2016

Determining Factors that Influence Smooth Cordgrass (Spartina alterniflora Loisel) Transplant Success In Community-Based Living Shoreline Projects

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DETERMINING FACTORS THAT INFLUENCE SMOOTH CORDGRASS (*SPARTINA ALTERNIFLORA* LOISEL) TRANSPLANT SUCCESS IN COMMUNITY-BASED LIVING SHORELINE PROJECTS

by

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A thesis submitted in partial fulfillment of the requirements
for the Honors in the Major Program in Interdisciplinary Studies,
in the College of Undergraduate Studies
and in The Burnett Honors College
at the University of Central Florida
Orlando, Florida

Spring Term, 2016

Thesis Chair: Dr. Linda Walters

ABSTRACT

Efforts to mitigate shoreline erosion through living shoreline methods along the USA Atlantic seaboard have often incorporated the cultivation and transplantation of smooth cordgrass, *Spartina alterniflora*. Assessments of these transplants at several sites in the Indian River Lagoon have shown that survival is variable after a year (survival: 10-93%). Lower survival has been attributed to environmental variables such as dislodgement by wave energy, and transplant shock due to salinity changes from cultivation to estuarine conditions. To improve living shoreline projects, we examined the effects of cultivation salinity (0 ppt, 15ppt) on transplantation success, and the success of anchoring plants to biodegradable mats (Jute mesh, 5 individuals per 50 cm^2) and utilizing oyster bags as breakwaters in facilitating reestablishment of new transplants. *Spartina alterniflora* individuals were grown under salinity treatments for 20 weeks; plants grown in 15 ppt produced new shoots with significantly greater heights than those grown in freshwater. The plants were then transplanted to two sites in the IRL, and monitored after four weeks. After four weeks there was a greater net increase in stem density and larger decrease in plant height for plants grown in 15 ppt. Jute-mesh mats and oyster bags did not impact growth or survival of transplants. Low-saline (15 ppt) conditions increased shoot growth of the project by 50% in four weeks at a cost of 30 cents per additional shoot produced by an individual. Longer-term monitoring will determine if benefits persist or decrease over time, and if the cost is justified by the benefits.

DEDICATION

I would like to dedicate my thesis to my parents, thank you for your encouragement and continual support, I would not have been able to go into higher education in the United States without it. And for my past and present professors and mentors, who have served as guides and helped me grow academically and personally to one day become an exceptional scientist.

ACKNOWLEDGMENTS

I wish to thank the volunteers with the Mad Scientists' Research Society that assisted with collection of *Spartina alterniflora* plugs for this research: Heidi Waite, Ricardo Torres, Bowen Ding, Katie Tutt, John Weng, and Alexis Ghersi. I also want to extend a thank you to Andrea Carrion, Maria Barragan, Samantha Mensah, Alberto Carrion, and Suzanne Connor for assistance in experiment set-up. Thanks to Elissa Zapata and Kelly Kirk from Knights for Marine and Wildlife Conservation for helping with data collection. I wish to thank my professor, Dr. Linda Walters for guidance and supporting me through not only the thesis process but also in numerous academic endeavors. I also want to thank my committee members Dr. Melinda Donnelly and Dr. Peter Jacques for their guidance and suggestions throughout the course of this thesis. This research was made possible through the use of the UCF Biology greenhouse facilities, and Fellers House Field Station and Seminole Rest Historic site under management by Canaveral National Seashore (National Park Service).

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INTRODUCTION

Coastal erosion presents a multi-million dollar economic problem to a growing population of residents along shorelines (Heinz Center, 2000). Economic impacts arise from high costs required to remediate erosion damages through management and restoration efforts. These costs are expected to increase in the future as coastal erosion damages are exacerbated by changing climatic conditions resulting in more frequent storms and sea-level rise (Hanson and Lindh, 1993; Leatherman et al., 2000; Zhang et al., 2004). A large part of the U.S population is already burdened by this coastal erosion problem as 123.3 million people in the United States in 2010 (39% of the total population) resided within coastal shoreline counties (NOAA, 2013). The number of people affected by coastal erosion is expected to rise and population trends published by NOAA (2013) estimate an 8% growth in these coastal counties by 2020, resulting in a total of 134 million coastal residents. The Heinz Center (2000) predicts this rising population should expect, over the course of sixty years, a 25% loss of homes located within 500 feet (152 m) of shoreline due to coastal erosion, and determined damages related to coastal erosion would surpass $530 million annually. The projected costs and rising number of people affected by coastal erosion has galvanized governmental and community efforts to produce improved coastal erosion mitigation methods. These efforts have included the creation of novel coastal infrastructure, and non-ecologically intrusive alternatives that employ natural and artificial infrastructure to mitigate coastal erosion.

Hard-Armoring and Ecological Impacts

Erosion control methods have historically centered on hard-armoring coasts using bulkheads, breakwaters, jetties, riprap, revetment, and groynes to prevent erosion (Bulleri and Chapman, 2010; Charlier et al., 2005). However, an increasing body of research has demonstrated evidence for adverse, unintended environmental and ecological impacts of hard-armoring technologies. Bulkheads, for example, do not absorb wave energy, they instead reflect wave energy. This reflection causes scouring which erodes the shoreline and the structure itself, which increases adjacent water depth while decreasing intertidal habitat (**Figure 1**; Riggs and Ames, 20013; Douglas and Pickel, 1999; Swann, 2008; Bilkovic and Roggero, 2008). Bulkheads can also prevent inland migration of marsh habitat as an adaptive response to sea-level rise, which can have longer-term impacts (Bulleri and Chapman, 2010). Along with decreasing habitat, hard-armoring is linked to significant shifts in community assemblages on and surrounding these structures (reviewed in Bulleri and Chapman, 2010). Decreases in population densities of commercially important species such as blue crab, fish, penaeid shrimp, and anchovies, along with general decreases in species diversity, have also been documented (Seitz et al. 2006; Goodsell and Chapman, 2007; Pearson et al., 2000). While diversity of native assemblages decreases, invasive species have been known to exploit these artificial coastal infrastructures (Airoldi et al., 2005; Mineur et al., 2012). These impacts have shifted some interest from hard-armoring technologies to natural and hybrid infrastructure that integrate ecological impacts into planning and erosion control design such as "living shoreline" projects (**Figure 1**; Kochnower, 2015).

