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Estimating Seed Bank Responses to Changing Environmental Conditions in the Louisiana Coastal Zone

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DEDICATION

I would like to dedicate this thesis to my parents, Alfred and Geraldine Horaist. They have provided me with an immense amount of resources and support to be able to pursue this dream. Without their support, the path would have been much rougher and more difficult. I can only hope that I will be able to provide for them the same love and support when they are in need. I would also very much like to thank my academic advisors, without whom this opportunity for graduate school and the experiences would not have been possible. They have provided important knowledge, materials, and guidance to complete the necessary trials and tribulations. I now feel comfortable in referring to Scott and Jenneke as my friends. I am excited and thankful to be where I am today. I am very proud of what I have been able to accomplish and look forward to the challenges of tomorrow. A graduate degree is a team effort, often more than I could have ever imagined.

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LIST OF ABBREVIATIONS

AICc	Akaike Information Criterion corrected for small sample size
Br	Brackish marsh type
ΔAICc	change in the AIC corrected for small sample size
CRMS	Coastal Reference Monitoring System
CWPPRA	Coastal Wetlands Planning, Protection and Restoration Act
F	Flood water level condition
Fr	Fresh marsh type
Int	Intermediate marsh type
IPCC	Intergovernmental Panel on Climate Change
K	parameters used in a statistical model
LAVegMod	Louisiana Vegetation Model
m	meter of the metric system
M	Marsh type
N	Non-flood water level condition
S	Salinity treatment
W	Water level condition
Sal	Saline marsh type
Sign. Code	Significant Codes
Spp	Species
***	Significance of data ranging between 0 and 0.001
**	Significance of data ranging between 0.001 and 0.01
*	Significance of data ranging between 0.01 and 0.05

. (dot)	Significance of data ranging between 0.05 and 0.1
Blank space	Significance of data ranging between 0.1 and 1.0

INTRODUCTION

The coastal zone of Louisiana has been losing wetlands at some of the highest rates found in the United States. Approximately 4877-km^2 net of wetlands have been lost in Louisiana between 1932 and 2010, with most of the land converting to an open-water condition (Mitsch and Gosselink, 2007; Couvillion et al., 2013; Barras, 2006). This means that the Mississippi River delta has lost land at a rate of 102-km^2 per year (Couvillion et al., 2013). About 25% of the Mississippi River delta has been lost and expectations are for an additional 10,000 to 13,500 km^2 of land to be converted to open water by 2100 (Blum and Roberts, 2009). While the rate of loss has begun to slow in recent years, the flat low-lying topography of the coastal zone could make the forecasted changes in salinity or flood regime affect larger areas (Sasser *et al*., 2014).

The coastal zone of Louisiana has already been altered to a very high degree. There are a number of factors that need to be evaluated to understand the future of the Louisiana coastal zone. The vertical sediment and organic accretion (Nyman, 2006) has not been able to keep up with subsidence. The lack of available sediment is caused in part by an 80% reduction in the sediment load carried by the Mississippi River relative to the levels of the 1850s (Kesel and Reed, 1995). The river flow has also been contained within levees and its sediment restricted by dams (Belt, 1975). Because of this, the once annual flooding events have not been able to reach and provide the wetlands with needed river borne sediments. It has been estimated that the sediment load required to reconstruct the Mississippi River delta exceeds the current capacity of the river (Blum and Roberts, 2009). The post-glacial isostatic adjustment of the North American continental plate and, to a lesser degree the compaction of the soil has also contributed to subsidence of the land relative to sea level.

Eustatic sea-level rise refers to the change in sea level caused by the fluctuations in the amount of water taken up or released by the polar icecaps and glaciers. The sea level can also be affected by thermal expansion or contraction. For every change of 1°C, the sea level will change by 2 meters (IPCC, 2014). The factors contributing to eustatic sea-level rise could be affected abnormally depending upon future CO_2 concentration increases. The upper bound of expected rises in sea levels was estimated to be between 0.44 to 0.74 meters for the 21st century (IPCC, 2014). The actual measurements of sea-level rise and global temperatures have closely tracked the highest levels of these predictions (IPCC, 2014). The combined factors of eustatic sea levels and the isostatic changes in land elevation are now referred to as the relative sea level. The Louisiana Gulf Coast has experienced the highest rates of relative sea-level rise in the entire gulf region. The rates of relative sea-level rise have been measured between 1.04 cm/year up to 1.77 cm/year in the Teche Basin (Penland and Ramsey, 1990). The combination of these factors paint a bleak picture for the coastal zone of Louisiana and there has been a growing concern about what ecological and environmental impacts may occur as the environment deteriorates (Olivieri and Gouyon, 1997). Even with efficient selection of diversion projects and the capture of 100% of the sediment from the modern sediment load of the Mississippi River, an inevitable retreat of the coastline is almost certain (Blum and Roberts, 2009). While predictions vary widely, there is the possibility of creating a sustainable coast by leveraging vegetative accretion, but to defer decisions based on the uncertainty of current predictions make any possible defense even more difficult (Blum and Roberts, 2009).

The Louisiana coastal zone has four marsh types: fresh, intermediate, brackish and saline (Sasser et al., 2014; Gosselink, 1984; Chabreck, 1972). A fifth wetland type, forested swamp (Chabreck, 1972) is often included, but was not included in this study. The forested swamps are a wetland classification that simply fell outside the focus of this research. The studied marsh types are described in more detail below.

