Interactions among flood regime, sediment type, and bioturbation within salt marsh systems in the context of relative sea-level rise

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#### Abstract

Interactions within temperate intertidal salt marshes were studied on three scales: micro, meso, and, macro. In the literature, it is commonly stated that *Uca* spp. (fiddler crab) burrows aerate sediment. Oxygen concentrations surrounding individual burrows were measured to assess the potential of burrows to aerate bulk sediment. Contrary to convictions in the literature, this study determined that, within poorly drained muddy sediments, oxygen penetration depth only had a significant increase up to a distance of 2mm from the burrow wall, and the increase in oxygen depth penetration was only 0.5mm. Therefore, generalizations should not be made concerning the ability of burrows to aerate surrounding sediments. The capacity of sediments to drain is an important factor in determining the potential for sediment aeration.

A rise in sea-level relative to the marsh surface will result in sediments gradually becoming more poorly drained as the extent and duration of inundation increases. Many studies concerning the interactions between *Uca* spp. burrows and sediment chemistry or *Spartina alterniflora* growth have been conducted in well-drained sediments. In a mesocosm study that contained both well-drained (sand) and poorly drained (mud) sediments, it was determined that burrows have different effects on pore water sulfide concentrations and *S. alterniflora* production depending on the sediment type and flood regime. Opposite to the conventional paradigm, within poorly drained sediments, the presence of burrows significantly increased pore water sulfide concentrations. Increased flooding significantly decreased *S. alterniflora* monthly production by the end of the growing season. Within the mud treatment, the presence of burrows significantly

decreased production with increased flooding compared to the mud treatment with current sea-level.

Six intertidal salt marshes were studied within one watershed within the VCR-LTER site. Differences among marshes could not be attributed to geomorphic setting, topographic location, nor sediment type; this determined that salt marshes within small geographic areas (<100km<sup>2</sup>) can be quite variable in terms of their characteristics.

These results show that it is necessary for experiments within salt marshes to consider and document marsh characteristics including sediment type, hydroperiod, and spatial location in the marsh in order to place research results into the proper context.

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# Chapter 1. Introduction to the Dissertation. Investigating interactions within salt marshes on three scales, micro, meso, and macro.

Salt marshes have many important roles that directly benefit humans and the environment. These marshes act as nursery habitat for many juvenile fish and shellfish species, many of which have commercial value. Wading birds and some mammalian predators use salt marshes as foraging grounds and migratory birds use them as stopovers when migrating along coasts. The marshes protect coastal areas by attenuating wave energy and taking the brunt of storm damage along coastlines, including those inhabited by large human populations. Salt marshes act as water filters that may lessen eutrophication of coastal waters by removing excess nutrients that enter from rivers, streams, and adjacent uplands and assimilating the nutrients within their above- and belowground biomass. This crucial habitat is continually being threatened by encroachment and associated coastal development caused by expanding human populations (Day et al. 1989, Mitsch & Gosselink 1993).

#### **Goals of Dissertation**

The goals of this dissertation were to explore the effects of fiddler crab burrows on other components of salt marsh systems, and to explore these relationships on different scales and within different types of intertidal salt marsh environments.

#### **Introduction to Research Chapters**

In order to determine the interactions among tidal regime, sediment characteristics, presence or absence of fiddler crab burrows, *Spartina alterniflora* production, and pore water ion concentrations, I investigated the relationships between these variables in natural salt marsh systems as well as a constructed marsh system. Through two field studies and one large-scale mesocosm experiment I investigated these interactions on different spatial scales (millimeter scale to ecosystem scale) in natural and controlled systems.

#### Chapter 2. Sediment oxygen concentrations surrounding Uca pugnax burrows

To determine how fiddler crab burrows alter pore water chemistry on the millimeter scale, individual crab burrows were studied. In order to study the effects of individual fiddler crab burrows on surrounding sediment pore water chemistry, an oxygen electrode was used *in situ* to determine oxygen concentrations surrounding individual burrows. Oxygen concentrations were measured adjacent to the burrows to a distance of 10 mm away from the burrows to a depth of 10 mm. In addition to measuring oxygen concentration surrounding natural burrows, oxygen concentrations were measured surrounding artificial burrows, and as a control they were measured surrounding a point with no burrow present. These measurements were done in three different marshes within the Machipongo Box Transect of the VCR-LTER.

