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FELLOWSHIP APPLICATION COVER PAGE

APPLICANT TYPE ☐ Postdoctoral Researcher ☒ Ph.D. Graduate Student

PROJECT NUMBER _____

PROJECT TITLE Frequency, distribution and ecological impact of cryptic hybrid invaders: management tools for eradication of invasive Spartina

FINANCIAL SUMMARY

First Year CALFED Funds Requested:	$ 42,264
Total CALFED Funds Requested:	$ 86,785
Duration:	2 years
Proposed Start/Completion Dates:	09/01/08-08/31/10

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Will animal subjects be used? ☐ Yes ☒ No

APPROVAL DATE: _____ PROTOCOL #: _____ PENDING: _____

Does this application involve any recombinant DNA technology or research? ☐ Yes ☒ No

FREQUENCY, DISTRIBUTION AND ECOLOGICAL IMPACT OF CRYPTIC HYBRID INVADERS:
Management tools for eradication of invasive *Spartina*

INTRODUCTION

History of *Spartina* hybrids in San Francisco Estuary

Primeval San Francisco Estuary salt marshes are vegetated with fields of pickleweed and California cordgrass in the mid to high intertidal, while the low intertidal consists of vast expanses of open mudflats. *Spartina alterniflora*, endemic to the eastern United States, was introduced into the range of native California cordgrass, *S. foliosa*, in south San Francisco Bay ca 30 years ago (USACE 1978; Faber 2000). Extensive hybrid swarms have arisen through reciprocal hybridization (Daehler and Strong 1997, Ayres et al 1999, Anttilla 2000). Hybrids have the potential to alter salt marshes in a multitude of ways. They outcompete California cordgrass and swamp native stigmas with their prodigious pollen output, threatening native cordgrass with extinction (Ayres, Strong et al. 2003). They grow above the upper limit of native *Spartina*, outcompeting the *Sarcocornia pacifica* (Pacific pickleweed) that normally dominates marshes above mean high water, invade formerly open mudflats in the low intertidal, and fill in the tidal channels that interlace marshes. (Ayres, Smith et al. 2004). Projecting from the rate of hybrid expansion in the 1990s, *S. alterniflora x foliosa* was predicted to cover all 28,098 hectares of marsh and mudflat in San Francisco Bay within 200 years (Ayres, Smith et al. 2004).

By altering salt marsh habitat, invasive *Spartina* harms the native fauna as well. Alameda Song Sparrows, a California Species of Special Concern, have a 30% lower success rate when they build their nests in hybrid cordgrass (Nordby, Cohen et al. 2008). Ornithologists are concerned that the hybrids' potential to fill in marsh channels could reduce foraging opportunities for the endangered California Clapper Rail (Project 2000). And whereas native *Spartina* provides habitat for a diverse and abundant benthic invertebrate community, the hybrids' dense rhizomes exclude the benthic invertebrates that constitute the basis of the food web for shorebirds and fishes (Strahlberg, Toniolo et al. 2004; Brusati and Grosholz 2006).

The California Coastal Conservancy initiated the San Francisco Estuary Invasive *Spartina* Project (ISP) to reverse the spread of invasive *Spartina*. In 2004, the year region-wide control began, hybrid *Spartina* covered 704 acres, up from 470 in 2000 (I.S.P. 2008). As of 2006, the last year for which data is available, that figure had declined to 599, or slightly less than 1% of San Francisco Bay's marshes and mudflats. These figures are for "net cover;" that is, the acreage that would be covered if all areas ranging from 1-100% cover in hybrid *Spartina* were condensed into one continuous area of 100% cover. The gross area covered by invasive *Spartina* of any cover class is much higher, i.e. 1631 acres in 2006.

Although the acreage of *Spartina* is declining in response to the control program, managers are encountering increasing difficulty in distinguishing hybrids from natives (Peggy Olofson and Ingrid Hogle, pers. comm.). While net acreage of all field and/or genetically identified hybrids declined by 22% from 2005 to 2006, the net acreage of unresolved hybrids (morphologically ambiguous plants that either were not tested in the lab, or had inconclusive laboratory results) increased by 33%, from .94 to 1.27 net acres.

The cause of the increase in unresolved hybrids is unknown. Possible factors are that morphologically ambiguous hybrids have a fitness advantage over evident hybrids because they are less likely to be herbicided, or that the hybrid population increasingly resembles the native plants as they repeatedly backcross to *S. foliosa.* Regardless of the cause, given the potential of hybrids to spread rapidly and profoundly alter the ecology of San Francisco Estuary, the possibility that even a small percentage of the hybrid population is being overlooked by the control program is of serious concern.

