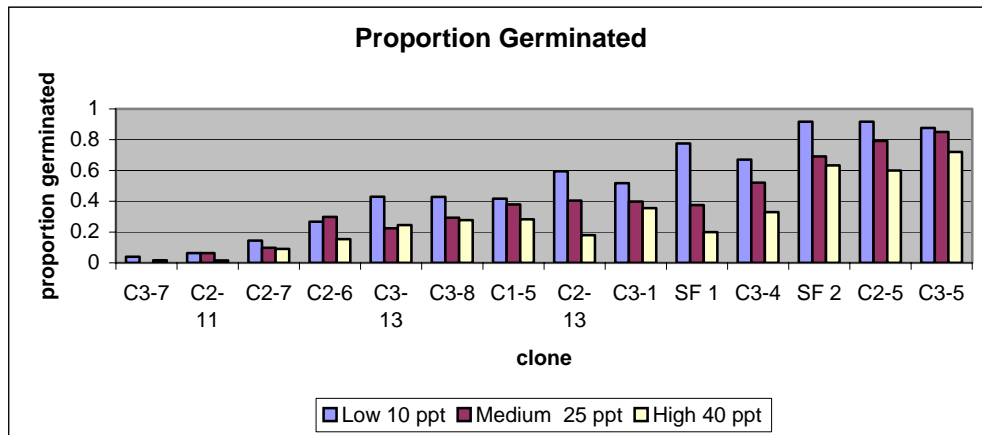


Figure 5  
Proportion of seeds germinated  
C=*S. alterniflora* x *foliosa* hybrid clones, SF=*S. foliosa* clones

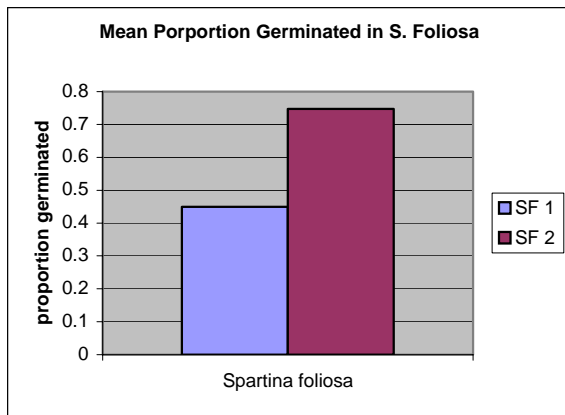


Figure 6

The mean proportion germinated was determined and plotted for *S. foliosa*, unlike previously hypothesised, *S. foliosa* was one of the better germinators overall.

The plant genotype has an affect on the salinity tolerance of the seeds, but it is not the only factor contributing to the salinity tolerance observed in the seeds. This can be observed when the salinity tolerance of the parent plants is compared to the salinity tolerance found in the seeds (Table 2).

Clone ID	% Hybrid in Parent Species	Parent Relative Tolerance*	Seed Relative Tolerance*
C2-11	57	H	L
C2-6	50	H	M
C2-7	50	H	H
C3-8	43	Hest	H
C1-5	93	M	H
C3-5	50	M	Hest
C3-1	92	M	H
C3-13	14	M	M
C2-13	93	MH	L
C2-5	46	L	H
C3-7	71	L	M
C3-4	0	L	M
SF-1	#0	N/A	L
SF-2	#0	N/A	H

Table 2

Relative Salinity Tolerance of Parental Species and Seeds

C=*S. alterniflora* x *foliosa* hybrid clones, SF=*S. foliosa* clones

\*Relative (Salinity) Tolerance=Total proportion Low germinated/Total proportion High germinated

\*L<0.4, M=0.4-0.6, H>0.6, and Hest>0.8 ,

#Previous genetic analysis revealed unhybridized *S. foliosa* marsh at collection site

## Discussion

The results attained in this experiment could have enormous implications for native *S. foliosa* and the habitat it supports. The fact that the pre-germination solution does not

affect germination in the seeds lends itself to the idea that *Spartina* seeds are viable, for at least 8 months (Mooring et al 1971), regardless of floating in either; hyper-saline, estuarine, or brackish waters. *Spartina alterniflora* x *foliosa* hybrids are not limited by the salinity of the water that the seeds float in prior to colonization.

The general germination trends for all three germination treatments were consistent with those attained by Callaway and Josselyn (1992), as time increased the proportion germinated decreased. Both studies involved a refrigeration period prior to germination simulating the natural overwintering environment of floating on tidal waters. Germinating quickly after depositing in the tidal marsh would be most advantageous to colonization, explaining the trends found in the three germination environments.

The germination treatments did have an effect ( $p < 0.05$ ) on germination indicating that the location, or elevational zone, where the seeds deposit is a significant factor in germination and colonization. In the germination environments: low (10ppt) yielded 50% germination  $\pm 1.8\%$ , medium (25ppt) = 38.5%  $\pm 1.8\%$ , and high (40ppt) = 29.3%  $\pm 1.8\%$ . Increases in salinity lead to decreases in germination, a trend that was also observed by Mooring *et al.* (1971) in *S. alterniflora* from North Carolina. The linear regression equation was calculated to predict how changes in salinity would affect germination within the observed range, for example if there was an extremely wet year resulting in an overall decrease in salinity. A high coefficient of determination,  $R^2 = 0.99463$ , the fitted regression accounts for, or explains, 99.463% of the total variation in proportion germinated. The equation, Proportion Germinated =  $0.569943 - 0.0070397 * \text{Salinity}(\text{ppt})$ , explains how a 10% decrease in salinity from, for example 20ppt to 18ppt, increases the percent germinated by 1.4%, from 42.9% to 44.3% (respectively).

It should be noted that mean proportion germinated for *S. foliosa* was determined and plotted to yield mean percent germination = 45% in SF1 and 74.7% in SF1. This large percent germination is not consistent with the maximum germination of 46% for *S. foliosa* (D.R. Ayres, unpublished data). This indicates that the native *S. foliosa* is not a poor germinator as previously believed.

Plant genotype definitely has an effect on the proportion germinated and the salinity tolerance exhibited in the seeds, however; it is not the only contributing factor involved (Table 2) and, unfortunately, no genetic analysis was performed on the seeds in this study.

This objective of this study was to determine the salinity tolerance of the invasive *Spartina alterniflora* x *foliosa* hybrids and the native *S. foliosa*.

Recent hybridization and polyploidization involving hexaploid species resulting from the introduction of the exotic *S. alterniflora* hybridizing with native sister species *S. foliosa*, results in introgressant hybrid swarms (Ainouche et al 2003). To date all species are polyploids, with no known diploid species, making accurate counts of the numerous small chromosomes particularly difficult, resulting in a need for additional investigations at the population level on most of the American taxa (Ainouche et al 2003). *S. alterniflora* differs from *S. foliosa* in ecological characteristics such as; *S. alterniflora* exhibits a higher tolerance to tidal submersion, size of the plants vary, and the flowering precocity of *S. foliosa* (Daehler and Strong, 1997). Only one chloroplast haplotype, CCT, is documented in *S. foliosa* populations and little information exists on the level of nuclear genetic diversity (Anttila et al, 2000). The lack of chloroplast diversity found in *S. foliosa* can be explained by it's geologically younger habitat of this Pacific species and the much smaller geographic range compared to *S. alterniflora*. *S. alterniflora* has much greater molecular diversity in both the nuclear genomes (Perkins et al., 2002) and the chloroplast haplotypes CAT, TAA, and TAT (Anttila et al, 2000).

Hybridization is bidirectional (Ayres et al., 1999) and occurs during the overlap in flowering period (*S. foliosa* begins flowering a few weeks before *S. alterniflora*, in June, and both species continue flowering through September) (Anttila et al 1998). *S. alterniflora* has a greater male fitness than *S. foliosa*, producing 21 times more viable pollen than the native and the *S. alterniflora* pollen increased the seed set of native plants almost eightfold over native pollen, resulting in hybrid swarms with up to 90% nuclear markers specific to *S. alterniflora* and displaying the chloroplast haplotype of *S. foliosa* (Anttila et al, 2000). This hybridization and recurrent backcrossing of closely related sister taxa results in new genotype combinations (Ainouche et al., 2003), but so little is currently known about this new invasive species.

*Spartina alterniflora* x *foliosa* hybrids have the potential to greatly alter the estuarine ecosystem and hydrology of the San Francisco Bay by accreting sediment and changing tidal marshes into cordgrass meadows (Daehler and Strong 1996). *Spartina* seeds are not limited by the salinity, or lack of, salinity in the water prior to germination. Decreases in

salinity lead to increases in germination. Colonization in both higher and lower elevational zones is possible due to the wide range of salinities that the hybrid seeds exhibited. If colonization were to occur it could have enormous repercussions on the millions of shorebirds that use the San Francisco Bay for foraging and nesting environments, including the California Clapper Rail (*Rallus longirostris obsoletus*). Other federally listed endangered species that inhabit the marsh zones at risk for colonization are the Salt Marsh Harvest Mouse (*Reithrodontomys raviventris*) and the Soft Birds-beak (*Cordylanthus mollis ssp. Mollis*), however; the affect of invasion by *Spartina alterniflora* x *foliosa* hybrids on these species has not been determined (Ayres *et al.* 2003). *Spartina alterniflora* x *foliosa* hybrids are a great threat to estuarine ecosystems of the Pacific Coasts because of the unique ecosystem engineering abilities they posses (Ayres *et al.*, 1999). It is imperative that these hybrid populations are monitored and the genetics driving this evolution are fully understood before the genetic makeup of native *S. foliosa* completely evolves and hybridizes into this new invasive species.

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