Fresh marshes are generally characterized by plants that can only live and complete their lifecycles in salinities that are at or very near 0.0 ppt (Chabreck, 1972). Salinities up to 3.0 ppt can be tolerated, but only for very short periods of time (Visser et al., 1998). With salinities at or near zero, the water levels in the marsh will most often dictate the dominant species of a freshwater marsh or wetland (Mitsch and Gosselink, 2007). Species like *Panicum hemitomon, Sagittaria spp.*, *Typha spp.* and *Leersia hexandra* are common and many others like *Polygonum spp.* are found in abundance indicating the marsh may be in a process called a drawdown. A drawdown occurs as waters recede from flooded conditions. New sediment and nutrients are deposited and encourages growth of plants useful to waterfowl and small mammals. Marshes dominated by species like *Sagittaria spp.* and *Panicum hemitomon* sometimes create floating marshes. Floating marshes create a tight mesh of roots and rhizomes to create a separate false floor to the marsh (Sasser et al., 1996). Plants in a floating marsh are often able to maintain themselves above the water level through buoyancy from aerenchyma in the roots and gasses released from microbes (e.g. methane). Whereas only a few centimeters of water are usually visible in floating marshes, the actual surface of the sediment is often a 0.5 to 1.0 meter beneath.

An intermediate marsh has vegetation that can tolerate salinity levels up to about 5 ppt (Visser et al., 1998). Intermediate sites have many of the same species as the fresh marshes, but the addition of some salt tolerant species creates a more diverse species mix. The marshes can be dominated by *S. lancifolia* in the lower salinities to *Spartina patens* in the saltier areas (Visser et al., 1998). Other prominent species, like *Phragmites australis, Panicum dichotomiflorum* and *Schoenoplectus americanus,* are often characteristically found in an intermediate marsh.

A brackish marsh represents a considerable change in the vegetation. The salinity could reach 18 ppt and the number of species begins to diminish significantly relative to the fresher marsh types. A few dominant species such as *Spartina patens* and *Schoenoplectus robustus* can create complete monoculture meadows (Visser et al., 1998). *Distichlis spicata* is often present and *Eleocharis parvula* can be found in open patches (Visser et al., 1998). *Spartina alterniflora* and *Juncus roemanianus* usually indicate a saltier marsh, and often their existence is scattered. A brackish marsh usually had a regular inflow of freshwater and a slightly higher elevation than the salt marsh (Visser et al., 1998).

The coast of Louisiana experiences a diurnal tidal pattern. The diurnal tidal pattern means that there are usually only one high tide and one low tide in a 24-hour period. The diurnal tidal pattern with the numerous rivers bringing sediment to the coast has developed a string of barrier islands. The barrier islands naturally protect the low-lying coastal marshes from salt-water intrusion. Although regular ocean salinities range from 32 to 35 ppt, the Louisiana salt marshes rarely have salinities over 25 ppt. The vegetation in these marshes is better able to withstand regular flooding and higher salinities. The combination of these stresses pared the list of resident species to a hardy few. The Louisiana saline marshes do not usually have a great deal of species diversity and are often monocultures of tall grassy species (Visser et al., 1998). Saline marshes are most identifiable by the dominant presence of *Spartina alterniflora*, *Juncus roemerianus* and *Schoenoplectus robustus* (Visser et al., 1998).

Objectives:

Marshes could use vegetative accretion and sediment trapping to prevent land from devolving to open water (Reed and Cahoon, 1992). My research was designed to provide data to estimate the establishment response of seeds to changes in the water level and salinity conditions found in the Louisiana coastal zone. The design of my seed bank study approximated how areas made available for colonization, resulting from natural accretion, coastal restoration, or other natural or man-made processes, could become colonized. I hypothesized that soil samples subjected to less water and salinity stress would generate more seedlings and by extension have greater species richness. The objective of my research was to examine how species might respond to environmental changes due to relative sea-level rise. The importance of understanding how the vegetation of the Louisiana marshes may respond to the described environmental changes is important for future planning of protection and restoration projects (Visser et al., 2013). Hence, the study area was made large enough so that the results could be reasonably applied to make inferences about the entire Louisiana coast.

My study also provided some insights into the contribution of seed dispersal to the response of plant communities to environmental drivers. I compared the list of species that appeared in my seed bank study to the species observed in the vicinity of my study sites. I compiled lists of species that were found in progressively larger areas surrounding my sample plots. The comparison of historical and research lists of species were used to estimate possible seed dispersal distances from the parent plant.

METHODS

My research was conducted in three major phases: site selection, field data collection and a seed bank study. Once all the data was gathered from the field and the seed bank study, a statistical analysis was conducted to determine how the source of soils, and growing conditions influence germination. This data was further augmented with data from the Coastwide Reference Monitoring System (CRMS) project data to estimate possible seed dispersal distances.

Site Selection:

The coastal zone includes all or part of 20 of the 64 Louisiana parishes and covers about 40,728 square kilometers (15,725 square miles). I chose to locate each of my field sites near one of the CRMS monitoring stations. The CRMS program maintains about 393 randomly selected sites to monitor the status of Louisiana's coastal wetland communities as well as the progress of coastal restoration projects (CRMS, 2014). I first had to exclude some sites because the expense of difficulty associated with access. For example, CRMS sites located east of the Mississippi River were too far, or those only accessible by airboat had added associated machinery and expertise expenses. There were 89 CRMS sites excluded because they were east of the Mississippi River. Four of the originally selected sites were rejected because of airboat requirements. Two other sites were removed from consideration because of legal hurdles or safety concerns.

