FINAL REPORT

Project Name: CMP 15 – Identifying ecologically effective wetland restoration techniques in coastal wetlands.

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Cover photo: Aerial view of wetland restoration site in the Lower Neches Wildlife Management Area, taken August 2011.

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Background

Wetland restoration: objectives and challenges. Mitigation for wetland loss frequently involves habitat restoration. These projects often create terraces or mounds from dedicated excavations of adjacent soil or from dredge spoil pumped from channel maintenance projects. The outcome is a narrow terrace with low plant diversity that is adjacent to relatively deep open water habitats (Figure 1). Natural marshes typically have more expansive vegetated areas, include micro-variations in elevation that promote plant diversity, and are adjacent to shallow mudflat habitat (Feagin & Wu 2006). Terraces will likely increase habitat for certain marsh-dependent fishery species (Rozas et al. 2005), but this design may be unable to fulfill other important marsh functions, such as augmenting species diversity, increasing productivity and carbon sequestration, and providing support for higher trophic levels (Thom et al. 2004). In order to optimize restoration of the bulk of these important wetland functions, it is critically important to identify the most ecologically successful restoration techniques.

Figure 1: Marsh terraces

What are the most effective restoration techniques? It is often logistically challenging to quantitatively compare the ecological success of different restoration methods. However, we had the unique opportunity to compare several restored brackish tidal wetlands that were constructed in different formations from a variety of soil sources. These restored wetlands, located in the Old River Unit within the Lower Neches Wildlife Management Area (Texas Parks and Wildlife Department, Port Arthur, Texas), are in close vicinity to each other and are of similar age, providing an ideal scenario for comparisons among marsh restoration techniques. A set of wetlands constructed in spring 2008 varied in soil source and construction design, and another site constructed in 2011 utilized dredge material to fill a large (500 acre) continuous area to emergent marsh elevation. Our primary objective was to assess which restoration methods provide habitat that closely resembles natural habitat and contains the most complete set of ecological values on near-term and long-term time scales. We sought to provide quantitative comparisons of restoration techniques that could inform the local and state agencies (e.g., Texas Parks and Wildlife Department, Texas General Land Office, city and county governments) as they plan and design future restoration projects in the region.

Restoration and carbon sequestration. In wetland ecosystems, carbon is absorbed from the atmosphere by the microbial and plant communities. A portion of this carbon is respired, but the remainder is converted into biomass that may become sequestered in the soil (Bridgham et al. 2006). This carbon sequestration role of wetlands is of paramount importance in their ability to help maintain global atmospheric carbon levels. Therefore, a critical function of restored coastal wetlands is to sequester carbon from the atmosphere at the same rate as reference wetlands. There has been little previous work on patterns of carbon storage in restored coastal wetlands, so we sought to assess the carbon sequestration potential of various restoration construction techniques.

Invasive species management. Another challenge facing wetland restoration is the invasion of exotic species that may disrupt the ecological recovery of the site. Aquatic habitats of restored brackish wetlands along the northwestern Gulf of Mexico coast have been extensively invaded by a non-native plant, Eurasian watermilfoil (*Myriophyllum spicatum*). The ecological consequences of this invasion are unclear. This plant may provide some benefits, such as improvement of water clarity (James et al. 2004), but it may also be a less palatable food source for consumers (Boland et al. 2008). This project built on previous work that monitored the nature of the spread of this invasive plant and evaluated the ecological consequences of its displacement of native species such as widgeon grass (*Ruppia maritima*). Our work sought to determine whether or not the watermilfoil is a problem species that needs to be more intensively managed.

Project Tasks

We identified five discrete tasks for this project, and this report provides detailed results for each of the tasks.

Task 1: Monitor early development of a wetland restored with beneficial use dredge material and compare the ecological effectiveness of engineered restoration techniques

Task 2. Identify ecological consequences of aquatic exotic species invasion and develop management techniques

Task 3. Quantify the carbon sequestration potential of restored wetlands

Task 4. Web site for dissemination of data

Task 5. Education and outreach

Task 1: Monitor early development of a wetland restored with beneficial use dredge material and compare the ecological effectiveness of engineered restoration techniques

Task 1: Approach
Monitoring
We initiated a monitoring program in January 2009 in restored marshes in the Old River Unit of the Lower Neches Wildlife Management Area (see Cover Photo and Figure 2), administered by the Wildlife Division of the Texas Parks and Wildlife Department. The monitoring program continued through April 2012. Our surveys enabled us to assess differences in plant community development in the following habitat types:

1. *Excavated mounds*. Excavated mounds were constructed from material excavated from adjacent bottom sediment (Figure 3). These mounds were surrounded by water with an average depth of 1 to 1.5 meters. Mounds were planted with *Spartina alterniflora* cv. Vermilion stems transplanted from the National Resources Conservation Service (NRCS) Golden Meadow plant material center (Louisiana, USA) in fall 2007-early 2008.

2. *Filled mounds*. This approach first excavated mounds from adjacent bottom sediments. Then, open water areas between mounds were filled to a shallow depth (< 0.5 m) with dredge spoil pumped directly from a Gulf States Utilities (GSU) canal (Figure 3). Mounds were planted with *Spartina alterniflora* cv. Vermilion stems as above.

3. *Pumped mounds*. Pumped mounds were constructed with material from an area where dredge spoil had accumulated on the upland area along the GSU canal for several years. The dredge material was excavated, mixed with water, and pumped into the restoration area to form mounds (Figure 3). These mounds were surrounded by water with an average depth of <0.5 m. Mounds were planted with *Spartina alterniflora* cv. Vermilion stems as above.

4. *Terraces*. Terraces were constructed from material excavated from adjacent bottom sediment in a manner similar to excavated mounds. Terraces were built along the outer boundary of each of five subdivisions within the site as containment levees in order to reduce water movement and erosion potential. Mounds were planted with *Spartina alterniflora* cv. Vermilion stems and *Schoenoplectus californicus* stems as above.

5. *Reference marsh*. Reference marshes were adjacent areas with relatively dense stands of salt-tolerant intermediate marsh vegetation such as *Spartina patens*, *Spartina alterniflora*, and *Schoenoplectus robustus*.

6. *Beneficial use marsh*. A new restoration site was constructed by the Port of Orange (TX) in 2011 (completed December 2011; Figure 4). This new site was a beneficial use project that utilized dredge material to fill a large (500 acre) continuous area to emergent marsh elevation. This created a relatively homogeneous topography that closely resembled natural marshes in the area. This site was not actively planted; natural plant colonization was allowed to occur.
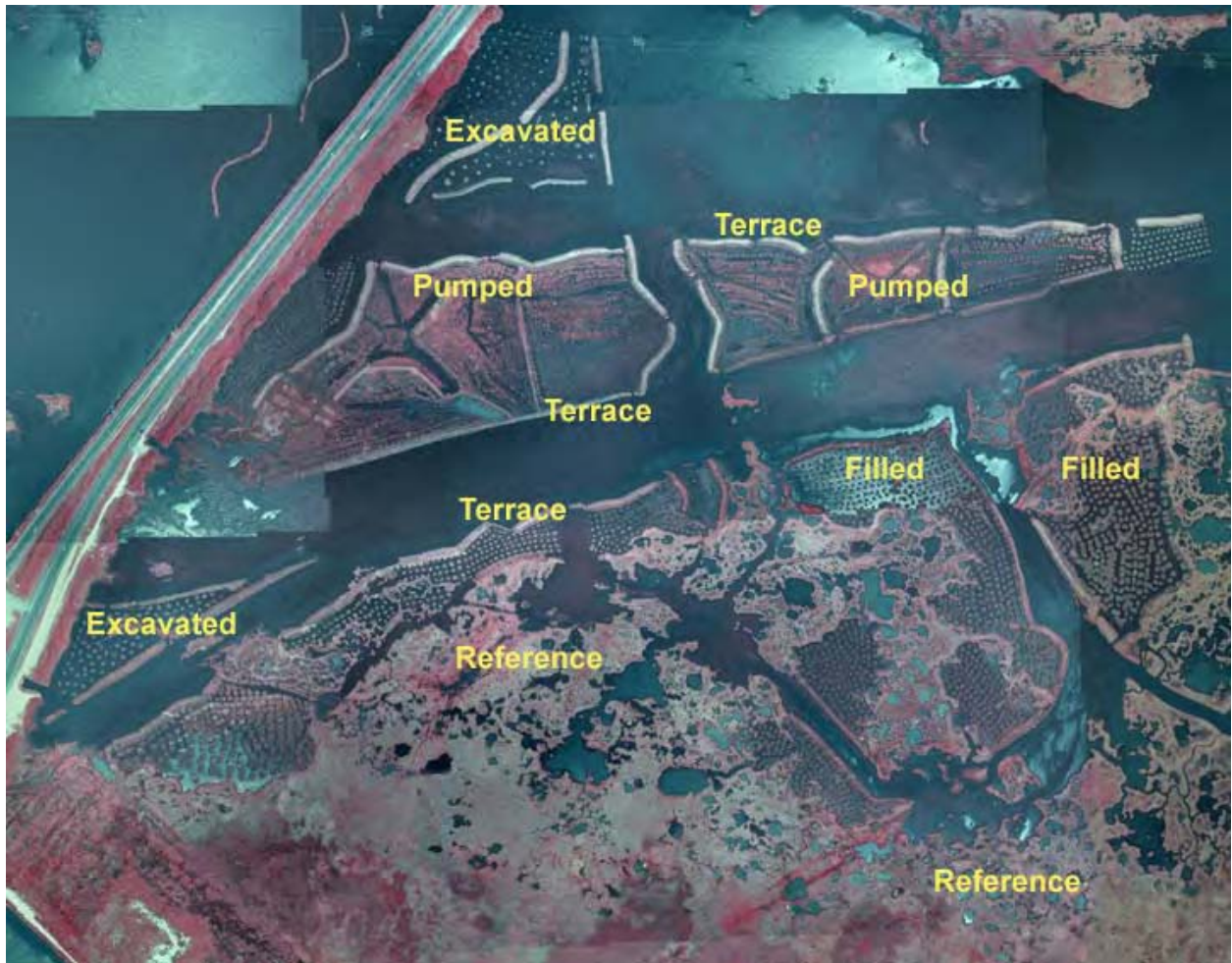
Figure 2: Aerial view of wetland restoration site in the Lower Neches Wildlife Management Area, taken September 2009. Areas of excavated, filled, and pumped mounds are noted; each mound area is surrounded by terraces. The reference site is located south of the constructed area.
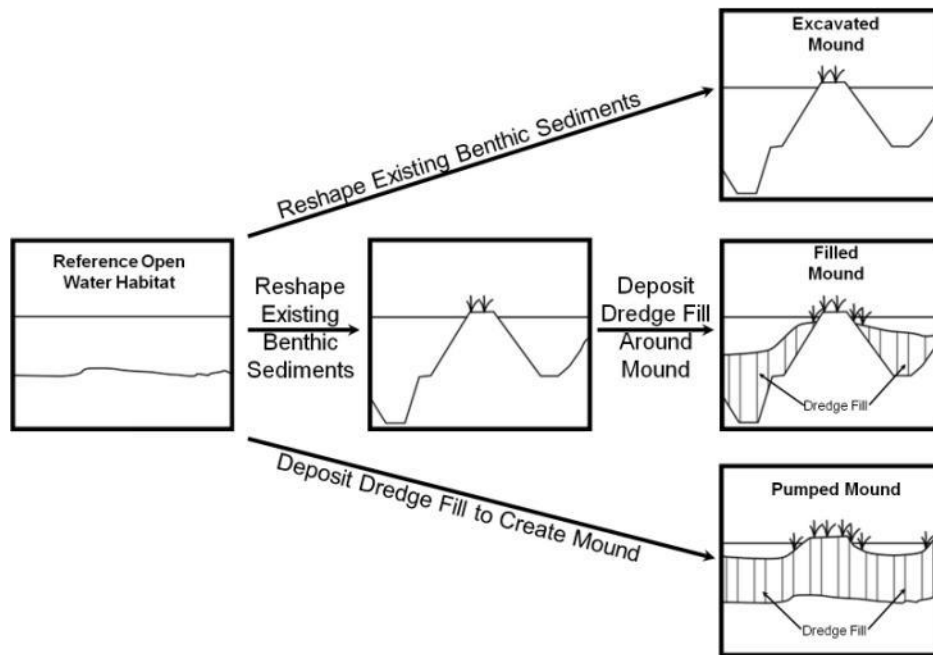
Figure 3: Graphic depiction of excavated, filled, and pumped mound construction design. Terraces were constructed in a similar manner as excavated mounds.

Figure 4: Hashed area depicts the area where dredge material was placed for the beneficial uses marsh along Highway 73 in Port Arthur, TX. The "Chevron Restoration Site" is the location of the engineered marsh described above.

At the first five habitat types, monitoring occurred at least four times per year (through April 2012) at 7-10 stations in each habitat type. The following information was collected on each sampling date:

- Photos were taken of each plot and percent plant cover was calculated using a spectral analysis program called VegMeasure.
- All of the stems within a representative 10 x 20 cm quadrat were clipped at the sediment surface and brought back to the lab. In the lab we:
    i. Counted the number of stems and then calculated stem density per m^2.
    ii. Measured the height of all stems and determined average stem heights in centimeters.
    iii. Rinsed and dried all tissue and calculated aboveground biomass as dry weight (kg/m^2).
- We measured nitrogen content of *Spartina alterniflora* leaf tissue with a CHNSO analyzer.
- We measured the phosphorus content of *Spartina alterniflora* leaf tissue using a dry-oxidation acid hydrolysis extraction followed by colorimetric analysis of the extract (Fourqurean et al. 1992).
- We measured the chlorophyll *a* content of *Spartina alterniflora* leaf tissue with a SPAD-502 portable leaf meter (Konica Minolta Corporation, USA). This device measures the transmission of 650 and 940 nm red light through leaves and is a reliable tool for inferring chlorophyll content (Netto et al. 2002).
- A throw trap was used to measure aquatic fauna species composition and density.

Additional metrics that were measured once/year included:

- Cores were collected to determine belowground plant biomass was collected on a subset of the sampling dates (June 2009, September 2010, September 2011).
- Soil characteristics that were recorded include:
    i. Grain size with the hydrometer method of Bouyoucos (1962).
    ii. Organic content by loss on ignition
    iii. Nitrogen content using a CHN elemental analyzer.
    iv. Phosphorus content using a dry-oxidation acid hydrolysis extraction followed by colorimetric analysis of the extract (Fourqurean et al. 1992).

Data from all sampling events are graphed for illustration purposes and important trends and patterns are highlighted in the text. Patterns during the contract period (Feb 7, 2011 – June 30, 2012) are emphasized, but in some cases, data from the monitoring period prior to the contract period are provided for context.

Beneficial uses marsh
Dredge material placement began on 8/16/11 and was completed in mid-December 2011. The dredge material remained unconsolidated through March 2012, limiting our ability to safely access the site. We conducted qualitative observations from the perimeter of the site, and the site remained unvegetated throughout the remainder of the project period (Figure 5, Figure 6, Figure 7). The original design of this particular site was intended to create relatively homogeneous emergent marsh habitat. Although this beneficial uses marsh is more homogeneous than the engineered marsh (see Figure 2), variations in dredge material placement resulted in a visible amount of topographical diversity, with some tidal channels for faunal access (Figure 5). Therefore, we expect that the site will eventually develop large expanses of emergent plant habitat that are interspersed with tidal channels that will allow aquatic fauna access to the area.

During a site visit in January 2012, we noted hundreds of waterfowl and shorebirds using the site. Natural plant colonization will likely occur over the next several years, and may be the focus of future work. Further monitoring of the beneficial uses marsh is not currently funded, but we will submit grant applications to continue our work at the site. In the meantime, we focused our detailed analyses on the other aspects of Task 1 – specifically, the ongoing monitoring of restored areas constructed in different mound and terrace configurations in 2008.

Figure 5: Aerial image of the beneficial uses site in November 2011, near the completion of dredge placement. View is looking north from Highway 73; containment terraces are visible along the edge of the site.

Figure 6: View of dredge material in January 2012, showing microvariations in topography and unvegetated terrain. Vegetation at the edge of the picture is in the adjacent reference area.

Figure 7: View of dredge material in the beneficial uses site in June 2012, showing the unvegetated terrain.

The following describes key results from the engineered marshes that were constructed in mound and terrace formations.

Percent cover

The restored areas generally had similar total live plant cover (all species combined) as the reference site (Figure 8). Representative pictures of each mound type are shown below (Figure 9, Figure 10, Figure 11). The lowest plant cover occurred in the winter senescence periods (January of each year). Emergent plant biomass did not appear to be affected by the extreme drought in the region in 2011 that dramatically raised salinities at the site (Figure 12); live biomass was higher in 2011 than on previous sampling dates.
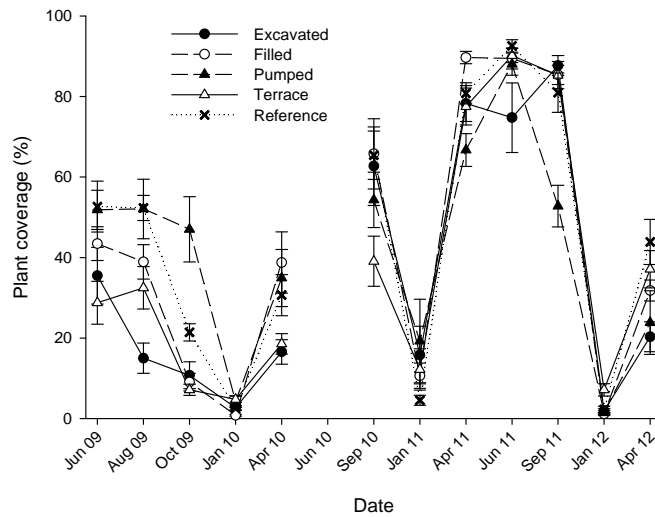
Figure 8: Total live plant cover at various restoration sites and a reference area. Bars represent standard error. No plant cover data were collected in June 2010. $6 \leq n \leq 10$

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Figure 9: Representative excavated mound area in June 2012.

Figure 10: Representative filled mound area in winter, showing shallow channels at low tide.

Figure 11: Representative pumped mound area, showing mound expansion into shallow channels.
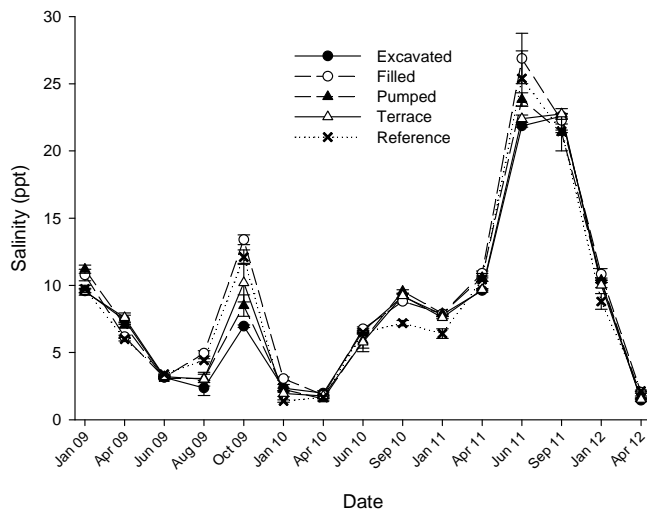
Figure 12: Water column salinity at various restoration sites and a reference area. Bars represent standard error. $6 \le n \le 10$

Stem density

The reference area has higher total live emergent plant stem density (all species combined) than all restored areas in 2009, but in 2010 and 2011, stem density was generally similar among all habitat types (Figure 13). No strong seasonal patterns were apparent, suggesting that stem density may be space-limited throughout the year.
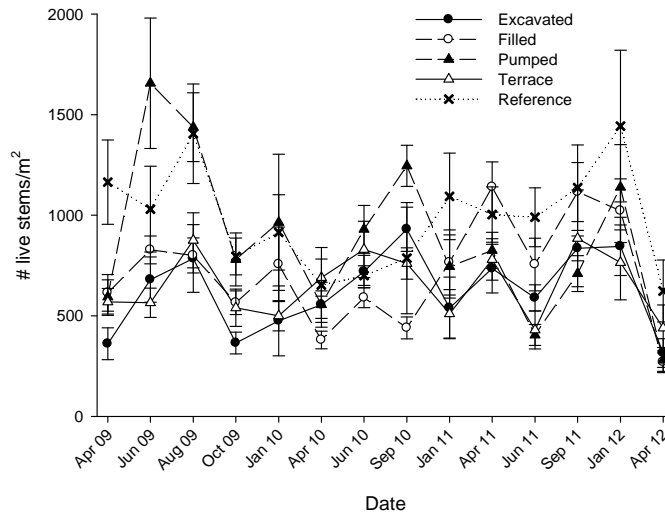
Figure 13: Total live plant stem density at various restoration sites and a reference area. Bars represent standard error. $6 \leq n \leq 10$

Canopy height

For the first two years of the study, the live canopy height was tallest on the terraces, but in 2011, the terrace canopy height was much lower (Figure 14). This change was likely driven by a decrease in the density of the tall species *Schoenoplectus californicus*. This pattern may have been driven by a die-off of that species or a sampling artifact. In either case, live stem height in restored areas tended to be lower in the winter (January) of each year, likely reflecting winter senescence.
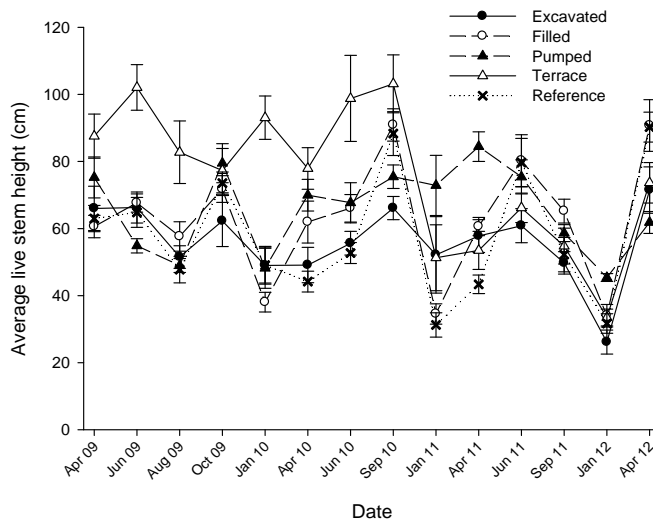
Figure 14: Average canopy height of all live species at various restoration sites and a reference area. Bars represent standard error. $6 \leq n \leq 10$

Aboveground plant biomass
Live aboveground plant biomass (all species combined) was generally similar among habitat types (Figure 15). Biomass was consistently lower in January and April than at other times of year, and peaks of biomass occurred during the summer growing seasons. Aboveground plant biomass was probably driven by environmental factors that vary seasonally, such as light, temperature, and rainfall.
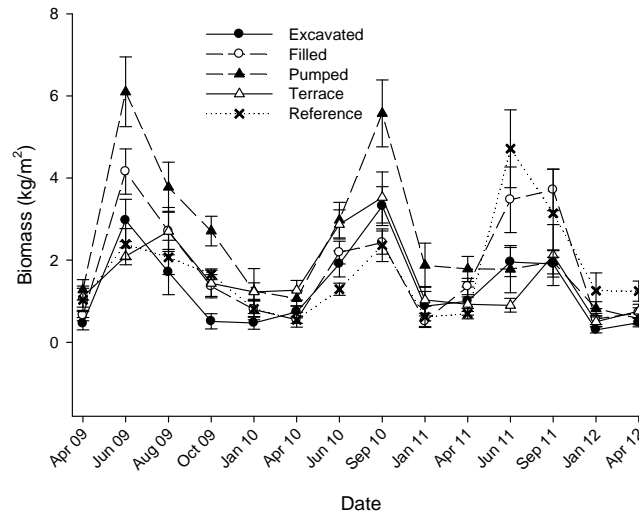
Figure 15: Total live aboveground plant biomass at various restoration sites and a reference area. Bars represent standard error. $6 \leq n \leq 10$

Belowground plant biomass
We collected cores to measure belowground plant biomass in September 2010 and September 2011 (data collected prior to the project period in June 2009 are included for reference). On all dates, total belowground biomass (pooled for all species) was substantially higher in the reference area than in all restored areas (Figure 16). All restored areas had similar belowground biomass. There were few changes in belowground biomass between June 2009 (Figure 16a) and September 2011 (Figure 16c), suggesting that there has been little development of the belowground root systems in the restored areas over the study period.
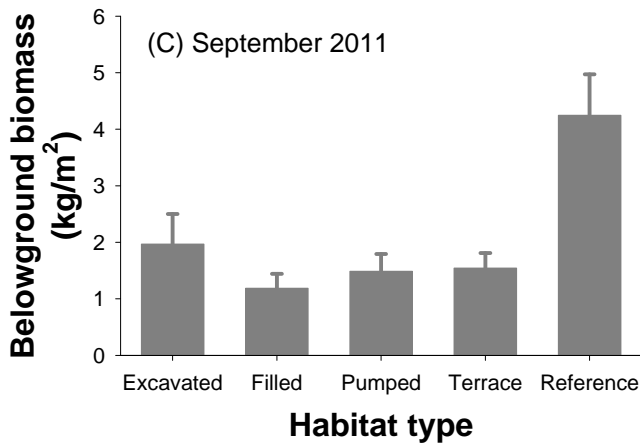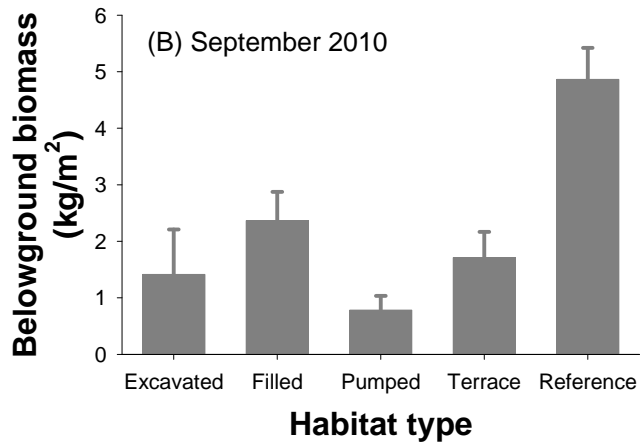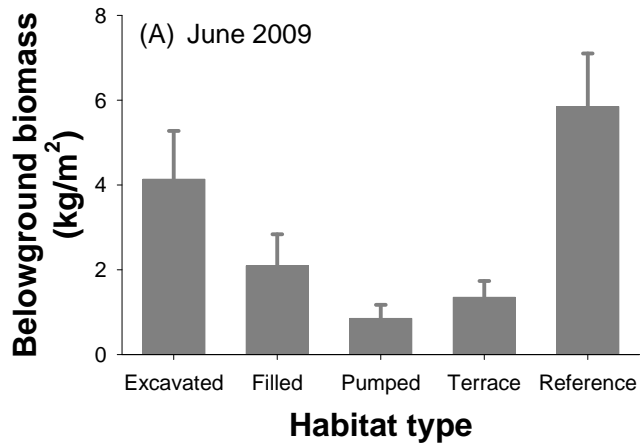
Figure 16: Total belowground plant biomass at various restoration sites and a reference area in (a) June 2009, (b) September 2010, and (c) September 2011. Bars represent standard error. $6 \le n \le 10$

Plant tissue nutrient content

The ratio of *Spartina alterniflora* leaf nitrogen to phosphorus (N:P) was measured through January 2012. The N:P ratio was generally similar among reference and all restored areas (Figure 17). In addition, the N:P ratio was consistently near or below the 30:1 ratio that suggests N limitation (Atkinson & Smith 1983), indicating that N may be limiting at all reference and restored areas.
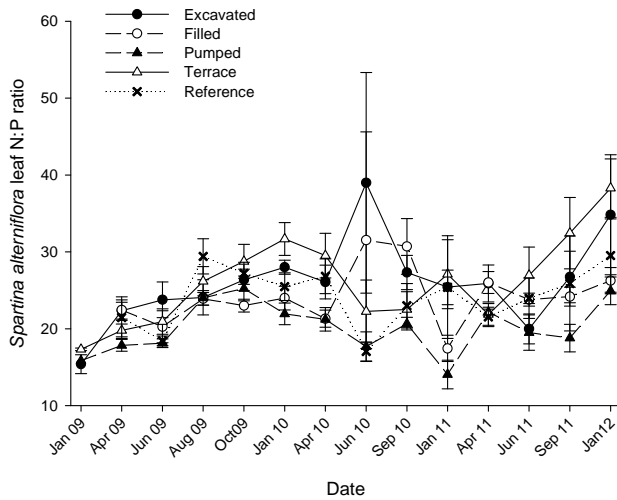
Figure 17: *Spartina alterniflora* leaf nitrogen: phosphorus content at various restoration sites and a reference area. Bars represent standard error. $6 \leq n \leq 10$

Spartina alterniflora leaf chlorophyll
Spartina alterniflora chlorophyll *a* content was variable and similar among all habitat types (Figure 18), suggesting that *S. alterniflora* productivity was similar at all reference and restored areas. Productivity was higher in 2009 than in subsequent years, perhaps reflecting the rapid growth and lack of space limitation in the newly restored areas. Alternatively, weather conditions may have been particularly conducive to growth that year.
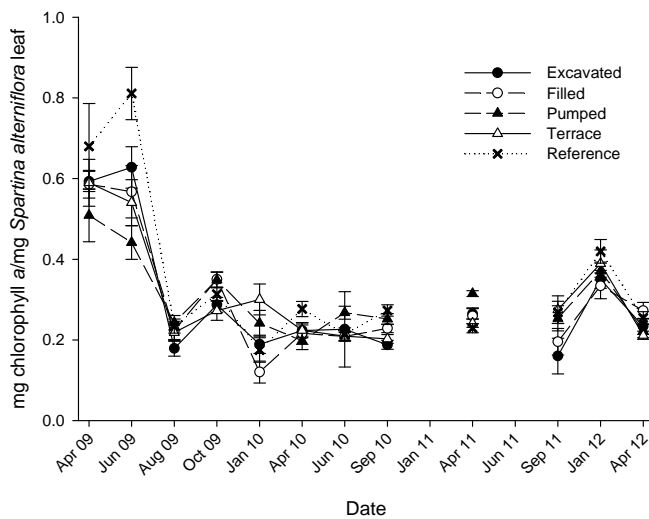
Figure 18: Chlorophyll *a* content of *Spartina alterniflora* leaves at various restoration sites and a reference area. Bars represent standard error. $6 \leq n \leq 10$. No data were collected in January or June 2011.