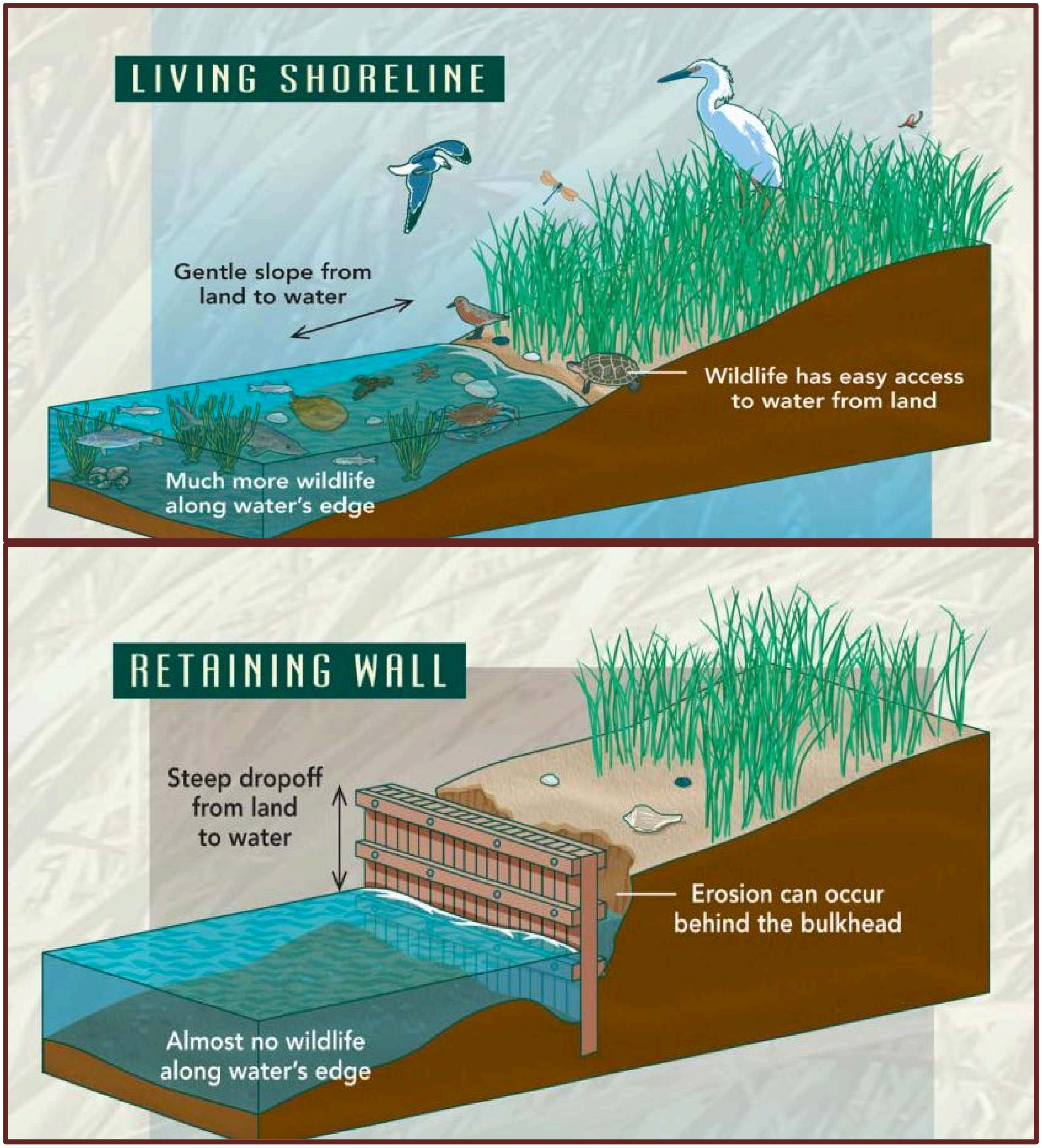

Figure 1. Depiction contrasting a living shoreline and hard-armoring. Courtesy of Frank McShane in collaboration with Partnership for the Delaware Estuary Inc.

Living Shoreline Theory and Methods

A living shoreline is defined by National Oceanic and Atmospheric Administration as a coastal erosion control method that works through "bank stabilization and habitat restoration techniques", and has become effective alternatives to hard-armoring in low-wave energy systems (Mitsova and Esnard, 2012). Living shorelines function on the premise that reducing hard-armoring technologies that can bring negative ecological consequences and increasing the use of cost-effective natural infrastructure, such as creation or restoration of natural vegetation, will bring positive ecological benefits along with the reduction of coastal erosion (Seitz et al., 2006; Subramanian et al., 2008). Artificial and natural infrastructures such as oyster reefs used as breakwaters provide three-dimensional structures that counter wave forces, resulting in reduction of wave energy (Meyer et al., 1997; Piazza et al., 2015; Manis et al., 2014).

Creating or restoring natural coastal vegetation of foundation species, such as *Spartina alterniflora* (or smooth cordgrass*),* also mitigates coastal erosion through direct mechanisms involving aboveground and belowground biomass. High stem densities attenuate wave energy and reduce wave height though creating friction and drag forces against tidal forces, and reallocating energy through the stem (Leanard and Luther, 1995; Knutson et al., 1982; Anderson and Smith, 2014). Roots also directly reduce erosion through stabilizing and strengthening shoreline sediment, and allow plants to resist tidal forces producing mechanisms for erosion abatement aboveground (reviewed in Gedan et al., 2010). *Spartina alterniflora*, commonly-used in living shoreline projects accounted for an 80% decrease in wave energy in a Chinese estuary, and an empirical model created by Knutson et al. (1982) demonstrated a 40% reduction in wave energy with 94% reduction in wave height over 2.5 m of vegetation (Yang et al., 2012; Knutson, 1982). In a Florida estuary, a combination of *S. alterniflora* and oyster reef habitat as part of a living shoreline design dissipated 64% of wave energy hitting the shoreline (Manis et al., 2014).

Overall, these erosion mitigation methods restore or maintain natural habitat, which preserve ecosystem services of these coastal ecosystems. These services include their role as nursery grounds for commercially or recreationally important species, pollutant and excess nutrient removal, and improvement in water quality (Rosaz et al., 2005; Davis et al., 2006; Nelson et al., 2004; Bilkovic and Mitchell, 2013). Living shorelines can also provide educational benefits where management is community-based or educational material is created, while the overall cost of implementing these methods reduces the associated cost of erosion mitigation (Swan, 2008).

Living Shoreline Methods in the United States

Various living shoreline designs and methods can be found in the United States. The method used by Swan (2008) to mitigate coastal erosion in Dauphin Island, Alabama, United States, included the use of *S. alterniflora* plantings and pyramidal concrete breakwaters called "coastal havens" that were designed to increase colonization by native eastern oysters. Their living shoreline method was successful in allowing sediment accretion at targeted shorelines, and increasing natural populations of eastern oyster (Swann, 2008). Several living shoreline designs along the Atlantic coast in states like North Carolina, Virginia, Maryland have hybrid methods including the use of stone sills and groins, and wooden breakwaters along with creation of marsh habitat (Burke, 2005; Currin et al., 2010). Others have used oyster reefs as a natural and effective breakwater to reduce shoreline erosion (Myer et al., 1997; Piazza et al., 2005; Currin et al., 2010; Scypher et al, 2011). The size of these oysters and height of reef have positively correlated to reduction of shoreline erosion (Scypher et al, 2011). In the southern Atlantic coast, mangroves have been deployed as part of natural vegetation restoration alongside the use of oyster reefs as breakwaters (Walters et al., unpublished data).

Living Shoreline and Hard-Armoring Cost Comparisons

Cost associated with these living shorelines have been documented in some studies. These costs vary depending on method and ratio between the use of artificial structures (e.g. cement or rocks for breakwaters) and natural "structures" such as oyster reefs and habitat restoration. In a study lead by Swann (2008), hybrid living shoreline (including artificial "coastal haven" infrastructure and habitat restoration) resulted in an expenditure of approximately US $335 per meter of shoreline. However, Davis and Luscher (2008) estimated that living shoreline costs can be less at US $150 per meter of shoreline when utilizing only natural materials. This contrasts the costs of hard-armoring coasts with bulkheads or revetments, which have average installment expenditures between US $630 and $752 per meter (Grabowski, 2012). Other assessments have placed the cost between $1800 to $7600 per linear foot, and even $3,000 to $10,000 per foot (Stamski, 2005; Caldwell and Segall, 2007). It is evident that living shoreline projects can be less expensive and cost-effective alternatives to hard-armoring coasts in areas when wave energy can be significantly reduced by living shorelines.

Living Shorelines in the Indian River Lagoon, Florida

Indian River Lagoon (IRL) is one of the most biologically diverse estuaries in the United States. It constitutes approximately 40% of the eastern coastline of Florida. The estuary is located within the ranges of salt-marsh species like *Spartina alterniflora*, and more tropical species like the red mangrove (*Rhizophora mangle*), which creates mixed mangrove and salt-marsh coastal ecosystems (Schmalzer, 1995). In this ecosystem, *S. alterniflora* occurs lower in zonation within the intertidal zone than *R. mangle*. Below the fringes of *S. alterniflora,* natural populations of oysters can be found. However, the lagoon has experienced losses of natural oyster reef populations, partly attributed to the negative effects of boat wakes that dislodge them and move them above the intertidal zone, eventually leading to their death (Garvis et al. 2015). Interests in mitigating coastal erosion and restoring oyster reefs in this area have focused on using living shorelines that are planned and designed considering ecological knowledge of this estuarine system.

Our research group's living shoreline design includes using the addition of eastern oyster shell as breakwaters in the lower intertidal zone, and transplantation of *S. alterniflora* and *R. mangle* in the middle and upper intertidal zones, respectively. To prepare for living shoreline projects oyster shells are collected from shucking facilities. These shells are then dried in the sun for a minimum of six months to ensure minimal chance of transferring any diseases to natural oyster populations. After this period, community and partner groups, along with our research group, create shell bags and oyster mats. Shell bags are created by filling mesh bags with loose oyster shells, and mats are made by vertically tying 36 shells to a 0.25 m^2 Vexar™ mat (mesh diameter of 1.5 cm) with cable ties. Mats and shell bags are then deployed to targeted shoreline areas where they are attached to each other with cable ties, and sprinkler weights. *Spartina alterniflora* plugs and *R. mangle* propagules are collected from the estuary and grown for a minimum of six months under freshwater conditions to allow for sufficient root growth before transplanting them in their respective zones behind oyster mats or bags. *Spartina alterniflora* plantings with the addition of stabilized oyster shell used as breakwaters have shown to decrease wave energy more then when these two methods are used separately (Manis et al., 2014). Overall, this vegetation restoration and stabilized oyster method has been demonstrated to be effective in attenuating wave energy, reducing coastal erosion, and increasing eastern oyster densities, despite low survival of transplants in some cases (Donnelly et al., unpublished data; Manis et al., 2014; Manis, 2013).

Improving Living Shorelines in the Indian River Lagoon

Oyster mats and shell bags have shown success in creating substrate for oyster recruitment leading to oyster reef restoration, while providing coastal shoreline erosion control (Garvis et al., 2015; Manis et al., 2015). *Spartina alterniflora* transplantation, on the other hand, has seen variable success. Assessments of these transplants at several sites have shown that survival is variable after a year (10%-93%), with a cumulative average survival rate of 70% (Donnelly et al., unpublished data). Lower survival has been attributed to environmental variables such as dislodgement by natural and boat-driven wakes, and transplant shock due to salinity changes from cultivation to estuarine conditions is also suspected. This has created interest in determining factors that influence the transplant success of this species, and use this knowledge to increase the effectiveness of our shoreline stabilization projects.

It is important to consider physiological and ecological constraints of *S. alterniflora* when setting guidelines for cultivating it for living shoreline projects. This species is a perennial C_4 plant that is considered a foundation species in salt-marshes of the Gulf of Mexico and Atlantic coast (Dayton, 1972; Gedan et al, 2010). It is found in the lower zones of the shoreline were it is exposed to high salinities through daily tidal inundation (Bertness et al., 1991; Pennings et al., 2005). In order to tolerate high salinity, this facultative halophyte employs a series of salt-tolerance mechanisms that include salt secretion through specialized glands, reduction of osmotic potentials, and increased tissue rigidity (Baisakh et al., 2006; Touchette et al., 2009). *S. alterniflora*'s ability to tolerate saline and sometimes anoxic conditions allows it to survive, and dominate in lower intertidal zone where other salt-marsh species cannot (Bertness and Ellison, 1987; Bertness, 1991; Pennings et al., 2005; Wigert and Freeman 1990). However, like most facultative halophytes, this species germinates at higher rates and has higher survival and growth in lower salinities (Witje and Gallagher, 1996; Carrion et al., unpublished data).

In current living shoreline methods *S. alterniflora* plugs are grown in freshwater pools for six months. Freshwater allows for increased belowground biomass, survival, and number of new shoots than when grown in high salinity (Carrion et al., unpublished data). Following this period, they are transplanted into targeted shoreline sites. It is suspected that while *S. alterniflora* has a remarkable ability to tolerate salinity changes, long-term cultivation in freshwater followed by sudden transplantation into higher salinities may result in transplant shock that can lead to lower survival. Certain halophytes like *S. alterniflora* can experience reduction in their salt-tolerance or avoidance responses when exposed to high salinities when they are not acclimated to high salinities and are older in age (Hwang and Morris, 1994; Touchette, 2009). It is also possible that stress created by a sudden shift from freshwater to estuarine conditions, along with transplant damage, can hamper growth of root biomass that traps and bind sediment, leaving transplants vulnerable to wave energy during the first weeks of transplantation. Although areas with established populations of *S. alterniflora* can reduce wave energy to allow other individuals to establish, many areas targeted for living shoreline projects have little or no established populations (Bruno, 2000). Thus, I hypothesize salt-stress and wave energy may be factors in transplant success.

It is possible that growing this species at salinity levels lower than those at estuarine sites can reduce salinity-induced stress when potted in the greenhouse for rearing, and after transplantation in the field. An optimum salinity level would allow for maximizing survival and growth during rearing, while still reducing chances of transplant shock after transplanting into targeted restoration sites. To facilitate reestablishment of *S. alterniflora* transplants, the use of a stabilizing substrate such as a "mat" to anchor transplants, similar to "oyster mats", can be a possible solution to dislodgment by wave energy. This method could prevent dislodgment and allow fine root hairs and overall root biomass to grow and hold transplants. A mat made from biodegradable geotextile mesh such as jute fiber could assist in *S. alterniflora* transplant reestablishment and require little maintenance as it would eventually biodegrade. Jute mesh has been previously used in soil erosion control and natural vegetation reseeding projects (Krenitsky et al., 1998, and Ghosh et al., 2003). Addition of stabilized oyster shell, which have been shown to reduce wave energy could have a positive effect on plant reestablishment after transplanting. Thus, I aimed to experimentally determine if: (1) Growth and survival of *S. alterniflora* plugs differ when reared in freshwater compared to low-saline conditions; (2) Transplantation success differ in *S. alterniflora* reared in freshwater or low-saline condition; (3) The use of biodegradable jute-mesh mats to anchor transplants subjected to wave energy has a positive impacts on *S. alterniflora* reestablishment; and (4) The presence of oyster bags used as breakwaters have a positive role in shoot height, stem density, transplant survival, and retention of *Spartina alterniflora* at restoration sites.

METHODS

Collection and Rearing

I tested the effects of salinity on *Spartina alterniflora* growth, survival, and asexual reproduction during rearing prior to transplantation into the field to later determine the effects of rearing salinity on transplantation success as part of the second stage of the study. I used rearing methods currently employed by various community-based living shoreline stabilization projects with the addition of salinity treatments. In late summer of 2015, I collected *S. alterniflora* individuals for rearing from seven robust and distinct strands of salt-marsh in Mosquito Lagoon, a micro-tidal estuary located in the northern section of the Indian River Lagoon system. Only individuals with recently initiated culms were collected in order to reduce the likelihood of plant senescence during winter leading to lower observed survival rates independent to the effects of rearing salinity treatments.