I classified each of the remaining CRMS sites as either representing a fresh, intermediate, brackish or saline marsh. The original classification was based on the environmental and species composition reported by CRMS for each site. Similar to vegetation maps for coastal Louisiana (Sasser et al. 2013), my study used the dominant vegetation. The dominant mix of vegetation was considered a better indicator of levels of water and salinity most commonly found in an area over time. The site marsh classifications were then adjusted to what was experienced in the field. Four CRMS sites from each marsh classification were randomly selected for a total of 16 study sites. Three sites were re-classified to a different marsh type, after conditions were found in the field to be different than those found the previous year in the CRMS data.

Study Site Location:

Each one-kilometer^2 CRMS site has a restricted 200-m^2 area. The restricted area is used for CRMS monitoring and research. My field study site had to be located within the one-kilometer CRMS site, and maintain a safe distance from the restricted research area (Figure 2). By conducting my research within the CRMS site, I was able to use the CRMS historical and vegetation data in comparison with my field survey and seed bank study data.

Field Data Collection:

Vegetation surveys and soil sample collections were conducted at each of my selected CRMS sites. I selected the center of each study plot by blindly throwing a meter stick into the marsh and using its landing point as the southwestern corner of the 2x2 meter sample plot.

I surveyed the vegetation coverage within the 2x2 meter sample plot before the taking of soil samples disturbed the sample plot. The vegetation survey provided a list of the species present as well as their coverage. The coverage was based on an ocular estimate and was recorded to the nearest 5%. Within the 2x2 meter sample plot, thirty 5x5 cm (diameter x depth) soil sample cores were taken using a sharpened 5 cm diameter (2”) PVC pipe. A cutting edge was ground on one end of the PVC pipe to facilitate plunging it into the marsh bed. I collected a total of about 7.6 liters (2 gallons) of soil material. The depth of the soil cores was selected because it best reflected the most recently settled seeds used for recruitment (Baldwin et al., 1996; van der Valk and Davis, 1978; Keddy and Reznicek, 1982). The soil samples were stored on ice in the field before being stored in refrigeration (2 - 4°C).

Sixteen 1x1 meter survey plots were established every five meters along twenty-meter transects that extended in all four cardinal directions from the sample plot. All the vegetation was identified to the species level and their percent coverage recorded in the same way as the sample plot was surveyed. I will use the term sample plot to refer to the 2x2 meter plot from which soil samples were taken, and survey plot to refer to the 16 1x1 meter plots where only species cover was surveyed.

Seed Bank Study:

The soil samples were kept refrigerated to reduce the chance of seed germination until all soil samples were collected, and the seed bank study could begin (Baldwin et al., 1996, van der Valk and Davis, 1978). The refrigeration was also intended to increase the germination for seeds that may require some stratification to break dormancy (Baldwin et al., 2007). Stratification is a process where through a period of cold promotes the end of an embryonic dormancy phase and allows the seed to germinate (Baldwin et al., 2007). The normal refrigeration temperatures of 2 to 4°C were not considered abnormal for a Louisiana winter in the coastal zone where temperatures rarely dip below freezing. The seeds were kept in those conditions for between 4 to 7 weeks. The temperatures and time of storage were deemed to be adequate to break their dormancy, as the duration of Louisiana winters in the marsh are very short and comparatively mild.

I processed the collected soil samples by removing all the large stems and roots. This procedure reduced the potential for vegetative establishment and allowed the seed bank study to focus on the seeds. A soil sample of 45 ml was spread in a thin layer upon a 200 ml sand base in labeled germination cups. The resulting soil sample layer measured approximately 1 cm in depth (Baldwin et al., 1996; van der Valk and Davis, 1978; Keddy and Reznicek, 1982). The soil samples germinated seedlings over a 28-day period that took place from the 27th of July to the 23rd of August 2014 at the University of Louisiana at Lafayette Ecology Center greenhouses. The greenhouse provided a controlled environment reducing the chances of contamination due to precipitation or wind. The study tested eight environmental conditions each with four replicate pools. The soil samples from the 16 study sites were represented in each replicate pool in a randomized pattern. Therefore, the seed bank experiment had a total of thirty-two replicate pools and 512 germination cups (8 conditions x 4 replicates x 16 sites).

The soil samples in the seed bank experiment were tested with salinity levels at 0, 5, 10 and 20 parts per thousand (ppt). A common artificial seawater mix (Instant Ocean®, Aquarium Systems Inc., Mentor, Ohio, USA) was used to create the different salinities. I also subjected the soil samples to either flood or non-flood conditions. The flood treatment samples had a constant 4-5 cm depth of water above the soil surface. The non-flood treatment maintained the water level 1-2 cm below the soil sample surface. These pools were checked every other day for 28 days. At each visit, the first and second new plant germinations were recorded, water levels corrected and salinities adjusted.

At the completion of the 28-day germination period, the seedlings were counted, recorded, and transplanted into prepared growth trays. The growth trays were labeled with the CRMS site number and contained non-fortified potting soil. The growth trays were prepared at the beginning of the 28-day study and no seedlings were ever found during that period. Hence, the potting soil was considered not to be a source of any seeds or produce any seedlings that could confuse my species identification data. The potting soil was kept moist with fresh well water. The growth trays were maintained and periodically supplied with a mild nutrient supplement (Miracle-Gro®, The Scotts Company LLC, Marysville, Ohio, USA). The seedlings were maintained until being identified to the species level (Baldwin et al., 1996). After six months, all the remaining unidentified plants were identified to the genus level. The labels for three seedlings had faded making the site unknown. The seedlings were known to belong to one of the fresh sites, but certainty of which site was the problem. These unassigned seedlings would not have altered the study's findings or overall results. Therefore, they were excluded from the results. Only 65 of the 208 (31.3%) of the seedlings had to be identified to the genus level, all other seedlings were identified to the species level.

Statistical Methods:

The data collected from the seed germinations and the seedlings later identified provided seed germination and species richness data. The collected data was tested on how salinity, water level and the marsh type, from which soil was collected, for their effects upon the variation in the germination and species richness data. The factorial model combinations of the three covariates were compared using Akaike Information Criterion (AIC). The model with the combination of covariates that best explained the variation of the data earned the lowest AIC value. Only models with a ΔAIC value less than 7 were considered in the analysis.

The selected models were tested for significance of variation between each covariate and the variance data within each covariate. The salinity covariate had four levels (0, 5, 10 and 20 ppt), water level had two levels (flood and non-flood) and marsh type had four levels (fresh, intermediate, brackish and saline). Because the data contained an extensive number of zeros, the data failed the requirements of normality. Hence, a Poisson model with a log-linked function was used to test the significance between each of the levels within the model. The Poisson model was run for all the factorial combinations of the salinity, water level and marsh type using R version 3.1.1. The data from the seed bank study germination and species richness counts were processed in this way. Significance was measured by finding a P-value < 0.05 between the tested intercept and the next level of data. All of the main affects and the interactive affects of the models were tested for significance.

I tested the species data for similarity in the data between the species found in the CRMS data and the species observed in the field, and between the species found in the field and species observed in the seed bank with the Wilcoxon signed-rank test. The Wilcoxon signed-rank test had to meet three assumptions; 1) data can be paired and came from the same population, 2) each pair was chosen randomly and independently, and 3) the data were at least ordinal. If the test found support for the null hypothesis with a P-value > 0.05, the test indicates the two tested sets of data had a similar shape and distribution.

Knowing the diversity and evenness of species within a plant community was important in being able to compare the study sites. A comparison of species found in the data was used to estimate potential dispersal distances. A Simpson Index was calculated for the species found in the sample plots, the survey plots and my seed bank study. The Simpson (1949) index was used to explain and compare the species diversity and to a lesser extent evenness of individual marsh sites and marsh types. The values of the index increased as diversity (D) decreased and it provided a probability value on a scale between 0.0 and 1.0

where, $D = 1 - \frac{\sum_{i=1}^{S} n_i(n_i-1)}{N\,(N-1)}$ (DeJong, 1975). In the equation, S was the number of species, n_i was the number of plants of a species, and N was the total number of individual plants (DeJong, 1975). The calculation represented the probability that two individuals, picked independently and randomly from a population, belonged to different species (DeJong, 1975).

The number of days to the first and second germinations was tracked. All the records with zeros were suppressed. The mean days of the first and second germination were calculated for each treatment combination.

Seed Dispersal:

Species identified from the seedlings were classified for dispersal according to where its nearest possible parent may be found. The locations of the potential seed parents were classified as resided in: 1) the sample plot, 2) the survey plots, 3) the CRMS site, or 4) outside the CRMS site. A simple presence absence evaluation of species found through my seed bank study with lists created through the field surveys and the CRMS data

RESULTS

I collected 336 individual seedlings from the 512 treatment cups used in my seed bank study and identified 17 different species. Seed banks from the fresher marshes germinated more seedlings than those from saltier marshes. My results found that salinity was the most important factor driving seed germinations and species richness. Water level and marsh type were only significant when salinity levels were zero. My study was able to use the species richness data to approximate the dispersal distances of seed from their parental seed provider data.

Seed Germinations:

AIC scores for models combining the three variables of salinity, water level and marsh type were compared to find the model that best describe the variation in the response of the soil samples with seed germinations (Table 2). The model that best explained the variation in the data included all three variables and their two-way interactions. The second and third models with scores of 0.36 and 2.61 respectively and meet the ΔAIC of 7 threshold value.

The models used a reference of 5 ppt salinity, fresh as the marsh type, and non-flood for the water level to see some of the interactions only seen in the 0.0 salinity treatments. The model that best explained the variation in the seed germination data used all three factors and their two-way interactions (Table 3). The statistics clearly showed that salinity was the most dominant factor. The limited number of germinations found in the salinity 20 ppt treatment and the saline marsh types could have affected the significance calculations. There was significance between the flood and non-flood water level conditions and the marsh types were able to explain significant variation in the data. The two-way interactions of the salinity treatments and water levels only showed significance when salinity was at 0.0 ppt. None of the marsh type interactions with salinity showed any more significance. Only intermediate marsh types showed significance in the flooded water level conditions. The second best model to explain variation in the seed germination data used the salinity and water level interaction with the additive affect of marsh type (Table 4). Salinity 20 ppt treatment did not show any significance. The salinity treatments show significance in the other three treatments, and significance for all marsh types. Only salinity 0.0 ppt in the flood water level condition showed significance, otherwise there was no significance between the flood and non-flood water level conditions. The third and last model to be considered for analysis tested the marsh type and water level interaction with the additive affect of salinity (Table 5). This model showed the differences seen between flood and non-flood were the most significant. All levels of salinity, except salinity 20 ppt, and the four marsh types showed significant differences in the data. Only brackish seed banks in 0.0 ppt salinity almost showed significance with all the salinity treatments and the marsh types. In conclusion, the evaluation of all three of these models showed that salinity was the most dominant factor in seed germination data. Marsh type also showed significant effects, but only when salinity was absent. The water level conditions were only significant when salinity was held as an additive affect or at the 0.0 ppt treatment.

A summary of the seed germination data (Table 6) showed seedling count totals for each site under all eight studied treatments. The soil samples from fresh marsh sites were able to significantly generate more seedlings than any other marsh type. The soil samples that generated the most seedlings were from the fresh marsh sites with a mean value of 44.75 seedlings site^{-1} (Table 6). Intermediate and brackish marsh sites, with mean values of 31.33

and 12.20 respectively, showed a steady decrease to the saline sites mean value of only 0.50. Seed germinations in the two different water level treatments showed a similar pattern with the fresher sites germinating more seedlings than the saltier sites. Most marsh types showed better seed germinations in the non-flood treatment than the flood treatment, with the only exception found in the saline marsh type. Typically, the fresher marsh types had more germinations than the saltier marsh types. The only exception was that the fresh marsh type had a slightly smaller mean value for seed germinations than the intermediate marsh sites in the flood treatment and in the marsh type and water level interaction. Almost twice as many seed germinations were found in the soil samples under the non-flood treatment than those in the flood treatment by 219 to 117 (Table 6). The significance of the salinity effects upon seed germinations for each marsh type can be seen by the mean germinations found in the 0 ppt salinity treatment and the means steadily reduced as the level of salinity increased. A soil sample from CRMS0581 was the only site to germinate any seedlings in the 20 ppt salinity treatment, and only two seed germinations were found in any soil samples from a saline marsh site. Once germination conditions were met, seeds usually germinate within a few days (Espinar et al., 2005; Ungar, 1996; Thompson, 1979). My soil samples produced the mean days to their first seed germinations were mostly near the end of the second week, and any second seed germinations followed and mostly during the third week (Figure 3). This basic pattern of days to the first and second germination was consistent for all the marsh sites in all the treatments.

Species Richness:

The species richness data gained from the seedlings that survived long enough and grew large enough from the seed bank study for identification were represented in 17 species.

Two of the 17 species were not found in the list of 60 species (Appendix A) amalgamated from my field surveys. I used AIC to find the most parsimonious model that best explained the variation. AIC compared different combinations of salinity, water level and marsh type as predictors for the response of species richness. The lowest AIC score (Table 7) found the model using the water level and marsh type interactions with salinity as an additive effect had the lowest AIC score (Table 8) and was the best fit for the data. The models were tested using varying lines of reference, but they all told the same story. The reference that best showed the differences in the data was with salinity at 0.0 ppt, the water level in non-flood and a fresh marsh type.

Salinity showed to be the most determining factor in finding significance in the data for all the models. The best-fit model (Table 8) showed no significance between the 0.0 ppt and 5 ppt salinity treatment, but significance between the other salinity treatments. Significance was found between the water level conditions. Significance was found between all the marsh types except the saline marsh type. Data was very scarce for the saline marsh type and mathematical calculations could have been hampered. Only the interaction between the intermediate marsh type and the flood condition showed significance. The second model (Table 9) only used salinity and marsh type as linear effects. All the salinity treatments, except 5 ppt, and the marsh types showed significant differences in the data with the reference. The third best model (Table 10) tested all three variables as linear effects. The model showed the same lack of significance for the 5 ppt salinity treatment and no significance existed between the two water level conditions. The fourth and last model considered had the salinity and water level interaction with marsh type held as an additive effect (Table 11). The model showed how flooding showed not significance in the presence of salinity above 0.0 ppt.

I created a summary of all the data collected from the CRMS data, field site and my seed bank study (Table 12). While dominant species were used as the most distinguishing characteristic in the marsh type classification process, the salinity information does provide a good foundation when comparing how individual sites and marsh types responded to the tested treatments. The mean salinities generally fell within the normal expected ranges for the marsh type classifications and annual maximums sometimes reached many times higher than the annual mean value. Frequency and period of the fluctuations were not a part of my research, but the frequency and period of salinity fluctuations does make a difference in the vegetation found in a marsh. Therefore, the vegetation was used as the most distinguishing characteristic in the initial and final classification of the study sites. The mean species richness found in the CRMS data was higher than the mean species richness found at the field study sites and in the seed bank study. The higher species richness found in the CRMS data should be expected as it is generated from a larger area. The mean species richness values followed a consistent reduction from the fresher to the saltier marsh sites in the CRMS data, the field site and in the seed bank study.

Simpson indices were calculated for the species richness found in each sample plot, the combined survey plots and the seed bank study (Table 12). When space on the chart became limited, the column showing the Simpson index values became labeled as “index” for the data column to its left. The mean Simpson Index for each of the marsh types showed the fresher sites had higher scores than the saltier sites in each category of sample plot, survey plots and seed bank study. The mean Simpson Index scores of the fresh marsh sites for the sample plot and survey plots were 0.78 and 0.82 respectively. The mean Simpson Index scores decreased, as the general salinity of the sites increased, with the saline sites only getting a mean index of 0.03 for the sample plot and a 0.38 for the survey plots. The highest Simpson Index in the seed bank study was found in the fresh marsh calculation with a score of 0.67 and down to 0.0 for the saline sites.