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Soil grain size
Soil grain size analyses were conducted in September 2011 (data collected prior to the project period in September 2010 and June 2009 are included for reference). On all sampling dates, soil percent sand was higher in the reference site than in all restored sites (Figure 19). The lower sand content in the restored areas likely reflects the loosely consolidated clay dredge material that was used in construction of the site.
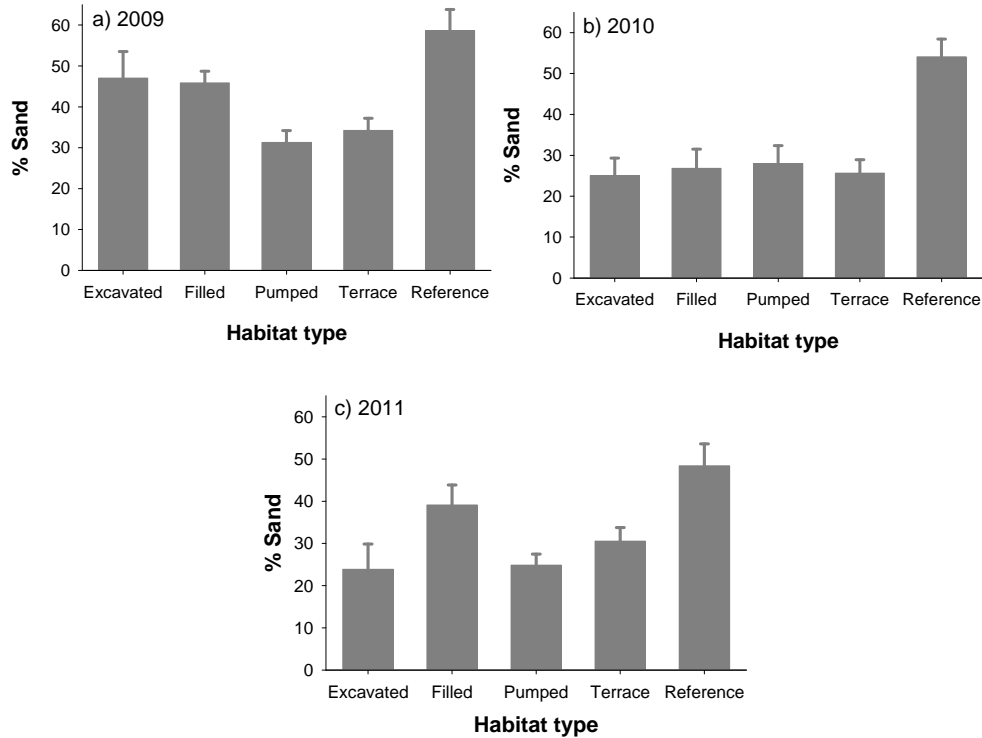
Figure 19: Percent sand content of soil at various restoration sites and a reference area in a) June 2009, b) September 2010, and c) September 2011. Bars represent standard error. $6 \le n \le 10$

Soil organic content
Soil organic content analyses were conducted in September 2011 (data collected prior to the project period in September 2010 and June 2009 are included for reference). In 2009, soil organic content was higher in the reference site than in all restored areas (Figure 20a). By 2010, however, the differences among sites were much less pronounced, suggesting that plant detritus and other organic matter were accumulating in the restored areas (Figure 20b). In September 2011, soil organic content was lower in filled, pumped, and terrace sites than in excavated and reference areas (Figure 20c).
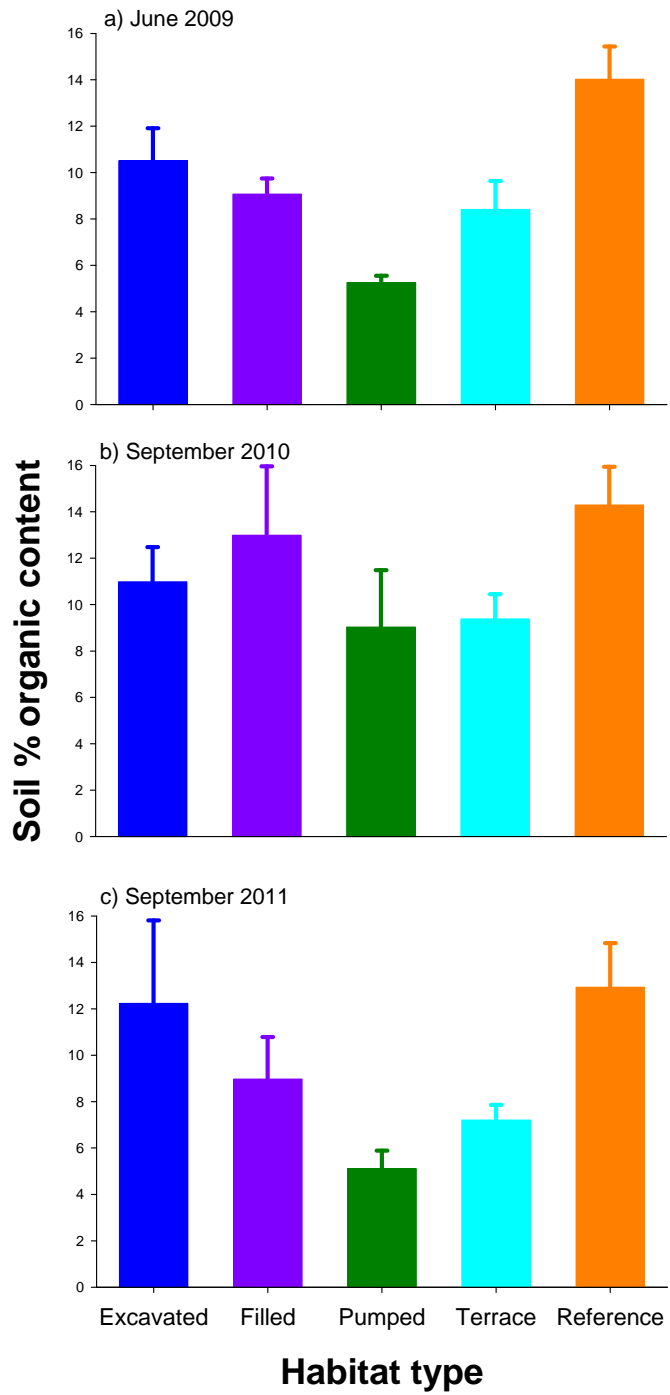
Figure 20: Percent organic content of soil at various restoration sites and a reference area in a) June 2009 and b) September 2010. Bars represent standard error. $6 \leq n \leq 10$

Soil nutrients

Soil nutrients were recorded in September 2011 (data collected prior to the project period in September 2010 and June 2009 are included for reference). On all sampling dates, soil percent nitrogen (N) was higher in the reference site than in all restored sites (Figure 21). Soil nitrogen levels were lowest in the pumped mounds in all years. Although the reference site had higher nitrogen content than all restored areas, N concentrations were relatively low at all sites, which may drive the low N:P ratios in the plant tissue (suggesting N limitation) (Figure 17). Soil percent phosphorus (P) was significantly higher in the reference site than in all restored sites in 2009 and 2010 but not 2011 (Figure 22). In general, soil %P was relatively low across all sites throughout the study period.
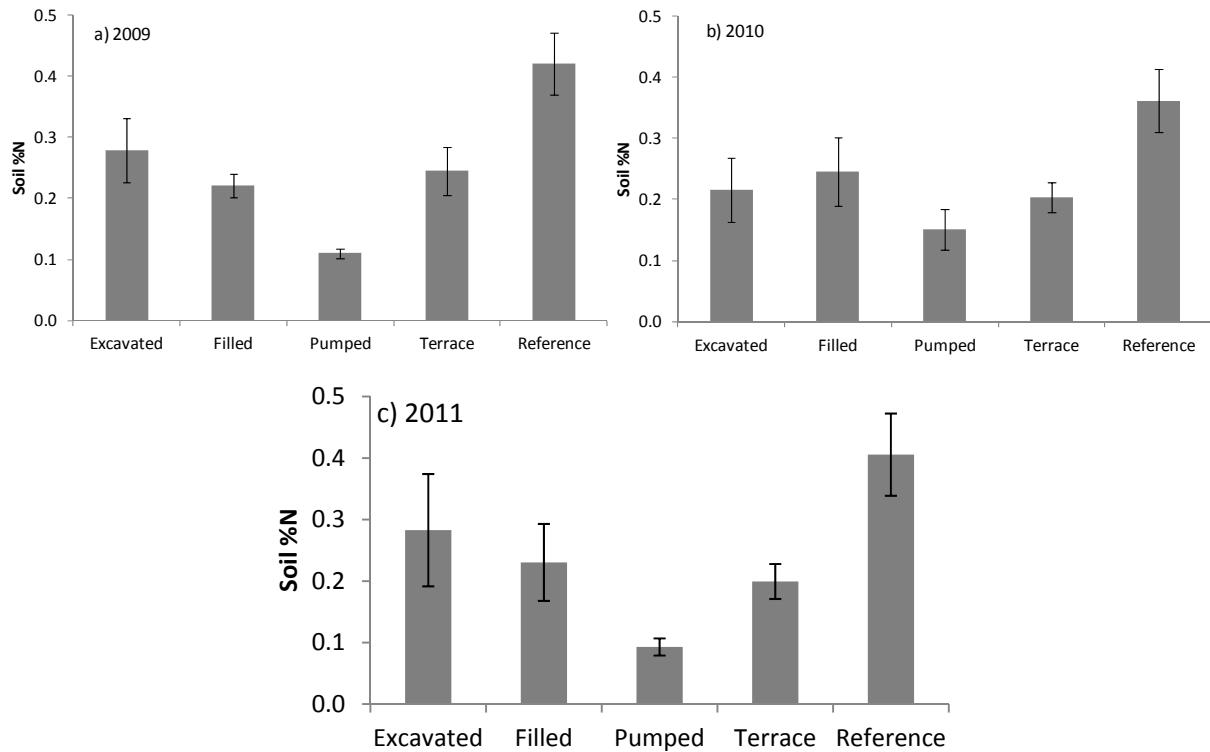
Figure 21: Soil percent nitrogen (N) at various restoration sites and a reference area in a) June 2009, b) September 2010, and c) September 2011. Bars represent standard error. $6 \leq n \leq 10$
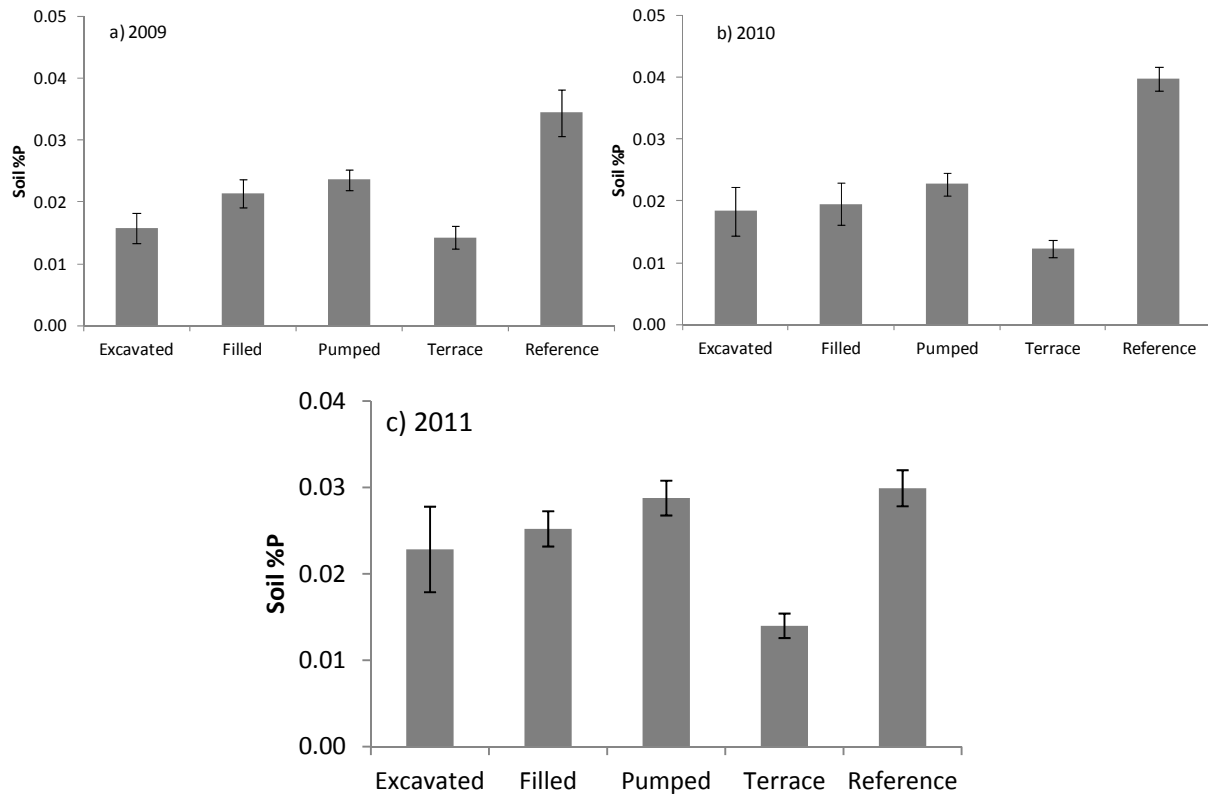
Figure 22: Soil percent phosphorus (P) at various restoration sites and a reference area in a) June 2009, b) September 2010, and c) September 2011. Bars represent standard error. $6 \leq n \leq 10$

Photosynthetic response to soil salinity and nutrients

The photosynthetic response of many wetland plant species to soil salinity and nutrients has been described in the laboratory, but less is known about the cumulative effect of these abiotic factors in the natural environment. We correlated field measurements of chlorophyll fluorescence with simultaneous measurements of soil nitrogen and phosphorus content, soil salinity, and relative leaf nitrogen content in four species (*Spartina alterniflora*, *S. patens*, *Schoenoplectus californicus* and *S. robustus*), that are common in brackish and salt marshes of the northwestern Gulf of Mexico. We found that the range of environmental conditions experienced by each species in the field was greater than what has been considered in laboratory investigations, and that saline soils may elicit an inverse photochemical response that has not been documented in the laboratory. The relationship between chlorophyll fluorescence and soil N:P ratio was not significant in *S. alterniflora*, nonlinear in *S. patens* and *S. robustus*, and significant and positive in *S. californicus*. The relationship between leaf nitrogen content and effective quantum yield was significant and positive in *S. alterniflora* and *S. robustus*, but only *S. alterniflora* appeared to be able to increase relative leaf nitrogen content over a wide range of soil nutrient and salinity regimes. *S. californicus* had the greatest potential for photosynthetic light capture but also had the narrowest distribution. Thus, species adapted to high levels of abiotic stress (*S. alterniflora*) were less dominant when the stress was removed, and species with the highest potential for photosynthetic performance (*S. robustus* and *S. californicus*) could only be found in locations with favorable abiotic conditions.

The complete methods and results were compiled in a manuscript that is in press at the *Journal of Coastal Research*. The article text is appended in Appendix A.

Aquatic fauna (secondary production)
Detailed measurements of the aquatic faunal community were conducted through September 2011 at excavated, filled, and pumped mounds and at reference sites. Terraces surrounded each of the different types of constructed areas (see Cover Photo and Figure 2), so the aquatic habitat around the terraces was not distinct from the aquatic habitat surrounding the immediately adjacent mounds. Therefore, we selected mounds on the interior of the study area and away from the terrace formations for aquatic faunal sampling, and terraces were excluded from the aquatic fauna analyses.

Aquatic fauna were collected using a throw trap. The throw trap apparatus consisted of two horizontal square 1-m^2 frames (one PVC frame and one metal rod frame), connected in parallel by a fine mesh net. Each sampling station was approached by an airboat drifting towards the station with the motor turned off. The trap was tossed from the airboat into the water on top of a haphazardly selected submerged aquatic vegetation (SAV) aggregation at each station. The SAV aggregation selected was typical of the assemblage in the area and usually included both *Ruppia maritima* (widgeongrass) and *Myriophyllum spicatum* (Eurasian milfoil). A two-person team entered the water behind the trap and slid another square mesh net underneath the throw trap; the whole trap and its enclosed contents were raised on to the deck of the airboat for cleaning and sorting. All fishes were removed from the vegetation on-site and placed in plastic bags containing water from the study site that was chilled to < 4°C. These bags were placed in a large cooler containing an ice slurry to euthanize all fishes (in accordance with TAMU Animal Use Protocol 2009-31). All samples were transported back to the lab in coolers and frozen pending further analysis. In the lab, all invertebrates that were not removed in the field were manually removed from the SAV and tallied.

Invertebrate densities were not consistently higher or lower in any particular habitat type, but some temporal patterns were evident. Invertebrates tended to be most abundant in fall 2009 and fall 2010 (Figure 23a), but densities dropped dramatically in 2011, probably due to the sudden increase in water salinity (Figure 12) during the exceptional drought that year. In general, the most abundant invertebrate species were grass shrimp *Palaemonetes pugio*, marsh snails *Probythinella louisianae*, and gammarid amphipods. *Probythinella* were particularly abundant in November 2009 and September 2010, and were largely responsible for the strong seasonal patterns in total invertebrate density. *Probythinella* also appeared to be very drought sensitive: snail density decreased from > 500/m^2 in most areas in September 2010 to 0/m^2 in September 2011. During the 2011 drought, some marine species such as *Penaeus aztecus* (brown shrimp) appeared in the study area (3-24/m^2).

There were no clear seasonal patterns in fish density, but, like invertebrates, fish density declined in the 2011 drought year (Figure 23b). The most abundant vertebrate species were rainwater killifish *Lucania parva*, sailfin molly *Poecilia latipinna*, and sheepshead minnow *Cyprinodon variegatus*. *Poecilia* appeared to be particularly sensitive to drought conditions, and exhibited a

ten-fold reduction in density in 2011 relative to previous years. During the 2011 drought, some marine species such as *Brevoortia patronus* (Gulf menhaden) appeared at low densities in the study area.
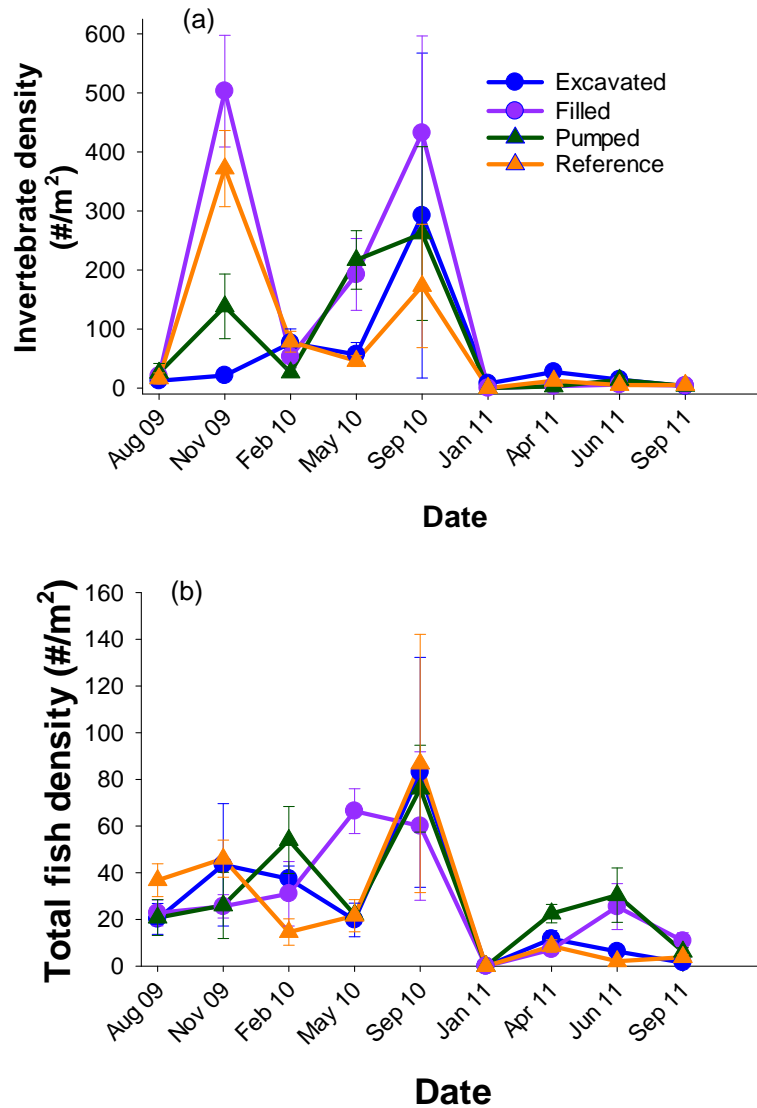
Figure 23: Aquatic animal density over time at various restoration sites and a reference area. (a) Total invertebrate density; (b) total fish density. Bars represent standard error. $6 \leq n \leq 10$

Benthic infauna

Two undergraduate interns investigated how construction type and soil characteristics (particularly soil organic content) influenced the infaunal community. Cores were collected from each habitat type from the marsh edge in October 2010 and from multiple elevations in July 2011 and November 2011. All cores were returned to the laboratory on ice and rinsed through a 500 µm sieve in 2010 and a 250 µm sieve in 2011. Material remaining on the sieve was fixed with a 10% formalin-rose bengal solution. After 1-2 weeks, the fixed organisms were sorted, identified to the Lowest Practical Taxonomic Level (LPTL), counted, and preserved in 70% EtOH.

Infauna were identified from nine distinct taxonomic groups (Figure 24). In 2011, the relative abundance of capitellids increased, which caused a qualitative shift in community structure. Capitellids are typically considered to be stress tolerant, and their high densities in November 2011 may have been a response to the higher salinity at the site (Figure 12).

