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**THE ECOLOGICAL FACTORS INFLUENCING THE MARSH-UPLAND  
ECOTONAL PLANT COMMUNITY AND THEIR USE AS PART OF AN  
EFFECTIVE RESTORATION STRATEGY**

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of the requirements for the degree of

DOCTOR OF PHILOSOPHY

in

ECOLOGY AND EVOLUTIONARY BIOLOGY

by

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## ABSTRACT

Carla Fresquez

### **THE ECOLOGICAL FACTORS INFLUENCING THE MARSH-UPLAND ECOTONAL PLANT COMMUNITY AND THEIR USE AS PART OF AN EFFECTIVE RESTORATION STRATEGY**

The following research explores how abiotic and biotic processes interact to shape the distributions of the marsh-upland ecotone, a characteristic high marsh plant community in Pacific coast salt marshes that forms the transition zone between vegetated marsh plain and upland habitats. Understanding how abiotic and biotic ecological features interact to structure the marsh upland ecotone is necessary for predicting how the boundaries and distributions of this plant community will respond to disturbance, both human and natural, and for the design of effective strategies to restore and conserve degraded habitats. The abrupt boundaries, relatively simple community composition, and rapidly transitioning abiotic gradient of the marsh-upland ecotone make both observational and manipulative approaches feasible for addressing these research goals. Here both approaches are used to quantify the abiotic and biotic factors responsible for setting species distributions, to test how the relative influence of these factors changes across the underlying abiotic gradient resulting from variable tidal influence, and to design an effective restoration strategy for habitats degraded by disruption of the natural abiotic regime. These results challenge the applicability of a classic theoretical framework commonly applied to describe the

structure of the marsh-upland ecotone, increase our understanding of the ecological processes, both biotic and abiotic, structuring the plant community of the marsh-upland ecotone, and optimize a time- and cost-effective restoration strategy to restore degraded ecotone habitats. This body of research significantly enhances our understanding of the complex abiotic and biotic processes structuring the marsh and also contributes to the understanding of how these processes structure species distributions in general.

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## INTRODUCTION

The following research explores how abiotic and biotic processes interact to shape the distributions of the marsh-upland ecotone, a characteristic high marsh plant community in Pacific coast salt marshes that forms the transition zone between vegetated marsh plain and upland habitats. Understanding how abiotic and biotic ecological features interact to structure the marsh upland ecotone is necessary for predicting how the boundaries and distributions of this plant community will respond to disturbance, both human and natural, and for the design of effective strategies to restore and conserve degraded habitats. The abrupt boundaries, relatively simple community composition, and rapidly transitioning abiotic gradient of the marsh-upland ecotone make both observational and manipulative approaches feasible for addressing these research goals. Here both approaches are used to quantify the abiotic and biotic factors responsible for setting species distributions, to test how the relative influence of these factors changes across the underlying abiotic gradient resulting from variable tidal influence, and to design an effective restoration strategy for habitats degraded by disruption of the natural abiotic regime.

One of the classic paradigms explaining how species distributions are structured in stressful habitats, the stress gradient hypothesis (SGH), predicts that along a gradient of potentially stressful physical conditions the distributional boundary of a species at the low-stress end is set by interspecific interactions, often competition. At the high-stress end of the gradient, unless facilitative interactions mitigate the stressor, the distributional boundary is set by intolerance to the stressful abiotic

conditions beyond that boundary (Bertness and Ellison 1987, Bertness 1991). In Chapter 1 *Testing the stress gradient hypothesis in a salt marsh-upland ecotonal plant community*, I use an experimental outplant to test the applicability of this framework for identifying whether intolerance to stressful abiotic conditions or interspecific interactions set the abrupt distributional boundaries of three dominant ecotonal species. Typically, in salt marsh plant communities the boundary set by stressful abiotic conditions is generally found where the frequency and duration of tidal inundation are highest. Conversely, competitive interspecific interactions influence boundaries and zonation under the less stressful, low-inundation conditions. However, the applicability of the stress gradient hypothesis framework for explaining the abrupt boundaries and narrow transitional area of the marsh-upland ecotonal plant community, located where overall inundation frequencies are relatively low to nonexistent, has not been explored. In addition, the location of the relatively more stressful area and, therefore, the direction of the underlying stress gradient, had not been examined for this species assemblage. By outplanting established cuttings of the three dominant species of the marsh-upland ecotone beyond their existing distributional boundaries, both seaward and landward of their observed elevational distributions, I was able to examine how abiotic conditions dictate where these species persist along the marsh to upland transition zone. The results of this experimental outplants indicate that when outplanted into the seaward marsh habitat, two out of the three focal ecotonal species are not physiologically excluded by the abiotic conditions, performing as well or better than when outplanted at the ecotonal

(control) elevation treatment. In contrast, at the upland boundary, outplanted individuals experienced high mortality rates, suggesting intolerance to the physical conditions of the upland. These results suggest that the marsh-upland ecotonal plant community is indeed excluded from persisting in the landward upland community by physiological intolerance to those abiotic conditions, while these species are tolerant of the conditions found seaward of their distribution in the marsh plain habitat. A competition treatment at each outplant elevation further showed no evidence that competition from either the adjacent marsh or upland vegetation sets boundaries between the ecotone and marsh or upland. These results suggest that in contrast to the classical paradigm for intertidal salt marsh systems, at the higher elevations of the marsh-upland ecotone the stress gradient is actually inverse to the elevational gradient. While these results suggest that the SGH is not quite an applicable framework for predicting the factors determining distributional boundaries, they suggest that, in contrast to lower elevational marshes, a few predictions of the SGH do apply to the marsh-upland ecotone community, albeit in an inverse manner.

Identifying the ecological factors maintaining the distributions of the ecotonal species community is important from both research and management perspectives. By quantifying the abiotic conditions across the transition from marsh to ecotone to upland, the nature of the abiotic gradient and its influence on the biotic community may be explored. From a management perspective, this information directly informs the design and construction of restored salt marshes and, indeed, this information is currently being used to assist in restoration of salt marshes in Elkhorn Slough. In

Chapter 2, *Edaphic and biotic features determining structure and boundaries of an ecotone and adjoining habitats*, I use soil samples collected over the course of two years to document seasonal and interannual variation in the soil moisture and salinity above, below, and within the ecotone. In addition, a multivariate approach is used to characterize the abiotic gradient in edaphic conditions and determine how this gradient covaries with elevation. In addition, a neighbor removal manipulation found that, in comparison to the complex abiotic conditions that vary strongly across habitat types, competition from vegetation of the adjacent habitats plays a relatively small role in determining the distribution of the marsh-upland ecotone. Overall, these results concur with the findings in Chapter 1 and provide a fuller picture into the nature of the gradient across this dynamic transition zone.

In the final chapter, *Restoration through reintroduction of an abiotic stressor: Salinization as a marsh restoration tool*, I used a series of experimental manipulations to test the effectiveness of salt addition as a method to both suppress cover by non-native upland species and increase cover by marsh species in marshes degraded by tidal restriction. Alterations of underlying abiotic conditions through tidal restriction eliminates the environmental gradient critical for maintaining the structure and composition of the marsh-upland ecotonal community and facilitates encroachment of the primarily non-native upland plant community into areas previously occupied by the ecotone. By using salt addition to restore one component of the abiotic gradient eliminated by tidal restriction, I observed major reductions in cover by upland non-natives and, over time, was able to see a significant increase in

cover by marsh species. This restoration approach is an effective, efficient, and low cost restoration strategy to reverse some of the effects of tidal restriction increase the area available for ecotonal species colonization.

The following dissertation research challenges the applicability of a classic theoretical framework commonly applied to describe the structure of the marsh-upland ecotone, increases our understanding of the ecological processes, both biotic and abiotic, structuring the plant community of the marsh-upland ecotone, and uses this information to optimize a time- and cost-effective restoration strategy to restore degraded ecotone habitats. This body of research significantly enhances our understanding of the complex abiotic and biotic processes structuring the marsh and also contributes to the understanding of how these processes structure species distributions in general.

Bertness, M. D. 1991. Zonation of *Spartina patens* and *Spartina alterniflora* in a New England salt marsh. *Ecology* 72:138–148.

Bertness, M. D., and A. M. Ellison. 1987. Determinants of pattern in a New England salt marsh plant community. *Ecological Monographs* 57:129–147.

## CHAPTER ONE:

### **TESTING THE STRESS GRADIENT HYPOTHESIS IN A SALT MARSH- UPLAND ECOTONAL PLANT COMMUNITY**

#### ABSTRACT

The stress gradient hypothesis (SGH) predicts that distributional boundaries along environmental gradients are set by intolerance to abiotic conditions at the stressful end of the gradient and biotic interactions at the less stressful end. We quantified the elevational distributions of three plant species of the marsh-upland ecotone, *Distichlis spicata*, *Frankenia salina*, and *Jaumea carnosa*, characterized the tidal inundation gradient, and tested the influence of this gradient and of biotic interactions on the distributions of each of these species. We transplanted each species to zones landward and seaward of their observed boundaries, either with neighboring plants removed or left in place. For all three species, we found no evidence that distributional boundaries are set by interactions with neighbors, contrary to the expectations of the SGH. While other applications of SGH to marsh systems identify the seaward portion of the gradient as more stressful and the landward portion more benign, we found the opposite. All three species tolerated conditions seaward of their observed distributions at least as well as those in their observed range, and two species had increased mortality landward of their observed range. Overall, responses of the three taxonomically unrelated species were similar, supporting consideration of the ecotonal plant community as a conservation unit. Our results illustrate that

ecotone plants can tolerate far more tidal inundation than they currently experience, suggesting the ecotonal community may prove resilient to sea level rise.

## INTRODUCTION

Identification of the factors that control the distributions of species is a basic, but challenging goal of ecology. It is well understood that a species' distribution and boundaries are, at least in part, dependent on the abiotic conditions of the local environment. Underlying abiotic conditions both directly influence species distributions through physiological tolerance limits (Mahall and Park 1976, Barbour 1978, Cooper 1982a) and indirectly by affecting the outcome of biotic interactions (Pennings and Callaway 1996, Levine et al. 1998, Bockelmann and Neuhaus 1999, Hacker and Bertness 1999, Emery et al. 2001, Pennings et al. 2005, Greenwood and MacFarlane 2006, Crain 2008). These ecological processes have strong impacts on the distribution of species and communities across environmental gradients, often leading to abrupt boundaries and zonation patterns between adjacent habitats and species patches (Smith and Huston 1989). However, due to the complexity of both the biotic and abiotic processes responsible for setting distributional boundaries and the underlying environmental gradients, the development of a generalizable model for predicting the dominant processes has proven especially challenging (Pennings et al. 2003, 2005, Fariña et al. 2009).

The stress gradient hypothesis (SGH) is one conceptual framework used to predict how abiotic gradients interact with biotic interactions to determine species distributions. It predicts that along a gradient of potentially stressful physical conditions, the distributional boundary of a species at the low-stress end is set by interspecific interactions, often competition, while at the high-stress end of the

gradient, unless facilitative interactions mitigate the stressor, the distributional boundary is the result of intolerance to the stressful abiotic conditions beyond that boundary (Bertness and Ellison 1987, Bertness 1991). As a result, species whose distributions coincide with gradients in environmental stress are often competitively excluded from colonizing adjacent benign habitats due to their inferior competitive ability relative to the species found in less stressful areas. For example, forbs tolerant of the ionically stressful soils in serpentine habitats are competitively excluded from adjacent non-serpentine soils by competitively superior non-serpentine plants (Kruckeberg 1954) and salt marsh species tolerant of the physical stress of tidal inundation are outcompeted in adjacent non-tidal areas (Crain et al. 2004). Conversely, these superior competitors are limited from expanding into the stressful serpentine and intertidal habitats due to intolerance of the physically stressful conditions.

Ecotones, transition zones between adjacent ecological systems, reflect the integration of the biotic and abiotic properties of the adjacent habitats (Risser 1995). Habitat overlap creates steep gradients in abiotic conditions across ecotones (Wiens et al. 1985, Risser 1995, Peters et al. 2006, Kark and van Rensburg 2006, Hufkens et al. 2009). The distribution of each species along a gradient—and therefore the structure of the community—depends on the conditions of the underlying physical gradient. Therefore, the plant communities in these areas are often found to be highly sensitive to alterations of adjacent habitats that disrupt the transitional gradient (Wiens et al. 1985, Gosz 1992). In physically narrow ecotones, such as those found at the marine-

terrestrial interface, conditions of the abiotic gradient may shift so rapidly across small spatial scales that even neighboring individuals are potentially subject to differing physical conditions (Risser 1995). This co-occurrence of steep abiotic gradients and distributional boundaries of species make ecotonal systems subject to the theoretical predictions of the SGH; allowing us to examine the abiotic and the biotic factors influencing species distributions and boundaries across relatively small spatial scales. Both ecotones (Gosz and Sharpe 1989, Delcourt and Delcourt 1992, Noble 1993, Risser 1995) and wetland margins are considered especially sensitive to processes that disrupt underlying gradients because the species that occupy these areas are close to the limits of their physiological tolerances (Wiens et al. 1985, Gosz 1992). This sensitivity to abiotic and biotic shifts in adjacent ecological systems also makes ecotones accurate potential bioindicators of environmental change, such as global change, sea level rise, and anthropogenic disturbance.

The marsh-upland ecotone is the narrow transition zone between the salt marsh plain and adjacent upland vegetation (Callaway et al. 1990). The plant community in this zone consists of salt-tolerant plant species that can withstand infrequent inundation by the highest tides and storm surges. In intertidal salt marsh plant communities, like the marsh-upland ecotone, frequency of inundation plays a major role in establishing plant zonation patterns and community structure (Adams 1963, Chapman 1978, Snow and Vince 1984, Bertness and Ellison 1987, Bertness 1991). Variation in the frequency of tidal inundation creates strong environmental gradients which correlate with elevation and various potential hydrologic and edaphic

stressors, including soil salinity, anoxia, and waterlogging (Adams 1963, Callaway et al. 1990). Given the position of these plant communities along tidal gradients, the ability of salt marsh plant communities to respond to predicted sea level increases has received significant recent attention, with emphasis on the stress of excessive tidal inundation setting the seaward boundaries of vegetation (e.g. Morris et al. 2002, Kirwan et al. 2010).

The empirical evidence used to formalize the conceptual framework of the stress gradient hypothesis is based largely on studies of zonation patterns in *Spartina* dominated New England salt marshes (Bertness and Ellison 1987, Bertness 1991, 1992, Hacker and Bertness 1999). In these systems the distributional boundaries of vegetation zones are generally set by intolerance to abiotic conditions at the seaward boundary and biotic interactions at the landward boundary. The physiologically stressful conditions caused by frequent inundation, including waterlogging and high salinity (Adams 1963, Callaway et al. 1990), and the relatively benign conditions near the landward boundary create the steep gradient in abiotic conditions that the SGH predicts influences species distributions. In these systems, competitively dominant species escape the more stressful conditions found near the seaward boundary by excluding less competitive species to lower elevations. In contrast to the abundance of studies examining how well the drivers of zonation patterns are predicted by the SGH in New England and Southern Atlantic marshes, examination of the applicability of the SGH in Pacific coast marshes of North America has been limited, especially in California (but see Pennings and Callaway 1992; Morzaria-Luna and

Zedler 2014). Similarly, though empirical research on the drivers of zonation patterns in the plant communities of the high salt marsh are uncommon in both Atlantic (Pennings and Moore 2001) and Pacific geographic regions, to our knowledge the SGH has never been explicitly tested in the marsh-upland transition zone of Pacific coast marshes. Regardless, this general paradigm is often used to anecdotally explain zonation patterns and boundary dynamics of the transition zone in Pacific coast estuaries by both land managers and researchers.

The overarching goal of this study was to test the predictions of the SGH for the distribution of the marsh-upland ecotonal plant community and determine whether this paradigm appropriately predicts the physical and biotic drivers of this plant community. First, we characterized the elevational distributions of the three most common ecotonal species. Second, we investigated how one key abiotic factor, tidal inundation duration, varied across the upland, ecotone, and marsh. Third, we determined the mechanisms responsible for the measured distributions of each species by manipulating the inundation frequency experienced by each ecotonal species. Here we compared the differential survival over time of individual ecotonal plants planted within and outside of their observed distributions. Finally, we tested the influence of biotic interactions, specifically competition and facilitation, on the distribution of each species through neighbor removal manipulations.

## STUDY SYSTEM AND METHODS

This research was conducted between March and October 2012 at Elkhorn

Slough estuary in Monterey Bay, California. Elkhorn Slough experiences a Mediterranean climate, with the majority of annual precipitation occurring between October and May. This 1200-ha estuary contains the second largest tract of contiguous salt marsh on the California coast. Maximum tidal range is 2.5m and minimal freshwater inputs result in salinity levels similar to the adjacent marine environment (Caffrey et al. 2002). Terrestrial habitats directly adjacent to the salt marshes of Elkhorn Slough are a mix of *Quercus* woodlands, *Baccharis* scrub, invaded grasslands, and native grassland remnants (Caffrey et al. 2002). All experiments were conducted at two relatively undisturbed sites with no history of anthropogenic tidal restriction, Azevedo Marsh and Yampah Island, located in the upper- and mid-estuary, respectively. Sites in different parts of the estuary were specifically chosen to investigate estuarine scale variation in ecotone distributional elevations but this variation was not found. Both sites are bordered by similar upland habitats made up of a mix of native and invasive grasses and forbs. Common upland species adjacent to the marsh include *Danthonia californica*, *Sisyrinchium bellum*, *Taraxia ovata*, *Baccharis pilularis*, *Conium maculatum*, and *Brassica* species.

As it does at other regional estuaries (Mahall and Park 1976), the salt marsh dominant, *Sarcocornia pacifica*, forms a monoculture approximately between mean high water (MHW) and mean higher high water (MHHW), which at Elkhorn Slough correspond to 1.5m and 1.7m NAVD88, respectively (Van Dyke 2012). Unlike the salt marshes in the nearby San Francisco Bay, there is no *Spartina* in this system. In contrast to the *Sarcocornia* zone, which can extend for dozens of meters horizontally,

the plant community of the marsh-upland ecotone is constrained to a narrow area typically only a few meters wide (Caffrey et al. 2002, Wasson and Woolfolk 2011). Elkhorn Slough marshes are very low in the tidal frame relative to those of other estuaries in the region, likely a result of increased tidal range resulting from the 1946 opening of an artificial mouth to the estuary to accommodate Moss Landing Harbor (Caffrey et al. 2002). Steep hills surround the marsh plain, and the marsh-upland ecotone appears to form a “bath tub ring” at the base of these hillsides. As with other California coast salt marshes, despite the fact that the areal extent of the marsh-upland ecotone translates to only a small fraction of total salt marsh area, this ecotone plant community supports the majority of native marsh plant diversity (James and Zedler 2000), providing habitat for several species whose distributions are limited to the ecotone (Traut 2003).

We chose to investigate three taxonomically diverse co-occurring ecotonal species, *Distichlis spicata* (L.) Greene (Poaceae), *Frankenia salina* (Molina) I.M (Frankeniaceae), and *Jaumea carnosa* (Less.) Gray (Asteraceae). *Distichlis*, *Frankenia*, and *Jaumea* dominate the marsh-upland ecotone plant community, accounting for 43% of vegetation cover in this transitional zone (Wasson and Woolfolk 2011). We selected these three co-occurring but taxonomically unrelated species to provide generality to our understanding of how the predictions of the SGH might apply to the marsh-upland ecotone. All three species are perennial and expansion of patches occurs primarily through vegetative growth.

#### Elevational Distribution Surveys & Duration of Tidal Inundation

Prior to conducting experiments to identify the mechanisms setting distributional boundaries of the three species, it was necessary to identify and characterize these boundaries. The elevational distributions of *Distichlis*, *Frankenia*, and *Jaumea* were measured using a surveyor's level at both study sites. The average elevational range of each species' distribution was quantified by surveying the most landward and most seaward individuals of each species approximately every 2m along transects paralleling the wetland-upland boundary at both sites (Azevedo Marsh: 110 m transect, Yampah Island: 155 m transect). Absolute elevations were calculated by comparing relative elevation measure collected using a surveyor's level to benchmarks established with a Trimble 5800 RTK survey. Orthometric heights collected using the Trimble were post-processed using the National Oceanic and Atmospheric Administration's Online Positioning User Service (OPUS) using the GEOID09 model and NAVD88 vertical datum.

We characterized the stress gradient across the ecotone created by variation in tidal inundation. To quantify the duration of tidal inundation across the distribution of each species, we used data on water levels obtained from a long-term water monitoring station continuously deployed within the slough. Using data logged by a sonde (Yellow Springs Instruments 6000 series) that measured water depth every 15 minutes using a pressure sensor, at a known vertical deployment elevation, we converted water depth data to height in NAVD88. Field ground-truthing revealed that measured water levels correspond very closely to surveyed elevations on the marsh plain at sites throughout the estuary, i.e. the elevation of the water line on the marsh

plain as surveyed by RTK GPS was the same as the elevation of that tide level measured simultaneously by the sonde. We calculated the percent time spent inundated and the number of hours per year inundated at both the observed mean seaward boundary and the mean landward boundary for each species, averaging inundation data from three years, 2010 to 2012. We also used this method to calculate the percent time spent inundated and the number of hours per year inundated at each of the three outplant treatment elevations (described below) over the course of the experiment, March to October 2012.

### Outplant Experiment

We used an outplant experiment to test whether these three ecotonal species are limited to their narrow elevational ranges due to physiological intolerance of the environmental conditions associated with tidal inundation or by biotic interactions with the plant communities of the adjacent habitats. We manipulated both the location of outplants along the tidal influence gradient and the presence of neighbors using a blocked factorial design.

All individuals included in the study were propagated in the greenhouse in November 2011 from field-collected individuals. Starting four weeks before outplanting plants were watered every two to three days with seawater. Plants were transplanted to their target elevations in the field in early spring, March 2012. The experiment ended in early October 2012. We outplanted all three species at Yampah Island (*Distichlis spicata*, *Frankenia salina*, and *Jaumea carnosa*), but only two species at Azevedo Marsh (*Distichlis* and *Jaumea*) due to an insufficient number of

greenhouse stock of *Frankenia salina*. Replicates per treatment combination were as follows, Yampah Island (*Distichlis* n=18, *Frankenia* n=18, *Jaumea* n=12), and Azevedo Marsh (*Distichlis* n=17, *Jaumea* n=16). All replicate blocks were planted at least 3 meters apart.

Individuals of each species were outplanted at three elevational locations: landward of (Upland), seaward of (Marsh), and within (Ecotone, as a control) that species' observed distribution. At each of the treatment elevations, we planted two individuals of similar size. One was planted directly into the existing vegetative community (+neighbors), while the other was planted into the center of an area 30cm diameter circular where all neighboring plants had been removed manually through clipping (-neighbors). Neighbor removals were maintained weekly or as needed throughout the course of the experiment. Plants were watered manually every other day for the first two weeks to reduce losses due to transplant stress.

Elevational treatments were determined as follows (Figure 1). Upland: approximately 20 cm higher than one standard deviation from the mean elevation of the landward boundary of each species. Marsh: 15 cm lower than one standard deviation from the mean elevation of the seaward boundary. Ecotone: half-way between the mean seaward boundary and mean landward boundary. Slight variation in the elevational distributions of each species between sites required Upland, Ecotone, and Marsh elevation treatments to be established based on measurements collected at each site. Therefore, treatment elevations for each species varied slightly

across sites. The elevation of landward and seaward boundaries also varied by species, so the elevation of treatment transplant locations did as well.

Unexpectedly, approximately two weeks after outplanting several plants disappeared, apparently due to herbivory. To avoid further losses, every plant was caged using 20 cm cylindrical cages with 0.6 cm mesh. These cages were staked into the ground using 7 cm lawn staples. During the first 20 day period, all plants that disappeared due to apparent herbivory or died due to transplant stress were replaced. A separate caging experiment was done later to investigate the potential consequences of herbivory for the distribution of these species (Fresquez unpublished data).

The first mortality assessment was conducted approximately 3 weeks after planting. Assessments were subsequently performed every 7 to 15 days over the course of the experiment, for a total of 13 assessments between April and September. A final mortality assessment was performed in late October, the end of the salt marsh growing season. During this final mortality assessment, the aboveground biomass of all remaining living individuals was collected. Samples were washed, dried, and weighed.

We analyzed survivorship over the course of the 300-day experiment using a Cox Proportional Hazards model, with individuals surviving to the last assessment right-censored. Neighbors, elevation, and their interaction were included as fixed effects. We analyzed log-transformed aboveground biomass data with two-way ANOVA, again examining effects of neighbors, elevation, and their interaction. For

both the survival analysis and the ANOVA, sites were combined as there were no significant differences between sites for either of the factors or their interaction. All statistics were performed using JMP V. 10 (SAS Institute).

## RESULTS

### Elevational Distribution Surveys & Duration of Tidal Inundation

Surveys revealed that all three species have narrow and fairly similar elevational ranges, spanning about 40-80 vertical cm (Table 1). The landward boundary of *Distichlis* extended considerably further into the adjacent upland than either *Jaumea* or *Frankenia*, due to the presence of a few sparse individuals well into the upland. Relative to the other two species, the seaward boundary of *Distichlis* was highest and the seaward boundary of *Jaumea* was lowest.

Individual plants at different positions along the elevational gradient of the ecotone experience very different abiotic conditions as a result of tidal inundation (Table 1). Specifically, *Frankenia* individuals at the observed seaward boundary experience over 22 times more hours of inundation per year compared to *Frankenia* individuals at the landward boundary, *Jaumea* individuals at the seaward boundary experience over 57 times more inundation than those at the landward boundary, and *Distichlis* at the seaward boundary are inundated over 500 more hours than those individuals at the landward boundary, which are never inundated at all. In addition, very small differences in elevation correspond to large differences in inundation regime. For example, though the location of the landward boundaries of *Jaumea* and

*Frankenia* differed by only 8cm, this difference corresponds to 22 more hours of inundation per year experienced at *Frankenia*'s landward boundary.

As intended, the inundation frequencies experienced at both the Marsh and Upland elevation treatments over the course of the experiment were outside of the typical range of inundation frequencies experienced by each species across their natural distribution. Marsh treatment inundation frequencies exceeded seaward boundary inundation frequencies by 4.45% (67 hrs), 4.89% (32hrs), and 11.69% (467 hrs), for *Distichlis*, *Frankenia*, and *Jaumea*, respectively. For all three species, Upland treatment elevations were outside of the zone inundated by tides, therefore all inundation frequencies were 0% and 0 hours. The Ecotone (control) treatment elevations represent inundation frequencies experienced at the midpoint of the natural distribution of each species: *Distichlis*, 0.08% (6.5hrs); *Frankenia*, 0.80% (45hrs); *Jaumea*, 1.96% (64hrs) (Table 1).

### Outplant Experiment

Overall, the outplant experiment yielded numerous significant results, with effects differing by species and between the landward and seaward boundaries. For *Distichlis* and *Jaumea*, there was a significant effect of elevation treatment over time (*Distichlis*:  $\chi^2=68.27$ ,  $df=2$ ,  $p<0.0001$ ; *Jaumea*:  $\chi^2=76.69$ ,  $df=2$ ,  $p<0.0001$ ; Table 2), though there was no effect of neighbors or interaction between the two factors. Survivorship curves demonstrated significantly higher mortality over time at the Upland elevation as compared to other treatments (Figure 2). On average across both neighbor removal treatments, only 11% of *Distichlis* individuals at the Upland

elevation survived to the end of the season compared to 54% and 79% of individuals at the Ecotone or Marsh elevations, respectively. Similarly, only 31% of Upland *Jaumea* individuals survived to the end of the season compared to 89% and 94% of individuals in the Ecotone or Marsh elevation treatments, respectively. For both *Distichlis* and *Jaumea*, the risk of mortality was significantly higher at the Upland elevation treatment compared to Marsh or Ecotone elevations (*Distichlis*: Upland x Marsh: Risk ratio 8.13,  $p < 0.0001$ ; Upland x Ecotone: Risk ratio=3.14,  $p < 0.0001$ , Table 2) (*Jaumea*: Upland x Marsh: Risk ratio=15.54,  $p < 0.0001$ ; Upland x Ecotone: Risk ratio=13.49,  $p < 0.0001$ , Table 2).

At the end of the season, the proportion of *Jaumea* individuals that survived at the Marsh elevation treatment was significantly greater than the survivorship observed at the Ecotone (control) elevation treatment. Survivorship of *Distichlis* individuals at the Marsh elevation was the same as survivorship at the Ecotone (Figure 2). Survivorship of *Distichlis* individuals at the Marsh elevation were almost 30% lower than those at the Ecotone (control) elevation (Figure 2). The risk of *Distichlis* mortality at the Ecotone elevation treatment was over twice as great as the mortality risk at the Marsh elevation (Risk ratio 2.59,  $p = 0.0013$ ; Table 2). For *Jaumea*, survivorship was similar between Ecotone (89%) and Marsh (93%) elevations (Figure 2). The risk of *Jaumea* mortality at the Ecotone elevation was equal to the mortality risk in the Marsh elevation (Risk Ratio=0.82,  $p = 0.32$ ; Table 2), indicating that the survival of *Jaumea* individuals planted well seaward of their

natural occurring distributions was similar to the survival of individuals planted within their observed distributions.

Outplanted *Frankenia* individuals were not significantly affected by elevation, neighbors, or their interaction (Table 1). All three treatment elevations experienced final mortality percentages ranging from 50-90% (Figure 2). Similar to the results seen for *Distichlis* and *Jaumea*, final mortality was not lower at the Ecotone (control) elevation than at the Marsh elevation.

For all species, biomass at the end of the experiment showed no effect of elevation, neighbors, or their interaction, except for a significantly negative effect of Elevation on *Jaumea* biomass (*Distichlis*: Elevation  $F_{2,96}=0.67$ ,  $p=0.52$ , Neighbors  $F_{1,96}=1.80$ ,  $p=0.18$ , Elevation\* Neighbors  $F_{2,96}=0.85$ ,  $p=0.43$ ; *Frankenia*: (note insufficient sample size due to high mortality throughout experimental period.) Elevation  $F_{2,17}=3.49$ ,  $p=0.08$ , Neighbors NA, insufficient sample size, Elevation\* Neighbors  $F_{2,17}=0.25$   $p=0.63$ ; *Jaumea*: Elevation  $F_{2,109}=10.58$ ,  $p<0.0001$ , Neighbors,  $F_{1,109}=2.25$ ,  $p=0.14$ , Elevation\* Neighbors  $F_{2,96}=0.09$ ,  $p=0.91$ ). Individual Student's tests indicate that the significant effect of elevational treatment on *Jaumea* biomass was due to significantly smaller Marsh individuals as compared to Ecotone or Upland individuals ( $t_2=1.98$ , Marsh-Upland  $p=0.0018$ ; Marsh-Ecotone  $p<0.0001$ , Ecotone-Upland  $p=0.81$ ).

## DISCUSSION

We found that the landward boundary of the marsh-upland ecotone is likely set by inhospitable abiotic conditions and found no evidence of biotic interactions setting distributional boundaries at either the seaward or landward portions of the ecotone gradient. In addition, contrary to our hypothesis, conditions were not prohibitively stressful at the seaward, frequently inundated portion of the tidal gradient. These results contrast with studies that support the application of the SGH in salt marsh systems (Bertness and Hacker 1994, Pennings and Moore 2001, Pennings et al. 2005, He et al. 2011), which generally find that biotic interactions set landward boundaries with benign higher elevation habitats, while the intense physical stress of high salinity and anoxic soils sets boundaries in physiologically stressful lower elevation areas. In contrast to the findings in other geographic locations, in this California salt marsh, we found that from the perspective of the marsh-upland ecotonal plant community the directionality of the underlying stress gradient is reversed, indicating a negative relationship between inundation stress and elevation. We also found no evidence that biotic interactions set distributional boundaries under low stress conditions, as predicted by the SGH; there was no effect of neighbor removal anywhere along the ecotonal distribution. Our results lend support to an increasing body of literature that has found the simple paradigm of the SGH difficult to apply when environmental gradients are complex or when it is not clear which abiotic conditions are the most stressful (Pennings and Callaway 1992, Costa et al. 2003, Fariña et al. 2009, Guo and Pennings 2012).

At the seaward boundary, the surprising results of this outplant experiment indicate that the frequent inundation of the lower marsh is not responsible for excluding ecotonal species from lower elevations. Both *Distichlis* and *Jaumea* survived as well or better when outplanted seaward of their current distribution. Therefore, the seaward boundary of each species is not the result of intolerance to the abiotic conditions lower along the tidal gradient. In addition, despite increasing evidence for the prevalence of facilitative interactions in frequently inundated salt marsh habitats (Bertness and Callaway 1994), there was no effect, positive or negative, of neighbor removal on either mortality rates or biomass of outplanted individuals, thus eliminating support for the possibility that the enhanced survivorship at the Marsh elevation treatment was the result of facilitation by neighboring *Sarcocornia*.

*Distichlis* and *Jaumea* individuals outplanted landward of their natural distributions (Upland) had higher rates of mortality than those planted at either the Marsh or Ecotone treatment locations, indicating that the abiotic conditions at the most landward portion of the tidal influence gradient are more physiologically stressful than at either of the other treatment elevations. This suggests that the landward boundary is the result of physiological intolerance to the abiotic conditions of this zone. This intolerance to the conditions landward of the ecotone's upper boundary may be the result of a Mediterranean climate with limited rainfall, where desiccation poses more of a threat to marsh plants than inundation.

Biotic interactions with the species of the adjacent upland habitat do not appear to set the location of the landward boundary in this system. Although we cannot rule out the possibility that competition might be important under different conditions or for different life stages, we found no effect of neighbor removal at the Upland elevation treatment. This result was surprising both because it contrasts with the predictions of the SGH and because the adjacent upland community is dominated by weedy species, such as *Brassica nigra*, *Conium maculatum*, and *Cirsium vulgare* that are actively managed because of their aggressive capacity to outcompete other species.

Colonization of the adjacent upland habitat appears to be limited by abiotic conditions and not by biotic interactions. The greater role of abiotic factors in setting this boundary provides a potential mechanistic explanation for the result of a recent ten year study by Wasson et al. (2013), where significant landward migration of the ecotone-upland boundary was found to be correlated to increased inundation at the landward end of the tidal frame. When considered in the context of our findings, the observed landward migration of the landward boundary is likely the result of expansion of the ecotonal community's fundamental niche due to the landward expansion of the tidal gradient. The landward migration is likely the consequence of amelioration of abiotic conditions at higher elevations by the tides and not due to a reduction in competitive interactions by weedy upland species, as previously thought.

Results for *Frankenia salina* are difficult to interpret because of low sample size. Because of propagation problems only one site and half of the total number of plants could be included in the transplant experiment for this species. In addition,

subsequent mortality made it even more difficult to ascertain differences among the treatments. Patterns in this species tended to be similar to the other two focal species, but results were not significant.

The absence of a negative effect of increased inundation on Marsh treatment individuals indicates that a factor besides intolerance to frequent inundation is responsible for preventing most ecotonal species from successfully colonizing marsh elevations. The SGH predicts interspecific interactions to set and maintain boundary distributions under physiologically stressful conditions and much research on salt marsh plant distribution patterns reflect this. However, our results show no evidence that seaward boundary distributions are determined by competitive or facilitative interactions and, furthermore, no evidence that conditions seaward of the ecotone result in increased physiological stress. One possibility is that sublethal effects of frequent inundation may contribute to excluding ecotonal species. However, only the biomass of *Jaumea* individuals was significantly reduced at the Marsh elevation treatment; both *Distichlis* and *Frankenia* showed no reduction in biomass. Another ecological process that may regulate species distributions is herbivory. Though the influence of top down forces in structuring salt marsh plant communities has generally been considered minor, a notable exception demonstrating strong top down control of *Spartina alterniflora* by *Littoratia irrorata* suggests that herbivory can sometimes be important (Silliman and Zieman 2001). Herbivory by crabs, rodents, or other herbivores, which may have been responsible for the loss of transplanted ecotonal plants at the initiation of this experiment, may contribute to the maintenance

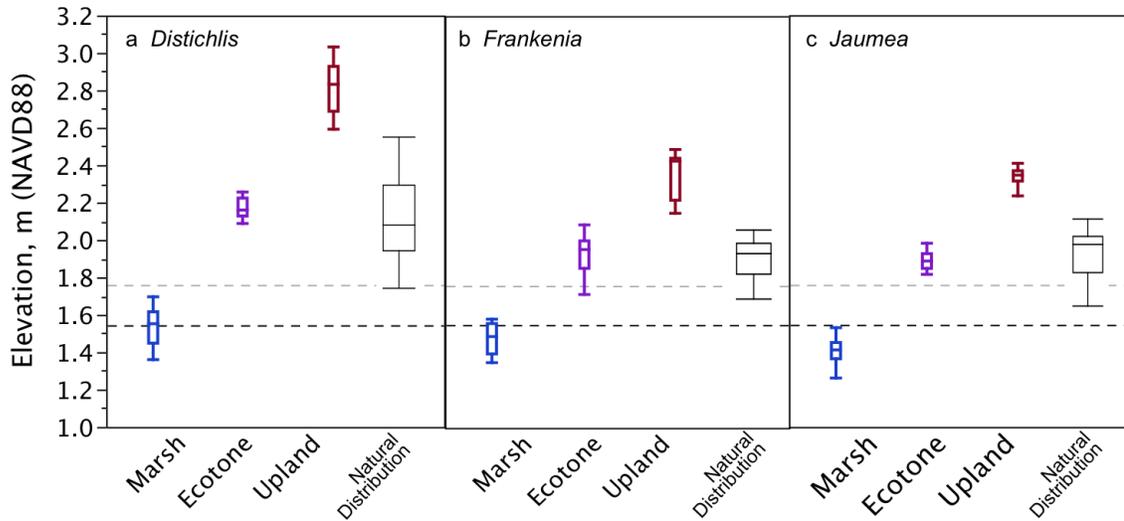
of the seaward ecotonal boundary. We tested this possibility with a follow-up caging experiment examining the influence of herbivory on survivorship of these species, but no significant herbivory was detected in that experiment (Fresquez, unpublished data). However, episodic herbivory cannot be ruled out. Alternatively, the narrow distribution of these ecotonal species may also be the result of recruitment limitation in the marsh plain. These three ecotonal dominants colonize bare space primarily through vegetative expansion. However, all three species infrequently set viable seed which, when produced, is likely deposited just above the low marsh through wrack deposition (Minchinton 2006).

Previous research has shown that the distribution of the high marsh-upland ecotone plant community is sensitive to anthropogenic disturbances such as sediment deposition (Allison 1995), freshwater runoff (Callaway and Sabraw 1994), tidal restriction and disturbance from cattle (Woolfolk 1999; Wasson and Woolfolk 2011), invasion by non-native species (Callaway and Zedler 1998), and nutrient addition (Traut 2005, Martone and Wasson 2008). Our results indicate notable similarity among all three dominant ecotonal species in the ecological processes setting distributional boundaries and elevational distributions. We explicitly examined three taxonomically unrelated species to determine whether distributions are set by similar or contrasting ecological processes. Our findings suggest that because the ecological processes structuring the distributions of each species are relatively similar consideration of the ecotonal plant community as a single unit may be appropriate from both an ecological and conservation perspective. Therefore, this threatened plant

community may benefit from strategies that manage the whole community rather than using an individualistic approach.

Understanding how current abiotic conditions influence species distributions and boundaries is critical for anticipating how species distributions will respond to changes in abiotic conditions that accompany climate or land use change. Our investigation of the ecological processes controlling the distribution of this plant community is especially informative as it illustrates how similar marshes on the Pacific coast and other places may respond as sea levels rise. Fortunately, our results suggest that there is room for limited optimism regarding conservation of this ecotonal community in the face of sea level rise. This plant community may be able to adjust to increased sea levels due to the high inundation tolerances demonstrated by individuals planted seaward of the ecotone-marsh boundary and the ability of these species to migrate landward in response to increases in inundation (Wasson et al. 2013). While the SGH suggests that the stresses of increased inundation caused by sea level rise and the competitive interactions limiting the landward boundary will lead to a contraction of the zone available to many salt marsh plant species in other systems, the reversed nature of the stress gradient experienced by the marsh-upland ecotone in this estuary means that these species may actually benefit from increased inundation. This reverse stress gradient may thus be the key to providing the marsh-upland ecotone plant community with resilience in the face of sea level rise.

TABLES AND FIGURES



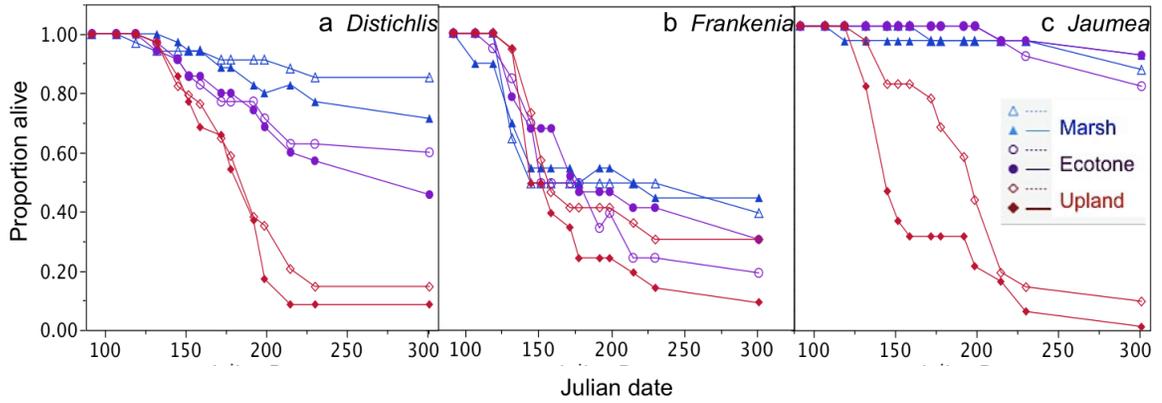
**Figure 1.** Outplant treatment elevations and natural distributions of a) *Distichlis spicata*, b) *Frankenia salina*, and c) *Jaumea carnosa*. For each species, experimental outplant treatments were established by planting individuals at elevations seaward, Marsh treatment (blue), or landward, Upland treatment (red) of observed distribution of that species. Control treatments, Ecotone (purple), are defined as the midpoint of the species natural distribution. Box plots indicate mean and interquartile range, whiskers drawn to furthest point within 1.5 X interquartile range. All elevations in meters relative to NAVD88; 0 NAVD88 corresponds approximately to Mean Lower Low Water in this estuary.

**Table 1.** Mean Elevations and Inundation Durations at Boundaries and Outplant Treatment Elevations

	Observed species distributions, 2010-2012			Outplant treatment elevations, March - October 2012		
	Marsh Boundary	Upland Boundary	Percent Time Inundated, %	Marsh	Ecotone	Upland
	Mean Elevation, m (st. dev)	Mean Elevation, m (st. dev)	Percent Time Inundated, %	Percent time inundated, %	Percent time inundated, %	Percent time inundated, %
<i>Distichlis spicata</i>	1.71 (0.25)	2.51 (0.40)	6.42%	10.87%	0.08%	0%
<i>Frankenia salina</i>	1.65 (0.23)	2.07 (0.22)	8.46%	13.35%	0.80%	0%
<i>Jaumea carnosa</i>	1.70 (0.08)	2.15 (0.09)	6.75%	18.44%	1.96%	0%

**Table 2.** Results of Proportional Hazards Survivorship Model: Significance of fixed effects Elevation, Neighbors (+ or -), and their Interaction

	Cox Proportional Hazards Model			Risk Ratios, Prob>Chisq			
	Elevation	Neighbors	Elevation x Neighbors	Upland x Ecotone	Ecotone x Marsh	Upland x Marsh	Neighbors
<i>Distichlis spicata</i>	<b>&lt;0.0001</b>	0.1506	0.7773	<b>&lt;0.0001</b>	<b>0.0013</b>	<b>&lt;0.0001</b>	0.1506
<i>Frankenia salina</i>	0.3723	0.9897	0.2437	0.4854	0.4569	0.1599	0.9897
<i>Jaumea carnosa</i>	<b>&lt;0.0001</b>	0.4860	0.4540	<b>&lt;0.0001</b>	0.8216	<b>&lt;0.0001</b>	0.4860



**Figure 2.** Survivorship over time for all three species (*Distichlis spicata*, *Frankenia salina*, and *Jaumea carnosa*) expressed as proportion of individuals remaining alive at each Julian date. Open circles indicate neighbors removed (-neighbors) and closed circles indicate no removal (+neighbors).

## CHAPTER TWO:

### **EDAPHIC AND BIOTIC FEATURES DETERMINE ECOTONE STRUCTURE AND BOUNDARIES WITH ADJOINING HABITATS**

#### ABSTRACT

Examinations of species distributions along abiotic gradients suggest that these distributions are the product of both differential species tolerances of the underlying conditions and of biotic interactions between adjacent species. Examination of the abiotic and biotic factors structuring the marsh-upland ecotone allows the determination of the relative influence of these factors on the distributional boundaries of each of the dominant species of the ecotonal community and of the ecotone itself. Over the course of two years, soil samples were collected from transects that extended across the marsh, ecotone, and upland. Seasonal measurements of the soil moisture and pore water salinity reveal significant variability seasonally and both within and between habitat types and indicate that the relationship between elevation and soil salinity across the ecotone can be both linear or hump-shaped depending on seasonal and interannual precipitation conditions. SIMPER analyses on eleven characteristics of soils collected along each transect indicate that vegetation defined habitat types show significant patterns of dissimilarity in underlying edaphic conditions. Principal components analysis further indicates that while several of these edaphic variables covary strongly along the primary axis (PC1 55% variance) the loading of salinity, a stressor with large implications for distributional patterns, on the secondary axis (PC2 15.5%) suggests that the abiotic

factors setting distributions may not covary with elevation. To quantify the impact of competitive interactions with the vegetation of the adjacent habitats on ecotonal species, a neighbor removal manipulation where all non-ecotonal species were removed was paired alongside control transects. Removals were maintained for two growing seasons but there was minimal indication that competitive interactions limit the boundaries of the ecotonal community from expanding into the adjacent habitat types. However, significantly higher coverage by ecotonal species within the ecotone, especially near the boundaries with the adjacent habitats, provide insight into the individualistic processes limiting the distributional boundaries of the component species of the ecotone and concur with previous research on the factors limiting the distributions of these ecotonal species.