Two fresh and one intermediate CRMS sites (3054, 463 and 188) consistently had the highest number of seedlings under all treatments (Table 12). *Eleocharis spp.* was the most prolific species found under the tested conditions. Two seed bank species, *Eleocharis equisetoides* and *Cyperus esculentus* (Appendix A)*,* were not seen in any field site. Intermediate marsh site CRMS0188 yielded 57 seedlings with *Eleocharis spp.* and *Sagittaria lancifolia* being the most common (Appendix B). CRMS0188 site had the lowest annual mean salinity of the intermediate sites and the field site was dominated by *S. lancifolia*. Fresh marsh site CRMS0463 yielded 51 seedlings with *Bidens laevis*, *Eleocharis spp.,* and *S. latifolia* as the most common species. CRMS0463 site was dominated by *Leersia hexandra*, *S. latifolia*, *Typha domingensis* and *B. laevis*. Ninety-four seedlings of eight species were germinated from the fresh site CRMS3054 soil samples. The most common species were *Ludwigia spp., Typha spp.,* and *S. lancifolia*. CRMS3054 also had the lowest species richness of the fresh marsh sites where *S. lancifolia* and *T. domingensis* dominated the field site. Some individual intermediate sites had nearly the same species richness and Simpson indexes as the fresh sites. Intermediate site CRMS0520 had noticeably lower species richness found in the seed bank study, the survey plots, and in the sample plot (Table 9). Brackish CRMS0399 and CRMS0581 showed rather strong species richness and Simpson Index scores.

Dispersal:

The data was evaluated for seed dispersal by determining where species were found, as well as where they were not found. Most of the species seen in the sample plot were not found in the seed bank study (Table 12). The mean species richness for the fresh sites was 9.75 and the mean number of species not seen in the seed bank study was 7.0, and none of the species seen at saline marsh sites were reflected in the seed bank study. It was also less likely a species found in the seed bank study was also seen in the survey plots. Only a species found in the seed bank study for the fresh marsh sites was more likely to have also been seen in the survey plots. The mean value of species found fresh marsh site of their seed bank was 6.0 and only 1.75 species were not seen in the study area. All other marsh types had less of their species found in the seed bank study than were seen in the survey plots.

The species found in the seed bank study were compared with the species surveyed within the sample plot, the survey plots and the CRMS data (Table 13). The closest match of the species determined the closest possible parent seed provider. The comparison was a simple closest proximity evaluation. After combining species records to be able to make inferences for the entire coastal zone, there were 41 records of species grouped by marsh type. The analysis found that about 75% or 29 of the 41 species records showed a possible parent within the study sites. My data showed that 8 species records, with at least one record in each marsh type, could have had their parent seed provider reside outside the CRMS site.

DISCUSSION

My study has attempted to estimate how different marsh type vegetation could react to changes in their abiotic conditions of salinity and water level. Soil samples collected from randomly selected sites of each of the four marsh types provided data on how they may react when confronted with new levels of salinity and water level stress. The compiled results from the seed germination count data and the species comparisons were used to determine seed dispersal distances. The results from my study could be used to make inferences about the entire Louisiana coastal zone.

Seed Germinations:

Salinity was clearly found to be the most dominant factor driving seed germination in my study, and the marsh type and water level interactions with salinity were only significant or almost significant when the salt was absent. The odd results found in the data for saline marsh types and results from the 20 ppt treatment were probably a manifestation of the number of zeros found in the data effecting the calculations. The data clearly showed that the water level and marsh type variables were only able to create significance in the data when salinity was absent.

The seed bank samples subjected to the 0.0 ppt salinity and non-flood treatments consistently generated more seedlings than any other treatment for all marsh types and CRMS sites. In general, the fresher sites had more seedlings than the saltier marsh sites. The trend could indicate support for my original hypothesis that the seed banks subjected to less stress would be able to generate more seedlings. However, germinations from the fresh marsh sites in 0.0 ppt and flood treatment generated less plants than the same soil samples in 5.0 ppt or the intermediate sites. The low number of germinations by the fresh marsh type in

0.0 ppt and flood treatment revealed that high water levels could adversely affect fresh marshes more than other marsh types under similar conditions. The flood regime does allow submergents to thrive in flooded conditions, emergents in periodically flooded sites, and flood-tolerant annuals in drawdowns (Mitsch and Gosselink, 2007). I did see some species, like *Ludwigia spp.* and *Polygonum punctatum*, which usually characterize drawdown conditions.

The saline and brackish seed banks did not generate many seedlings. Saline and brackish marshes do not usually maintain seed banks (Thompson and Grime, 1979). Most saline and brackish marsh plants are perennial and therefore opt for the less risky strategy of asexual reproduction and expands vegetatively. Seeds usually are developed and not deposited until conditions are favorable and germinate quickly. Several environmental factors have to be correct for a seed to successfully germinate. Most wetland species seeds will have a prolonged period of dormancy once released from their parent plant (Bliss and Zedler, 1998). Seed dormancy permits seeds to wait and germinate when local conditions become more suitable (Bliss and Zedler, 1998). Examples of the cues that can break dormancy include changes in moisture, temperature, light, or salinity. Some seed may require a process called stratification. Stratification requires a seed of some species to have a period of cold before germination (Schutz and Rave, 1998). A similar process is scarification. Scarification could be caused by chemical changes in the soil or by passing through an animal's digestive tract (Mueller and van der Valk, 2002; Reinecke and Hartke, 2005). These processes have evolved to increase germination rates for some species by weakening, scaring, or breaking their protective coating of the seed. Temperature and light were conditions not addressed in my study, but could account for some of the variation in the number of species being expressed in my seed bank study by some marsh types. Other studies have found that the optimum temperature for *Phragmites communis* to germinate was 20°C (Gorai et al., 2006). Hence, normal summertime temperatures of 30-35°C under which my study was conducted may have been too high for some species to germinate. The proper light cycle or temperature may never have been reached to make the most conducive conditions for seed germination. It was entirely possible that there were many viable seeds in many of the soil samples.