Overall, the influence of habitat type was greatest during environmentally benign conditions and reduced under environmentally stressful drought conditions. Construction design ("habitat type") significantly influenced infaunal density but not richness in October 2010; there was no significant effect of habitat type in July 2011 or November 2011 (Figure 25, Figure 26). Density and richness were highest in the pumped habitat type on all sampling dates except October 2010. However, the influence of construction type was not due to sediment organic content; there was no significant relationship between infaunal density and soil organic content (Figure 27).
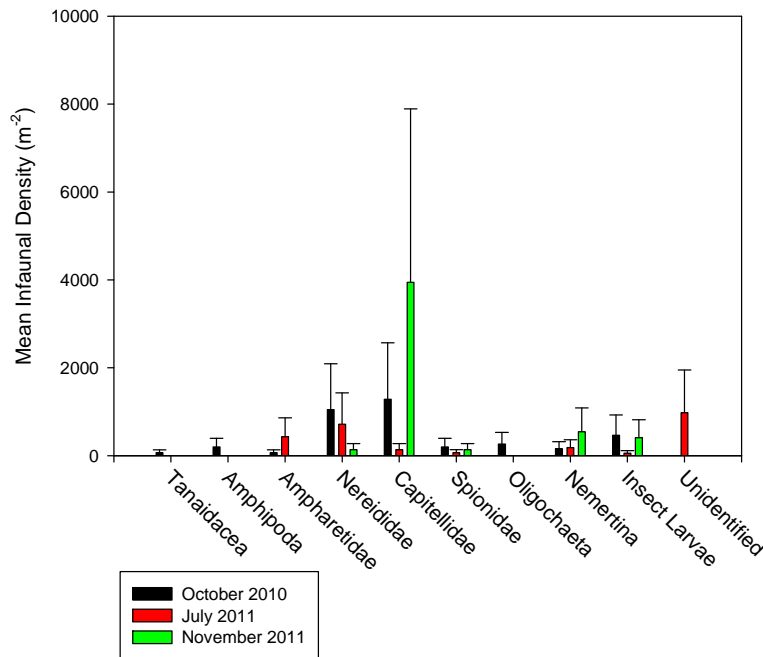
Figure 24: Total mean density (no. m^{-2}) for each taxonomic group in the reference, excavated, filled, pumped, and terrace sites. Data were pooled among elevations and habitat types. Error bars represent standard deviation.
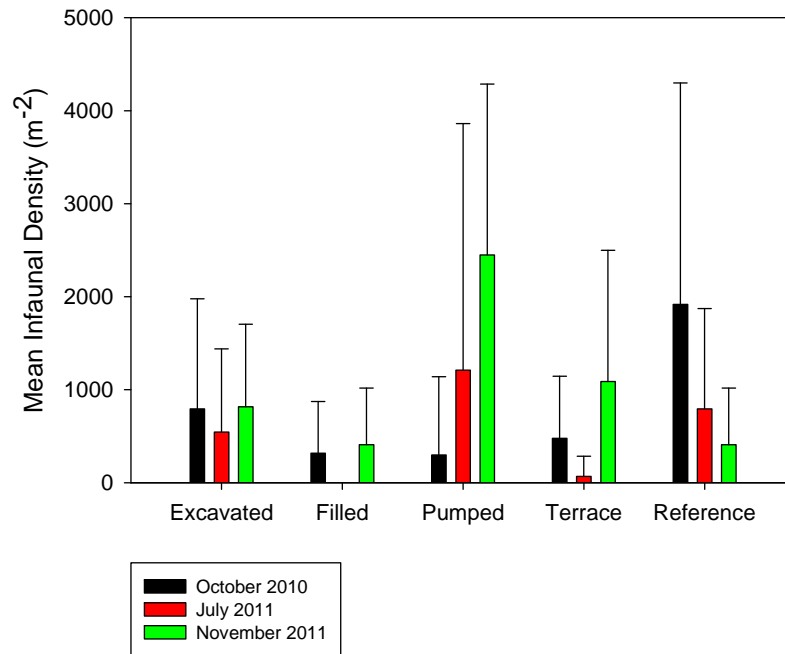
Figure 25: Mean infaunal densities (# m^{-2}) for the reference, excavated, filled, pumped, and terrace sites in October 2010, July 2011, and November 2011. Data were pooled among elevations. Error bars represent standard deviation.
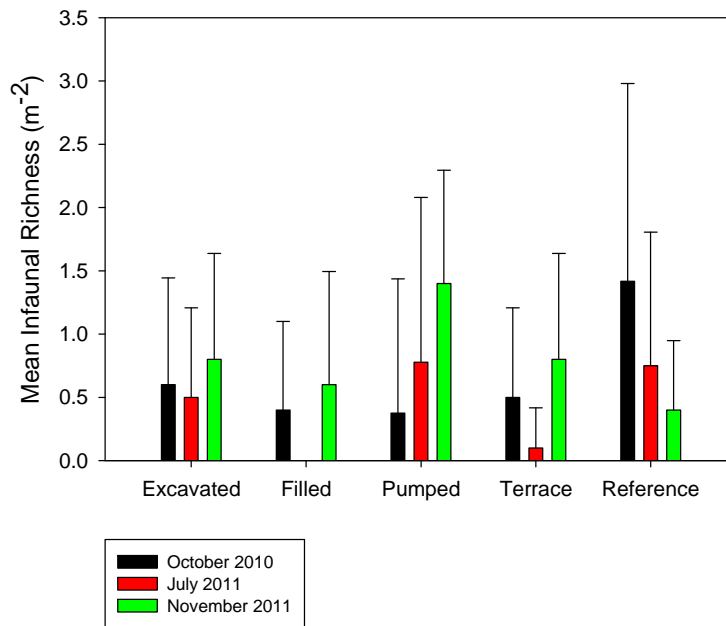
Figure 26: Mean infaunal richness (# taxonomic groups m^{-2}) for the reference, excavated, filled, pumped, and terrace sites in October 2010, July 2011, and November 2011. Data were pooled among elevations. Error bars represent standard deviation.
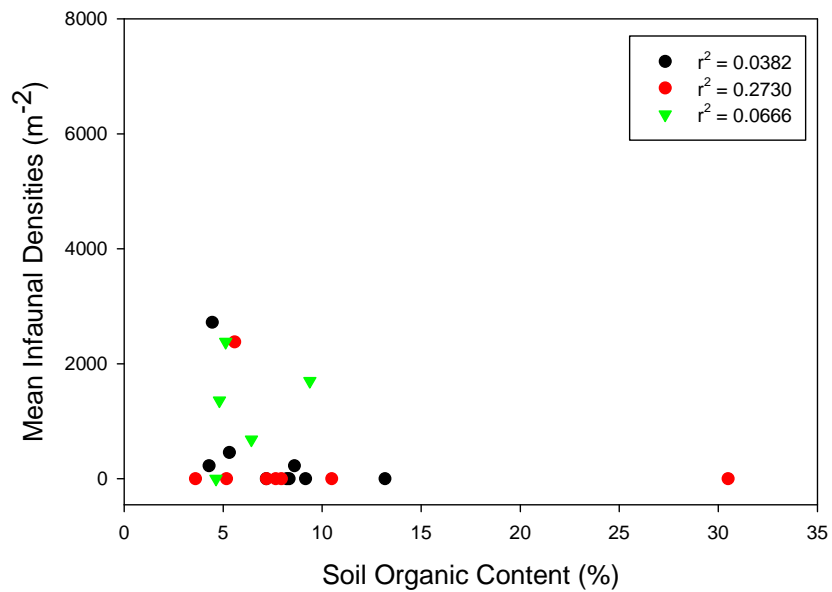
Figure 27: The relationship between infaunal density (# m^{-2}) and soil organic content (%) for the pumped sites in October 2010, July 2011, and November 2011. Similar trends were observed on all sampling dates for all habitat types. Data were pooled across elevations.

Task 1: Synthesis
In order to compare the development of overall ecosystem structure among habitat types, discriminant function analyses were performed on the June 2009 and September 2011 datasets. These analyses incorporated suites of variables to help identify the characteristics that were most similar among reference and restored areas. These analyses included emergent plants, water, and soil characteristics.

In 2009, discriminant function 1 (51.1% of the explained variability) was most strongly related to emergent plant metrics, especially plant cover (Figure 28). Function 2 (38.4%) was related to soil characteristics, particularly soil phosphorus content. In 2011, discriminant function 1 (89.1%) was most strongly related to the concentration of suspended solids, and function 2 (5.6%) was related to emergent plant cover, plant belowground biomass, and water column chlorophyll a concentration. Along the discriminant function 1 axis, pumped mounds were closest to the reference area.

The DFA analyses had 100% classification success throughout the study period. This indicated that all of the habitat types were unique relative to each other. Furthermore, none of the restored areas appeared to be converging on the reference area during the early development of this marsh (Figure 28).

Task 1: Implications for restoration
At the emergent plant level, each construction approach successfully yielded at least some plant characteristics that were similar to the reference site (Figure 8 – Figure 18), demonstrating that brackish marsh restoration is achievable on the short-term. However, as the suite of measured variables increased in complexity, the dissimilarity between the reference and restored sites increased. At the ecosystem level, each of the restoration types was unique relative to the others, and none of them resembled the reference site (Figure 28). It is not yet clear if marsh recovery is on a trajectory towards reference conditions, but ecological succession in restored marshes may take many years (Craft et al. 1999). In the meantime, there is clearly a productive plant and animal community in the restored marshes, so at the organismal level, restoration efforts have been successful.

Ecosystem-level analyses of the various construction techniques indicated that pumped mounds were most similar to the reference area in 2011 (Figure 28). However, differences among construction types were relatively small, and there was no clear stand-out in terms of total ecological performance at this point in the early development of the restored marshes. Therefore, creation of an appropriate marsh elevation appears to be a more important consideration than fine-scale construction design.
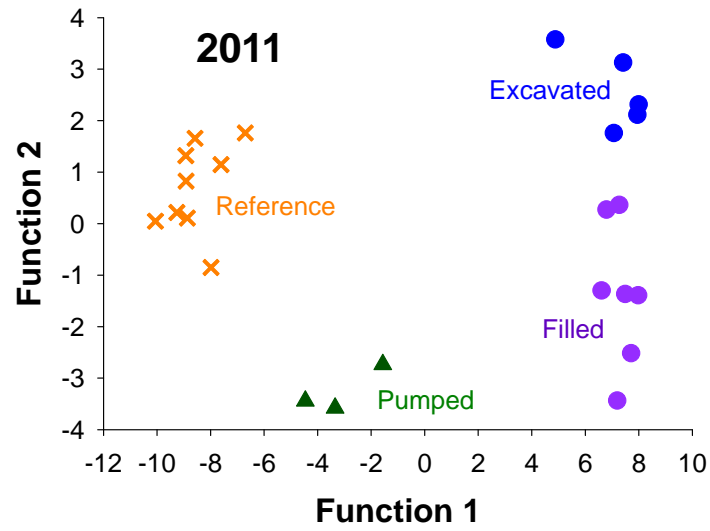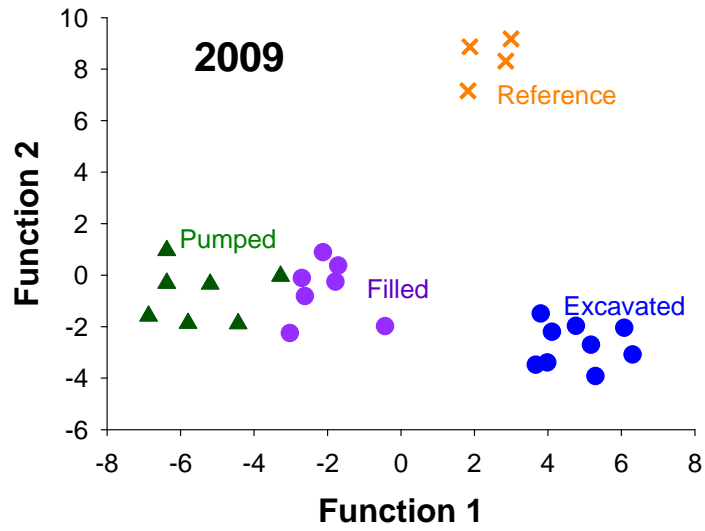
Figure 28: Discriminant function analysis of emergent plant, soil, and water characteristics in reference and restored areas in June 2009 and September 2011.

Task 2: Identify ecological consequences of aquatic exotic species invasion and develop management techniques

Task 2: Approach

At excavated, filled, and pumped mounds and at reference sites, we monitored the biomass of native and invasive submerged aquatic vegetation (SAV) species at least four times per year (through April 2012) at 7-10 stations in each habitat type. Terraces surrounded each of the different types of constructed areas (see Cover Photo and Figure 2), so the aquatic habitat around the terraces was not distinct from the aquatic habitat surrounding the immediately adjacent mounds. Therefore, we selected mounds on the interior of the study area and away from the terrace formations for aquatic SAV sampling, and terraces were excluded from the SAV analyses.

Submerged aquatic vegetation biomass was recorded by dragging the head of a 16-tine metal rake for two meters from the side of a non-motorized boat (modified from Spears et al. 2009).

We also conducted a series of herbivory and predation experiments with native and exotic SAV species. Key results are described below; detailed methods and results were published in the appended manuscript.

Task 2: Key Results

SAV biomass

Two submerged aquatic vegetation (SAV) species were frequently encountered at the study site: *Ruppia maritima* (widgeongrass) and *Myriophyllum spicatum* (Eurasian milfoil). For much of the study period, the non-native *Myriophyllum* dominated the study area, regardless of restoration status (Figure 29). In 2011, exceptional drought in the region (http://droughtmonitor.unl.edu/) raised salinities to unusually high levels at the site (Figure 12). Concurrently, *Myriophyllum*, which is typically a brackish or freshwater species, virtually disappeared from the study area (Figure 29). As *Myriophyllum* declined, the native SAV *Ruppia maritima*, which is more tolerant of saline conditions, became much more common (Figure 30). *Myriophyllum* is apparently a superior competitor at low salinities, but *Ruppia* can grow in salty waters in the absence of competitors. Small amounts of macroalgae, mostly green filamentous genera including *Cladophora* and *Spirogyra*, were occasionally noted at the study site.
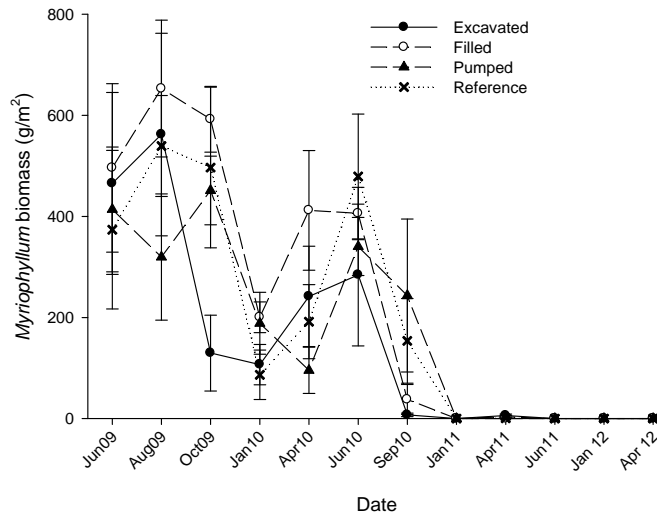
Figure 29: *Myriophyllum spicatum* dry biomass at various restoration sites and a reference area. Bars represent standard error. $6 \leq n \leq 10$
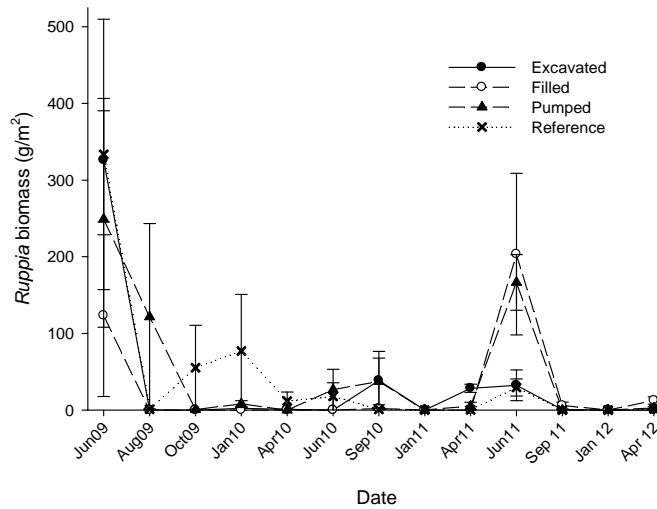
Figure 30: *Ruppia maritima* dry biomass at various restoration sites and a reference area. Bars represent standard error. $6 \leq n \leq 10$

SAV herbivory and predation experiments

Macroinvertebrates use SAV to meet nutritional requirements and seek refuge from predators. Shifts in SAV dominance can alter patterns of SAV use by associated fauna, but the dynamics of these alterations following *Myriophyllum spicatum* displacement of *Ruppia maritima* are not well understood. In this portion of the study, we examined the trophic implications of shifts between native (*Ruppia*) and exotic (*Myriophyllum*) SAV. We addressed the following questions: 1) Do aquatic invertebrates directly consume *Myriophyllum*, and 2) do predator–prey interactions differ in native and exotic SAV canopies? We collected *Ruppia*, *Myriophyllum*, *Palaemonetes* spp. (grass shrimp), and juvenile *Callinectes sapidus* (blue crabs) from the field

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and ran a series of three mesocosm experiments in the laboratory. In a "no-choice" grazing experiment, *Palaemonetes* consumed 13% of the *Ruppia* biomass but had no impact on *Myriophyllum* biomass. In a second study, when given a choice of plant canopies, *Palaemonetes* selected *Ruppia* over *Myriophyllum* 60% of the time when *Callinectes* was absent, but selected *Myriophyllum* more frequently in the presence of *Callinectes*. The selection of *Myriophyllum* was likely due to its refuge value; a third mesocosm experiment resulted in 60% *Palaemonetes* mortality due to *Callinectes* predation in *Ruppia* canopies but only 20% mortality in *Myriophyllum* canopies. *Myriophyllum* has a more structurally complex canopy that probably provides better protection from predation. Our studies suggest that native and exotic plants provide substantially different values as food sources and as refuges from predators for lower trophic levels.

The complete methods and results were compiled in a manuscript that was published in the *Journal of Experimental Marine Biology and Ecology.* The article text is appended in Appendix B.

Task 2: Implications for restoration
Native *Ruppia* and invasive *Myriophyllum* have very different ecological values, but the net ecological benefit for common herbivores may be balanced – *Myriophyllum* provided better protection from predators and *Ruppia* was more palatable for consumption. The long-term fitness implications of *Myriophyllum* proliferation are less certain, but our short-term studies suggest that *Myriophyllum* does provide some benefits for herbivores. Additional work is needed to investigate the benefits and costs of *Myriophyllum* displacement of *Ruppia* for other marsh organisms (e.g., waterfowl, juvenile fish).

Active management of *Myriophyllum* in this system may not be necessary in this particular brackish marsh because periodic (and sometimes prolonged) inundations by salt water may limit *Myriophyllum* biomass (Figure 29), reducing the need for physical removal or chemical or biological controls.

Task 3: Quantify the carbon sequestration potential of restored wetlands

Task 3: Approach
Coastal marshes play an important role in global carbon cycles, yet coastal development has led to global losses of marsh habitat. To address this problem, many coastal wetlands have been restored or created over the past several decades using a variety of construction techniques, but it is unclear if carbon capture in constructed marshes is equal to that of reference marshes or if rates of carbon capture are influenced by marsh construction techniques. To comparatively assess carbon capture by emergent and submerged vegetation in constructed and reference marshes, we made quarterly measurements of standing biomass and carbon content in above- and belowground emergent plant tissue and submerged vegetation in three constructed areas (2-3 years old) and one reference area in a brackish marsh in the northwestern Gulf of Mexico in 2009 and 2010. We also used aerial photographs to construct a GIS database of emergent and submerged vegetation coverage. These data were combined to estimate net annual carbon capture per square meter of marsh vegetation in each constructed and reference area.

Task 3: Key Results
Rates of carbon capture by emergent aboveground vegetation and submerged aquatic vegetation were similar in constructed and reference areas. However, the carbon production of submerged vegetation ($0.01 - 0.03$ kg m^{-2}) was much less than emergent vegetation ($0.20 - 1.67$ kg m^{-2}), and constructed areas contained an order of magnitude less emergent habitat than the reference area. Consequently, the annual carbon production of entire constructed areas (emergent + submerged vegetation; $0.13 - 1.24$ kg m^{-2}) was always less than half that of the reference area ($0.84 - 2.54$ kg m^{-2}).

Task 3: Implications for restoration
Although productivity of emergent and submerged vegetation in constructed and reference areas was similar, the smaller ratio of land to water in the constructed areas reduced their annual rate of carbon capture at a larger spatial scale. To more closely mimic rates of carbon capture in reference marsh habitats, constructed marsh designs should aim to replicate the ratio of land to water in adjacent reference marshes.

The complete methods and results were compiled in a manuscript that was published in *Ecological Engineering*. The article text is appended in Appendix C.

Task 4: Dissemination of data

<u>Task 4: Approach and Key Results</u>
The PIs have maintained TAMUG lab websites (e.g., see http://www.tamug.edu/armitage/) that describe their currently funded wetland ecology and restoration projects. In addition, PI Armitage manages a research blog (see http://marshdispatch.blogspot.com/) to periodically provide updates on project findings and related topics.

The PIs also actively inform the public of the availability of the web site via attendance at scientific conferences, meetings with community clubs and organizations, attendance at education and outreach events, and participating in the State of the Bay meetings (see Accomplishments: Presentations).

Task 5: Education and outreach

<u>Task 5: Approach and Key Results</u>
The PIs have actively integrated internal and external educational and outreach opportunities into the framework and execution of this project.

This project has supported numerous graduate and undergraduate students and postgraduate scholars. In addition, this project has provided technical training for research assistants at many levels, including undergraduate, baccalaureate, and masters (M.S.) level technicians. We have been particularly active in providing hands-on research experiences for undergraduates: in the last year, this project has included one undergraduate honors student, two undergraduate research scholars, and one NSF Research Experience for Undergraduates Fellow, as well as several volunteer research interns. A complete list of personnel supported or trained (fully or partially) by this project during the contract period (February 2011-June 2012) is included in the following section (see Accomplishments: Students and scholars supported).

This project has contributed to nine presentations at scientific conferences and 11 presentations for secondary students and community groups. A complete list of presentations related to this project during the contract period is included in the following section (see Accomplishments:

Accomplishments
<u>Degree awarded</u>
1. Michael Bell defended his M.S. thesis (2011) based on this project. His thesis was titled "Aquatic animal and macrophyte communities in a recently restored brackish marsh: possible influences of restoration design and the invasive plant species *Myriophyllum spicatum*".

<u>Students and scholars supported</u>
This project has supported numerous graduate and undergraduate students and postgraduate scholars. In addition, this project has provided technical training for research assistants at many

levels, including undergraduate, baccalaureate, and masters (M.S.) level technicians. A list of personnel supported or trained (fully or partially) by this project follows.

Postdoctoral Research Associates (2)
  Dr. Eric Madrid, 1/2009 – 8/2011
  Dr. Erin Kinney, 11/2011 – present

Graduate students (5)
  Michael Bell, M.S., 2011
  Allison Parnell, Masters of Marine Resource Management, 2011
  Carolyn Weaver, current Ph.D. candidate
  Courtney Lee, current M.S. student
  Cara Valinoti, current M.S. student

Undergraduate students (10)
  Lorenda Archer – Undergraduate research intern (2012)
  Brittney Davis – Texas Institute of Oceanography Fellow and Undergraduate Research Scholar (2010-2012)
  Theodore Driscoll – Texas Institute of Oceanography Fellow and Undergraduate Research Scholar (2011-2012)
  Danielle Gaucher – Undergraduate research intern (2012)
  Jenny Lam – Student worker (2011-2012)
  Clint Lee – Undergraduate research intern (2012)
  Caitlin Roush – Undergraduate research intern (2012)
  Melissa Tuttle – Student worker (2011)
  Cara Valinoti – NSF Research Experience for Undergraduates Fellow (2010-2011)
  Kate Wiemers – Honors program scholar (2011-2012)

Non-student technicians (2)
  Nicole Ekstrom, Masters of Marine Resource Management (2010-2012)
  Cody Hales, B.S. (2010-2011)

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Articles in press or in print in peer-reviewed scientific journals are listed below.

Madrid, E.N., A.R. Armitage, and A. Quigg. In press. The response of photosystem II to soil salinity and nutrients in wetland plant species of the northwestern Gulf of Mexico. *Journal of Coastal Research*.

Madrid, E.N., A. Quigg, and A.R. Armitage. 2012. Marsh construction techniques influence carbon capture by emergent and submerged vegetation in a brackish marsh in the northwestern Gulf of Mexico. *Ecological Engineering* 42: 54-63.

Valinoti, C.E., C.-K. Ho, and A.R. Armitage. 2011. Native and exotic submerged aquatic vegetation provide different nutritional and refuge values for macroinvertebrates. *Journal of Experimental Marine Biology and Ecology* 409: 42-47.

Following is a list of nine oral and poster presentations related to this project given at local, national, and international scientific conferences during the contract period, listed in reverse chronological order.

1. Armitage, A.R., C.-K. Ho, E.N. Madrid, M.T. Bell, E.L. Kinney, A. Quigg. June 2012. Drought and construction techniques influence ecosystem-level restoration of a brackish marsh. 9th INTECOL International Wetlands Conference.
2. Kinney, E.L., Armitage A.R., Quigg, A. June 2012. Effects of drought on restored and reference salt marshes in the Northwestern Gulf of Mexico. 9th INTECOL International Wetlands Conference.
3. Davis, B., Driscoll, T.G., IV, Armitage, A.R. April 2012. Infaunal abundance in restored and reference marshes in the northwestern Gulf of Mexico: the impact of habitat design and drought. TAMUG Student Research Symposium.
4. Wiemers, K. And A.R. Armitage. April 2012. Determination of successful brackish marsh restoration through use of aquatic fauna density and population size structure. TAMUG Student Research Symposium.
5. Davis, B., Driscoll, T.G., IV, Armitage, A.R. March 2012. Infaunal abundance in restored and reference marshes in the northwestern Gulf of Mexico: the impact of habitat design and drought. 41st Benthic Ecology Meeting.
6. Armitage, A.R., M.T. Bell, C.-K. Ho, E.N. Madrid, A. Quigg. November 2011. Metapopulation dynamics in estuarine habitat restoration: Do reference areas act as source populations? Coastal and Estuarine Research Federation 21st Biennial Conference.
7. Armitage, A.R., C.-K. Ho, E.N. Madrid, M.T. Bell, A.S. Quigg. August 2011. Built for success: Brackish marsh construction techniques influence ecosystem-level restoration success. 96th Ecological Society of America Annual Meeting
8. Armitage, A.R. and A.S. Quigg. May 2011. Defining restoration success in brackish marshes on the Gulf of Mexico coast. International Oil Spill Conference.
9. Bell, M.T. and A.R. Armitage. March 2011. It's an invasion! The competitive relationship between two submerged macrophytes, *Myriophyllum spicatum* and *Ruppia maritima*. 40th Benthic Ecology Meeting.

Following is a list of 11 oral presentations related to this project given at local community meetings or to other public audiences during the contract period, listed in reverse chronological order.
1. June 2012. Coastal marshes: What are they worth? Lecture to Lafitte's Cove Nature Society.
2. June 2012. The science of restoring coastal wetlands. Lecture to NSF Research Experience for Undergraduates interns at TAMUG.
3. June 2012. What is a marine biologist? Presentation at TAMUG New Student Conference.
4. March 2012. Restoring coastal wetlands: How? And why? Lecture to Armand Bayou Nature Center volunteers.
5. February 2012. Ecological assessments of wetland restoration success at multiple spatial scales. Rice University Department of Ecology and Evolutionary Biology seminar.

6. January 2012. Exploring and restoring coastal wetlands. Presentation at TAMUG Sea Aggie Preview.
7. November 2011. Exploring and restoring coastal wetlands. Presentation at TAMUG Sea Aggie Preview.
8. June 2011. What is a marine biologist? Presentation at TAMUG New Student Conference.
9. April 2011. A virtual trip to an estuary. Texas Envirothon Competition for high school students.
10. April 2011. Using ecological principles to define habitat restoration success. University of Texas Marine Science Institute seminar.
11. February 2011. Exploring and restoring coastal wetlands. Presentation at TAMUG Sea Aggie Preview.

Problems encountered

The exceptional drought in Texas in 2011 dramatically altered the study site. In particular, the lack of freshwater input from rainfall caused the salinity at the site to approach marine levels. Accordingly, there was a loss of aquatic vegetation and shifts in the aquatic animal communities towards more marine assemblages. Despite the unusual weather conditions, we were able to maintain our monitoring program and fulfill the original project objectives.

Construction of the beneficial uses material site was originally scheduled for summer 2010, but construction initiation was delayed for reasons beyond our control until August 2011. Multiple agencies, including the Texas Parks & Wildlife Department, the Port of Orange, and the Army Corps of Engineers were involved in the planning, permitting, financing, and execution of the project, but the bureaucratic reasons for the delays were not communicated to TAMUG. Construction was completed in December 2011, but no plant colonization occurred during the contract period. Despite the delays in the construction of this site, the beneficial uses study was a relatively small portion of our project, and we were able to address our original objectives by focusing our efforts on completing the other project tasks.