# <u>Chapter 3. Interactions among flood regime, sediment type, and fiddler crab burrows</u> within a salt marsh mesocosm experiment

A controlled framework where tidal flooding could be manipulated was necessary to determine the effects of increased flooding due to relative sea-level rise on salt marsh systems. Therefore, a flood regime manipulation experiment was conducted in which I was able to study interactions and relationships among a variety of parameters within a controlled salt marsh system. Interactions among flood regime, sediment type, and the presence or absence of burrows were determined for many chemical and biological parameters within salt marshes.

#### Chapter 4: Intertidal marsh field survey

To understand relationships among parameters within natural systems, I conducted a field survey of salt marshes within the Machipongo Box Transect (VCR-LTER IV & V Proposals 2000, 2006) of the VCR-LTER site on the Eastern Shore of Virginia. Six marshes were chosen for their differences in geomorphic setting, topographic location, and sediment type to encompass the variety among salt marshes within this coastal barrier island system.

# Chapter 2: Sediment oxygen concentrations surrounding *Uca pugnax* burrows Introduction

It is commonly thought that the presence of Uca spp. (fiddler crab) burrows within salt marsh sediments generally aerate the sediment (Katz 1980, Bertness 1985, Genoni 1991, Nomann & Pennings 1998) allowing oxygen (O<sub>2</sub>) to penetrate deeper into the sediments much like mechanical core aeration of turf grass and lawns (Beard 1982, Aveni & Chalmers 2009, Sachs 1996). Diffusion is the main process by which O<sub>2</sub> enters bulk sediments surrounding a Uca spp. burrow, therefore it is the physical presence of the burrow and not the activity of the crab that affects sediment O<sub>2</sub> dynamics. Uca spp. do not irrigate their burrows when they inhabit them, but some species often plug the opening of their burrow with sediment during high tide to maintain an air chamber within the end of the burrow (Pearse 1914, Dembowski 1926, Teal 1959, Crane 1975, de la Iglesia et al. 1994, Koretsky et al. 2002). While in their burrows, Uca spp. utilize different strategies for respiration; they either remain in a chamber of air (Dembowski 1926, Teal 1959, de la Iglesia et al. 1994) or remain in a flooded burrow causing increased respiration rates (Teal 1959) and for some species a decrease in activity (Teal 1959, de la Iglesia et al. 1994). Because Uca spp. respire in their burrows at high tide some O<sub>2</sub> within the burrow water is consumed, but it is only the physical presence of the burrows themselves that affects O<sub>2</sub> diffusion into surrounding sediments. The effect of Uca spp. burrows on O<sub>2</sub> penetration has been speculated (Valiela et al. 1978, Montague 1982, Katz 1980, Bertness 1985, Howes et al. 1981), but has not been studied explicitly. A few studies have shown that *Uca* spp. burrows can oxidize the sediment increasing the redox potential and increase sediment drainage (Katz 1980, Howes et al. 1981, Montague

1982, Bertness 1985, Hughes et al. 1998), but  $O_2$  within the pore water of sediment surrounding *Uca* spp. burrows has not been measured.

As a result of the sediment characteristics, many intertidal salt marsh sediments remain water-logged at low tide, leaving the water table at or near the sediment surface at low tide (Howes et al. 1981). Transport of solutes or O<sub>2</sub> within sediment or across the sediment-water interface occurs by molecular diffusion caused by concentration gradients (Berner 1980, Kristensen & Kostka 2005). Sediment that retains pore water can gain dissolved O<sub>2</sub> at the sediment surface or exposed burrow wall through molecular diffusion from either contact with the atmosphere at low tide or contact with seawater from the incoming tide. When the water table remains at the sediment surface, crab burrows remain filled with water. When a burrow is filled with water the burrow wall is not exposed to the atmosphere, therefore  $O_2$  is not able to move more deeply into the sediment through the burrow wall. Even if the burrow drains partially, the surrounding sediment retains interstitial pore water due to capillary action. Molecular diffusion is 10,000 times more slow through water than through air (Haynes 2011) therefore these water-logged sediments gain O<sub>2</sub> very slowly compared to well-drained sediments (i.e. sandy sediments). Vertical diffusion at the sediment surface allows greater depth penetration into the sediment than diffusion radially away from a burrow wall into surrounding sediments (Fenchel 1996). Geometry of the interface at the burrow wall is not linear but curved; so for diffusion at the burrow wall, there is a greater volume of sediment associated for the same amount of surface area, therefore the depth of penetration is less (Fenchel 1996). In the presence of burrows, the added radial diffusion

at the burrow wall makes diffusive transport a three-dimensional process (Kristensen & Kostka 2005).

In the intertidal zone where salt marshes exist there are a limited number of animals that inhabit burrows (Daiber 1982); Uca spp. burrows are the most abundant and conspicuous (Teal 1958, