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Analysis of the Facilitative Interaction between *Batis Maritima* and *Avicennia Germinans* as a Mangrove Restoration Strategy

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ANALYSIS OF THE FACILITATIVE INTERACTION BETWEEN *BATIS MARITIMA* AND *AVICENNIA GERMINANS* AS A MANGROVE RESTORATION STRATEGY.

A Thesis

by

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ABSTRACT

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The multiple experiments conducted in an *Avicennia germinans* forest in Laguna Vista, TX was focused on determining if facilitation theory could explain the behavior observed between the herbaceous halophyte *Batis maritima* and *A. germinans*. I hypnotized that a positive interaction exists but through which mechanisms of facilitation I did not know. The overarching theme of the study is the presence or absence of *B. maritima*. Facilitation theory tells us that positive interactions are most often found under stressful conditions such as those on the coastal tidal flat of the Laguna Madre. Locally *B. maritima* has been found to colonize tidal flats following a disturbance or prior to the arrival of mangrove seedlings. To elucidate this idea, I conducted multiple seedling and propagule experiments. I found significant interactions that correspond with several of the mechanism of facilitation theory. Microclimatic conditions were shown to have been significantly affected by the presence of *B. maritima*. Air temperature was reduced by *B. maritima* foliar shading which was confirmed by the reduction in light intensity from April – September. Growth responses for *A. germinans* height showed a significant preference for the *B. maritima* treatment. Survivability of *A. germinans* seedlings was greatly increased within *B. maritima* patches. All seedlings growing in the absence of *B. maritima* died before the experiment was over.

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CHAPTER I

ECOSYSTEM RESTORATION

Restoration of a degraded coastal ecosystem has been shown to be less expensive than the costs associated with replacing it (Alexander et al., 2011). When the focus is placed on the multitude of services provided by a rehabilitated coastal wetland vs. a single service replacement facility, restoration makes economic sense in natural-capital benefit value (Groot et al., 2013). Funding for restoration projects can vary greatly depending on the approach taken. Spurgeon (1999) applied a “benefit-cost analysis” to quantify various restoration projects e.g., coral reef rehabilitation in the Florida Keys National Marine Sanctuary, and sea grass rehabilitation in Galveston, TX, while the National Oceanic and Atmospheric Administration (NOAA, 1997) indicated mangrove planting materials costs. The valuation of projects varies depending on the ecosystem and life stage of plant materials used (NOAA 1997; Spurgeon, 1999). Cost-effective projects are essential to restoring the most habitat with a limited budget (Spurgeon, 1999). Wetlands have a value estimated at $4.9 trillion/yr. worldwide from the ecosystem services they provide (Constanza et al., 1996). The services that have received the most attention include water filtration, flood control, recreation and wildlife (U.S. Environmental Protection Agency (US EPA), 2006).

Mangroves are a common feature on the U.S. Gulf Coast wetlands and contribute to the ecosystem services provided by them. For example, building a water treatment plant to offset a degraded wetland ignores the multitude of free services that wetlands provided such as the revenue the recreation industry generates, more than $100 billion/year in the U.S. alone (US EPA, 2006). Restoration of degraded ecosystems can be done through passive restoration at a lower cost through biotic interactions and natural succession of the local flora. Examples of successional facilitation have been noted in Belize where saltwort (*Batis maritima* Linnaeus, 1759) was shown to be an early colonizer after disturbance on mangrove stands (Whigham et al., 2008). Incorporation of such mechanisms could reduce cost by reducing the age and thus price of planting target material.

The coastal zone of Texas. was estimated to have converted 1,902,022 ha of wetland (Cooley and Slayter, 2015). This anthropogenic threat to coastal wetlands has been replaced by urban development and chemical industries (Cooley and Slayter, 2015). Chemical industries have become all too common along the U.S. coastline. In South Texas, for example, along the Brownsville Ship Channel, construction has begun on various Liquefied Natural Gas (LNG) facilities, pipelines, and ports. They are adjacent to a recently restored 10,000 ha of estuarine wetland, Bahia Grande (Moore, 2019). A justification for supporting the development of this coastal land by one local manager was that the LNG Company would help pay for continuing restoration and prove themselves as “good neighbor” (Clark, 2017). However, the threat to the wetlands located down the surge of these proposed sites has risen from nearly zero to real potential as was seen in 2017 during hurricane Harvey in the Houston-Galveston area. Florida is currently leading the way in LNG production as transportation and marine fuels seeking to expand towards exporting through investments from companies such as Eagle LNG Partners and American LNG (Corkhill, 2016). Unfortunately, the reality of chemical plants is they are largely situated in an all-encompassing concrete facility. The displacement of wetlands and the services they provide cannot be mitigated by the construction of holding ponds while expecting a continuation of services. Therefore, restoration of coastal wetlands where and when possible should be carried out to maintain ecosystem services and their economic benefits. Restoration has been shown to not only be cost effective, it can generate internal rates of return by as much as 3% (de Groot et al., 2013). According to Zedler (2000), succession theory is the beginning of restoration, and as such we must look towards the floral communities of the disturbed sites.

Costal wetland ecosystem services can be divided into two categories: tangible and intangible (Wallace, 2007). Tangible services can serve as indicators of estuaries' health (Whitfield and Harrison, 2014). As such, increases and decreases in fishes catch or changes in water quality are quickly felt by the local communities and industries. This almost instantaneous action-reaction system allows for the adjustment of management strategies. Intangible ecosystem services, likely to be overlooked or neglected, include nutrient cycling and storm surge protection as the effects are only observed after accumulation or sudden need respectively (Domingos, Lopes, and Struffaldi, 2000). Reduced nutrient cycling as an intangible service requires time for the cumulative effect of the change before it is acknowledged as a disturbance effect (Wallace, 2007). The seasonal occurrence of the Gulf of Mexico's "dead zone" is in part due to the inability of the coastal waters to proportionally cycle the excessive nutrient influx (NOAA, 2019). Storm surge protection is provided by the absorption and retention of water across the wetlands (Baird et al., 2009; Wells, Ravilious, & Corcoran 2006). The energy produced by storms is absorbed and reduced by the topography of the land as well as the existing vegetation (Baird et al., 2009; Wells, Ravilious, & Corcoran 2006). Storm surge protection is not deferred due to a temporal component but likely for the ambiguity of the service it provides as well as the economy of coastal communities.

Assessing an environment for restoration should be the first concern at the inception of a project (Spurgeon, 1999). Lewis (2005) writes that instinctually when one hears restoration their focus and assumptions are to begin planting. Lewis and Marshall (1997) listed five critical steps for planning a restoration project of mangrove, first autecology knowledge of the mangrove species, second knowledge of the normal hydrology of the site, third assessment of the disturbance, fourth restore the normal hydrology and allow for natural recruitment. The final step is planting, implemented only once all disturbance factors or stressors have been mitigated. This is an important guideline as Lewis and Gilmore (2007) reiterates that solely planting mangroves for example, will not restore the system. Restoration projects have specific criteria for success. Sometimes success is represented in terms of target species survival, amelioration of an ecosystem service or a goal set by stakeholders (Lewis, 2005). The proper approach and realistic goals must be a priority if success is realistically expected (Erwin, 1990; Lewis, 2005). However, often project success is not defined and the lack of a central database of all restoration projects makes quantification of success difficult (Suding, 2011).

Holland et al. (2005) gave examples of mutualistic relationships across various species and ecosystems that could be harnessed in restoration efforts. Lewis (1982) reported early colonizing species such as smooth cordgrass (*Sporobolus alterniflora* (Loisel); formally S*partina alterniflora*) will often facilitate the establishment of mangroves in a primary succession throughout climax community process. Evidence has shown that if a benefit can be obtained for a target species it should be encouraged (Halpern et al., 2007).

The original zonation of Florida mangroves by Davis (1940) describes a community type expanded on by Lewis et al. (1985) to include four variations to the original pattern of zonation including smooth cordgrass and saltwort. These patterns allow restoration projects to implement facilitative effects into their planning. The knowledge obtained from the natural zonation of a coastal ecosystem can allow for the direct intervention on edaphic stressors through successional species that can ameliorate these conditions creating a more hospitable environment (Bertness, Gough, and Shumway, 1992; Bertness and Hacker, 1994; Chapman, 1974). Although, positive interactions can be found from neighboring species (Silliman et al., 2006) it may not be as economically feasible as would be an early colonizing approach. For example, the cost-benefits obtained from propagules vs. seedlings could be a costly approach (NOAA, 1997). Lewis (1981) reported that costs associated with a small red mangrove (*Rhizophora mangle* Linnaeus, 1753) project could include propagules purchased for $1,600/ ha compared with 6-month old seedlings at $12,103/ha, and $70,000/ha for 3-year-old trees all with the same planting density of 0.91/m^2 (Mangrove Systems Inc., 1979).Variations in cost continue as Lewis (2001) acknowledges that a ~1,200/ha shrimp pond project could range from $240,000 – 840,000. In a report titled Primary Restoration, NOAA (1997) indicated that when adjusted to the parameters of their report Teas (1977) gathered project data for red mangroves propagules purchased for $2,649/ha 6-mo. old seedlings, $5,273/ha, and 3-year-old trees for $454,055/ha.

Positive Interactions

Original-thinking ecological theory accounted not only for the competitive (negative) interactions but the positive interactions as well (Bertness and Callaway, 1994; Clements, Weaver, and Hanson, 1926). However, Gleason (1939) held a more individualistic view of plant communities. Decades later, Connell and Slayter (1977) would only add to the dissention from classical ecological theory. Modern theory has embraced negative interactions in the face of mounting evidence of the benefits of positive interactions (Bruno, Stachowicz, and Bertness, 2003; Dighton, 1986; Muscatine, 1990). Lortie et al. (2004) argued for a “dualistic” ideology to be adapted. It has been suggested by Silliman and Gedan (2009) that the commonly used plantation style of restoration projects be replaced using clustered planting. Cluster planting allows for mitigation of stressors through intraspecific facilitation (O'Brien and Zedler, 2006). In a five-seedling cluster planting experiment, Toledo, Rojas, and Bashan (2001) recorded an initial survival of 86% after 6 mo. and a 74 % survival of seedling clusters planted in bare ground after 2 years. Increasing the density of a target species can be costly depending on the increase and life stage of the material as previously mentioned. However, generally speaking if edaphic and other stressors are not ameliorated before seedling planting, mortality rates could remain high (Pezeshki and DeLaune, 2012). Nurse plant facilitation has been documented as producing similar responses as clusters of a monotypic planting (McKee, Rooth, and Feller 2007). Literature on the modifications and ameliorations that early colonizers have on environmental stress has been well documented (Bertness and Yeh, 1994; Nobel, 1980; Silander and Antonovics, 1982). Facilitations can occur through the physical presence of a nurse plant or facilitator plant. Facilitating plants can provide a structure that encourages propagule stranding (Callaway, 1995; Huto, McAuliffe, and Hogan 1986). The physical structure of the facilitator (e.g., leaves, branches, culms of grass or trunk) can provide structural support and shelter for establishing seedlings by protecting them from wind damage and shading increasing soil moisture (Aguiar, Soriano, and Sala, 1992; Callaway & Walker, 1997). Surface soil salinity can be ameliorated through the reduction of evaporation through shading (Bertness, Gough, and Shumway, 1992).