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Appendix A

The response of photosystem II to soil salinity and nutrients in wetland plant species of the northwestern Gulf of Mexico.

The response of photosystem II to soil salinity and nutrients in wetland plant

species of the northwestern Gulf of Mexico

Eric N. Madrid[a], Anna R. Armitage[b], and Antonietta S. Quigg[c, d]

Running Head: PSII, salinity and nutrients in wetland plants

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ABSTRACT

The photosynthetic response of many wetland plant species to soil salinity and nutrients has been described in the laboratory, but less is known about the cumulative effect of these abiotic factors in the natural environment. In this investigation we correlated field measurements of chlorophyll fluorescence with simultaneous measurements of soil nitrogen and phosphorus content, soil salinity, and relative leaf nitrogen content in four species (*Spartina alterniflora*, *S. patens*, *Schoenoplectus californicus* and *S. robustus*), that are common in brackish and salt marshes of the northwestern Gulf of Mexico. We found that the range of environmental conditions experienced by each species in the field is greater than what has been considered in laboratory investigations, and that saline soils may elicit an inverse photochemical response that has not been documented in the laboratory. The relationship between chlorophyll fluorescence and soil N:P ratio was insignificant in *S. alterniflora*, nonlinear in *S. patens* and *S. robustus*, and significant and positive in *S. californicus*. The relationship between leaf nitrogen content and effective quantum yield was significant and positive in *S. alterniflora* and *S. robustus*, but only *S. alterniflora* appeared to be able to increase relative leaf nitrogen content over a wide range of soil nutrient and salinity regimes. *S. californicus* had the greatest potential for photosynthetic light capture but also had the most narrow distribution. Thus, species adapted to high levels of abiotic stress (*S. alterniflora*) were less dominant when the stress was removed, and species with the highest potential for photosynthetic performance (*S. robustus* and *S. californicus*) could only be found in locations with favorable abiotic conditions.

ADDITIONAL INDEX WORDS - *Photosynthesis, Chlorophyll Fluorescence, Nitrogen,*

Phosphorus, Marsh, Gulf of Mexico

INTRODUCTION

The primary production of brackish and salt marshes plays a crucial role in global carbon cycles (Sahagian and Melack, 1988; Dixon and Krankina, 1995; Chmura et al., 2003) and contributes to ecological processes such as accretion and trophic support in coastal systems (Dawes, 1998; Mitsch and Gosselink, 2007). Primary production in brackish and salt marshes is high for a terrestrial ecosystem, which is somewhat paradoxical because these environments experience extreme temperatures, have salty soils that often contain sulfuric compounds, and are frequently submerged, making them an inhospitable habitat for most plant species (Koch et al., 1990; Cronk and Fennessy, 2001). Marsh plants have evolved specialized anatomical and regulatory features that help them tolerate this extreme environment, including salt-excreting glands on their leaves, aerenchyma tissues that transport oxygen to roots, and pathways for the production of soluble proteins that help cope with fluctuating water potentials (Raven et al., 1999; Cronk and Fennessy, 2001). However, even with these adaptations, marsh plants experience substantial physiological stress at nearly all times (Drake, 1989; Mendelssohn and Morris, 2002).

Coastal marshes are dominated by a handful of halophytic emergent and intertidal vascular plants. The relationship between abiotic environmental factors and photosynthesis in these plant species has been explored primarily in the laboratory under precisely controlled environmental conditions (ex. Longstreth and Strain, 1977, Pezeshki et al., 1987, Bradley and Morris, 1992, Maricle et al., 2007). These experiments have successfully identified many key aspects of the photosynthetic response of the most common marsh plant species, *Spartina alterniflora*, to soil salinity

and soil nutrients including nitrogen and phosphorus (reviewed by Mendelssohn and Morris, 2002).

While these treatments are necessary for the dissection of physiological pathways, they do not represent the full range of conditions experienced in the field. For example, Longstreth and Strain (1977), Pezeshki et al. (1987), Pezeshki and DeLaune (1993), Ewing et al. (1995), Nieva et al. (1999), and Maricle et al. (2007) for example, measured photosynthesis under constant porewater salinity treatments < 30 ppt. In the field, marshes are tidally-influenced and experience daily fluctuations in submergence and porewater salinities that can range from 0 ppt to > 100 ppt due to evaporation of standing water at low tide (Cronk and Fennessy, 2001; Mitsch and Gosselink, 2007).

Laboratory and greenhouse experiments have also identified complex and simultaneous interactions between soil salinity and soil sulfide concentration that influence nutrient absorption (Morris, 1984; Morris and Dacey, 1984; Bradley and Morris, 1990, 1992; Brown, 2006). Yet when the photosynthetic response of marsh plants to nutrients has been collected in the field, it has either not been measured simultaneously with soil salinity (Giurgevich and Dunn, 1979; Dai and Wiegert, 1997; Baerlocher et al., 2004), sediment electrical conductivity has been used as a proxy for soil salinity (Castillo et al., 2000) or direct measurements of soil nutrients have not been recorded (Dai and Weigert, 1997; Castillo et al., 2000; Baerlocher et al., 2004; Wigand et al., 2004). Thus, while laboratory-based photosynthetic research has provided a foundation to understand the physiological mechanisms influencing photosynthesis in marsh plants, less is know about the cumulative real-world response of these species to

abiotic stressors in the field. It is important to develop a better understanding of

photosynthesis in marsh plant species in light of expected climate change events such

as rising sea levels and changes in temperature and rainfall that may impact the

physiology of the vascular plants responsible for most primary productivity in brackish

and salt marshes.

In this investigation, we simultaneously measured chlorophyll fluorescence, leaf

nitrogen content, soil nitrogen content, soil phosphorus content, N:P ratio, and soil

salinity in individuals of *Spartina alterniflora* Loisel. (Poaceae), *Spartina patens* (Aiton)

Muhl (Poaceae), *Schoenoplectus californicus* (C.A. Mey.) Steud. (Cyperaceae)*,* and

Schoenoplectus robustus (Pursh) M.T. Strong (Cyperaceae)*.* These plants are common

in brackish and salt marshes of the northwestern Gulf of Mexico and represent both C3

(*Schoenoplectus* sp.) and C4 (*Spartina* sp.) photosynthetic pathways. We believed that

the dry climate and extreme temperatures in the northwestern Gulf of Mexico would

produce soil salinities and nutrient loads in the field that were greater than what has

been tested in laboratory experiments. We also expected that because many individual

abiotic environmental factors have competing physiological effects (i.e. salinity generally

decreases photosynthetic potential while nitrogen additions have been shown to

increase wetland biomass production) that the cumulative magnitude of their response

would be small or perhaps even undetectable in the field.

MATERIALS AND METHODS

Study sites and plant species

Data were collected from six salt and brackish marshes near Galveston Bay in east Texas, United States at the peak of the growing season between July 2nd and August 31st of 2009 from 08:00 in the morning until noon (12:00) (Fig. 1). Sunset Cove (N29° 9.034', W95° 2.462'), Reitan Marsh (N29° 19.181', W94° 55.805'), and East Beach (N29° 19.916', W94° 44.777') are marine and had low plant diversity; only *S. patens and S. alterniflora* were found in these marshes. Sunset Cove is in west Galveston Bay in a relatively undisturbed location. East Beach is an anthropogenically impacted site on the south shore of the Houston Ship Channel that has restricted tidal flow and a high level of foot traffic. Reitan Marsh includes restored and reference marshes; our field sites were in the remnant reference marsh area. The Anahuac National Wildlife Refuge (Anahuac; N29° 35.198', W94° 29.893') and Armand Bayou Nature Center (Armand Bayou; N29° 35.052', W95° 4.590') had brackish and marine areas. Brackish marshes in Anahuac and Armand Bayou were the most diverse and contained all four plant species included in this investigation, while the salt marsh areas at these sites were comprised almost entirely of *S. alterniflora*. Marshes within the Lower Neches Wildlife Management Area (LNWMA; N30° 00.228', W93° 51.539') near the west shore of Sabine Lake are brackish. Restoration efforts in this area were completed in early 2008 and most restored marshes were planted with *S. alterniflora* and *S. californicus.* We collected data from reference and restored areas of the marsh.

Our aim was to examine each plant species across the most broad range of abiotic conditions, rather than test differences between each marsh. Therefore, marshes with multiple elevations and diverse salinity and nutrient regimes (ex. Anahuac and Armand Bayou) contained a larger number of field sites than marshes of a more

homogenous composition (ex. Sunset Cove). The number of field sites in each marsh

are indicated in Figure 2.

Soil sample collection and analysis

At each field site, 0.04 m^2 quadrats were haphazardly placed on the ground

within two meters of the waterline. Because each marsh was tidally influenced, each

field site was within the intertidal area. One soil core (10 cm deep) was taken from the

center of the quadrat and the section between 2.5 and 5.0 cm below the soil surface

was removed for analysis. We chose to analyze this section of the core because earlier

trials with this technique demonstrated that the topmost layer of soil (0.0 - 2.5 cm)

contained small depressions that led to inconsistent levels of salinity and nutrients

between replicate samples collected from within a single quadrat. Soil from deeper

portions of the core (> 5.0 cm) contained less root biomass and measurements of soil

salinity could be reproduced. Soil samples were dried at 60°C for 48 hrs, after which

nitrogen content was determined by oxidation in a Perkin Elmer (Waltham,

Massachusetts, USA) series II CHNS/O analyzer. Nitrogen standards were run after

every ninth soil sample. Particulate total phosphorus content was determined in soil

samples by a dry-oxidation, acid hydrolysis extraction followed by a colorimetric

analysis of phosphate concentration of the extract as described in Solorzano and Sharp

(1980).

Relative salinity of soil samples was measured by filtering a portion of a

saturated soil paste (Richards, 1954) through two layers of no. 2 Whatman filter paper

onto a Leica® temperature-compensated refractometer. The saturated soil paste

technique was preferred over measurements of pore water salinity because salinities in tidally influenced brackish and salt marshes frequently change and we believe the soil paste-derived measurements of soil salinity provide a more long-term indication of relative salinity history rather than single "snapshot" measurements of porewater salinity.

Fluorescence measurements

Chlorophyll fluorescence was measured on intact leaves of three to five plants per species per site using a MINI-PAM (H. Walz, Effeltrich, Germany) with leaf-clip holder (2030-B). In all cases measurements were performed using the procedures described in the MINI-PAM manual (Heinz-Walz 1999) and essentially as described in Bilger et al. (1995) and Rascher et al. (2000). All data were collected from the third leaf from the top of the plant, just as in previous investigations where comparative photosynthetic measurements have been made on leaves at similar stages of development (ex. Maricle et al., 2007).

In situ chlorophyll a fluorescence measurements were performed on leaves at ambient temperature regimes. The distance between the leaf and fiber optics on the leaf-clip holder was kept at 8 mm such that the light source settings gave a signal between 200 and 500 units. The fiber optic axis was positioned at a 60° angle with the leaf plane so that shading was avoided when external actinic illumination was applied and so that the illumination across the leaf was homogenous. The same instrument settings for light intensity, light sensitivity and signal gain / amplification factor were used throughout the period of data collection.

Photosynthetic yield and rapid light curve (RLC) analysis

The effective quantum yield (Φ_{PSII}) of each leaf was taken at ambient

photosynthetic active radiation (PAR). Then, a RLC was generated for each leaf. A

different section of the same leaf used to measure ambient Φ_{PSII} was used to create

each RLC so that artifacts of the previous Φ_{PSII} measurement would not be included in

new measurements. For each RLC, Φ_{PSII} was measured at nine increasing actinic light

intensities of ten seconds each at 0, 55, 81, 122, 183, 262, 367, 616, and 1115 of PAR.

Leaves were shaded while generating an RLC so that as little ambient light as possible

contributed to the measured PAR. After the leaf was placed in the leaf-clip holder and

before any measurements were taken, the auto-zero function was applied to suppress

any background signal from remaining ambient light.

PSII response parameters including the electron transport rate (ETR), which is a

relative measure of electron transport through the photochemical reactions leading to

carbon fixation, were estimated using the default calculations of the MINI-PAM

instrument and were derived with the formula:

$$ETR = \Phi_{PSII} \times PAR \times AF \times 0.5$$

Where Φ_{PSII} is the effective quantum yield measured by the MINI-PAM after each

pulse of PAR, the standard light absorption factor of leaves (AF) is 0.84 as determined

by Björkman and Demmig (1987), and photon absorption is assumed to be distributed

equally between photosystems I and II (0.5; Heinz-Walz 1999). ETR was plotted

against PAR and fit to a photosynthesis-irradiance (PI) curve with SigmaPlot (Version 10.0) using the hyperbolic tangent function of Platt et al. (1980):

$$ETR = P_{max} (1 - e^{-(\alpha PAR) / Pmax}) (e^{-(\beta PAR) / Pmax})$$

Typical R^2 fits were > 0.99. PI curve fitting allowed for the calculation of the initial linear slope of light response, α, which describes the efficiency of photosynthetic energy conversion in leaves at sub-saturating light intensities.   The PI curve fit also allowed for calculation of P_{max}, which is the theoretical maximum rate of photochemical energy conversion. Together, α and P_{max} provide a means to compare light capturing potential; plants with the greatest α and P_{max} are capable of the highest rates of photosynthetic energy conversion. Φ_{PSII} has an average maximum of 0.83 and is a measure of the ratio of light absorbed by PSII that is used in further photochemical reactions (Bilger et al., 1995; Maxwell and Johnson, 2000). Φ_{PSII} describes the overall efficiency of photosynthetic light capture and larger values of Φ_{PSII} are associated with greater levels of overall photosynthetic health (Genty et al., 1989; Rasher et al., 2000; Maxwell and Johnson, 2000). It should be emphasized that α, P_{max}, and Φ_{PSII} are not quantitative measures of photosynthesis, but rather allow for qualitative comparisons of light capture potential and efficiency (Ralph and Gademan, 2005).

Leaf meter measurements

We estimated levels of leaf nitrogen with a SPAD-502 portable leaf meter (Konica Minolta Corporation, USA). This device measures the transmission of 650 and 940 nm

red light through leaves and is a reliable tool for inferring nitrogen and chlorophyll

content (Markwell et al., 1995; Bullock and Anderson, 1998; Netto et al., 2002). The

significant correlation between SPAD value and chlorophyll concentration is

summarized in Appendix Figure 1. These measurements were only possible in *S.*

alterniflora and *S. robustus* because the meter is designed to work with flat leaves that

are at least three mm wide. Leaves of *S. californicus* are thick and round, and those of

S. patens are too narrow.   Three SPAD measurements were recorded on the same

area of the same leaves used to measure RLC response and the average of these three

measurements was used for statistical analysis.

Statistical analyses

Linear regression analyses between soil nutrient or soil N:P ratio and PAM or

SPAD measurements were performed with SigmaPlot version 10.0 (Systat Software,

Chicago, Illinois, USA).

The main and cumulative effects of soil salinity and N:P ratio on PSII were

analyzed with multiple regression analysis for each species. Soil salinity, soil %N, and

soil %P were independent variables in three separate linear models that included P_{max},

α, and Φ_{PSII} as dependent variables. All statistical analyses were performed with SPSS

version 16.0 (IBM, Chicago, Illinois, USA).

To qualitatively compare rapid light curves (RLC) among sites with different soil

salinities, we averaged RLC data for sites with mostly brackish soils (salinity <20),

primarily marine soils (salinity 20-40), moderately hypersaline soils (salinity 40-60), and

extremely hypersaline soils (salinity >60). Likewise, to compare RLC among sites with

different levels of nutrients, we grouped RLC data according to Redfield nutrient ratios:

sites with nitrogen limited soils (N:P <15), relatively balanced soils (N:P 15-30), and

phosphorus limited soils (N:P >30).

RESULTS

Typical ranges of soil salinity and nutrients

The range and median soil salinity, %N content, and %P content were different in

each marsh (Fig. 2). Anahuac NWR, Armand Bayou, East Beach, and the LNWMA had

generally lower salinities than Reitan Marsh and Sunset Cove (Fig. 2A). The LNWMA

had the largest range of soil nitrogen content (Fig. 2B) and Anahuac NWR had the

largest range of soil phosphorus content (Fig. 2C).

Different environmental characteristics were associated with each plant species

(Fig. 3). *S. alterniflora* was found across the greatest range of soil conditions with soil

salinities from 7 ppt to 103 ppt (Fig. 3A), %N concentrations from 0.05 to 0.62 (Fig. 3B),

%P concentrations from 2.68×10^{-3} to 4.70×10^{-2} (Fig. 3C), and N:P ratios from 5.6 to

93.1 (Fig. 3D). *S. patens* and *S. robustus* grew in similar environments with soil

salinities from 7 ppt to 52 ppt (Figs. 3B, 3C), %N concentrations from 0.06 to 0.62 (Figs.

3B, 3C), %P concentrations from 8.34×10^{-3} to 0.06 (Figs. 3C, 3E), and N:P ratios from

2.2 to 39.2 (Figs. 3B, 3C). *S. californicus* was found in the least saline soils (< 23; Fig.

3D) with %N concentrations from 0.09 to 0.5 (Fig. 3D), %P concentrations from 6.15 x

10^{-3} to 5.63×10^{-2} (Fig. 3D), and N:P ratios from 5.3 to 27.3 (Fig. 3D).

PSII response of *S. alterniflora*

P_{max} and α in *S. alterniflora* significantly decreased in soils with elevated salinities (Table 1). Elevated soil %P was also associated with a decrease in α (Table 1). The cumulative effects (R^2) of the interactions between photosynthesis and soil salinity, %N, and %P were generally low (P_{max}:0.11, α: 0.12) and they likely had limited functional significance. When RLC data were grouped by soil salinity and averaged, differences in P_{max} and α were most obvious between plants growing in brackish soils (<20 ppt) and any other salinity (Fig. 4A). We did not observe a significant photosynthetic response to soil %N in *S. alterniflora* (Table 1).

PSII response of *S. patens*

In *S. patens*, increasing soil salinity correlated with lower values of P_{max} and α. Higher levels of soil %N were associated with significantly lower P_{max} and α (Table 1). The cumulative R^2 values of these relationships (P_{max}: 0.32, α: 0.38) accounted for ≈ 1/3 of the variability observed in the data.

Averaged RLC data suggest that the P_{max} of *S. patens* was highest in soil salinities <20 ppt (Fig. 4B). P_{max} was also highest in plants growing in soils with intermediate N:P ratios (16-30; Fig. 5B). This suggested that the relationship between P_{max} and N:P ratio may have been nonlinear in *S. patens*. When analyzed with linear and quadratic functions, a quadratic function was a slightly better fit for the data (P < 0.00; R^2 = 0.15).

PSII response of *S. robustus*

In *S. robustus*, P_{max} and α had an inverse relationship with soil salinity. We did not observe a PSII response to soil %N or %P in *S. robustus*, and cumulative R^2 values for this species were relatively low (P_{max}: 0.16, α: 0.29; Table 1).

RLC data suggest that P_{max} of *S. robustus* was highest in soil salinities <40 ppt (Fig. 4C). Although there were no significant relationships between soil nutrients and any measure of PSII, the highest levels of P_{max} appeared to occur in *S. robustus* with intermediate ratios of soil N:P (Fig. 5C), just as in *S. patens* (Fig. 5B). Therefore, PSII response data and soil N:P ratios were also analyzed with linear and quadratic models. A significant quadratic relationship was found between P_{max} and soil N:P ratio (P = < 0.00; R^2 = 0.18). Both soil salinity and soil N:P ratio thus appear to interact with photosynthesis in *S. robustus*. This relationship is linear between soil salinity and P_{max} and soil salinity and α (Table 1) and quadratic between P_{max} and soil N:P ratio.

PSII response of *S. californicus*

S. californicus was only found in a narrow range of soil conditions (Fig. 3D), but a significant positive interaction was observed between soil %N and P_{max}, soil %N and α (Table 1). A significant negative interaction was observed between soil %P and P_{max}, and soil %P and α (Table 1). The cumulative effects of soil salinity and nutrients on P_{max} accounted for about a third of the variability observed in the data and were generally greater than in *S. alterniflora* and *S. robustus* (Table 1). We were not able to compare average RLC data among different salinities because *S. californicus* occurred in only brackish sites (Fig. 4D). P_{max} of *S. californicus* was highest in soils with the greatest ratios of N:P (Fig. 5D).

Relationship between soil and leaf nitrogen

Increased levels of soil nitrogen significantly correlated with increased levels of

leaf nitrogen in *S. alterniflora* as indicated by SPAD measurements (Fig. 6A) but not in

S. robustus (Fig. 6C). SPAD measurements did not correlate with P_{max} or α in *S.*

alterniflora (P_{max} Linear Regression - P = 0.17, R^2 = 0.02; α Linear Regression - P =

0.82, R^2 = 4.0 x 10^{-4}) or *S. robustus* (P_{max} Linear Regression - P = 0.96, R^2 = 4.6 x 10^{-5};

α Linear Regression - P = 0.31, R^2 = 0.02). SPAD measurements significantly

correlated with Φ_{PSII} in both species (Fig. 6B, 6D), indicating that PSII was more efficient

when leaf nitrogen content was high.

DISCUSSION

Field measurements of chlorophyll fluorescence

Collecting data from plants in the field under "natural" conditions allowed us to

observe the real-world cumulative effect of abiotic environmental factors on

photosynthetic light capture potential. Our data demonstrate that typical field conditions

for marsh plants in the northwestern Gulf of Mexico are more saline and variable in

nutrients than those that have been examined in the laboratory. This broad sampling

was effective in identifying significant relationships between chlorophyll fluorescence,

soil salinity, and soil nutrients in each plant species, including an inverse relationship in

some species between soil salinity and P_{max} / α (Table 1) that has not been reported in

previous investigations (Longstreth and Strain, 1977; Maricle et al., 2007). To our

knowledge, the results we present from *S. robustus* and *S. californicus* are the first

photosynthetic measurements in these species.

Saline soils decrease photosynthetic light capture potential

Previous laboratory-based investigations have found saline soils to either inhibit

(Smillie and Nott, 1982; Belkhodja et al., 1994; Moradi and Ismail, 2007) or have no

effect on PSII in non-halophyte crop plants (Mishra et al., 1991; Brugnoli and Björkman,

1992; Jimenez et al., 1997; Misra, 2001; James, 2002; Morant-Manceau et al., 2004;

Ranjbarfordoei et al., 2006; Sixto et al., 2006). In brackish and salt marsh plant species

saline soils have been shown to have no effect on chlorophyll fluorescence (Nieva et al.,

1999; Castillo et al., 2000; Lu et al., 2002; Maricle et al., 2007), yet we observed a

significant and negative relationship between soil salinity and P_{max} and soil salinity and

α in the field, particularly for *S. alterniflora*, *S. patens*, and *S. robustus* (Table 1). We

propose two hypotheses to explain this difference between our data and those of

previous investigations.

The first explanation is that because the soil salinities we sampled span a larger

range than what has been included in previous data sets, we have observed salinity-

induced responses that are only detectable at very high salinities. We inferred long

term porewater salinity history with different techniques than those used in previous

investigations, and direct quantitative comparisons of our measurements and those of

past researchers are not possible. Nevertheless, the soil salinities we included from *S.*

alterniflora and *S. patens* were as high as 103 ppt and 52 ppt, respectively (Figs. 2D,

3A), while the highest water salinity used to irrigate those same species in laboratory

investigations has been 30 ppt (Nieva et al., 1999; Castillo et al., 2000; Lu, 2002; Maricle et al., 2007).

An alternate explanation is that another environmental factor or a combination of environmental factors that directly correlate with soil salinity and that we did not measure drove the changes in chlorophyll fluorescence that we observed. Soil redox potential is a strong candidate. Previous investigations of photosynthesis in marsh plant species of the northwestern Gulf of Mexico demonstrate that when redox potentials are low, soils are often times reducing and anaerobic (Mendelssohn and Postek, 1982; Pezeshki et al., 1988; Bradley and Morris, 1992; Mitsch and Gosselink, 2007). These conditions have been shown to decrease rates of photosynthetic gas exchange in *S. patens* (Bandyopadhyah et al., 1993; Pezeshki and DeLaune, 1993) and *S. alterniflora* (Mendelssohn and Postek, 1982; Pezeshki et al., 1988; Bradley and Morris, 1992) but their effect on chlorophyll fluorescence has not been investigated. If soil redox potential was inversely related to soil salinity in the marshes included in this investigation, then soil redox potentials would have decreased in more saline soils and anaerobic conditions would have become more prevalent. Thus, the interactions we have observed between soil salinity and chlorophyll fluorescence may describe a cumulative response to soil salinity and soil redox potential (rather than soil salinity alone), and this may explain the differences between our field collected data and previous laboratory experiments.

Soil salinity, N:P ratio, and other soil conditions synergistically constrain photosynthetic light capture in wetland plants

Nitrogen is limiting in coastal marshes of the western Atlantic Ocean (Broome et al., 1974, Gallagher, 1975, Chalmers, 1979, Mendelssohn, 1979, Valiela et al., 1975, Levine et al., 1998) and northern Gulf of Mexico (Patrick and DeLaune, 1976, Pezeshki and DeLaune, 1988, Pezeshki et al., 1988, Pennings et al., 2002). *S. alterniflora* has been the subject of most fertilization experiments in marshes and its response to nitrogen addition can vary from insignificant (Valiela and Teal, 1974; Gallagher, 1975; Patrick and Delaune, 1976) to gross changes in morphology (Valiela et al., 1975) and biomass production (Broome et al., 1974, Gallagher, 1975, Valiela et al., 1975, Chalmers, 1979, Mendelssohn, 1979, Pennings et al., 2002). Meticulous examination of this species by several researchers including Mendelssohn (1979), Mendelssohn and Postek (1982), Morris (1984), Morris and Dacey (1984), Pezeshki et al. (1988), and Bradley and Morris (1990, 1992) concluded that the fertilization response of *S. alterniflora* depends on its ability to absorb nitrogen from the soil in the face of other confounding abiotic factors including soil sulfide concentration, soil redox potential, soil aeration, and soil salinity. These abiotic factors can either preclude nitrogen from being absorbed or require diversion of nitrogen from building biomass to such a degree that the minimum concentration needed for survival negates any beneficial impacts of elevated soil nitrogen levels (Bradley and Morris, 1992). Thus, soil salinity works synergistically with other soil abiotic factors to determine nutrient availability in salt marsh species, and soil nitrogen levels alone may not directly correlate with photosynthetic output or biomass production (Mendelssohn and Morris, 2002).

We did not observe a change in P_{max} or α in response to soil nitrogen or N:P ratio in *S. alterniflora* (Table 1; Fig. 5A), but SPAD and soil nitrogen content

measurements indicate that when more nitrogen was available in the soil, *S. alterniflora*

incorporated more of it into its leaves (Fig. 6A), and this correlated with an increase of

Φ_{PSII} (Fig. 6B). Elevated Φ_{PSII} is indicative of an efficient and overall healthy,

undamaged PSII reaction center (Maxwell and Johnson, 2000). The minimal level of

nitrogen needed to maintain photosynthetic potential across a broad range of soil

salinities must have been met in the *S. alterniflora* we sampled because even when soil

nitrogen content was limiting, P_{max} and α were the same (Table 1; Fig. 5A). Similar

results indicating that *S. alterniflora* can continue to absorb nitrogen as it becomes

available in the soil under a wide range of environmental conditions have been obtained

along the Atlantic and Gulf coasts of the U.S. (Mendelssohn, 1979; Pennings et al.,

2002).