## INTRODUCTION

Species distributional boundaries are the products of the differential influence of the conditions within and beyond the distribution of that species. Identifying the factors controlling the distribution of a species requires a detailed understanding of both the abiotic conditions experienced by that species and of those outside of its range. In addition to the direct influence of abiotic factors on species distributions, the abiotic conditions may also indirectly affect distributions by impacting the outcome of biotic interactions. In species where distributional ranges coincide with large variations in the underlying abiotic conditions, as with species oriented along steep gradients, understanding the relative influence of abiotic and biotic factors on their distributions is a major challenge and has generated a significant body of research. Close examinations of the distributions of species along abiotic gradients have been pivotal in the generation of theories regarding the biotic and abiotic processes structuring species distributions (Whittaker 1956, Connell 1961, Bertness and Ellison 1987). These investigations have especially focused on the distributions of species in stressful habitats because of the relatively strong influences of abiotic conditions and the distinct patterns of zonation across the environmental gradient (Wilson and Keddy 1986, Bertness and Hacker 1994, Callaway and Pennings 1998).

Ecotonal systems are excellent model systems to examine the processes that structure community distributions. Ecotones, the narrow areas of transition between larger adjacent ecological systems, are the result of overlap between habitats with disparate physical and biotic properties. These transitional areas, wedged between

broader habitat zones with more uniform conditions, reflect the integration of the biotic and abiotic properties of the adjacent habitats, creating steep gradients of rapid change in physical conditions. The rapid transitions in vegetative communities and steep underlying gradients allow us to examine both the abiotic conditions and biotic interactions potentially responsible for setting distributions. In physically narrow ecotones, such as those found at the marine-terrestrial interface, the conditions of the gradient shift so rapidly that even neighboring individuals are subject to differing physical conditions (Risser 1995). This often leads to abrupt boundaries across the ecotone, as species remain limited to areas within their range of physiological tolerance.

The marsh-upland ecotone is the narrow transition zone between the salt marsh plain and adjacent upland vegetation (Callaway et al. 1990). In intertidal salt marsh plant communities, like the marsh-upland ecotone, frequency of inundation plays a major role in establishing plant zonation patterns and community structure (Adams 1963, Chapman 1978, Snow and Vince 1984, Bertness and Ellison 1987, Bertness 1991). The influence of strong environmental gradients on community structure in salt marsh systems is well established (Adams 1963, Chapman 1978, Snow and Vince 1984, Bertness and Ellison 1987, Bertness 1991). In the high marsh-upland ecotone the abrupt boundaries, relatively simple community composition, and steep changes in abiotic conditions all occur within a small spatial scale, making this system especially amenable to use as a model system. Using this simple community and complex abiotic gradients, it is possible to test ecological theory on the factors

responsible for setting species distributions and investigate how the relative influence of each of these factors depends on the variable conditions within and outside of those distributions.

Across the transition from marsh to upland, the ecotone experiences broad variations in the frequency of tidal inundation, which in turn creates a strong gradient in edaphic stressors (Adams 1963, Callaway et al. 1990). This environmental gradient structures the distribution of the species of these habitats both directly, by limiting species to areas within their physiological tolerances (Mahall and Park 1976, Barbour 1978, Cooper 1982b), and indirectly, by influencing the outcomes of biotic interactions among the species of the salt marsh community (Pennings and Callaway 1992, Levine et al. 1998, Bockelmann and Neuhaus 1999, Pennings et al. 2005, Greenwood and MacFarlane 2006). The stress gradient hypothesis (SGH) is one conceptual framework typically used to predict the relative influence of direct abiotic conditions and indirect biotic interactions on the distributions of species in salt marsh systems. It predicts that along a gradient of potentially stressful physical conditions, the distributional boundary of a species at the low-stress end is set by interspecific interactions, often competition, while at the high-stress end of the gradient, unless facilitative interactions mitigate the stressor, the distributional boundary is the result of intolerance to the stressful abiotic conditions beyond that boundary (Bertness and Ellison 1987, Bertness 1991). As a result, species whose distributions coincide with gradients in environmental stress are often competitively excluded from colonizing adjacent benign habitats due to their inferior competitive ability relative to the species

found in less stressful areas. However, the abiotic gradient influencing species distributions is not static. Therefore, the shape and intensity of the underlying abiotic gradient across the marsh to upland transition zone is affected by many external factors, including interannual, seasonal and diurnal variation in tides and precipitation. This dynamic nature of the abiotic gradient increases the complexity of predicting the relative influence of abiotic and biotic factors on species distributions patterns.

Using this predictive framework I quantify and compare the abiotic conditions found within the marsh, ecotone, and upland habitats to examine how the abiotic conditions differ between habitats and to identify whether variability in abiotic conditions at the thresholds between habitats influences the biotic interactions setting the distributional boundaries of the ecotonal plant community. I also employ a neighbor removal manipulation to determine how biotic interactions between the non-ecotonal upland or marsh species and the ecotonal plant community influence the distribution and abundance of the marsh-upland ecotone.

## METHODS

All experimental manipulations were conducted within the Elkhorn Slough estuary in Monterey Bay, California. Elkhorn Slough, located along the Central California coast, contains 1,147 ha of salt marsh habitat, one of the largest remaining tracts of salt marsh in California (Caffrey et al. 2002). This region has a mild Mediterranean climate, with dry, warm summers and most precipitation occurring

from mid-October through May in the form of rain and fog. It is an open estuarine system with a maximal tidal range of 2.5m. Salinity levels within the estuary are similar to the adjacent marine environment due to minimal freshwater inputs. The upland vegetation around Elkhorn Slough is a mix of oak-woodland, *Baccharis* chaparral, invaded grasslands, and native grassland remnants (Caffrey et al. 2002). Elkhorn Slough salt marshes are dominated by *Sarcocornia pacifica* (Standley) Scott (Chenopodiaceae), hereafter *Sarcocornia*. This perennial species forms a monoculture from the frequently inundated mudflat to the high marsh, where its upper boundary corresponds with the lower boundary of the marsh-upland ecotonal plant community.

The marsh-upland ecotone forms the transition zone between the adjacent upland and the *Sarcocornia* dominated marsh plain. The ecotonal plant community consists of a mix of mostly perennial native salt marsh species tolerant of some tidal inundation and the abiotic conditions associated with tidal inundation. The native ecotonal plant community at Elkhorn Slough is dominated by three species, *Distichlis spicata* (L.) Greene (Poaceae), *Frankenia salina* (Molina) I.M (Frankeniaceae), and *Jaumea carnosa* (Less.) Gray (Asteraceae), with less abundant patches of *Triglochin maritima* (L.) (Juncaginaceae), and *Spergularia marina* (L.) (Caryophyllaceae). *Atriplex triangularis* (Willd.) (Chenopodiaceae) [renamed *Atriplex prostrata* DC. and designated as non-native after the completion of this experiment, (Baldwin et al. 2012)]. The species of the marsh-upland ecotone, with the exceptions of *A. triangularis*, *S. marina*, and *T. maritima*, propagate most readily through vegetative

expansion, though each does set viable seed and seedling recruitment is not extremely rare (Bree Candiloro and Rob de Bree native propagation specialists).

Transects were established in early fall of 2010 at four sites distributed across Elkhorn Slough: Azevedo, Coyote, Packard and Yampah (Figure 1). Sites were selected based on accessibility and history of human use. Each site contains salt marsh with no history of tidal restriction, an adjacent upland area with no recent history (<50 years) of human use, and an intact ecotone wedged between these two plant community types. Permanent transects were established at four sites; four transects at three sites and three transects at the fourth site, n=15. Transects were 1-m wide, separated by >30-m, and oriented perpendicularly to the ecotone. They begin in the marsh, extend across the entire ecotone, and end in the upland. The absolute length of each transect is a function of slope so (due to variation in the slope from marsh to ecotone to upland) transects varied in length. Transect lengths are defined as twice the length of the ecotone, as each transect extends 50% of the ecotone length into both the adjacent marsh and the adjacent upland (Figure 2). Therefore, although transects are of different lengths, they contain the same proportional amounts of each habitat type. Transects were marked using two permanent PVC pipes, one at the marsh-ecotone boundary and one at the upland-ecotone boundary. The boundaries of the ecotone plant community are defined as the point where ecotonal vegetation transitions to 100% cover by the vegetation of the adjacent habitat. In other words, the marsh-ecotone boundary is the location of the most seaward ecotonal species individual and the upland-ecotone boundary is the location of the most landward

ecotonal species individual. The boundaries of the ecotonal plant community are relatively abrupt. However, the boundaries of the adjacent upland and marsh plant communities are more gradual, overlapping with the upper and lower, respectively, portions of the marsh-upland ecotone.

For assessment of how abiotic, specifically edaphic, and biotic characteristics differ between marsh, ecotone, and upland, each transect was divided into nineteen equally sized contiguous plots. Plot numbers 1 through 4 were located in marsh habitat, 5 through 10 were low ecotone habitat, 11 through 14 were high ecotone habitat, and 15 through 19 were in upland (Figure 2). Due to variation in transect lengths described above, plot length varied across transects from 0.36-m to 1.04-m, averaging 0.68-m.

Absolute elevation at the center of each plot was calculated by comparing relative elevation measures collected using a surveyor's level to benchmarks established with a Trimble 5800 RTK survey. Orthometric heights collected using the Trimble were post-processed using the National Oceanic and Atmospheric Administration's Online Positioning User Service (OPUS) using the GEOID09 model and NAVD88 vertical datum.

#### Seasonal Variation in Soil Pore Water Salinity and Moisture Content

Seasonal variation in air temperature leads to varying rates of evaporation of soil pore water, which can have especially large impacts on the infrequently inundated elevational zones within the ecotone. Therefore, monitoring seasonal variation in pore water salinity and soil moisture is critical to accurately quantify the

influence saline soils may have on the distribution of the ecotonal community. Pore water salinity and percent moisture of each plot within the transect was measured by collecting soil samples from all plots in Summer 2011 (mid-July), Fall 2011 (mid October), Spring 2012 (early May), and Fall 2012 (late October). In addition, a soil sampling event in early October 2011 was unable to be completed prior to the first rain and only four transects from two sites (three from Yampah and one from Azevedo) were sampled. This incomplete sample set will be referred to as Pre-rain 2011. Samples were collected from every plot in Summer 2011, Fall 2011, and Pre-rain 2011 and only collected from odd numbered plots in Spring 2012 and Fall 2012. Soil samples were collected from along the boundary between paired removal and control transects (see Figure 2). Soil cores were collected using a 3-cm diameter soil core to a depth of 10-cm. Each core was homogenized, weighed, then dried for a minimum of 24 hours at 60° C, and weighed again to quantify soil moisture content. Soil salinity was measured using a modified dilution method (U.S. Salinity Laboratory 1954). The entire soil sample was rehydrated using a gravimetric ratio of 3:1 deionized water to soil, stirred, and allowed to settle for 24 hours. The salinity of the mixture was measured using an YSI 10/30 conductivity, temperature and salinity meter. Pore water salinity (ppt) was calculated based on the original water content of the soil sample. The percent moisture of each sample is the weight of water in each sample by the total sample weight.

MANOVA was used to test how percent moisture and pore water salinity varied by season, habitat type, and their interaction. Site was included as a factor but

was non-significant for both percent moisture and pore water salinity so it was excluded from further analysis. Tukey tests were used to examine how percent moisture and pore water salinity differed among habitat types within each season.

#### Edaphic Characteristics of the Marsh, Ecotone, and Upland

Subsamples of soils collected from odd numbered plots in Fall 2012 were analyzed for organic matter content (%), phosphorous (ppm), potassium (ppm), magnesium (ppm), calcium (ppm), sodium (ppm), sulfate (ppm), pH, and cation exchange capacity (CEC) by A&L Western Analytical Laboratories in Modesto, California. These data and previously described pore water salinity (ppt) and percent moisture were included in multiple multivariate analyses to examine whether edaphic characteristics vary between each habitat type and to identify the edaphic variables that drive similarity or differences within and among habitat types.

All multivariate analyses were done using Primer v. 6 and edaphic variables were normalized to minimize variability due to differing measurement scales. To graphically examine dissimilarity in edaphic conditions among habitat types (marsh, low ecotone, high ecotone, and upland), an NMDS plot based on a Euclidean distance resemblance matrix was used. Analysis of similarities (ANOSIM) was used to determine whether the habitat types were significantly different and to assess pairwise dissimilarity between each habitat type. A SIMPER analysis was then used to determine how dissimilarity within habitat types compared to dissimilarity between habitats and to identify which edaphic variables contributed most to dissimilarity between habitat types. To prevent pseudoreplication in the ANOSIM and SIMPER

analyses, plots from each habitat type were averaged prior to normalization and generation of resemblance matrix.

A principal components analysis (PCA) using the eleven edaphic variables was performed to reduce the dimensionality of the edaphic variables. The composite principal component scores of principal component axis 1 (PC1) and principal component axis 2 (PC2) were then used to test how they correlate with plot elevation.

#### Neighbor Removal Manipulation

To examine how interactions with species from the adjacent upland and marsh habitats influence colonization by ecotonal species into these habitats, all non-ecotonal species were removed from a 0.5-m wide area directly adjacent to each transect, creating paired removal and control plots (Figure 2). Neighbor removal manipulations were initiated in early fall of 2010 when marsh and ecotonal plant communities are at peak aboveground biomass. All species were removed across the length of the entire transect except for the native ecotonal plant community, namely *D. spicata*, *F. salina*, *J. carnosa*, *A. triangularis*, *S. marina*, and *T. maritima*. Transects were maintained monthly or as needed over the course of the two-year experiment.

Cover by all species within each plot in paired removal and control transects was assessed in both summer and fall of 2011 and 2012. However, all analyses will be on data collected during the final fall 2012 assessment. The percent cover by every species within each removal and control plot was visually estimated using the modified Daubenmire cover scale method (Daubenmire 1959). Though the stature of

species in these transects rarely exceed 0.4 m, plants often overlapped, allowing for total species coverage greater than 100%. Where indicated the difference in cover between paired removal and control plots ( $\% \text{Cover}_{\text{Removal}} - \% \text{Cover}_{\text{Control}}$ ) was used as a response variable.

ANOVA was used to test for differences in cover between removal and control plots across habitat types for each of the three dominant ecotonal species, *D. spicata*, *F. salina*, and *J. carnosa*, and by all ecotonal species summed (the three dominant species plus *T. maritima*, *S. marina*, and *A. triangularis*). The response variable was the difference in percent cover between paired removal and control plots ( $\% \text{Cover}_{\text{Removal}} - \% \text{Cover}_{\text{Control}}$ ). Site was included as a random effect but only significantly affected coverage by *F. salina* and *J. carnosa* so it was dropped from the ANOVA for *D. spicata* and for all ecotonal species.

To ensure our analyses captured the effect of removal treatment at the boundaries, the analyses are focused onto the plots near the boundary where vegetative expansion or rhizomatous growth by ecotonal species would be expected to occur. Recall, the definition of the upland-ecotone boundary is the location of the most landward ecotonal individual. Similarly, the definition of the marsh-ecotone boundary is the location of the most seaward ecotonal individual. The plot containing the boundary individual is referred to as the “boundary plot” and the plot adjacent to the boundary is the “boundary adjacent plot” (Figure 2). These boundary designations reflect the location of the marsh and upland boundaries at the time of experimental set-up. Matched pairs analyses were done on these plots at the marsh and upland

boundaries. A matched pairs analysis was used to test for differences between percent cover in removal and control plots in the boundary plot and boundary adjacent plots of marsh and upland boundaries. These analyses were performed separately for all ecotonal species and for *D. spicata*, *F. salina*, and *J. carnososa*.

## RESULTS

### Seasonal Variation in Soil Pore Water Salinity and Moisture Content

The percent moisture in marsh, ecotone, and upland soils ranged from over 80% in marsh soils to close to 0% in upland soils (Figure 3). Season significantly affected the amount of moisture in soils ( $F_4=7.01$ ,  $p=0.0004$ ), percent moisture varied broadly depending on habitat type ( $F_3=137.77$ ,  $p<0.0001$ ), and there was a significant interaction between these two factors ( $F_{12}=4.43$ ,  $p<0.0001$ ). Post hoc tests examining how moisture varied between habitat types revealed that, across all seasons, moisture levels consistently differed between marsh and low ecotone habitat types, but soil moisture in the high ecotone and upland were not significantly different (Figure 4 A-E).

Both the mean pore water salinity and the shape of the relationship between pore water salinity and location along the transition from marsh to upland varied widely (Figure 5). Across all seasons, soils in the marsh were the least variable and relatively close to the salinity of the water in the estuarine main channel, mean = 43.9ppt  $\pm$ sd 3.2. In the low ecotone, mean pore water salinity was very similar to the marsh, but was much more variable, 44.2ppt  $\pm$ 13.6. The high ecotone was on average much less salty, but also very variable, 26.1ppt  $\pm$ 19.8, as were upland soils, 12.8ppt

±13.9. Pore water salinity varied significantly by both season ( $F_4=15.43$ ,  $p<0.0001$ ) and habitat type ( $F_3=31.65$ ,  $p<0.0001$ ). In addition, the effect of season depended on the habitat type ( $F_{12}=3.71$ ,  $p=0.0002$ ). Post hoc tests indicate that differences in salinity among habitat types varied widely depending on the season (Figure 4 F-J).

#### Edaphic Characteristics of the Marsh, Ecotone, and Upland

The NMDS plot (stress=0.09, 50 iterations, Figure 6) shows that habitat types differ based on edaphic characteristics (ANOSIM  $R=0.517$ ,  $p=0.001$ ). In addition, the edaphic environment in each pairwise habitat comparison was significantly different from each other (Table 1). A SIMPER analysis also indicated that the Upland and High Ecotone edaphic environments are very similar, more so than the Low Ecotone and High Ecotone habitats (Table 1). Not surprisingly, the dissimilarity between the most distinct habitats, the Upland and Marsh, was due to differences in edaphic variables strongly correlated with tidal inundation.

The principal component analysis revealed strong covariance among edaphic variables across the gradient from marsh to upland (Figure 7). More than half (55%) of the variability in the edaphic conditions was explained by the primary axis of the PCA (PC1), which was driven by Na, Mg, S, and K abundances, moisture (%), organic matter (%), and CEC in approximately equal proportions (Table 2). The second axis, PC2, explained an additional 15.5% of variance and was driven by Ca abundance and pore water salinity (Table 2). The relationship between the scores of principal component 1 and the mean elevation (m) of plots from which each Fall 2012 soil sample was collected is well described by a quadratic relationship ( $r^2=0.65$ ,

$f=123.23$ ,  $df=2$ ,  $p<0.0001$ )(Figure 8A). In addition, the mean PC 1 score decreases linearly from the marsh to upland (Figure 8B). PC2 and elevation are significantly linearly correlated ( $r^2=0.20$ ,  $f=33.05$ ,  $df=1$ ,  $p<0.0001$ ), though the correlation is not as strong as the PC1 and elevation relationship (Figure 8C). Mean PC2 scores indicate that the low ecotone and upland habitats are most different and the marsh and high ecotone are most similar (Figure 8D).

### Neighbor Removal Manipulation

The plot-level abundances of each of the component salt marsh species (including both ecotonal and marsh species) in the control plots varied across the elevational gradient from marsh to ecotone to upland (Figure 9A). At the end of the two-year removal experiment, the relative abundance of ecotonal species in removal plots was similar to control plots (Figure 9A,B). The effect of neighbor removal on the difference in cover by ecotonal species between removal and control plots varied depending on the habitat type ( $F_3=22.06$ ,  $p<0.0001$ , Figure 10A). The effect of neighbor removal was consistently positive across habitat types, indicating an increase in cover by the ecotonal plant community in response to the removal of non-ecotonal species. Post hoc tests indicate that in both the high ecotone and the low ecotone neighbor removal led to similar increases in cover by ecotonal species (high ecotone= $13.70\%$  st. dev.  $\pm 26.31$ , low ecotone= $21.72\% \pm 26.06$ ). However, the nearly negligible increases in cover by ecotonal species due to neighbor removal in the upland and marsh habitat types were significantly less than the effect observed in the ecotone (upland= $1.22\% \pm 7.23$ , marsh= $0.17\% \pm 0.63$ ). There was a significant effect of

neighbor removal on the difference in cover by *D. spicata* ( $F_3=9.99$ ,  $p<0.0001$ ), *F. salina* ( $F_3=6.55$ ,  $p=0.0003$ ), and *J. carnososa* ( $F_3=4.20$ ,  $p=0.0063$ )(Figure 10 B-D). Coverage by both *F. salina* and *J. carnososa* were significantly affected by site, (*F. salina*  $F_3=6.25$ ,  $p=0.0004$ ; *J. carnososa*  $F_3=4.25$ ,  $p=0.0059$ ) but the variance attributable to site was still small for both species (*F. salina* 6.9%, *J. carnososa* 4.9%). For individual species, removals had, on average, a positive effect on cover within all habitat types. For *D. spicata*, post hoc tests indicate that this species increased similarly in response to removals in both the high ecotone (high ecotone= $9.89\% \pm 20.90$ ) and the low ecotone (low ecotone= $10.03\% \pm 17.38$ ). However, increases in cover by *D. spicata* in the upland and marsh habitat types were both very small (upland= $1.56\% \pm 6.55$ , marsh= $0.05\% \pm 0.32$ ) and significantly less than the increases observed in the ecotone (Figure 10 B). For *F. salina*, increases in cover in response to removals were greatest in the low ecotone (low ecotone= $7.88\% \pm 20.29$ ). There was no increase in cover in the upland (upland= $-0.33\% \pm 2.89$ ) and a minimal increase in the marsh (marsh= $0.08\% \pm 0.45$ )(Figure 10 C). Across habitat types, neighbor removal had the least positive effect on *J. carnososa*, with the greatest increase observed in the low ecotone (low ecotone= $3.62\% \pm 11.30$ )(Figure 10 D).

In upland boundary adjacent plots there was consistently no increase in cover in removal plots by the ecotonal plant community overall or by any of the individual species (Table 3, Figure 11). However, in upland boundary plots there was a significant increase in cover in response to neighbor removal by grouped ecotonal species (Figure 11A) and by *D. spicata* (Figure 11B)(Table 3). The effect of neighbor

removal on the marsh boundary was quite different than that observed at the upland boundary. In contrast to the absence of a treatment effect in upland boundary adjacent plots, cover by ecotonal species was marginally greater ( $p=0.060$ ) in marsh boundary adjacent plots (Figure 11A). In marsh boundary plots grouped ecotonal species (Figure 11A), *F. salina* (Figure 11C), and *J. carnosus* (Figure 11D) all had significantly greater coverage in removal plots (Table 3). Interestingly, the species most responsive to removals at the marsh and upland boundaries were consistently those species that are typically most abundant near those boundaries under natural conditions (Figure 9).

## DISCUSSION

The abiotic conditions found within in each habitat type vary widely and, overall, the distinct composition of the biotic community of each habitat seems to reflect these large differences in abiotic conditions. This investigation into to the relative influence of abiotic and biotic ecological processes on the distribution and composition of the marsh-upland ecotone transition suggests that the boundaries between the ecotone and the adjacent habitat types are strongly influenced by these edaphic conditions, while the distributions of each of the component species of the ecotonal community within the ecotone are influenced by a combination of both biotic and abiotic processes.