The problem of detecting hybrids

The efficacy of the control program depends on the ability to distinguish hybrid *Spartina* from the native. The means for discriminating hybrids from natives are twofold. The primary tool is field identification based on morphology. However, my work shows that hybrid morphology is highly variable. In 2007 I sampled 89 plants in three marshes where there was a mixture of native and hybrid *Spartina*. I measured each plant for four diagnostic characters: height (from the soil to the top of the tallest stretched leaf), stem diameter, intensity of pinkness on stem (on a scale of 0-7), and width of widest leaf in proportion to its length. I then tested each sample in the lab for its genetic identity using the methods described in Daehler and Strong (1997). Of the 89 samples, 55 were native and 34 were hybrids. The two taxa showed statistically significant differences in the means for all four characters (Student's t-test, $p<.05$); however, 11 of the 34 hybrids (32%) were within one standard deviation of the mean for native plants in all four characters. I classified these plants as "cryptic hybrids." Because these plants are phenotypically similar to California cordgrass, they are likely to be overlooked by control efforts.

The second means of distinguishing hybrids from natives is genetic testing using RAPD markers (Daehler and Strong 1997). In cooperation with the Invasive *Spartina* Project, the lab at U.C. Davis administered by Dr. Debra Ayres, and staffed by myself andother research assistants, processes samples of *Spartina* to determine their genetic identity. We use nine RAPD markers for these tests. A sample is characterized as native based on the presence of four *S. foliosa* markers and the absence of five *S. alterniflora* markers. If at least one *S. foliosa* marker is absent, or at least one *S. alterniflora* markers is present, the plant is diagnosed as non-native.

However, there is a probability that some hybrids will display only *S. foliosa* bands. This probability increases as hybrids continually backcross to native plants. If, for example, a hybrid plant that has three of the four *S. foliosa* markers and no *S. alterniflora* markers crosses with a native *S. foliosa*, on average one in eight offspring will appear to be a native plant on the basis of the nine RAPD markers. Given that 49 of 216 hybrid samples tested in 2007 were returned as hybrid based solely on the absence of one foliosa marker, we can infer the existence of hybrids that were falsely identified as native by our present genetic methods.

In addition to being difficult to distinguish with genetic tests, hybrids that are highly backcrossed to California cordgrass are also more likely to be morphologically cryptic. Previous work has shown that hybrids with a higher percentage of their genome derived from *S. foliosa* phenotypically resemble the native. Ayres *et al.* (2004) showed that hybrids with 40% or less of their genome derived from *S. alterniflora* resemble *S. foliosa* in their height, width, biomass and stem color (Figure 1), whereas hybrids with a higher percent of their parentage derived from *S. alterniflora* had means far outside the variance observed in native cordgrass. In conclusion, hybrids that are highly backcrossed to *S. foliosa* may be more likely to escape detection by visual identification and have a higher probability of having a RAPD profile characteristic of *S. foliosa.*

The overlapping difficulties in detecting hybrids using visual and genetic identification create an obstacle for the control program: how can eradication of hybrids be achieved unless they can all be detected? The California Coastal Conservancy is investing heavily in the *Spartina* control program, but they may not have the tools they need to guarantee that the program will be successful.

Classifications of *Spartina* spp. relevant to this proposal

Spartina foliosa: The native cordgrass of the west coast of North America, also called California cordgrass.
Spartina alterniflora: The species introduced from the east coast to San Francisco Bay.
S. alterniflora x foliosa hybrids: The hybrid of the two taxa; highly invasive in SF Bay.
- evident hybrids: Hybrids that can be visually distinguished from *S. foliosa* by their morphology.
- cryptic hybrids: Hybrids with morphological measurements that lay within one standard deviation of the means of *S. foliosa*, making accurate visual classification problematic.

- genetically undetected hybrids: Hybrids that possess the nine RAPD markers characteristic of *S. foliosa* currently used to screen *Spartina* samples, causing them to be inaccurately identified as native plants by current genetic analysis methods. These can be morphologically evident or cryptic hybrids.

Questions

Overarching question: can we provide managers with the tools they need to detect hybrid *Spartina*, and given that some cryptic hybrids are likely escape eradication, what danger do they represent to tidal wetlands?

Specific questions:

1. Can we improve our genetic screening to virtually eliminate the number of hybrid plants falsely identified as natives?
2. What is the frequency and distribution of cryptic hybrids, and what spatial and environmental variables predict where they are they likely to occur?
3. If cryptic hybrids do escape control efforts, are they likely to spread rapidly or invade the low intertidal?

Objectives

Given that detecting hybrids is of paramount importance to the success of hybrid *Spartina* control efforts, this proposal suggests a three-pronged approach to the problem.

1. Reduce or eliminate the problem of genetically undetected hybrids.
2. Determine the patterns governing the density of morphologically cryptic hybrids and identify areas that require intense genetic screening to guarantee hybrid detection.
3. Given that some cryptic hybrids may remain after high-level control efforts have ceased, evaluate the potential for cryptic hybrids to invade and alter tidal marshes, thereby giving managers the information they need to weigh the costs and benefits of attempting to eradicate every cryptic hybrid in the Estuary.

Goal 1. Develop a model using microsatellite markers and Bayesian statistical algorithms to improve the accuracy of DNA tests for hybrids.

The use of nine fixed, species-specific RAPD markers to identify hybrid *Spartina* allowed Drs. Donald Strong and Debra Ayres to document the hybridization of *Spartina alterniflora* with the native *Spartina foliosa* (Daehler and Strong 1997; Ayres, Garcia-Rossi et al. 1999). The Invasive *Spartina* Project continues to use this method in testing samples for genetic confirmation of their field identification. RAPDs are inexpensive and useful for classifying the vast majority of hybrid plants. However, other methods hold the potential for greater accuracy in detecting highly backcrossed individuals. Specifically, we can use the 35 microsatellite primers developed for *Spartina* (Blum, Sloop et al. 2004; Sloop, McGray et al. 2005). These are a more complex tool to use for distinguishing taxa because, unlike the RAPD markers we employ, they require sophisticated statistical analysis to use in the diagnosis of hybrids, but hold the potential for greater accuracy.

The 35 microsatellite markers we have available for *Spartina* have different properties from the RAPD markers. The RAPD markers amplify loci that are diagnostic and species-specific; that is, one allele occurs in all members of *S. foliosa* and *S. alterniflora*, respectively. The presence of any combination of alleles from both species in a single individual is conclusive evidence of hybridity. In the microsatellite markers, there are multiple alleles (also called polymorphic loci) that occur in both species. However, the frequencies of the alleles differ by species. As a result, the application of the microsatellite markers to the diagnosis of hybrids becomes one of statistical probability. A simple example follows this logic. If the A allele is present in 80% of *S. foliosa*, but only 20% of *S. alterniflora*, an individual with the A allele is more

likely to have originated from *S. foliosa.* The problem as a whole is in fact more complex than averaging probabilities across loci, because we can also apply population genetic models of hybridization. When two populations in Hardy-Weinberg equilibrium for a neutral gene such as microsatellites hybridize, the hybrids display a higher rate than expected of possessing two alleles derived from the same parental population, a phenomenon called linkage disequilibrium. Through a series of iterations, the software program STRUCTURE (Pritchard, Stephens et al. 2000) employs Bayesian statistics to construct a model of subpopulations in Hardy-Weinberg equilibrium and assigns a probability to every individual that it belongs to a given population (in this case, either *S. foliosa* or *S. alterniflora*). Individuals that are assigned to both populations are designated as hybrid individuals.

The use of polymorphic microsatellite loci in conjunction with STRUCTURE has been shown to be an extremely powerful tool in conservation projects targeted at eliminating hybrid invaders in protected populations (Adams, Lucash et al. 2007; Meraner, Baric et al. 2008; Oliveira, Godinho et al. 2008; Randi 2008; Schwartz and Beheregaray 2008). These methods proved highly effective even when using far fewer than 35 microsatellite loci; for example, Adams *et al.* (2007) used 18 loci to identify and remove coyote hybrids from the endangered red wolf population in North Carolina.

In order to test the efficacy of using microsatellite markers and STRUCTURE in the identification of *Spartina* hybrids, I generated hypothetical microsatellite genotype data that resembled that of *S. alterniflora* and *S. foliosa* using EASYPOP software (Balloux 2001). I simulated the circumstances of the hybridization as documented in Ayres *et al.* (2003), with a relatively small number of *S. alterniflora* crossing with *S. foliosa* to generate a small number of first generation hybrids, followed by subsequent mixing of hybrids with a large population of *S. foliosa.* My hypothetical data is biased to generate a conservative result, that is, present a lower rate of correct hybrid identification than real data. I accomplished this by two means. First, I generated hypothetical parental populations with a lower Fst than the true value: .5 instead of .61 (Fst between *S. alterniflora* and *S. foliosa* provided by C. Sloop, pers. comm.), and employed 17 instead of the full suite of 35 microsatellite markers we have available for *Spartina*. A lower Fst and reduced number of microsatellite markers are the two major factors that handicap the accuracy of STRUCTURE in distinguishing hybrids (Vaha and Primmer 2006).

I have found that even with these conservative parameters, after four generations of hybridizing in my hypothetical data, STRUCTURE can distinguish hybrids and *S. alterniflora* from *S. foliosa* 100% of the time. The method could use further testing with more generations of backcrossing between the hybrids and *S. foliosa* and a higher Fst. Varying the number of microsatellite markers used could also indicate the minimum number necessary for the desired level of accuracy. However, these promising preliminary results indicate that microsatellites and Bayesian statistical methods of population assignment hold the potential to make our genetic screening nearly 100% accurate, virtually eliminating the probability of falsely identifying hybrid plants as natives.

