AQUATIC MACROPHYTE AND ANIMAL COMMUNITIES IN A RECENTLY RESTORED BRACKISH MARSH: POSSIBLE INFLUENCES OF RESTORATION DESIGN AND THE INVASIVE PLANT SPECIES *Myriophyllum spicatum*

A Thesis

by

MICHAEL THOMAS BELL

Submitted to the Office of Graduate Studies of Texas A&M University
and the Graduate Faculty of The Texas A&M University – Corpus Christi
in partial fulfillment of the requirements for the joint degree of

MASTER OF SCIENCE

May 2011

Major Subject: Marine Biology

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Approved by:

Chair of Committee,	Anna R. Armitage
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ABSTRACT

Aquatic Macrophyte and Animal Communities in a Recently Restored Brackish Marsh: Possible Influences of Restoration Design and the Invasive Species *Myriophyllum spicatum.* (May 2011)

Michael Thomas Bell, B.A., Texas A&M University at Galveston

Chair of Advisory Committee: Dr. Anna R. Armitage

The numerous benefits that wetlands provide make them essential to ecosystem services and ecological functions. Historically, wetland losses have been caused by natural and anthropogenic changes. In Texas, nearly 50% of coastal wetland habitat has been lost since the 1930s and losses in the Lower Neches watershed have been some of the most extensive. Restoration is a way to mitigate these losses and can be accomplished in many ways. Each restoration design creates different aquatic habitats that can influence both submerged aquatic vegetation (SAV) and faunal communities. The restoration of the Lower Neches Wildlife Management Area (LNWMA) has created the conditions for the growth of the invasive submerged macrophyte, *Myriophyllum spicatum* (Eurasian watermilfoil) which may be competing with the native aquatic grass, *Ruppia maritima* (widgeongrass) for essential nutrients. In this study, an attempt was made to link restoration design with both SAV and aquatic fauna community structures by using a throw trap to characterize assemblages observed in three different types of restored marshes. We also performed two controlled mesocosm experiments in 0.5 gal aquariums to determine growth inhibition by *M. spicatum* on *R. maritima*. Analyses using Kruskal-Wallis non-parametric test determined that temporal variations in fauna and SAV community composition was greater than any restoration effect. Discriminant Function Analyses (DFAs) determined two to three key faunal species that best predicted association among restoration designs, but linear regressions could not determine any consistent relationship between individual species density and biomass of the dominant SAV species, *M. spicatum*. For the mesocosm experiments, *M. spicatum* inhibited the biomass production and branch count of *R. maritima* when the two species are grown together (ANOVA, $p = 0.004$ and 0.003, respectively). Changes in SAV assemblages due to competition and habitat characteristics could play a major role in determining faunal community. In order to minimize the temporal effect observed and better determine any habitat pattern that may be present, a much longer study is necessary.

The world is mud-luscious and puddle-wonderful.

~e.e. cummings

To my parents

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1. INTRODUCTION

The numerous benefits that wetlands provide make them essential to ecosystem services and ecological functions. Wetlands harbor high levels of biodiversity and support complex food webs (Mitsch and Gosselink, 1993; Nedland *et al.*, 2007). They provide habitat for numerous plant and animal species, both marine and freshwater, including some threatened or endangered species (Nedland *et al.*, 2007). Wetlands also improve water quality within a watershed, provide flood protection, and sequester carbon, subsequently regulating climate (Hassan *et al.*, 2005; Verhoeven *et al.*, 2006; Verhoeven and Setter, 2010). From a human economic viewpoint, it is estimated that wetland ecosystem services are valued at nearly $3.4 billion a year in the United States (Schuyt and Brander, 2004).

1.1 Wetland loss

The western Gulf of Mexico, including Texas, contains nearly half of the salt and brackish marshes in the United States and is experiencing rapid rates of anthropogenically-driven coastal marsh loss (Gagliano *et al.*, 1981; White and Tremblay, 1995). This makes the area an ideal study site to look at unnatural changes in coastal habitats. Since the 1930s approximately 10,700 ha of Texas'

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coastal wetlands, including saltwater, brackish, and freshwater habitats, have been lost mainly due to dredging for navigational purposes, levee construction (Morton and Paine, 1990; Tuhus, 2008), residential development (Adair *et al.*, 1994; Katovich, 2008), and agriculture (Mitsch and Gosselink, 2000). The loss of these complex systems results in the elimination of many important functions that reach across habitats and trophic levels. Mechanisms of loss include subsidence (accelerated by withdrawal of groundwater) and upstream diversion of sediment and water, which results in the submergence of marsh habitats and the conversion of marsh to open water (Tornqvist *et al.*, 2008; Ravens *et al.*, 2009).

1.2 Mitigation of loss through restoration

Restoration is a way to mitigate the loss of these habitats. In many coastal regions of the United States, restoration is accomplished through the creation of new marsh habitat by importing sediment and emergent plants to a degraded marsh habitat (Rozas *et al.*, 2005; Baustian *et al.*, 2008). This method can maintain groundwater pressures as well as combat subsidence (Weinstein *et al.*, 2002; Wills *et al.*, 2008).

Coastal marsh restoration has been discussed in the scientific literature since the 1980s, but has been more about re-establishing a marsh’s emergent plant canopy. It was Zedler and Powell (1993) and Zedler (1996) that have given us a better understanding for restoration work and subsequent health of restored marshes based on both faunal and vegetation communities. Accordingly, restoration must be evaluated from a community-level rather than a species-level perspective. Later studies have discussed certain structural attributes of a marsh that should be examined such as soil texture and nutrients, vegetation structure, and invertebrate and fish populations, to help assess ecosystem functioning (Weinstein *et al.*, 2002).

Restoration of some biotic and abiotic ecosystem components is not always successful (Minello *et al.*, 1994). Challenges in marsh restoration include avoiding extreme spatial heterogeneity of chemical and physical properties of the soil (Lindau and Hossne, 1981), identifying the beneficial effects of restored marsh edge on specific, less motile, macrofauna species (Minello *et al.*, 1994), and understanding functional differences, such as lower species richness and diversity, faunal trophic interactions, and habitat quality between restored and natural marshes that are structurally similar (Levin, 1996). Understanding these challenges and shortcomings of past marsh restoration is beneficial to restoration work in the future.

Restoration efforts usually focus on marsh edge. The edge of a habitat is essential to overall aquatic faunal productivity because it increases habitat complexity and creates refuge (Peterson and Turner, 1994; Dionne, 1998; Rozas, 2009). Restoration projects with marsh edge habitat that mimics natural marshes typically have higher aquatic fauna abundance and richness relative to restored marshes that lack such edge habitat (Minello *et al.*, 1994; Able *et al.*, 2004). Current work also usually targets emergent marsh vegetation (Chow-Fraser, 1998; Delaney *et al.*, 2000; Rozas and Minello, 2001; Baustian *et al.*, 2008; Miller and Fugii, 2010) and commercially and recreationally important species that utilize that microhabitat (Minton, 1998; Rozas and Minello, 2001; Gray *et al.*, 2002; Minello and Rozas, 2002; Rozas *et al.*, 2005).

1.3 Restoration techniques

In Texas, widespread subsidence has led to the submergence and degradation of coastal marshes, and restoration generally involves raising the substrate back to emergent marsh elevation (Baustian *et al.*, 2008). There are many different techniques for raising marsh elevation with the two most common being backfilling (“dredge pumping”) and excavation.

Backfilling involves a hydraulic dredge that pumps sediment from a spoil bank back into open water, increasing the submerged sediment elevation (Baustian *et al.*, 2008). This technique can lead to recolonization of marsh vegetation by facilitating natural plant recruitment from the natural seed bank in the sediment, but can be expensive and its success is dependent on the dredge operator’s skill (Neill and Turner, 1987; Turner *et al.*, 1994; Baustian and Turner, 2006; Baustian *et al.*, 2008; Tuhus, 2008). Furthermore, backfilling broad open areas does not create an equivalent amount of marsh edge relative to reference marshes consisting of tidal creeks and ponds (Minello *et al.*, 1994; Delaney *et al.*, 2000).

The most commonly used excavation technique in restoration is terracing, which involves the use of benthic sediment to create ridges at marsh elevation. These ridges are arranged in such a way to allow the flow of water and movement of organisms throughout the terrace field (Rozas *et al.*, 2005). After construction, emergent vegetation is then planted on the terrace.

One way to compensate for a lack of edge habitat in a restoration project is mound building. This technique involves creating circular formations constructed in an area of open water. Sources of soil include dredge material from off-site or excavated benthic sediment. Backfilling does not necessarily need to create a planar surface, but can be used to create “pumped mounds” of variable sizes and height above the water surface. Success of such technique is still dependent on the operator’s skill but its design can potentially match the amount of marsh edge relative to a reference marsh. In the same respect, the creation of mounds through excavation can have a similar effect, by returning marsh elevation to a natural state and create large amount of edge habitat for aquatic fauna. Methods can also be combined. Excavated mound formations tend to create deeper (>1 m) aquatic habitats than pumped mounds. By excavating mounds and filling in the deep water with dredge material, the depth of adjacent aquatic habitat can be adjusted to a more shallow (<0.5 m) level. Mound building is a construction design that is not commonly used, so the advantages and disadvantages of this approach are not as well understood (Rozas *et al.*, 2005).

1.4 Submerged aquatic vegetation (SAV) in restoration

Submerged aquatic vegetation (SAV) is an often overlooked component of ecosystem restoration, which usually focuses on emergent plant canopies (Gu, 2008). Submerged aquatic vegetation provides important habitat for foraging and reproduction of juvenile and small-bodied fishes and macroinvertebrates (Kemp *et al.*, 1990; Pelicice *et al.*, 2008). It also provides aides in the protection of small prey fauna from predators (Gleason and Wellington, 1988). Benefits of increasing SAV biomass in wetland restoration include increasing faunal densities and lowering water nutrient content and turbidity (Zimmer *et al.*, 2003). Submerged aquatic vegetation also increases the amount of habitat per unit area of benthos (Crowder and Cooper, 1982; Humphries, 1996), stabilizes sediments (Han *et al*., 2009) and actively takes up nutrients from the water column (Barko and James, 1998). The growth and distribution of SAV is controlled by a multitude of environmental factors from water quality to the conversion of water to land, making them measurable indicators for important environmental characteristics (Janauer, 2001; James *et al.*, 2005; Clayton and Edwards, 2006). Therefore, integration of SAV into restoration design can substantially augment the ecosystem functions provided by the project.

Common submerged vegetation species in brackish marshes on the Texas Gulf coast include *Myriophyllum spicatum* (Eurasian watermilfoil)*, Ruppia maritima* (widgeon grass), and filamentous green algae (e.g., *Spirogyra* spp., *Cladophora* spp.). *Myriophyllum spicatum* is a non-native macrophyte that is considered an “invasive” or “nuisance” species in the United States (Hoagland and Jin, 2006; Modley, 2008). It grows in dense monocultures in freshwater and brackish habitats at water depths of one to three meters (Boylen *et al.*, 1999; Burlakova and Karatayev, 2007; Madsen *et al.*, 2008). It has the ability to propagate using three different methods: stolon production, fragmentation and seed production (Smith and Barko, 1990; Madsen and Smith, 1997). Structure of this species consist of highly complex shoot stem with wide leaf whorls arranged around both the main stem and multiple branches as well as an extensive, fibrous branching root system. *Ruppia maritima* and the filamentous green algae, *Spirogyra* sp. and *Cladophora* sp., are native to coastal marshes in Texas. *Ruppia maritima* can exhibit annual or perennial growth patterns (Bigley and Harrison, 1986) and is generally found in shallow coastal and inland brackish waters in Texas between a salinity of 10-30; although it has been observed in fresher waters (Brayshaw, 1985; Adair *et al.*, 1994). The structure of *R. maritima* consists of branching underground rhizomes with shallow, narrow non-branching roots. As flowering occurs, the macrophyte creates vegetative shoots off the primary axis and forms lateral branches (Bigley and Harrison, 1986). *Spirogyra* is a genus of filamentous green algae that annually form floating masses between late spring and mid-summer, which can grow intertwined with submerged macrophytes (Bold, 1967; Townsend and Padovan, 2005). Its growth is usually due to its ability to take up and use nitrogen faster than other vascular species when increased levels of nitrogen in both the water column and sediment are present (Han et al., 2009). *Cladophora* is another late spring to early fall annual that forms floating mats on the water surface with attachment to benthic sediment up to eight meters deep (Power *et al.*, 2009).

In restored coastal marshes that incorporate SAV habitat, SAV species composition of SAV may depend on the restoration technique that was employed. Restoration construction designs can vary in water characteristics (clarity, nutrient concentration, depth, salinity), all of which may impact SAV growth and competitive dynamics. *Myriophyllum spicatum* root and shoot growth is lower in deeper (1.7 m) water than in shallow (0.2 m) habitats, suggesting high light requirements (Strand and Weisner, 2001), but has also been found to grow in shallow, low light areas as well. *Ruppia maritima* abundance and distribution can increase when both water clarity and salinity increase, but when salinity and clarity decreased, *M. spicatum* tend to flourish (Cho, 2005). When *Spirogyra* is present, it competes with submerged macrophytes for nutrients and light (Han *et al.*, 2009).

