The Effects of NaCl on Germination in *Suaeda californica* (Chenopodiaceae)

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Abstract Wetlands benefit the environment in many ways by helping with flood control, water quality, and providing habitat. Many wetlands were lost because of direct conversion by humans or threat by invasive species. Recent concerns have now called for the reversal of these damages by restoring native vegetation. Salinity is a critical environmental factor in salt marsh wetlands for determining seedling survival for many species. The effect of salinity on germination on an endangered native California plant, *Suaeda californica*, was addressed in this research. *S. californica* seeds were exposed to concentrations of 100 to 500 mol m^{-3} NaCl to determine the optimal salinity range for germination. Seeds showed no appearance of a radicle in any of the salt concentrations. Seeds were then recovered in distilled water for 10 days. Percentage recovery of germination ranged from 15-30%. The implication of this research shows that more research needs to be done on seed dormancy to understand the requirements of breaking dormancy. Moreover, wetland restoration efforts for *S. californica* do not seem promising when seeds are not germinating in salt concentrations of the habitat.

Introduction

Wetlands benefit the environment by helping with flood control, water quality, and providing habitat for many endangered species (Mitsch and Gosselink 2000). Estimates of global wetland area range from 5.3 to 12.8 million km^2, (less than 9% of the global land area) (Zedler et al 2005). However, about half of the global wetlands have been lost. In 1971 an international treaty called the Ramsar Convention was signed in Ramsar, Iran. Initially started for 18 nations, it is currently comprised of 154 nations to cooperate in wetland conservation and sustainability. It designates 149.7 million hectares of wetlands with 1,651 sites globally as Wetlands of International Importance with 22 sites listed for the United States (Ramsar 2007).

At the time of European settlement, the United States had 221 million acres of wetlands, but only an estimated 105.5 million acres of wetlands were recorded in 1997 (Dahl 2000 and Dahl and Allord 1996). This is because wetlands are being converted to agricultural (26%), silviculture (23%), rural development (21%), and urban development (30%) with an estimated wetland loss rate of 58,500 acres per year (Dahl 2000). Current events like Hurricanes Katrina and Rita reveal that coastal communities become vulnerable when wetlands are degraded. They are not able to provide protection from storm surges crashing inland because of human establishment of dams and levees that reduce the amount and texture of sediment going into the wetlands resulting in accretion deficit (Walker et al 1987; Templet and Meyer-Arendt 1988). Wetlands along the Mississippi River are now only able to store 12 days of floodwater when they were able to store 60 days (US EPA 1995).

California is no exception; about 90% of its wetlands have been lost over the last 200 years (Zedler 1996). This dramatic loss, as well as potential future loss, has caused a movement in efforts to preserve wetlands and restore them to their original condition. A national policy has been implemented as a goal for the nation to have no net loss of wetlands (Conservation Foundation 1988). To not have any more wetland loss means that conservation of current wetlands and the restoration of degraded wetlands must be implemented. Ecological Restoration is defined by the Society for Ecological Restoration International as, “the process of assisting the recovery of an ecosystem that has been degraded, damaged, or destroyed” (SER 2004). Wetland restoration focuses on rebuilding the functions of the wetlands that have been damaged or destroyed either by increasing flood storage, water quality and/or wildlife habitat.

Currently, one goal of the Golden Gate Audubon is to restore species' habitat at Pier 94, a salt marsh of approximately six acres in San Francisco, California (Conservancy 2005) (Figure 1; Figure 2). Pier 94 is a wetland that is located in an industrialized area of the Bay and is threatened by a non-native invasive cordgrass, *Spartina alterniflora* Loisel (Conservancy 2005). Wetlands that are threatened by invasive species can disrupt biodiversity. In southern California, *Agrostis avenacea* J.F Gmel. (Pacific bentgrass) is an Australian annual invasive that has permanently altered the wetlands. It has become dominate because of its tumbleweed dispersal and its ability to grow taller making it more robust compared to other native species, permanently altering the composition of the wetlands (Zedler and Kercher 2004).

Figure 1 Map of Bay Area in Northern California

Figure 2 Map of Pier 94 in San Francisco, Ca

Because of concerns over irreparable impacts from cordgrass at Pier 94, efforts were made to reintroduce a California native plaint, *Suaeda californica* Watson (California Sea-blite) that use to be present in the San Francisco Bay area, but was listed Federally Endangered on December 15, 1994. Historical records only begin in 1866 showing that *S. californica* was distributed throughout California in counties Alameda, San Luis Obispo, and San Diego with frequencies of localities decreasing with time (Figure 3). It is a halophyte, a plant adapted to living in a saline environment, in the Chenopodiaceae family. *S. californica* became endangered because it is a sensitive species that is easily threaten by human activities and any natural processes that adjust the microtopographic gradient or hydrologic conditions of the habitat, including sedimentation, dredging and bluff erosion (NatureServe 2006). Moreover, *Suaeda* is taxonomically confusing because the genus has wide-ranging polymorphic species and thought to consist of 90 species; twice as many names have been published (Fisher 1997).