The next step is to apply this method to *Spartina* samples (detailed in Approach, below). The first stage would be to run samples with both RAPDs and microsatellites to compare results. The new method may replace RAPDs for genetic screening of all *Spartina* samples. Alternatively, it can be used as a supplement to the present, simpler methods, by further testing only those samples that are designated as *S. foliosa* by the RAPD markers, thereby reducing the percentage of hybrid plants that are falsely characterized as natives.

Goal 2. Determine the frequency and distribution of cryptic hybrids, identify spatial and environmental variables that correlate with their occurrence, and predict the areas that are likely to host a high density of cryptic hybrids.

The second major obstacle to hybrid detection is the difficulty of correctly classifying plants in the field. Last year, 8.24% of 461 samples that were field identified with high or medium confidence in the field as *S. foliosa* were identified as hybrid by means of RAPD markers. This percentage does not take into account that some plants identified as *S. foliosa* in the lab may have also been hybrids, but were undetectable with the nine RAPD markers. The major source of misidentification may be morphologically

cryptic hybrids of the sort that I documented in my 2007 field work: hybrid plants that possess phenotypes indistinguishable from *S. foliosa* (see "The problem of detecting hybrids," above). Testing plants genetically, especially with the improved methodology outlined above, can eliminate the problem of misidentification based on visual characters. However, it is impossible to test every *Spartina* plant in the Bay for a hybrid genotype. Cordgrass is a dominant salt marsh species that occurs in large monospecific swards in every salt marsh in the Estuary; furthermore, it is virtually impossible to distinguish one clone from the next in *S. foliosa* meadows, and the formidable sampling effort and expense that would be required to test the genetic identity of every tiller of grass in a *Spartina* meadow is obvious. Given that hybrid plants exist that are difficult to distinguish visually from their native congeners, and testing every plant in the Estuary genetically is impossible, it is necessary to identify those areas where cryptic hybrids are likely to occur and flag them for intense genetic screening. In this way, limited resources can be targeted where they are most useful.

The focus of this portion of the project is to determine trends in the density of cryptic hybrids. To do so we must answer a number of questions. Is most of the variability in the occurrence of cryptic hybrids between marshes (i.e. some marshes have many, others few to none), within marshes (i.e. certain zones in marshes have many, others few), or some combination of inter and within marsh variation? Is variability in the occurrence of cryptic hybrids explained by time since colonization (i.e. marshes that were colonized more recently have a more highly backcrossed, and therefore more cryptic, population of hybrids), or by environmental variables (i.e., certain growing conditions cause a convergent morphology in hybrid and native plants).

Previous work on the East Coast of North America has shown that the morphology of *Spartina Alterniflora* is highly plastic. Identical genotypes transplanted to different zones in the same marsh can display tremendous variation in height and stem width (Mendelssohn 1979; Anderson and Treshow 1980), dependent on soluble nitrogen concentration, pore water salinity and the redox potential of the soil. This body of work suggests that shorter, more delicate growth forms of hybrids in San Francisco estuary may be the result not just of genetic factors (e.g. hybrids with a high percentage of their genome derived form *S. foliosa* are smaller, shown by Ayres *et al.* 2004), but that environmental factors interacting with genetic variation may produce high frequencies of cryptic hybrids. Given that pore water salinity, redox potential, and soluble nitrogen and sediment texture have all been shown to covary with height and biomass in both *S. alterniflora* and *S. foliosa* (Mendelssohn 1979; Anderson and Treshow 1980; Lindig-Cisneros, Desmond et al. 2003; Strong and Ayres 2005; Tyler, Lambrinos et al. 2007), these factors merit investigation as possible factors that could produce a short, foliosa - like growth form in *S. alterniflora x foliosa* hybrids.

These environmental factors also vary within marshes along the intertidal elevation gradient and by location in the Bay (Ustin, Pearcy et al. 1982; Mitsch and Gosselink 2000; Tyler, Lambrinos et al. 2007). By identifying trends in hybrid morphology, and the resulting variability in density of cryptic hybrids, I will be able to project the areas that are most likely to host high numbers of cryptic hybrids as the zones requiring the highest intensity of genetic screening.

Goal 3. Evaluate the potential of cryptic hybrids to spread rapidly and colonize the low intertidal.

Given that cryptic hybrids exist, detection and eradication of them will require a disproportionate amount of resources compared to the control of morphologically evident hybrids. Careful screening by field biologists and genetic testing in the laboratory require expenditures of time and money. Even with the best efforts to eliminate all hybrids from the estuary, it is likely that some exotic genes will remain in the population. However, it is necessary to ask, what are the risks posed by cryptic hybrids? If they morphologically resemble *S. foliosa*, do they interact with the ecosystem like *S. foliosa*? If the answer is yes, organizations such as the California Coastal Conservancy and CALFED may choose to direct funds elsewhere when evident hybrids are extirpated.