1.5 Aquatic faunal communities in SAV

Fish density and species richness are often positively related to SAV biomass (Cry and Downing, 1988; Kemp *et al.*, 1990; Duffy and Baltz, 1998; Pelicice *et al.*, 2008). An increase in macrophyte biomass (e.g., higher stem and leaf density) can increase the availability of microhabitats that provide associated aquatic fauna with food, spawning sites, and protection from predation (Gleason and Wellington, 1988; McTigue and Zimmerman, 1991; Chaplin and Valentine, 2008). Chaplin and Valentine (2008) discovered that macroinvertebrate production was highest on the exotic SAV, *M. spicatum*, due to its high level of structural complexity, compared to native SAV species (*Heteranthera dubia* and *Vallisneria americana*) studied in the Mobile-Tensaw Delta. This high level of complexity decreased predation on macroinvertebrates by small fish (Chaplin and Valentine, 2008; Martin and Valentine, 2011). In a study performed by Humphries (1996), total abundance and species richness of macroinvertebrates were higher in the more structurally complex and shallowest growing macrophyte, *Myriophyllum simulans*, than in two native, less complex SAV species, *Triglochin procera* and *Eleocharis sphacelata*.

1.6 Effects of invasive SAV

Ecosystem restoration may be disrupted by the colonization of non-native, invasive species. Invasive plant species can impact coastal marsh restoration by outcompeting native species and altering water column and sediment nutrient availability (Windham and Lathrop, 1999). Invasive plant species can also lower soil salinity and create erosion dominated disturbance regimes leading to the degradation of natural habitats (Vitousek *et al.*, 1997; Windham, 2001).

Myriophyllum spicatum is a highly competitive invasive species in many regions of the world, usually creating large, dense monocultures and displacing native species within the first three years of its introduction into brackish habitats (Trebitz *et al.*, 1993; Madsen and Smith, 1997; Valley and Newman, 1998). Much of the previous work on *M. spicatum* is from freshwater habitats in Europe (Van Wijck *et al.*, 1994), the Middle East (Ali and Soltan, 2006), and both the northern (Titus and Adams, 1979; Nichols, 1994) and southern regions (Newbolt *et al.*, 2008) of the United States. Due to its spread into Texas in recent years, *M. spicatum* has begun to interact with native SAV species, particularly *Ruppia maritima.* Very little is known about interactions between these species in brackish marshes on the Gulf Coast.

1.7 Goals of this study

There have been few direct comparisons among different mounding restoration designs, but each construction technique has the potential to create different aquatic habitats, which can subsequently influence the SAV and aquatic animal communities. To date, the links among restoration techniques, SAV, and aquatic animal communities have not been concurrently quantified adequately. Therefore, the goal of my study was to evaluate the aquatic plant and animal communities within a recently restored coastal brackish marsh where several different construction techniques were utilized. I also explored some of the interactions between *M. spicatum* and *R. maritima* at this site. Objectives of this study were threefold:

1. Characterize the effect of restoration construction technique on the SAV biomass and composition of SAV and aquatic animal communities
    - H_0: SAV biomass and composition of SAV and aquatic animals will not be different among restoration construction techniques
    - H_1: SAV biomass is higher and composition more diverse in the marsh habitats shallow water.
    - H_2: Aquatic animal density and diversity is greatest in marsh habitats with shallow water.
2. Characterize the relationship between SAV biomass/composition and faunal density/diversity
    - H_0: There is no change in aquatic fauna density/diversity with a change in SAV biomass/composition
    - H_1: Aquatic fauna density/diversity is greater in marsh habitat with greater SAV biomass/compostion.

3. Determine if *M. spicatum* may be inhibiting *R. maritima* biomass production

    H_0: An increase in *M. spicatum* biomass does not affect *R. maritima* growth

    H_1: Increased *M. spicatum* biomass has a negative effect on *R. maritima* by inhibiting its growth.

2. METHODS

2.1 Study site

My study site was located within the Lower Neches Wildlife Management Area (LNWMA) (30° 0.437’ N, 93° 51.507’ W) near Port Arthur, TX (Fig. 1a). This area is part of the Lower Neches watershed, located on the northern Gulf of Mexico along the coast of Texas. This region has experienced extensive loss of wetlands, with approximately 5,000 ha lost since the 1950s (Field *et al.*, 1991; White and Tremblay, 1995).

The site was historically part of the Chenier Plains freshwater marsh system, but the introduction of salt water through drainage canals connected to the Intracoastal Waterway and Entergy (formally Gulf State Utilities) outfall canals in the 1950s caused the freshwater marsh vegetation to die off. Subsequent subsidence converted the emergent marsh into open water. In 1997 and 2007 plugs were installed in the canals to reduce saltwater input, and large-scale vegetation restoration was initiated in 2007. Construction methods used resulted in three distinct habitat types: 1) “Pumped” mounds (P1-P5), using a backfilling technique, were created with off-site dredge material and surrounded by shallow water habitat (0.25 – 0.50 m depth); 2) “Excavated” mounds (E1-E5) were created from dedicated sediment (adjacent to the mound construction) and surrounded by a water depth of 1 – 2 m; and 3) Excavated with fill mounds (EF1-EF5), using excavation a backfilling methods, were constructed with dedicated sediment and surrounded by off-site dredge material, creating shallow (< 0.5 m) aquatic habitat. A reference marsh (R1-R5) with similar tidal influence and remnant brackish marsh vegetation was selected near the restored areas to act as a standard of comparison, although this marsh was not truly natural, but not actively managed or altered.

On an aerial photo of the site, all mounds within each habitat type were assigned a number (1-100+), and five were chosen using a random number generator. Google Earth was used to determine the GPS coordinates of those five mounds (Fig. 1b). The mounds were at least 1 m apart and spread throughout each of the habitat types in order to encompass the heterogeneity within each restoration method. This sampling station distribution should have captured the range of conditions within each habitat type and limited the potential for pseudoreplication. Sampling stations were located in the aquatic habitat immediately adjacent to the randomly selected mounds in a water depth of 0.25 - 2 m. Areas that appeared to have reference vegetation conditions were identified on aerial photos, and points were haphazardly selected within those areas.

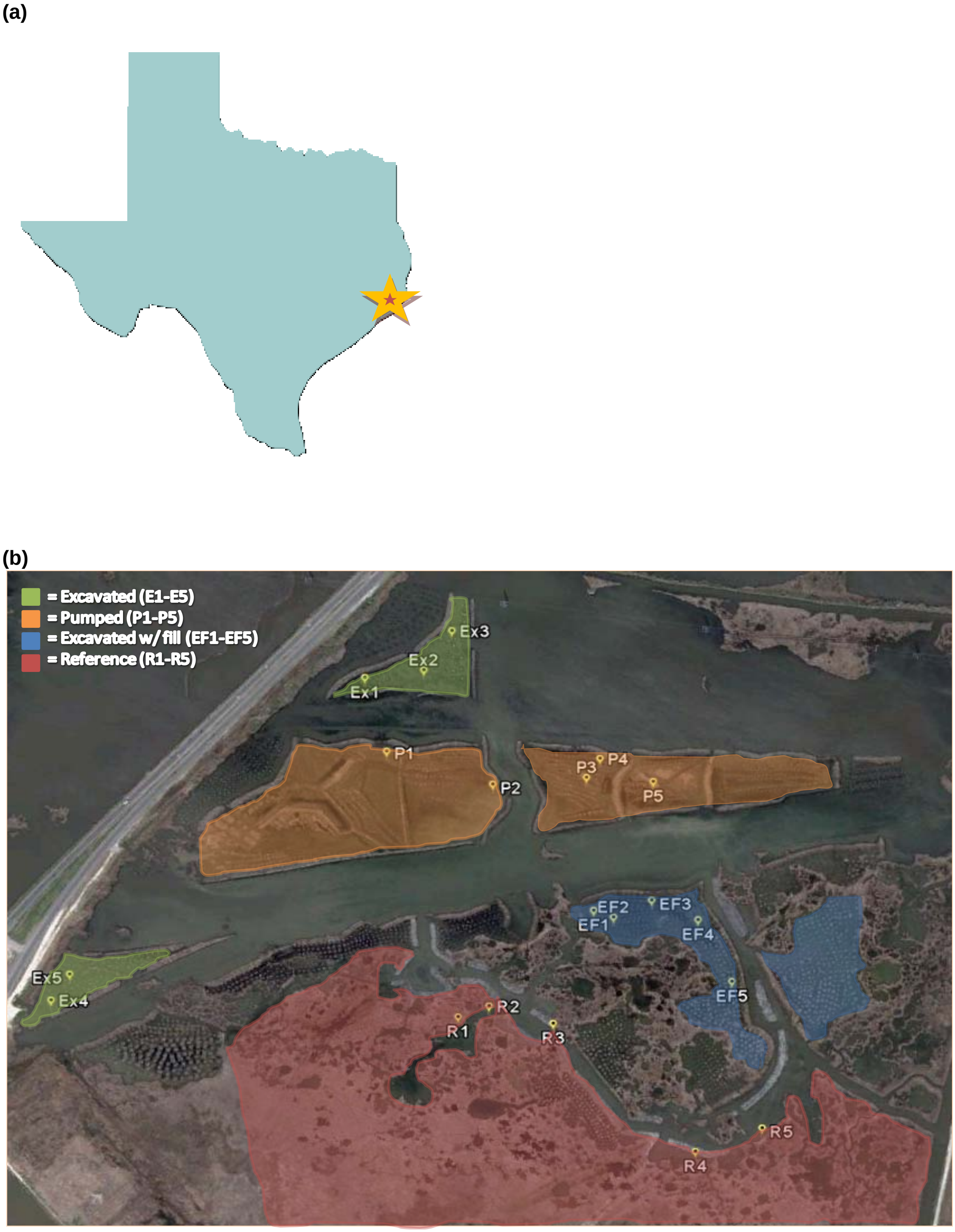

Fig. 1. Study site located 30° 0.437' N, 93° 51.507' W. (a) State map of Texas with Port Arthur designated by the star near the Louisiana border. (b) Aerial photograph of the restored area within the Lower Neches Wildlife Management Area (LNWMA). All stations surveyed within the area are marked with a yellow dot and alphanumeric code. (c) Cross-sectional representation images of the different construction designs. Representation images courtesy of Dr. Eric Madrid.

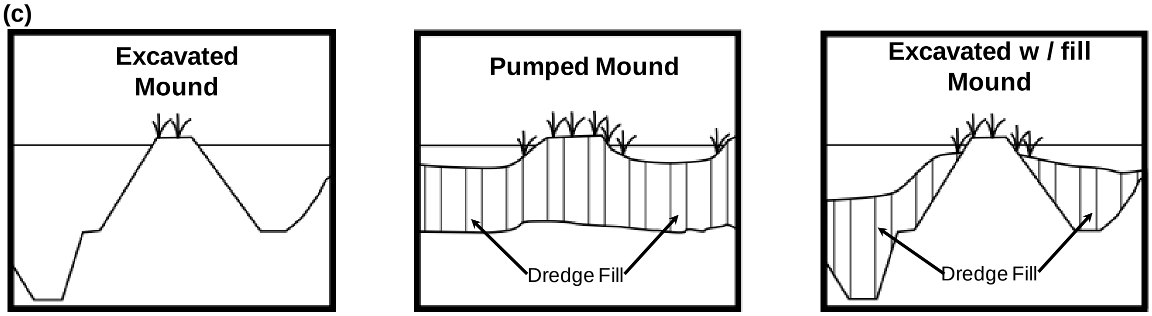

Fig. 1. continued

2.2 Restoration influence on SAV

In order to evaluate the influence of different restoration methods on the SAV community, I performed six bimonthly field surveys between August 2009 and August 2010 at each of the twenty stations described above. SAV composition and biomass was quantified by combining 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). Each mound was slowly approached in a non-motorized boat and the head of a 16-tine metal rake was cast two meters away from the side of the boat. The rake was dragged towards the boat and across the bottom in a downward sweeping motion that collected all the vegetation within an area 0.041 meters wide by two meters long. Vegetation trapped in the rake was placed in a one gallon plastic bag, transported on ice to the lab, and stored at -20°C in a freezer prior to processing. In the lab, plants were thawed, separated by species, rinsed to remove adhered sediment, dried at 60°C, and weighed to determine biomass. In conjunction with the SAV collections, water column salinity was measured with a YSI salinometer. Salinity measurements were collected at the surface and just above the benthos within one week of the survey.

SAV biomass and salinity could not be transformed to conform to the homoscedastic variance assumption of ANOVA, and thus a Kruskal-Wallis non-parametric test (Chan and Walmsley, 1997) was used to test for differences in SAV biomass and salinity among the four habitat types within each sampling period.

2.3 Interaction between *Myriophyllum* and *Ruppia*

Based on qualitative observations, submerged macrophyte growth in the field was dominated by *M. spicatum*, and where *M. spicatum* did not grow, *R. maritima* appeared to grow well. Therefore, I conducted two experiments to explore the interactions between *M. spicatum* and *R. maritima*. The goal of the first experiment was to determine if *M. spicatum* inhibited the growth of *R. maritima* and if there was a biomass threshold at which *M. spicatum* limited *R. maritima* growth. The second experiment examined if growth inhibition of *R. maritima* was due to competition with *M. spicatum* or attributable to general (intraspecific) density dependence. The design was a modified version of the study done by Agami and Waisel (1985). Sterilized sediment, autoclaved at 121°C for 30 minutes on two consecutive days, as described by Carter et al. (2007), was placed into 0.5 gal aquaria. Filtered water from the field was then added, and D.I. water supplemented any loss of water in the aquaria due to evaporation. In the first experiment, each container received two grams (wet weight, roots removed) of *R. maritima* along with one, two, three, or zero grams of *M. spicatum* (wet weight, roots removed) (n = 5 per treatment). Each species was planted in the sediment. In the second experiment, to maintain similar total biomass levels as the first experiment, one gram (wet weight) of *R. maritima* was initially added to each aquarium. Then, instead of *M. spicatum*, one, two, three, or zero grams of extra *R. maritima* (wet weight) was added (n=5 per treatment). Wet weights were determined after spinning each plant in a low velocity centrifuge for one minute. All plants were of similar age, growth stage, and reproductive status. Total wet weight, stem length and number of branches for each plant were measured. After 6 weeks, all plants were removed and final wet weight was determined for each species. I also recorded stem length, number of leaves and branches for each plant. The experiment was performed outside in indirect sunlight at the University of Houston’s Coastal Center in Texas City, TX from April to June 2010. Aerators were added to prevent stagnation of the water and wire mesh was placed on top of each aquarium to prevent the intrusion of insects.

Homoscedasticity of variances was confirmed and a one-way ANOVA was used to evaluate competitive dynamics between the two SAV species and the potential for intraspecific density dependence for *R. maritima* growth. In experiment #1, initial wet weight (g) of *M. spicatum* was the fixed factor and change in *R. maritima* biomass and plant morphometrics – length and branching, as the response variables. A Bonferroni post-hoc test was used to determine any significant differences among the four habitat types. In the second experiment, the biomass of the extra *R. maritima* added to each aquarium was the fixed factor in a one-way ANOVA. To correct for differences in initial biomass, relative changes in biomass were calculated as $\mathrm{abs}[((WW_i / WW_f) / WW_i) * 100]$, where WW_i was the initial wet weight and WW_f was the final wet weight. This relative change in biomass was then used as the response variable.

2.4 SAV and restoration influence on aquatic fauna

To evaluate the influence of different restoration methods on the aquatic animal community, I performed a series of quarterly faunal surveys from August 2009 to May 2010. Fauna were assessed using throw traps, which effectively quantify the population structure of small, abundant invertebrates and demersal fishes (Rozas and Minello, 1997). 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 station was approached by an airboat drifting towards the station against the wind with the motor turned off. The trap was tossed from the airboat into the water on top of a haphazardly selected SAV aggregation at each station. 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. Trap deployment was repeated on aggregations of each species of SAV present at a station (up to three SAV species were present: *M. spicatum*, *R. maritima*, and green algal mats). A marine bilge pump was used to rinse any sediment and debris from the throw trap on-site. All SAV that was both attached to the benthic substrate and floating on the surface within the 1 m^2 area of the throw trap was gathered, put into labeled 1-gallon plastic bags and put over ice to be transported back to the lab. All fishes were removed from the vegetation on-site and placed in plastic bags containing water from the study site that has been chilled to < 4°C. These bags were placed in a large cooler containing an ice slurry to euthanize all fishes (in accordance with AUP 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. All invertebrates and fishes were identified to the lowest practical identification level and counted. For each station and restoration method, I determined faunal density, species richness (S) and, Shannon-Wiener diversity (H^l):

$$\mathrm{H}' = \sum_{i=1}^{S} (p_i \ln p_i)$$

S = total number of species in the community
p_i = relative abundance of each species

Once the macroinvertebrates were removed, the SAV underwent a secondary rinse to remove any remaining sediment. The dry weights of each SAV species were determined as described above.

Since inter-annual and inter-seasonal variations in estuarine fauna populations can be large (Akin *et al.*, 2003; Rozas *et al.*, 2007; Shervette and Gelwick, 2008; Mendoza and Zarate-Hernandez, 2009), all response variables had heteroscedastic variances and could not be transformed to conform to ANOVA assumptions. Therefore, I used Kruskal-Wallis non-parametric tests (Chan and Walmsley, 1997) to examine the effects of habitat type on total faunal density, species richness, and species diversity within each sampling period.

To investigate which faunal species would best predict associations among the four habitat types, discriminant function analyses (DFA) were performed for each sampling period on the most prevalent invertebrate and fish species in the marsh (Morrison, 1984; Windle *et al.*, 1993). Species that occurred in fifty percent or more of the throw trap tosses were considered prevalent in the marsh during that sampling period and were used as predictors in the DFA. The relative importance of each predictor was determined by their standardized discriminant function coefficients (r) (Morrison, 1984) and helped to determine how well each faunal species predicted habitat type groupings in the DFA. A predictor species' r-value greater than 0.50 indicated a strong correlation with the first discriminant function (df1), so I only reported those predictors with an $r > 0.50$ in the analyses.

M. spicatum was the dominant SAV species in the marsh throughout most of the study duration, so I ran linear regressions across all habitat types within each sampling period to determine if *M. spicatum* biomass (used as the predictor variable) influenced the aquatic fauna community composition within the marsh. The response variables used in these analyses were total fauna density, the density of the most common aquatic faunal species (species that occurred in fifty percent or more of the throw trap tosses), species richness (S), and species diversity (H’).

3. RESULTS

3.1 Submerged macrophyte & algal biomass

There was no consistent effect of habitat type on plant biomass, and temporal variation appeared to be much larger than the effect of habitat type (Fig. 2). The only significant habitat effect occurred in October 2009 (Kruskal-Wallis $X^2_3 = 8.023$, $p = 0.046$), when *M. spicatum* biomass was substantially lower in the excavated than the other three marsh habitats (Fig. 2c). Each SAV species peaked in biomass at different times of the study: *M. spicatum* was most abundant in August 2009, *R. maritima* in June 2009 and January 2010, and algae in April 2010.

Salinities in the marsh were between 1 and 4 in four of the six sampling periods (Fig. 3). In October 2009 and May 2010, salinities were substantially higher, ranging from 7 to 14. A non-parametric Kruskal-Wallis test showed a weak statistical difference in salinity levels among the four habitat types in the August 2009 sampling period ($X^2_3 = 8.220$, $p = 0.042$), most likely driven by lower salinity in the excavated with fill habitat. There was a strongly significant difference in salinity among habitat types in October 2009 ($X^2_3 = 15.423$, $p = 0.001$), primarily due to higher salinities in the excavated with fill and reference marsh habitats. A significant difference in salinity among habitat types in January 2010 ($X^2_3 = 13.603$, $p = 0.003$) was driven by lower salinity in the reference marsh habitat.

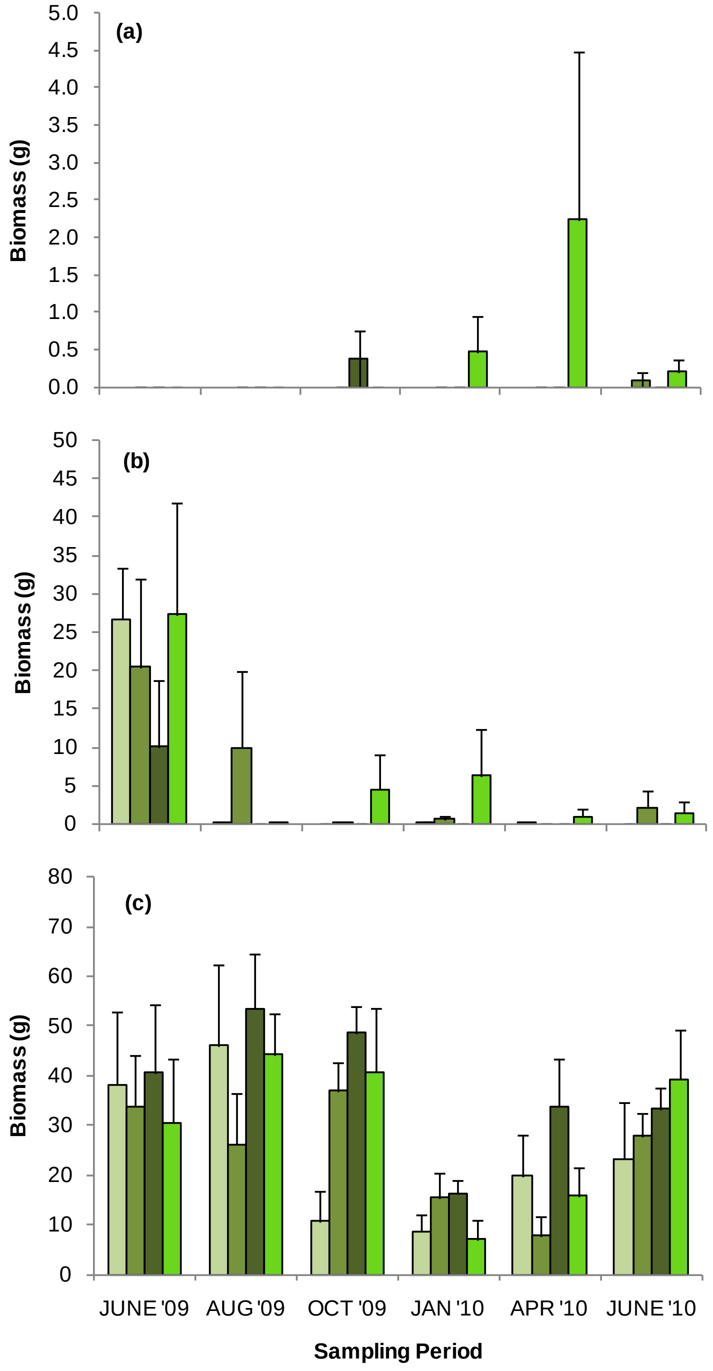

Fig. 2. Submerged aquatic vegetation (SAV) biomass. Mean (a) algae, (b) *Ruppia maritima*, and (c) *Myriophyllum spicatum* biomass (g) in different habitat types at six times of year. Habitat types consisted of three restored areas and one reference area. Excavated marsh (E) = 🟩, Pumped marsh (P) = 🟩, Excavated with fill marsh (EF) = 🟩, Reference marsh (R) = 🟩. Error bars indicate one SE.

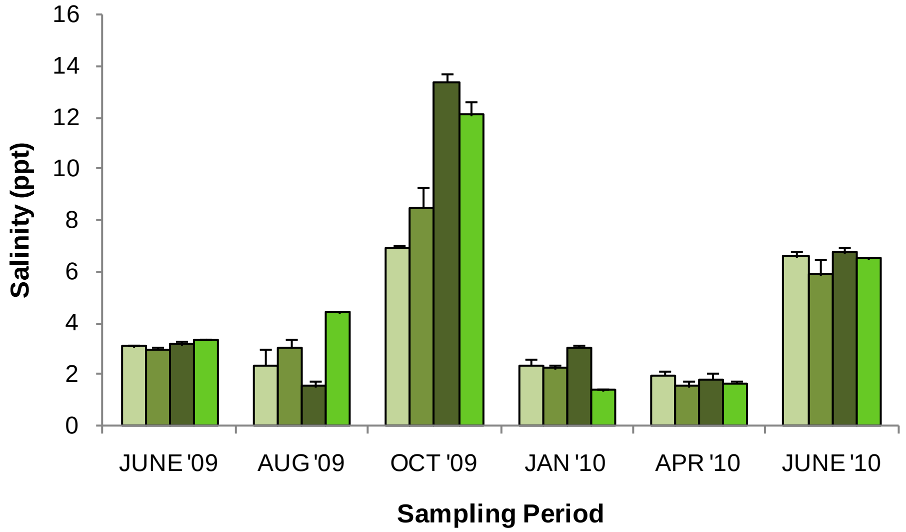

Fig. 3. Mean salinity (ppt) in different habitat types at six times of year. Habitat types consisted of three restored areas and one reference area. Excavated marsh (E) = 🟩, Pumped marsh (P) = 🟩, Excavated with fill marsh (EF) = 🟩 , Reference marsh (R) = 🟩. Error bars indicate one SE.

3.2 Effects of *Myriophyllum* on *Ruppia* biomass production

Myriophyllum spicatum significantly reduced *R. maritima* biomass production in the controlled mesocosm experiments (Table 1, Fig. 4). Relative to the control, *R. maritima* biomass was significantly lower in all *M. spicatum* addition treatments, regardless of the amount of *M. spicatum* added, suggesting that the presence of *M. spicatum*, even in low amounts, suppresses *R. maritima* biomass (Bonferroni p = 0.01). Control aquariums containing *R. maritima* growing alone had an average biomass increase of 1.50 grams, whereas, relative to the control, *R. maritima* biomass decreased by an average of 0.51 (SE = 0.39) grams in all treatment aquaria with *M. spicatum* (Fig. 4a). *Myriophyllum spicatum* also significantly decreased *R. maritima* branch count

(Table 1, Fig. 4b). *Ruppia maritima* in control aquariums developed an average of 7.3 new branches while the treatment aquariums had an average loss of 6.4 branches. Stem lengths of *R. maritima* were not affected by the addition of *M. spicatum* (Table 1, Fig. 4c).

The addition of different amounts of conspecifics in the *R. maritima* density-dependence study did not affect biomass production of this species (Table 1, Fig. 5). *Ruppia maritima* in all of the mesocosm aquariums increased in relative biomass. Control and treatment aquariums experienced a relative increase in *R. maritima* biomass of well over one-hundred percent.

Table 1. Results of biomass inhibition and density dependence mesocosm experiments. Three separate one-way ANOVA analyses of the relationships between *M. spicatum* or *R. maritima* biomass (g wet weight) and change/% change in biomass (g wet weight), stem length, and branching of *R. maritima.*

Factor	**Response**	***F***	**Sig.* (*p*)**
M. spicatum Biomass (g)	Δ *R. maritima* Biomass (g)	6.847	0.004
	Δ *R. maritima* stem length (cm)	1.714	0.204
	Δ *R. maritima* branching (# branches)	7.284	0.003
R. maritima Biomass (g)	%Δ *R. maritima* Biomass (g)	1.130	0.367

*$p < 0.05$

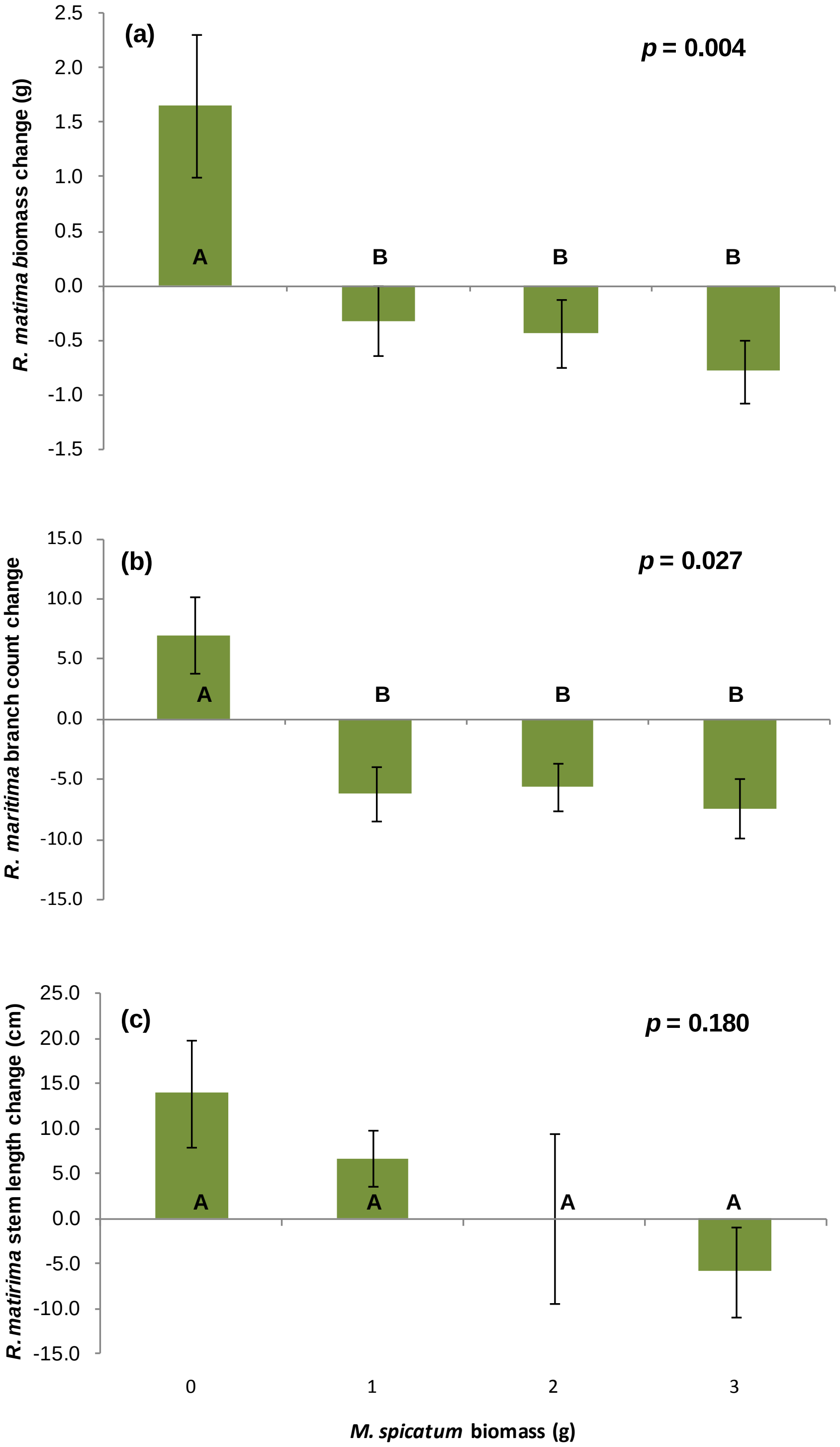

Fig. 4. Growth inhibition of *R. maritima* by *M. spicatum.* Mean change in (a) biomass and plant morphometrics of *R. maritima* (b and c) in aquariums with differing initial *M. spicatum* biomass. Error bars signify one S.E. Letters indicate significant differences based on Bonferroni post-hoc tests.

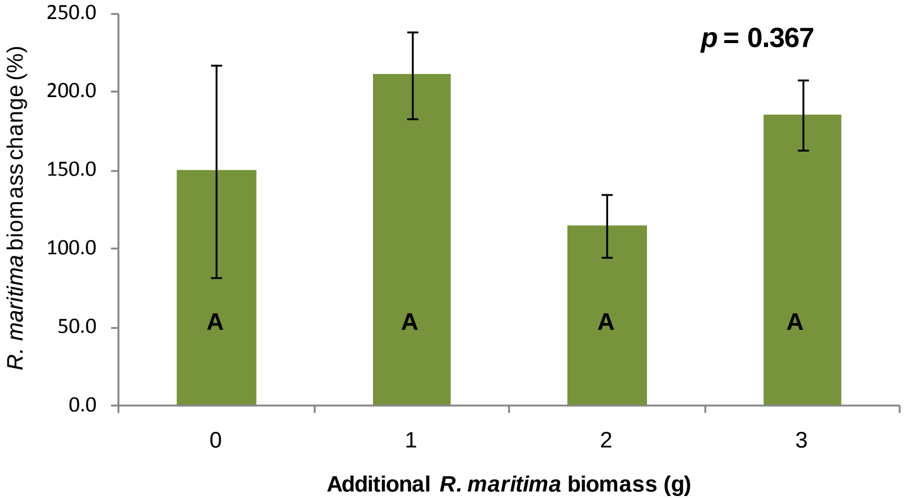

Fig. 5. Mean relative change in biomass of *R. maritima* in aquariums with differing amounts *R. maritima* biomass added. Error bars signify one S.E. Letters indicate significant differences based on Bonferroni post-hoc tests.

3.3 Faunal densities

Fifteen fish species and 12 invertebrate species were identified from the 154 samples taken over the four sampling periods. Three of the 12 invertebrate species could only be identified to genus and one only to family (Table 2).

Table 2. Total number pooled for all twenty stations of all fishes and invertebrates collected from the marsh in four sampling periods from August 2009 through May 2010. The percent of the stations in which each species was present during each sampling period is also provided.

		Tot. no. of individuals				% of stations species present			
Common names	Scientific names	Aug.	Nov.	Feb.	May	Aug.	Nov.	Feb.	May
Invertebrates									
Daggerblade grass shrimp	*Palaemonetes pugio*	303	1305	992	818	76.5	90.0	92.5	97.5
Pink shrimp	*Farfantepanaeus duorarum*	18	11	0	21	14.7	10.0	0	27.5
Brown shrimp	*Farfantepanaeus aztecus*	13	0	0	0	17.6	0.0	0	0.0
Stone crab	*Menippe adina*	2	0	0	0	5.9	0.0	0	0.0
Blue crab	*Calinectus sapidus*	15	3	5	7	35.3	7.5	7.5	20.0
Olive nerite snail	*Neritina reclivata*	1	0	0	0	2.9	0.0	0	0.0
Marsh snail	*Probythinella louisianae*	80	8952	368	3888	11.8	75.0	37.5	85.0
Dragonfly larvae	*Anisoptera sp.*	13	41	59	7	8.8	37.5	40	20.0
Water boatman	*Corixa sp.*	0	10	0	15	0.0	20.0	0	27.5
non-biting midge larvae	Chironomidae	0	11	375	2	0.0	7.5	70	5.0
Amphipod	*Amphipoda sp.*	0	13	495	339	0.0	7.5	57.5	30.0
No common name	*Hydrobiomorpha casta*	0	0	0	28	0.0	0.0	0.0	20.0
Fishes									
Rainwater killifish	*Lucinia parva*	280	438	715	740	82.4	77.5	92.5	92.5
Gulf killifish	*Fundulus grandis*	1	0	0	0	2.9	0.0	0	0.0
Sailfin molly	*Poecilia latipinna*	490	598	190	319	85.3	82.5	80	75.0
Sheepshead minnow	*Cyprinodon variegatus*	54	41	182	132	44.1	22.5	57.5	35.0
Naked goby	*Gobiosoma bosc*	19	18	28	16	29.4	22.5	27.5	22.5
Clown goby	*Microgobius gulosus*	40	22	13	26	38.2	27.5	12.5	17.5
Code goby	*Gobio robustum*	8	0	0	0	14.7	0.0	0	0.0
Striped mullet	*Mugil cephalus*	1	0	1	0	2.9	0.0	35	0.0
White mullet	*Mugil chrema*	5	0	2	1	5.9	0.0	2.5	5.0
Pinfish	*Lagodon rhomboides*	7	0	0	0	14.7	0.0	5	0.0
Inland silverside	*Medinia beryllina*	4	52	32	20	8.8	7.5	0	27.5
Bluegill	*Lepomis macrochirus*	0	0	1	0	0.0	0.0	7.5	0.0
Dusky pipefish	*Sygnathus floridae*	18	3	7	10	20.6	5.0	2.5	12.5
Gizzard shad	*Dorosoma cepedianum*	0	0	0	10	0.0	0.0	7.5	15.0
Spotted worm eel	*Myrophis punctatus*	4	0	0	0	11.8	0.0	0.0	0.0

There was strong seasonal variation in total faunal density (Fig. 6). Total faunal density was statistically different among habitat types in February (X^2_3 = 19.330, p = 0.000) and May 2010 (X^2_3 = 19.525, p = 0.000). In February, faunal densities were substantially higher in the reference area relative to all restored habitat types. In May, faunal densities were higher in the pumped and excavated with fill habitat types than in the excavated and reference habitat types. In general, excavated mounds had consistently low animal density and the reference area usually had densities as high as or higher than in all restored habitat types; an exception was May 2010, when faunal densities were relatively low in the reference habitat type. Densities were uniformly low in August 2009, and there was high variability in November 2009, obscuring statistical differences among habitat types.

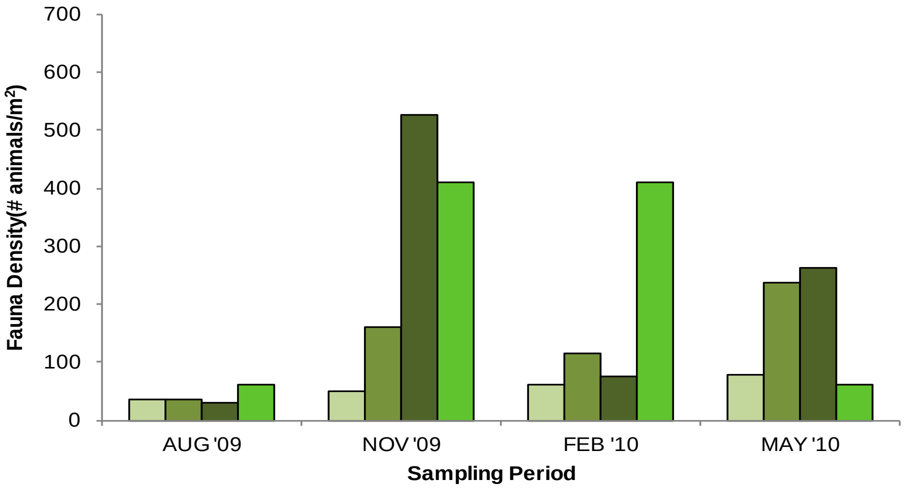

Fig. 6. Mean fauna (fish and invertebrate) densities in each habitat type across four sampling periods. A Kruskal-Wallis non-parametric test was performed due to unequal variances in the fauna densities. Excavated marsh (E) = 🟩, Pumped marsh (P) = 🟩, Excavated with fill marsh (EF) = 🟩, Reference marsh (R) = 🟩. Error bars indicate one SE.

In August 2009 there was one significant discriminant function (eigen value > 1) that accounted for most of the variance (89.1%) (Table 3). The standardized discriminant coefficients indicate that *Poecilia latipinna, Palaemonetes pugio*, and *Lucania parva* were most strongly related to discriminant function 1 and defined the separation between the reference area and all restored habitat (Fig. 7a). *P. latipinna* and *P. pugio* had higher densities in the reference habitat than any other area, and *L. parva* had lower densities in the reference area.

In November 2009, there was one significant discriminant function, which accounted for the 86.9% of the variance (Table 3). The excavated with fill habitat separated from the excavated and pumped habitats along the first discriminant function, but all restored areas overlapped with the reference habitat (Fig. 7b). *Probythinella louisianae* was the only species that was strongly correlated with DF1 and was found in higher densities in the excavated with fill and reference habitats.

Table 3. Discriminant function analysis (DF1) and densities of prevalent fish and invertebrate species among three restored areas and one reference area in all sampling periods.

August 2009 Variables (individuals/m^2)	Excavated Area (*n* = 8)		Pumped Area (*n* = 9)		Excavated with fill Area (*n* = 9)		Reference Area (*n* = 8)		Standardized discriminant coefficient
	Mean	*St. Dev.*	*Mean*	*St. Dev.*	*Mean*	*St. Dev.*	*Mean*	*St. Dev.*	
Lucania parva	3.375	3.461	15.666	19.339	8.125	6.151	5.222	6.260	-0.873
Poecilia latipinna	11.125	8.131	6.111	7.253	7.625	6.885	31.666	21.142	0.667
Palaemonetes pugio	4.250	5.625	6.777	7.102	5.375	5.578	18.333	19.085	0.852
Goby species	2.250	2.764	2.333	1.936	0.750	1.164	2.666	2.783	-0.242
Eigenvalue = 1.613									
Percent of variance = 87.0*									

November 2009 Variables (individuals/m^2)	Excavated Area (*n* = 10)		Pumped Area (*n* = 10)		Excavated with fill Area (*n* = 10)		Reference Area (*n* = 10)		Standardized discriminant coefficient
	Mean	*St. Dev.*	*Mean*	*St. Dev.*	*Mean*	*St. Dev.*	*Mean*	*St. Dev.*	
Lucania parva	8.000	11.709	8.900	10.836	9.500	7.043	17.400	12.659	-0.102
Poecilia latipinna	14.000	35.026	10.900	18.537	14.200	9.818	20.700	22.075	0.119
Palaemonetes pugio	18.500	21.412	22.200	26.460	36.600	32.680	53.200	27.446	-0.135
Probythinella louisianae	1.100	1.911	114.400	167.488	464.200	295.895	315.500	202.636	0.998
Eigenvalue = 0.930									
Percent of variance = 75.0**									

February 2010 Variables (individuals/m^2)	Excavated Area (*n* = 10)		Pumped Area (*n* = 10)		Excavated with fill Area (*n* = 10)		Reference Area (*n* = 10)		Standardized discriminant coefficient
	Mean	*St. Dev.*	*Mean*	*St. Dev.*	*Mean*	*St. Dev.*	*Mean*	*St. Dev.*	
Lucania parva	30.000	16.186	17.200	14.335	13.800	14.265	10.500	15.966	0.761
Poecilia latipinna	2.100	2.469	9.400	6.040	5.100	9.503	2.400	3.025	-0.756
Palaemonetes pugio	36.600	28.964	9.300	8.042	17.000	11.274	36.300	48.244	-0.138
Amphipoda spp.	29.500	35.264	0.200	0.421	6.200	9.693	13.600	17.379	0.526
Chironomidae	9.500	18.337	2.400	3.405	13.500	30.248	12.100	17.597	0.065
Cyprinodon variegatus	1.000	1.333	14.700	21.515	1.100	1.663	1.400	2.221	-0.360
Eigenvalue = 1.018									
Percent of variance = 59.5**									

May 2010 Variables (individuals/m^2)	Excavated Area (*n* = 10)		Pumped Area (*n* = 10)		Excavated with fill Area (*n* = 10)		Reference Area (*n* = 10)		Standardized discriminant coefficient
	Mean	*St. Dev.*	*Mean*	*St. Dev.*	*Mean*	*St. Dev.*	*Mean*	*St. Dev.*	
Lucania parva	12.300	12.789	13.800	8.916	37.600	24.327	10.300	11.851	0.579
Poecilia latipinna	3.400	4.926	2.800	4.467	23.000	16.931	2.700	3.743	0.875
Palaemonetes pugio	20.200	21.446	12.900	11.694	27.800	16.995	20.900	21.609	-0.104
Probythinella louisianae	34.700	50.046	194.000	132.708	148.200	177.753	11.900	18.722	0.317
Eigenvalue = 2.142									
Percent of variance = 84.5*									

*$P = 0.000$

**$P = 0.001$

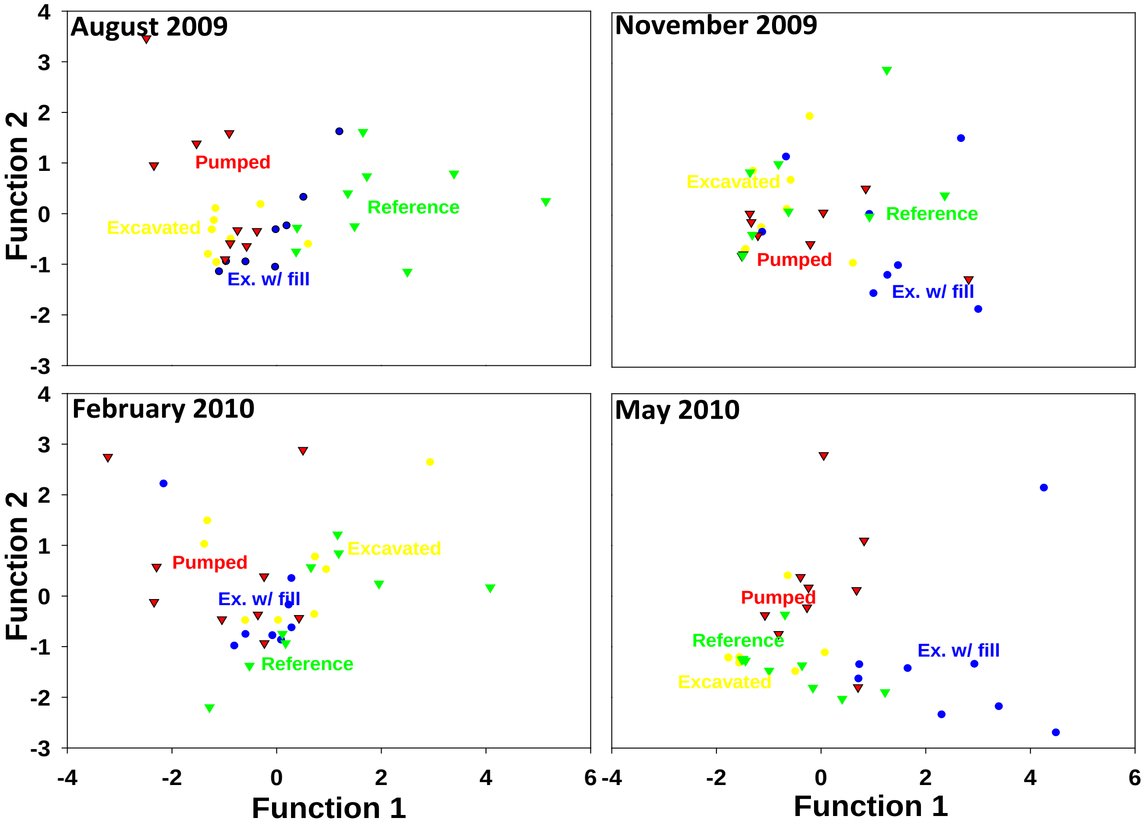

Fig. 7. Discriminant scores of function 1 and 2 for each station in all habitats. All four sampling periods are displayed. (●) Excavated area, (▼) Pumped area, (●) Excavated with fill area, and (▼) Reference area.

In February 2010 there was no clear separation among the four areas along the first discriminant function, which accounted for only 59.5% of the variance (Table 3, Fig. 7). The standardized discriminant coefficients determined that *L. parva* and *Amphipoda* sp. were positively significant in distinguishing the groups and were found in higher densities in the excavated area. *Poecilia* *latipinna* was negatively significant in distinguishing the groups and was found in low abundances across all areas.

In May 2010, there was one significant discriminant function; it accounted for the 82.5% of the variance (Table 3). The excavated with fill habitat separated from the other three habitats along the first discriminant function (Fig. 7). *Probythinella louisianae* and *L. parva* were the only species that was strongly correlated with DF1 and was found in higher densities in the excavated with fill area.

Classification success was similar among all sampling periods. The overall classification rate for the discriminant functions was highest in November 2009 and February 2010, with 45.0% of the cross-validated grouped cases correctly classified. The lowest overall classification rate occurred for the May 2010 sampling period, with 43.6% of the cross-validated grouped cases correctly classified.

3.4 Species richness (S) and Shannon diversity (H')

Species richness was also significantly different among habitat types in February and May 2010 ($X^2_3 = 14.389$, $p = 0.002$ and $X^2_3 = 9.288$, $p = 0.026$, respectively) (Fig. 8). In February 2010, species richness was highest in the excavated area and lowest in the reference area. In May 2010, excavated with fill mounds had higher species richness than the other three habitat types.

Faunal Shannon diversity (H’) was significantly different among the four habitat types in August 2009. (X^2_3 = 8.226, p = 0.042). In this sampling period, the excavated area had the highest diversity score and the reference area had the lowest. Other significant habitat effects occurred in February 2010 and May 2010 (X^2_3 = 12.180, p = 0.007 and X^2_3 = 13.005, p = 0.005, respectively). In February 2010, there was lower Shannon diversity in the reference area than the restored areas. In May 2010, the lowest diversity score occurred in the pumped area (Fig. 9).

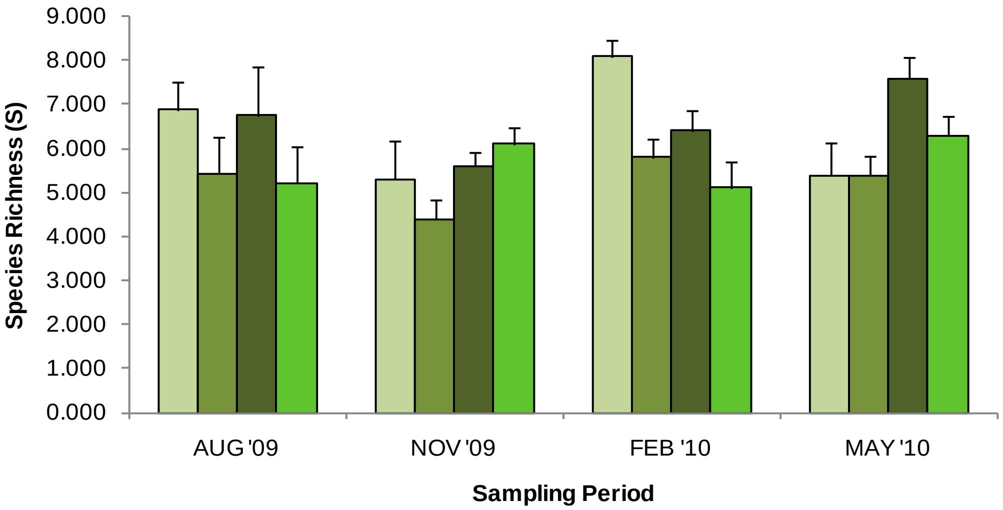

Fig. 8. Mean species richness in each habitat type across four sampling periods. Excavated marsh (E) = 🟩, Pumped marsh (P) = 🟩 , Excavated with fill marsh (EF) = 🟩, Reference marsh (R) = 🟩. Error bars indicate one SE.

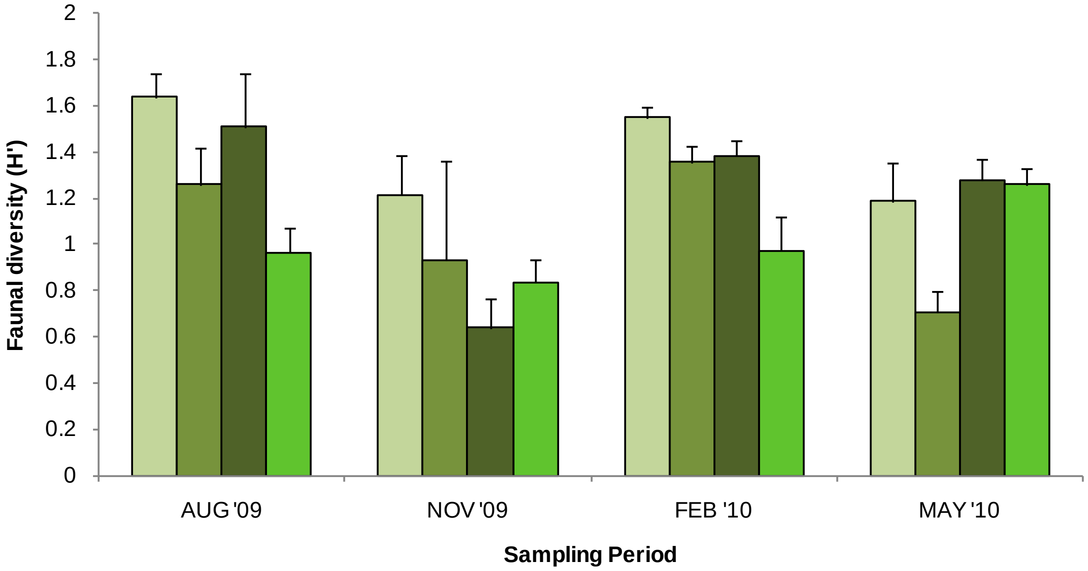

Fig. 9. Mean diversity scores in each habitat type across four sampling periods. Excavated marsh (E) = 🟩, Pumped marsh (P) = 🟩, Excavated with fill marsh (EF) = 🟩, Reference marsh (R) = 🟩. Error bars indicate one SE.

3.5 *Myriophyllum* biomass effects on aquatic fauna

Linear regressions examining relationships between *M. spicatum* biomass (g/m^2) and faunal diversity were only significant in November 2009. There was a significant positive relationship between total faunal density and *M. spicatum* biomass, and *M. spicatum* biomass explained almost 40% of the variation in total fauna density (Fig. 10b). There were no significant relationships between *M. spicatum* and total faunal density in any other sampling period (Fig. 10).