Milbrandt and Tinsley (2006) reported that various colonizers such as sea-blights (*Suaeda* spp.) and oxeye (*Leucanthemum vulgare* (Lamarck, 1778)) were common after a mangrove disturbance. Howard et al. (2015) found that desert saltgrass (*Distichlis spicata*

(Linnaeus, 1753)) and gulf coast spikerush (*Eleocharis cellulosa* (Torrey, 1836)) prevented or diminished black mangrove (*Avicennia germinans* (Linnaeus, 1759)) propagule establishment. As a process of natural succession, recovery of structure and/or function can be slow even with intervention (Zedler, 2000). Succession as a process of passive restoration may be a sustainable practice that could allow for rehabilitation of damaged mangroves.

Early colonizing halophytic herbaceous plants such as saltwort can ameliorate conditions and provide a more suitable soil conditions for subsequent successional species (Bertness, 1991). Wrack and the arrival of herbaceous plants and nitrogen (N) fixers can mitigate some existing environmental conditions (Whigham et al., 2008). A positive interaction has been observed for mangrove seedlings recruiting, and apparently growing better in patches of herbaceous halophytes compared to those growing in bare mudflats (Milbrandt and Tinsley, 2006). Conversely, Howard et al. (2015) found that black mangroves were outcompeted by herbaceous halophytes in a mesocosms experiment. However, mangrove seedlings have been observed to be visibly healthier when established within non-competing herbaceous primary colonizers (Milbrandt & Tinsley, 2006).

Mangroves

Mangroves are commonly thought of as any halophytic woody shrub or tree that populate the tidal ranges of tropical and subtropical coasts (Tomlinson, 1986). Currently mangroves have an expanding range (Armitage et al., 2015), but in general their vast distribution occurs within the latitudes of 30˚ N and 30˚ S (Giri et al., 2011). The four occurring species of mangroves in the subtropical Gulf-Atlantic coastal region of the U.S. are red mangrove, white mangrove (*Laguncularia racemosa* (Linnaeus, 1807)), buttonwood mangrove (*Conocarpus erectus* (Linnaeus, 1753)), and black mangrove (Tomlinson, 1986). The occurrence of each species is governed by a range of factors chief amongst them are hydrology, salinity, temperature and tidal range (Elster et al., 1999; Hester and Pickens, 2010; Walsh, 1974).

Avicennia germinans

The black mangrove is a tree that can reach 30 m in height (Tomlinson, 1986). It is more commonly found as a small multi-stemmed shrub in cooler latitudes due to winter die-back (Osland et al., 2014), as well as in areas of poor nutrient soil and high salinity (Twilley, Lugo, and Patterson-Zucca, 1986). A member of the Acanthaceae family, the black mangrove has expanded its' northern distribution greater than any other American mangrove species (Hester and Pickens, 2010). However, the warmer winters of Bermuda at 32° N (Johnston, 1983) have allowed black mangrove stands to establish beyond the contiguous U.S. range. The black mangrove is the focus species of this study.

The flowering period for black mangroves in South Texas. begins in late summer through early winter (Figure 1) (pers. obs.) A viviparous reproducer, black mangroves release propagules into the natural hydrology, undergoing a buoyancy period (Hester, Aleman, and Willies, 2008) (Figure 2). Along the coast of Laguna Vista, TX. (Holly Beach study site) mangrove propagules begin to drop in October and continue through February depending on the conditions at any given site (pers. obs.) The propagules are encased in a fleshy testa supporting a maturing embryo (Lonard, 2016). The buoyancy period allows for pericarp shedding and the initial development of the root system (Delgado et al., 2001; Hester, Aleman, and Willies, 2008). Black mangrove propagules develop three types of roots as they establish themselves: anchoring, horizontal and aerial roots (Lonard, 2016). Anchoring (primary) roots are essential for initial propagule rooting after stranding the horizontal roots also called radial roots are shallow growing roots that give rise to the aerial roots (pneumatophores) (Lonard, 2016). These roots are a characteristic species trait and can grow to a maximum length of 30 cm, rising above high tide level and allowing for direct gas exchange (Dawes, 1981).

Figure 1. A) Black mangrove (*Avicennia germinans*) flower buds prior to inflorescence bloom, May 2019; and B) black mangrove flowers blooming, November 2019 in Holly Beach, TX (see text for geographic location).

Figure 2. A). Black mangrove (*Avicennia germinans*) propagules prior to releasing into local hydrology, April 2019; and B) stranded propagules of black mangrove at the height of the tide range, October 2018; in Holly Beach, TX.

Disturbances affecting mangroves can result in various patterns of destruction such as aboveground biomass destruction or complete plant uprooting (Schaetzl et al., 1988). The aboveground biomass destruction or die back retains high resilience allowing for passive restoration implementation (Zedler, 2000; Zhu, Woodall, and Clark, 2012). Uprooting or complete mangrove forest destruction provides managers with the single option of active restoration with likely mangrove reintroduction programs. However, the coastal tidal mudflat is generally rich in organic material (Krauss et al., 2008) and yet nutritionally provides little balance in N or phosphorous (P) availability (Patterson and Mendelssohn, 1991). A passive restoration program can be implemented by influencing the natural successional processes of the local flora (Milbrandt and Tinsley, 2006).

Batis maritima

Saltwort is a member of the Family Bataceae, a C_3 herbaceous perennial also known as turtle weed, or pickle weed (Debez et al., 2010; Milbrandt and Tinsley, 2006). Saltwort is an obligate halophilic succulent, which propagates clonally and through seed dispersal (Lonard, Judd, and Stalter, 2011). As a dioecious plant it produces unisexual flowers that bloom in late spring and early summer locally (Figure 3.) (pers. obs.). The vegetative propagation ability has been suggested as the mechanism by which saltwort comes to initially colonize disturbed black mangrove forest (Milbrandt and Tinsley, 2006). Saltwort can be found between 30˚ N to 3˚ S and in countries such as Brazil (Lonard, Judd, and Stalter, 2011), Venezuela (Medina et al., 1989), Mexico (Zedler, 1977), Belize (Whigham et al., 2008), and the USA (Choi et al., 2001). The zonation of saltwort has been noted in Georgia (Pennings and Callaway, 2000), and Florida (Choi et al., 2001) to be in the mid- to high- intertidal zones in salt marshes. On the South TEXAS. coast, it is found along a salinity gradient in salt marshes (Lonard, Judd, and Stalter, 2011). Hypersaline soils will not sustain saltwort (Dunton, Hardegee, and Whitledge, 2001; Zedler, Williams, and Boland, 1986). Salt barrens can support saltwort colonies but only on the marginal transition zones (Choi et al., 2001).

Figure 3. A) Blooming saltwort (*Batis maritima*) in May 2019, 30 mins. after sunset, and B) enlargement of the foreground flower in A; in Holly Beach, TX.

The saltwort-mangrove interaction has been observed in Florida and Belize (Whigham et al., 2008), as well as in South Texas. (pers. obs.). Understanding and harnessing the successional interaction between saltwort and black mangrove as an approach to restoration could be key to rehabilitating mangrove habitats more successfully. Milbrandt and Tinsley (2006) describe saltwort as a fugitive species surviving on disrupted intertidal areas and as colonizer species. This study will address the plant-to-plant interaction between saltwort and the black mangrove. Locally, saltwort is the dominant colonizer of tidal flats (pers. obs.). Other herbaceous halophyte appears to associate with mangroves, Rützler and Feller (1988) reported sea purslane (*Sesuvium portulacastrum* Linnaeus, 1753) as an associate occurring on higher elevations in a Belizean cay. Weaver and Armitage (2018) also observed an association while studying the expansion of black mangrove into salt marsh dominated by *Sporobolus alterniflora*.

Overarching Hypothesis

1. A facilitative interaction exists between saltwort and black mangrove seedlings and propagules, and this interaction is multifaceted.

Derived Hypotheses

1. The presence of saltwort improves the microclimatic conditions under its canopy, specifically light intensity and temperature for black mangrove establishment and growth.
2. Saltwort alters the growing substrate providing a more favorable environment for the establishment of black mangrove seedlings.
3. Survival and growth of black mangrove seedlings is improved when growing within stands of saltwort.

Derived Objectives

1. To assess the effect of saltwort on surface and soil temperatures, light intensity, and selected soil parameters (redox potential; electrical conductivity; pH; and carbon (C), N, and P availability).
2. To confirm the increased stranding of propagules and their survival in stands of saltwort.
3. To determine if black mangrove seedlings have increased survival, growth, and leaf chlorophyll content when growing surrounded by saltwort.

CHAPTER II

MATERIALS AND METHODS

Study Site

The site for this study is at Holly Beach, found in Laguna Vista, TX. (Figure 4). The experimental plots were located at 26°07’19” N and 97°17’33” W in a private preserve managed by The Conservation Fund, a non-profit organization. Holly Beach is a 450.8 ha coastal preserve encompassing three ecosystems: coastal prairie, Tamaulipan thornscrub and estuarine habitat with mangroves (pers. obs). The site supports a diverse community of quadrupeds such as javelinas (*Pecari* sp.), nilgai (*Boselaphus tragocamelus* Pallas, 1766), Texas tortoise (*Gopherus berlandieri* (Agassiz, 1857)) and white tail deer (*Odocoileus virginianus* (Zimmermann, 1780)). As estuarine fish nursery, plovers (*Pluvialis* spp.), gulls (*Larus* spp.), great egrets (*Ardea alba* (Linnaeus, 1758)), and great blue heron (*Ardea herodias* (Linnaeus, 1758)) are always present. The flora of Holly Beach is just as diverse as the fauna (pers. obs). The prairie is dominated by gulf cordgrass (*Spartina spartinae* (Trinius) Merrill ex Hitchcock, 1913), the Tamaulipan thornscrub is comprises of a diverse group of woody species (pers. obs).

Figure 4. Holly Beach preserve (outlined in red) located just north of Laguna Vista, TX. The location of experimental plots is denoted by a yellow star (modified from Google Earth, 2019).