The significant variation in moisture content of soils across the marsh, ecotone, and upland habitat types reflects the dynamic nature of intertidal habitats and of ecotones in general. By definition ecotones represent the overlap of the conditions of the adjacent habitats and any variability in the conditions of these adjacent habitats is

reflected and potentially magnified within the ecotone itself (Delcourt and Delcourt 1992, Risser 1995). Interestingly, while the moisture content of soils within each habitat type is relatively consistent seasonally, the relative salinity of each habitat type varies widely by season, especially within the ecotone. This pattern of decreasing pore water salinity from marsh to upland was only observed during the rainy season when there is sufficient precipitation or runoff from adjacent slopes to mitigate the effects of evaporation in the infrequently inundated ecotone. This indicates that for a significant portion of the year the salinity gradient is relatively linear as in higher latitude and non-Mediterranean climate marshes. However, in the remaining portions of the year when precipitation is low or in drier years, the marsh-upland ecotone exhibits a hump-shaped relationship between elevation and salinity similar to those observed in drier climate marshes, including Southern California marshes (Pennings and Callaway 1992). Seasonal and interannual variation in pore water salinity across the transition from marsh to upland is particularly important for restoration and management of ecotonal plant communities. The relative differences in soil salinity between each habitat type only parallels the relative differences in soil moisture during the Spring where precipitation is highest meaning that soil moisture may only accurately represent the soil salinity of this transition zone during the rainy season.

Multivariate edaphic analyses indicate that the edaphic environments significantly differ among habitats and quantify the magnitude of dissimilarity using Euclidean distances. While the strong dissimilarity between marsh and upland habitats is not

unexpected, this analysis did yield interesting insights into the relative dissimilarities between habitats. Interestingly, the low ecotone habitat is quite dissimilar to the marsh habitat and is actually more similar edaphically to the upland than it is to the marsh. This reinforces the fact that the edaphic environments within the ecotone and the marsh are quite distinct. In addition, while both the low ecotone and high ecotone were more similar to the upland than to the marsh, the high ecotone was more similar to the upland than to the low ecotone. Interestingly, this break between the low ecotone and high ecotone reflects the abrupt decline in abundance of the two ecotone dominants that are most abundant in the low ecotone, *F. salina* and *J. carnosa* (Figure 9). These results lend support to the long held beliefs that this high marsh ecotonal plant community is physiologically excluded from the prohibitively stressful habitat of the marsh plain and tolerant of the conditions of the upland habitat. However, as will be described below, multiple lines of evidence indicate that the reverse is true—the ecotonal community is actually physiologically intolerant of the upland conditions and tolerant of the conditions of the marsh.

A primary goal of this investigation was to investigate the shape of the relationship between the underlying gradient and elevation. Quantifying the strength and shape of this relationship is not only important for determining how abiotic conditions influence the ecotonal distribution, but is also of particular interest from a conservation and restoration perspective. Understanding how the abiotic conditions vary with elevation helps land managers predict how the plant community will respond to disturbances, such as tidal restriction, that alter these underlying

conditions and also inform the design of effective restoration strategies. By using a multivariate approach to collapse the complex abiotic gradient of this transition zone into axes that account for over 70% of the variance, it was possible to quantify the relationship between the potentially most influential edaphic variables and elevation. Interestingly, if only the primary principal component axis was considered when collapsing the edaphic variables into a composite axis, the strongly linear correlation between PC1 and elevation would lead to the incorrect conclusion that elevation is an appropriate proxy to describe the abiotic gradient. However, the secondary principal component (PC2) illustrates that an additional 15.5% of the variance is attributed to other edaphic variables, including pore water salinity, and this axis is orthogonal to PC1. Negative values of PC2 indicate high salinity measures and the ecotonal soil samples are negatively correlated with PC2. The Fall 2012 soil samples included in this analysis were collected when soil salinity across the marsh to upland transition is both highest and most variable within the ecotone (Figure 5). Therefore how the variability captured by PC2 influences the distributions of the plants across this transition is unclear.

The relatively consistent absence of expansion by the ecotonal plant community into the adjacent habitats suggests strongly that these species are not competitively limited by upland plant species. Though I found some significant increases in cover by ecotonal species due to neighbor removal in the plots that coincide with the upland and marsh boundaries of the ecotone, removing non-ecotonal species generally did not result in significant colonization of the areas beyond the ecotonal boundaries.

This absence of expansion indicates that it is unlikely that competitive interactions play a major role in setting the boundaries between the ecotonal plant community and the adjacent habitats. However, the abundance and elevational distributions of each of the dominant ecotonal species varies across the ecotonal transition zone and each occupies a slightly different but overlapping elevational range (Figure 9). While the overall results of the neighbor removal manipulation do not show a strong pattern of competitive exclusion by marsh and upland species, the species-level responses of each of the ecotone dominants reflects individualistic processes that control the distributions and abundance of each species across the ecotone.

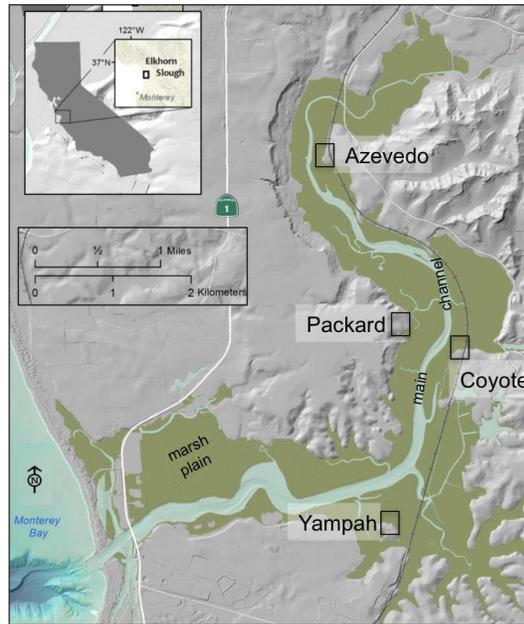
Though there was a significant increase in coverage within the upland boundary plots, the ecotonal community failed to expand into the open space adjacent to the upland boundary in response to removal. This lack of landward expansion of ecotonal species into the upland contrasts with the general paradigm that high marsh species are excluded from colonization of the adjacent upland habitat by competition from upland plants. However, it concords with the findings of a previous experiment, where experimentally outplanted *D. spicata*, *F. salina*, and *J. carnosus* individuals planted landward of their natural distributions did not thrive in the abiotic conditions above the ecotone and appeared to be physiologically (not competitively) excluded from the upland (Chapter 1). Among the individual species, *D. spicata* was the only species where an increase in cover was observed within the upland boundary plot. This increase coupled with the natural peak in *D. spicata* abundance in the high ecotone (Figure 9) suggests that the high ecotone is likely the physiologically optimal

zone for this species. In addition, in combination with the results of the experimental outplant (Chapter 1), it also suggests that though abiotic factors may have a greater influence on *D. spicata*'s upland boundary, competitive interactions from upland species do limit the abundance of this species at the landward end of its distribution.

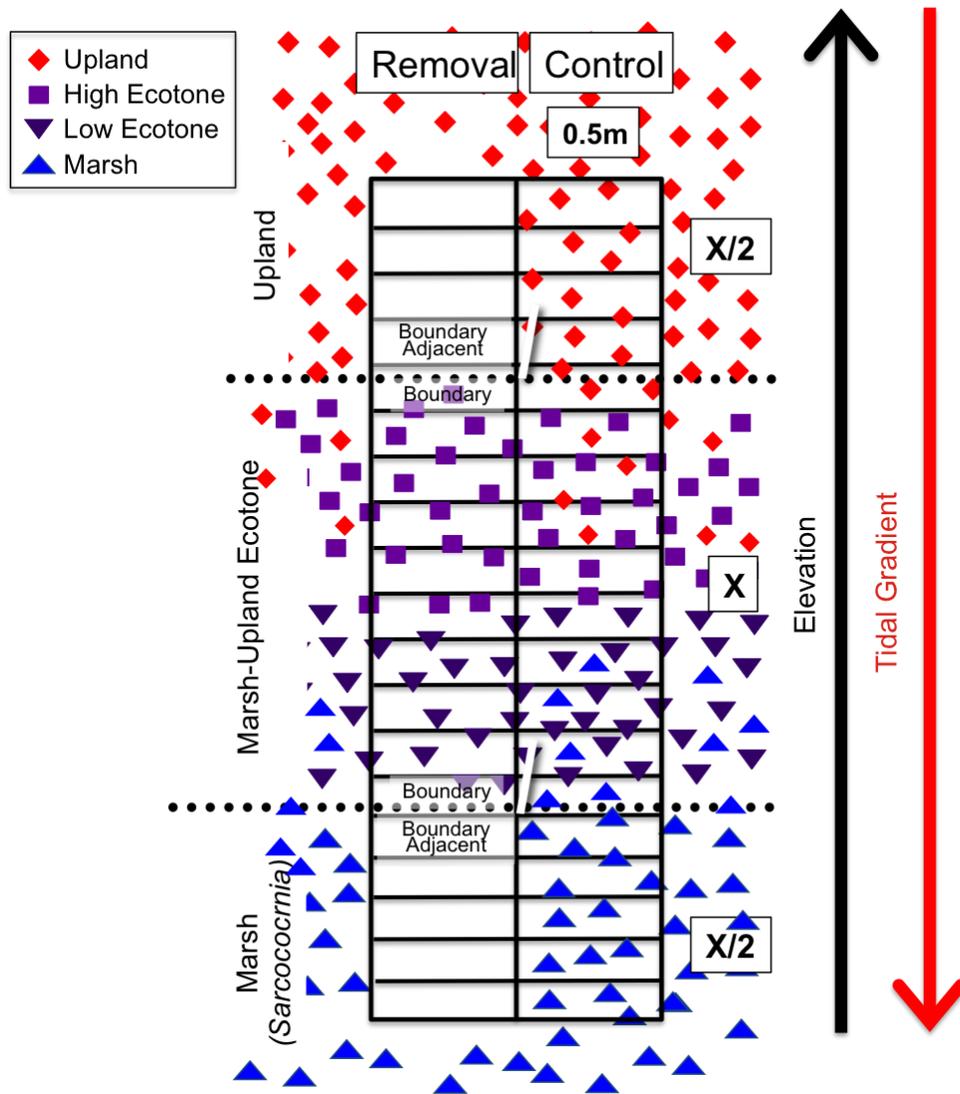
*Frankenia salina* and *J. carnosus* responded to neighbor removal at the marsh boundary similarly to the way *D. spicata* responded to removal at the upland boundary. Both these species are most abundant near the marsh boundary and though they did not exhibit significant expansion into the plots adjacent to their marsh boundaries, both species increased in cover within their respective boundary plots when neighbors were removed. These results provide some insight into the unexpected experimental outplant results (Chapter 1) that showed that outplanted *J. carnosus*, and to a lesser degree *F. salina*, can tolerate the abiotic conditions seaward of their natural distributions and, indeed, survive better in the marsh than within their own distributions (Chapter 1). However, given the enhanced survival observed when outplanted into the marsh the absence of expansion into those habitat is surprising. Overall, the cumulative evidence from this and the outplant experiment indicates that the abiotic conditions within the marsh are tolerated by the majority of the ecotonal species. Though the absence of strong evidence makes it difficult to definitively conclude that competition is relatively more influential on the location of the marsh-ecotone boundary, it is clear that the abiotic conditions are likely not the most influential ecological factor.

The factors limiting the distribution of the marsh-ecotone plant community are both complex and variable and may vary from year to year due to stochastic influences on the abiotic conditions and biotic community. Indeed, the particular ecological factors driving distributional patterns may be occurring at an entirely different ecological scale than the habitat level comparisons examined here. The marsh-upland ecotone is a transitional community that links terrestrial and aquatic systems, regulates nutrient flows, and also contains the majority of the salt marsh plant diversity. By characterizing the edaphic conditions experienced by this transitional community and determining the influence competitive interactions from the adjacent vegetation have on the dominant ecotone species, this research provides an evidence-based framework upon which to base wetland restoration strategies.

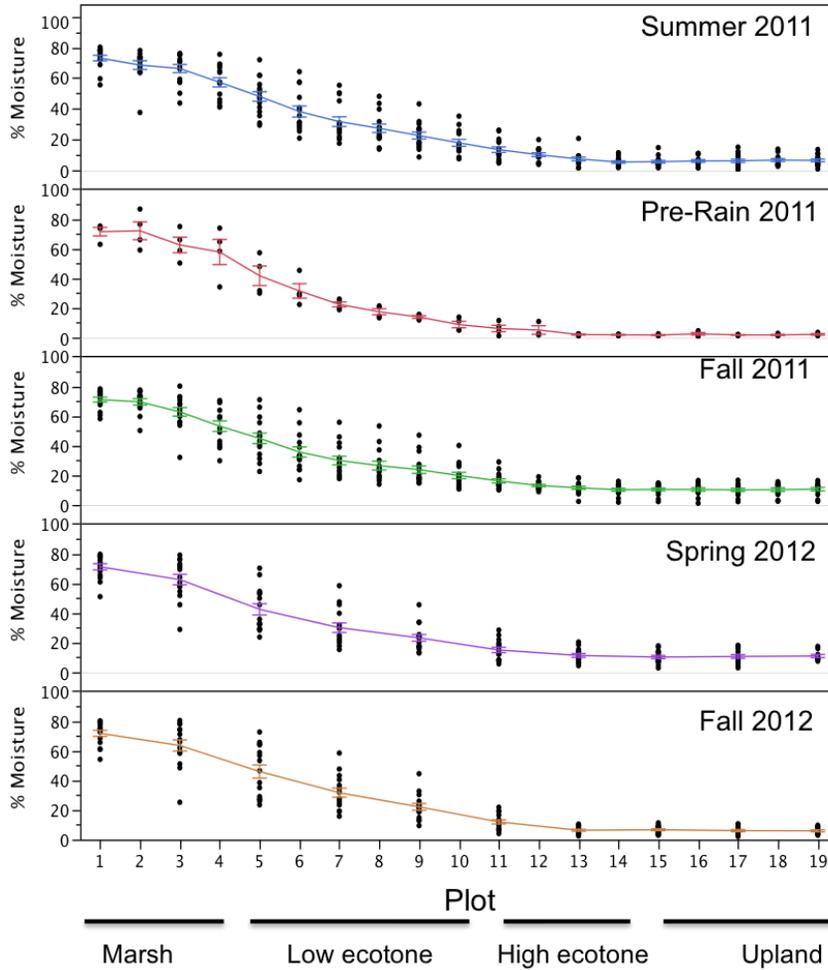
FIGURES AND TABLES



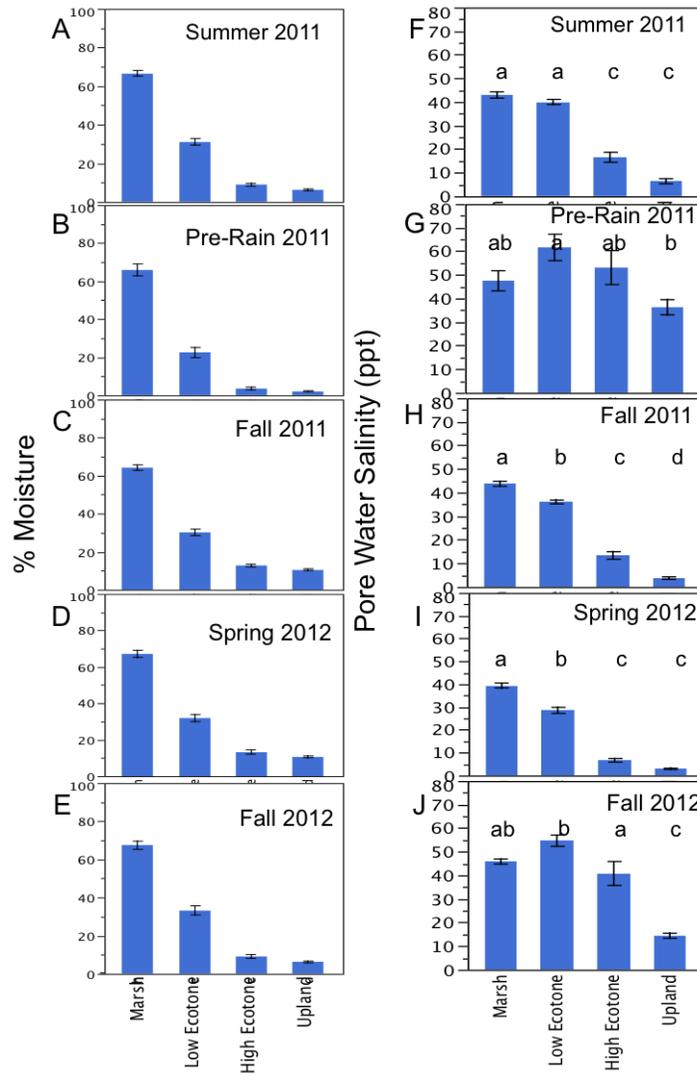
**Figure 1.** Map of Elkhorn Slough in Moss Landing, California. All transects established in early Fall 2010 were located within Azevedo (n=3), Coyote (n=4), Packard (n=4), and Yampah (n=4) marshes. Green areas indicate elevational zones that support *Sarcocornia* dominated marsh plain habitat.



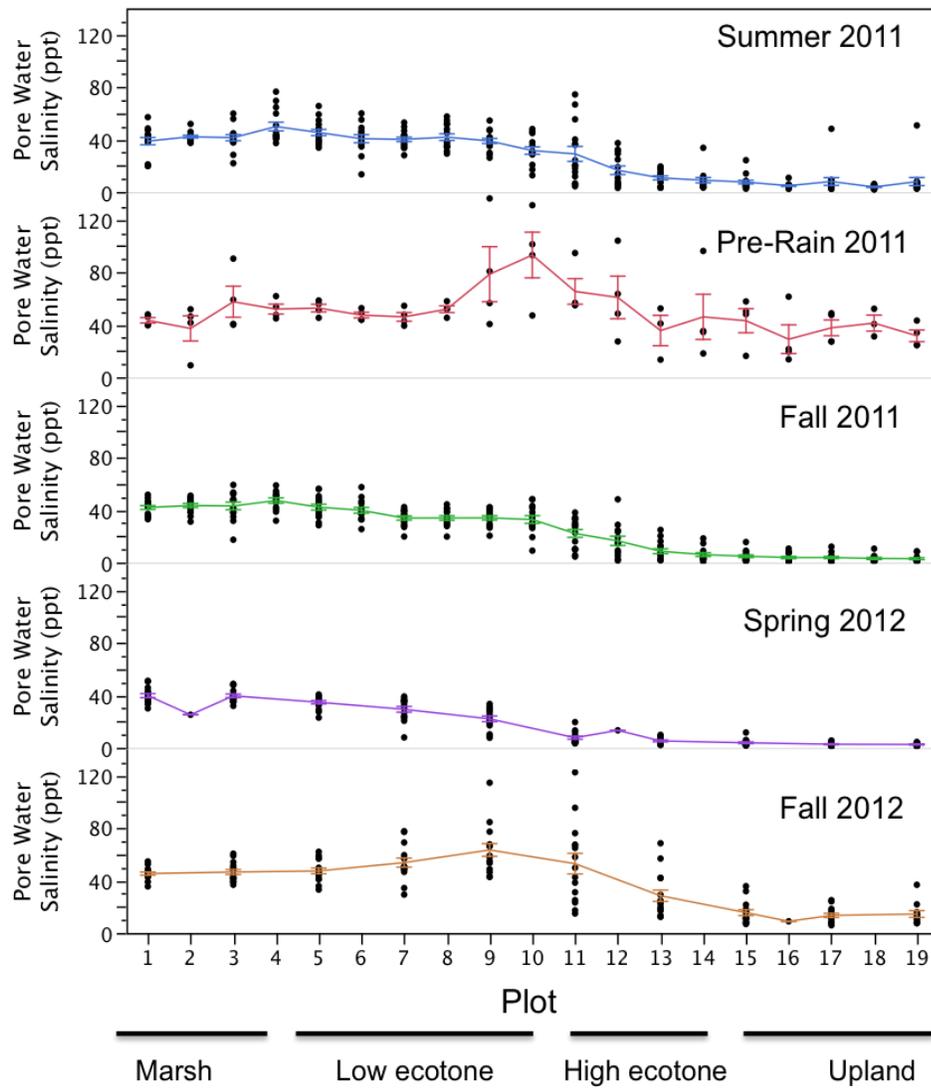
**Figure 2.** Diagram of transect orientation and experimental design. Total transect length is equal to twice the length of the ecotone ( $x$ ), while the upland and marsh portions of each transect are equal to half the length of the ecotone ( $x/2$ ). The broken horizontal lines illustrate marsh- and upland-ecotone boundaries. Boundary plots and boundary adjacent plots at the marsh- and upland-ecotone boundaries are indicated. Experiments initiated Fall 2012,  $n=15$ .



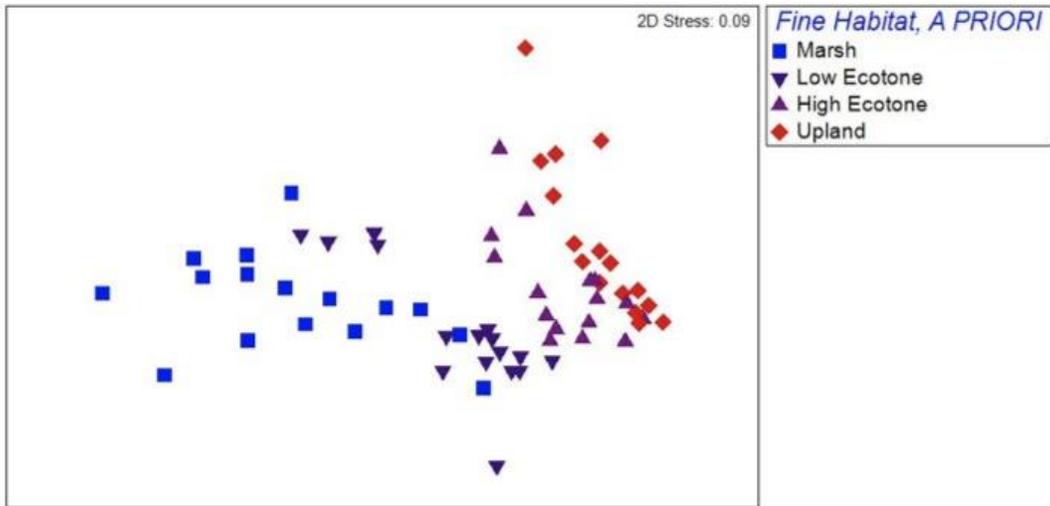
**Figure 3.** Soil moisture (%) of samples collected from plots within each transect. Soil sampling scheme varied depending on date of sample. Summer 2011 and Fall 2011 plots were sampled from each of the 15 transects, n=15 per plot. Pre-rain 2011 sampling was interrupted by first rain of the season, only five transects from two sites sampled per plot, n=5. Spring and Fall 2012 only odd numbered plots were sampled, n=15 per odd numbered plot. Error bars indicate one standard error.



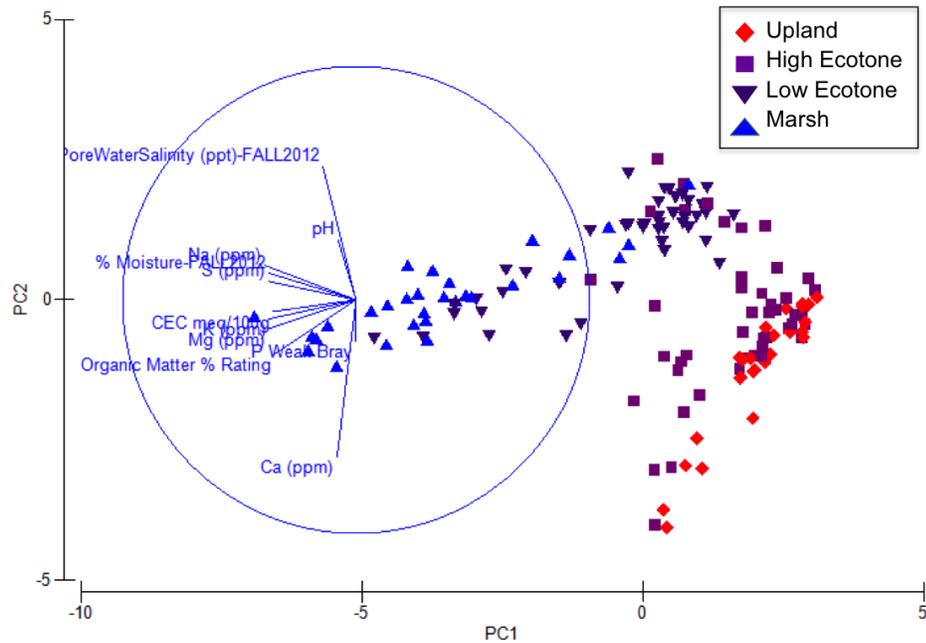
**Figure 4.** Soil moisture (%) (left panels A-E) and pore water salinity (ppt) (right panels F-J) by habitat type. Soil moisture differed similarly across habitats between seasons: Marsh and Low Ecotone habitats were significantly different from each other and from the other habitats. High ecotone and upland were not significantly different. Error bars indicate one standard error.



**Figure 5.** Pore water salinity (ppt) of samples collected from plots within each transect. The number of samples per plot at each date described in Figure 3 legend. Error bars indicate one standard error.



**Figure 6.** NMDS plot of eleven abiotic edaphic variables: organic matter content (%), phosphorous (ppm), potassium (ppm), magnesium (ppm), calcium (ppm), sodium (ppm), sulfate (ppm), pH, cation exchange capacity (CEC), soil moisture (%), and pore water salinity (ppt). Euclidean distances used to visualize dissimilarity between plots of each habitat type. Stress 0.09.



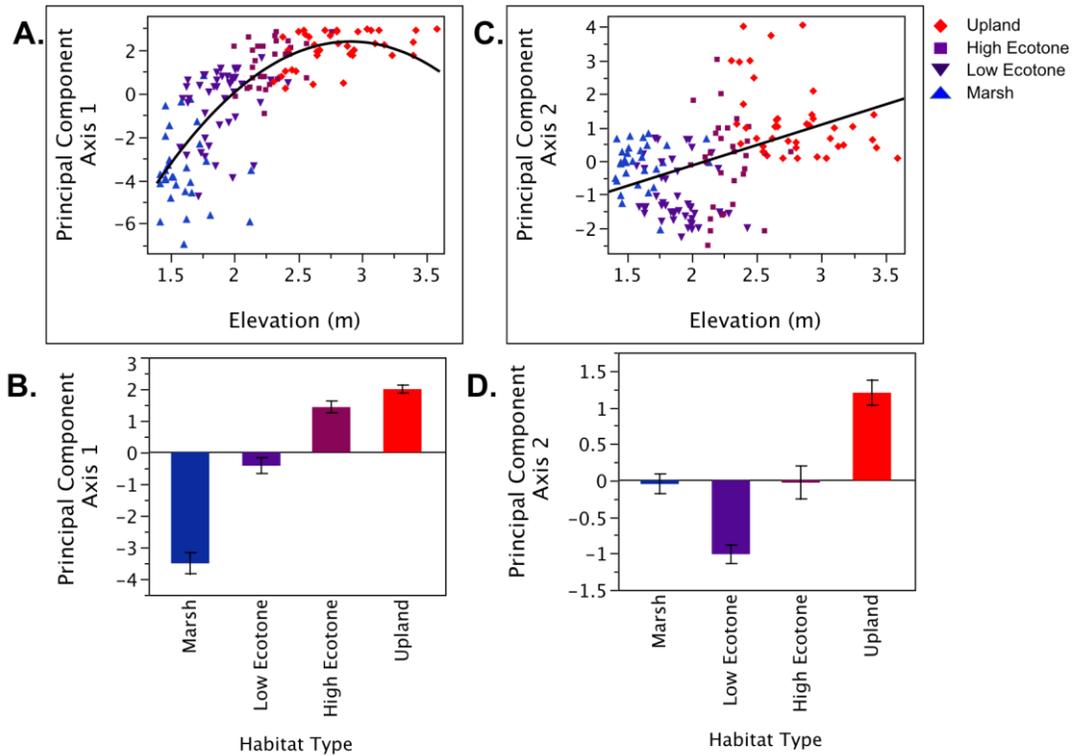
**Figure 7.** Principal components analysis grouped by habitat type. Eleven edaphic variables (listed in text) measured from samples collected Fall 2012. Principal component axis 1 explains 55% of the variance, while principal component 2 explains an additional 15.5%. Differing symbols and colors indicate the habitat type from where each soil sample was collected.

**Table 1.** Results of Analysis of Similarity (ANOSIM) and Similarity Percentages (SIMPER). Eleven abiotic edaphic variables used to calculate global R, where completely dissimilar habitats would have a value of 1, and a p-value, which indicates statistically differences between habitats. Average squared distance, a measure of dissimilarity where low numbers indicate very similar habitats, and the four edaphic variables that contribute most to the variation between habitat types.

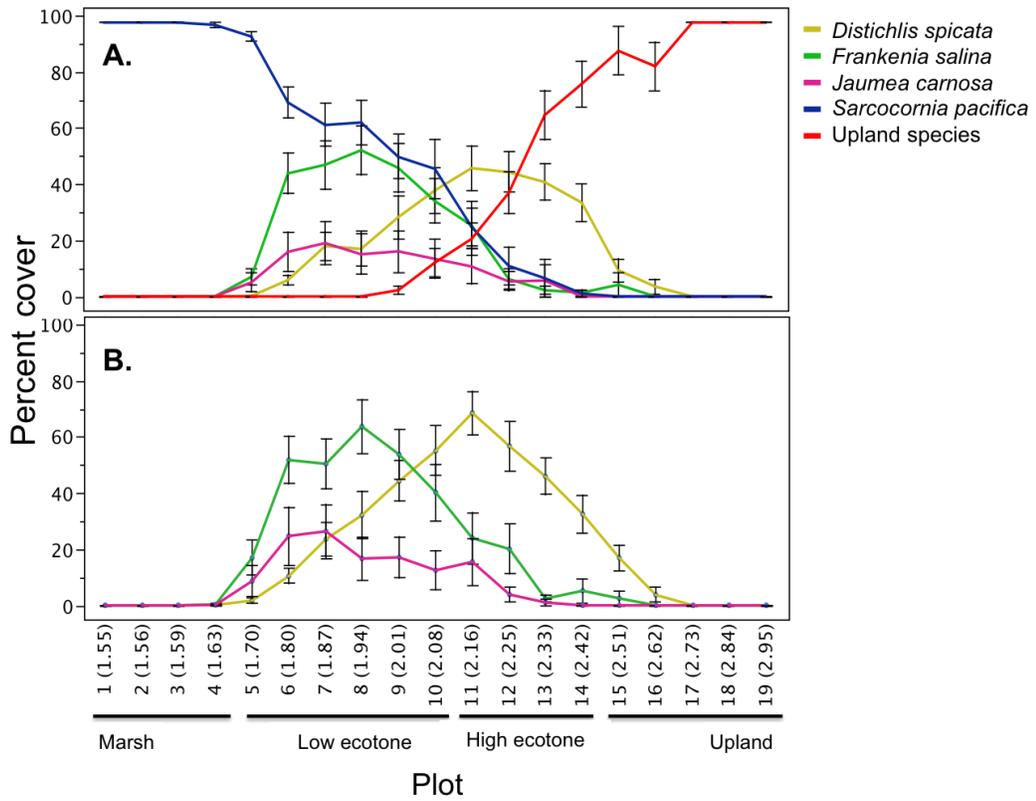
ANOSIM			SIMPER			
<i>Pairwise Comparison</i>	<i>R statistic</i>	<i>p</i>	<i>Average squared distance</i>	<i>Edaphic variables</i>	<i>Variance contribution</i>	<i>Cumulative percent of variance</i>
Marsh x Low Ecotone	0.39	0.001	23.59	Organic matter (%) CEC S (ppm) pH	16.06% 13.23% 11.99% 11.13%	52.42%
Marsh x High Ecotone	0.80	<b>0.001</b>	36.44	% moisture S (ppm) CEC Na (ppm)	14.32% 14.11% 12.84% 12.66%	53.93%
Marsh x Upland	0.87	<b>0.001</b>	43.96	S (ppm) Na (ppm) % moisture CEC	12.71% 12.54% 12.52% 11.45%	49.22%
Low Ecotone x High Ecotone	0.43	<b>0.001</b>	15.65	Pore water salinity pH P (ppm) K (ppm)	15.49% 14.23% 11.31% 10.93%	51.96%
Low Ecotone x Upland	0.63	<b>0.001</b>	23.11	Pore water salinity Ca (ppm) P (ppm) pH	23.03% 15.11% 11.70% 11.40%	61.24%
High Ecotone x Upland	0.09	<b>0.029</b>	9.75	Ca (ppm) P (ppm) Pore WaterSalinity K (ppm)	30.88% 21.58% 16.26% 9.60%	78.32%

**Table 2.** Eigenvalues, variance explained, and variable loadings in a principal components analysis of eleven abiotic variables of soils in marsh, ecotone, and upland habitats.

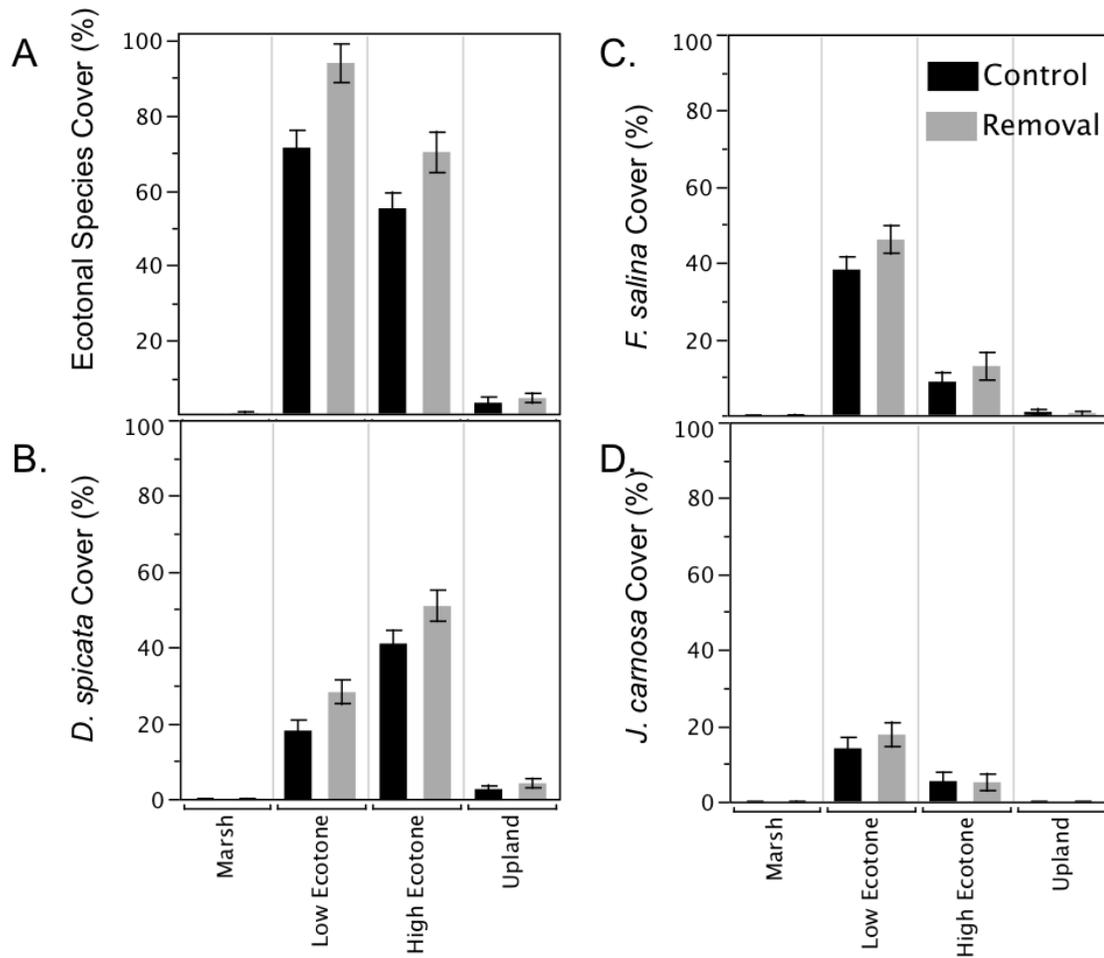
Variable	Principal Component				
	1	2	3	4	5
Eigenvalues	6.05	1.71	1.35	0.84	0.47
Cumulative variance (%)	55.0	70.5	82.8	90.4	94.7
Na (ppm)	-0.391	-0.147	-0.01	0.04	0.143
Mg (ppm)	-0.377	0.124	0.089	0.223	-0.167
S (ppm)	-0.377	-0.079	-0.21	-0.16	0.143
Moisture (%)	-0.375	-0.115	-0.044	-0.021	0.399
K (ppm)	-0.371	0.081	0.157	0.086	-0.215
CEC meq/100g	-0.359	0.051	-0.263	-0.179	-0.136
Organic matter (%)	-0.349	0.233	0.025	-0.046	0.122
Pore water salinity (ppt)	-0.142	-0.573	0.177	-0.226	-0.673
Ca (ppm)	-0.081	0.673	0.194	0.159	-0.396
pH	-0.077	-0.258	0.668	0.494	0.223
P (ppm)	-0.003	0.178	0.58	-0.749	0.183



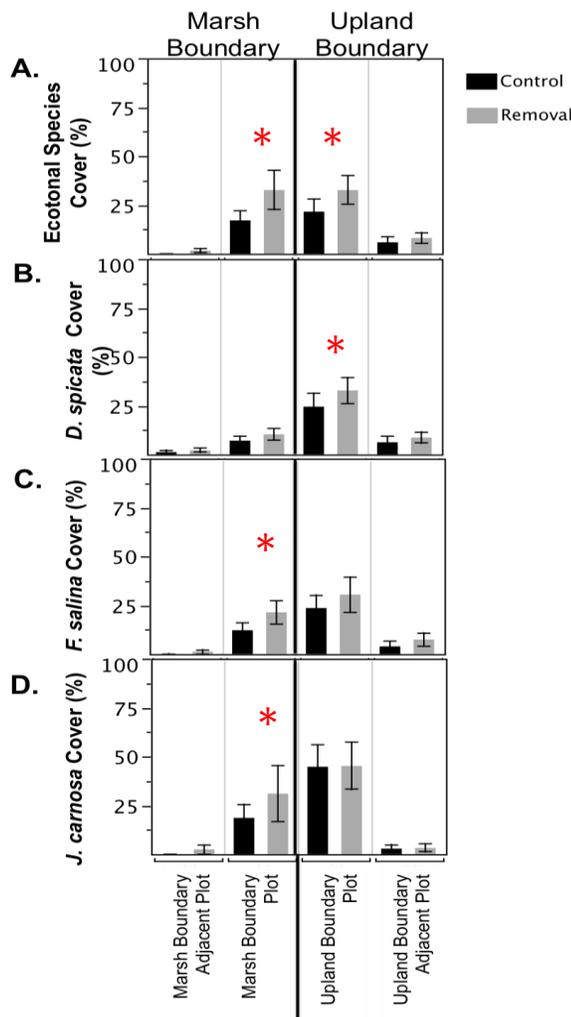
**Figure 8.** Panel A displays the quadratic relationship between PC1 and plot elevation (m) ( $R^2=0.55$ ,  $f=166.74$ ,  $df=1$ ,  $p<0.0001$ ). Panel B illustrates the mean difference in PC1 among habitat types. Panel C displays the cubic relationship between PC2 and plot elevation (m) ( $R^2=0.30$ ,  $f=18.8$ ,  $df=3$ ,  $p<0.0001$ ). Panel D illustrates the similarity in PC 2 between the marsh and high ecotone and the large differences between the low ecotone and upland.



**Figure 9.** Panel A. displays the distribution and plot-level cover by each ecotonal species, the marsh species dominant, *Sarcocornia pacifica*, and the summed cover by upland species. Data collected after two growing seasons in Fall 2012. Panel B. displays the cover by each ecotonal species in each plot after two years of removal treatments. Error bars indicate one standard error.



**Figure 10.** Coverage by A) all ecotonal species, B) *D. spicata*, C) *F. salina*, and D) *J. carnosa* in removal and control plots by habitat type after two growing seasons. Data collected in Fall 2012. Error bars indicate one standard error. (Note: Though analysis described in the text tested effects of habitat type on the *difference* between removal and control,  $\% \text{Cover}_{\text{Removal}} - \% \text{Cover}_{\text{Control}}$ , for ease of visual comparison this graph displays mean percent cover in both removal and control plots.)



**Figure 11.** Four left bars indicate cover in control (black) and removal (gray) plots directly adjacent to the marsh boundary (*Marsh Boundary Adjacent Plot*) and in plots that contain the marsh boundary (*Marsh Boundary Plot*). Four right bars indicate percent cover in control and removal plots directly adjacent to the upland boundary (*Upland Boundary Adjacent Plot*) and in plots that contain the upland boundary (*Upland Boundary Plot*). Graphs show mean percent cover by all ecotonal species (A), and by the individual species, *D. spicata* (B), *F. salina* (C), and *J. carnosa* (D). Data collected in Fall 2012. Error bars indicate one standard error.

**Table 3.** Results of matched pairs analysis of coverage by all ecotonal species, *D. spicata*, *F. salina*, and *J. carnososa* in boundary adjacent plots and boundary plots.

	All Ecotonal Species				<i>D. spicata</i>			<i>F. salina</i>			<i>J. carnososa</i>		
	<i>df</i>	<i>t</i>	<i>p</i>	<i>df</i>	<i>t</i>	<i>p</i>	<i>df</i>	<i>t</i>	<i>p</i>	<i>df</i>	<i>t</i>	<i>p</i>	
Boundary adjacent plots	14	1.65	0.060	14	1.02	0.16	14	1.13	0.14	6	1.18	0.14	
Upland boundary	14	0.92	0.19	14	1.41	0.09	14	0.88	0.20	7	-0.68	0.74	
Boundary plots	14	2.29	<b>0.019</b>	14	1.59	0.13	14	1.58	<b>0.07</b>	7	2.07	<b>0.04</b>	
Upland boundary	14	3.03	<b>0.0045</b>	13	2.32	<b>0.019</b>	12	0.67	0.26	7	0.16	0.44	

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## CHAPTER THREE:

### **RESTORATION THROUGH REINTRODUCTION OF AN ABIOTIC STRESSOR: SALINIZATION AS A MARSH RESTORATION TOOL**

#### ABSTRACT

Alterations of abiotic conditions due to human development often degrades community structure, diversity, and resilience, and may lead to the establishment and spread of non-native species. Tidal restriction structures alter the abiotic conditions associated with full tidal influence and significantly degrade the marsh upland ecotone, a transitional plant community located at elevations between the salt marsh plain and the adjacent upland habitat. These altered conditions lead to increases in invasion by non-native upland species, major reductions in native diversity, and significant changes in community structure. This ecotonal community is especially vulnerable to alterations of its gradient in tidally influenced abiotic conditions, including soil salinity, due to the large role these stressors play in setting its boundaries and elevational distribution. Through targeted application of salt crystals, we tested how effective, efficient, and persistent restoring one component of the tidal gradient is for suppressing non-native upland species, increasing cover by ecotonal and marsh species, and reversing the effects of upland encroachment. In all three independent field experiments we consistently observed significant suppression of upland plant species in response to salt addition. A randomized block experiment where salt was applied at dosages of 0 g/m<sup>2</sup> (Control), 600 g/m<sup>2</sup>, 850 g/m<sup>2</sup>, 100 g/m<sup>2</sup>, and 1350 g/m<sup>2</sup> found that the three lower salt addition dosages restored pore water

salinity to levels indistinguishable from those in reference ecotone soils and, though there was rarely an immediate effect of salt addition on cover by marsh species, the effects of salt addition persist through subsequent growing seasons, leading to delayed increases in cover by marsh and ecotonal species. A combined mowing and salt addition experiment found that combining these restoration strategies not only reduces upland plant cover, but also is effective at reversing encroachment of the upland plant community through landward migration of the ecotone-upland boundary. A final experiment with paired salt addition and control transects also demonstrated the effectiveness of salt addition in reversing the upland plant community encroachment and expanding the area available for marsh-upland ecotonal species to persist. Salt addition is an effective, low cost, and relatively low effort strategy for restoring the native diversity and structure of the high marsh and marsh-upland ecotone, especially in cases where the restoration of full tidal flow is impossible.

## INTRODUCTION

Human development has significantly altered the diversity, stability, and resilience of existing ecosystems and is frequently implicated in their degradation by facilitating the establishment and spread of non-native species (Hobbs and Huenneke 1992). This degradation often occurs as a result of development practices that alter existing abiotic conditions. Alterations of abiotic conditions can have both direct and indirect impacts on the establishment of invasive species and the invasibility of altered habitats (Alpert et al. 2000). Invasibility may be *directly* increased when physical conditions are altered in ways that allow establishment and spread of non-native species formerly excluded due to physiological intolerance of pre-disturbance conditions. Invasibility may also be *indirectly* increased when the outcomes of environment-dependent biotic interactions change in ways that benefit the invader.

Habitats associated with highly stressful abiotic conditions may be disproportionately vulnerable to increased invasion when changes in abiotic conditions lead to the relaxation of abiotic stressors. Typically, stressful abiotic environments are relatively uninvaded and are regarded as less invasible than more benign habitats for two reasons (Alpert et al. 2000). First, introduced species may not tolerate the stressful conditions associated with the habitat, thereby indefinitely preventing their establishment or persistence. Second, the stressful abiotic conditions may provide a competitive advantage to stress-tolerant natives in interactions with introduced non-natives. However, when abiotic conditions are altered in ways that dampen ecological processes that maintain stressful conditions, the competitive

advantage enjoyed by native species is reversed. For example, artificial increases in nutrient levels allowed weedy non-native annuals intolerant of edaphically stressful serpentine soils to dominate previously uninvaded native serpentine grassland communities (Huenneke et al. 1990). In habitats where an underlying stress gradient creates variability in the underlying stressor, the structure of plant communities frequently depends on the outcome of biotic interactions that depend on the abiotic conditions (Wilson and Keddy 1986, Pennings and Callaway 1992, Bertness and Hacker 1994, Crain et al. 2004). Environmental stress gradients influence the distributions of species; frequently leading to the displacement of stress-tolerant competitively inferior species to more stressful areas by competitively dominant species (Hacker and Bertness 1999, Gerhardt and Collinge 2003). Though in benign environments competition from non-natives may lead to competitive exclusion of native species, in the context of a stressful habitat intolerance of the stressor by the non-native species may tip the competitive advantage towards native species. Abiotic processes play a very large role in both structuring plant communities and limiting invasion by non-native species in these stressful habitats. The large influence of the underlying abiotic conditions may magnify the effect alterations of the abiotic environment will have on these plant communities.