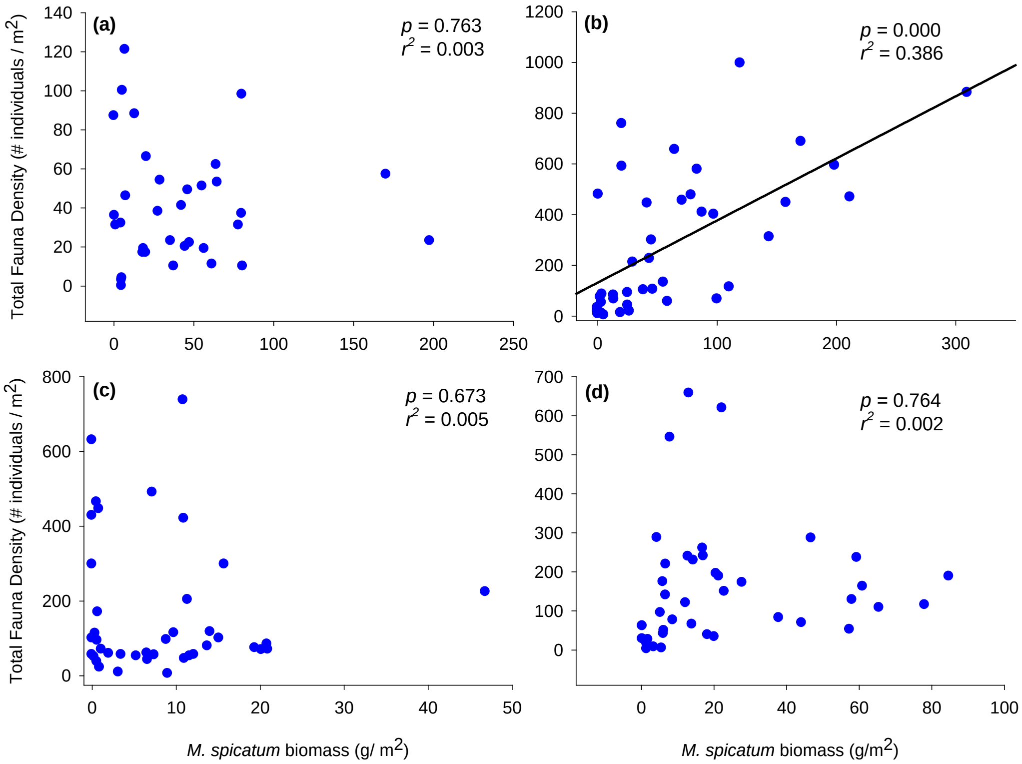

Fig. 10. Total faunal density plotted against *M. spicatum* biomass across all four habitats within each sampling period. (a) August 2009, (b) November 2009, (c) February 2010 and (d) May 2010). Plots with a significant relationship between the predictor and response variable are illustrated with a best fit regression line in.

To further evaluate the influence of aquatic vegetation biomass on aquatic fauna, I repeated regression analyses using the most common (i.e., occurred in >50% of the throw trap deployments) fish or invertebrate species or families as the response variables. In August, the three most common species were *Lucania parva*, *Poecilia latipinna*, and *Palaemonetes pugio*. Three species in family Gobiidae (Goby) were common but low in abundance, so I grouped all three Gobiidae species (*Microgobius gulosus, Gobiosoma bosc, Gobiosoma* *robustum*) together for this analysis. None of these species were significantly related to *M. spicatum* biomass (Fig. 11).

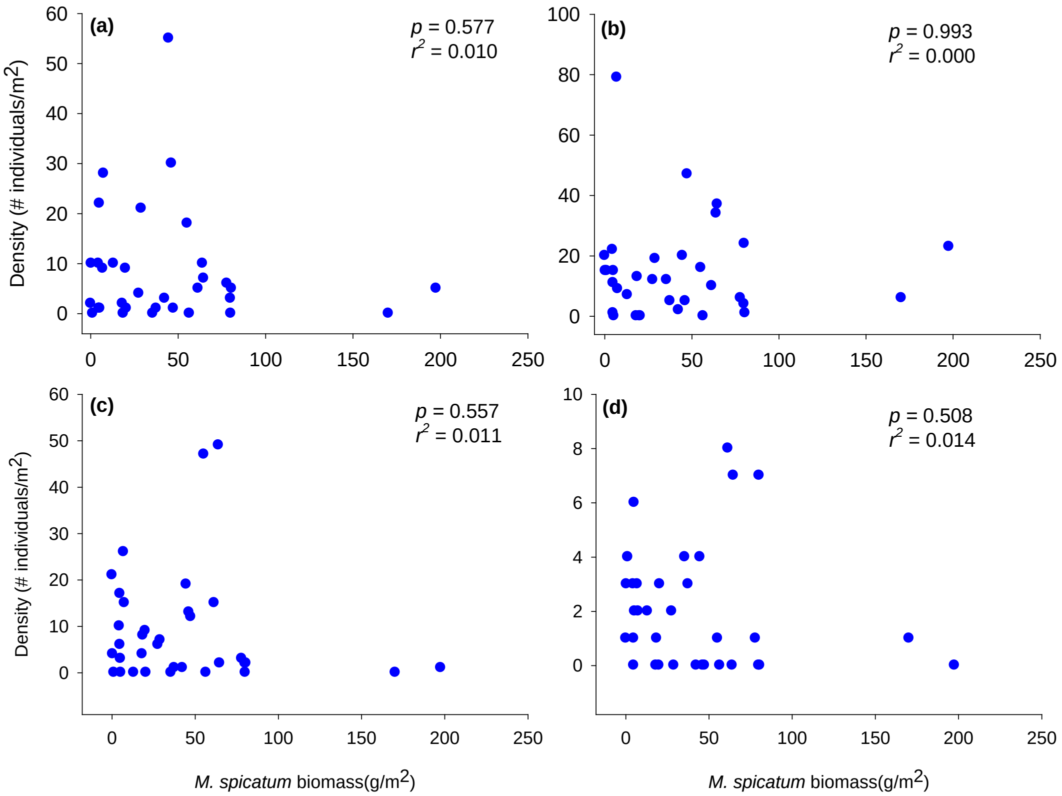

Fig. 11. The influence of *M. spicatum* biomass on the densities of the most common fauna collected in August 2009. Common fauna include: (a) *L. parva*, (b) *P. latipinna*, (c) *P. pugio* and (d) gobies. Densities are cumulative across all four habitats within the marsh.

In November 2009, the four most common species were *Lucania parva*, *Poecilia latipinna*, and *Palaemonetes pugio* and the Gobiidae species. Densities of two invertebrate species, *P. pugio* and *P. louisianae* were positively related to *M. spicatum* biomass (Fig. 12c, d), but macrophyte biomass only explained 17%

of the variation in *P. pugio* density and 37% of *P. louisianae* density. The two fish species, *L. parva* and *P. latipinna*, were not significantly related to *M. spicatum* biomass (Fig. 12a, b).

Of the six common species, *L. parva*, *P. latipinna*, *P. pugio*, *Amphipoda* sp., Chironomidae, and *C. variegatus*, in February 2010, only the Chironomidae were significantly positively related to *M. spicatum* biomass (Fig. 13e). However, *M. spicatum* explained less than 25% of the variability in *Chironomidae* density. This positive relationship was largely driven by a single station with high *M. spicatum* biomass and high Chironomidae density (Fig. 13e). The removal of the outlier would result in a non-significant relationship between *M. spicatum* biomass and Choronomidae density. None of the other species were significantly related to *M. spicatum* biomass (Fig. 13).

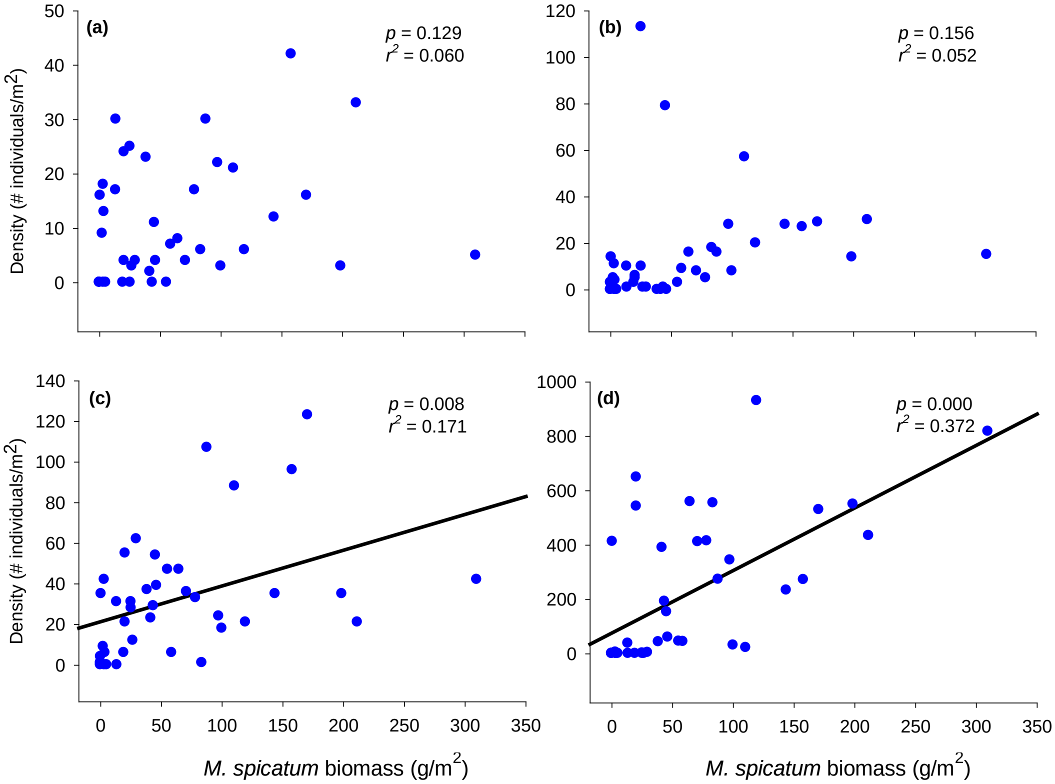

Fig. 12. The influence of *M. spicatum* biomass on the densities of the most common fauna collected in November 2009. Common species include: (a) *L. parva*, (b) *P. latipinna*, (c) *P. pugio* and (d) *P. louisianae*. Densities are cumulative across all four habitats within the marsh. Plots show significant relationships with a best fit regression line.

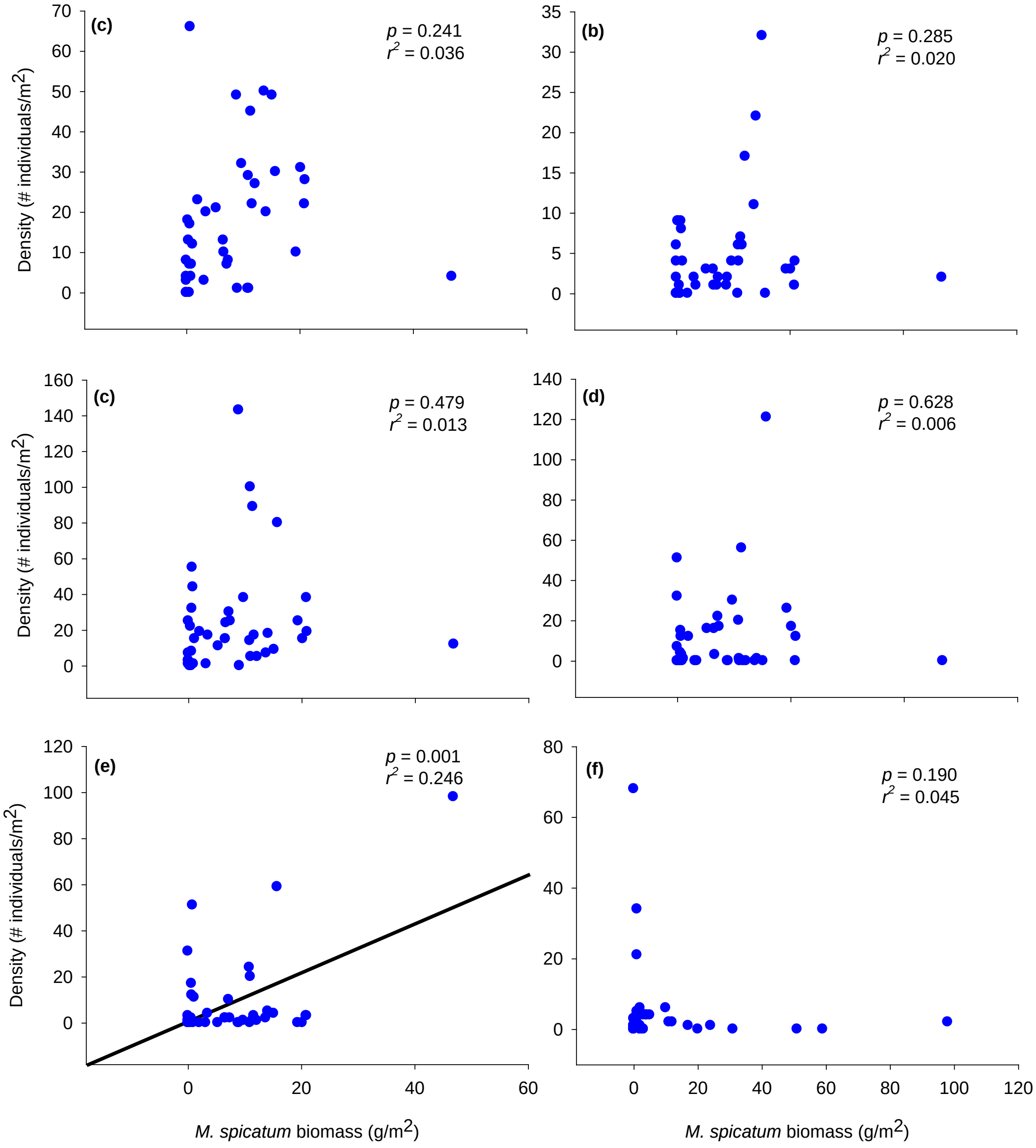

Fig. 13. The influence of *M. spicatum* biomass on the densities of the most common fauna collected in February 2010. Common fauna include: (a) *L. parva*, (b) *P. latipinna*, (c) *P. pugio*, (d) *Amphipoda* sp., (e) Chironomidae, and (f) *C. variegates*. Densities are cumulative across all four habitats within the marsh. Plots show significant relationships with a best fit regression line.