The mudflat supports about 26 ha of fringe mangroves dominated by saltwort and black mangrove. The mangroves are hydrologically maintained by a saline creek linked to the lower Laguna Madre. During the late summer and throughout the fall, the mangrove stands and the tidal basin experience frequent flooding (pers. obs.). The zonation of the research around the tidal creek includes a belt of about 10 m wide of black mangroves ranging from 1-3 m tall. The mangrove heights lessen the further from the creek, giving way to an ~ 2 m wide border of continuous saltwort followed by patches of saltwort that open to the mudflats (Figure 5).

Figure 5. View of the Holly Beach, TX. research site showing natural zonation. Tidal creek fringe black mangroves (*Avicennia germinans*, darker green vegetation in background) diminish landward where saltwort (*Batis maritima*, lighter green vegetation in foreground) dominates giving way to mudflats.

Experimental Designs

Plot Experiment

A stratified randomized design was adopted for this study. Twenty-eight plots were established for seven treatments (Table 1), and one control with four replicates each. The treatments were divided to measure two stages of the establishment process, the propagule and the seedling stages. The division was expected to provide information on mangrove seedling recruitment and subsequent establishment and growth. Dividing the experiment into two early life stages, propagule and seedling, allows simultaneous observations of stranding and anchoring effects on propagules and the assumed facilitative effect of saltwort on the seedling stage.

Table 1. Plot experiment treatment identification.
Black mangrove (*Avicennia germinans*) and saltwort (*Batis maritima)*.

Treatment No.	Treatment
1	Black mangrove seedlings on bare-mud (experimental control)
2	Black mangrove seedlings in natural saltwort
3	Black mangrove seedlings in planted saltwort
4	Black mangrove propagules placed on bare-mud
5	Black mangrove propagules placed in planted saltwort
6	Black mangrove propagules placed in natural saltwort
7	Black mangrove established adult trees (reference control)

On December 22, 2017, plots were established as 1.5 x 1.5 m squares, while the sampling area was the 1 x 1 m interior quadrat providing a 25 cm surrounding buffer to account for edge effects. The densities for the seedling planting were determined using data obtained at San Martin Lake (26°00'25"N 97°18'03"W) on natural saltwort patches bordering mangrove stands (detailed below), and the available area of the target quadrat. Propagule placement was determined at the time of deployment based on propagule availability. In the end, 20 mangrove seedlings and nine propagules were planted/located within the sampling area (1 m^2) of each plot.

The natural density of saltwort was estimated at San Martin Lake using a 0.25 m^2 quadrat in 10 replications on a 30 x 100 m continuous saltwort stand. Results indicated that on average an established saltwort patch has 284 plants/m^2. Having calculated for spacing and planting and accounted for mangrove seedling planting, a density of 120 saltwort per 2.25 m^2 plot (one every 20 cm) was adopted. This planting density also allowed for less disturbance/trampling of the soil and algal mat within and around the plots during manual transplanting. Having determined a planting density for both saltwort and mangrove seedlings, the focus moved on to the propagation of the required plant material (for nursery propagation details see Appendices A & B).

The saltwort seedlings were transplanted on January 9, 2018 and were approximately 25 cm tall with a tight root ball (Figure 6). All nursery grown mangrove seedlings, which were transplanted to the field site on March 5, 2018, perished within 2 weeks. Therefore, on March 25, 2018, recently rooted seedlings on-site were collected. These seedlings were ~ 15 cm height and were placed in peat moss container cells. The seedlings were maintained in the shade of resident mangroves for 1 week then were transplanted into the plots on March 31, 2018. However, due to limitations in available seedlings and propagules the planted saltwort treatment for seedlings was eliminated.

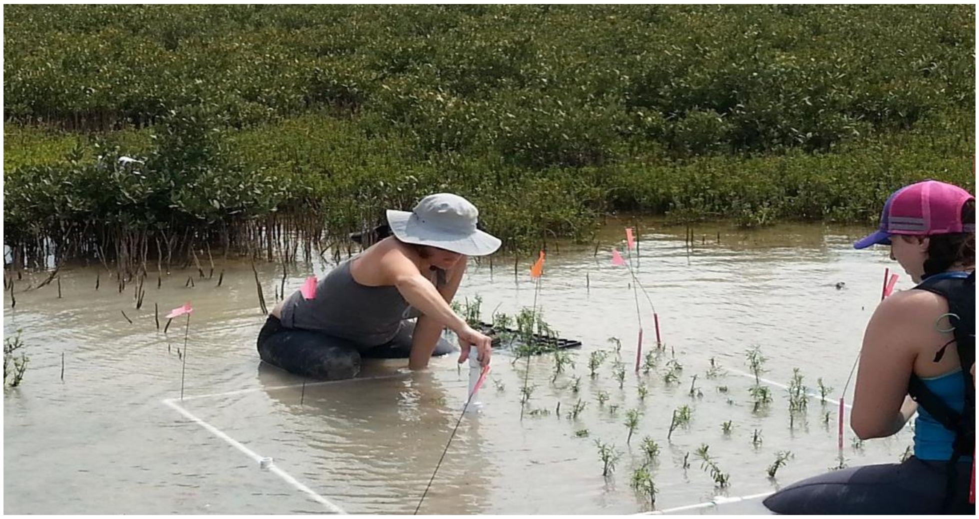

Figure 6. Saltwort (*Batis maritima*) seedling planting on January 2018 at a rate of 53 plants/m^2 at Holy Beach, TX. (see text for details).

A propagule stranding experiment was conducted from October 2018 to February 2019 (112 d). The purpose was to determine the effect if any that natural saltwort had on capturing propagules vs bare-mud and planted saltwort seedlings. Beginning at the start of propagule drop season, bare-mud and natural and planted saltwort plots were initially monitored every 2 weeks and had originally set out to monitor every 28 d, but the schedule became fluid due to weather and tidal fluctuations. After the initial 2 weeks measurement monitoring varied from every 22-30 d for a total of seven surveys looking for stranded propagules. The propagules were removed from the plots once counted and data were compiled cumulatively, to best illustrate and analyze the impact of natural saltwort towards propagule stranding. A propagule anchoring experiment was initiated at the end of the propagule drop season (March 2019). The stock of natural/local propagules was scarce at the time of experimental set up. The original intention of 20 propagules placed in the center quadrat was no longer feasible. Based on the stock of propagules collected 1 week earlier, it was determined nine propagules would be placed in the center quadrat of each bare-mud and natural/ planted saltwort plots. Originally monitoring for this experiment was to be 21 d but weather and tidal fluctuations delayed counting by 2 d. After 23 d, all anchored propagules within the 1 m^2 center quadrat were counted and assumed to be of the original nine propagules. The initial step of this experiment could have been omitted as the origins of the propagules found to be rooting in any given treatment could not be accounted for. The nine propagules originally placed in the treatment quadrats were not tethered, marked or weighed down in any matter. Natural anchoring/rooting should have been and by all accounts is likely what was observed and reported.

Seedling Pairs Experiment

A pair design was adopted for this experiment. On August 31, 2018, a total of 30 pairs of existing black mangrove seedlings representing two life stages were selected: 1) 15 pairs were of seedlings establishing from the most recent current year seedlings; and 2) 15 pair were of 2- or 3-yrs.-old seedlings. The pairs represented two treatments: a seedling growing within saltwort patch and a seedling growing in bare-mud. Both seedlings were in proximity (< 5 m apart) and of comparable size and general appearance. This pairing allowed for discerning if survivability and growth is affected by the presence or absence of saltwort. This design allowed for control of unwanted variables such as nursery planting residues and severe algal mat disturbance. The current year seedlings in this design were from the same stock of seedlings transplanted in the plot experiment and allowed for some comparisons.

The average height of the first set of pairs (the current) year seedlings was 10 cm and had no more than the first pair of true leaves and both cotyledons intact. The second set of pairs were estimated *in situ* to be between 2-3 years old and still lacking pneumatophores. The average height of these recruits was 37 cm (± 1.44 SE) and supported multiple branches. The overall criteria used for selection of the pairs were: 1) that they had similar physical characteristics; 2) be within 5 m of each other; 3) one set of the pair is found to be growing in a natural saltwort patch; and 4)the other set was growing in bare-mud.

Data Collection

Plot Experiment

The plot experiment measured seedling survival and plant growth responses: basal diameter, height and chlorophyll content as measure of overall plant health were recorded four times over the course of a year. The seedlings were measured for height (± 0.1 cm), basal diameter (mm) (Pittsburgh digital caliper, model 47257 (± 0.03 mm)), and *in vivo* leaf chlorophyll content (mg/m^2) (Opti-Sciences, model CCM-300 (±0.03)). Microclimate parameters (soil and air temperature and light intensity) were continuously monitored every 4 – 6 hrs. for 12 mos. Microclimate parameters were evaluated at each plot placing one HOBO logger (Onset, model UA-002-08 (± 0.53 $^{\circ}$C)) at the center of each plot to record temperature 5 cm above substrate surface, and light intensity at the soil surface. One i-button (Cooper-Atkins Corp., model GI 100, (± 1 $^{\circ}$C)) was placed in the center of each plot at ~ 5 cm in depth to record soil temperature. The HOBO loggers and i-buttons were used to assess the effect of the presence of saltwort on the microclimate for black mangrove seedlings and propagules. Equipment malfunction in the HOBO loggers and i-buttons failed to produce any replications for the adult mangrove base line treatment and was thus excluded from data analysis. However, the data collected from the adult mangroves was still included in the figures for overall visual comparison. Pore-water sampling, redox potential and total C and N contents were measured once. Composite pore-water samples were obtained at a depth of 10 cm using a suction sampler (M. H. E. products, 35 mm Push Point

Sediment Research Sampler) for each plot, three 100 ml samples were combined in a clean 1 l thermos rinsed between collections with DI water . The composite sample yielded a 50 ml sample for analysis. The pore-water samples were analyzed for nitrates (NO_3), phosphates (PO_4), pH and salinity. Nitrate and PO_4 test kits (Hach Pocket Colorimeter II, model 58700-02) were used to measure nutrient contents of the pore-water samples. A handheld tester (Hanna instruments, model HI98121, (± 0.05 pH/ ± 2mV) was used for pH. Salinity was measured as parts per thousand (‰) with a handheld refractometer. Although included in the table (as an indication that samples were collected without surface water contamination) surface water collected was not included in the data analysis. Redox measurements were obtained with a field redox meter built following Vepraskas (2002). Three soil composite samples per plot were collected to a depth of 13 cm utilizing a core sampler with a diameter of 2.5 cm. The soil samples were dried for 72 h at 60 °C, grinded and analyzed with an elemental combustion system (Costech, model ECS 4010 (for C, hydrogen (H), N ± 0.5%) to determine total C and N contents. Propagule stranding was recorded seven times over 121 d. while propagule anchoring was measured once. The data was cumulatively combined per treatment and the final collection data was subjected to analysis.