Following collection, these individuals were potted in 1-gallon pots with the local county yard-compost soil, and placed in outdoor pools (114 cm x 19 cm depth) of standing water with one of two salinity treatments: freshwater (0 ppt) or low-saline artificial seawater (15 ppt; Instant Ocean, Spectrum Brands, Inc., Atlanta, GA). This low salinity was chosen as it is medial to this species salinity tolerance and only a 10 ppt difference from ambient salinity conditions in proposed transplantation sites. Plants were grown under ambient environmental conditions and salinity treatments for 10 weeks to maximize rhizome, root, and aboveground growth prior to transplantation into field conditions. In order to maintain salinity levels, water salinity was measured three times a week and after rainfall events, and adjusted as needed. This was done three times a week during dusk when the majority of evaporation had occurred, and after precipitation events.

Transplantation Treatments

Two shoreline sites in Mosquito Lagoon, Fellers House Field Station (N 28° 54' 23.90", W 80° 49' 16.05") and Seminole Rest (N 28° 54' 9.44", W 80° 49' 12.94"), were identified and used as sites for *S. alterniflora* transplantation in the first week of February following cultivation **(Figure 1).** Our site at the Fellers House was selected because this shoreline has experienced losses of *S. alterniflora* due to anthropogenic disturbance, which has created a demand for restoring the smooth cordgrass population in the area. At Seminole Rest, a previous community-based living shoreline project was established that included *S. alterniflora* transplants and oyster bags as breakwaters, but the success of these transplants was limited due to low survival and suspected washing-out of plants by tidal forces. The site was chosen to allow us to compare the success of prior transplant efforts, and that of our transplants under experimental treatments, including the effects of the presence of oyster bags as breakwaters on transplantation success.

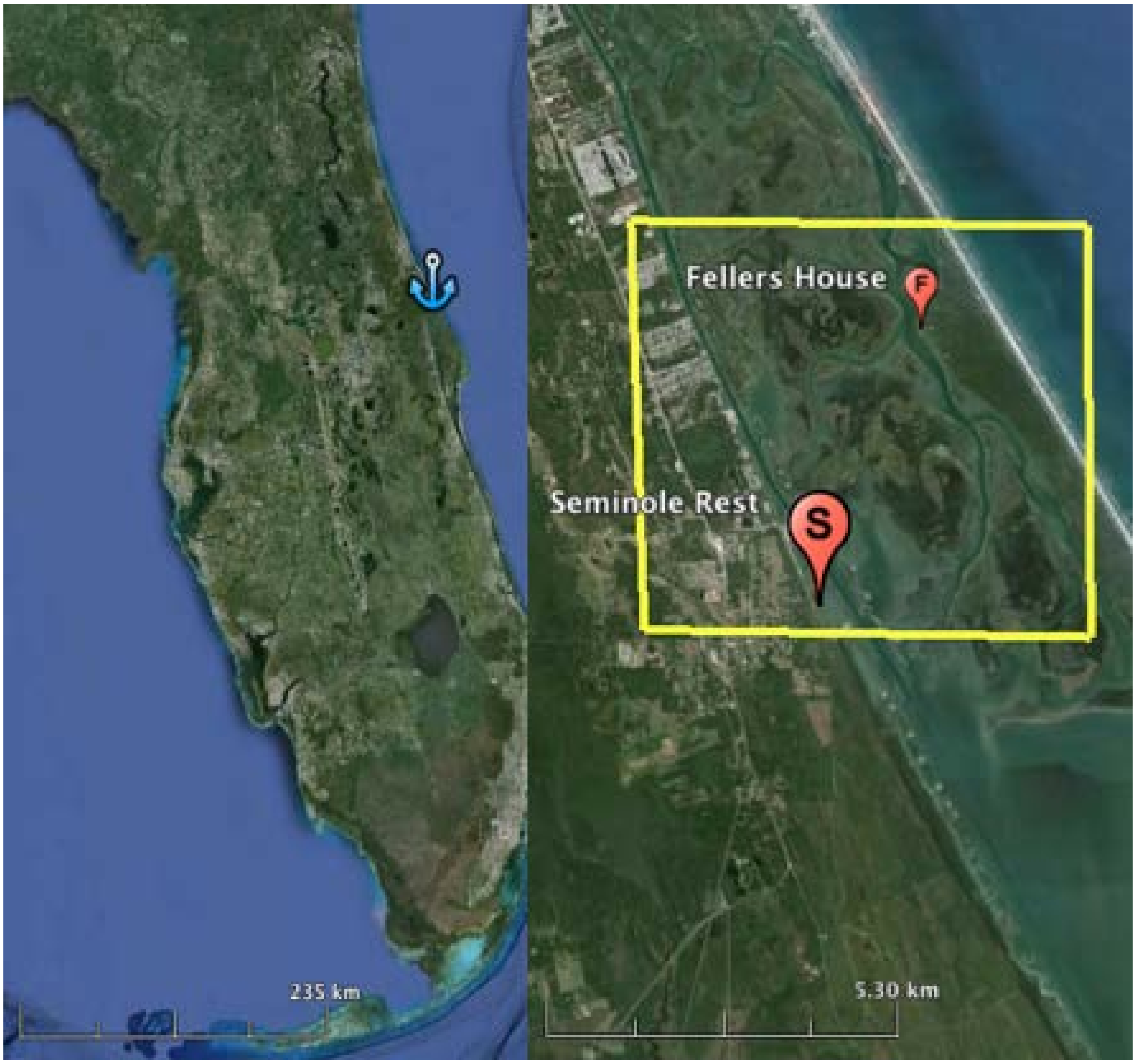

Figure 2. Map of study sites within the Mosquito Lagoon, in Indian River Lagoon, Florida.

To test the effects of cultivation salinity and the use of a stabilizing substrate on transplant establishment success, *S. alterniflora* individuals that received the same salinity treatments were transplanted together in the upper intertidal zone in groups of 5 transplants per m^2 with one of two substrate treatments: the use of a stabilizing mat with 50x50 cm dimensions, "mat", or the exclusion of a mat, "no-mat". In total, there were four possible treatments at each site (freshwater x mat/no-mat, and saltwater x mat/no-mat). Each site served as an additional treatment (Fellers House: absence of oyster bags, Seminole Rest: presence of oyster bags). At each shoreline site I deployed 4 experimental plots, one of each salinity x mat treatments formed one experimental plot along the shoreline placed next to each other in randomized order (**Figure 2**).