On the other hand, cryptic hybrids may represent as serious a threat to local salt marsh ecosystems as do the evident hybrids. Since *S. alterniflora* and *S. foliosa* are known to display highly variable growth forms in response to the environment (Mendelssohn 1979; Anderson and Treshow 1980; Lindig-Cisneros,

Desmond et al. 2003; Tyler, Lambrinos et al. 2007), cryptic hybrids may resemble their native parental species because of the characteristics of the local microhabitat. In that case, cryptic hybrids could develop into highly robust clones as they spread vegetatively into different microenvironments within the marsh, or the offspring of cryptic hybrids could colonize habitats that produce evident hybrids. Furthermore, it is unknown if the short, delicate morphology is a fixed genetic characteristic of cryptic hybrids that restricts them to the same intertidal elevations as *S. foliosa*, or if cryptic hybrids are, like the evident hybrids, capable of invading low elevation marsh channels and mudflats. Lastly, we do not know if cryptic hybrids display the high pollen production, large seed set, and rapid vegetative growth that has been documented in the general hybrid population (Ayres, Strong et al. 2003; Ayres, Smith et al. 2004).

An assessment of the potential of cryptic hybrids to spread and the effects they may have on the ecology of San Francisco Estuary is a prerequisite for managers to be able to make informed decisions about the wisest expenditure of resources. Is detecting and exterminating every cryptic hybrid in the Estuary of vital importance for the health of the ecosystem, or are cryptic hybrids so similar to native plants that resources will be better directed elsewhere?

APPROACH

Improving the accuracy of genetic tests for hybrids

Part 1. Finalize testing the use of microsatellite loci in conjunction with Bayesian statistics on simulated data. Testing this method on simulated population data is useful because in generated data sets we know the *a priori* taxonomic identity of each individual and can model the likely efficacy of the method in detecting hybrids in real data when the true identity of a sample is unknown. Test method using simulated data with 15 generations of hybridization, Fst of .61, and data for 10, 15, 20, 25, 30 and 35 microsatellite loci.

- Determine the relationship between the number of loci used and the level of accuracy in detecting hybrids.
- Determine the minimum number of loci necessary for the desired level of accuracy in hybrid detection.

Part 2. Run 200 DNA samples with both RAPDs and microsatellites. A large number of samples is necessary since individuals that are misidentified by either method are likely to be rare. Fortunately, the Invasive *Spartina* Project sends U.C. Davis large number of samples each year (1000+ in 2007) for genetic analysis.

Construct a matrix of the following form:

	Identified as native with microsatellites	Identified as non-native with microsatellites
Identified as native with RAPDs	**N** # of individuals identified as native with both tests	**H_M** # of individuals identified as hybrid only with microsatellites
Identified as non-native with RAPDs	**H_R** # of individuals identified as hybrid only with RAPDs	**H** # of individuals identified as hybrid with both tests

Possible outcomes:
H_R=0: RAPDs do not identify any individuals as hybrid that cannot be detected with microsatellites. Consider replacing RAPD screening with microsatellites, or using microsatellite testing on all samples identified as native by RAPDs.
H_M=0: Microsatellites do not identify any individuals as hybrid that cannot be detected with RAPDs. Increase the number of microsatellite loci used in tests to improve hybrid detection rate and re-run tests. If H_M does not improve, retain RAPD screening and do not institute microsatellite testing.
H_R>0: RAPDs identify different individuals as hybrids from microsatellites, and should be retained as a method for genetic screening.
H_M>0: Microsatellites identify different individuals as hybrids from RAPDs, and should be instituted as a method for genetic screening.

Three different management decisions could result from this comparison of RAPDs to microsatellites: the continued use of RAPDs alone, the replacement of RAPDs with microsatellites, or the use of RAPDs and microsatellites in conjunction with each other. Knowing the different rates of detection by each method would allow the California Coastal Conservancy and the Invasive *Spartina* Project to determine the best method for genetically screening *Spartina*.

Predicting areas with high densities of cryptic hybrids

Sample, measure, and map native and hybrid *Spartina* in the field to determine means and standard deviations of native and hybrid plants for four diagnostic characteristics. Sample seven salt marshes that contain a mixture of native and hybrid *Spartina* around San Francisco Estuary, prior to the annual application of herbicide, over a three year period (2008 – 2010). Marshes have been selected in consultation with the Invasive *Spartina* Project from those where the field biologists found identification of hybrids to be difficult, and to span the geographic range of the estuary's salt marshes. See Figure 2 for sample sites.

Walk transects parallel to water's edge; walk multiple transects at least 10 meters apart at 2-3 intertidal elevations in wide marshes. Sample approximately every 30 paces to prevent the possibility of sampling the same clone twice for a total of 25 samples in each marsh. Within 30 centimeters of boot, select the tallest tiller and the shortest tiller (assuming tillers within 30 centimeters are the same clone). Map location with GPS unit with subfoot accuracy (Trimble Geo XH, Trimble, Sunnyvale CA). Measure four morphological characters:

1. Total stretched length (from sediment to tip of tallest leaf) of tallest tiller
2. Culm diameter (diameter of stem above sediment) of tallest tiller
3. Leaf allometry (width of widest leaf/length of widest leaf) of tallest tiller
4. Intensity of stem pinkness of shortest tiller

These characters were selected because they are in use by the field biologists working as who annually map the extent of invasive *Spartina* for the Invasive *Spartina* Project, and 1, 2 and 4 were shown to vary between native and hybrid plants in Ayres (2004).

Adjacent to the tallest tiller, also measure:

1. Soil pore water salinity (using a refractometer)
2. Redox potential (using platinum electrodes and an mV meter)
3. Soil pore water ammonium (lab analysis)
4. Soil texture (using sieve)
5. Intertidal elevation (using survey equipment)

Sample leaf of tallest tiller for genetic identification in the laboratory (method of DNA analysis will depend on the outcome of the comparison on microsatellites and RAPDs). Run four two-way ANCOVAs, one for each measured morphological character as the response variable, and with Taxa, (native versus hybrid), Marsh of Origin and Taxa * Marsh of Origin interaction as the model effects. Use

environmental measurements as covariates. Calculate the mean and standard deviation of for each morphological character for hybrid and native plants. Calculate the percent of hybrids that fall within one standard deviation of the mean of *S. foliosa* for all four measurements. Classify these as cryptic hybrids. Compare the frequency of cryptic hybrids across marshes. Test the following hypotheses:

1. The mean values for each of the four morphological characters differs by taxa
2. The mean values for the four morphological characters differs by marsh
3. There is a taxa * marsh interaction; that is, hybrids and natives will look more similar in some marshes compared to others.
4. Environmental covariates will explain a significant portion of the variance in morphological characters. Identify the covariates that have the greatest effect on *Spartina* morphology. Propose a model for predicting locations that will host high frequencies of cryptic hybrids based on environmental characteristics.

Evaluate the potential of cryptic hybrids to spread and alter the ecology of San Francisco Estuary salt marshes

Part 1. Vegetative growth will be calculated by the change in stem density in a .25 meter squared quadrat cover the three year sampling period. For clones with visible borders (not growing in a meadow), lateral spread can also be calculated by walking the perimeter of the clone with the GPS unit. Maternal fitness will be measured by revisiting sample locations in September and October when *Spartina* is in bloom. Density of flowering tillers will be measured in a .25 meter squared quadrat and the longest inflorescence will be sampled. I will count seed set by sub-sampling a high, mid, and low branch along the length of the inflorescence and counting percent seed set as # filled seeds divided by total seeds. Variation between plants in their fitness will depend not just on genetic and environmental variability, but also on the likelihood that they are herbicided by the control program.

Part 2. The potential for cryptic hybrids to invade tidal channels and mudflats will be investigated experimentally in a mesocosm experiment at Bodega Marine Laboratory. Due to the strict directive of the management program to eradicate hybrids, it is impossible to maintain a common garden in the field that includes non-native *Spartina.* However, similar work investigating the response of another species of cordgrass (*Spartina pectinata)* to flooding regimes in a mesocosm experiment showed significant responses of growth to flooding depth, suggesting that the mesocosm approach here will also prove illuminating.

The experiment will have two treatment types: deep and shallow flooding. Seawater is available at Bodega Marine Laboratory as well as outdoor tanks. I have been collecting tillers from the samples I measured in the field in 2007 and 2008 and am cultivating them in the greenhouse. Eleven native plants, eleven cryptic hybrids, and eleven evident hybrids, for a total of thirty-three genotypes, will be selected to represent the range of morphologies observed in native and hybrid *Spartina*. Six tillers from each genotype will be repotted, for a total of 198 pots, in containers of approximately 9 cm cubed, filling two tanks of a meter cubed each, and half will be randomly assigned to the deep or shallow flooding treatment. Deep flooding will be simulated by placing the pots on the floor of the tanks and filling them to a depth of 60 centimeters twice daily. Shallow flooding will be simulated by placing pots on top of PVC pipe 30 centimeters tall. Interspersing the shallow and deep flooding treatments within the tanks will prevent the problem of pseudoreplication. I will run the experiment from May – October to give the plants time to flower.