Designing efficient and effective habitat restoration strategies are increasingly recognized as a priority for both researchers and land managers when altered physical conditions have led to increased invasibility, degradation of native plant communities, and loss of ecosystem services. One way land managers address these challenges has

been through simulation of specific abiotic conditions determined to have large impacts on community structure, ecological function, and other ecological characteristics. This strategy is especially useful in areas where changes in the physical environment contribute to invasion and where restoration of pre-disturbance processes, such as natural fire regimes, is infeasible (Hobbs and Humphries 1995). Grazing by domesticated cattle in areas where wild grazers have been removed is used to simulate the disturbance and herbivore pressure that promotes native species cover. Carbon additions (Blumenthal 2003, Perry 2004) are used to suppress non-native invasions in response to elevated nitrogen levels from fertilization (Vitousek et al. 1997), atmospheric N deposition (Bobbink 1991), and invasion by N-fixing shrubs (Vitousek et al. 1987, Maron and Connors 1996). These approaches restore some aspect of pre-disturbance abiotic conditions to hinder non-natives and stimulate native regeneration.

Coastal salt marshes, vegetated areas in the intertidal zones of relatively sheltered coastal embayments, are physiologically stressful environments due to intense hydrologic and edaphic stressors, including frequent submergence, high soil salinity, waterlogging, and anoxic soil conditions (Adams 1963, Callaway et al. 1990). The distributions of species from the low-elevation salt marsh plain to the high marsh plant communities are structured by an underlying gradient in abiotic stressors caused by tidal inundation. This stress gradient structures species distributions both directly by limiting species to areas within their physiological tolerances (Mahall and Park 1976, Barbour 1978, Cooper 1982a) and indirectly by influencing the outcomes of

competitive interactions among the species of the salt marsh community (Pennings and Callaway 1992, Levine et al. 1998, Bockelmann and Neuhaus 1999, Pennings et al. 2005, Greenwood and MacFarlane 2006). Though, as described above, the intense physical conditions of the salt marsh are expected to limit potential for invasive establishment, disruption of natural tidal regimes, through dikes, levees, and other tidal restriction structures, can have strong impacts on their abiotic environment. Tidal restriction dampens the amplitude of tides, reduces the frequency and duration of tidal inundation, causes changes in the distribution of the underlying stress gradient, and eliminates or reduces many abiotic stressors, including soil salinity (Roman et al. 1984, Portnoy and Giblin 1997, Portnoy 1999). These alterations have been shown to promote colonization by non-native invasive species; specifically, reductions in soil salinity caused by tidal restriction can lead to colonization by less salt-tolerant non-natives (Beare and Zedler 1987, Zedler et al. 1990, Callaway and Zedler 1998) and permit invasion by non-native species previously excluded by pre-disturbance abiotic conditions (Sun et al. 2003, Ritter et al. 2008, Martone and Wasson 2008, Wasson and Woolfolk 2011, Mora and Burdick 2013).

The changes caused by tidal restriction may disproportionately affect high marsh plant communities like the marsh-upland ecotone, a characteristic feature of Californian salt marsh systems (Callaway et al. 1990). This high marsh plant community is found at elevations between mean higher high water (MHHW) and the reach of the highest tides (James and Zedler 2000, Traut 2005b), where tidal inundation is already infrequent under full tidal flow and would be most reduced

under tidal restriction. Habitats directly adjacent to upland plant communities, like the marsh-upland ecotone, are especially vulnerable to invasion by weedy upland invasives (Roman et al. 1984, Beare and Zedler 1987, Sun et al. 2003). Though this ecotonal plant community translates to only a small fraction of total marsh area, it contains the majority of native salt marsh plant diversity (James 2001), including ecotonal specialists whose distributions are limited to this zone (Callaway et al. 1990, Traut 2003). Preservation of the biodiversity concentrated within the narrow elevational distribution of the marsh-upland ecotonal plant community and the potential vulnerability of these species to displacement by weedy upland species make the marsh-upland ecotone an important conservation target (James and Zedler 2000, Traut 2005b, Wasson et al. 2013). Previous work has demonstrated that tidal restriction can result in intrusion of upland species, both native and non-native, into the marsh-upland ecotone and conversion of ecotone to purely upland habitat (Wasson and Woolfolk 2011, Wasson et al. 2013), effectively reducing habitat for the narrowly distributed ecotonal plant community and causing significant losses in native marsh plants and salt marsh biodiversity in general (Kennish 2001, Zedler et al. 2001, Zedler and Kercher 2004).

The restoration of full tidal flow to tidally restricted areas is often suggested as a solution for restoring salt marsh plant communities negatively impacted by the altered hydrology of tidal restriction. However, logistical constraints of reintroducing tidal flow often make the restoration of full tidal flow impossible. Tidal restriction structures are often used in wetland reclamation, to impound water for waterfowl

hunting or livestock use, and to protect infrastructure such as roads, railroads, and other coastal developments from storm surges (Kennish 2001, 2002) and are therefore unlikely to be removed for restoration purposes. Under these circumstances, targeted applications of salt crystals are a viable alternative to restoring full tidal flow. Salt addition is a potential strategy for the restoration of pre-tidal restriction soil pore water salinity in marsh-upland ecotones degraded by tidal restriction. In salt marshes, pore water salinity plays a major role in determining the outcome of biotic interactions between many salt marsh halophytes and non-native invaders and directly limits establishment by salt intolerant non-natives (Beare and Zedler 1987, Kuhn and Zedler 1997, Konisky and Burdick 2004). By selectively restoring this particular characteristic of the pre-invasion abiotic environment we may be able to effectively reduce coverage by non-native species, restore native salt marsh species, and reverse encroachment by the upland plant community. In southern California, researchers have begun to investigate how restoration of soil salinity and the timing and frequency of salt applications may reduce establishment of annual non-native species, primarily annual grasses (Kuhn and Zedler 1997, Uyeda et al. 2013). However, the use of salt addition in tidally restricted marshes of northern California, where the precipitation necessary for delivering salt into soils is much greater, has not been quantified. Investigations into the efficacy of targeted salt addition in tidally restricted marshes are especially important because this practice would circumvent numerous logistical problems associated with restoring full tidal flow to restricted marshes and would also minimize the need for intensive invasive management practices, such as

mechanical removals and herbicide applications.

Here I used a series of experimental manipulations to examine whether the targeted application of salt crystals effectively excludes non-native species and contribute to the restoration of native salt marsh species where tidal restriction has altered the tidal influence gradient. In the first manipulation, I tested the effectiveness and longevity of various salt addition dosages on both marsh and upland species, with special focus on two common upland invaders. I also quantified the effect of salt addition dosage on pore water salinity and compared the salinity of experimental soils to those of a fully tidal reference ecotone. Second, I assessed the effectiveness of salt addition in combination with mowing, another restoration technique known to depress non-native species at the marsh-upland transition zone (Roman et al. 1984). Finally, I tested how well salt addition can reverse the conversion of marsh-upland ecotonal habitat to upland and examined variation in the responses of native and non-native upland species to salt addition.

## STUDY SYSTEM AND METHODS

Elkhorn Slough, located along the Central California coast in Moss Landing, CA, contains 1,147 ha of salt marsh habitat, one of the largest remaining tracts of salt marsh in California (Caffrey et al. 2002). Elkhorn Slough experiences a Mediterranean climate, with the majority of annual precipitation occurring between October and May. As a result of a long history of intensive human use, the slough has been dramatically impacted by human perturbations (Silberstein et al. 2002). Over

30% of the Slough's salt marsh community is located behind tidal restriction structures, constructed to control erosion, prevent flooding of low-lying roads, and, historically, for agricultural reclamation (Dyke and Wasson 2005). At Elkhorn Slough the marsh-upland ecotone is made up primarily of *Distichlis spicata* (L.) Greene (Poaceae), *Frankenia salina* (Molina) I.M (Frankeniaceae), and *Jaumea carnosa* (Less.) Gray (Asteraceae). In this system, these species are found almost exclusively within the ecotone. The salt marsh plain dominant is *Sarcocornia pacifica* (Standley) Scott (Chenopodiaceae).

All experimental manipulations occurred at two sites within tidally restricted marshes of Elkhorn Slough. At both sites, the marsh-upland ecotone is significantly invaded by non-native upland weeds and the areal extent of the marsh-upland ecotone is significantly different than that of ecotones with full tidal flow. Porter-Blohm marsh (36°51'9"N, 121°45'1"W) is managed to entirely exclude tidal exchange, but receives limited tidal water through leaking water control structures and occasional flood events that overtop the water control structures (Wasson and Woolfolk 2011). In addition to impounded tidal flow, during the rainy season freshwater from adjacent hillsides accumulates, inundating lower elevations with fresh to brackish water for several weeks a year. Whistle Stop lagoon (36°49'27"N, 121°44'19"W) is also tidally restricted. However, the water control structures limiting tidal flow from adjacent unrestricted tidal areas permit 15-20% of the full tidal range that occurs at adjacent full tidal sites. At this site, the ecotonal plant community is reduced to remnant patches of *Frankenia*, *Jaumea*, and *Distichlis*, and a few individuals of *Atriplex*. In

most areas all ecotonal species are completely absent and individuals of *Sarcocornia*, the marsh plain dominant, abut directly with upland weeds.

The salt marshes of Elkhorn Slough that receive full tidal flow are adjacent to terrestrial habitats that include *Quercus* woodlands, *Baccharis* scrub, invaded grasslands, and native grassland remnants. However, the tidally restricted marshes examined in this study are adjacent to upland communities made up of over 80% weedy non-native plants on average (Wasson and Woolfolk 2011). These include *Conium maculatum* (Poison hemlock), *Carduus pycnocephalus* (Italian thistle), *Cirsium vulgare* (Bull thistle), *Brassica nigra* (Black mustard), *Brassica rapa* (Field mustard), and *Raphanus sativus* (Wild radish) (Zimmerman and Caffrey 2002).

In all cases salt applications were done manually by sprinkling a premeasured mass of salt crystals over each plot. Care was taken to apply an even layer to all parts of the plot and to avoid depositing salt crystals onto anything other than the soil, such as leaves or stems. Salt crystals obtained directly from Cargill Incorporated solar saltworks in south San Francisco Bay contain over 99% sodium chloride and can be purchased widely as Diamond Crystal® Solar Naturals™ Salt Crystals. Salt crystals fully dissolved into the soil through ambient precipitation in between applications. All statistical analyses were performed using JMP V. 11.

#### Salt Addition Dosage and Response

To test the effectiveness of salt addition as restoration method, salt crystals were applied at five salt addition dosage treatments in the tidally restricted Porter-Blohm marsh. Each of six blocks contained five 1 x 1-m treatment plots arranged

parallel to the marsh-upland boundary. Each treatment plot was separated by a 1-m buffer, resulting in 10 x 1-m blocks (Figure 1). In February 2009, blocks were established in areas that were previously part of the marsh-upland ecotone (Wasson and Woolfolk unpublished data) but were currently dominated by the common non-native upland invaders Italian thistle, *Carduus pycnocephalus*, and Poison hemlock, *Conium maculatum*. Each block still contained a minimum of 30% coverage by native marsh species, including *Sarcocornia pacifica*, *Distichlis spicata*, and *Frankenia salina*. Five salt addition treatment dosages, 0 g/m<sup>2</sup> (Control), 600 g/m<sup>2</sup>, 850 g/m<sup>2</sup>, 100 g/m<sup>2</sup>, and 1350 g/m<sup>2</sup>, were applied monthly in February, March, and April during the 2008-2009 rainy season.

In May 2009, after three months of salt addition, the effect of salt addition was quantified using a 1x1m quadrat to obtain visual estimates of percent cover by upland species, marsh species, and initially dominant upland species within each plot.

Soil samples were collected from each treatment plot (n=30) prior to salt addition (Feb 2009) and from the midpoint of the marsh-upland ecotone of an adjacent fully tidal reference marsh to test for a difference in soil salinity between tidally restricted and fully tidal marshes. After three months of salt addition dosage treatments (May 2009), soil samples were collected again from each treatment plot to assess how the soil salinity achieved via salt addition compared to the salinity of soils in the adjacent fully tidal marsh. All samples were collected to a depth of 10-cm using a 3-cm diameter soil core. Each core was homogenized, weighed, then dried for a minimum of 24 hours at 60° C, and weighed again to quantify soil moisture content.

Soil salinity was measured using a modified dilution method (U.S. Salinity Laboratory 1954). The entire soil sample was rehydrated using a gravimetric ratio of 3:1 deionized water to soil, stirred, and allowed to settle for 24 hours. The salinity of the mixture was measured using an YSI 10/30 conductivity, temperature and salinity meter. Pore water salinity was then calculated based on the original water content of the soil sample.

The specific impacts of salt addition on the two initially most abundant non-native upland invaders, *C. vulgare* and *C. maculatum*, were further investigated by measuring the effect of salt dosage on aboveground biomass and on various life stages. The aboveground biomass response after one, two, and three salt additions, in February, March, and April, respectively, was estimated using a proxy due to the need for non-destructive sampling over the course of the experiment. The aboveground biomass of *C. vulgare* was estimated by summing the diameter of all rosettes rooted within three randomly selected 30 x 30-cm quadrats within each 1 x 1-m treatment plot. Similarly, the heights of each *C. maculatum* were measured and summed from three randomly selected quadrats within each plot. Using the same subsampling scheme as the biomass proxy, I tested the effect of salt addition on various life stages remaining within each plot after three months of salt addition. I counted the total number of individuals and then categorized each as seedling, adult, or reproductive adult.

In March 2010, close to a year after the final April 2009 salt addition treatment was applied, plots were revisited to quantify the persistence of the effects of

salt addition on the percent cover of all upland species, marsh species, and *C. maculatum* and *C. vulgare* within each plot.

ANOVA was used to test whether the initial (pre-treatment) pore water salinity of experimental plots was indeed different from the pore water salinity of samples collected from within the midpoint of the marsh-upland ecotone plant community in an adjacent fully tidal marsh. Similarly, ANOVA was also used to assess whether the pore water salinity achieved through three applications of salt was significantly different from the pore water salinity of an adjacent fully tidal marsh. For both analyses, a student's t-test was used to compare means at each salt addition dosage.

The effect of salt addition on percent cover by upland and marsh species was tested using a mixed model nested ANOVA with salt addition dosage treatment (Control, 600g/m<sup>2</sup>, 850 g/m<sup>2</sup>, 1100 g/m<sup>2</sup>, or 1350 g/m<sup>2</sup>) as a fixed factor and dominant non-native upland invaders (*C. maculatum* or *C. vulgare*) nested within block as a random factor. Percent cover by both upland and marsh species were log+1 and square root transformed, respectively, to achieve normality. For both upland and marsh species cover, I performed blocked planned contrasts, Dunnett's tests, to quantify the effectiveness of each salt addition dosage relative to the control. Due to non-homoscedasticity the effect of salt addition on the initially dominant non-native upland invaders was tested using a blocked nonparametric Kruskal-Wallis test. Blocked nonparametric planned contrasts, Steel tests, were used to test for differences between the effect of each salt addition dosage and the control.

The effect of salt addition on the biomass of the two dominant invasive upland weeds after one, two, and three salt additions was analyzed using a blocked, nonparametric Kruskal-Wallis test. Identity of the dominant upland species was initially included in the model but was removed because it was not significant. For all three variables Steel tests were used to test how effective each salt addition dosage was in comparison to the control.

To assess how dosage treatment affected the distribution of age classes, the total number of individuals, seedlings, reproductive adults, and adults were analyzed using blocked nonparametric Kruskal-Wallis tests. Steel tests were used to test how effective each salt addition dosage was in comparison to the control.

To test the persistence of the effect of salt addition on upland species and initially dominant upland species in March 2010, ten months after the application of the final salt addition, I used blocked nonparametric Kruskal-Wallis tests. The effect of salt addition dosage on percent cover by marsh species was tested using a Welch test due to unequal variances between treatments. For all three variables, Steel tests were also used to test how coverage in each plot compared to the control.

#### Effects of Salt Addition, Mowing, and Their Combination in a Tidally Restricted Lagoon

In October 2009, a manipulative experiment testing the effectiveness of combining salt addition and mowing on the restoration of native marsh plant communities was implemented at a tidally restricted site, Whistlestop lagoon in Elkhorn Slough National Research Reserve. This experiment was part of a larger

restoration effort by land managers at Elkhorn Slough NERR. To enhance native diversity on the Reserve, a large area was mowed to reduce cover and, ultimately, the seed bank of invasive forbs, such as *C. maculatum*, *B. nigra* and *B. rapa*, *C. pycnocephalus*, *C. vulgare*, and *Silybum marianum* (Milk thistle). By combining mowing with salt addition, the effectiveness of each can be assessed individually and in combination to determine which method or combination of methods is most effective in restoring the marsh-upland ecotonal plant community.

Twenty transects were established in October 2009 within both a mowed (n=10) and an un-mowed area (n=10). Within these two areas transects were alternately assigned to the *-salt* (control) or *+salt* treatments. Transects were oriented perpendicularly to the water's edge and established by placing a permanent PVC marker 0.5-m below the most landward marsh species (in all cases this was *Sarcocornia*). Transects were 2-m long x 0.5-m wide and extend 0.5-m below and 1.5-m above the PVC marker. To determine if distance from the water's edge influenced the response of the marsh and upland plant communities to salt addition or mowing, transects were divided into two 1 x 0.5-m quadrats, lower (seaward) and upper (landward). The seaward lower quadrat extended from 0.5-m below the PVC marker to 0.5-m above the marker, while the landward upper quadrat extended from 0.5-m to 1.5-m. Over the course of the 2009-2010 and the 2010-2011 rainy season, the *+salt* treatments were sprinkled with salt crystals at a dosage of 600g/m<sup>2</sup> every 4-6 weeks depending on the amount of rainfall, for a total of 3 salt applications per

rainy season. Mowing occurred twice during each rainy season and was intermittently maintained using a weed-eater as needed.

The effectiveness of each treatment on suppressing cover by non-native upland species and augmenting cover of native marsh species was assessed in February 2010, October 2010, April 2011, and June 2011. The effectiveness of salt addition, mowing, and their combination was assessed in two ways. First, I monitored the location of the marsh-upland boundary, by measuring the distance from the PVC marker to the most landward marsh plant individual greater than 20-cm high. Second, I measured the percent cover of both marsh species and upland species within the lower and upper quadrats of each transect.

In July 2011, after two seasons of salt addition, soil samples were collected from the middle of each plot (at the boundary of the upper and lower quadrats) to determine the impact of salt addition on pore water salinity. Samples were collected and analyzed as described above.

Because this experiment was implemented within another restoration project, a fully factorial design was not possible. Thus in all cases the mowed and unmowed areas were analyzed separately. I used t-tests to test each assessment date for a positive effect of salt addition on the location of the marsh-upland boundary. A two-way ANOVA was used to test for the effects of salt addition, quadrat location (lower or upper), and their interaction on percent cover by marsh species and on percent cover by upland species over the course of the two-year experiment.

Salt Addition in a Seaward Migrating Tidally Restricted Marsh-Upland Ecotone

To test the effectiveness of salt addition in reversing the significant seaward migration of the marsh-upland boundary in Porter-Blohm marsh, where the restoration of tidal influence is not possible, I expanded upon an existing long-term marsh-upland ecotone monitoring project described in Wasson et al. 2013 (in Wasson et al. 2013 Figure 2, Site D1). In this study Wasson et al. found that at this site the marsh-upland boundary migrated an average of 4-m seaward over the course of ten years (2001-2011). This horizontal migration corresponds to an 11-cm decrease in elevation. In October 2010, I established plots in 7 of the pre-existing transects to act as controls for 7 new salt addition plots. Both pre-existing control plots and the new salt addition plots were 2 x 0.5-m and centered at the ecotone-upland boundary (Figure 2) so that the plot extended 1 meter landward into the upland vegetation and seaward into the ecotonal plant community. This boundary is defined as the last landward marsh species individual. Salt was applied 5 times to the 7 salt addition plots monthly during the rainy season from November 2010 to March 2011 at a dosage of 600g/m<sup>2</sup>.

The effect of salt addition on the location of the ecotone-upland boundary and on percent cover by upland species and by marsh species was assessed in June 2011. The location of the marsh-upland boundary was calculated by subtracting the initial October 2010 boundary location from the final June 2011 boundary location. Percent cover by upland species, not including bare space, was measured using the point intercept method at three points across the width of the plot every 20cm, a total of 33 intercept points.

T-tests were used to test for an effect of salt addition on both percent cover by upland species and on percent cover by marsh species. Field observations and examination of the raw data suggested that the only abundant native present in treatment plots, the perennial grass Creeping wild rye (CWR), *Elymus triticoides*, was responding differently than other upland species to salt addition. To test whether salt addition had an effect on coverage by the native CWR I used a t-test. The effect of salt addition on the location of the marsh-ecotone boundary in June 2011 was also tested using a t-test.

## RESULTS

### Salt Addition Dosage and Response

Prior to salt addition, initial pore water salinity of all soil samples collected from the tidally restricted Porter-Blohm marsh (mean 2.89 ppt) was much lower than pore water salinity of soils collected from the reference fully tidal marsh (mean 29.25 ppt)( $F=36.64$ ,  $df=5$ ,  $p<0.0001$ ). Post hoc tests indicated that there was no within treatment difference in pore water salinity among treatment plots prior to salt addition ( $p>0.05$  in all cases) (Figure 3A). Each of the salt addition dosage treatments resulted in an increase in pore water salinity ( $F=4.97$ ,  $df=5$ ,  $p=0.0012$ , Figure 3B). Post hoc tests indicated that the pore water salinity achieved by 600 g/m<sup>2</sup>, 850 g/m<sup>2</sup>, and 1100 g/m<sup>2</sup> salt addition dosages were statistically similar to the pore water salinity of samples from the reference site (Figure 3B).