Only two seedlings were found in soil samples taken from saline marshes, and both occurred in a flood treatment. None of the marsh types showed any significant advantage when their seed banks were subjected to salinity treatments of 10 or 20 ppt. The water level conditions did not have an additive effect when salinities were at 10 or 20 ppt. Others have found that high salinity conditions tend to inhibit germinations, even for some halophytes (Gorai, 2006; Espinar et al., 2005; Ungar, 1996). I tested salinity levels regularly during the study to insure consistent saline levels were maintained throughout the study. I found that the salinity measurements of the non-flood treatment pools generally fluctuated more than the pools in the flooded treatment. The 20 ppt salinity in the non-flood treatment tended to fluctuate most of all. The salinity fluctuations were usually about 10% of the target salinity. The result of my study agreed with those findings as more seedlings were found when salinity conditions were at the lowest levels. The germination data showed a clear advantage of soil samples exposed to lower salinity and non-flooded conditions (Figure 4) (Baldwin and Mendelssohn, 1998). The data strongly favored seed germinations found in the fresher marsh sites over saltier ones, and the two seedlings from saline soil samples were in 0.0 and 5 ppt salinity treatments. Even species that were more tolerant of saline conditions were found to have higher germination success when exposed to lower salinity levels during the germination period (Espinar et al., 2005). The four sites with no seed germinations were from either a brackish or a saline marsh site. Salt and brackish marshes are often perennial dominated and monotypic systems where most reproduction is asexual or performed through vegetative expansion (Mitsch and Gosselink, 2007). They usually produce high numbers of seeds, but quickly germinate as conditions dictate. Therefore, it appears that high salinity marshes may not maintain seed banks.

Species Richness:

Salinity again was determined to be the dominant factor. Salinity and marsh type consistently showed significance in the models when considered as linear variables. The only interaction to show significance was the flood condition in for intermediate marsh seed banks. This showed how salinity and water level could equalize conditions for species and only a short list of species may perform under the high conditions presented through my study.

The species found in many of the soil samples did not reflect the species seen in the corresponding sample plot. Intuitively, one would expect that most of the species found in the seed bank study would have been seen in the sample plot or in one of the surrounding survey plots. Yet, this was not always the case. Only the fresh sites had a higher than average chance of finding species in the survey plots. The evaluation of the species richness data collected in the field and through my seed bank study began the process of developing data to determine possible dispersal distances. The identification of species found in the individual seed banks helped determine the possible seed dispersal distances, but it is sometimes equally as important to find out where the species were not found. My study showed support for seeds having difficulty germinating when all the vegetation was cleared and in flood conditions (Baldwin and Mendelssohn, 1998). While the number of seedlings was lower under flooded conditions, my research showed that the species richness was not significantly different between the flood and non-flood treatments. Many studies have found that wetland seed banks should reflect the dominant surface vegetation (van der Valk and Davis, 1978; Leck and Graveline, 1979; Thompson and Grime, 1979; van der Valk, 1981; Hopkins and Parker, 1984). My study found that the species present in the sample plot reflected very few of the species identified in their seed banks. Expanding the area to the entire study site, only the fresh marsh sites were more likely than not to express the same species found in their seed bank. My findings agree with those of previous studies. The limitation of time could have kept my seed bank study from continuing through the primary period of germination for many species, the spring.

The differences in life histories found in wetland plants could explain some of these differences in the findings of my study. Plants, like *S. lancifolia* have been documented in fresh to intermediate marsh types, have mostly negative growth responses to increases in salinity (Baldwin and Mendelssohn, 1998). The negative growth responses of more salt tolerant species, like *Spartina patens*, to salinities and water levels increase could indicate how a marsh might respond newly cleared or created soil surfaces become available for colonization (Baldwin and Mendelssohn, 1998). My results support the fact that most plants had negative responses to higher salinities and water levels. My data also illustrated some of the variation in the responses different seed banks could exhibit when confronted with changing environmental conditions.

The more saline sites often had a few species that were found in great abundance throughout the study sites. Fresher sites universally had greater diversity and evenness. For example, *Polygonum punctatum*, *Sagittaria spp.* and *Typha spp.* were nearly universally found in all the fresh site seed banks and survey plots. Some variety of *Eleocharis spp*. was found in most sites regardless of the marsh type. The brackish or saline marsh types mostly had only a few dominant species including *Spartina spp.* or *Schoenoplectus spp.* with *Distichlis spicata* often interspersed. The most common fresh species seen in the field also made a strong presence in the seed bank study, but the common or even dominant halophytic species did not. The common denominator for all the species that did appear in my seed bank study was the ability to produce a large number of small viable seeds.

My field surveys often showed a small presence of *Eleocharis spp.,* but *Eleocharis spp.* was seen with much higher frequency in many of my seed bank study soil samples. Similar quicker responding pioneer species often persist in seed banks because only a few plants are needed to produce numerous small seeds. The seed from these species can remain viability for a longer period of time (Mitsch and Gosselink, 2007; Thompson and Grime, 1979). They only germinate when new open areas become available (Schutz and Rave, 1998). Therefore, the finding of non-dominant or weedy species in the seed bank study was not an unexpected discovery (Hopkins and Parker, 1984).