I will measure total stretched length (from sediment to tip of longest leaf), culm diameter, number of living stems, and leaf area (leaf width*leaf length*# of leaves) at the beginning and end of the experiment. Covering inflorescences with plastic tubes will prevent the accidental dispersal of hybrid seed. At the end of the experiment, I will measure the number of flowering stems, and harvest the above- and below-ground biomass. The initial biomass of each tiller will be estimated by destructively sampling extra tillers at the beginning of the experiment to develop a regression of biomass by total stretched length and culm diameter. I will also take a cross section of a leaf from one tiller per pot at the beginning and end of

the experiment to measure the change in leaf area dedicated to aerenchyma. The number of living tillers, number of inflorescences, and percent increase in biomass can be used as indicators of fitness under the different flooding regimes. Total stretched length, leaf area and percent of leaf cross section dedicated to aerenchyma will be used to achieve a mechanistic understanding of why fitness varies by flooding regime. Height, leaf area and leaf area dedicated to aerenchyma can affect flooding tolerance: greater height increases the above-water time for photosynthesis and for channeling oxygen to the roots through aerenchyma, more leaf area maximizes photosynthetic capacity in plants that spend long durations of time under water, and a higher percentage of the leaf cross section dedicated to aerenchyma can channel more oxygen to roots in waterlogged, anoxic soils (Castillo, Redondo et al. 2005).

Statistical analysis will be performed by constructing three-way ANOVAs with flooding treatment (deep versus shallow), taxa (native, cryptic hybrid, or evident hybrid) and genotype (with six replicates per genotype) as the model effects. Total stretched length, culm diameter, number of living stems, leaf allometry, and number of inflorescences will be the model responses. I will test the hypotheses that the responses will vary by flooding treatment, taxa, genotype, taxa * treatment interaction, and genotype * treatment interaction. Comparisons to make include:

1. The fitness of natives, cryptic hybrids and evident hybrids across flooding regimes. Do evident hybrids differ from natives (as would be expected if the mesocosm experiment accurately reflected the marsh environment)? Do cryptic hybrids differ from natives and/or evident hybrids in their fitness under different flooding regimes? If cryptic hybrids are not statistically different from natives but are different from evident hybrids, this would suggest that they are unlikely to be able to invade the low intertidal. On the other hand, if they show higher fitness in deep flooding than the natives, they may represent a threat to mudflats and marsh channels.
2. The morphological plasticity of genotypes under different flooding regimes. Do genotypes display a fixed morphology in their height, leaf area and aerenchyma area, or do they respond plastically to flooding regime? Plasticity would be indicated by a significant genotype * treatment interaction.

TIMELINE

Summer 2008

- Refine hypothetical data set and test the accuracy of STRUCTURE in conjunction with microsatellite data at identifying hybrids.
- Train with Christina Sloop, a graduate of the U.C. Davis PhD program in Ecology who used *Spartina* microsatellites in her dissertation, in the appropriate laboratory techniques.
- Sample *Spartina* in the field to determine the mean and standard deviation of native and hybrid morphological characteristics and the frequency of cryptic hybrids. Bring samples into the greenhouse in preparation for mesocosm experiment. Measure size of clones, stem density, density of inflorescences, and seed set.

Winter 2008-2009

- Run microsatellites on frozen DNA samples from summer 2008 that have already been tested for RAPD markers. Compare the detection of hybrids between microsatellites and RAPDs. Select the best method of genetic screening for subsequent work. Prepare report for the Invasive *Spartina* Project on the advantages of each method. Write up investigation of applicability of microsatellites and Bayesian methods of population assignment for publication.

Spring/Summer 2009

- Revisit field samples from 2008 to measure changes in phenotype and vegetative spread. Repeat measurement of inflorescences and seed set. Sample new transects in marshes to increase sample

size for mean and standard deviation of morphological characters. Measure environmental covariables (porewater ammonium, soil texture, porewater salinity, and intertidal elevation).
- Run mesocosm experiment at Bodega Bay Marine Laboratory.

Winter 2009-2010
- Perform statistical analysis of data on frequency and distribution of cryptic hybrids and the environmental covariables that correlate with more convergent morphologies in native and hybrid plants. Write report on predicting areas of high cryptic hybrid density for the Invasive *Spartina* Project.
- Begin statistical analysis of first year of mesocosm experiment data.

Summer 2010
- Repeat mesocosm experiment if more replication is necessary to see significant results.
- Revisit field samples from 2008 and 2009 to continue to measure rate of vegetative spread and fecundity.

Fall 2010
- Perform final data analysis of mesocosm experiment. Prepare report on fitness in the field of cryptic hybrids and their potential for invading the low intertidal.

Winter 2010 – Spring 2010 (after the end of the CALFED fellowship time span)
- Write up dissertation, submit results of field study on the distribution and environmental trends underlying the occurrence of cryptic hybrids and mesocosm experiment for publication.

OUTPUT

Benefits to community mentors
The Invasive *Spartina* Project will receive written reports on three topics of interest to them:

1. the potential for improved hybrid detection with new methods of genetic analysis;
2. distribution and frequency of cryptic hybrids, and the environmental variables that covary with their occurrence;
3. the fecundity and rate of vegetative spread of cryptic hybrids, and the potential of cryptic hybrids to invade the low intertidal.