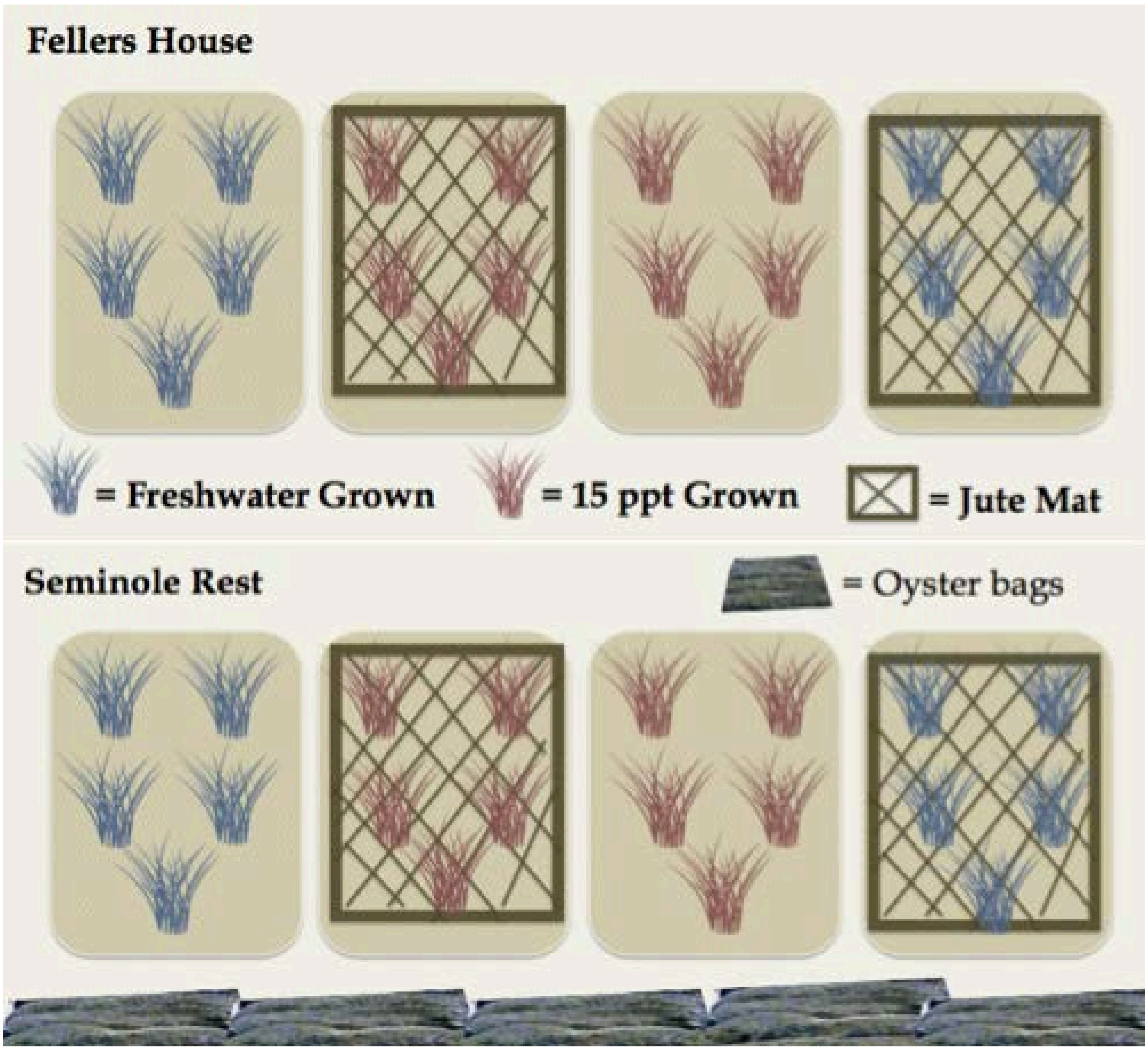

Figure 3. Depiction of one experimental plot design at our living shoreline deployment sites. Treatments within experimental plots were randomized.

In total, 8 m^2 of shoreline for each site was utilized for a total of 16 m^2. Individuals that received "mat" treatment were put through a jute-mesh erosion control mat in a randomized pattern. Mats themselves were secured to the shoreline sediment using 10 cm metal garden staples, and covered with sediment. This mat was used as a stabilizing substrate aiming to facilitate reestablishment of new transplants facing wave energy. Jute-mesh was utilized for its biodegradable properties, its successful use in controlling erosion, and ample mesh-hole size (2-2.5 cm) allowing for new shoot growth from vegetative reproduction.

Growth, Survival, and Dislodgement Measurements

After 20 weeks of rearing, and immediately prior to transplantation, I measured and calculated mean height for the initial culm, number of new shoots (tillers) produced and their respective heights, and recorded flowering and survival rates of each *S. alterniflora* "unit" (culms and tillers). *S. alterniflora* units were declared dead if their rhizome was soft and had deteriorated, and the main culm had not produced new tillers. Main culms that had flowered and died, but produced new shoots were considered alive. Only *S. alterniflora* "unit" considered alive were used for the transplantation study. After transplantation, I had weekly monitoring for a period of 4 weeks to measure and calculate mean change in stem density as a proxy for new shoot growth, survival rates of *S. alterniflora* units, and increases in shoot heights. Individuals that were dislodged and "washed-out" by wave energy were considered dead and percentages of "washed-out" plants were calculated for each treatment.

Data Analyses

A one-way ANOVA was used to evaluate the significance between the growth measurements (number of new shoots produced, new shoot heights, flowering, and survival) of plants grown in freshwater and those grown under low-saline treatments (15 ppt) during cultivation. In the transplant experiment, initial and final measurements of *S. alterniflora* individuals were compared at 4 weeks through a standard, full-factorial 3-way ANOVA (Salinity x Mat x Site) to determine statistical significances and interactions. Dead *S. alterniflora* units were excluded for these statistical analyses, except when calculating mean survival and flowering rates.

RESULTS

Affects of Salinity During Rearing

After 20 weeks of rearing in outside pools, we observed differences in new shoot heights between plants grown in freshwater and low-saline conditions (**Table 1 and Figure 3**). *Spartina alterniflora* individuals grown in 15 ppt produced new shoots with higher mean heights than those produced by plant grown in freshwater (**Figure 1**). Although mean new shoot heights differed, there were no differences in the mean number of shoots produced between plants grown in 15 ppt and those grown in freshwater (**Table 1**). Likewise, there were no differences in survival between our salinity treatments (**Table 1**). Flowering was determined to have resulted from a factor independent to our salinity treatments as flowering rates between 15 ppt and 0 ppt treated plants were not significant (**Table 1**).

Table 1. One-way ANOVAs were use to determine significant differences in flowering, mean new shoots produced, mean new shoot height, and survival between the two salinities. Decimals rounded to thousandths place. All measurements in following format (Mean ± S.E). Shoot heights are in centimeter units.

Treatment	N*	Flowering (Dec.%)	#New Shoots	Shoot Heights	Survival (Dec%)
0 ppt	6	0.344 ± 0.02	4.16 ± 0.068	10.709 ± 0.270	88.333 ± 0.069
15 ppt	6	0.317 ± 0.013	4.04 ± 0.065	13.552 ± 0.177	93.89 ± 0.044
P-value		0.642	0.6258	0.005**	0.128