Seedling Pair Experiment

Growth responses were measured four times over the course of a year. Selected individuals were measured for height (cm), basal diameter (mm), and *in vivo* leaf chlorophyll (mg/m^2) content as above. The final data collection for growth responses (basal and height) was negatively affected by sedimentation and scouring the data was removed from the analysis and not displayed in the figures presented. Survival numbers were determined as a default process while recording growth responses; however, initial survival measurements were included in data set.

Statistical Analyses

A one-way ANOVA was used to analyze all data collected. To assess the assumptions of the ANOVA test, independent variable categories, independence of observations, visual inspection of Q-Q plots, Shapiro-Wilk test for normality and Levene's test for homogeneity of variances were used. All significant differences were followed up with a post hoc analysis using Tukey's HSD test, except in the case of the seedling experiment where only two independent variables were present. An additional post hoc test (Duncan's Multiple Range Test (MRT)) was included in the analysis of total N as a more sensitive test of significance (Carmer and Swanson, 1973) when Tukey's HSD failed to identify the point of significance.

CHAPTER III

RESULTS

Plant Responses

Seedling Stranding and Root Anchoring

Stranding of black mangrove seedlings within the established plots was documented over 112 d, between October 2017 and February 2018, when propagules were present and abundant in South TEXAS. A very large and significant difference in the ability of naturally occurring saltwort to retain propagules in comparison to bare-mud plots and planted saltwort plots was found ($F_{(0.05)\,(2)} = 52.31$, $p < 0.001$) (Figure 7). The total cumulative propagule stranding in saltwort covered plots was 17 times greater compared to propagule stranding in bare-mud. Propagule stranding in planted saltwort plots was also very low, only slightly higher than on bare-mud (Figure 7).

The root anchoring experiment showed almost twice as many propagules were able to anchor their root in natural saltwort than in bare-mud plots and planted saltwort plots ($F_{(0.05)\,(2)} = 1.13$, $p = 0.366$) (Figure 8). Although the differences among treatments was not statistically significant, the differences in averages indicates that 1-year-old planted saltwort at 53 plants/m^2 does not possess the canopy or branching density needed to facilitate root anchoring.

Figure 7. Effects of the presence of saltwort (*Batis maritima)*, naturally occurring and planted, on black mangrove (*Avicennia germinans)* propagule stranding; counts ± SE (bars). Cumulative stranding is presented for experimental plots at Holly Beach, TX. after 112 d, between October 27, 2018 and February 16, 2019. Significant differences ($p<0.05$) are indicated by different letters.

Figure 8. Anchoring (rooting) of black mangrove (*Avicennia germinans*) propagules after 23 d in the established plots (detailed in Figure 7), March 2019. Counts ± SE (bars) and letters as in Figure 7.

Pair Seedling Survival

Pair Experiment. Of the 30 individuals originally marked (15 growing in bare-mud and 15 black mangroves in saltwort patches), the bare-mud seedlings experienced 100% mortality within 4 months while the saltwort patch seedlings experienced 27% mortality (Figure 9). After this high initial mortality, seedlings mortality in the saltwort patch was maintained at 0% until the final sampling date 12 months later resulting in an overall 60% mortality for the saltwort patch seedlings (Figure 9).

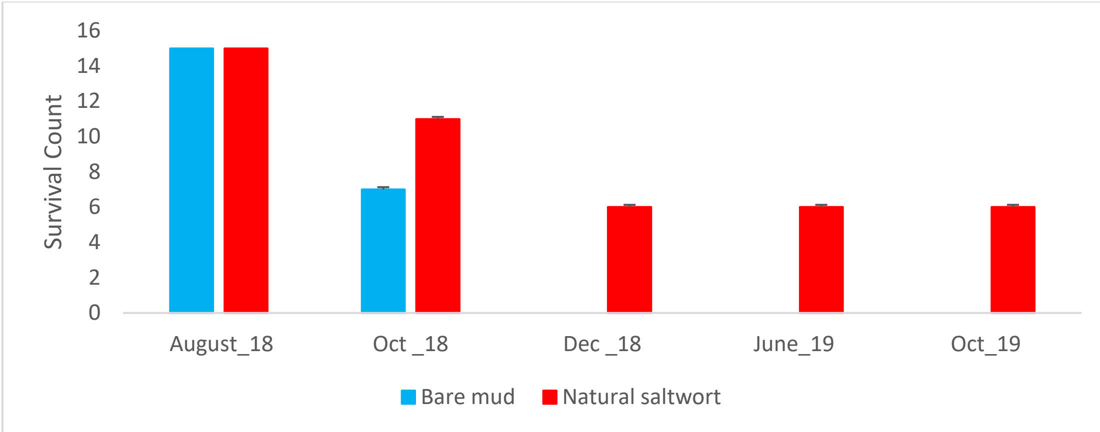

Figure 9. Black mangrove (*Avicennia germinans*) current year seedlings survival in the pair experiment, with 15 individuals per treatment. No statistical analysis was conducted due to complete mortality of bare-mud seedlings. Data are counts ± SE (bars).

Overall, these older (two-year-old) seedlings had a higher survival rate; bare-mud seedlings experienced 20% mortality while the saltwort patch seedlings survived for the initial 10 months. At the final measurement, these older seedlings experienced 40% mortality when growing in bare-mud and 33% mortality when growing within saltwort patches (Figure 10).

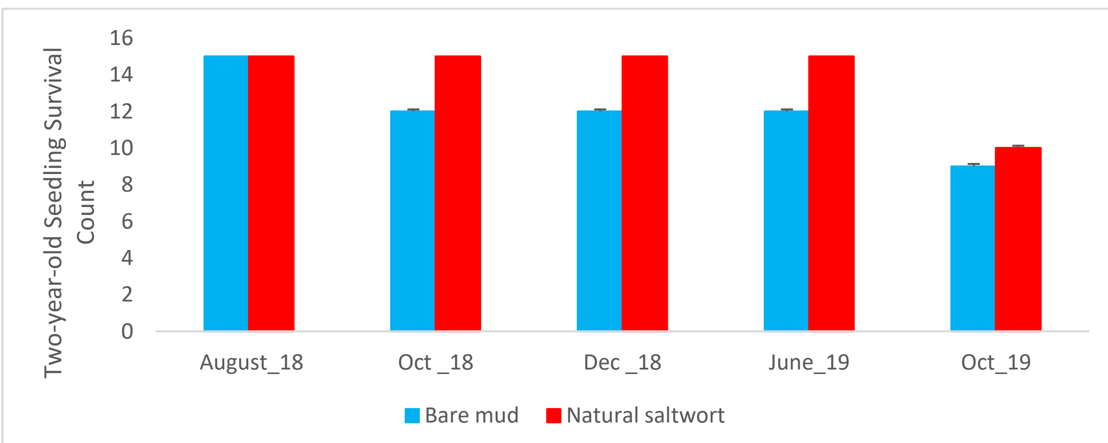

Figure 10. Black mangrove (*Avicennia germinans*) 2-3 years old seedling survival, pair experiment, with 15 individuals per treatment. Data are counts ± SE (bars). No statistical analysis was conducted.

Plot Seedling Survival

Plot experiment. The transplanted nursery seedlings were sampled one month after transplantation, the bare-mud seedlings experienced 12% mortality while the saltwort patch seedlings experienced 17% mortality (Figure 11). A trend that would not last. About 6 mos. after transplantation, mortality was 100% for seedlings in bare-mud plots compared to 81% mortality for seedlings in natural saltwort patches. The experiment continued for 10 more months during which time the natural saltwort patches remained at 81% mortality. The seedlings used in this plot experiment were of the same age and vicinity as the seedlings used in the pair experiment and of these all the bare-mud seedlings experienced the same level of mortality (100%).

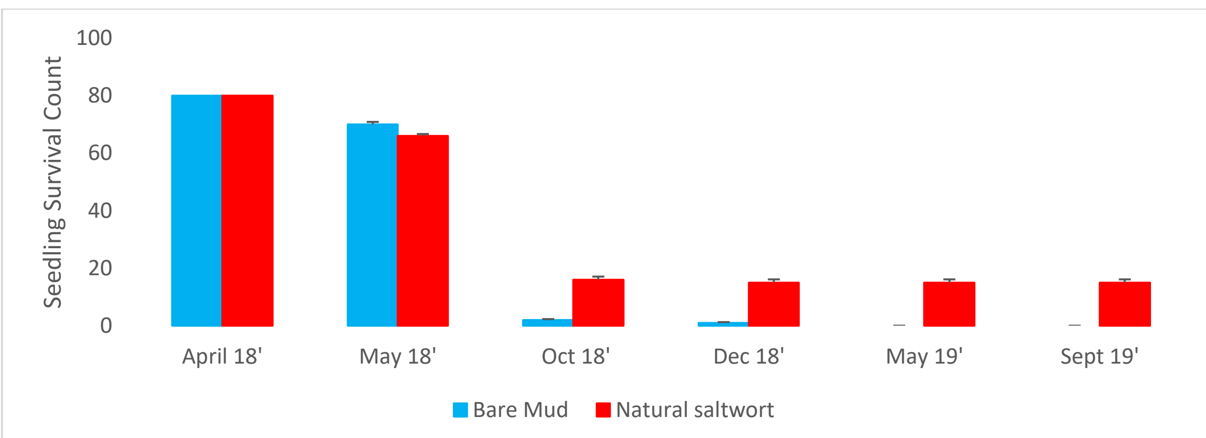

Figure 11. Survival of transplanted black mangrove (*Avicennia germinans*) seedlings in the plot experiment, with 80 individuals per treatment. Data are counts ± SE (bars). No statistical analysis was conducted due to complete mortality of bare-mud treatment seedlings.

Seedling Growth and Leaf Chlorophyll Content

Growth responses to saltwort treatment resulted in no significant differences in basal diameter between the 2-3-year old black mangrove seedlings in October 2018, December 2018 and June 2019 ($F_{(0.05)(1.)}$ = 0.154, p = 0.700; $F_{(0.05)(1)}$ = 0.176, p = 0.681; $F_{(0.05)(1)}$ = 1.026, p = 0.326, respectively) (Figure 12). However, there was a visible trend of greater gains in biomass for the natural saltwort 2-3 years old seedlings for most of the measuring period. Over the course of the experiment, seedling height responses trended towards a statistically significantly difference between treatments with no significant differences in(October 2018 ($F_{(0.05)(1)}$ = 1.742, p = 0.204), but significant differences in December 2018 and June 2019 ($F_{(0.05)(1)}$ = 5.002, p = 0.039; $F_{(0.05)(1)}$ = 14.701, p = 0.001, respectively). (Figure 13). *In vivo* leaf chlorophyll content resulted in no significant differences between treatments for the samplings of October 2018, December 2018, June 2019 and October 2019 ($F_{(0.05)(1)}$ = 0.036, p = 0.852; $F_{(0.05)(1)}$ = 0.123, p = 0.730; $F_{(0.05)(1)}$ = 1.967, p = 0.179; $F_{(0.05)(1)}$ = 0.183, p = 0.674), respectively). (Figure 14).

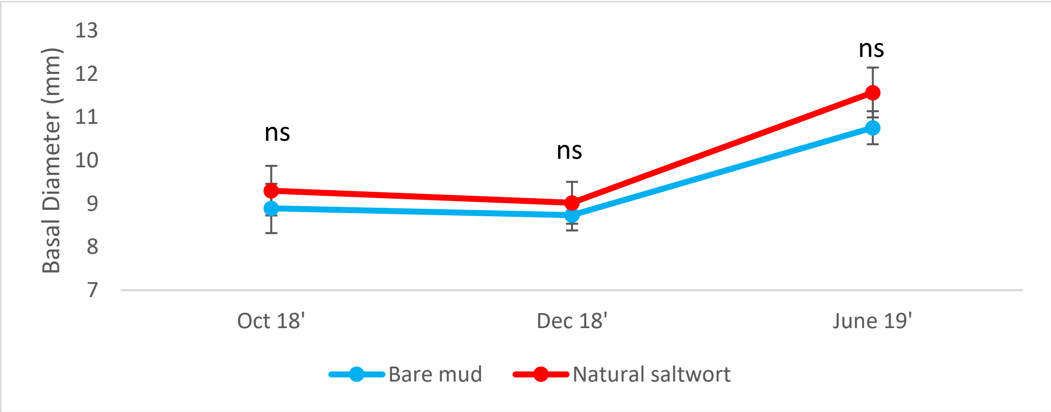

Figure 12. Mean ± SE (bars) basal diameter (mm) over time of black mangrove (*Avicennia germinans*) 2-3 years old seedlings. Sample size = 19; ns = not significant.

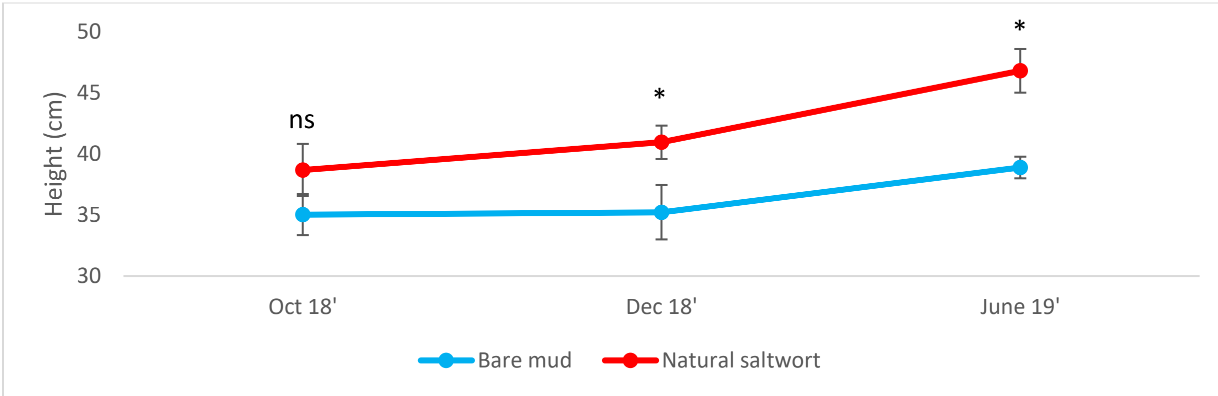

Figure 13. Means ± SE (bars) height (cm) over time of black mangrove (*Avicennia germinans*) 2-3 years old seedlings. Sample size = 19; ns = not significant; * = P ≤ 0.05.

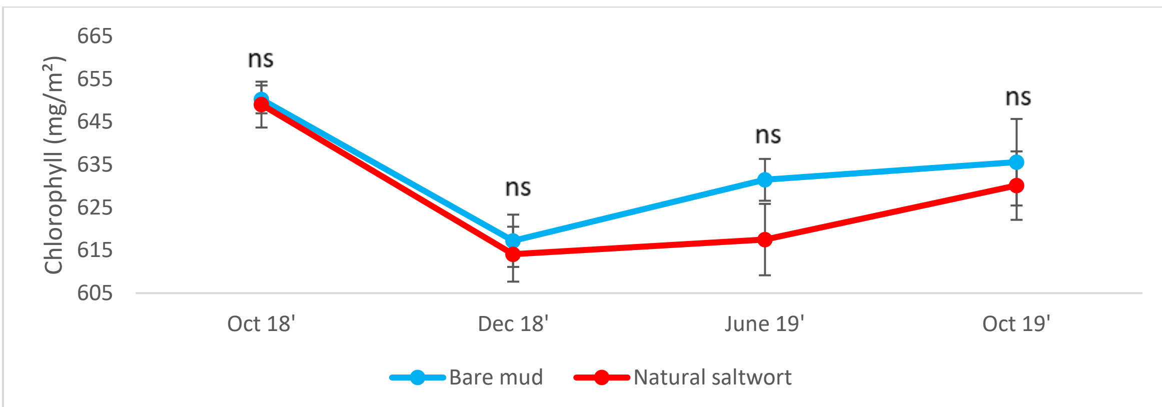

Figure 14. Means ± SE (bars) leaf chlorophyll (mg/m^2) content over time of black mangrove (*Avicennia germinans*) 2-3 years old seedlings. Sample sizes = 19; ns = not significant.

Soil Variables

Soil total C content was significantly different beneath natural saltwort and the adult black mangrove plots ($F_{(0.05)\,(3)}$ = 2.76, p = 0.054) (Figure 15). Total N content was marginally significantly different among treatments ($F_{(0.05)\,(3)}$ = 2.82, p = 0.050) (Figure16). A Tukey’s post hoc test followed by a Duncan's post hoc test was applied to detect which treatment showed a statistically significant difference when the former failed to. The mean N concentrations were

20% lower in natural saltwort soil compared to adult mangroves (Figure 16) as was the case for total C but with a smaller difference (Figure 15).

Figure 15. Mean ± SE (bars) total soil carbon contents (%) as affected by soil cover treatments in the plot experiment. Sample size = 8 per treatment, different letters represent significant differences (p<0.05) as per the Tukey HSD test.

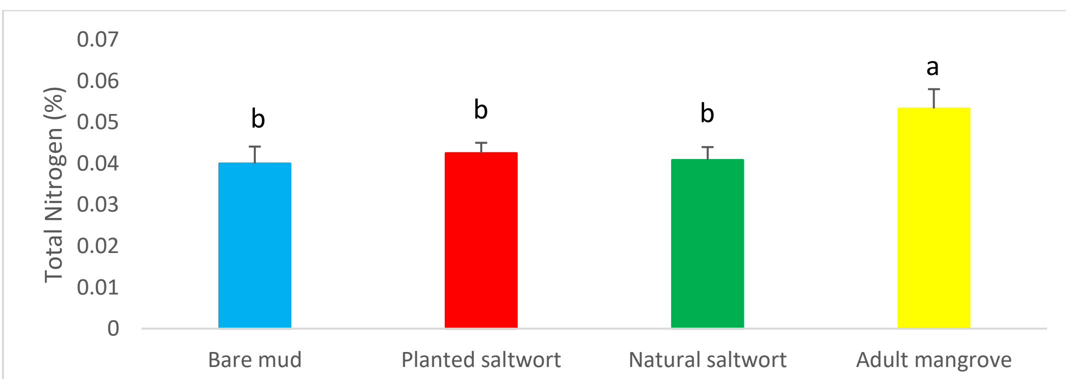

Figure 16. Mean ± SE (bars) total soil nitrogen contents (%) as affected by soil cover treatments in the plot experiment Sample size = 8 per treatment, different letters represent significant differences (p<0.05) as per Duncan's MRT post hoc test.

Pore-water analysis resulted in a significant difference among treatments for pH ($F_{(0.05)(3)} = 6.29$, $p = 0.008$), with more neutral values recorded under saltwort cover, both planted and natural (Table 2). Phosphate concentrations among treatments were not significantly different ($F_{(0.05)\,(3)}$ = 2.06, p = 0.160), however; a slightly higher PO_4 concentration was recorded in the planted saltwort treatment (Table 2). Nitrate concentrations were also not significantly different ($F_{(0.05)\,(3)}$ = 3.13, p = 0.066), a higher NO_3 concentration was however observed for planted saltwort (Table 2). Pore-water salinity showed no significant differences ($F_{(0.05)\,(3)}$ = 0.66, p = 0.607) although the levels were higher than the surface tidal water. Similarly, redox potential measurements indicated no statistically significant differences among treatments ($F_{(0.05)\,(3)}$ = 2.82, p = 0.084) (Figure 17). A more oxidized state of the soil beneath natural saltwort and to a lesser degree under adult mangrove was observed, compared to bare-mud and the planted saltwort plots.

Table 2. Pore-water analyses variables from treatment plots (n = 20) 10 cm depth, except for surface water, which is included to contrast against pore-water profiles. Values are mean ± se. Different letters represent significant differences as per the Tukeys HSD test and * denotes exclusion from statistical analyses (see text for details).

Treatment	pH	Salinity (ppt)	PO_4 (mg/l)	NO_3 (mg/l)
Bare-mud	7.41 (± 0.03) [a]	52.75 (± 2.56)	0.33 (± 0.03)	0.52 (± 0.16)
Planted saltwort	7.35 (± 0.03) [b]	55.00 (± 1.29)	0.43 (± 0.05)	0.60 (± 0.05)
Natural saltwort	7.20 (± 0.06) [b]	54.50 (± 0.29)	0.32 (± 0.04)	0.25 (± 0.14)
Adult mangrove	7.40 (± 0.04) [a]	55.75 (± 1.25)	0.33 (± 0.03)	0.23 (± 0.03)
Surface water*	7.85 (± 0.05)	49.75 (± 0.25)	0.03 (± 0.01)	0.23 (± 0.04)

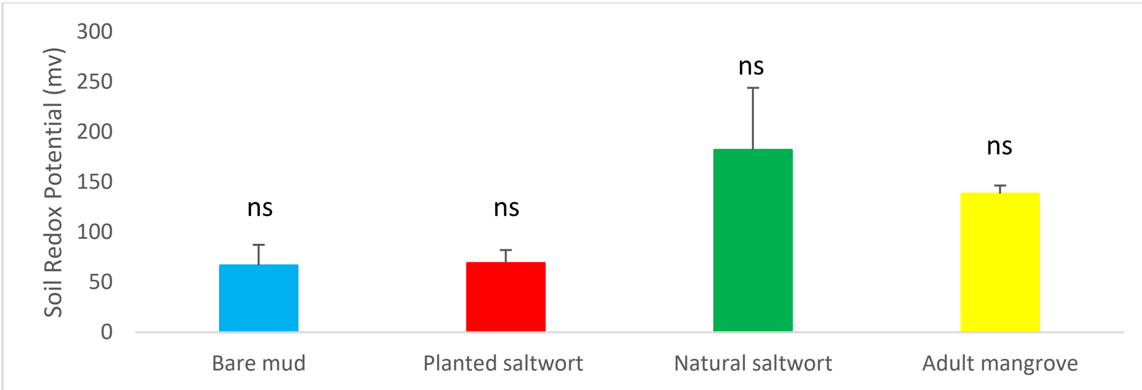

Figure 17. Mean ± SE (bars) soil redox potential (mv) under various plant cover in the plot experiment. Sample size = 16, ns = not significant.

Effects on Microclimate

Microclimatic measurements were continuously recorded at least four times a day for a year. The adult mangrove plots were excluded from the data analyses due to equipment failure, resulting in lack of replications. The soil temperature measurements were found to have had a significant difference among treatments for the last six data collections of the experiment (Figure 18; Table 3). The late spring to early fall months (Figure 18) shows a pattern of cooler soil temperatures under natural saltwort and adult mangrove cover and correspond to the most thermally stressful periods at the study site. Air temperature was significantly impacted by treatment for almost the entire measuring period the exception being January 2019 ($F_{(0.05)(3)}$ = 7.781, p = 0.065) (Figure 19; Table 4). Similar to the late spring to early fall months trend seen in soil temperatures, is evident in air temperatures despite data highly overlapping. Light intensity differences were significant among treatments on July 2018 and August 2018 ($F_{(0.05)(3)}$ = 29.756, p = 0.011; $F_{(0.05)(3)}$ = 347.405, p = 0.000, respectively) (Figures 20; Table 5). This coincides with the highest light intensity readings (94,660 lumens) for bare-mud plot compared to 23,630 lumens for the natural saltwort plots.

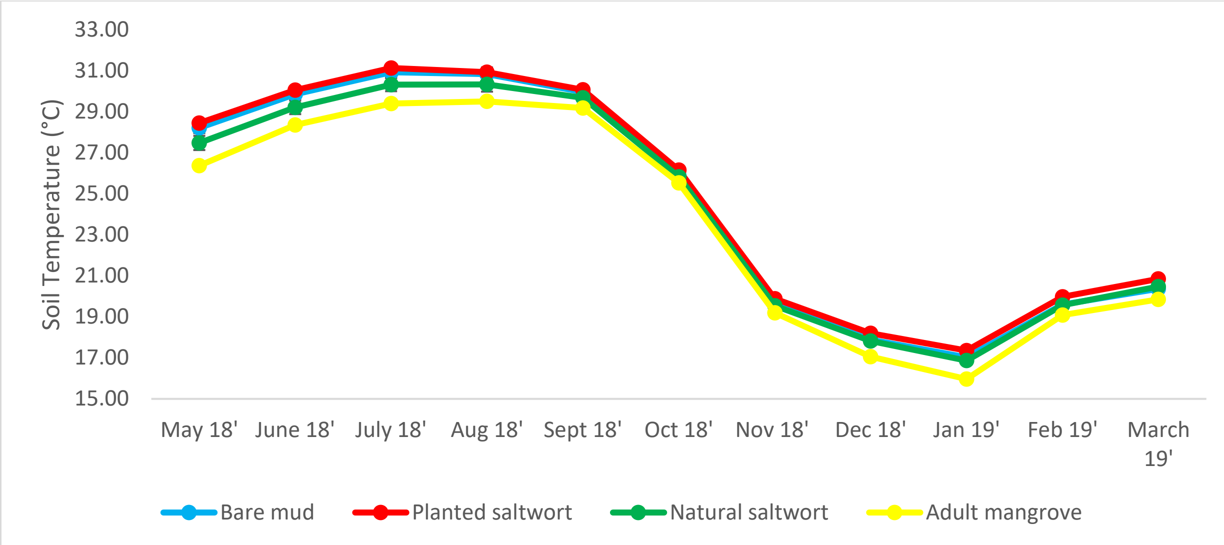

Figure 18. Mean ± SE (bars) soil temperature (°C) at 5 cm depth under various plant cover in the plot experiment. Error bars too small to be visible. Sample size = 6 / treatment.

Table 3. Soil temperatures (°C) per month, values are mean ± SE, F and p values and Tukey's HSD Post Hoc (different letters represent significant differences, p<0.05).

	STATISTICS				TUKEY'S HSD POST HOC		
Month	Mean	SE	F	P value	Bare Mud	Planted Saltwort	Natural Saltwort
May 18	26.88	.19	2.210	0.191	a	a	a
June 18	28.70	.17	1.945	0.223	a	a	a
July 18	29.64	.18	1.565	0.284	a	a	a
Aug 18	29.75	.17	0.927	0.446	a	a	a
Sept 18	29.15	.16	1.026	0.414	a	a	a
Oct 18	25.21	.18	5.227	0.048	b	a	ab
Nov 18	19.01	.24	8.125	0.020	b	a	b
Dec 18	17.20	.23	7.358	0.024	b	a	ab
Jan 19	16.51	.26	9.140	0.015	b	a	b
Feb 19	19.40	.18	8.440	0.018	b	a	b
Mar 19	20.10	.18	9.429	0.014	b	a	b

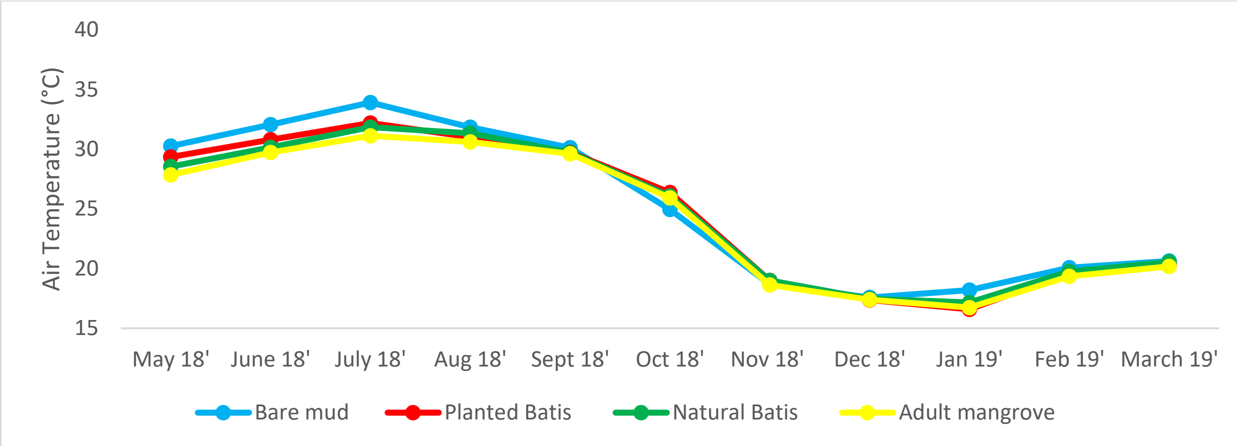

Figure 19. Mean ± SE (bars) air temperature (°C) at 5 cm height under various soil cover in the plot experiment. Error bars too small to be visible. Sample size = 6 / treatment.

Table 4. Air temperatures (°C) per month, values are mean ± SE, F and p values and Tukey's HSD Post Hoc (different letters represent significant differences, p<0.05).

	STATISTICS				TUKEY'S HSD POST HOC		
Month	Mean	± SE	F	P value	Bare Mud	Planted Saltwort	Natural Saltwort
May 18	29.94	.21	15.040	0.027	a	a	b
June 18	31.49	.25	17.017	0.023	a	a	b
July_18	33.42	.42	25.481	0.013	a	a	b
Aug 18	31.66	.24	47.289	0.005	b	a	c
Sept 18	30.01	.08	24.854	0.014	a	b	c
Oct 18	26.36	.06	9.424	0.051	a	a	a
Nov 18	19.24	.10	13.092	0.033	a	a	b
Dec 18	17.58	.07	9.616	0.050	a	a	a
Jan 19	17.00	.12	7.781	0.065	a	a	a
Feb 19	19.90	.07	18.950	0.020	a	a	b
Mar 19	20.57	.06	5.168	0.107	a	a	a

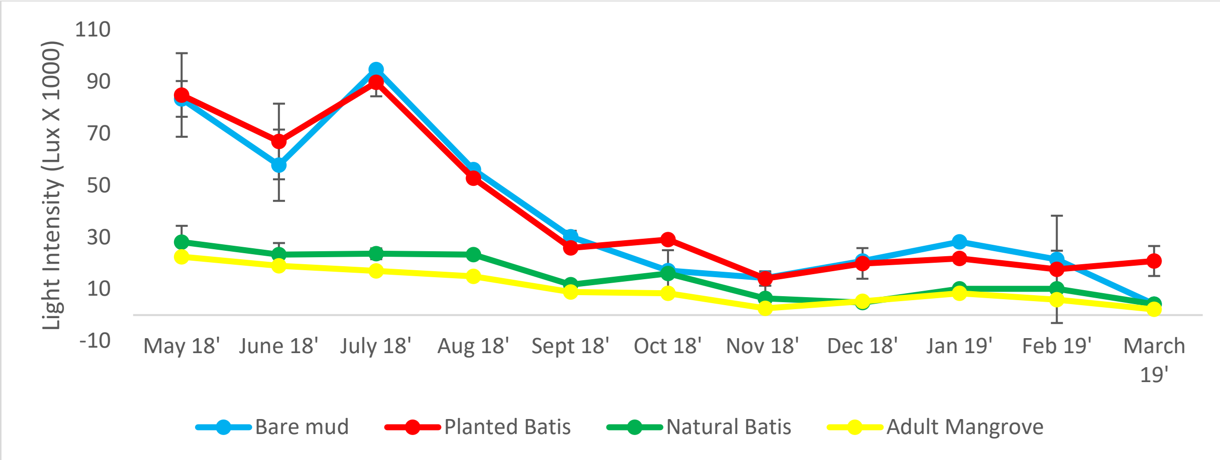

Figure 20. Mean ± SE (bars) light intensity (Lux) at 5 cm height. Error bars too small to be visible in some points. Sample size = 6 / treatment.

Table 5. Light intensity (Lux) per month, values are mean ± SE, F and p values and Tukey's HSD Post Hoc (different letters represent significant differences, p<0.05).