After three months of salt addition in May 2009, salt addition significantly reduced cover by upland species ( $F_{9,20}=11.19$ ,  $P<0.0001$ , Figure 4A) and there was no effect of dominant species ( $F_{9,20}=0.11$ ,  $P=0.77$ ). Percent cover by upland plants was significantly reduced at all four treatment dosages compared to the control, with increasingly significant reductions in cover by upland species as treatment dosage increased (Control mean=69%; 600g mean=23.7%,  $p=0.0013$ ; 850g mean=21.7%,  $p=0.0030$ ; 1100g mean=9.2%,  $p=0.0002$ ; 1350g mean=3.2%,  $p<0.0001$ , Figure 4A). In contrast to the significant effects of salt addition on cover by upland species, there was no effect of salt addition ( $F_{9,20}=0.10$ ,  $p=0.45$ ) and no effect of dominant species ( $F_{9,20}=0.10$ ,  $p=0.77$ ) on cover by marsh species (Figure 4B). Coverage by the two dominant upland species, *C. vulgare* and *C. maculatum*, was significantly reduced by salt addition ( $\chi^2=18.20$ ,  $df=4$ ,  $p=0.0011$ ) and each treatment dosage significantly reduced coverage as compared to the control (Control mean=57.2%; 600g mean=21.8%,  $p=0.030$ ; 850g mean=19.3%,  $p=0.030$ ; 1100g mean=8.7%,  $p=0.020$ ; 1350g mean=2.3%,  $p=0.020$ ).

The effect of salt addition treatment on the aboveground biomass of *C. vulgare* and *C. maculatum* was significant after only one salt addition treatment ( $\chi^2=14.00$ ,  $df=4$ ,  $p=0.0073$ , Figure 5A). After the first salt addition in February, the 1100 g/m<sup>2</sup> and the 1350 g/m<sup>2</sup> dosages resulted in significant reductions in biomass compared to the Control (Table 1). Similar significant and marginally significant reductions in aboveground biomass were also found after two ( $\chi^2=11.92$ ,  $df=4$ ,  $p=0.018$ , Figure 5B) and three salt additions ( $\chi^2=9.21$ ,  $df=4$ ,  $p=0.056$ , Figure 5C), respectively. Later in the

season in March and April only the 1100 g/m<sup>2</sup> and the 1350 g/m<sup>2</sup> dosages resulted in mean biomasses that were significantly less than the biomass in the control treatment (Table 1). The two lowest dosages, 600 g/m<sup>2</sup> and 850 g/m<sup>2</sup> did not lead to reductions in the aboveground biomass of these two non-native upland species regardless of the number of treatments applied (Table 1).

Overall, salt addition had the greatest negative effect on the seedling life stage ( $\chi^2 = 14.66$ ,  $df = 4$ ,  $p = 0.0055$ , Table 2), with the 850 g/m<sup>2</sup>, 1100 g/m<sup>2</sup> and 1350 g/m<sup>2</sup> dosages resulting in significantly fewer seedlings than the control treatment (Table 2). In contrast, both the number of reproductive adults and the total number of adult *C. vulgare* and *C. maculatum* individuals were not significantly affected by salt addition at any dosage (Table 2). In addition, when age class was not considered, the total number of individuals in each treatment plot was significantly reduced by salt addition ( $\chi^2 = 16.09$ ,  $df = 4$ ,  $p = 0.0029$ , Table 2), with both the 1100 g/m<sup>2</sup> and the 1350 g/m<sup>2</sup> dosages resulting in significantly fewer individuals than the control treatment (Table 2).

Ten months after the final salt addition application, there was no longer a strong negative effect of salt addition on percent cover by upland species, but a weak trend towards a reduction in cover persisted ( $\chi^2 = 7.70$ ,  $df = 4$ ,  $p = 0.10$ , Figure 6A). In contrast to the lack of an effect of salt addition on percent cover by marsh species immediately after the final salt addition treatment, there was a significantly positive effect of salt addition on percent cover by marsh species after ten months ( $F = 3.41$ ,  $df = 4$ ,  $p = 0.043$ , Figure 6B). All salt addition dosage treatments had a significantly

or marginally significantly greater effect on marsh species cover compared to control plots (Control mean = 20%; 600 g/m<sup>2</sup> mean = 51.2%, p = 0.055; 850 g/m<sup>2</sup> mean = 56.7%, p = 0.044; 1100 g/m<sup>2</sup> mean = 34.2%, p = 0.051; 1350 g/m<sup>2</sup> mean = 57.8%, p = 0.044). The significantly negative effect of salt addition on the initially dominant upland species also persisted ( $\chi^2 = 13.20$ , df = 4, p = 0.010, Figure 6C). Coverage by the initially dominant species was significantly less than in control plots at every salt addition dosage except the lowest (Control mean = 42.6%; 600 g/m<sup>2</sup> mean = 11.8%, p = 0.10; 850 g/m<sup>2</sup> mean = 8.2%, p = 0.023; 1100 g/m<sup>2</sup> mean = 5.7%, p = 0.023; 1350 g/m<sup>2</sup> mean = 6.7%, p = 0.023).

#### Effects of Salt Addition, Mowing, and Their Combination in a Tidally Restricted Lagoon

In the spring of the second year of salt addition, April 2011, salt addition had a significantly positive effect on the position of the marsh-upland boundary in both the mowed ( $t_{5,12}=2.81$ , p=0.018; Figure 7C, Table 3) and the unmowed ( $t_{7,14}=2.16$ , p=0.034; Figure 7G, Table 3) areas. At this assessment, the addition of salt to mowed plots resulted in migration of the marsh-upland boundary three times further landward than in plots where no salt was added). There was also a positive effect of salt addition in the unmowed area in the summer of the second year, June 2011 ( $t_{6,04}=2.05$ , p=0.043; Figure 7H, Table 3). At all other times (February 2010, October 2010, and June 2011), salt addition did not result in landward migration of the marsh-upland boundary in either the mowed or unmowed areas (p>0.05; Figure 7, Table 3).

The response of coverage by upland and marsh plants to salt addition, quadrat location, and their interaction differed depending on the time of year and whether plots were located in mowed or unmowed areas. In mowed areas, the general pattern was that upland plant cover was significantly reduced by salt addition, the upper quadrat had significantly more upland plant coverage, and there was an interaction between these two main effects (Figure 8 Mowed, Table 4), though the last assessment date June 2011 was an exception (Figure 8D, Table 4). In unmowed areas, the pattern was similar (Figure 8 Unmowed, Table 4), though there was no significant interaction in October 2010 (Figure 8F, Table 4) and a marginally significant effect of quadrat location in April 2011 (Figure 8G, Table 4).

The marsh plant community responded differently than the upland plant community. In both mowed and unmowed areas, percent cover by marsh species was not significantly affected by salt addition, the lower quadrat had significantly more coverage, and there was no interaction between these two main effects (Figure 9, Table 5). The only exception to the consistent lack of an effect of salt addition on coverage by marsh species occurred in the spring following the second year of salt addition, April 2011 where plots in mowed areas that received salt had significantly higher cover by marsh species in both upper and lower quadrats (Figure 9C, Table 5).

#### Salt Addition in Seaward Migrating Tidally Restricted Marsh-Upland Ecotone

Salt addition had a negative effect on percent cover by upland species ( $t_{20.8}=2.26$ ,  $p=0.017$ , Figure 10A). In contrast, there was no effect of salt addition on percent cover by marsh species ( $t_{25.9}=0.49$ ,  $p=0.69$ , Figure 10B) or on coverage by the

native grass, Creeping wild rye (CWR) ( $t_{147}=0.74$ ,  $p=0.46$ ). There was a marginally significant effect of salt addition on landward boundary migration ( $t_{9,92}=1.72$   $p=0.06$ ).

## DISCUSSION

Overall, by restoring one of the primary stressors responsible for excluding non-native upland species, salt addition effectively reduced the ability of non-marsh species to colonize the marsh-upland ecotonal community thereby curtailing the loss of this threatened habitat. Salt addition effectively increased pore water salinity to levels that were comparable to the soil salinity of an equivalent fully tidal reference marsh. This increase in salinity led to significant and rapid reductions in coverage by most upland species. In addition, in contrast to other restoration approaches, this method requires exceptionally low temporal and financial investment and no special training to implement. It is also efficient, requiring relatively low amounts of salt to elicit significant changes in community composition. Salt addition is an example of the growing number of effective restoration strategies that rely on simulation of pre-disturbance abiotic conditions as a critical step in restoring communities degraded by anthropogenic influences. Though the specific mechanism was not tested, the observed reduction in coverage is likely due to salt toxicity and the persistence of this effect in following seasons may be due to germination inhibition or seed inviability due to high salinity levels. However, examination of the effect of salt addition on two especially ubiquitous invaders of the marsh-upland ecotone, *C. maculatum* and *C. vulgare*, demonstrated that after one, two, and three salt addition treatments the only

effective dosages were the two highest dosages. In addition, after three months of salt addition only the abundance of seedlings was affected by salt addition. Both *C. maculatum* and *C. vulgare* exhibit biennial life history strategies. They sequester resources in deep taproots their first year, then bolt and flower in their second year. These species, and any others that rely on stored resources, may be able to at least partially avoid the salt toxicity stress that prohibits resource acquisition in non-salt tolerant species.

Today, the upland habitats adjacent to the marsh-upland ecotone are dominated by non-natives and these species are the ones encroaching upon the marsh and causing the net loss of habitat for the marsh-upland ecotonal plant community. Prior to the disturbance of tidal restriction, native upland species would have been much more abundant in the adjacent uplands and those with greater salt tolerance would likely have occurred, to some degree, in the ecotone. The few upland natives that do persist in these areas, including Creeping wild rye, Coyote bush (*Baccharis pilularis*), Stinging nettle (*Urtica dioica*), and various *Juncus* species, have also migrated seaward into areas previously occupied by marsh-upland ecotone. In contrast to other non-native upland species the native perennial grass, Creeping wild rye, did not respond to salt addition, both demonstrating the known salt tolerance of this species (Hughes et al. 1975) and illustrating how native upland species that naturally occur in marsh-upland ecotonal habitats are unlikely to be negatively affected by salt addition.

Tidal restriction and the resulting invasions by upland weeds have led to net loss of wetland habitats. However, salt addition had a positive effect on the location of the

marsh-upland ecotone boundary, defined as the location of the most landward marsh individual. Though the effect of salt addition on the location of this boundary was milder than its reduction of coverage by upland plants, migration of this boundary in response to salt addition is more promising from a wetland conservation perspective because official wetland delineation criteria typically define wetlands by the presence of wetland species. The significant landward migration of this boundary indicates that salt addition reverses the pattern of marsh-upland ecotone loss and increases the size of this tidal wetland habitat.

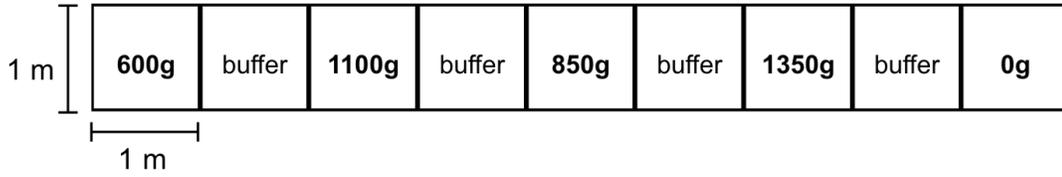
Though coverage by marsh plant species was generally increased by the addition of salt, the effects were subtle and much weaker than the effect of salt addition on the upland plant community. In addition, the positive effect of salt addition on marsh species was often delayed, becoming apparent only in the second growing season after salt addition or after multiple years of salt addition. The delayed response of the marsh species indicates that salt addition can have both immediate effects on marsh species abundance and continuing effects in subsequent years. However, this delayed response also indicates that direct competition from competitively dominant invading upland species may only be one of many factors contributing to the conversion of marsh-upland ecotone into upland habitat.

Salt addition is an effective, realistic option for reducing the abundance of upland species in the marsh-upland ecotone, thereby priming the area for increased colonization and spread by native marsh species. However, solely increasing the pore water salinity of soils is clearly not sufficient to restore marsh-upland ecotone

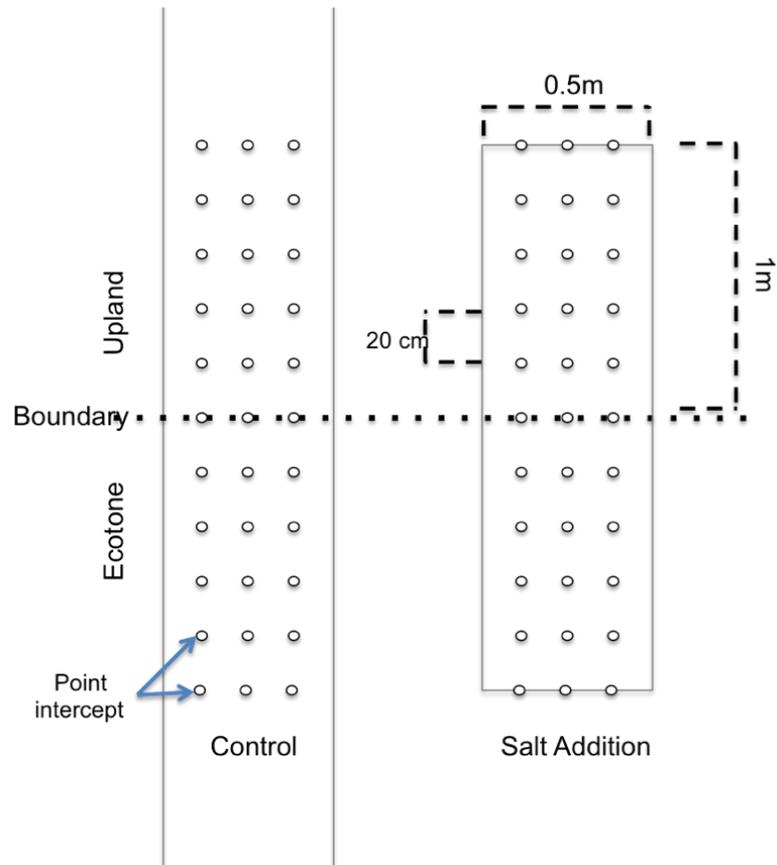
habitats. There are likely many other complex edaphic and hydrologic factors associated with full tidal influence that are important for restoring marsh habitat. Restoration of regular tidal inundation by removing tidal restriction structures is the best option for restoring all these complex factors to pre-tidal restriction conditions. Indeed, there are numerous examples where tidal flow has been shown to reverse upland plant community encroachment, increase coverage by native marsh species plants, reduce coverage by both non-native and non-marsh species, and increase total marsh area to pre-disturbance levels (Streever and Genders 1997, Roman et al. 2002, Warren et al. 2002, Buchsbaum et al. 2006, Smith et al. 2009, Wasson and Woolfolk 2011). In our study, a possible factor leading to the absence of a significant recovery of native marsh species in response to salt addition is that establishment in tidally restricted areas is likely hindered by severe recruitment limitation. However, even in areas where tidal flow has been reintroduced and regular tidal inundation can potentially deliver marsh plain and ecotonal species seeds, insufficient seed rain can delay reestablishment of marsh and ecotonal species for many years. In a nearby south San Francisco bay marsh, natural establishment of ecotonal species following the reintroduction of tidal flow took between four and fourteen years (Faber 2004). Ultimately, tidal restoration guidelines recommend that as part of an effective marsh restoration plan it is necessary to plant diverse arrays of ecotonal and marsh species plants to increase biomass accumulation and accelerate the restoration of ecosystem function (Callaway et al. 2003). It is clear that whether or not restoration of tidal flow is possible, restoration of tidally restricted marsh habitats requires land managers to

integrate multiple restoration approaches. As coastal development managers continue to recognize the value of preserving and restoring marsh diversity, salt addition is an excellent restoration tool to be integrated into tidal wetland restoration plans.

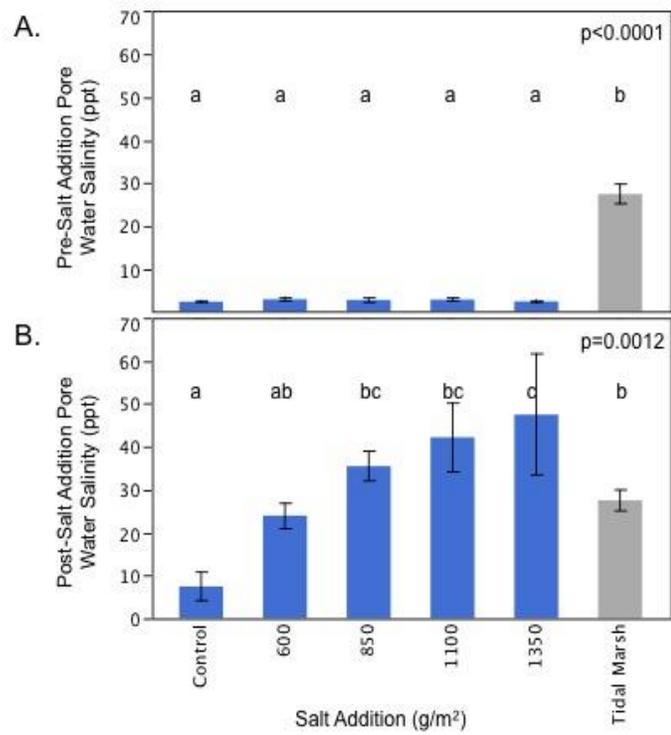
FIGURES AND TABLES



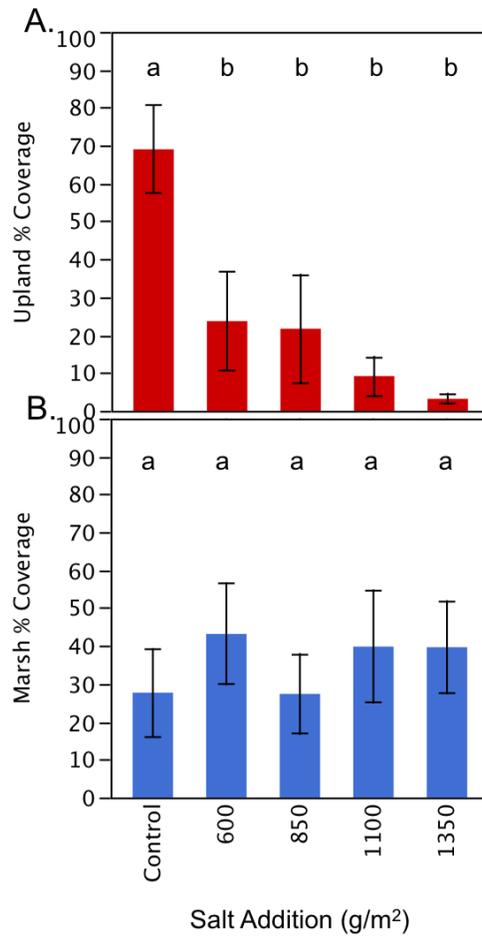
**Figure 1.** Salt Addition Dosage Response Experiment: Arrangement of 1x1m treatment plots within experimental blocks (n=6). Treatment was assigned randomly within each block. Each treatment plot was separated by a 1x1m buffer. Blocks were oriented parallel to the marsh-upland boundary. All blocks were complete. All blocks established February 2009.



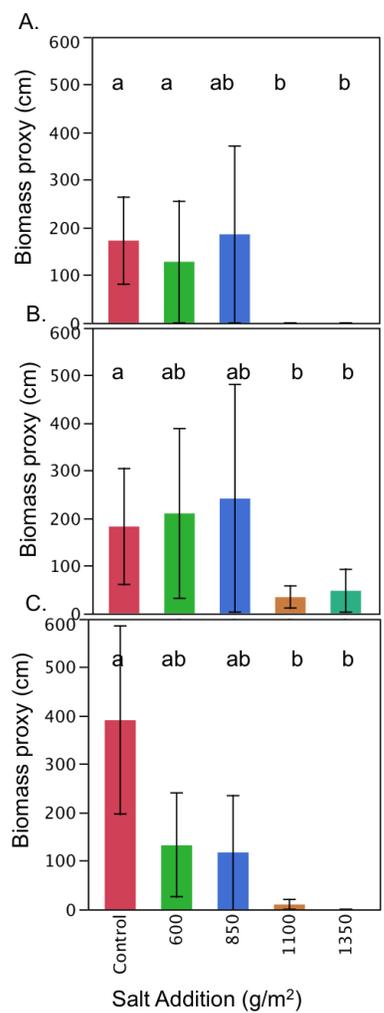
**Figure 2.** Salt Addition in Seaward Migrating Tidally Restricted Marsh-Upland Ecotone: Control plots (n=7) were located within transects established in 2001 as part of a long term monitoring study (Wasson et al. 2013). Salt addition plots (n=7) established in 2010. All 0.5m x 2.0m plots were centered at the marsh-upland boundary, defined as the location where percent cover by upland species is 100%. Open circles represent point intercepts collected at 20cm intervals across each plot. Salt was applied to salt addition plots at a dosage of 600g/m<sup>2</sup>.



**Figure 3.** Pore water salinity (ppt) of soil samples collected from treatment plots (A) before and (B) after salt addition as compared to pore water salinity in a reference fully tidal marsh. Letters indicate significant differences and error bars indicate standard error.



**Figure 4.** Effect of salt addition dosage treatment on (A.) coverage by all upland plant species and (B.) coverage by native marsh species (n=6). Treatments were applied three times in February, March and April 2009. Data was collected in May 2009, one month after the final salt application. Letters indicate significant differences and error bars indicate standard error.



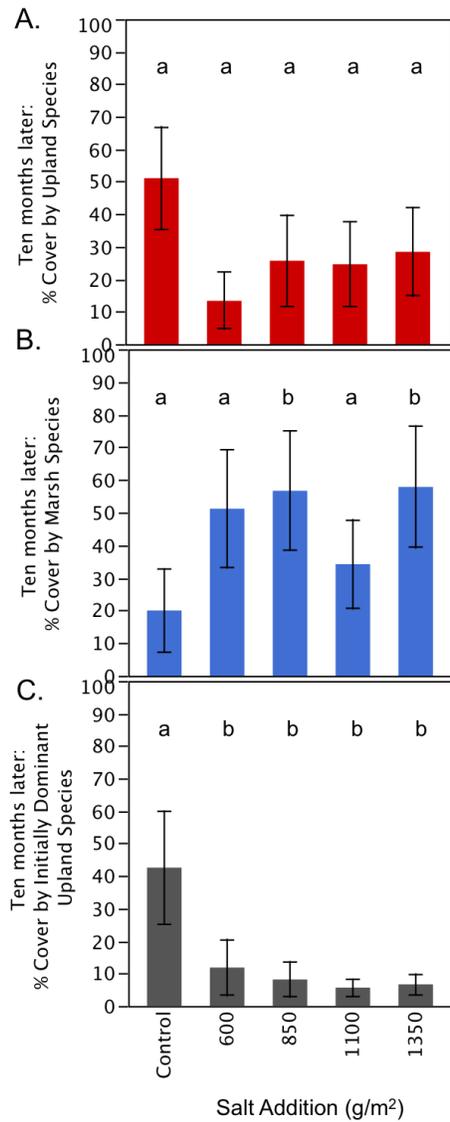
**Figure 5.** Effect of salt addition dosage treatment (g/m<sup>2</sup>) on aboveground biomass of initially dominant non-native upland species, *C. maculatum* and *C. pycnocephalus*, after (A) one, (B) two, and (3) salt addition treatments during the rainy season of 2008-2009. Aboveground biomass approximated using a biomass proxy (cm) due to the need for non-destructive sampling. Letters indicate significant differences and error bars indicate standard error.

**Table 1.** Effect of multiple salt applications on the aboveground biomass of initially dominant upland non-native species (*C. pycnocephalus* and *C. maculatum*). First two columns indicate significance levels of multiple salt additions. Following columns indicate mean aboveground biomass at each salt addition dosage treatment (g/m<sup>2</sup>) and planned contrasts comparing effectiveness of each treatment dosage to the Control.