The relationship we describe between soil nitrogen, leaf nitrogen, and

photosynthetic health helps to explain why P_{max} and α were maintained in *S. alterniflora*

in soils that were nitrogen limited (Table 1; Fig. 5A). However, other investigations in *S.*

alterniflora have measured increased biomass production in response to nitrogen

fertilization (Broome et al., 1974, Gallagher, 1975, Valiela et al., 1975, Chalmers, 1979,

Mendelssohn, 1979, Pennings et al., 2002), so then why didn't we observe increased

P_{max} and α in less nitrogen-limited soils?

It is important to remember that our data measured only the PSII light capturing

processes of photosynthesis (P_{max}, α, and Φ_{PSII}). In most plant species, rates of carbon

fixation and photosynthetic light capture are directly related (Krall and Edwards, 1992;

Edwards and Baker, 1993), however in marsh plant species rates of carbon fixation and

light capture can change independently (Maricle et al., 2007). Maricle et al. (2007)

discovered this unique photosynthetic property in *S. alterniflora* and has hypothesized

that alternative e⁻ sinks including leakage of CO_2 from bundle sheath cells are

responsible for the uncoupling of photosynthetic reactions in C4 marsh plants. Perhaps

when nitrogen becomes more available in *S. alterniflora* leaves, the contribution to

these sinks is reduced and photosynthesis becomes more efficient. This would allow for

greater rates of carbon fixation that would potentially translate to increased biomass

production even if photosynthetic light capture had not changed. Simultaneous

measurements of light capture and carbon fixation under diverse nutrient loads would

test this hypothesis and be an interesting area of future investigation.

In *S. robustus,* leaf nitrogen content correlated with Φ_{PSII} (Fig. 6D) just as in *S.*

alterniflora (Fig. 6B). However, leaf nitrogen content in *S. robustus* did not increase with

soil nitrogen load (Fig. 6C). These data suggest that nutrient absorption processes in *S.*

robustus may not be able to adapt to changing soil characteristics as well as *S.*

alterniflora, and this may ultimately explain why these two species inhabit soils with a

different range of nutrient concentrations (Figs. 2E, 3A, 3B, 3E, 3F).

Different ecophysiological response strategies correlate with different spatial

distributions of marsh plant species

We observed the most disparate response to salinity and nutrients in *S.*

alterniflora and *S. californicus*. P_{max} in *S. alterniflora* changed little across a broad

range of soil salinities (Fig. 4A) and nutrient regimes (Fig. 5A) with an average value of

26 μmol m^{-2} s^{-1}. Conversely, *S. californicus* could not be found at all in soils with

salinities > 23 ppt, but when nitrogen was not limiting average P_{max} increased to 28

μmol m^{-2} s^{-1} from an average value of 15 μmol m^{-2} s^{-1} in more nitrogen limited soils

(N:P ratios <11). We even saw average P$_{max}$ values as high as 51 μmol m^{-2} s^{-1} in an

outlying sample of *S. californicus* in soils with an N:P ratio of 99. Thus, the

ecophysiological response strategy of *S. californicus* is more sensitive to environmental

conditions, but when conditions are favorable there is a greater capacity for

photosynthetic light capture to increase in *S. californicus* than in *S. alterniflora* as

indicated by P$_{max}$ and α.

The different photosynthetic response strategies of *S. alterniflora* and *S.*

californicus almost certainly play a role in the range of environmental conditions in

which each species is found within the marsh. *S.alterniflora* is more broadly distributed

and completely dominant in high salinity and low marsh habitats (Mendelssohn and

Morris, 2002) while *S. californicus* can only survive within a narrow range of soil

salinities (Figs. 2D, 3D). If species distribution is considered a measure of success for

each of these strategies, then the conservative response of *S. alterniflora,* in which

moderate photosynthetic potentials are maintained across a broad range of soil

salinities and nutrient regimes, appears to be more effective than the aggressive yet

less adaptive strategy of *S. californicus*.

CONCLUSIONS

Our data indicate that the actual environmental conditions experienced by

wetland plants in the northwestern Gulf of Mexico are more saline than what has been

simulated in the laboratory. Previous investigations have not correlated measurements

of soil %N and %P content with photochemical light capture, but our data show a

disparate nutrient response in wetland plants. Our data also demonstrate that even in

the uncontrolled conditions of the field, complex physiological response pathways

originally described in the laboratory can be readily detected in simultaneous

measurements of abiotic conditions and photosynthetic processes. These sorts of data

provide an ecological context for the mechanistic findings of laboratory investigations

and demonstrate that the species in this investigation adapted to the highest levels of

abiotic stress (*S. alterniflora*) were less dominant in the field when those stressors were

removed, while species with the highest potential for photosynthetic performance (*S.*

robustus and *S. californicus*) could only be found in locations with favorable abiotic

conditions.

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Table 1 - Results of multiple linear regression analyses of soil salinity, %N, and %P on P_{max} and α. Indicated value is the standardized coefficient of interaction. Asterisks indicate P-value; *p≤0.05; **p≤0.01; ***p≤0.001. Cumulative R^2 values of analyses are indicated in the last row of each species group.

	P_{max}	α
Spartina alterniflora		
Soil Salinity	-0.32***	-0.29***
Soil %N	0.13	-0.09
Soil %P	-0.21	-0.30**
Cumulative R^2	0.11	0.12
Spartina patens		
Soil Salinity	-0.41**	-0.56***
Soil %N	-0.58***	-0.62***
Soil %P	0.09	0.21
Cumulative R^2	0.32	0.38
Schoenoplectus robustus		
Soil Salinity	-0.26*	-0.41***
Soil %N	-0.07	-0.12
Soil %P	-0.18	-0.180
Cumulative R^2	0.16	0.29
Schoenoplectus californicus		
Soil Salinity	0.11	0.26
Soil %N	0.30*	0.37*
Soil %P	-0.62***	-0.39*
Cumulative R^2	0.29	0.18

FIGURE CAPTIONS

Figure 1 - Field sites were in the northwestern Gulf of Mexico (A), in east Texas (B),

near Galveston Bay, Texas (C).

Figure 2 - Soil salinities (A), %N concentrations (B), and %P concentrations (C) at field

sites in the six marshes included in this investigation. The number of field sites in each

marsh are listed in parenthesis on the left.

Figure 3 - Soil salinities (A), %N concentrations (B), %P concentrations (C), and N:P

ratios (D) at field sites sampled in this investigation for *S. alterniflora*, *S. patens*, *S.

robustus*, and *S. californicus*.

Figure 4 - Rapid light curve data for *S. alterniflora* (A), *S. patens* (B), *S. robustus* (C),

and *S. californicus* (D). Each point is a mean, errors bars indicate standard error, and

results are grouped according to the salinity of the soil in which the plants were growing

at the time of measurement. Trend lines are the averaged PI function for the dataset.

Figure 5 - Rapid light curve data for *S. alterniflora* (A), *S. patens* (B), *S. robustus* (C),

and *S. californicus* (D). Each point is a mean, errors bars indicate standard error, and

results are grouped according to the N:P ratio of the soil in which the plants were

growing at the time of measurement. Trend lines are the averaged PI function for the

dataset.

Figure 6 - SPAD vs. soil %N and SPAD vs. ϕPSII in *S. alterniflora* (A, B) and *S.

robustus* (C, D).

Appendix Figure 1 - SPAD value strongly correlates with leaf chlorophyll a

concentration ($P < 0.0001$; $R^2 = 0.81$) and chlorophyll b concentration ($P < 0.0001$; $R^2 =$

0.82) in *Spartina alterniflora*.
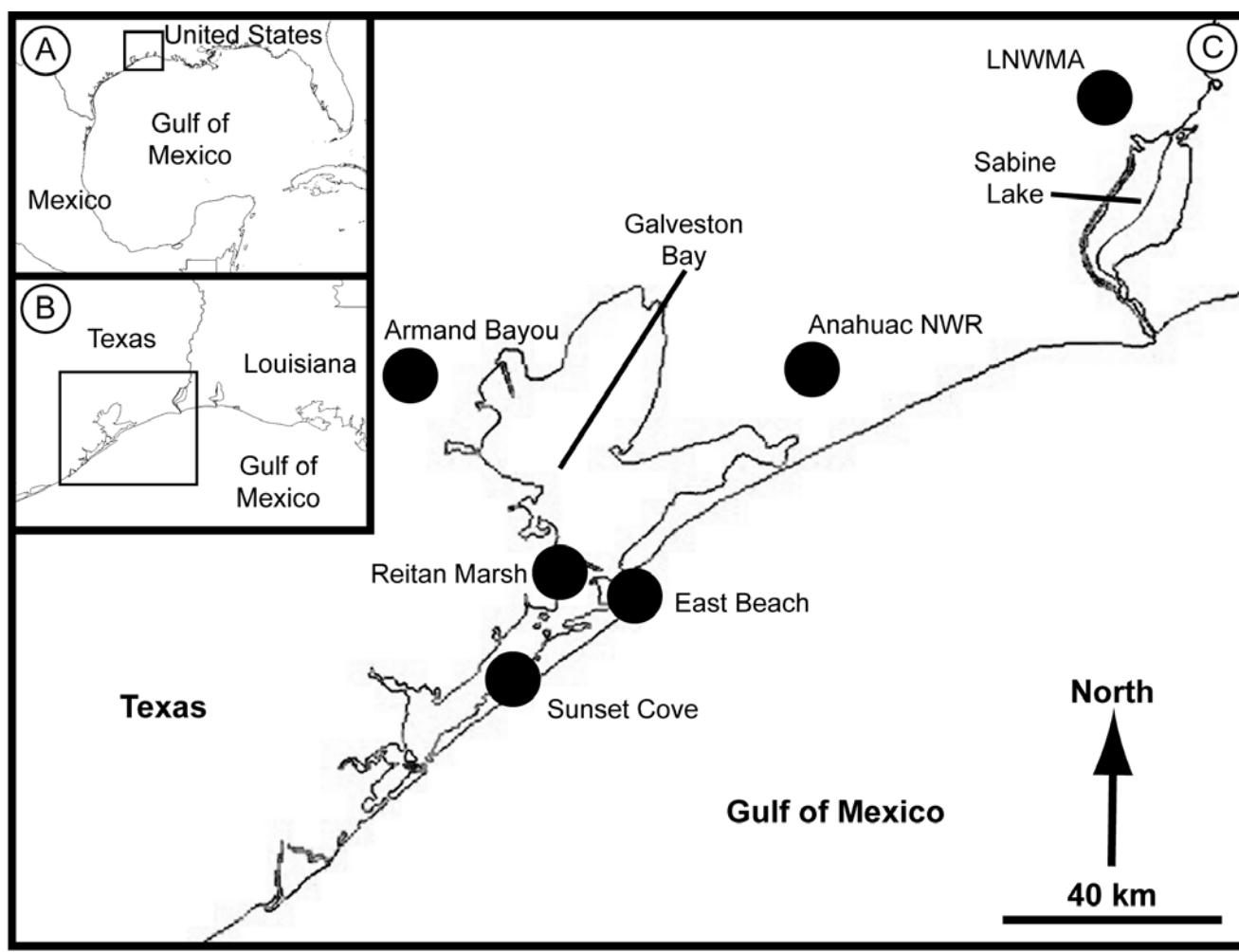
Figure 1

Figure 2
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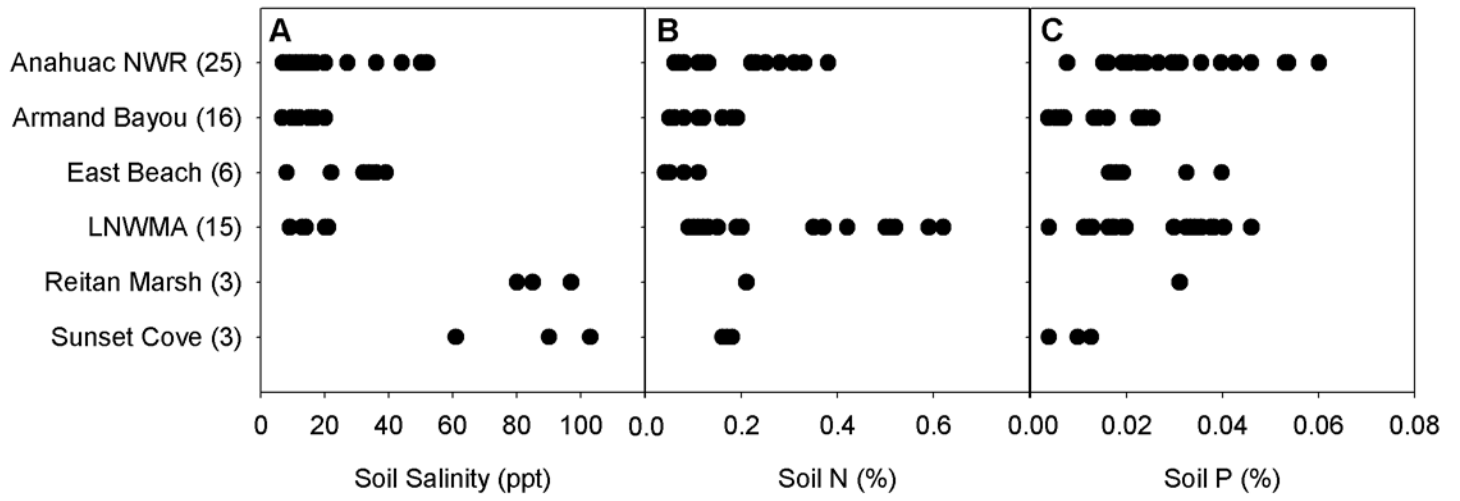
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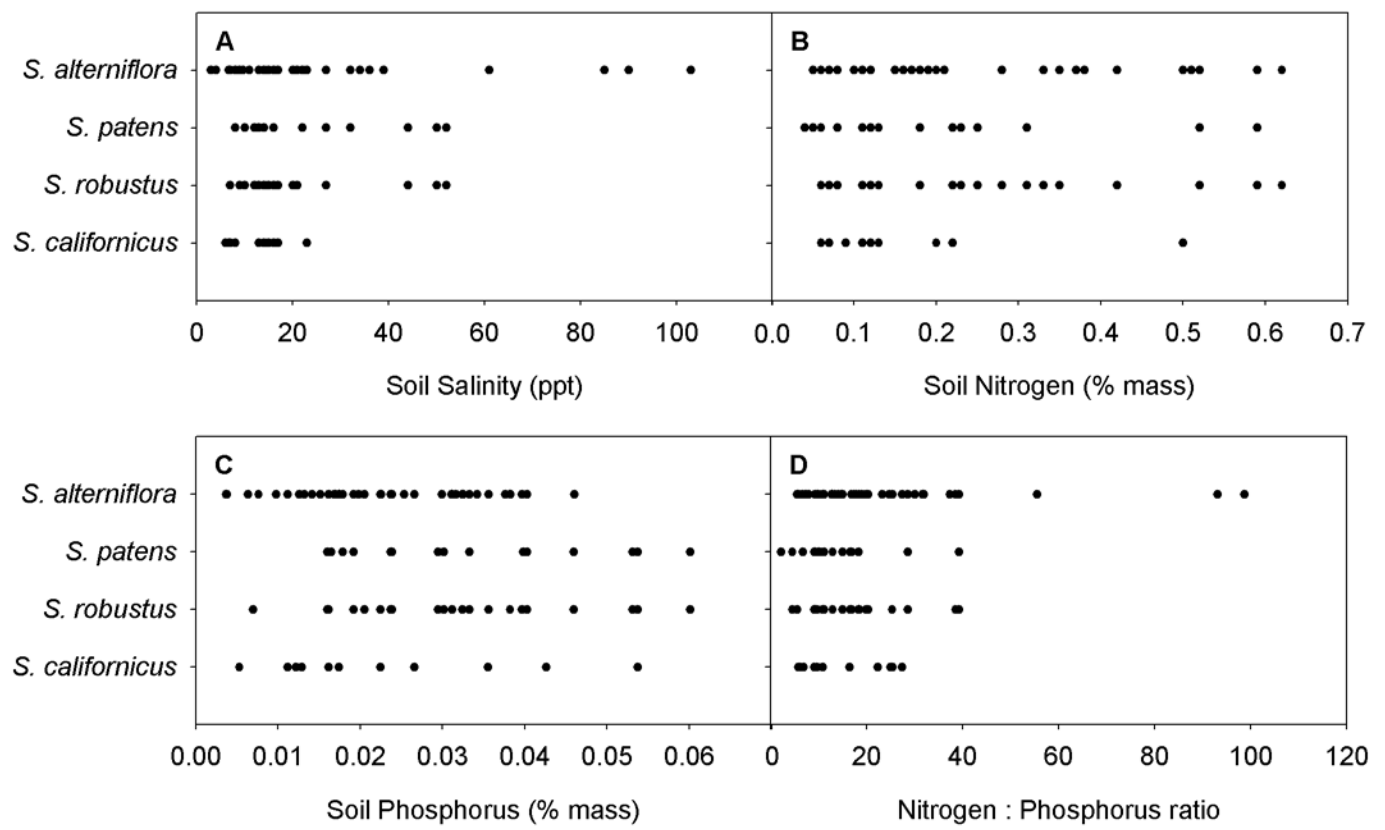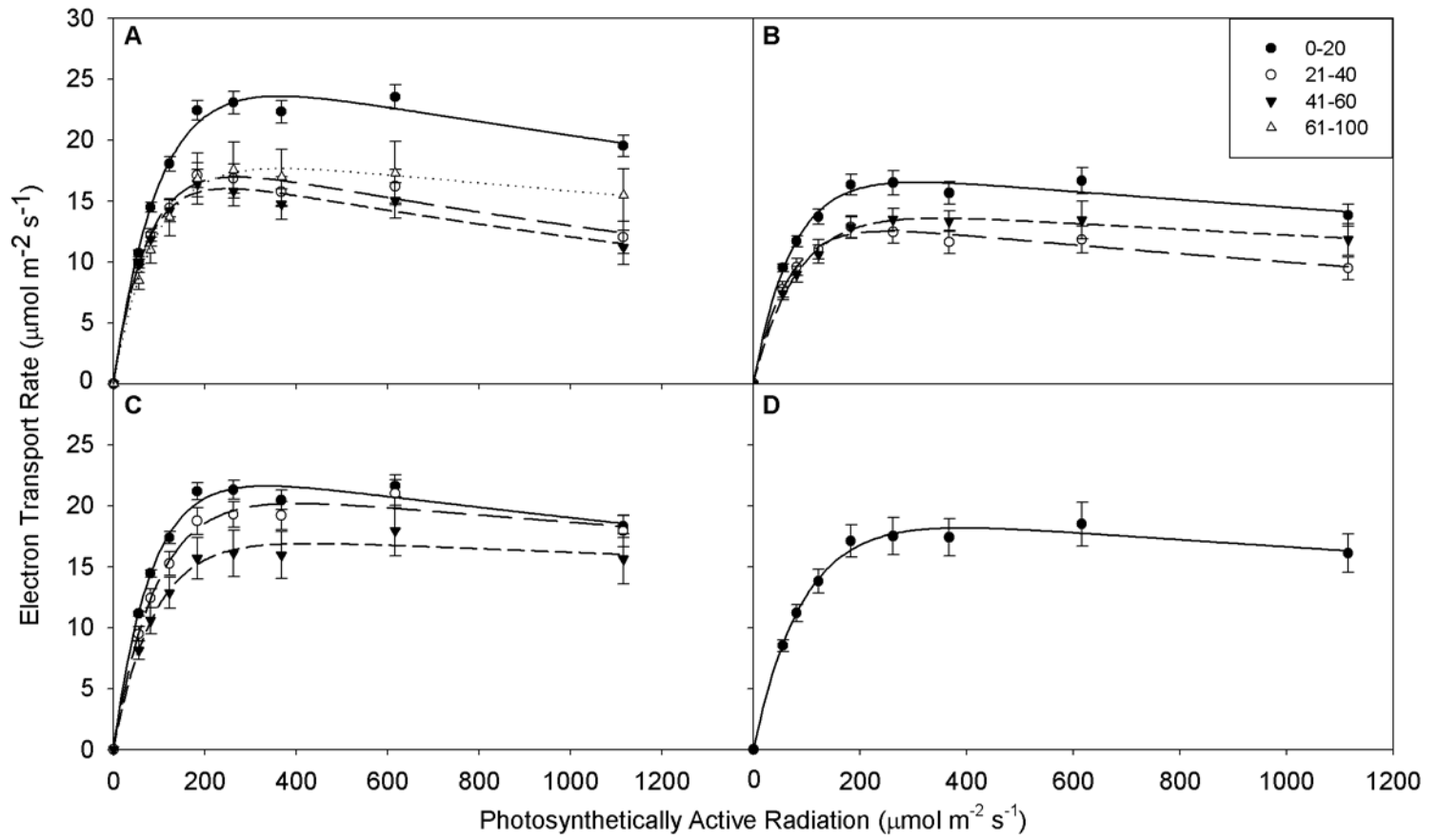
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Figure 6
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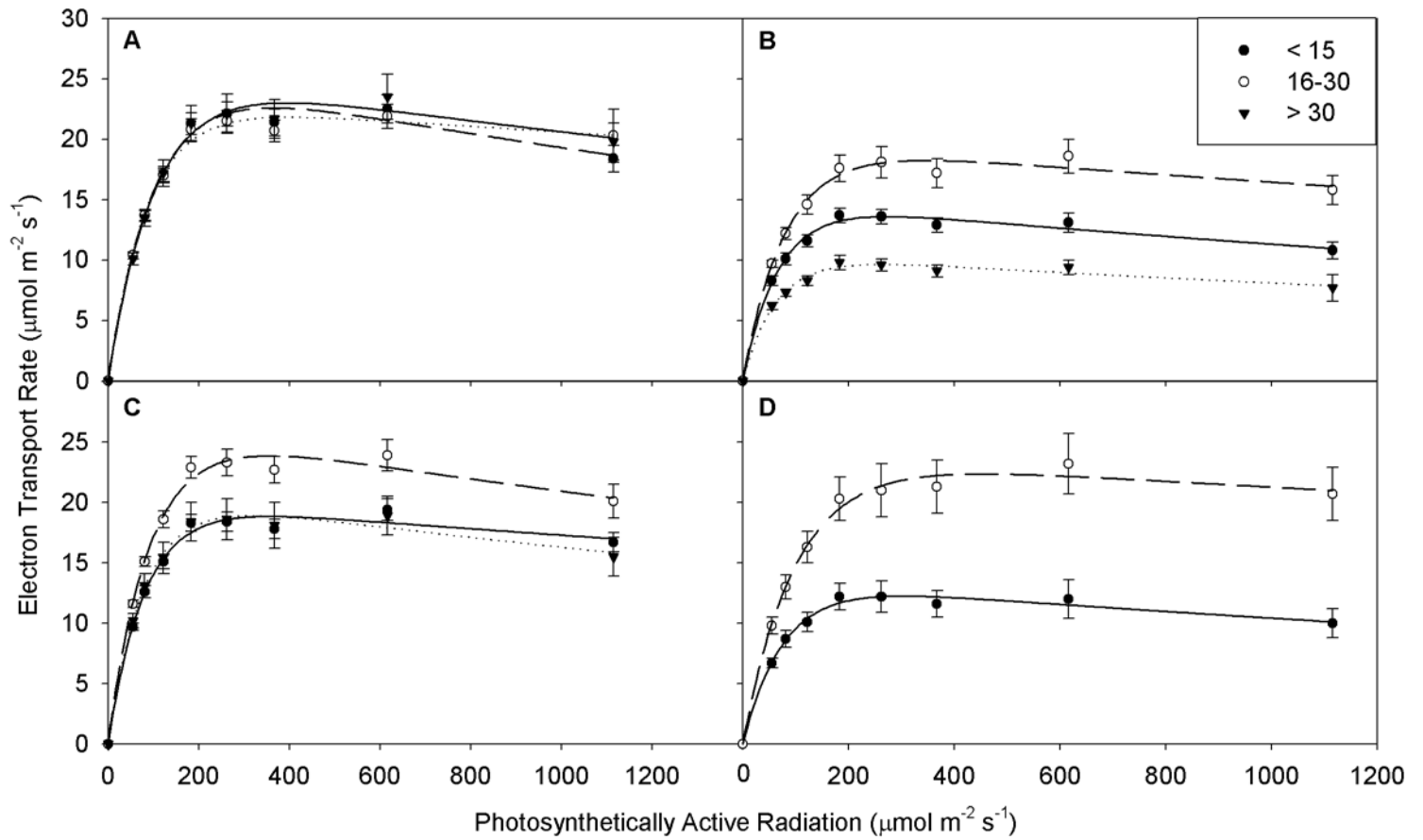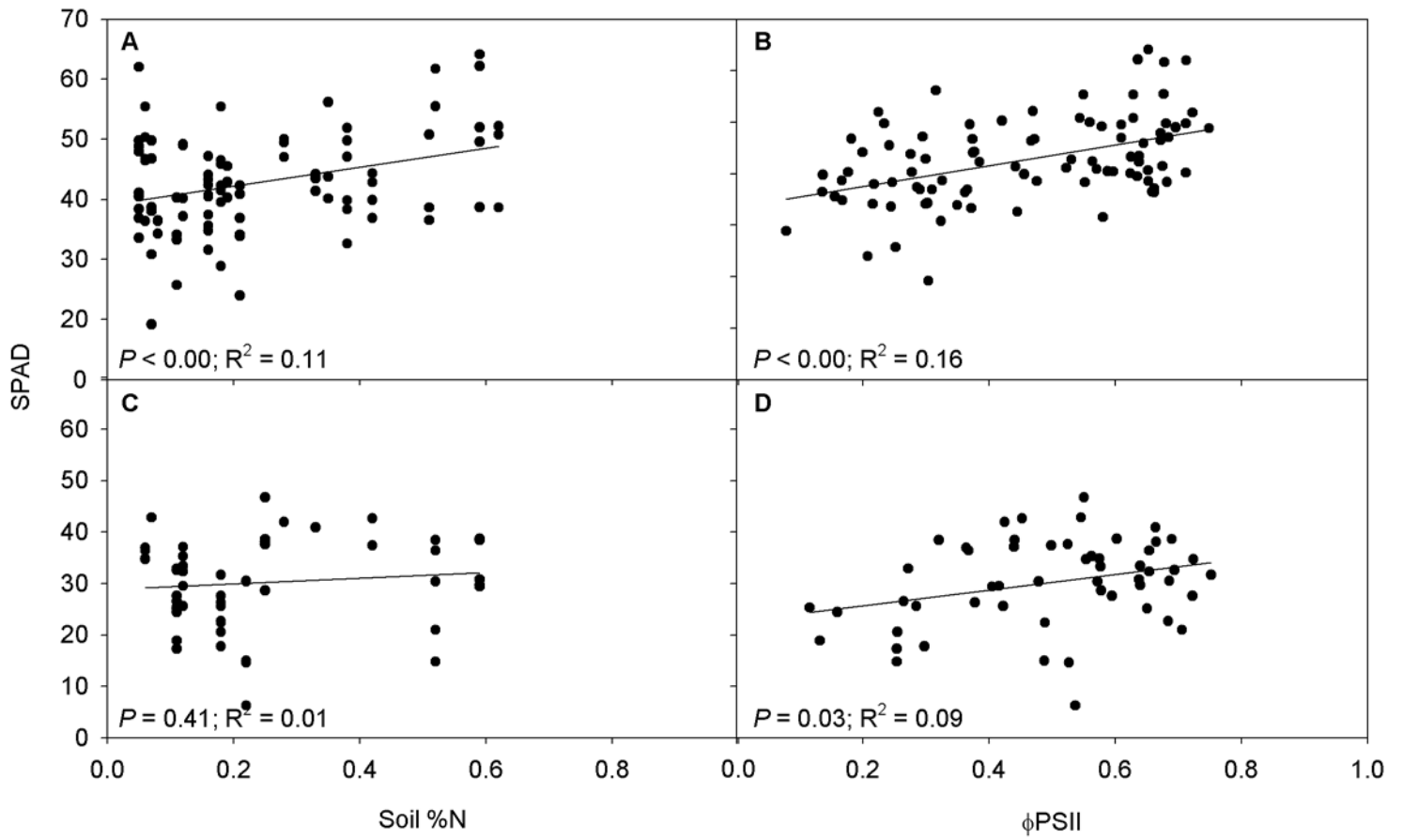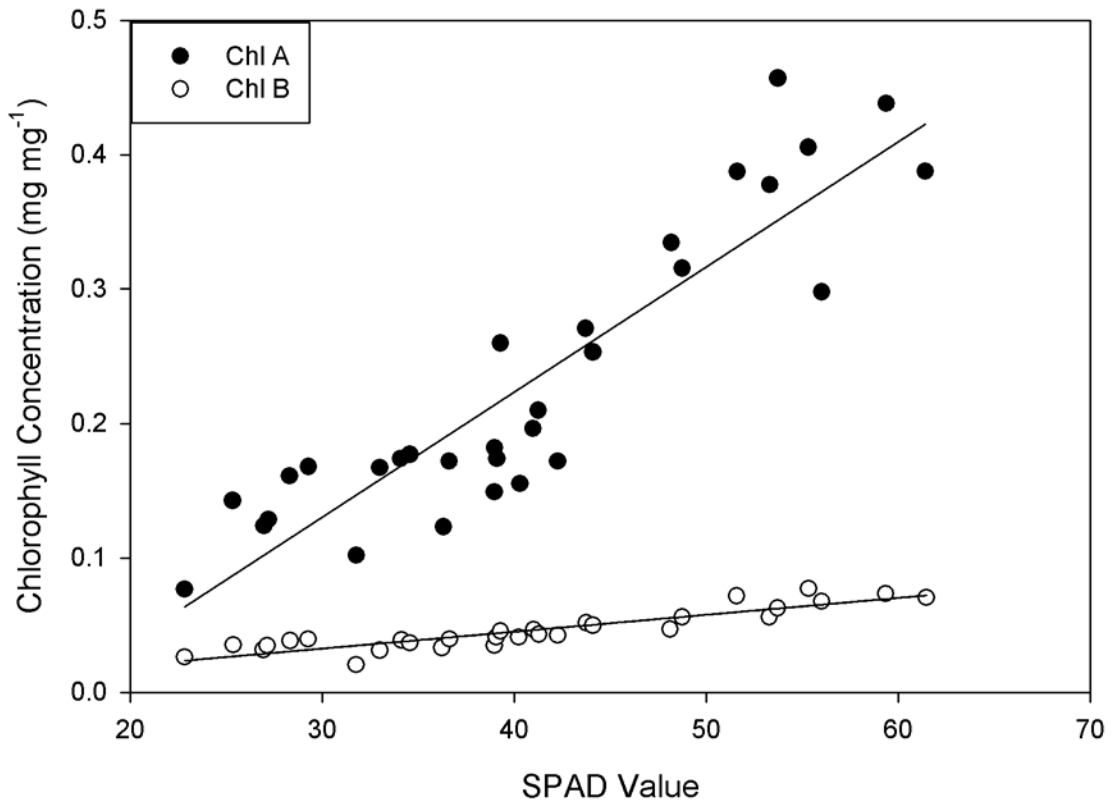
Figure 6