** N refers to number of averages used in calculations. N=1 is equal to 30 individuals grown together in outside pools.*

***Statistically significant at p<0.05*

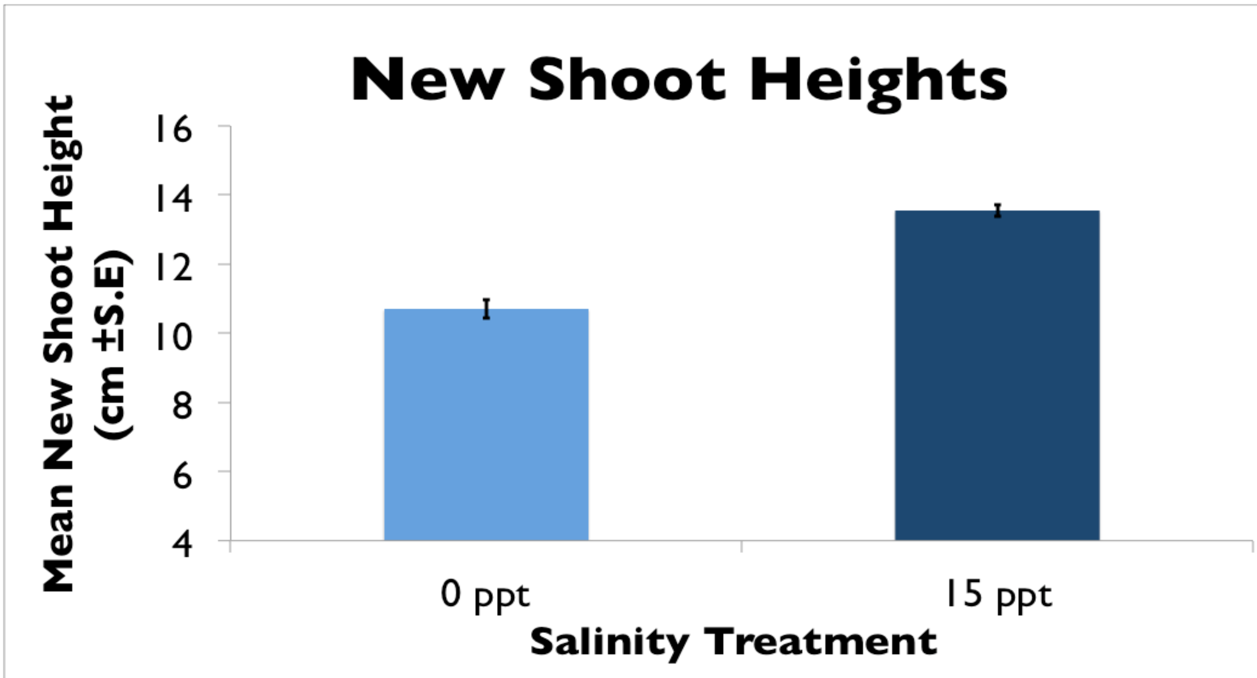

Figure 4. Mean heights of shoots produced by our *S. alterniflora* plugs after 20 weeks of rearing in outdoor pools of either freshwater or under low-saline (15 ppt) conditions.

Effects of Rearing Salinity on Transplantation Success

At four weeks after transplantation, there were differences in stem density between our salinity treatments. Both treatments resulted in positive increases to stem density following transplantation. *Spartina alterniflora* grown in low-saline conditions experienced a significantly higher increase in stem density (± S.E) with a mean of 3.23 ± 0.42 new shoots, compared to 2.09 ± 0.06 mean number of shoots for plants grown in freshwater (p=0.003; **Figure 4**). There were no interactions between salinity treatment and other treatments for this factor (**Table** 2; 3-way ANOVA, Salinity x Mat x Site). Despite a statistically significant increase in stem density in *S. alterniflora* grown in 15 ppt, these plants experienced a greater decrease in mean shoot height four weeks after transplantation than plants grown in freshwater (p=0.02; **Figure 5**).

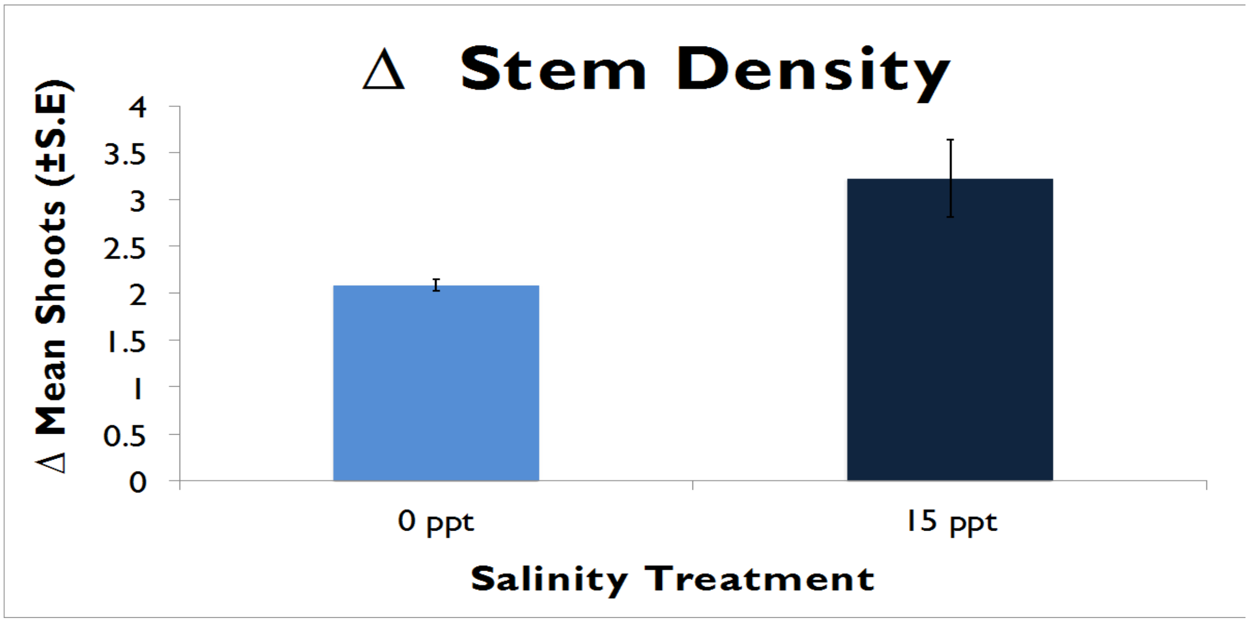

Figure 5. Mean change in stem density at four weeks after transplantation for *S. alterniflora* treated with freshwater and low-saline (15 ppt)

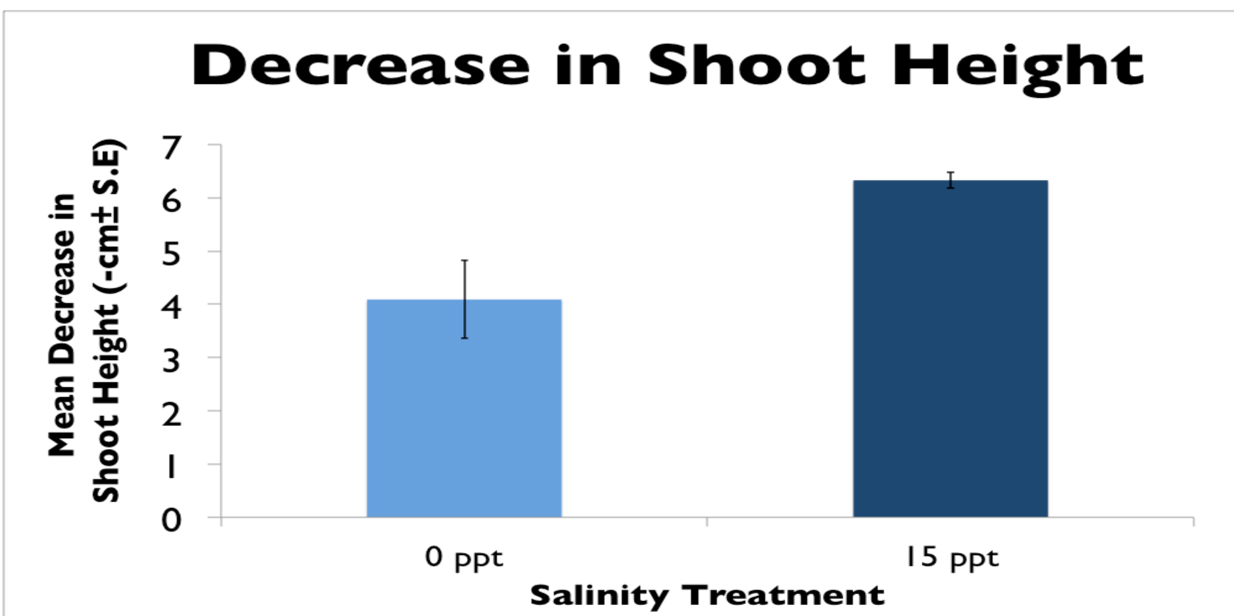

Figure 6. Mean negative change in shoot height at four weeks after transplantation for *S. alterniflora* treated with freshwater and low-saline (15 ppt).