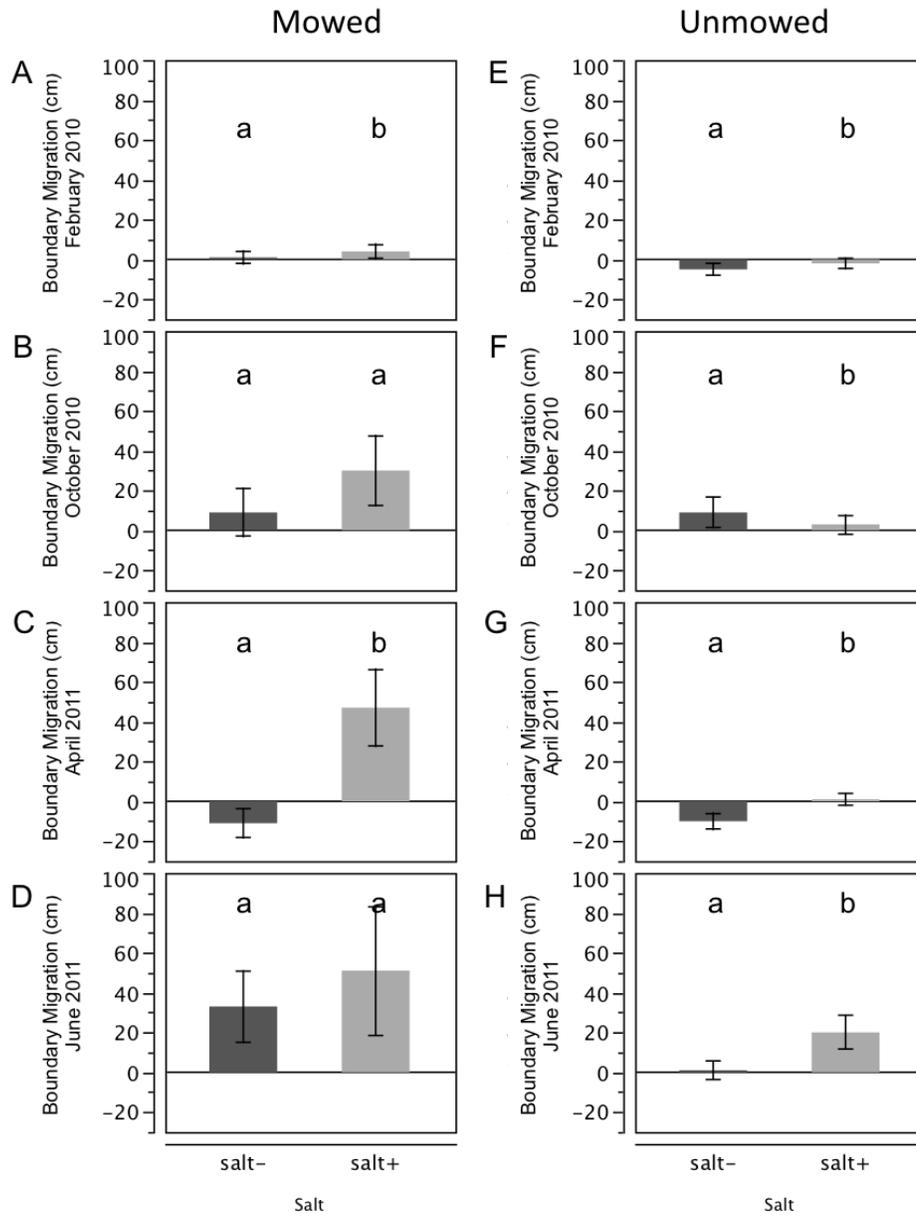
	Treatment df=4	$\chi^2$	Control		600 g/m <sup>2</sup>		850 g/m <sup>2</sup>		1100 g/m <sup>2</sup>		1350 g/m <sup>2</sup>	
			mean aboveground biomass proxy (cm)	z (p-value)								
One Salt Application	<b>0.0073</b>	14.00	183	-1.04 (0.68)	210	-1.84 (0.20)	241	-1.84 (0.20)	34	-2.80 (0.018)	48	-2.80 (0.018)
Two Salt Applications	<b>0.018</b>	11.92	173	-1.85 (0.20)	128	-1.85 (0.20)	186	-1.85 (0.20)	0	-2.81 (0.018)	0	-2.81 (0.018)
Three Salt Applications	<b>0.056</b>	9.21	391	-1.20 (0.57)	132	-1.60 (0.31)	118	-1.60 (0.31)	10	-2.41 (0.054)	0	-2.41 (0.054)

**Table 2.** Abundance of various life stage categories after three salt applications (May 2009) under varying salt addition dosage treatments. First two columns indicate significance levels of the effect of salt addition dosage treatment on abundance at each life stage. Following columns report mean number of individuals from each life stage for each dosage treatment and the results of planned contrasts comparing the effectiveness of each dosage to the Control. Bold terms indicate significance at the alpha 0.05 level.

	Treatment df=4	$\chi^2$	Control		600 g/m <sup>2</sup>		850 g/m <sup>2</sup>		1100 g/m <sup>2</sup>		1350 g/m <sup>2</sup>	
			mean # of individuals	z (p-value)	mean # of individuals	z (p-value)	mean # of individuals	z (p-value)	mean # of individuals	z (p-value)	mean # of individuals	z (p-value)
# Seedlings	<b>0.0055</b>	14.66	28.33	8.33	3.0	<b>-2.16 (0.010)</b>	<b>-2.81 (0.018)</b>	1.17	<b>-2.80 (0.018)</b>	1.17	<b>-2.80 (0.018)</b>	
# Reproductive Adults	0.26	5.30	5.67	7.50	12.67	-0.17 (0.10)	-0.34 (0.10)	0	-1.37 (0.45)	0.3333	-1.37 (0.45)	
# Adults	0.19	6.20	6.67	7.67	12.67	-0.16 (0.10)	-0.73 (0.88)	0.17	-1.71 (0.35)	0.3333	-1.71 (0.35)	
Total # Individuals	<b>0.0029</b>	16.09	35.00	16.00	15.67	-1.84 (0.20)	-2.00 (0.14)	1.33	<b>-2.80 (0.018)</b>	1.5	<b>-2.80 (0.018)</b>	



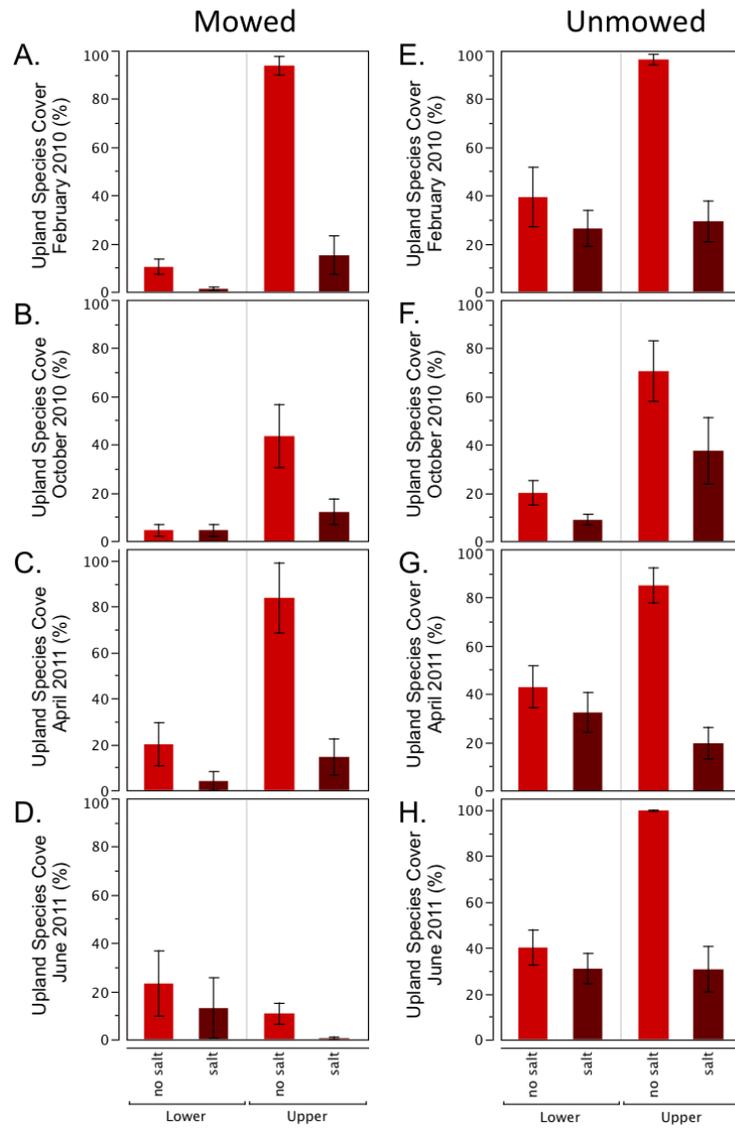
**Figure 6.** Persistence of the effect of salt addition dosage treatment (g/m<sup>2</sup>) on percent cover by (A) upland, (B) marsh, and (C) initially dominant upland species. Data collected March 2010 ten months after final salt application in April 2009. Letters indicate significant differences and error bars indicate standard error.



**Figure 7** Effect of salt addition on the location of boundary between marsh and adjacent upland plant communities in Mowed (A, B, C, D) and Unmowed (E, F, G, H) areas in Spring (February 2010), Fall (October 2010), Spring (April 2011), and Summer (June 2011). Measured in cm from a permanent marker. Positive numbers indicate expansion of the marsh-upland ecotonal community into areas previously dominated almost exclusively by non-native upland species. Letters indicate significant differences and error bars indicate standard error.

**Table 3** Effect of salt addition on the location of the marsh-upland boundary in plots located within mowed and unmowed areas. Bold terms indication significance at the alpha 0.05 level.

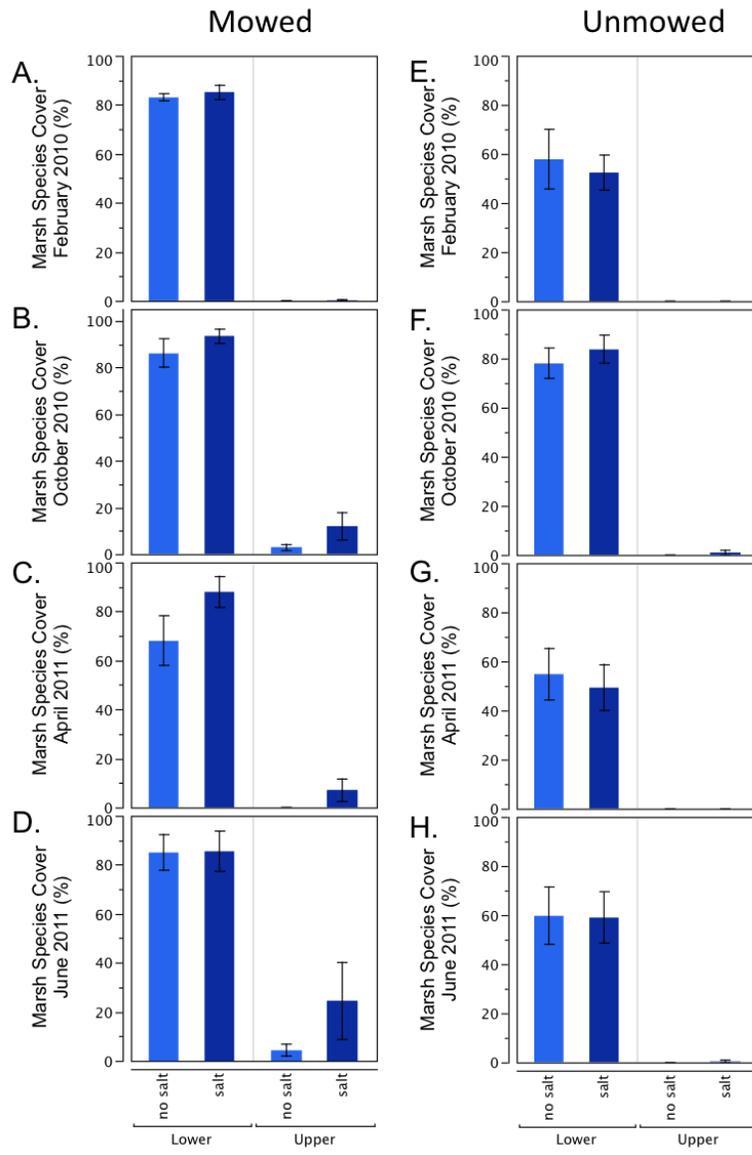
		February 2010 F (p-value)	October 2010 F (p-value)	April 2011 F (p-value)	June 2011 F (p-value)
Mowed	Salt Addition df=1,16	<b>87.24 (&lt;0.0001)</b>	4.65 (0.050)	<b>17.98 (0.0006)</b>	1.18 (0.30)
Un-Mowed	Salt Addition df=1,16	<b>22.55 (0.0002)</b>	<b>5.26 (0.040)</b>	<b>23.62 (0.0002)</b>	<b>30.06 (&lt;0.0001)</b>



**Figure 8.** Effect of salt addition on percent cover by upland species in Mowed (A, B, C, D) and Unmowed (E, F, G, H) areas in Spring (February 2010), Fall (October 2010), Spring (April 2011), and Summer (June 2011). Error bars indicate standard error.

**Table 4.** Effect of salt addition, quadrat location (lower or upper), and their interaction on percent cover by upland species in mowed and unmowed areas. Bold terms indicate significance at the alpha 0.05 level.

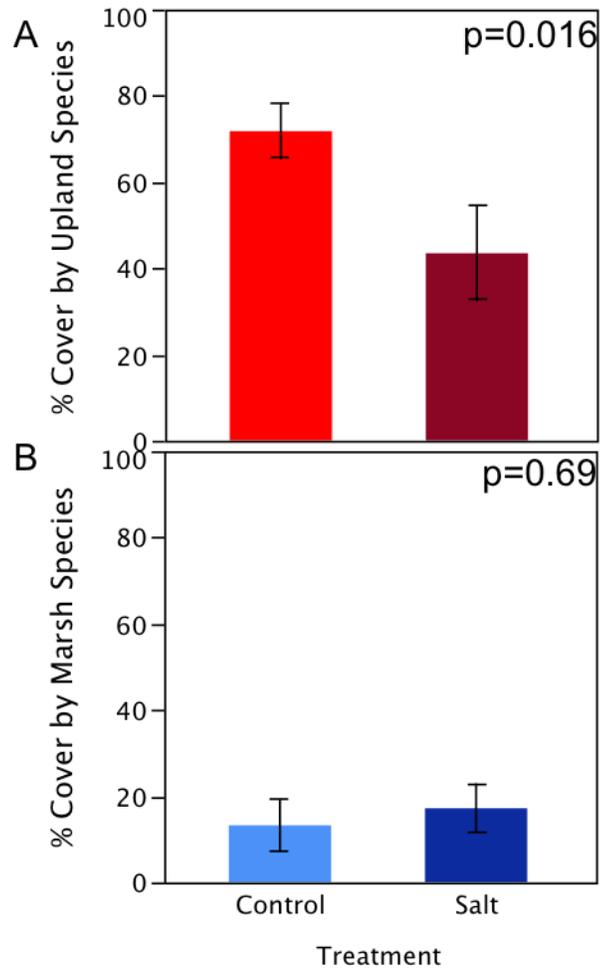
		February 2010 F (p-value)	October 2010 F (p-value)	April 2011 F (p-value)	June 2011 F (p-value)
Mowed	Salt Addition df=1,16	<b>87.24 (&lt;0.0001)</b>	<b>4.65 (0.050)</b>	<b>17.98 (0.0006)</b>	1.18 (0.30)
	Quadrat Location df=1,16	<b>107.49 (&lt;0.0001)</b>	<b>10.12 (0.0060)</b>	<b>13.64 (0.0020)</b>	1.74 (0.21)
	Salt * Quadrat df=1,16	<b>54.88 (&lt;0.0001)</b>	<b>4.65 (0.050)</b>	<b>7.02 (0.018)</b>	0.00 (0.99)
Un-Mowed	Salt Addition df=1,16	<b>22.55 (0.0002)</b>	<b>5.26 (0.040)</b>	<b>23.62 (0.0002)</b>	<b>30.06 (&lt;0.0001)</b>
	Quadrat Location df=1,16	<b>12.70 (0.0030)</b>	<b>16.90 (0.0008)</b>	3.66 (0.080)	<b>17.25 (0.0007)</b>
	Salt * Quadrat df=1,16	<b>10.29 (0.0060)</b>	1.28 (0.30)	<b>12.37 (0.0030)</b>	<b>17.60 (0.0007)</b>



**Figure 9.** Effect of salt addition on percent cover by marsh species in Mowed (A, B, C, D) and Unmowed (E, F, G, H) areas in Spring (February 2010), Fall (October 2010), Spring (April 2011), and Summer (June 2011). Error bars indicate standard error.

**Table 5.** Effect of salt addition, quadrat location (lower or upper), and their interaction on percent cover by marsh species in mowed and unmowed areas. Bold terms indicate significance at the alpha 0.05 level.

		February 2010 F (p-value)	October 2010 F (p-value)	April 2011 F (p-value)	June 2011 F (p-value)
Mowed	Salt Addition df=1,16	0.53 (0.50)	3.33 (0.090)	<b>4.50 (0.050)</b>	1.14 (0.30)
	Quadrat Location df=1,16	<b>2606.10 (&lt;0.0001)</b>	<b>330.51 (&lt;0.0001)</b>	<b>135.37 (&lt;0.0001)</b>	<b>43.48 (&lt;0.0001)</b>
	Salt * Quadrat df=1,16	0.37 (0.55)	0.028 (0.87)	1.0090 (0.33)	1.034 (0.32)
Un-Mowed	Salt Addition df=1,16	0.15 (0.71)	0.62 (0.44)	0.15 (0.70)	0.0004 (0.98)
	Quadrat Location df=1,16	<b>60.99 (&lt;0.0001)</b>	<b>357.73 (&lt;0.0001)</b>	<b>53.62 (&lt;0.0001)</b>	<b>57.33 (&lt;0.0001)</b>
	Salt * Quadrat df=1,16	0.15 (0.71)	0.31 (0.60)	0.15 (0.70)	0.005 (0.95)



**Figure 10.** Percent cover by upland species (A) and marsh species (B) in paired control and salt addition transects. June 2011. P-values indicate significance level. Error bars indicate standard error.

## SYNTHESIS

This dissertation research was motivated by the desire to understand the ecological processes structuring the abrupt boundaries and narrow distribution of a unique plant community fringing the salt marshes of Elkhorn Slough. In this central coast salt marsh the majority of plant diversity is concentrated to this narrow marsh-upland ecotone so identifying what combination of abiotic and biotic factors are responsible for the distinct zonation of this high marsh plant community was not only important from an ecological research perspective but also from a conservation and restoration perspective. This relatively diverse plant community is attached to an estuarine salt marsh, an ecosystem that has historically been degraded and destroyed by various types of human developments and disturbances. In addition, the position of the marsh upland ecotone in the upper intertidal zone means that this infrequently inundated plant community is likely to be sensitive to imminent changes in sea level. Indeed, recent research has already indicated that changes in inundation due to sea level have already begun to alter the structure and distribution of this ecotonal community (Wasson et al. 2013). For all these reasons, using multiple manipulative approaches to test the applicability of existing ecological theory, characterizing the underlying abiotic conditions, and testing the effectiveness of a cost effective strategy for restoration were necessary and informative steps towards the development of useful and evidence based adaptive management and restoration strategies for the marsh-upland ecotone.

One of the primary goals of this research was to characterize the underlying gradient in order to address open questions about the shape of the abiotic gradient influencing plant community composition. Prior to this research critical assumptions about the ecotonal abiotic conditions used to design restoration strategies and conceptualize the ecological processes structuring the marsh-ecotone plant community had been based primarily on the extensive research done on the high marsh plant communities of southern California. In southern California for much of the year the salinity within the ecotone is higher than the salinity of either of the adjacent habitats due to high rates of evaporation in this infrequently inundated zone. Infrequent periods of low salinity corresponding with rain and freshwater runoff are suggested to influence community composition, especially annuals, and drive species distributional patterns across the transition zone. In contrast, our investigations into salinity across the marsh to upland transition revealed that for most of the year salinity is relatively linear, decreasing gradually with increasing elevation, generally producing intermediate to low salinity levels within the ecotone. However, the gradient in salinity becomes hump shaped during a short period at the end of the growing season. During this period before the onset of the winter rains, the highest salinity levels are found in the ecotone. Identification of this relatively short window when salinity peaks in the ecotone is an important and novel contribution to our understanding of the marsh-upland ecotone in northern California and contrasts strongly with the conditions found in Southern California marshes.

In Chapter 1 the attempt to apply the classic model used to describe the processes structuring distributions, the stress gradient hypothesis (SGH), yielded several new insights into how the species of the marsh-upland ecotone are limited to their current relatively narrow zone and generated new questions about the processes structuring this community. First, one of the major findings of the experimental outplant that has repercussions for restoration came out of the survival patterns of three ecotonal species when experimentally outplanted outside of their natural distributions. We found that individuals outplanted into the high inundation conditions of the marsh survived as well or better than in the ecotone. Conversely, for two out of three species survival when outplanted into the upland was significantly worse than in the ecotone. These results indicate that, in contrast to the general notion, from the perspective of these ecotonal species the gradient in physiological stress is negatively correlated with elevation. It is not difficult to understand the origins of the assumption that elevation and stress are positively correlated in an intertidal system. The number of hours ecotonal species are inundated at the marsh boundary is approximately 6 times greater than near the upland boundary, creating a steep gradient in tidal influence across the ecotone. The physiological challenges associated with tolerating frequent inundation, include water logged and anoxic soils and salinity stress. However, our results indicate that the underlying stress gradient is, in fact, negatively correlated with elevation.

Fortunately, unexpectedly high tolerances for inundation by these ecotonal species combined with observations of increases in cover by ecotonal species when

upland species are removed *from within the ecotone* (Chapter 2 and 3) suggest that the marsh-upland ecotone may be less vulnerable to the “drowning” phenomena expected to threaten salt marsh species lower in the ecotone. Ecotonal individuals near the marsh boundary will likely tolerate increases in inundation, especially in the relatively well-drained soils of the ecotone, long enough for these perennial species to expand into areas near the upland boundary. This upland expansion is highly likely because the abiotic conditions at these higher elevations will become more similar to those within the ecotone due to increased inundation and ecotonal species will be able to colonize them. In addition, the effect of increased inundation by seawater would likely be similar effect to the effect of artificial salt addition, eliminating salt intolerant upland species and freeing space for eventual colonization by ecotonal species.

Overall, it is clear that the simplistic predictions of the stress gradient hypothesis (SGH) are insufficient to explain the distributions of these species along this complex and variable abiotic gradient. This research joins a growing body of literature that has found that the SGH, while sometimes accurate at describing the distributional controls across relatively simple linear environmental gradients, it is less appropriate when applied to complex abiotic gradients that vary non-linearly stochastically. However, this research does provide a basic understanding of how the distributional boundaries of each species are influenced by competitive interactions and abiotic conditions; information that has already begun to inform restoration practices in the Monterey Bay.

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