Appendix Figure 1

Appendix B
Native and exotic submerged aquatic vegetation provide different nutritional and refuge values for macroinvertebrates

Native and exotic submerged aquatic vegetation provide different nutritional and refuge values for macroinvertebrates

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A B S T R A C T

Proliferation of exotic submerged aquatic vegetation (SAV) has the potential to alter trophic interactions among native fauna. The invasion of *Myriophyllum spicatum* (Eurasian watermilfoil) into brackish and freshwater wetlands on the Gulf Coast of the United States has decreased the abundance of native plant species such as *Ruppia maritima* (widgeongrass). Macroinvertebrates use SAV to meet nutritional requirements and seek refuge from predators. Shifts in SAV dominance can alter patterns of SAV use by associated fauna, but the dynamics of these alterations following *Myriophyllum* displacement of *Ruppia* are not well understood. In our study, we examined the trophic implications of shifts between native (*Ruppia*) and exotic (*Myriophyllum*) SAV in a brackish wetland in Port Arthur, Texas, USA. This study addressed the following questions: 1) Do aquatic invertebrates directly consume *Myriophyllum*, and 2) do predator–prey interactions differ in native and exotic SAV canopies? We collected *Ruppia*, *Myriophyllum*, *Palaemonetes* spp. (grass shrimp), and juvenile *Callinectes sapidus* (blue crabs) from the field and ran a series of three mesocosm experiments in the laboratory. In a "no-choice" grazing experiment, *Palaemonetes* consumed 13% of the *Ruppia* biomass but had no impact on *Myriophyllum* biomass. In a second study, when given a choice of plant canopies, *Palaemonetes* selected *Ruppia* over *Myriophyllum* 60% of the time when *Callinectes* was absent, but selected *Myriophyllum* more frequently in the presence of *Callinectes*. The selection of *Myriophyllum* was likely due to its refuge value; a third mesocosm experiment resulted in 60% *Palaemonetes* mortality due to *Callinectes* predation in *Ruppia* canopies but only 20% mortality in *Myriophyllum* canopies. *Myriophyllum* has a more structurally complex canopy that probably provides better protection from predation. Our studies suggest that native and exotic plants provide substantially different values as food sources and as refuges from predators for lower trophic levels.

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1. Introduction

Biological invasions by exotic species can have adverse biotic and abiotic consequences that alter fundamental ecosystem properties (Vitousek, 1990). Second only to habitat destruction, species invasions pose the greatest threat to biodiversity in many ecosystems, particularly when the exotic species are ecosystem engineers that can directly modify physical habitat structure (Crooks, 2002; Jones et al., 1994; Wilcove et al., 1998). Exotic species often competitively displace native species, leading to shifts in dominance and species loss (Boylen et al., 1999; Duffy and Baltz, 1998). Alterations of plant community structure (Bertness, 1984; Hooper et al., 2005), food web

functioning (Zedler and Kercher, 2004), nutrient cycling (Ehrenfeld, 2003; Vitousek and Walker, 1989), and hydrology (Levine et al., 2003) following species invasions have been documented in terrestrial and freshwater habitats. Aquatic systems are particularly vulnerable to invasion as a result of anthropogenic activities such as urban development and international transport (Mack and Lonsdale, 2001; Ruiz et al., 1999).

The impacts of species invasion often cascade across multiple trophic levels. For example, the introduced shrub, *Mimosa pigra*, has converted sedge wetlands of tropical northern Australia to shrubland, leading to substantial loss of native floral and faunal species (Braithwaite et al., 1989). Likewise, the introduced grass, *Bromus tectorum*, has invaded the perennial bunchgrass communities of southern Utah, impacting native plant species richness, reducing fungi and invertebrate abundance, and increasing the abundance of active bacteria (Belnap and Phillips, 2001). Invasions of plant species can also alter trophic relationships by decreasing food availability for native herbivores (Demopoulos et al., 2007; Yoshioka et al., 2010) or, less frequently, by providing a new, labile food source (Levin et al., 2006; Whitcraft et al., 2008).

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Estuarine systems are particularly impacted by invasions of exotic submerged aquatic vegetation (SAV). Invasive SAV is capable of rapid expansion into dense monocultures, suppressing or eradicating native SAV due to resource competition (Boylen et al., 1999; Nichols and Shaw, 1986). As a result, dense exotic SAV canopies can decrease habitat quality and trophic support for associated fauna such as fish and waterfowl (Mack et al., 2000; Madsen et al., 1991). However, species invasions are not always associated with negative consequences. Some studies have shown positive correlations between exotic SAV and macroinvertebrate production (Humphries, 1996; Kilgore et al., 1989; Posey, 1988; Posey et al., 1993). This facilitation is often attributed to the increased structural complexity and presumably higher refuge value of exotic SAV (Chaplin and Valentine, 2009; Crooks, 2002; Toft et al., 2003).

As ecosystem engineers, SAV modify the physical state of the aquatic habitat (Jones et al., 1994), but two of the most important functions of SAV for aquatic macroinvertebrates are to meet their nutritional needs and to provide refuge from predators (Batzer, 1998; Brendock et al., 2003; Krecker, 1939), although exotic SAV is often less palatable to native herbivores (Dibble and Kovalenko, 2009; McKnight and Hepp, 1995; Xiong et al., 2008). The refuge value of SAV is largely driven by the structural nature, or architecture, of the plant, which is typically described by the number and arrangement of stems, branches, and leaves (Cheruvelil et al., 2000; Lillie and Budd, 1992). SAV with finely dissected architecture may provide macroinvertebrates with a more suitable refuge habitat (Krecker, 1939; Smith and Barko, 1990). Therefore, if an exotic submerged macrophyte has a more structurally complex architecture, then it may provide macroinvertebrates with better protection from predators.

Myriophyllum spicatum (Eurasian watermilfoil) is a common exotic SAV species that has spread rapidly in brackish and freshwater habitats in the southeastern US over the last 35 years (Smith and Barko, 1990). It has been a successful invader because it can tolerate fluctuations in a variety of environmental factors, including salinity, temperature, and light (Nichols and Shaw, 1986). It is considered a nuisance species because it can alter the canopies and composition of aquatic systems by outcompeting native SAV species (Madsen et al., 1991). *Myriophyllum* exhibits finely dissected architecture, creating a canopy that is nearly twice as complex as plant species with long, straight leaves (Chaplin and Valentine, 2009). *Myriophyllum* can support higher macroinvertebrate abundance relative to SAV canopies with less complex architecture (Chaplin and Valentine, 2009; Martin and Valentine, 2011), although the mechanisms driving this pattern are not well understood. We investigated some potential drivers of these patterns by comparing aquatic invertebrate use of exotic *M. spicatum* with native *Ruppia maritima*. In particular, we asked 1) Do aquatic invertebrates directly consume the exotic *Myriophyllum*, and 2) Are predator–prey interactions altered in the presence of exotic *Myriophyllum*? We expected that macroinvertebrate herbivores would consume more native *Ruppia* than exotic *Myriophyllum*. We also expected that macroinvertebrate herbivores would preferentially inhabit the structurally complex *Myriophyllum* over the comparably less complex *Ruppia*, and that this choice would be driven by higher predation rates in the simpler *Ruppia* canopy.

2. Methods

2.1. Study area and organisms

This study was conducted using plants and animals collected from a restored brackish (average salinity<5 ppt) wetland in the Lower Neches Water Management Area in Port Arthur, Texas, USA (30.005092° N and 93.855779° W). Restoration of the wetland created a mixture of emergent *Spartina alterniflora* marsh and aquatic habitat. Native aquatic vegetation included *Ruppia* (widgeongrass) and green algae (*Spirogyra* sp. and *Cladophora* sp.), but exotic *Myriophyllum*

dominated the SAV community throughout much of the site (A.R. Armitage, unpublished data). *Ruppia* has relatively uniform architecture, with alternating, threadlike leaves (10 cm long, 4–6 mm wide) on thin stems that can reach up to 1 m long (Correll and Correll, 1975; Godfrey and Wooten, 1979). *Myriophyllum* has relatively complex architecture, with feather-like leaves (12–24 mm long) whorled in groups of four at stem nodes every 2–4.5 cm along densely-growing stems that are up to 7 m long (Lillie and Budd, 1992). The dominant herbivorous macroinvertebrates in the SAV canopy were *Palaemonetes* spp. (grass shrimp), amphipods, and gastropods; we focused on the most abundant group, *Palaemonetes* spp. The dominant benthic predators within the SAV canopies were juvenile *Callinectes sapidus* (blue crab). All organisms will be referred to generically hereafter.

2.2. Sample collection and preparation

To examine relationships between aquatic invertebrates and native or exotic SAV, we performed a series of mesocosm experiments in the laboratory in June 2009. For all experiments, *Ruppia* and *Myriophyllum* were removed as whole plants from the marsh by hand. The plants were placed in Ziploc® bags with water from the marsh and stored in a cooler for transport to the laboratory.

We used dip nets to collect adult *Palaemonetes* and juvenile *Callinectes*. We used *Palaemonetes* with carapace lengths between 14 and 20 mm and *Callinectes* with carapace widths between 35 and 50 mm. These were the most common sizes collected from the field; we performed pilot studies to confirm that *Callinectes* in this size range were capable of capturing and consuming adult *Palaemonetes*. The animals were transported back to the laboratory in separate, aerated buckets containing water from the marsh.

In the laboratory, we rinsed *Ruppia* and *Myriophyllum* with tap water and removed the root material. Prior to each experiment, the plants were acclimated for 24 h in aquaria filled with 4 ppt salt water made from deionized water and Instant Ocean®. *Palaemonetes* were placed in a separate aquarium with prepared 4 ppt salt water for a starvation period of 24 h. Individual *Callinectes* were placed into separate 1-L jars with prepared 4 ppt salt water for 24 h.

2.3. Herbivory assay

We filled 24 1-L glass jars with 300 mL of prepared (deionized water + Instant Ocean®) 4 ppt salt water. The jars were placed in a 4×6 grid in an outdoor shelter with indirect sunlight. Sprigs of *Ruppia* and *Myriophyllum* were spun in a salad spinner for 45 s in order to obtain a constant wet weight. We placed bunches (1.5 g wet weight) of *Ruppia* into 12 randomly selected jars, and bunches (1.5 g wet weight) of *Myriophyllum* into the 12 remaining jars. We did not directly measure canopy architecture, but previous studies have demonstrated that when standardized to biomass, *Myriophyllum* has a more complex canopy than species with longer, flat leaves (Chaplin and Valentine, 2009). We then placed one *Palaemonetes* into six randomly selected *Ruppia* jars and six randomly selected *Myriophyllum* jars; the remaining 12 jars contained no *Palaemonetes* and served as controls.

After ten days, all plant material was removed from the jars, and the wet weight was determined as previously described. We calculated the percent change in wet weight as $100 * (\text{final} - \text{initial})/\text{initial}$. Homoscedasticity of variances was confirmed with Levene's test and change in wet weight was analyzed with a two-way analysis of variance, where the independent variables were plant species and herbivore presence.

2.4. Refuge experiment

To investigate *Palaemonetes* preferences for inhabiting native or exotic SAV, we conducted an experiment where *Palaemonetes* could

choose between the two plant species. We partitioned four ten-gallon aquariums into two equal halves with plastic lattice (1 cm² mesh). The lattice was secured inside the aquariums with silicone adhesive. Each aquarium was filled with 2 gal of prepared 4 ppt salt water and placed indoors near a window that provided even, indirect sunlight. We placed 20 g (spun to a constant wet weight) of *Ruppia* on one side of each aquarium, and 20 g of *Myriophyllum* on the other side. Although biomass was equivalent on both sides of the tank, the *Myriophyllum* qualitatively appeared to have higher percent cover than the *Ruppia*. Ten *Palaemonetes* were placed in each of the aquaria, five on each side; these shrimp were able to move freely through the 1-cm² grid of the partition. In two of the aquaria, we placed one *Callinectes* on each side of the aquarium; these crabs were unable to pass through the partition. The remaining two aquaria did not contain *Callinectes* and served as controls.

Due to limited aquarium space, this experiment was repeated three times with two replicates per treatment per trial. Plants and animals were released at the end of each trial and new specimens were used for each successive trial. Because the trials were conducted in succession within a short time period, we considered time period to be a random block. For each trial, we counted the number of *Palaemonetes* on each side of the aquariums five times a day for a period of seven days. For each aquarium, we pooled all observations over the study period and divided the total number of shrimp observed on *Ruppia* by the total number of shrimp counted to determine the percent of time that *Palaemonetes* spent on *Ruppia*. Homoscedasticity of variances was confirmed with Levene's test. We analyzed the data with a blocked one-way ANOVA, where time period was a random block, *Callinectes* presence or absence was the fixed factor, and the percent of time *Palaemonetes* spent on *Ruppia* was the dependent variable.

2.5. Predation experiment

We performed a third experiment to compare *Callinectes* predation rates on *Palaemonetes* in canopies of *Ruppia* or *Myriophyllum*. We added 1.3 L of prepared 4 ppt salt water to eight 1.5 gal aquaria. The aquaria were placed indoors near a window that proved even, indirect sunlight. We placed bunches (4 g standardized wet weight) of *Ruppia* into four randomly selected aquaria and added bunches (4 g standardized wet weight) of *Myriophyllum* to the remaining four aquaria. We then placed five *Palaemonetes* into each aquarium. One *Callinectes* was placed into each of two randomly selected *Ruppia* aquaria and two randomly selected *Myriophyllum* aquaria; the remaining four aquaria contained no *Callinectes* and served as controls.

Due to limited space we were unable to run more than two replicates at a time, so this experiment was repeated two times. New plant and animal specimens were used for each successive trial. Because the temporal replicates were conducted in rapid succession within a short time period, we considered time period to be a random block. We counted the number of live *Palaemonetes* at the end of each day, and calculated total percent *Palaemonetes* mortality after a period of seven days. Since no *Palaemonetes* mortality occurred in the treatments without crabs, we assumed that all mortality was due to *Callinectes* predation. Levene's test revealed that the variances were heteroscedastic and could not be transformed. Therefore, we performed nonparametric Kruskal–Wallis tests, where time period (block) and plant species (*Ruppia* or *Myriophyllum*) were independent factors and total percent mortality of *Palaemonetes* in tanks with *Callinectes* was the dependent variable.

3. Results

3.1. Herbivory assay

There was high variability among replicates, but there was a marginally significant interaction between plant species and *Palaemonetes*

Table 1
Summary of two-way ANOVA results for the herbivory experiment. Plant species (*Ruppia* or *Myriophyllum*) and *Palaemonetes* (shrimp) presence or absence were the factors and percent change in plant wet weight was the dependent variable.

	df	MS	F	p
Plant	1	128.8	0.6	0.446
Shrimp	1	871.5	4.1	0.057
Plant*Shrimp	1	667.1	3.1	0.092
Error	19	212.4		

addition (Table 1), which was driven by a larger herbivore effect on *Ruppia* than on *Myriophyllum* (Fig. 1). *Ruppia* biomass was an average of 13.0% ($\pm$4.5%) lower when *Palaemonetes* were present than in the control, where biomass increased by 2.5% ($\pm$7.9%). *Myriophyllum* biomass increased by 4–10% regardless of whether or not *Palaemonetes* was present (Fig. 1). We frequently observed *Palaemonetes* grazing directly on *Ruppia*, but never witnessed direct grazing on *Myriophyllum*.

3.2. Refuge experiment

The presence of *Callinectes* influenced *Palaemonetes* plant selection. *Palaemonetes* spent significantly less time on *Ruppia* (and therefore more time on *Myriophyllum*) when *Callinectes* were present (40.2% $\pm$ 2.3%) than when *Callinectes* were absent (59.9% $\pm$ 3.1%; Fig. 2, Table 2). Consequently, *Palaemonetes* preferred *Myriophyllum* when crabs were present and *Ruppia* when crabs were absent. There was no significant block (time) effect (Table 2).

Callinectes behavior was qualitatively similar between plant canopies: we observed *Callinectes* pursuing *Palaemonetes* in a predatory manner in both plant canopies, although our observation periods were not long enough to witness successful captures. *Palaemonetes* mortality in tanks with crabs averaged 45.0% ($\pm$5.0% SE); there was no mortality in tanks without crabs.

3.3. Predation experiment

There was no *Palaemonetes* mortality in treatments where *Callinectes* were absent. *Callinectes* predation on *Palaemonetes* was significantly lower in *Myriophyllum* canopies (20.0% $\pm$0.0 at the end of the study period) than in *Ruppia* canopies (60.0 $\pm$8.2; Kruskal–Wallis p = 0.026; Fig. 3). There was no significant block (time) effect (Kruskal–Wallis p = 0.711). We observed occasional grazing by *Callinectes* on *Myriophyllum*, but not on *Ruppia*.
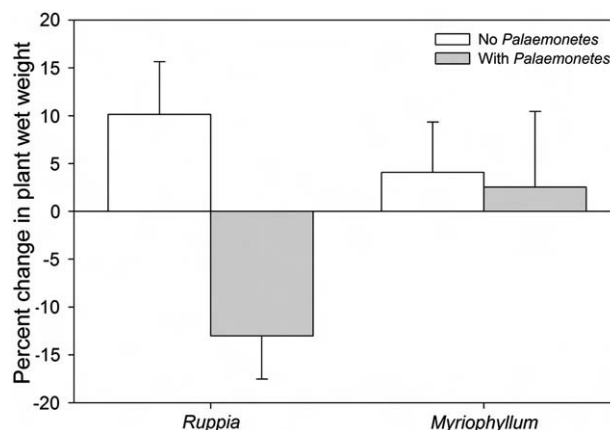

Fig. 1. Herbivory by *Palaemonetes* on *Myriophyllum* and *Ruppia*. Error bars represent standard error (n = 6).
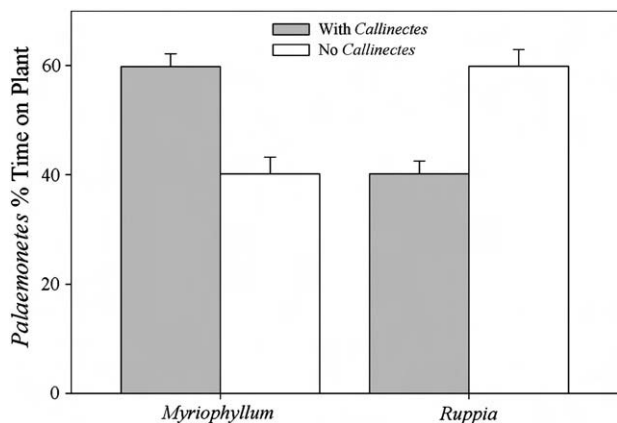
Fig. 2. Plant selection by *Palaemonetes* in the presence and absence of *Callinectes*. Error bars represent standard error (n = 6).

Fig. 3. Percent predation mortality of *Palaemonetes* in different plant canopies. Error bars represent standard error (n = 4).

4. Discussion

Despite higher macroinvertebrate productivity and abundances on *Myriophyllum* than on *Ruppia* (Chaplin and Valentine, 2009; A.R. Armitage, unpublished data), not all of the ecological values provided by the exotic *Myriophyllum* were superior to the native *Ruppia*. In particular, interactions across trophic levels, including herbivory and predation, were markedly different between native and exotic vegetation. The native *Ruppia* was a preferred food source for *Palaemonetes*, but the exotic *Myriophyllum* offered *Palaemonetes* better refuge from predation by *Callinectes*.

Our study suggests that *Palaemonetes* preferred to graze on the native SAV species, *Ruppia*. Preferences for native over exotic SAV have been documented for many aquatic invertebrate (Xiong et al., 2008) and vertebrate (Dibble and Kovalenko, 2009; McKnight and Hepp, 1995) grazers. Native SAV species may be more palatable than exotic species; *Myriophyllum* has high hydrolyzable tannin (phenolics) and lignin concentrations that act as herbivore deterrents (Choi et al., 2002; Marko et al., 2008; Smolders et al., 2000). Less is known about defenses against herbivory in *Ruppia*, but it is generally considered to be a highly palatable species (Burlakova et al., 2009). Furthermore, *Myriophyllum* may also have low nutritive value, as suggested by low growth and survivorship of herbivorous arthropods and gastropods on a *Myriophyllum* diet (Choi et al., 2002; Qiu and Kwong, 2009). Apical shoot tissue of *Myriophyllum* tissue has higher nitrogen concentrations than some other SAV genera (e.g., *Chara, Lemna, Potamogeton*; Chambers et al., 1991; McKnight and Hepp, 1998; Choi et al., 2002), suggesting that it is nutritious but not readily consumed by herbivores because of its substantial chemical defenses. Some aquatic plants exhibit compensatory growth in response to herbivory (McNaughton, 1983), providing an alternative explanation for why we did not detect any shrimp impact on *Myriophyllum* biomass. However, *Myriophyllum* is frequently avoided by aquatic herbivores (Dibble and Kovalenko, 2009; McKnight and Hepp, 1995; Xiong et al., 2008). Therefore, the lack of a shrimp effect on

Myriophyllum biomass in our study most likely reflects limited shrimp grazing on that species.

In areas like our study site where exotic *Myriophyllum* has displaced native *Ruppia*, *Palaemonetes* may be forced to graze in the exotic plant canopy. Longer-term studies are necessary to discern the fitness implications of this shift in diet, since low-quality foods can decrease herbivore fecundity, growth, and survivorship (Cruz-Rivera and Hay, 2000). Although the specific effects of *M. spicatum* on *Palaemonetes* fitness have not yet been determined, *Myriophyllum aquaticum* consumption can decrease gastropod fitness (Qiu and Kwong, 2009), and it is likely that *M. spicatum* will have a similar impact on *Palaemonetes* in our study area. However, in some cases, herbivores can compensate for a low-quality diet by consuming more plant tissue or more nutritious parts of the plant (McKnight and Hepp, 1998), though this compensatory herbivory is more frequently documented in larger, more selective herbivores such as waterfowl. Mesograzers such as small crustaceans are typically less able to survive on low-quality diets because for them, food quantity does not make up for nutritional quality (Cruz-Rivera and Hay, 2000). The preponderance of *Myriophyllum* at our study site (A.R. Armitage, unpublished data) suggests that *Palaemonetes* is in fact able to survive within *Myriophyllum* canopies. Sizable *Palaemonetes* populations may be able to persist on *Myriophyllum* canopies because, although the diet of *Palaemonetes* varies during its life cycle (Welsh, 1975), adults like those we studied typically graze on epiphytic assemblages associated with SAV, not the SAV tissue itself (Drury McCall and Rakocinski, 2007; Morgan, 1980).