We did not track the survival of every shoot within *S. alterniflora* units and all *S. alterniflora* units survived. Therefore, we determined that salinity was not a factor in survival of plugs.

Effects of Jute-Mesh Mat on Transplantation Success

After four weeks, the jute mesh at the Fellers House site had come apart and mostly washed away, a few stands of the fiber left over confirmed this fact. Some of the remaining jute-mesh had also washed over and smothered shoots. At Seminole Rest the jute-mesh fiber was still intact and visible. Data analyses showed that use of the biodegradable jute-mesh did not have significant effect on the increase in stem density or the decrease in mean shoot height (**Table 2;** P-values 0.867 and 0.557 respectively). This treatment did not interact with any other treatment significantly (**Figure 7, Table 2**). Although individual shoots within a *S. alterniflora* transplant unit were not tracked, no *S. alterniflora* transplants were dislodged leading to the conclusion that jute-mesh mats did not significantly affect survival of transplants four weeks after transplantation.

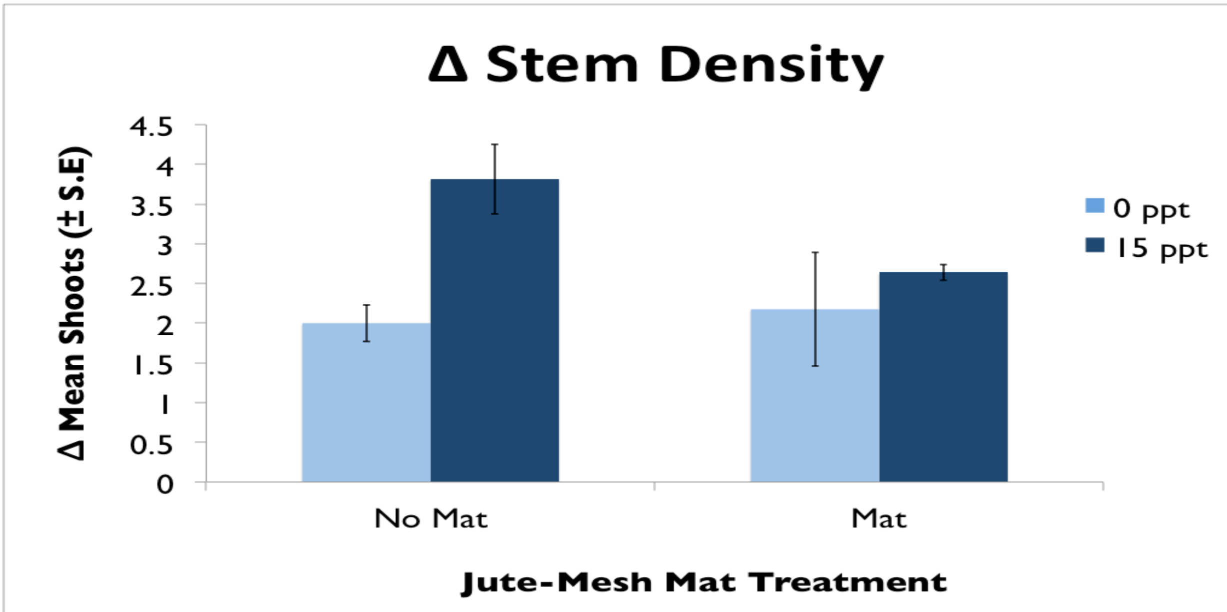

Figure 7. Mean change in stem density at four weeks after transplantation for *S. alterniflora* including both salinity and mat treatments.

Table 2. Three-way analyses results for jute-mesh mat and oyster bag treatments, along with salinity. Values listed are resulting p-values.

Treatment	N*	Δ Stem Density	Δ Heights
Site**	4	0.668	0.133
Mat	4	0.867	0.557
Site x Mat	4	0.723	0.907
Site x Salinity	4	0.188	0.395
Mat x Salinity	4	0.067	0.231
Site x Mat x Salinity	4	0.635	0.696

** N refers to number of averages used in calculations. N= 1 is equal to 5 individuals, N=4 is equal to 20 individuals.*
*** Refers to oyster bag treatment.*

Effects of Oyster Bags on Transplantation Success

Stem density and plant height were not affected by the presence or absence of oyster bags used as breakwaters, and oyster bags did not affect survival (**Table 2**). All of the *S. alterniflora* transplants survived at each site and none were dislodged, which determined that oyster bags did not play a significant role in retaining transplants at restoration sites after four weeks during the month of February.

DISCUSSION

The smooth cordgrass, *S. alterniflora*, grew average of 2.84 cm taller after 20 weeks of rearing in 15 ppt artificially-created seawater when compared to individuals grown in freshwater. Ma et al. (2013) experimentally determined that the invasive individuals of *S. alterniflora* along the Taijin coast had greater aboveground biomass at 5.33 ppt when compared to those treated with 30 ppt. They also found that *S. alterniflora* experienced an increase in belowground biomass at 5.33 ppt, and had higher chlorophyll production at 11.7 ppt. Several studies regarding salinity tolerances of other halophytes corroborate these findings. For instance, Clough (1984) found that *Avicennia marina* and *Rhizophora stylosa*, two species of mangroves found in Australia, grew poorly in the absence of NaCl, and had the highest growth in 8.5 ppt. Similarly, *Bruguiera parviflora*, a mangrove found in Southeast Asia was found to have optimal growth at 100 mM NaCl (approximately 5.3 ppt), and *Salicornia rubra*, a succulent halophyte, has its highest growth at 200 mM NaCl (approximately 11.7 ppt) (Khan et al., 2001; Parida et al., 2004). Halophytes as a whole benefit from small concentrations of sodium chloride. Egan and Ungar (1998) who studied the effects of potassium and sodium salts on the halophyte *Atriplex prostrata*, found potassium salts to be more malignant to growth than Na+ salts because of the specific ion toxicity of K+, and proposed Na+ was used to control water potentials. In a comparison of salinity between common reed, *Phragmites autralis*, and *S. alterniflora,* it was found indeed found that *S. alterniflora*'s higher salt tolerance was due to the use of Na+ to control osmotic pressures (Vasquez et al., 2006). Touchette et al. (2009) also noted that the increase in solute concentration within cell tissues, along with increased tissue rigidity, were responsible for the high salt-tolerance exhibited in *S. alterniflora*. It is possible that the main factor that contributed to differences we observed between our freshwater and 15 ppt grown *S. alterniflora* are associated with the use of near optimal levels of sodium ion concentrations for osmotic adjustment by the plant, which could have improved their growth.