Figure 3 County-level distribution of *S. californica* based on historical records

Because of its limited number of individuals there is currently not much knowledge about *S. californica*. Restoration efforts like Pier 94 deal with endangered plant species that are limited in quantity, but do not have background research on what salinity conditions are ideal for germination before introduction. Coastal wetlands have salinity gradients that vary, so it's important to know the salinity tolerance of the species. That is why currently at Pier 94 established *S. californica* plants are introduced into the wetland instead of seedlings because seeds are more vulnerable to the environment. Also, it will help guide future restoration efforts by selecting the appropriate zone where *S. californica* are suitable.

To help fill this hole in background knowledge of salinity tolerance in *S. californica*, information was gathered on salinity tolerance on the genus *Suaeda* from various species. Previous research on *S. fruticosa*, *S. calceoliformis* and *S. physophora* used concentration levels from 100 to 500 mmol m^{-3} of NaCl solution (Khan and Ungar 1997, Keiffer and Ungar 1997 and Song et al 2005). My hypothesis was that *S. californica* germination potential would be inhibited at 400 to 500 mmol m^{-3} NaCl concentrations as in previous studies of related plants (Katembe et al 1998 and Song 2005).

Methods

Seed Collection *S. californica* seeds were collected from San Francisco Port Pier 94 in San Francisco, California on the week of the 11th of December obtaining around 250 seeds. The background salinity levels at this site in the summer are approximately 28 to 34 parts per thousand (479 to 582 mmol m^{-3}) and in the winter approximately 11 to 15 parts per thousand (188 to 257 mmol m^{-3}). Dry seeds were stored in a refrigerator for about two months before use.

Germination To determine the effect of salinity on seed germination, five concentration levels of NaCl were prepared at 100, 200, 300, 400, and 500 mmol m^{-3}. In all the germination experiments, 20 seeds were sown in Petri dishes lined with two layers of filter paper and filled with 10mL of test solution. Two replicates of each solution were used in all experiments. All Petri dishes were placed in an incubator with constant temperatures (20°C). Every 2 days, the solution in each of the Petri dishes was removed and 10mL of test solution was added back again. Germinated seeds were recorded on a daily interval for 30 days and considered germinated when the emerging radicle was at least 2 mm. The rate of germination was determined by using a Timson index of germination velocity = $\Sigma G/t$, where G is percentage of seed germination, and t is total germination period (Timson 1965; Khan and Ungar 1997).

Recovery Test To recover seeds that failed to germinate in NaCl concentrations, ungerminated seeds were transferred to distilled water for treatment. Data was recorded daily for 10 days and considered germinated with the emergence of the radicle. The rate of germination was also determined by using Timson index of germination velocity.

Data Collection *S. californica* localities were collected from the Calflora (2007) database and the SMASCH database, provided by the University and Jepson Herbaria. A total of 44 records were found, but only half had either longitude and latitude coordinates or reference places that were specific enough to obtain coordinates using TopoZone.

Modeling Models were made using MAXENT, a software based on the maximum entropy of species geographic distributions. Two models were made to compare *S. californica* distribution in California based on climate data that was gathered from the Worldclim database. One model used current climate data that was averaged from 1950 to 2000 and a future climate data was based on present atmospheric carbon dioxide level of 335 ppmv with a doubling of 710 ppmv in 2100 AD using 16 climate variables in both models (Table 1) (Govindasamy et al 2003).

Current climate data was gathered at 2.5 arc min (~5 km^2) resolution and future climate data at 30 arc second (~1 km^2) resolution. The models were tested for their accuracy in predicting species distribution by setting aside 25% of the collection records to test if it predicted correctly an absence or a presence of the excluded collection records. The area under the Receiver Operating Characteristic curve (AUC) was used to explain the model's accuracy. It checks the chance that a random presence site will have a higher predicted value than a random absence site. An AUC value of 1.0 means perfect discrimination (Phillips et al 2006).

Table 1 Climate variables used in MAXENT

Variables
Annual Mean Temperature
Annual Precipitation
Isothermality
Maximum Temperature of Warmest Month
Mean Diurnal Range
Mean Temperature of Driest Quarter
Mean Temperature of Wettest Quarter
Minimum Temperature of Coldest Month
Precipitation of Driest Month
Precipitation of Driest Quarter
Precipitation of Warmest Quarter
Precipitation of Wettest Month
Precipitation of Wettest Quarter
Precipitation Seasonality
Temperature Annual Range
Temperature Seasonality

Results

Germination Seeds failed to germinate in all of the NaCl concentrations. Seeds showed no appearance of a radicle in any of the concentrations, so seeds were then transferred to distilled water for recovery. When transferred to distilled water some seeds were responsive and germinated. *S. californica* had a very poor recovery response with percentages ranging from as low as 15% in 100 mol m^{-3} NaCl to optimal recovery germination percentages at 30% in 400 and 500 mol m^{-3} NaCl (Table 2). A trend showed that recovery of germination increased with increased concentrations of NaCl (Figure 4).