The presence of *Callinectes* significantly increased the amount of time *Palaemonetes* spent on *Myriophyllum*. This is consistent with studies demonstrating that macroinvertebrates are more abundant on *Myriophyllum* (Chaplin and Valentine, 2009) and other SAV canopies with complex architecture (Cheruvelil et al., 2002; Krecker, 1939; Taniguchi et al., 2003). Similar patterns are described for littoral fish assemblages associated with vegetated aquatic systems (Troutman et al., 2007). The most likely factor influencing the selection of complex canopies is that they provide herbivores with superior protection from predators (Duffy and Baltz, 1998; Orth et al., 1984; Schofield, 2003). *Myriophyllum* has more finely dissected leaves than the native SAV genera *Heteranthera* and *Vallisneria*, creating a canopy that is nearly twice as complex (Chaplin and Valentine, 2009), and may therefore provide prey like *Palaemonetes* with a refuge from predation. The protection that the *Myriophyllum* canopy provides may be less effective at higher trophic levels, since predator presence did not influence rainwater killifish selection of *Myriophyllum* canopies in tidal freshwater systems in the Gulf of Mexico (Martin and Valentine, 2011). Therefore, the positive influence of *Myriophyllum* may be limited to lower trophic levels, though decreasing predation success could potentially decrease the transport of energy to higher consumers (Martin and Valentine, 2011).

Table 2
Summary of blocked one-way ANOVA results for the refuge experiment. The three temporal trials were random blocks, *Callinectes* presence or absence was the fixed factor, and the dependent variable was percent of time *Palaemonetes* spent on *Ruppia*.

	df	MS	F	p
Block	2	43.2	1.7	0.250
Block error	7	25.4		
Callinectes	1	1273.0	50.0	<0.001
Callinectes error	7	25.4		

Although our study focused on a few discrete interactions between and among native fauna and native and exotic SAV, we have provided direct evidence that exotic *Myriophyllum* alters trophic interactions in brackish wetland habitats. Some of the alterations were positive while others were negative, and the nature of the alteration varied with the number of trophic levels included in the study. Therefore, it is premature to determine whether the invasion of *Myriophyllum* into brackish wetlands will have a net positive or negative ecosystem effect. These trophic alterations should be examined further because the exclusion of a native species by an exotic species, particularly one with the potential to act as an ecosystem engineer (Crooks, 2002; Jones et al., 1994), is likely to alter food web dynamics and ecosystem processes such as energy and nutrient cycling (Grosholz, 2002; Madsen et al., 1991; Smith and Barko, 1990).

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Glossary

Brackish marsh: transitional zones between freshwater and salt marsh communities. These habitats are dominated by herbaceous (non-woody) vegetation and typically have salinities less than 5 ppt.

Herbivory: the consumption of primary producers.

Invasive species: floral or faunal species, often non-indigenous, that proliferate and adversely affect ecosystems and bioregions.

Submerged aquatic vegetation: Rooted plant species that grow vertically in the water column but do not extend above the water surface; generally excludes algae and floating plants.

Trophic: Pertains to nutrition; describes the position that an organism occupies in a food web.

Trophic interactions: describes direct and indirect nutritional relationships among species in an ecosystem.

Appendix C
Marsh construction techniques influence net plant carbon capture by emergent and submerged vegetation in a brackish marsh in the northwestern Gulf of Mexico

Marsh construction techniques influence net plant carbon capture by emergent and submerged vegetation in a brackish marsh in the northwestern Gulf of Mexico

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ABSTRACT

Coastal marshes play an important role in global carbon cycles, yet coastal development has led to widespread losses of marsh habitat. To address this problem, many coastal wetlands have been restored or created over the past several decades using a variety of construction techniques, but it is unclear if net plant carbon capture in constructed marshes is equal to that of reference marshes, or if rates of plant carbon capture are influenced by marsh construction techniques. To comparatively assess relative carbon capture by emergent and submerged vegetation in constructed and reference marshes, we measured standing biomass and carbon content in above- and belowground emergent plant tissue and submerged vegetation in three constructed areas (2–3 years old) and one reference area in a brackish marsh in the northwestern Gulf of Mexico in 2009 and 2010. We also used aerial photographs to construct a GIS database of emergent and submerged vegetation coverage. These data were combined to estimate net annual plant carbon capture per square meter of marsh vegetation in each constructed and reference area. This index of carbon input to wetland vegetation suggests that rates of carbon capture by emergent aboveground vegetation and submerged aquatic vegetation were similar in constructed and reference areas. However, submerged vegetation captured less carbon (0.1–$0.3\,\mathrm{kg\,m^{-2}}$) than emergent vegetation (0.2–$1.7\,\mathrm{kg\,m^{-2}}$), and constructed areas contained an order of magnitude less emergent habitat than the reference area. Consequently, the annual carbon production of entire constructed areas (emergent + submerged vegetation; 0.1–$1.2\,\mathrm{kg\,m^{-2}}$) was always less than half that of the reference area (0.8–$2.5\,\mathrm{kg\,m^{-2}}$). Therefore, although productivity of emergent and submerged vegetation in constructed and reference areas was similar, the smaller ratio of land to water in the constructed areas reduced their annual rate of plant carbon capture at a larger spatial scale. To more closely mimic rates of plant carbon capture in reference marsh habitats, constructed marsh designs should aim to replicate the ratio of land to water in adjacent reference marshes.

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1. Introduction

Coastal marshes are one of the most productive ecosystems on the planet (Brevik and Homburg, 2004; Dawes, 1998) and sequester large quantities of organic carbon (∼41 Tg/year; Chmura et al., 2003). Unlike freshwater inland wetlands (e.g., bogs and peatlands), saline coastal marshes generally have relatively low emissions of the potent greenhouse gas methane (Bartlett et al., 1987; Bartlett and Harris, 1993; Brevik and Homburg, 2004; Chmura et al., 2003; Connor et al., 2001; Ding et al., 2003; Pearce and Clymo, 2001; Thom et al., 2002; Poffenbarger et al., 2011). Coastal marshes thus play a vital role in the global carbon cycle (Chmura et al., 2003; Mitra et al., 2005; Sahagian and Melack, 1988), yet coastal development is causing an alarming rate of worldwide wetland loss. In the United States alone, an average of 160 acres of wetlands was lost every day between 1986 and 1997 (US Department of Agriculture, 2000a,b).

Wetland habitat loss is often mitigated through the restoration of degraded wetlands or the construction of new wetlands. In coastal areas of the Gulf of Mexico, constructed salt marshes are usually built from existing benthic sediments or dredge material that is shaped into mounds or terraces and surrounded by shallow water habitat (Costa-Pierce and Weinstein, 2002; Edwards and Proffitt, 2003; Streever, 2000; Turner and Streever, 2002). Coastal marsh restoration and construction projects are often considered

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successful if their emergent vegetation can meet benchmark levels of vegetation coverage and biomass production within a given period of time (Ainslie, 1994; Kentula, 2002). When threshold levels of plant coverage are achieved, it is often assumed that the ecological function and productivity of the constructed marsh is equal to that of reference marsh areas (Adamus et al., 1991; Ainslie, 1994; Edwards and Mills, 2005; Edwards and Proffitt, 2003; Short et al., 2000; Streever, 2000). A large body of literature has explored the merits of these assumptions; the majority of studies have focused on ecosystem functions like food web support and nutrient cycling (Craft et al., 2003; Streever, 2000; Thayer and Kentula, 2005; Zedler, 2000a,b; Zedler and Callaway, 1999). More recently, the standing carbon stock and carbon sequestration potential of restored and constructed coastal marsh habitats has received more attention (e.g., Irving et al., 2011; Miller and Fujii, 2010) because constructed coastal marshes that do not replicate the important carbon storage properties of reference marshes will ultimately contribute to the loss of an important global sink of organic carbon.

Given their capacity to sequester carbon, many researchers have explored the possibility of using coastal marshes as a natural means of carbon capture and storage (Connor et al., 2001; Dixon and Krankina, 1995; Irving et al., 2011; Miller et al., 2008; Miller and Fujii, 2010; Pelley, 2008; Santin et al., 2009; Shafer and Streever, 2000; Thom et al., 2002). Analyses of plant carbon capture in constructed marshes to date indicate that they generally contain less organic carbon than reference marsh habitats (Santin et al., 2009; Shafer and Streever, 2000) and that their construction would need to achieve an industrial scale to significantly impact global levels of atmospheric carbon (Irving et al., 2011). While newly constructed coastal marsh habitats may never attain the sequestered carbon stocks of reference marshes that are thousands of years old, it should be possible to construct marshes with annual rates of net plant carbon capture that are equivalent to reference marshes. To meet this goal, it is necessary to gain a better understanding of how marsh construction design influences plant carbon capture in constructed marshes.

A basic assumption about carbon storage in constructed wetlands is that carbon will become sequestered in the soil when carbon inputs exceed carbon loss. Levels of standing carbon and rates of net annual plant carbon capture in constructed marshes should thus be equal to or greater than reference areas in order to have equivalent rates of carbon sequestration. While many investigations have examined primary productivity of emergent vegetation in constructed, restored, and reference marshes, these comparisons typically occur among marshes at different sites or of different ages (e.g., Craft et al., 2003; Dai and Wiegert, 1996; Delaney et al., 2000; Edwards and Proffitt, 2003; Miller and Fujii, 2010; Short et al., 2000; Streever, 2000; Thayer and Kentula, 2005; Zedler, 2000a; Zedler and Callaway, 1999). Furthermore, to our knowledge, there have been no previous attempts to combine measures of emergent and submerged vegetation cover in constructed habitats in order to assess annual plant carbon capture at a landscape scale that integrates both emergent and subtidal habitats.

To explore how constructed marsh design may influence landscape-level rates of plant carbon capture by emergent and submerged vegetation, we measured biomass, carbon content, and vegetation coverage in constructed and reference areas of a brackish marsh in the northwestern Gulf of Mexico. Rather than quantifying absolute carbon capture by measuring carbon movement into recalcitrant, "captured" form, our approach provided a relative index of carbon input that could be compared among constructed wetlands that varied in structural design. All constructed areas in our study were engineered to contain circular mounds, but they varied in soil source and surrounding water depth. Our goals

Table 1
Soil characteristics of constructed (excavated, filled, pumped) and reference marshes in June 2009 and September 2010.

	Excavated	Filled	Pumped	Reference
% Organic carbon				
2009	4.2 ± 0.8	3.4 ± 0.4	1.2 ± 0.1	6.1 ± 0.8
2010	4.1 ± 0.7	4.3 ± 1.0	2.3 ± 0.4	5.6 ± 0.8
% Inorganic carbon				
2009	<0.1	<0.1	<0.1	0.2 ± 0.1
2010	0.1 ± 0.1	0.2 ± 0.1	0.1 ± 0.1	0.1 ± 0.1
% Nitrogen				
2009	0.28 ± 0.05	0.22 ± 0.02	0.11 ± 0.01	0.42 ± 0.05
2010	0.24 ± 0.05	0.25 ± 0.06	0.15 ± 0.03	0.36 ± 0.05
% Phosphorus				
2009	0.016 ± 0.002	0.021 ± 0.002	0.024 ± 0.002	0.034 ± 0.004
2010	0.018 ± 0.004	0.019 ± 0.003	0.023 ± 0.002	0.040 ± 0.002
% Sand				
2009	47.1 ± 6.5	45.9 ± 2.9	31.3 ± 2.9	58.6 ± 5.2
2010	23.4 ± 6.0	39.1 ± 4.7	24.9 ± 2.6	48.4 ± 5.2

were to identify which construction techniques were associated with the highest rates of primary production and plant carbon capture, and to compare how all constructed areas performed relative to an adjoining reference area.

2. Materials and methods

2.1. Study site and marsh construction

Constructed marshes were built in fall (September–November) 2007 in the Old River Unit of the Lower Neches Wildlife Management Area (N30°00.228′, W93°51.539′) near Port Arthur, TX, USA (Fig. 1). Three different construction techniques, termed excavated mounds (Fig. 2A), filled mounds (Fig. 2B), and pumped mounds (Fig. 2C), were used to create emergent marsh habitat within terraced containment areas (Fig. 1). Excavated mounds were formed from material excavated from adjacent bottom sediment that was shaped into circular mounds with an emergent area of ~27 m²/mound that were spaced approximately 12 m apart (Figs. 2A and 3A). Filled mounds were created by filling submerged areas surrounding excavated mounds with dredge material pumped directly from a nearby industrial canal (Figs. 2B and 3B). Filled mounds had an area of ~13 m²/mound and were spaced ~22 m apart. Pumped mounds were created by mixing soil from an upland dredge disposal site with water and then pumping the slurry into the restoration area (Figs. 2C and 3C). The slurry was pumped directly onto existing benthic sediments to create circular mounds with an average emergent area of ~23 m²/mound that were spaced ~14 m apart. The upland site used to create the slurry was 1.3 km to the southwest of the restoration area (Fig. 1C), and had been built from dredge spoil during periodic canal maintenance events between ca. 1980–2007. The final depth of the water surrounding filled and pumped mounds (~0.2 m) was less than around excavated mounds (~1.0 m; Fig. 2A–C). Tidal exchange occurred through multiple culverts into the area, such that all constructed and reference marsh areas experienced similar inundation frequencies and had water salinities that ranged from 1 to 6 ppt for most of the study period. Soil characteristics were similar among all constructed mound types; soils in the reference area were sandier and had higher organic and inorganic carbon, nitrogen, and phosphorus content (Table 1).

Following marsh construction, mounds were planted with *Spartina alterniflora* cv. Vermilion, a naturally occurring ecotype isolated by the Natural Resources Conservation Service that has been used extensively in marsh restoration in the northern Gulf
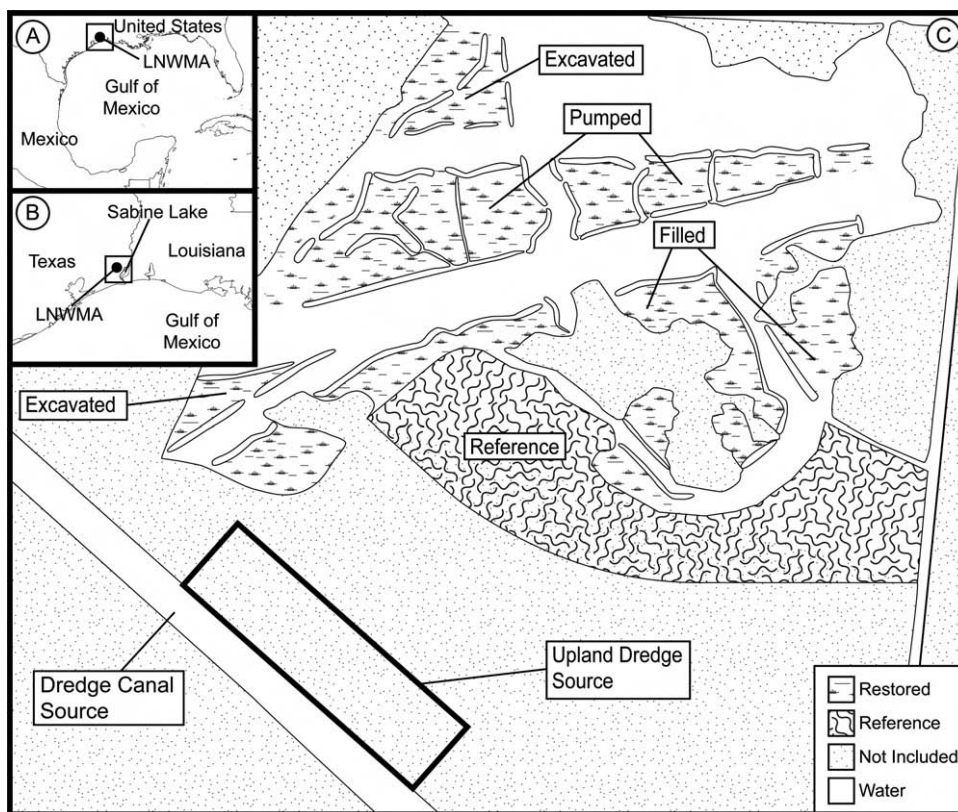
Fig. 1. The Lower Neches River Wildlife Management Area (LNWMA) is in the northwestern Gulf of Mexico (A) on the shore of Sabine Lake (B). The box in panel A surrounds the area depicted in panel B, and the area pictured in panel C is from a portion of the area surrounded by the box in panel B. Constructed and reference marsh areas in the LNWMA are indicated with shading; treatments and dredge sources are labeled. Containment terraces were built around each constructed area to protect the mounds from erosion.

of Mexico (USDA, 2000). Emergent habitat in the reference area primarily contained *S. alterniflora* and *Spartina patens*, along with small quantities of *Schoenoplectus robustus* and *Schoenoplectus californicus* (Fig. A.1). Submerged plant species in the reference and restored area included *Myriophyllum spicatum*, *Ruppia maritima*, and mats of green algae (primarily *Cladophora* sp. and *Spirogyra* sp.) (Fig. A.2).

Including submerged and emergent areas, a total of 0.45 km² of marsh habitat was built. The compartment containing excavated mounds (emergent + submerged areas) was 0.05 km², the compartment containing pumped mounds was 0.08 km², and the compartment containing filled mounds was 0.07 km² (Fig. 1C). The remaining constructed marsh areas that were not included in this study were built with a combination of these construction techniques. We established ten sampling stations at random locations in each of the excavated, filled, and pumped mound areas and in comparable reference habitat (Fig. 1C).

2.2. GIS calculations

We calculated the size of emergent and submerged areas in constructed and reference marshes with DeLorme XMap 7 GIS software (Yarmouth, ME). Base maps of the restoration area were constructed in a Geographic Information System (GIS) database from georeferenced aerial photographs of the study site taken on December 6, 2007, October 8, 2008, August 31, 2009, and August 5, 2010 (Fig. 3). The edges of mounds and shorelines were manually traced in each photograph to create vector format layers of emergent vegetation in constructed and reference areas. The outermost boundaries of each restoration area (where they made contact with surrounding terraces) and the comparable intertidal portion of the

reference area were also traced to create a vector layer of total emergent + submerged vegetation in each constructed (excavated, filled, or pumped) and reference area. The difference between the emergent (land) and the total area (land + water) of each constructed and reference area was the submerged area (water).

2.3. Plant sampling

All aboveground emergent plant tissue was harvested quarterly (April, June, September, January) from $10\,cm \times 20\,cm$ ($0.02\,m^2$) quadrats at each monitoring station from April 2009 through January 2011. Live and dead tissue was pooled and transported to the laboratory where it was rinsed, separated by species, dried at $70\,°C$ to a constant weight, and weighed to determine biomass ($kg\,m^{-2}$). The standing biomass of emergent aboveground vegetation (SB_{EAG}) at each monitoring station was the sum of live and dead plant biomass for all plant species.

Subsamples of dried plant tissue from each species were ground with a Thomas Scientific (Swedesboro, NJ) model 3383-L10 Wiley mini-mill and analyzed for carbon (C) content with a Perkin Elmer (Waltham, Massachusetts, USA) series II CHNS/O analyzer. *S. alterniflora* leaf %C content was measured at every sampling event, but the average leaf %C content changed <0.5% among all constructed and reference areas from April 2009 to January 2011. Since this temporal and spatial deviation was so small, the average %C content of all measurements (42.11 ± 0.20; $N = 347$) was used in further calculations of standing carbon for *S. alterniflora*.

Measurements of %C were not obtained at every sampling event for the other plant species included in this investigation. However, we assumed that their %C content also changed little over time. %C content was thus determined from the average of ten replicate
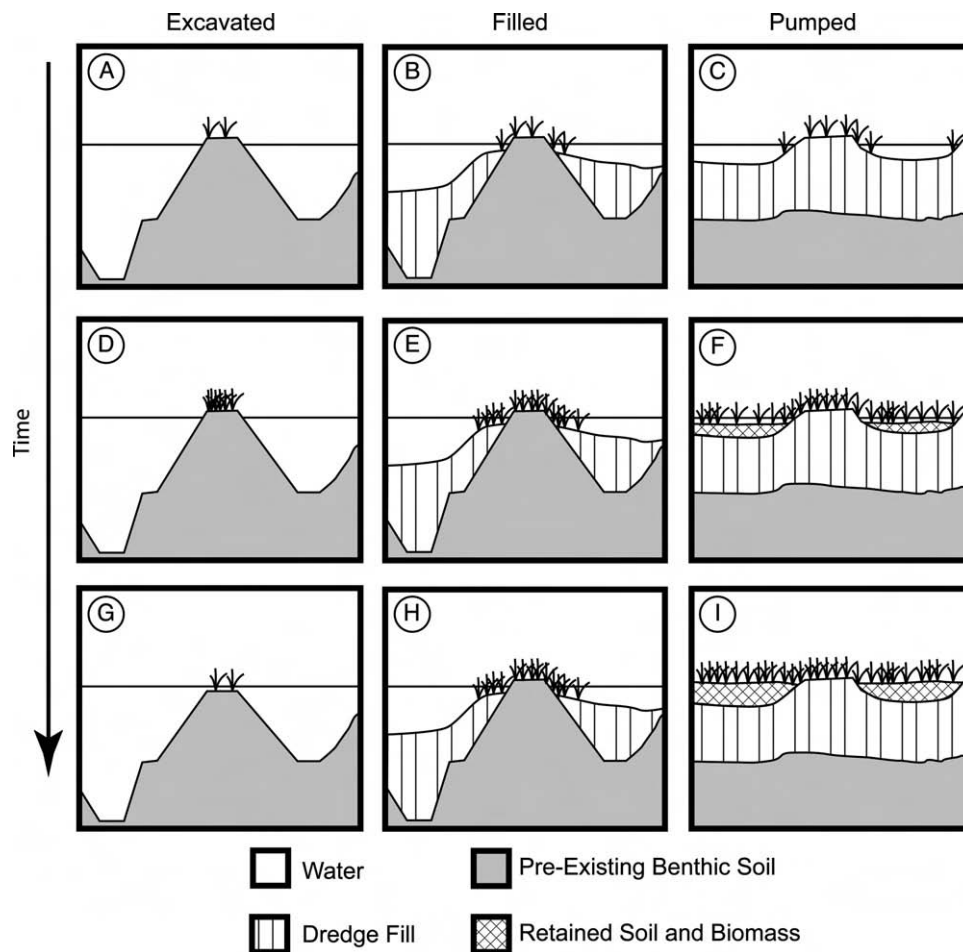
Fig. 2. Constructed mound designs. Soil source is indicated in legend and plantings of *S. alterniflora* are depicted on the mounds. The top row (A–C) depicts mounds following construction in 2007. The second and third rows depict changes over time based on data presented for the land to water ratio in Fig. 5 and net annual plant carbon capture in Fig. 6.

measurements of %C performed on dried and ground biomass samples collected in September of 2010 for *S. patens* (47.13 ± 3.09), *S. californicus* (46.43 ± 2.63), *S. robustus* (47.63 ± 5.92), *M. spicatum* (34.29 ± 0.48), *R. maritima* (33.73 ± 2.16), and algal mats (27.86 ± 0.99). The sum of the products of the standing biomass of each plant species multiplied by its corresponding %C content was the emergent standing carbon (SC_{EAG}) at each station.

Emergent belowground plant tissue was collected at each station at the peak of aboveground production in June 2009 and September 2010. Belowground biomass was extracted from soil cores (10 cm diameter, 20 cm deep) that were placed in the center of the quadrat used to collect aboveground biomass. Cores were rinsed in the laboratory over a 2 mm mesh sieve to isolate plant material from surrounding soil, dried at 70 °C to a constant weight, and weighed to determine standing biomass (SB_{EBG}). Carbon content was determined as described above, and total SB_{EBG} was multiplied by its %C content to determine emergent belowground standing carbon (SC_{EBG}) at each station.

To estimate standing biomass of submerged aquatic vegetation (SB_{SAV}), we combined the Rapid Survey Method of Deppe and Lathrop (1993) and Trebitz et al. (1993) with the Rake Method of Hansel-Welch et al. (2003) and Spears et al. (2009). Five stations in each constructed and reference area were approached in a non-motorized boat so as to not disturb the submerged vegetation. The head of a 16-tine, 0.04 m wide metal rake was dropped in the water

two meters away from the side of the boat. The rake was dragged across the bottom and towards the boat in a downward sweeping motion that collected all vegetation within a 0.08 m^2 area. Vegetation trapped in the rake was placed in a plastic bag, transported on ice to the laboratory, and stored at -20 °C until it could be processed. Once thawed, plants were separated by species, rinsed to remove adhered sediment, dried for at least four days at 70 °C to a constant weight, and weighed to determine biomass. Since submerged aquatic vegetation (SAV) biomass was relatively low, we pooled *M. spicatum*, *R. maritima*, and green algae biomass at each monitoring station to calculate standing biomass (SB_{SAV}). Carbon content and standing carbon of submerged aquatic vegetation (SC_{SAV}) was determined as described above.

2.4. Production estimates

We estimated net annual primary production (NAPP) for each type of vegetation (emergent aboveground, emergent belowground, submerged aquatic vegetation) in each of the three constructed areas and the reference area. Our estimate of emergent aboveground net annual primary production ($NAPP_{EAG}$) was modified from Milner and Hughes (1968) and Singh et al. (1975), where $NAPP_{EAG}$ was the difference between the maximum and minimum SB_{EAG} and negative values were replaced with zeros. In 2009, peak SB_{EAG} was recorded in June and end-of-season (minimum)
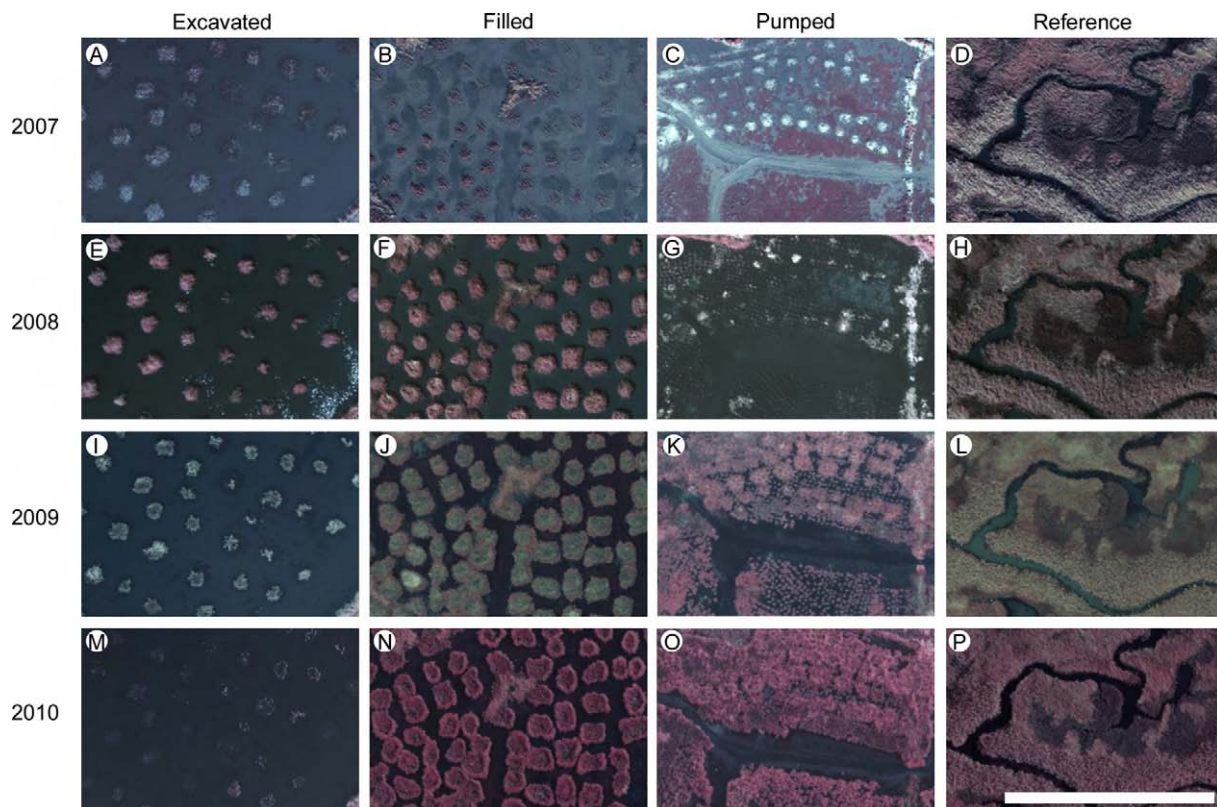
Fig. 3. A representative portion of each constructed and reference area viewed under high magnification in aerial photographs. Representative photos from excavated (A, E, I, and M), filled (B, F, J, and N), pumped (C, G, K, and O), and reference (D, H, L, and P) areas are shown over four consecutive years. Scale bar in P equals 75 m.