	STATISTICS				TUKEY'S HSD POST HOC		
Month	Mean	SE	F	P value	Bare Mud	Planted Saltwort	Natural Saltwort
May 18	29223.74	6989.34	1.667	0.326	a	a	a
June 18	23800.62	6551.36	0.733	0.550	a	a	a
July 18	34068.11	7463.018	29.756	0.011	a	a	b
Aug 18	20219.91	4081.06	347.405	0.000	b	a	c
Sept 18	9962.89	1574.07	7.631	0.067	a	a	a
Oct 18	13231.30	3872.51	1.384	0.375	a	a	a
Nov 18	13744.73	1823.33	0.234	0.805	a	a	a
Dec 18	6967.10	1936.55	0.776	0.535	a	a	a
Jan 19	3378.90	615.88	1.435	0.365	a	a	a
Feb 19	11502.62	6891.12	0.932	0.484	a	a	a
Mar 19	13169.44	3462.75	6.424	0.082	a	a	a

Discussion

This project was designed to confirm and elucidate positive plant-plant interactions between black mangrove seedlings and saltwort, an herbaceous halophyte. The apparent outcome of this interaction is the facilitation of seedling establishment and growth. This occurrence can be harnessed to enhance restoration efforts of mangrove habitats. Three possible avenues of facilitation, physical/mechanical, microclimatic, and soil conditions amelioration, were delved into.

The physical mechanisms of facilitation will be discussed first. Seedling survival is a key aspect of plant restoration programs. Several studies on exploiting positive plant interactions of co-occurring species have been documented (McKee, Rooth, and Feller, 2007; Moen, 1993; Nobel, 1980). Nobel (1980) reported that bare ground seedlings experience higher surface soil temperatures than their counter parts found in cushion plants. This mitigation of high temperature provides a "thermal refuge" (Arroyo et al., 2003) in harsh environmental conditions. Additionally, the presence of a nurse plant or cushion plant also lends support structures to rooting seedlings (McKee, Rooth, and Feller, 2007), and they can also provide a "hydric refuge" (Cavieres et al., 2006). The shading provided can help retain soil moisture by the reduction of evaporation forces playing upon the soil surface thus providing a more suitable environment for germination and seedling establishment (Callaghan, 1987; Moen, 1993). In this study, a large positive effect of saltwort on the physical stranding of mangrove propagules. Stranding or capture of a freely floating propagule by itself does not indicate ecological facilitation. However, if the rooting process is increased within saltwort then this will have been a positive interaction (Callaway, 1995). The anchoring or rooting experiment conducted revealed that twice as many propagules rooted successfully in natural saltwort patches than in bare-mud plots. Protection from desiccating winds on the tidal flats coupled with thermal (Arroyo et al., 2003) and hydric refuges (Cavieres et al., 2006) may explain this facilitation.

In the pair experiment with younger, current year seedlings, mortality was 100% for bare-mud plants compared to a 40% survival of the seedlings found in saltwort patches. The 2-3 years old plants growing in the bare-mud treatment had 60% survival compared to a 67% survival for the plants growing in saltwort patches. Survivability of the current year seedlings from the matched pair experiment coincided with results observed for the seedling plot experiment. The plot experiment recorded a 100% mortality for seedlings planted in bare-mud plots and 33% survival for those planted in natural saltwort. The lower mortalities of the seedlings growing in saltwort patches is an example of what Waughman, French, and Jones (1981) described in legumes of a terrestrial ecosystem as short-term facilitation. Waughman, French, and Jones (1981) wrote that as it pertains to the N fixation of legumes the benefits may only be available for a short period of time. Vallis (1978) postulated that it is in their death that legumes contribute the most N to surrounding grasses. Protection and structural support against the strong coastal winds as well as shielding seedlings from thermal stresses by the presence of saltwort are likely key for initial seedling survival. Although, Arroyo et al. (2003) dealt with cold environments in the Chilean Patagonian Andes the concept of cushion plants creating a microclimate more conducive to seedling establishment holds true for the microclimate created by saltwort at the study site. While experimenting with the facilitative effects of *D. spicata* and *Sesuvium portulacastrum* McKee, Rooth, and Feller (2007) found a 43-57% rooting of *R. mangle* seedlings in the herbaceous species opposed to 0% rooting in the bare substrate.

Although, seedling survival is paramount to restoration success, growth responses after survival is likely the second most important aspect to evaluate initial success. Two-3 years old seedlings growing with natural saltwort, on average tended to have larger basal diameter and greater height than their bare-mud counterparts. However, my final data collection for basal diameter and height were susceptible to error due to sedimentation and scouring between June-October based on observed patterns of erosion and hydrology during summer at Holly Beach. Saltwort patches experienced enough sediment accretion that resulted in shorter statures than previously recorded, and thus altered the initial basal diameter measuring location on the stem resulting in the elimination of one season (quarter) of data. This was not observed in bare-mud. Basal measurements were consistently larger for saltwort 2-3 years old seedlings, and in June 2019 the difference between treatments had increased. The height between treatments showed a greater response for 2-3 years old seedlings in saltwort. In October 2018, a significant effect in favor of the saltwort treatment was recorded. The effect increased in June 2019 with an even higher significance ($p < 0.001$) recorded. *In vivo* chlorophyll content, although non-significantly different between treatments, indicated a modest trend of increased photosynthetic activity for the bare-mud 2-3 years old seedlings throughout the study. This trend is surprising as shaded leaves tend to increase their chlorophyll content to offset reduced lighting (Cao, 2000).

The results obtained from the climatic instruments show an amelioration of both soil and air temperature respectively. During the hottest months a 0.5-1.0 °C reduction in soil temperatures between naturally occurring saltwort patches and the bare-mud plots was observed. The air temperature was affected to a greater extent recording a 1.5-2.0 °C reduction in the same treatments. McKee, Rooth, and Feller (2007) found a similar amelioration by *S. portulacastrum* and *D. spicata* in mangrove forests of the Caribbean. The amelioration ranged from 5-8 °C with *D. spicata* averaging 2 °C lower soil temperature than *S. portulacastrum* at the substrate surface. Notably, a cold mitigation effect from the saltwort patches during the winter months, an effect seen in alpine system cushion plants was not observed (Cavieres et al., 2006; Moen, 1993; Wilson, 1957). During this period, temperatures were almost identical in both bare-mud and saltwort patches, indicating little facilitative effects in the winter months. The shading ability of saltwort, which may seem minimal due to its short stature, was significant throughout the growing season reducing light intensity by at least 58%. Despite the low stature of saltwort, its ability to ameliorate light intensity, and soil and air temperature is a relevant facet of the facilitative interaction between saltwort and black mangrove seedlings. While saltwort will only grow to about 45 cm high before its branches topple over and become runners of the main plant, black mangrove seedlings will grow taller than their host plant after 2 or 3 yrs. thus eliminating any competition for sunlight in subsequent growth. McKee, Rooth, and Feller (2007) found that when planted in stands of *D. spicata*, black mangrove seedlings grew taller than their bare-mud counterparts. In addition, an increase in vigor and reproductive output was also documented for seedlings growing in *D. spicata*.

Effects of the presence of saltwort on soil conditions were examined through pore-water analyses for salinity, pH and concentrations of PO_4s and NO_3s, as well as soil C and N. Salinity was found not to be significantly different among treatments. However, plant shading can reduce soil salinity by reducing evaporation thus decreasing salt accumulation (Bertness and Hackner, 1994). A slightly lower salinity was recorded in the unshaded treatment, bare mud. Lacking underground root structures that can cause substrate elevation allowed the bare-mud plots to remain submerged for longer periods resulting in a lower salinity reading. The pH under natural saltwort was lower than for bare-mud and planted saltwort. This difference is likely an indication of oxidizing conditions occurring in the natural saltwort. Seybold et al. (2002) reported that in reducing conditions pH levels may rise as hydrogen ions are consumed in the process. Thus, because oxidation and reduction are reversible reactions it stands to reason that under oxidizing conditions pH values can decrease as hydrogen ion concentration increases. This observation is supported by the redox potential differences observed and discussed below.

Phosphates concentration in surface water were 10 times lower compared to concentrations in porewater at 10 cm depth in all treatments (Table 2). This coincides with the findings of Moore and Reddy (1994) in sediments of Lake Okeechobee, Florida, where surface oxidizing conditions resulted in lower concentrations soluble reactive P. Pore-water from planted saltwort plots had higher PO_4 concentrations than the bare-mud, natural saltwort, and adult mangrove plots. This may be a residual effect of the nursery propagation of the planted saltwort where commercial potting mix and soluble fertilizers were used. Comparing the PO_4 concentrations between the bare-mud and natural saltwort patches, there is no notable difference in available PO_4s. This observation suggest that P availability was not altered by the presence of saltwort.

Nitrate pore-water concentrations were not significantly different between the bare mud and natural saltwort plots. Surface water, natural saltwort and adult mangroves all displayed similar NO_3 concentrations. An explanation could be at 10 cm below the surface anaerobic conditions occur resulting in increased microbial fermentation and less available NO_3s (Moore and Reddy, 1994). If true, then one can conclude the bare mud treatment have less microbial activity than the vegetated plots.

Redox measurements indicate an oxidizing state (66-182 mV**)** for all treatments. Although no significant differences were found, a clear trend is apparent where the soil under natural saltwort (and adult mangrove to a lesser extent) is considerably more oxidized compare to bare-mud and planted saltwort. This finding supports the idea that abundant saltwort and mangrove roots maintain a less anaerobic soil, which is conducive for growth of young mangrove seedlings, in particular before they form their own pneumatophores. Radial oxidation of the rhizosphere can reduce stressors and prevent damage to root systems (Kludze and DeLaune, 1994; Pezeshki and DeLaune, 2012). Lonard (2016) describes how inundation and low soil redox can adversely affect black mangrove seedlings. Seedlings lacking aerenchyma tissue must rely on rhizosphere oxidation for metabolic process. In anerobic conditions mitochondrial respiration is halted and fermentation begins (Pezeshki and DeLaune, 2012). Fermentation is a costly process yielding only 2 ATP for every glucose molecule used while mitochondrial respiration can yield 36-38 ATP per glucose molecule (Pezeshki and DeLaune, 2012). Thus, one can see how an establishing seedling could be adversely affected by a reducing root environment.

Conclusions

The mechanisms of facilitation explored in this study imply physical, microclimatic and soil amelioration effects caused by the presence of saltwort. Facilitation in the case of saltwort and black mangrove seems to favor the later; the interaction is thus commensalism rather than mutualism. It can also be considered part of the ecological succession process in this particular habitat. This knowledge should be considered when mangrove restoration efforts are being devised in locations where both species coexist. Although the planted saltwort failed to show significant immediate effects that would result in facilitation as was documented for natural saltwort, it would be important to continue to monitor the planted saltwort plots to determine if conditions over time ameliorate for mangrove seedling growth as the canopy closes and root systems expand.