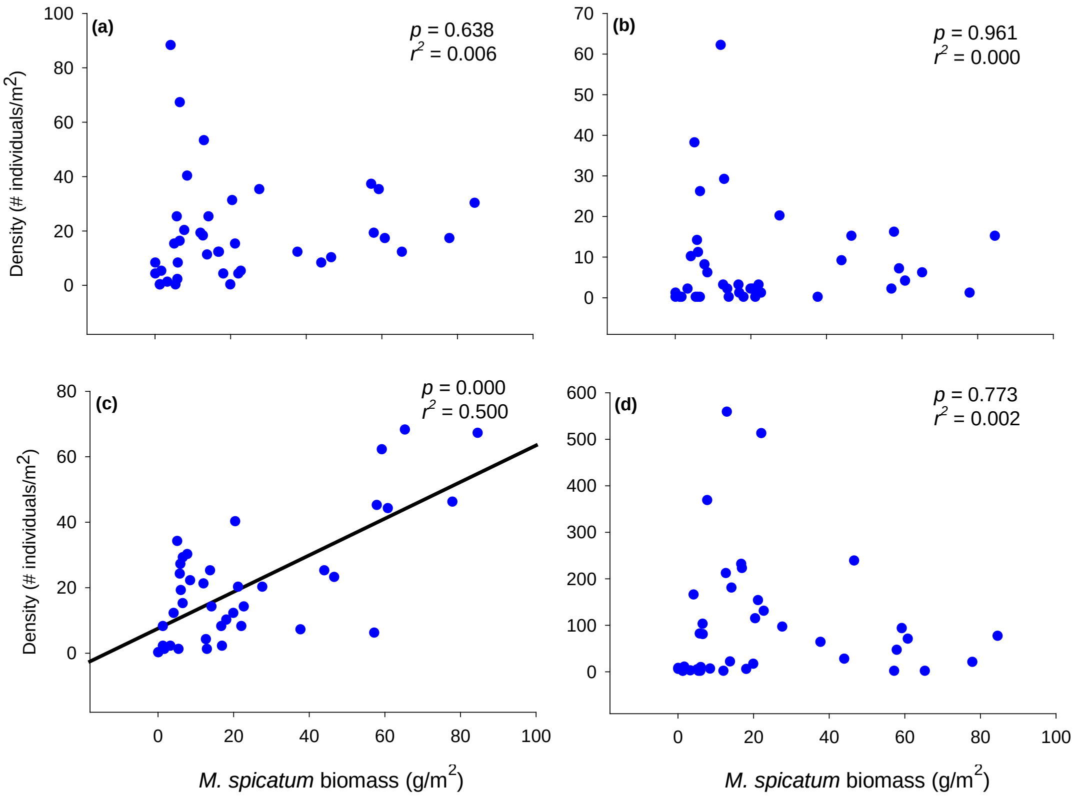

Fig. 14. The influence of *M. spicatum* biomass on the densities of the most common fauna collected in the May 2010. Common fauna include: (a) *L. parva*, (b) *P. latipinna*, (c) *P. pugio*, and (d) *P. louisianae.* Densities are cumulative across all four habitats within the marsh. Plots show significant relationships with a best fit regression line.

In May 2010, the most common species were *L. parva*, *P. latipinna*, *P. pugio*, and *P*. *louisianae*. *Myriophyllum spicatum* biomass explained 50% of the variation in *P. pugio* density (Fig. 14c). None of the other species were significantly related to *M. spicatum* biomass (Fig. 14).

Species richness (S) and Shannon diversity (H') were not significantly related to *M. spicatum* biomass within any of the sampling periods (Figs. 15 and 16)

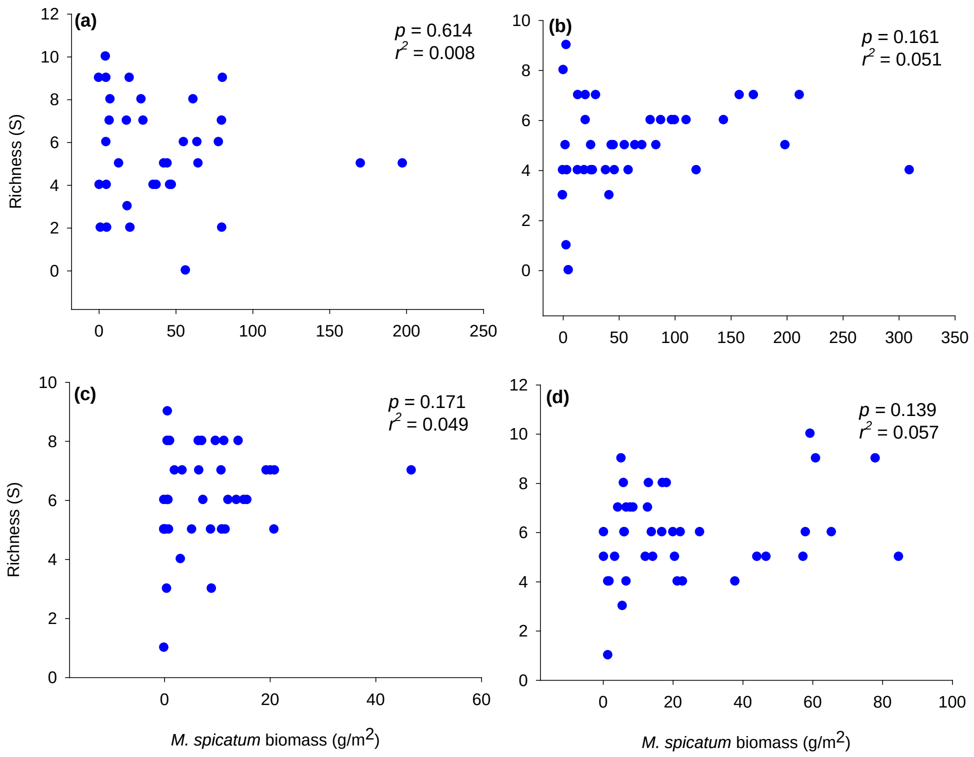

Fig. 15. The influence of *M. spicatum* biomass on species richness across all four habitat types within the marsh. Surveys occurred in: (a) August 2009, (b) November 2009, (c) February 2010 and (d) May 2010 sampling periods. Densities are cumulative across all four habitats within the marsh.

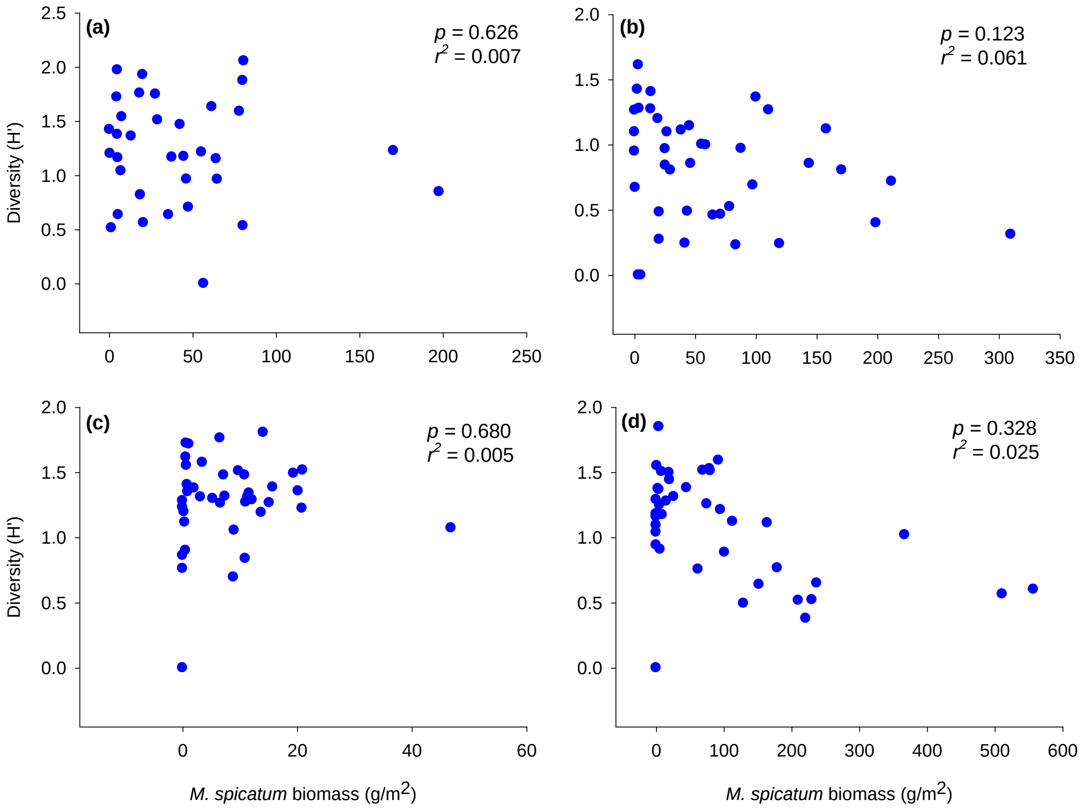

Fig. 16. The influence of *M. spicatum* biomass on total fauna diversity across all four habitat types within the marsh. Surveys occurred in (a) August 2009, (b) November 2009, (c) February 2010 and (d) May 2010 sampling periods. Densities are cumulative across all four habitats within the marsh.

4. DISCUSSION AND CONCLUSIONS

Overall, the high temporal variability in the faunal and SAV community was greater than any effect of restoration construction design. Seasonal variation in abiotic (e.g., water depth within each habitat, salinity) and biotic (e.g., grazing intensity on SAV) factors play a large role in both SAV and animal communities (Wright, 1992; Vermaat and Verhagen, 1996; Rehage and Loftus, 2007; Barletta *et al.*, 2008). In order to compensate for this seasonal variation and reveal any restoration effects, a longer multiyear study period is necessary. This study occurred during the initial colonization and early successional period of *M. spicatum* and the aquatic fauna. The patterns observed likely reflect the early colonization and recruitment in to the study site. A longer study would help to determine if the patterns observed in this study will continue in to the future. Rozas *et al*. (2007) determined significant increases in aquatic fauna diversity and abundance within a restored coastal marsh in Galveston, Texas from the time of initial marsh construction to ten years later. Despite high temporal variability in my year-long study, SAV and aquatic animal communities may be influenced by a multitude of factors (not measured in this study) created by different restoration designs and the invasion of a nuisance submerged macrophyte species.

4.1 Dominant SAV species

Myriophyllum spicatum was the only submerged macrophyte that was abundant throughout the study. The invasion of *M. spicatum* may have altered hydrological characteristics in the study site subsequently allowing for the species to flourish. Ali and Soltan (2006) measured an increase of *M. spicatum* in Lake Nasser, Egypt as well as increased soil alkalinity, sediment organic matter and water column nitrate availability. Higher levels of these abiotic factors can benefit *M. spicatum* growth and support its dominance within the area it invades (Buchan and Padilla, 2000).

In addition to the effects of *M. spicatum* on the growth of native submerged macrophytes, another explanation for reduced *R. maritima* biomass in my study area is likely low salinity. The study site maintained salinity that was consistently at or below the lower end of optimal growth (10) of this species, which may have limited *R. maritima* growth and reproductive output (Bonis *et al.*, 1993; Adair *et al.*, 1994).

Algal biomass was variable over space and time, and the mechanisms driving changes in biomass are not immediately clear. When algae mats were present, they appeared within sampling periods and habitats that had low levels of submerged macrophyte biomass, suggesting that these algae can tolerate more stressful abiotic conditions than the vascular SAV species (Han *et al.*, 2009). Alternatively, the algae may be an inferior competitor and can only grow in stressful abiotic conditions that exclude vascular SAV species (Hidding *et al.*, 2010)

Inter- or intraspecific competition among SAV can be related to many factors. Light is a well known factor influencing *M. spicatum* competition with other submerged macrophytes due to its fast growth and ability to form thick canopies (Smith and Barko, 1990; Herb and Stefan, 2006). *Myriophyllum spicatum* growth and subsequent shading might have suppressed *R. maritima* biomass production and branching. Negative interactions between *M. spicatum* and other submerged macrophytes such as *Najas marina* and *Myriophyllum exalbescens* is well documented (Nichols, 1994; Valley and Newman, 1998; Agami and Waisel, 2002). In contrast, intraspecific competition for *Ruppia maritima* intraspecific competition was minimal. This macrophyte's plant structure is less complex; therefore, shading may not be as influential.

An alternative explanation for *M. spicatum* inhibition of *R. maritima* biomass production may be the secretion of biochemicals by *M. spicatum* that negatively affect the growth, survival, and reproduction of the other species (allelopathy). Studies by Agami and Waisel (1985) and Nakai *et al.* (2000) observed allelopathic effects of *M. spicatum* on other SAV species. Their results determined allelopathic capability of *M. spicatum*. In the study, there was a decline in root and shoot density of submerged macrophytes added to controlled mesocosm aquariums previously containing *M. spicatum*. Other studies found secondary chemical secretion by *M. spicatum* in to the water column to be the cause of such decline (Leu *et al.*, 2002; Marko *et al.*, 2008).

Nitrogen competition could also have driven *M. spicatum* dominance in my study site. Nutrients often cause increases in SAV growth, but some SAV species are better than others at taking up available nutrients from water or soil. *Ruppia maritima* grows well with increased levels of both nitrogen and phosphorus (Thursby, 1984). Growth of *M. spicatum* also increases with a higher availability of nutrients (Wang et al., 2008). *Myriophyllum spicatum* is an aggressive competitor for nitrogen, using both foliar and root uptake at the same time (Shuskey et al., 2009). Seagrasses, similar to *R. maritima*, can acquire nitrogen in the water column through its foliage and pore water nitrogen through its root system (Touchette and Burkholder, 2000). The complex foliar and root structure of *M. spicatum* compared to *R. maritima* potentially makes this invasive species a more efficient competitor for both water column and pore water nutrients such as nitrogen.