Benefits to CALFED priorities and mission
CALFED will receive annual written reports on the progress of the proposed research and presentations at the CALFED/State of the Estuary conferences. The results of the research will improve the efficacy of hybrid *Spartina* eradication, a project that is underway in part because of CALFED funding. Hybrid *Spartina* is one of the most noxious aquatic pests in the Estuary, and eradication is important for the health of intertidal wetlands. Additionally, the proposed research will enable managers to evaluate the costs and benefits of detecting cryptic hybrid invaders, informing decisions about how to spend the limited funds available for control of invasive species.

Benefits to fellow
Receiving a stipend and tuition will enable me to focus full-time on my research, and funds for research expenses will cover those materials and supplies that cannot already be covered through another means.

This research project offers a launching pad for a career at the intersection of ecological theory and management applications.

Benefits to research mentors

My research mentors will continue their long-standing investigation of the *Spartina* invasion in the San Francisco Estuary. They also will likely be fellow authors on the papers I submit for publication.

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TABLES AND FIGURES

Figure 1.
The correspondence between percent of genome derived from *S. Foliosa* and morphological characteristics observed in plants grown in the greenhouse. From Ayres et al. (2004).

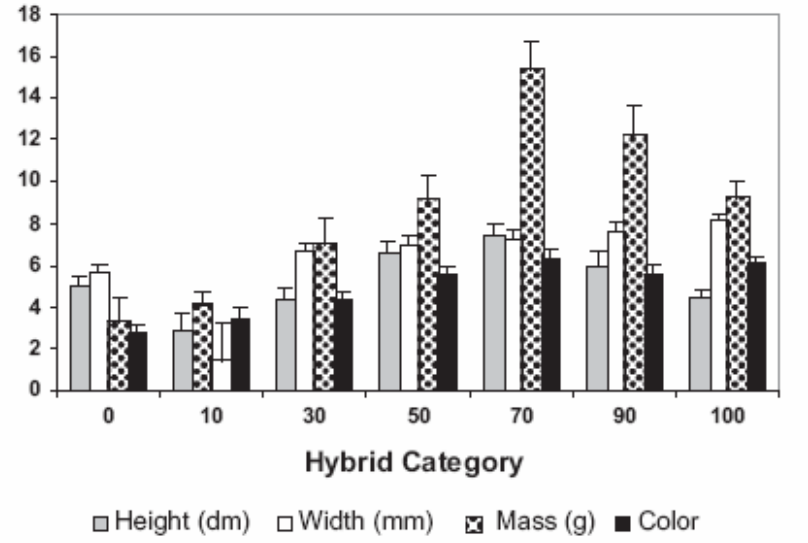
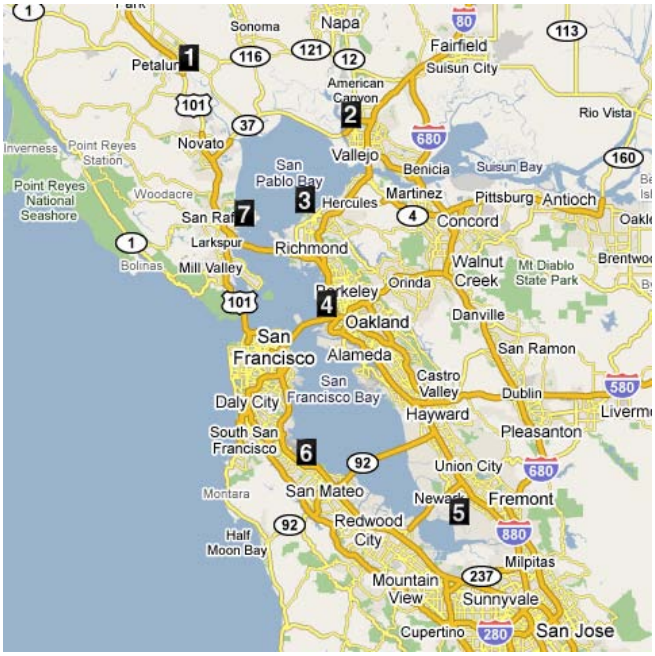


Figure 1. Appearance of greenhouse-grown plants averaged over each genetic category (color scale has been doubled to be on a scale comparable to the other measurements). Hybrid category is defined by the proportion of genetic marker states consistent with *S. alterniflora* (0% = *S. foliosa*; 100% = *S. alterniflora*).

Figure 2. Location and name of sample sites. From Google Maps (http://maps.google.com).



1. Schollenberger Park
2. White Slough
3. Point Pinole
4. Emeryville Crescent
5. Mowry Marsh
6. Sanchez Marsh
7. Loch Lomond Marina