Table 2 Percentage recovery of germination of *S. californica* seeds in five NaCl concentrations for 10 days

Number of Seeds	NaCl Concentration (mol m^{-3})	Germination (% Recovery)
20	100	15%
20	200	20%
20	300	25%
20	400	30%
20	500	30%

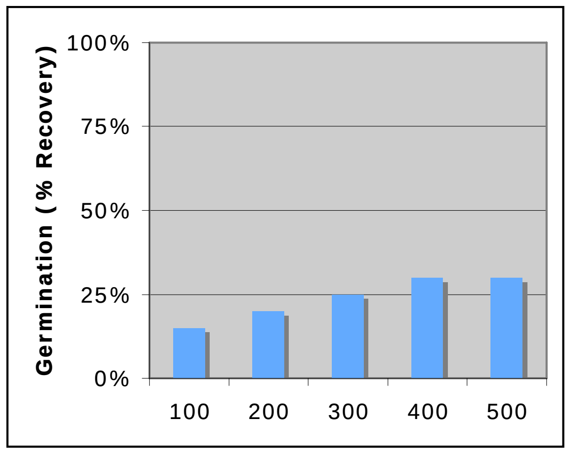

Figure 4 Percentage recovery of germination of *S. californica* seeds in five NaCl solution

Modeling The models yielded high values of AUC at .99 and standard deviation of 0.001. The model for current climate conditions showed that *S. californica* potential habitat is quite limited, but San Mateo and Monterey counties could be considered (Figure 5A). For future climate conditions potential habitat for *S. californica* spreads out more inland (Figure 5B).

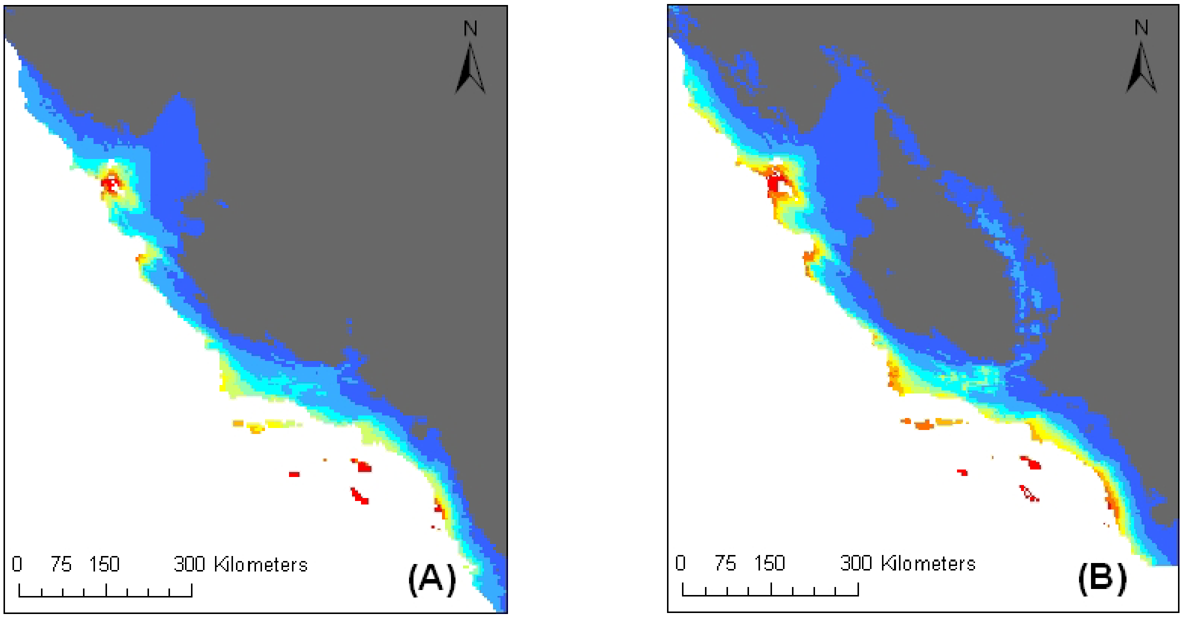

Figure 5 Distribution of *S. californica* from (A) current climate database and (B) future climate database. Hotter colors (eg. Red) indicate likely locations for *S. californica*, while cooler colors suggest less likely locations.