SB_{EAG} was in January of 2010. In 2010, peak SB_{EAG} was recorded in September and end-of-season SB_{EAG} was in January 2011. This technique is a conservative estimate of $NAPP_{EAG}$ because it does not account for biomass turnover/loss that may have occurred before peak biomass production.

The NAPP of belowground biomass ($NAPP_{EBG}$) was the difference between peak and end-of-season SB_{EBG}. Peak SB_{EBG} was directly measured in June 2009 and September 2010. End-of-season SB_{EBG} was estimated by multiplying end-of-season SB_{EAG} by the ratio of SB_{EAG} to SB_{EBG} at the peak of the growing season.

The NAPP of submerged aquatic vegetation ($NAPP_{SAV}$) was the difference between peak and end-of-season SB_{SAV}. These collections do not account for biomass turnover and are therefore a conservative estimate of $NAPP_{SAV}$.

We estimated net annual plant carbon capture (NACC) of the emergent aboveground plant community ($NACC_{EAG}$), emergent belowground plant community ($NACC_{EBG}$), and submerged aquatic vegetation ($NACC_{SAV}$) by calculating the difference between peak and end-of-season standing carbon (SC).

To integrate data from emergent and submerged areas and estimate net annual primary production at a larger landscape level ($NAPP_{AREA}$), we summed the products of the mean $NAPP_{EAG}$, $NAPP_{EBG}$, and $NAPP_{SAV}$ and their total respective areas (we assumed that emergent vegetation was only found on land and SAV was only found in the water), and divided this number by the size of each constructed or reference area in km^2. The net annual plant carbon capture of an entire constructed or reference area ($NACC_{AREA}$) was determined with a similar calculation except that mean NACC was used in place of mean NAPP. These values ($NAPP_{AREA}$ and $NACC_{AREA}$) describe the annual plant production and plant carbon capture per square meter of constructed or reference area.

2.5. Statistical analyses

Productivity and plant carbon capture were analyzed separately in 2009 and 2010 with one-way ANOVA, in which peak SB, end-of-season SB, NAPP and NACC for emergent aboveground, emergent belowground, and submerged aquatic vegetation were dependent variables and habitat type (excavated, filled, pumped, or reference) was the fixed factor. Homogeneity of variances for all response variables were confirmed with a Welch test. Variance was homogenous for each test and did not require transformation. Each ANOVA was followed with a post hoc Tukey test.

3. Results

3.1. Species composition of emergent and submerged areas

The emergent vegetation of each constructed area was dominated by *S. alterniflora* (Fig. A.1(A)–(C)). *S. alterniflora* biomass varied seasonally, and the highest values were in June 2009 and September 2010. In the reference area, there was more *S. patens* ($\leq 2.75 \, kg \, m^{-2}$) than in the constructed areas ($\leq 0.02 \, kg \, m^{-2}$), but *S. alterniflora* was still the dominant species (Fig. A.1D). *S. robustus* was absent from excavated and filled mounds and *S. californicus* did not occur in any of the constructed areas, but small amounts ($\leq 1.0 \, kg \, m^{-2}$) of both species were consistently found in the reference area (Fig. A.1D).

In all constructed and reference areas, *Myriophyllum* was the dominant submerged aquatic plant species (Fig. A.2). In both years, it was most abundant in late summer (August 2009 and September 2010), when it accounted for $\geq 65\%$ of all SAV standing biomass. Algal mats accounted for 41% of SB_{SAV} in filled mounds
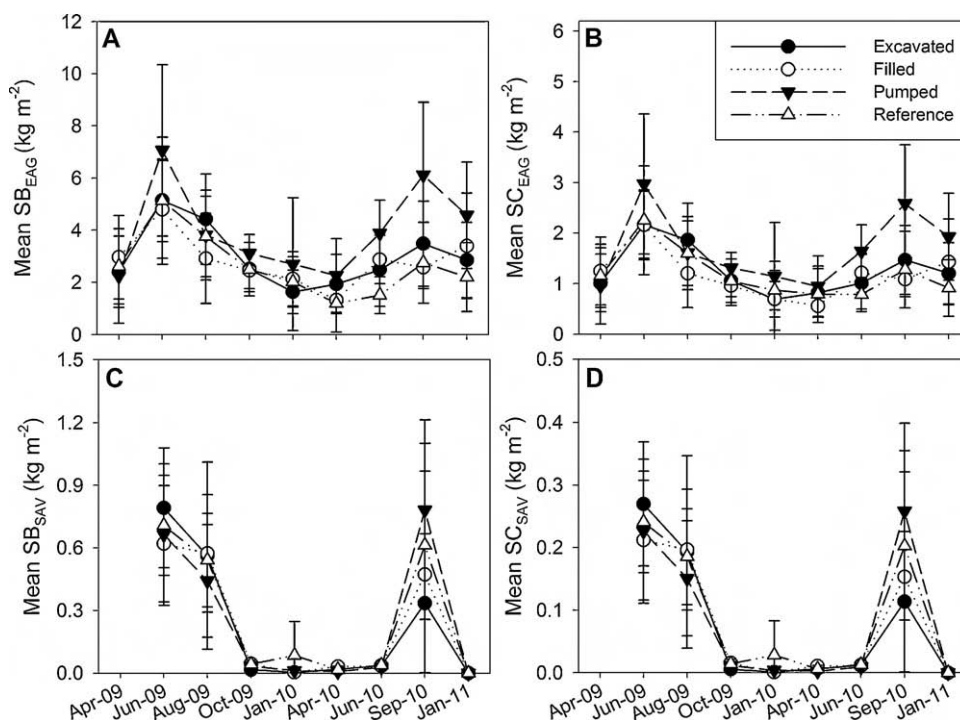
Fig. 4. Average standing biomass (A and C) and standing carbon (B and D) in emergent (A and B) and submerged (C and D) areas of constructed (excavated, filled, pumped) and reference marshes over time. Collection of emergent vegetation began in April 2009, and submerged aquatic vegetation collection began in June 2009. Error bars represent standard deviation.

in September 2010, but comprised a very small (<15%) portion of SB_{SAV} at all other sampling dates and locations (Fig. A.2).

3.2. Standing biomass and standing carbon

Mean values of peak SB_{EAG} were lower in September 2010 than in June 2009, and end-of-season mean values were slightly greater in January 2011 than in January 2010 for constructed and reference areas (Fig. 4A). Neither peak nor end-of-season mean SB_{EAG} varied significantly among habitat types in 2009 (One-way ANOVA; Peak – $P = 0.171$; end-of-season – $P = 0.507$; Table B.1). In 2010, peak SB_{EAG} was higher in pumped mounds relative to all other habitat types (One-way ANOVA, $P < 0.001$); end-of season SB_{EAG} was higher in pumped than in reference areas (One-way ANOVA, $P = 0.013$).

The SB_{SAV} reached peak levels in the same months as SB_{EAG} (Fig. 4C). Peak and end-of-season SB_{SAV} were not significantly different among constructed and reference areas in 2009 (One-way ANOVA; Peak – $P = 0.808$; end-of-season – $P = 0.351$; Table B.1) or 2010 (One-way ANOVA; Peak – $P = 0.578$; end-of-season – $P = 0.065$).

Because constructed and reference areas were dominated by the same plant species (Figs. A.1 and A.2) and differences in %C content among all plant species included in this investigation were small (see Section 2.4), the trends in SC_{EAG} and SC_{SAV} over time and among constructed and reference marsh areas (Fig. 4B and D) mirrored those observed for SB_{EAG} and SB_{SAV} (Fig. 4A and 4C).

3.3. GIS measurements

Differences in the dynamics of land expansion were clearly visible in aerial photographs of constructed and reference marsh areas from 2007 to 2010 (Fig. 3). When these images were incorporated into a GIS database, differences in the ratio of land to water in each area were quantified and the results are summarized in Table 2 and Fig. 5. The ratio of land to water in the reference area changed little

from 2007 (4.96) to 2010 (5.20) and was nearly always an order of magnitude greater than the land to water ratio of any of the constructed areas (Figs. 3 and 5; Table 2). As the ratio suggests, the reference area had the greatest amount of land coverage relative to the other sites. Excavated mounds, on the other hand, had the least amount of land and the most water surrounding each mound (Figs. 3 and 5; Table 2). Excavated mounds initially gained land mass, but by fall 2010, the ratio of land to water (0.08) was less than after the initial construction of the site in 2007 (0.09). Pumped and filled mounds had intermediate levels of land and water and experienced the greatest amount of change over the study period based on the ratio of land to water in each area (Figs. 3 and 5; Table 2). Filled mounds increased in the ratio of land to water from 0.30
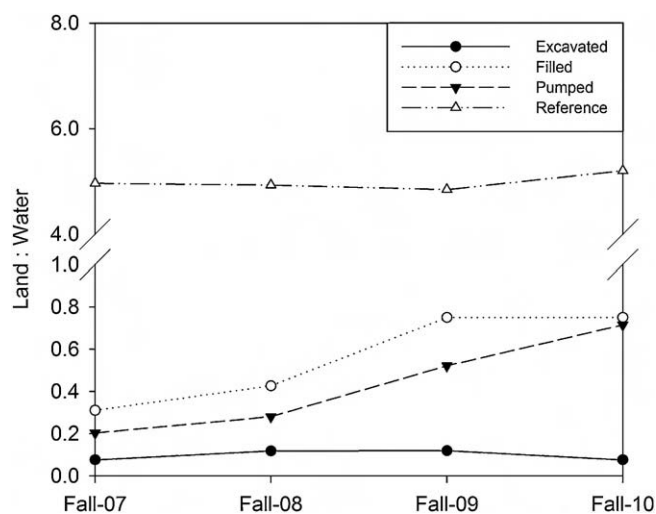

Fig. 5. Ratio of land to water within constructed (excavated, filled, pumped) and reference areas over four years after wetland construction in 2007.

Table 2
Land coverage, primary productivity, and carbon capture in constructed (excavated, filled, pumped) and reference marshes in 2009 and 2010. Each value was derived from measurements of productivity in Fig. 6 and Table A.1, and from a GIS database constructed from aerial photographs depicted in Fig. 3 and quantified in Fig. 5.

	2009				2010			
	Excavated	Filled	Pumped	Reference	Excavated	Filled	Pumped	Reference
Total area (km^2)	0.05	0.07	0.08	0.42	0.05	0.07	0.08	0.42
Total emergent area (km^2)	0.01	0.05	0.04	0.40	<0.01	0.05	0.06	0.36
Total water area (km^2)	0.04	0.02	0.04	0.01	0.05	0.02	0.02	0.06
Land: water	0.12	0.75	0.52	4.84	0.08	0.75	0.71	5.20
NAPP$_{AREA}$ (kg m^{-2})	1.61	3.57	2.96	6.75	0.33	1.96	2.17	2.96
NACC$_{AREA}$ (kg m^{-2})	0.56	1.47	1.19	2.71	0.13	0.30	0.34	0.84

in 2007 until fall of 2009 when land expansion stabilized near a ratio of 0.75. In pumped mounds, the ratio of land to water steadily increased from 0.21 in 2007 to 0.71 in 2010.

3.4. NAPP and NACC

NAPP$_{EAG}$, NACC$_{EAG}$, NAPP$_{SAV}$, and NACC$_{SAV}$ did not significantly differ among constructed and reference areas in 2009 and 2010 (Fig. 6A and C; Tables B.2 and B.3). NAPP$_{EBG}$ and NACC$_{EBG}$, however, were significantly higher in the reference area than in pumped mounds in 2009 and than in the excavated mounds in 2010 (Fig. 6B; Tables B.2 and B.3).

When the NAPP and NACC of all three kinds of vegetation (EAG, EBG, SAV) were combined with their surface area to estimate primary production and plant carbon capture of the entire constructed or reference area, large differences in NAPP$_{AREA}$ (Table 2) and NACC$_{AREA}$ (Fig. 6D; Table 2) became apparent. Both NAPP$_{AREA}$ and NACC$_{AREA}$ of the reference area were at least twice the value of any constructed area in both 2009 and 2010.

4. Discussion

4.1. Integrating landscape-level carbon storage into constructed marsh design

Although NAPP is influenced by local environmental conditions including latitude, climate, and soil chemistry (Mendelssohn and Morris, 2002), similar estimates of mean productivity have been reported in many other marshes on the Gulf of Mexico and East Coasts of the US. Mendelssohn and Morris (2002) have reviewed the productivity literature for marshes dominated by *S. alterniflora* and report a mean NAPP$_{EAG}$ of 0.8 kg m^{-2} for the northern Gulf of Mexico, which is well within the range of our estimates (0.2–1.7 kg m^{-2}). Fewer harvest-based reports of NACC$_{EBG}$ in coastal marshes are available, but our estimates of NAPP$_{EBG}$ (0.2–3.3 kg m^{-2}) are similar to those obtained in marshes on the East Coast of the US (1.7–7.6 kg m^{-2}; Dame and Kenny, 1986; Ellison et al., 1986; Schubauer and Hopkinson, 1984; Valiela et al., 1976), suggesting that our estimates of NACC$_{EBG}$ are also comparable. Submerged aquatic vegetation sometimes has a greater turnover rate than emergent vegetation (Engle et al., 2008; Miller and Fujii, 2010; Milsom et al., 2004), so our peak-season measurements may have underestimated SAV carbon capture. However, our estimates of NACC$_{SAV}$ (0.11–0.27 kg m^{-2}) are similar to previously published estimates for *Myriophyllum* spp. (0.12–0.26 kg m^{-2}; Adams and McCracken, 1974; Forsberg, 1959).

Overall, emergent marsh vegetation is more productive and less labile than submerged vegetation (Miller and Fujii, 2010) in many marshes on the Gulf of Mexico and East Coasts of the US. Under these conditions, the ratio of land to water in constructed marshes plays an important role in their capacity for plant carbon capture, which will be maximized in marshes with more emergent than submerged vegetation. However, under different conditions, submerged vegetation could contribute the same or more carbon to NACC$_{AREA}$ as emergent vegetation. Therefore, we do not call for a default increase in the ratio of land to water in constructed marshes, but stress the importance of duplicating the land to water ratio of references marshes in constructed marsh design.

Restoring rates of plant carbon capture is only one facet of the complex challenges associated with ecological restoration of coastal marshes (e.g., Biebighauser, 2007; Zedler, 2000b). Marshes that are built solely to duplicate the ratio of land to water in adjoining reference habitat may be at odds with other ecological goals. For example, marsh edge habitat increases nekton output in constructed marshes and consequently augments their fishery value (Minello and Rozas, 2002). However, the incorporation of edge habitat into constructed marshes typically results in more submerged marsh area that would lower the ratio of land to water. In brackish areas similar to the one we investigated, this would decrease the NACC$_{AREA}$. The need to restore plant carbon capture potential must be balanced with other ecological requirements, and continued integrative research like this study will help to identify construction designs that maximize both plant carbon capture and other ecological functions such as fishery value.

The capacity of emergent plants in constructed marshes to capture carbon is likely to change as the site develops over time. Our study occurred over a relatively short time period (three years) that immediately followed marsh construction, and may have therefore underestimated plant carbon capture potential in the constructed areas. Vegetation is often the fastest to develop in restored or constructed areas, often achieving cover and biomass comparable to reference areas in less than five years, particularly in temperate habitats like the Gulf of Mexico that do not have prolonged senescence periods (Craft et al., 2003; Edwards and Proffitt, 2003). However, recovery trajectories in coastal marshes are not always linear or predictable (Zedler and Callaway, 1999), and this is reflected in the different development patterns demonstrated in our constructed areas. The emergent habitat in pumped and filled mounds expanded substantially over the study period, but excavated mounds developed much more slowly (Figs. 3 and 5). Although the plant carbon capture potential in all of these habitats is likely to continue changing over time, our study clearly demonstrated the critical link between the ratio of land to water and the plant carbon capture potential in constructed marshes.

The absolute potential for plant carbon capture in brackish marshes like our study site may be somewhat offset by the production of the potent greenhouse gas methane. Methane emissions generally vary with salinity, with lowest production rates in saline (>18 ppt) wetlands (Bartlett et al., 1987; Bartlett and Harris, 1993; Ding et al., 2003; Pearce and Clymo, 2001; Poffenbarger et al., 2011). Brackish wetlands tend to have variable but relatively high methane emissions (Poffenbarger et al., 2011). However, our study focused the influence of constructed marsh design on landscape-level plant carbon capture, which is independent of marsh salinity. This study and previous works have demonstrated that emergent vegetation usually has a much higher net plant carbon capture
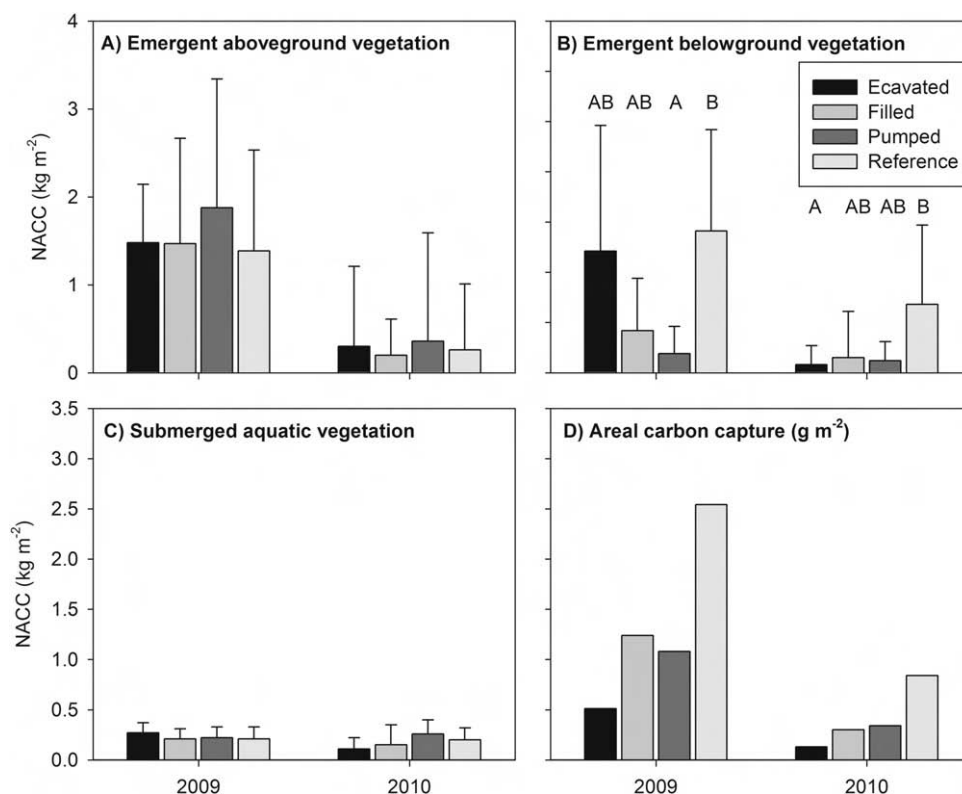
Fig. 6. Net annual plant carbon capture (NACC in kg m⁻²) of emergent aboveground vegetation (A), emergent belowground vegetation (B), and submerged aquatic vegetation (C) in constructed (excavated, filled, pumped) and reference marshes. Homogenous subsets from Tukey tests are indicated with letters where significant. Error bars represent standard deviation. Panel (D) depicts the total areal carbon capture in g m⁻² of each habitat type, including both emergent and submerged habitat, based on the values reported in Table 2.

rate than submerged vegetation (Adams and McCracken, 1974; Mendelssohn and Morris, 2002), and this relative difference may hold true across a range of salinities. Salt marsh restoration projects in Galveston Bay and other Gulf of Mexico estuaries often incorporate heterogeneous designs such as mounds or terraces (Rozas et al., 2005) that include a substantial amount of subtidal habitat. However, submerged aquatic vegetation such as seagrass in these habitats has declined substantially in the last 30 years, resulting in very low SAV biomass in these areas (Sheridan et al., 1998). Therefore, the difference in submerged and emergent plant biomass is pronounced in saline wetlands, and the impact of marsh design on plant carbon capture may be even more pronounced in salt marshes than in fresh or brackish marshes.

4.2. NACC was similar among constructed and reference marsh vegetation

Our approach provided a relative comparison of plant carbon capture among constructed wetlands that varied in design. Previously published analyses of constructed marsh performance typically include data from restored or constructed marshes that were engineered to have similar structural designs (Kuhn et al., 1999; Thom et al., 2004; Valiela et al., 1975). Comparisons among different construction designs are usually restricted to marshes at different sites that were built at different times (Craft et al., 2003; Delaney et al., 2000; Edwards and Mills, 2005; Edwards and Proffitt, 2003; Shafer and Streever, 2000). Our study was unique in that the constructed areas we examined were built within three months of each other and were in close proximity. Atmospheric conditions, tidal influence, anthropogenic disturbance, and freshwater inflow were similar in each constructed area and the reference area,

allowing for systematic comparisons among marsh construction designs and soil sources.

We expected NAPP and NACC of emergent and submerged vegetation in each marsh to be different. Instead, NACC was generally the same among constructed and reference marsh areas. Although we did not quantify absolute carbon capture by measuring carbon movement into recalcitrant forms in the sediment, we had expected that plant biomass and carbon content would vary among our wetland areas. The excavated, filled and pumped mounds were built from different soil sources (Figs. 1 and 2), and soil characteristics are usually an important determinant of restored and constructed marsh success, particularly in terms of plant biomass and productivity (Boyer and Zedler, 1998; Mitsch and Cronk, 1992). Aspects of the marsh construction effort that likely contributed to these similarities include: (i) the emergent portions of each area had roughly the same elevation, (ii) the emergent portions were planted with the same Vermilion clone of *S. alterniflora*, and (iii) even though the soil from the upland site used to create pumped mounds had been removed from the industrial canal up to 40 years ago, it was ultimately from the same source as the dredge spoil used to create the filled mounds, and ongoing monitoring at the site suggests that sediment grain size, nutrient concentrations, and organic content in dredge, upland, and existing bottom sediment are similar among all soil sources (A.R. Armitage, unpublished data). This homogeneity in elevation, plant composition, and soil composition likely contributed to the similar primary production and rate of carbon capture among emergent vegetation in constructed and reference areas.

NACC$_{SAV}$ was also similar among sites (Fig. 6C; Table A.1). This was unexpected because submerged plants were naturally recruited from the reference area and each constructed area was

a different distance from the source population. For example, the shortest distance between the northernmost plot of excavated mounds and the reference area was 0.79 km, while filled mounds were only 0.16 km from the reference area (Fig. 1). Both *Myriophyllum* and *Ruppia* apparently have rapid rates of dispersion that allowed for quick colonization of constructed areas.

4.3. The future dynamics of the land to water ratio may depend on water depth

Given the key importance of land to water ratio in NACC$_{AREA}$, it is important to understand why the ratio of land to water changed in each constructed area. We hypothesize that these changes were driven in large part by the depth of water surrounding each emergent mound. Emergent vegetation is capable of trapping sediment from surrounding water and creating soil through turnover of its own biomass (Castellanos et al., 1994; van Hulzen et al., 2007), which over time can contribute to the formation of emergent marsh. The brackish marshes we monitored in this investigation were dominated by *S. alterniflora*, which can grow in water-saturated soils but cannot survive long periods of submergence (Mendelssohn and Morris, 2002). If we assume that increases in emergent marsh area are from *S. alterniflora* expanding into surrounding submerged areas and creating emergent marsh habitat, then we would expect areas with shallow water habitat to gain land faster than areas with deeper water. This is exactly what we observed in the constructed areas.

Excavated and pumped mounds represent extremes of construction design in terms of water depth (Fig. 2A and C) and they also experienced the most disparate changes in the ratio of land to water from 2007 to 2010 (Fig. 5). Pumped mounds were surrounded by shallow water (Fig. 2C) and the ratio of land to water increased every year in the pumped mound area (Figs. 3 and 5). The shallow water surrounding pumped mounds likely provided ideal habitat for the creation of new land through the colonization of shallow water areas by *S. alterniflora* (Fig. 2F and I). In the case of excavated mounds, water depth was greater (Fig. 2A) and newly emergent areas did not develop (Fig. 3). Instead, the growth of *S. alterniflora* was constrained and the ratio of land to water gradually decreased over the course of this study, most likely due to sediment compaction that reduced emergent habitat (Figs. 2D, G, 5). In filled mounds, where water depth was intermediate (Fig. 2B), *S. alterniflora* appeared to expand into shallow water areas that were habitable but eventually these areas became occupied (Fig. 2E and H) and the ratio of land to water stabilized (Fig. 5). Over the next several years, we expect the ratio of land to water in both pumped and filled mounds to increase, but for this transition to occur more quickly in pumped mounds.

Between 2009 and 2010, the ratio of land to water increased by 0.19 in pumped mounds. If we assume that the conversion of submerged to emergent vegetation continues to increase at this same rate, then the ratio of land to water in pumped mounds will be equivalent to that of the reference area in 2033, an elapsed time of 24 years since construction. An interesting goal of future studies would be to identify the threshold water depth that determines if emergent vegetation will be able to lead to the production of new areas of emergent marsh habitat and consequently impact the NACC$_{AREA}$.

5. Conclusions

A large body of scientific literature has shown that constructed and reference marshes can obtain similar levels of emergent aboveground primary production (Costa-Pierce and Weinstein, 2002; Kentula, 2002; Shafer and Streever, 2000; Turner and Streever, 2002; Zedler, 2000a; Zedler and Callaway, 1999). However, the present investigation demonstrates that even when the productivity of a single species of vegetation is comparable between constructed and reference areas, the constructed site as a whole may not be performing as well on a landscape scale that integrates emergent and subtidal habitat. It is important for constructed and reference marshes to have comparable rates of plant carbon capture, and marsh construction designs should aim to duplicate the ratio of land to water in adjacent reference marshes to meet or exceed this goal.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ecoleng.2012.02.001.

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