High stem density is a goal for living shorelines as higher stem density increases sediment accretion and attenuation of wave energy resulting in decreased shoreline erosion (Gleason et al., 1979; Augustin, 2009; Leanard and Luther, 1995; Knutson et al., 1982; Anderson and Smith, 2014). When *S. alterniflora* individuals grown in 15 ppt were transplanted into our two restored sites they experienced higher net increases in overall stem density. This increase in net stem density implies greater production of new shoots and survival of initial shoots, and may have been possible for plants grown in 15 ppt due to the smaller difference in salinity ranges it experienced when transplanted into our restoration sites. Salinities in Mosquito lagoon usually range from 28-34 ppt, therefore transplanting *S. alterniflora* individuals grown in 15 ppt would experience a max increase of 19 ppt, while freshwater grown individuals would see a 28 ppt minimum increase in salinity. *S. alterniflora* has a remarkable ability to tolerate high salinity and changes in salinity levels, in one study, using osmotic adjustment and tissue rigidity to handle alternating weekly changes in salinity from 0 ppt to 30 ppt (Touchette et al., 2009). However, it is known that halophytes that have not been acclimated to salinities may have decreased ability to manage increased salinities, and is more evident in older plants (Munns, 2002; Hwang et al., 2004; Touchette et al., 2009). Hwang et al. (2004), found that *S. alterniflora* grown in 25 ppt and 40 ppt displayed structural modifications in response to a high salinity level, and this did not change after salinity was decreased, compared to individuals grown in 5 ppt and 15 ppt that were momentarily exposed to 25 and 40 ppt water. Is it possible that after a 20 week rearing period in lower salinity newer shoots were able to respond to salinity stress through structural modifications and were not required to make as significant physiological changes than plants with new shoots only acclimated to freshwater. As evidenced through this experiment, 15 ppt artificially-created seawater is not only beneficial during rearing *S. alterniflora*, but also beneficial after transplantation by increasing project success. It is important to note, however, that although 15 ppt plants experienced an increase in net stem density in four weeks, it is possible that *S. alterniflora* individuals may quickly adapt and stem densities may be equal to that of 15 ppt grown plants after a longer period of time. However, it appears more possible that an 15 ppt grown plants, due to increased shoot production and reduction of stress responses to elevated salinity, may have more shoots reach maturation and produce additional shoots with cascading effects that would more rapidly lead to stem densities equaling that of natural *S. alterniflora* strands found in Mosquito Lagoon than plants grown in freshwater.

Although our experiment found jute-mesh and oyster bags acting as breakwaters did not increase *S. alterniflora* transplant success, natural fibers have been successfully used in many living shorelines and stabilized oyster shells have evident shoreline erosion mitigation benefits (Partnership for the Delaware Estuary Inc., 2012; Myer et al., 1997; Piazza et al., 2005; Currin et al., 2010; Scypher et al, 2011; Manis et al., 2015). The Partnership for the Delaware Estuary Inc. is one organization that includes coconut fiber mats along with logs as part of their living shorelines design, however, they use wooden stakes to reinforce the structures. In my experiment, jute-mesh mats anchored with metal garden stakes at our Fellers House site may have not been sturdy enough to stabilize plants, and instead fell apart due to wave action in the heavily boat trafficked area. At our Seminole Rest site, the breakwaters stopped the jute-mesh from desintegrating, however there were no additional positive results due to the presence of jute-mesh mats. In contrast to the jute-mesh design in this experiment, oyster bags have been shown to be effective in reducing wave energy and mitigating shoreline erosion, however, my results suggest wave exposure does not play a significant role in survival, growth, or dislodgement of transplants at these sites four weeks after transplantation.

Although the use of 15 ppt artificially-created seawater resulted in benefits during rearing and four weeks after transplantation, it is important to determine costs and benefits to assist stakeholders in determining whether utilizing this method of rearing is favorable for their organization. The total cost for growing 250 *S. alterniflora* individuals in 15 ppt salinity over 20 weeks was $73.76, which results approximately

$0.30 per plant (**Table 3**). The average net increase in stem density after four weeks of transplantation was 3.23 ± 0.42 new shoots, compared to 2.09 ± 0.06 in individuals grown in freshwater. These values were rounded to nearest whole number for calculation. Using this net increase in stem

Table 3. Estimated marginal costs and benefits associated with moving from current to tested low-salinity (15 ppt) treatment according to our methods and results.

Treatment	Marginal Cost Per Plant	Net Increase in Stem Density Per Plant	Increase in Stem Density for Project*	Total Marginal Benefit **
0 ppt	$ 0	2	100%	0%
15 ppt	$ 0.30	3	200%	50%

** Percent increase in net stem density four weeks after transplantation.*

*** Percent gain in net stem density four weeks after transplantation when using 15 ppt rather than 0 ppt.*

density and the number of plants used for the experiment, it was concluded that plants grown in 15 ppt should experience an estimated 200% increase in stem density compared to 100% with freshwater-grown individuals. The total marginal gain from switching from 0 to 15 ppt artificial saltwater to rear plants is 50%. This means that after only four weeks, there were evident marginal benefits to transplant success by implementing the method used in this experiment. Because living shorelines in the Indian River Lagoon are largely community-based, the increase in hours of work due to the additional task of maintaining salinity was not included, as volunteers and school classrooms often grow and monitor *S. alterniflora* in outside pools. Although there may be additional time devoted, it is possible that there may be an educational component. For example, elementary school classrooms who grow these plants which may opt to do a lesson on the use of refractometers to measure salinity and the relevance of salinity to coastal species. Organizations who do not utilize volunteers or purchase their plants may have higher costs associated with using the 15 ppt method. Overall, this method increases transplant success and may be useful depending on the organization's goals, their perception of marginal benefits to compared to the additional costs, and the amount of total funding available for their project.

CONCLUSION

This research determined that growing *Spartina alterniflora* plugs in 15 ppt artificial seawater for community-based living shoreline projects had greater benefits to plant growth during rearing and stem density after transplantation than currently-used methods. After a 20 week rearing period, plugs demonstrated no difference in number of new shoots produced, however, there was an increase in shoot height for plants grown in 15 ppt compared to freshwater-grown individuals. This led to the conclusion that shoots produced by plants grown in a low-saline condition (15 ppt) grew faster, and that the use of added sodium ions for improved osmotic adjustment may have benefited their growth. Four weeks after transplantation, plants grown in 15 ppt also demonstrated a significant net increase in stem density, with an average of one additional shoot per plant, when compared to transplanted plants that had been grown in freshwater. It was concluded that shoots produced by 15 ppt-reared plants were able to respond to salinity stress through fixed, structural modifications developed during rearing and were not required to make as significant of physiological changes unlike freshwater plants with shoots that had only acclimated to freshwater. The goal of many living shorelines is to increase stem density of their plant transplants as this facilitates sediment accretion and reduces wave energy; this project showed that it is possible to increase project net stem density by 50% in only four weeks through growing *S. alterniflora* plugs in 15 ppt and transplanting to targeted restoration sites. It is possible that longer-term monitoring may demonstrate a positive correlation between time and net increase in stem density as newer shoots mature and produce additional shoots. Marginal costs from employing the best method found in this research was determined to be $0.30 per plant, a cost derived from the added use of salt to create artificial seawater. Other possible marginal costs were not included in this calculation. My research results determined jute-mesh and oyster bags as breakwaters had no impacts in the reestablishment of *S. alterniflora* plugs, and thus their costs and use were also excluded from the cost calculation. Using 15 ppt artificial seawater to grow plugs was determined to be a simple, yet effective method to increase project success, and should be considered by stakeholders for future living shoreline projects depending on budget limitations and set goals.

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