Dispersal:

Plants tend to follow two principal dispersal strategies (Mooney and Drake, 1986). First, a short-range strategy where the population makes steady advances through rhizome expansion or short-range seed dispersal. The second involves the dispersal of seeds that can quickly colonize new areas with small numbers of individuals sometimes at longer distances

(Mooney and Drake, 1986). The second of these colonizing methods seems to be most prevalent in North America (Mooney and Drake, 1986). Invading species most commonly use the same strategy, as they usually arrive with only a few individuals. These new pioneer species are initially rare and will encounter numerous barriers before they can become successfully establish (Mooney and Drake, 1986). These newly arriving and extremely small populations can be easily missed in normal sampling procedures. Often, the effects of genetic, demographic and stochastic environmental events will occur and the small or newly established population could be eliminated (Shigesada and Kawasaki, 1997). The same stochastic events may also create the changes necessary to provide those same small or rare species with the opportunity to become established and spread (Shigesada and Kawasaki, 1997). For these reasons, many of their seeds may persist in a seed bank waiting for more favorable conditions. Once the proper conditions are met, the population could expand. My research suggests how species could have been present in the field but not detected in the survey, or had persistent seeds in the seed bank and was not found in the seed bank study.

Plants have several strategies to take advantage of opportunities for survival. One of those strategies is to establish a seed bank. There are four types of seed bank relationships that have been used to illustrate the different ways seed banks respond to their abiotic and biotic conditions (Thompson and Grime, 1979). A Type I seed bank is common among a number of grasses and results in a limited and ephemeral seed bank. The seeds are dispersed during the warm weather germinate during the cooler and moister conditions found later in the year (Thompson and Grime, 1979). The character of these seeds is to germinate soon after they fall and consequently do not result in a persistent seed bank (Thompson and Grime, 1979). Type II seed banks are created throughout the summer and autumn with a winter dormancy pattern and with plants emerging in the warmer temperatures of the following spring (Thompson and Grime, 1979).

Type III is similar to Type I in that many seeds germinate soon after falling, but Type III seeds have the ability to persist and create a seed bank and wait for future opportunities to germinate (Thompson and Grime, 1979). Type IV seed banks have seeds that rarely germinate immediately after dispersal and maintain a considerable seed bank over time (Thompson and Grime, 1979). Any of these strategies can become dominant in the right conditions, and all can be found in wetlands and marshes as well. Spatial and temporal changes cause a great deal of variability in wetland seed germinations (Leak and Brock, 2000). Sometimes seed banks have been found to have the ability to reduce their seed production as a response to competition (Thompson and Grime, 1979). Perennial plants do not always need to reproduce sexually. Perennials can expand through vegetative growth using rhizomes in response to competition. My study provided sufficient resources and space to keep competition from being a factor.

The seed banks from the studied fresh and intermediate marshes mostly exhibited abundant seed banks and could be best described as a Type IV strategy. Perennials dominated these marsh types and have vegetative expansion capabilities, but their seed banks often showed the greatest species richness. There were also species that appeared to have dispersed from outside the field study site. The brackish and saline marsh site samples could be exhibiting Type II transient seed bank behavior. Seed can be found in abundance on vegetation in brackish and saline marsh vegetation. These seeds are often important food for wintering waterfowl. Perennials dominate brackish and saline marshes. These marshes have the opportunity to pursue the less risky asexual vegetative expansion strategy along with the added benefit of seed dispersal by wintering waterfowl.

The presence of a viable seed bank can allow for a quicker recovery after a disturbance. The interaction between disturbance, competition and stress are three conditions that should also be included in this discussion that will often dictate which species will succeed (Grime, 1977). J. P. Grime introduced a triangle with these three conditions to explain the primary strategies in plants (Figure 6). After a major disturbance, early pioneer or ruderal species will usually appear first on the landscape (Grime, 1977). Because resources would be readily available, competition would not be much of a factor, availability of open space would be considered high, and some low stress factors could be present. As time progressed, other species would begin to emerge and competition would become more acute. My research presented my seed banks with a highly disturbed soil surface and variable ranges of stress. Ruderal plants in general should have had the best strategy for dealing with a high disturbance and low stress environment. Ruderals are often annuals and pioneer species that can quickly react to a newly available location caused by disturbance. The mix and extent my research tested the abiotic factors of salinity and water level that, provided data for how other species in similar conditions may perform.

Seeds could have travelled distances over several hundred meters before settling into a seed bank. The evaluation of the closest proximity of a possible parent seed provider showed that it was more likely that a species found in my seed bank study had a potential parent seed provider within the study sites. There were species in each marsh type that did not have a match of a species from any one of the three species lists. Hence, those species could have travelled several hundred meters to the seed bank they were found. Of course the parent seed provider could have been an annual or simply died before I made my field surveys. Unfortunately, there were few species in my study that were also modeled by the LAVegMod (Table 14). The determination of seed dispersal distances found through my study is anticipated to be of value in future modifications of LAVegMod or similar programs. The results from my study show that in LAVegMod *Eleocharis* spp, *Sagittaria* spp, and *Typha* spp can establish from seed under the right environmental conditions.

Conclusion:

The responses of Louisiana marsh seed banks will vary widely under varying conditions of water level and salinity caused by the change in the relative sea level. Seed germinations responded best in seed banks exposed to no salinity and non-flooded conditions. Salt marsh soil samples showed the presence of little or no seed bank, with only a few seed germinations found at lower salinities. The species richness did not differ significantly between flood and non-flooded conditions. Some species showed a propensity to travel long distances, over hundreds of meters, and remain viable. The challenge for the Louisiana marshes will be to have the ability to adjust fast enough in a potentially very quickly changing environment of increased salinities and higher water levels. Until water flows and sediments can be restored to effectively nourish the Louisiana coastal zone, the marshes will not have the tools necessary for it to survive alone. My study has become a part of the collection of data to better understand how plants can change their dispersal strategies and distribution under these changing conditions.