4.2 Seasonal patterns in SAV

Submerged macrophyte surveys showed typical *M. spicatum* seasonal fluctuations (Stanley *et al.*, 1976), with its lowest biomass levels in the winter. This fluctuation can be due to a decrease in light attenuation. Eichler *et al* (1995) determined that the decline in *M. spicatum* abundance found in Lake George, NY, coincided with an increase in light scattering within the water column. Late fall and winter months experience shorter days and lower levels of light, thus decreasing the photoperiod for *M. spicatum* growth (Eichler *et al.*, 1995). Temperatures also fluctuate seasonally. Titus *et al* (1975) developed simulations that estimated a 10 percent increase in peak standing crop of *M. spicatum*, due to increased photosynthesis, with an 10°C increase in temperature. These simulations were then verified in subsequent laboratory experiments. *Myriophyllum spicatum* biomass within my study site followed similar patterns, with previous studies with a late spring increase, mid-summer peak and a early-winter dieback.

The SAV surveys in the marsh did not show seasonal fluctuations in *R. maritima* or algal biomass levels. Initially, *R. maritima* grew alongside *M. spicatum* and algal growth was not present. By the second sampling period, it was apparent that the marsh habitat was not supporting *R. maritima* growth for several possible reasons most likely stemming from the growth of *M. spicatum* or the low salinities found across the marsh (Bonis *et al.*, 1993; Buchan and Padilla, 2000; Nakai *et al.*, 2000; Ali and Soltan, 2006). Ephemeral algal growth within the marsh only began appearing in the October 2009 sampling period. *Myriophyllum spicatum* may have also limited algal production for similar reasons as *R. maritima*.

4.3 Restoration effects on SAV

The deepest water (excavated mounds) had consistently low *M. spicatum* biomass compared to the other areas. *Myriophyllum spicatum* often grows more vigorously, usually increasing its shoot density faster, in shallower water where higher water clarity contributes to increased photosynthetic rates (Titus *et al.*, 1975; Cho, 2005). Within the shallower areas of the Melton Hill, Tennessee reservoir, *M. spicatum* achieved greater shoot densities and among the entire area, did not experience biomass decline until October, November, and December (Stanley *et al.*, 1976).

Initially, *R. maritima* biomass was more abundant in the excavated and reference habitats, but as the sampling periods progressed, the only measurable *R. maritima* growth was in the pumped and reference habitats. Suppression of *R. maritima* growth by *M. spicatum* may have been less in these areas, as its abundance inversely shifted with changes in *M. spicatum* abundance levels. Depth could have had a similar effect on *R. maritima* as it did with *M. spicatum*. Like the invasive species, *M. spicatum*, *R. maritima* also experiences optimal growth in shallower water habitats (Adair *et al.*, 1994). The algal community did not vary as a function of habitat type, restoration effect, either, but was mainly found growing in patchy formations in the excavated with fill and reference habitats. Growth of algae could have been due to more stressful abiotic conditions, such as increase in nitrogen to toxic levels in the sediment and water in these areas (Han *et al.*, 2009; Hidding *et al.*, 2010).

It is important to note that the analysis of SAV biomass from the study site may contain possible errors. Measurements of water depth at each sampling station were not taken and therefore, water volume could not be calculated. Submerged macrophytes growing in deeper water may grow taller than their counterparts growing in much more shallow water, thus potentially increasing the SAV biomass (Nichols, 1994). In my study site, the deeper water areas generally contained the least amount *M. spicatum, R. maritima* and algal biomass. Including water volume in the analysis would determine that my surveys overestimated SAV biomass results.

Our sampling was capturing the initial colonization events and early competitive dynamics that will structure the aquatic community in the future. The time period between completion of marsh construction and my sampling of the SAV was no greater than one year. Prior to construction, no *M. spicatum* was identified in the area. At the start of the study in June 2009, *R. maritima* biomass was similar to *M. spicatum* biomass in all four habitats, but *R. maritima* was relatively uncommon during the remainder of the study period. *R. maritima* biomass may have decreased due to competition from the invasive exotic species, *M. spicatum.* Native macrophytes can be more susceptible to interspecific competition than canopy-forming, invasive species such as *M. spicatum* (Herb and Stefan, 2006). Many invasive species, including *M. spicatum*, can suppress native species growth over a range of water depths by reaching the surface early in the season and shading out competitors (Herb and Stefan, 2006).

4.4 Seasonal patterns in animals

Seasonal patterns in fauna at the study site are typical of estuarine communities. The density peaks in November and May are consistent with findings of fauna seasonal patterns in other parts of the Northern Gulf of Mexico. Through seining and gillnetting along the Mad Island marsh estuary in Texas, a study by Akin *et al (2003)* found peaks in total fauna levels in the spring, late summer and early winter. Rozas *et al* (2007) found multiple faunal species' peak levels throughout the spring and fall.

Seasonal patterns in diversity have been documented in many brackish marsh sites (Allen and Horn, 1975; Loneragan *et al.*, 1987). Salt tolerant *L. parva* and *P. latipinna* dominated all habitat types in every sampling period, causing diversity and richness to remain low throughout the study. Compared to freshwater and salt marsh systems, coastal brackish estuarine areas are difficult habitats for fish and invertebrates due to constant physical and chemical fluctuations, such as fluctuations in salinity (Hackney *et al.*, 1976). The salinity (average 1-4) at my study site may have been near the high optimal end for many freshwater animal species. Therefore, the faunal communities were constrained to a few salt tolerant species. In most cases, abiotic factors, such as salinity, constrain diversity by favoring fish and invertebrate species that are more tolerant of these fluctuations (Peterson and Ross, 1991). Species richness and Shannon diversity only varied slightly because salinity is always too high for other species.

The February faunal densities did not agree with previous studies. Amphipods and chironomids were unusually abundant during this sampling period. The high fauna density seen in the reference area in February 2010 may be explained by predator/prey interactions. It has been determined that Amphipods are a common prey species for fish in shallow coastal areas (Stål *et al.*, 2007); other common macroinvertebrate prey species including chironomid larvae increase in both range and abundance when their associated predator species, *Platichthys flesus* (European flounder) abundances are low (Williams and Williams, 1998). Although this species was not present at the study site, other estuarine fish that were present, including poecilids, have been determined to feed on chironomids (Darnell, 1961). Poecilids also prey on amphipods within tropical and subtropical estuarine habitats. Studies have shown decreases in abundance of these species within the winter season thus decreasing predation pressure on the amphipods (Nelson, 1979; Schaefer *et al.*, 1994).

4.5 Restoration effects on animals

Restored and reference habitats did not differ in seasonal animal abundance. Restored habitats experienced a decrease in the most common fauna species after their peaks in abundance; the reference habitat experienced similar shifts in abundance of the common fauna species through the sampling periods as well, but saw an increase in two other species groups, the Chironomidae and *Amphipoda* sp. This could be due to a functional difference between restored and reference marsh habitats.

Fauna densities were occasionally lower in shallowest habitat (pumped marsh) but consistently lower in the deepest water (excavated marsh). Diversity, however, was always high in the deepest water habitat. The marsh we examined is not only brackish, but it is also tidal. Water depths in our marsh sites are determined by both storm events and water levels of adjacent freshwater and ocean sources. The average water depth created by the different restoration techniques can influence the community composition of the aquatic fauna (Meyer and Posey, 2009). Deeper water habitats in the marsh generally contained more animal species. Densities for each species, though, were less than the shallower habitats. Kushlan (1976) determined that marshes in the Everglades with the most stable water depths contained the highest levels of faunal diversity. Studies linking fauna to submerged macrophytes have found higher abundance of fauna in areas containing denser macrophyte assemblages (McTigue and Zimmerman, 1991; Chaplin and Valentine, 2009). Deeper water, may limit the growth of *M. spicatum* (Strand and Weisner, 2001). Densities of *M. spicatum* at the study site were lowest in the excavated habitat. These low *M. spicatum* density levels may have resulted in low fauna density.

The four habitat types were associated with each other based on the identities of the common fauna. The discriminant function analyses indicated that assemblages of a few common species including the fresh and brackish water species, *L. parva* and *P. latipinna*, associated the reference area more with the excavated with fill and pumped habitats than the excavated habitat. These areas not only were closest to each other but also contained the shallowest water. It also indicates that the areas in the marsh varied in faunal assemblages over time. Several studies have attributed differences in faunal assemblages to water depth. Deeper water habitats in the Everglades maintained a more diverse faunal assemblage (Kushlan, 1976). In a study by Thiel *et al* (1995) not only found higher fish abundance in the shallow marginal regions of an estuary, but also more freshwater and brackish fish species.

4.6 SAV effects on animals

Assemblages of macrophytes in a marsh system can be just as important to the faunal community. The relationship between species density and SAV can be especially important in newly restored marshes (Castellanos and Rozas, 2001). Denser submerged macrophyte vegetation supports greater densities of *Lucania parva*, *Poecilia latipinna*, and *Palaemonetes pugio* densities by providing superior protection from predators (Chick and Mlvor, 1997; Castellanos and Rozas, 2001; Jordan, 2002; Kovalenko *et al.*, 2009). However, I did not detect consistently higher faunal densities in larger aggregations of *M. spicatum*, contradicting these studies.

One reason a majority of the fauna species did not appear in higher densities within larger aggregation of *M. spicatum* could be because the most common aquatic species in the marsh do not have strong preferences for more complex plant canopies. Other spatially and temporally variable habitat characteristics (Troutman *et al.*, 2007), not observed in this study, could have affected fauna density. Alternatively, *M. spicatum* may not provide any nutritional benefits and therefore does not attract the common aquatic fauna at my study site. In a controlled selective feeding experiment using four different submerged macrophytes, *Potamogeton pectinatus*, *Spirodela polyrhiza*, *Myriophyllum spicatum* and *Elodea canadensis*, *M. spicatum* contained the least amount of phosphorus and potassium and was subsequently least preferred species of the feeding fish (Setlikova, 2004).

4.7 Conclusions

The construction designs used in this restoration site can potentially create new and increased levels of refuge habitat for aquatic fauna, as well as establish habitat idea for the colonization of native and invasive SAV species. My study determined that the restoration of this area created three distinct aquatic habitats that have led to the development of a complex aquatic community, including *M. spicatum*, *R. maritima*, and aquatic fauna, but variability in recruitment succession patterns in the early development of this site obscured consistent habitat type effects. To better understand restoration designs, especially mound formations, in the future, restoration monitoring projects must include aquatic habitats. Including aquatic habitats in restoration and determining the overall effect of each new habitat on aquatic plant and animals will facilitate better community-level assessments of restoration success.

The decline in *R. maritima* abundance and subsequent rise in *M. spicatum* prevalence in the marsh could be explained by the abiotic history of the area. The site was formerly part of a freshwater marsh system. Hydrological modifications to the area accelerated the influx of salt water to this area, which caused salt-sensitive emergent vegetation to die off and led to subsidence, which allowed for salt-tolerant SAV species to thrive. *Ruppia maritima* may not have been historically native to this area, based on its affinity for a more saline environment; it may have colonized when salinity levels increased. This may be why *R. maritima* was found in the newly restored marsh; salinity levels were conducive to its growth. Since the salt plugs were installed in the drainage canals in 1997 and 2007, salt water levels began to gradually drop, creating an aquatic environment that was less conducive for *R. maritima* growth and more hospitable for the more freshwater invasive species, *M. spicatum* to colonize. This could be why I observed a decline in *R. maritima* and a proliferation of *M. spicatum. M. spicatum* is more structurally complex yet less nutritional SAV (Setlikova, 2004), which could have a drastic effect on the overall function of this newly restored marsh. The establishment and dominance of a single SAV species during such an early age of a restored marsh may inhibit further recruitment of other SAV species. It may also hinder further recruitment of aquatic animal species to the site.

Of the 27 aquatic animal species identified in the marsh, only seven were identified as "common" or found in 50% or more of my throw trap tosses. These species tended to be more salt-tolerant (Peterson and Ross, 1991).With such a high presence of only a relatively few species, this hints at an early stage of succession in the marsh. Early colonizing aquatic fauna are both pioneer and climax species. It is early to determine what shifts in the aquatic fauna communities might occur in the future since the physical conditions might prevent any other species from thriving. Unless the salinity goes down, it is unlikely to see any major changes in the composition of the faunal community.

4.8 Future directions

My mesocosm experiments determined that *M. spicatum* inhibited *R. maritima* biomass production. Growth of these two macrophyte occurred in filtered water from the study site with a natural salinity of 4 to 5. This salinity level is well outside the lower range of optimal growth of *R. maritima* but is within the higher range of optimal *M. spicatum* growth. These salinity levels possibly created mesocosm habitats that created a growth advantage for *M. spicatum*. To compensate for this potential experimental artifact in the study, another mesocosm experiments should be performed to observe the competitive dynamics of both *M. spicatum* growing alone and together within multiple salinity levels that are either conducive to *R. maritima* or *M. spicatum* growth. Lower salinities should allow *M. spicatum* to inhibit *R. maritima* growth even further than it has within salinity levels of 4 to 5. Higher salinities might provide an aquatic environment that allows for better growth and competition of *R. maritim*. This experiment can help predict the future presence or absence of the two SAV species within the marsh.

Potential sampling artifacts due to the different aquatic habitat structures throughout the marsh may have affected my results. Depending on how the mounds were created (excavated, backfilled, or combined) the aquatic habitats consisted of deep water with steep sloping mound sides or a shallow water habit with more gently sloping mound sides. In the deeper areas (particularly the excavated mounds), the throw trap may not have always landed flush with the benthic surface and the water depth may have been too great and the throw trap sank below the water surface. These issues may have allowed some of the more mobile fauna to escape, thus reducing the estimated faunal density in the excavated habitat type. Despite these artifacts, throw traps are still the best method because it effectively quantifies the population structure of small, abundant invertebrates and demersal fish like those primarily found in my study site, thus validating my results.

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