Restoration projects should explore the use of positive plant to plant interactions in their planning. In this study, black mangrove seedlings planted in naturally occurring saltwort patches outperform young black mangrove seedlings growing in bare mud by higher survivability 19% for the plot seedlings and 40% for the current year seedlings. The two-year old seedlings growing in natural saltwort showed better growth responses. Importantly, saltwort natural patches enhance propagule stranding and promote their anchoring and rooting. The incorporation of saltwort in restoration projects, either by planting prior to mangrove seedling transplantation or more effectively, by transplanting mangrove seedlings on existing saltwort patches would most certainly increase the success of mangrove restoration efforts. These findings can also be applied were other dominant herbaceous halophytes are present in the appropriate tidal range for this and other species of mangrove. Other herbaceous halophytes may facilitate survival and growth of mangrove seedlings but can also outcompete or otherwise negatively affect them. Site specific interactions should be the first consideration versus a cookie cutter approach to restoration.

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APPENDIX A

APPENDIX A

SALTWORT ROOTING EXPERIMENT

The literature on the propagation of saltwort is limited however it is clear that vegetative reproduction could result in greater success than seed germination (Johnson, 1935). Saltwort occurrence as a “fugitive species” with easy vegetative propagation, was suggested as a viable candidate for propagation by cuttings (Milbrandt and Tinsley, 2006.) In a preliminary test, five combinations of stem parts with and without a commercial rooting stimulant (Indole-3-butyric Acid powder) (Table 3) were compared. As this was an assessment of the best method to use for propagation a control group was not used. In a 50:50 mixture of peat moss and pearlite, saltwort cuttings were set for rooting, in a nursery table under full sun for 2 wks.

Table 6. Saltwort *Batis maritima* preliminary rooting experiment.

Treatment	Identification	Rooted cuttings %
1	Whole stem w/powder	100
2	Top stem w/powder	87.5
3	Bottom stem w/powder	100
4	Bottom stem w/o powder	87.5
5	Top stem w/o powder	37.5

The saltwort trays were placed in an accompanying basin tray that was kept flooded (10 cm) throughout the experiment. The cutting material was collected from a tidal flat area at Pier 19 in South Padre Island, Texas (26°04'48"N 97°10'07"W). Cuttings 25 cm long were prepared by removing all leaves. The diameter of stems vary thus cuttings were grouped into 3 categories:

whole, top and bottom stem with 8 replications per tray (n = 40) (Figure 21A). The average outside temperature during the experiment was 30.6 ˚C. On day 14, the cuttings and surrounding medium were gently washed away. The treatment results were photographed and compared (Figure 21B).

The rooting hormone powder was shown to be effective and was used in the large-scale propagation of saltwort material for the field experiment. Thus, 2,520 saltwort cuttings were produced at the nursery located in Sabal Palm Sanctuary (25°51’08.51’’ N 97°25’04.07” W), Brownsville, TX. The cuttings were grown for 3 ½ months under 50% shade cloth. The basin trays held 5 l of water with the growing trays of saltwort inserted. Being an obligate halophyte, saltwort cuttings may have an increased biomass when grown in moderate levels of salinity (Debez et al., 2010). Thus, 12 g/l of NaCl were added to each basin tray.

Figure 21. A) Saltwort (*Batis maritima*) vegetative rooting experiments and B) a comparison of root growth in *Batis maritima* cuttings. Labels at the bottom represent rooted cuttings detailed in Table 6.

APPENDIX B

APPENDIX B

Transplant Propagation

The literature on mangrove propagation through propagules is well documented (Tomlinson, 1986). Relying on proven techniques the procedure was simple, and no prior testing was conducted. Propagules were collected at San Martin Lake, TX. (Figure 22 A-C). Having prepared a 50:50 peat moss perlite medium, 360 cells were filled and placed in an individual clear plastic basin and kept flooded. The propagules used for seedling production were selected for size uniformity, general appearance, and shortest anchoring root extension. Those selected were placed atop of the growing medium in the cells and monitored.

Figures 22. A-C) San Martin Lake, TX. (26°00'25"N 97°18'03"W) black mangrove (*Avicennia germinans*) propagule gatherings.

Peat moss and perlite do not offer any nutrition to the emergent seedlings; thus, supplementation of mineral nutrition was essential. The saltwort and black mangrove seedlings received the following nutritional regimen. A nutrient solution following standard plant nutrient ratio N-P-K (9-58-08; 15 ml /4 l) was applied once to every tray at the 2nd week of rooting phase. On the 4th week and at every 2-week interval a nutrient solution following standard plant nutrient ratio N-P-K (20-20-20; 5 ml/4 l) nutrient solution was applied. Three weeks prior to field planting the mangrove seedlings were infested by whiteflies (*Trialeurodes vaporariorum* Westwood, 1856), spider mites (*Tetranychus* spp.), and aphids (Superfamily: Aphidoidea) for ~ 4 d before receiving a soapy water treatment. The damage was visually apparent through the blemishes remaining and the overall yellowing of those infected. While the saltwort and mangrove seedlings were growing at the nursery (Figure 23A, B), mangrove propagules were collected and stored in an open ice chest with a continual air stone in place to delay development. The ice chest water was completely replaced every 2 weeks for 2 months. These propagules were used to test stranding effect and not for any growth or establishment purposes.

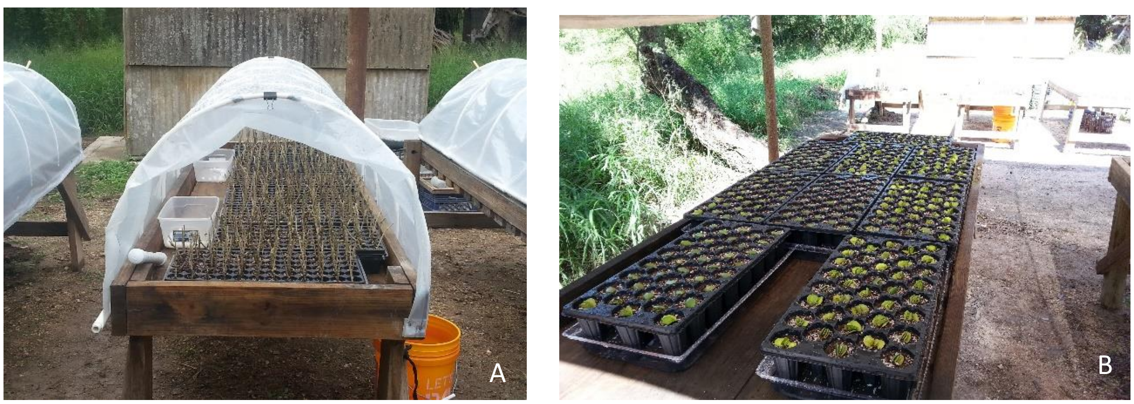
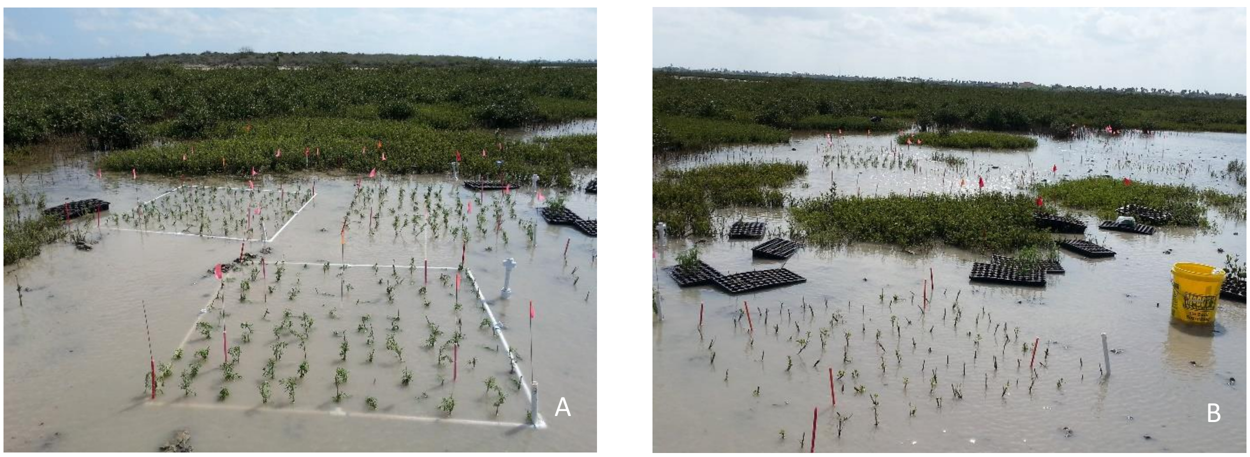


Figure 23. A) Saltwort (*Batis maritima*) cuttings growing on nursery tables, and B) black mangroves (*Avicennia germinans*) growing in water filled trays at nursery in Sabal Palm Sanctuary (25°51’08.51’’ N 97°25’04.07” W), Brownsville, TX.

Plot preparation began with the planting of nursery-grown saltwort (Figure 24A, B). A 1.5 m PVC quadrat equipped with stringed intervals set at 20 cm the assigned plots were marked and the saltwort seedlings were planted at each intersection. Using 20 cm lengths of 2.5 cm PVC shovels were crafted for minimal disturbance of the algal mat and matching the saltwort root ball. In practice, the shovels topped with a 1.9 cm PVC 4-way connector were best utilized to “core” the substrate for root ball insertion. The saltwort seedlings were monitored for the next 3 mo. and a 99% survivability was recorded. On the third month the saltwort, the mangrove seedlings and propagules were transplanted into their plots at Holly Beach. Using a 1 m^2 quadrat equipped with stringed intervals, 20 propagules were placed in position to their corresponding treatment plot. The mangrove seedlings were planted using the same quadrat as the propagules. The saltwort was planted using a separate 2.25 m^2 quadrat and intervals (one every 20 cm).



Figures 24. A and B) Post-planting of saltwort (*Batis maritima*) grown in nursery in Sabal Palm Sanctuary (25°51’08.51’’ N 97°25’04.07” W), Brownsville, TX.

BIOGRAPHICAL SKETCH

Javier Rene Navarro received an Associate of Art in Interdisciplinary Studies May 2014 from South Texas College. He received his Bachelor of Science in Biology from the University of Texas Rio Grande Valley in May 2017. In July 2020 he earned a Master of Science in Ocean Coastal and Earth Sciences from the University of Texas Rio Grande Valley. Correspondence: 10649 State Highway 107 Santa Rosa, TX. 78593; JavierNavarro300@gmail.com