Discussion

Germination *S. californica* is a halophyte that is federally endangered. With limited quantities of individuals available there is not much knowledge or research known about this California native plant. Therefore, optimal germination in NaCl concentrations would help future reintroduction efforts. Previously, experiments have been done on other species within the *Suaeda* genus, but none have been done on *S. californica*. Salinity concentrations on seed germination have been tested on *S. depressa, S. maritime, S. fruticosa, S. linearis, S. salsa, S. japonica and S. physophora* reporting a decrease in germination when placed in high salinity levels (Ungar 1962, Bakker *et al* 1985, Sheikh and Mahmood 1986, Shumway and Bertness 1992, Shen *et al* 2003, Yokoishi and Tanimoto 1994 and Song *et al* 2005). My study showed that germination was affected by salt treatment by failing to germinate in any of the NaCl concentrations. This raises questions as to why this happened.

One reason for failed germination could be because I did not do cold stratified treatment on the *S. californica* seeds. The *Suaeda* genus belongs in the Chenopodiaceae family and is known to have physiological seed dormancy. Cold stratification is used as a dormancy breaking treatment and helps increase seed tolerance to salinity (Baskin and Baskin 1998). In experiments with *S. maritime* and *S. linearis*, seeds were stratified in fresh water for one month before starting germination (Bakker et al 1985; Shumway and Bertness 1992). In other studies, *S. physopora*, *S. salsa* and *S. depressa* were refrigerated either for one month to four months before beginning germination, as in my study (Song et al 2005, Shen et al 2003, Ungar and Williams 1972 and Ungar 1962). In the past studies, both treatments produced seeds that germinated with no failed germination. However, general requirements to break physiological dormancy are hard to state because researchers did not test freshly mature seeds before storing them for one to several months and during this storage period the amount of dormancy lost is not known (Baskin and Baskin 1998).

Another reason for failed germination could be because of the type of salt used. Even when the level of osmotic stress is kept the same, some salts inhibit germination more than other salts. For example, *S. japonica* germinated at a lower percentage in NaCl and KCl than in NaGluc or KGluc showing sensitivity to chloride ions (Yokoishi and Tanimoto 1994). These two facts could explain why *S. californica* seeds did not perform that well.

When *S. californica* seeds were then placed in distilled water for recovery they germinated. The percentage recovery of germination of the seeds was not as high as in the other studies, but followed the trend of percent recovery increasing when NaCl concentrations increased (Shen et al 2003; Khan and Ungar 1997). This study and other studies have shown this trend to be true because germination is inhibited under high salt concentrations due to osmotic inhibition. When the salt stress is removed, seeds can then germinate because short-term exposure to salinity does not have an affect on seed viability (Baskin and Baskin 1998).

This still leaves many questions unanswered and more research needs to be conducted to fill in the gap of our knowledge of *S. californica*. There are complications in understanding the requirements of dormancy-breaking, so future research should be conducted on fresh mature seeds and given cold stratification over a long period of time to determine the time required to break dormancy (Bakker et al 1985; Shumway and Bertness 1992). Moreover since seeds failed to germinate, lower concentrations ranging from 0 to 100 mmol m^{-3} of NaCl should be implemented instead (Shen et al 2003).

This means that reintroduction efforts for *S. californica* at Pier 94 might be a lost cause because the species cannot germinate in salt concentrations higher than 100 mmol m^{-3} based on this research. Pier 94 salt levels ranges from 188 to 582 mmol m^{-3} and currently has been productive by planting established *S. californica* plants into the habitat for it to propagate. This method will have to continue indefinitely for *S. californica* survival. If salt concentrations are lower than 100 mmol m^{-3}, then maybe the species might be able to germinate in the habitat. Also, it could still be able to germinate even at higher concentrations. There are limitations as to how widely these data can be applied because other factors were not taken into account when conducting this experiment in the greenhouse that does occur in the habitat including salt flushes, moist-chilling for different lengths of time, soil organic matter, and nitrogen.

Modeling The two models of current and future climate distribution of *S. californica* showed that climate change along the coast of California is possible. The reason why distribution currently is limited to only San Luis Obispo county (Morro Bay) and San Francisco county (Pier 94) is because of the spread of urbanization near wetlands. With recent awareness and actions to reverse this phenomenon more wetland restorations are underway. By looking at the future distribution map, wetlands around the Central Coast and the Bay Area in Northern California can be considered for reintroducing *S. californica* into the habitat. Moreover, the future distribution map predicts that *S. californica* moves more inland with double CO_2 concentrations. This does not seem like an accurate prediction since this species only occurs in wetlands. A variable that was not taken into account when running MAXENT was elevation, so inland areas of the future distribution map are not appropriate for this species.

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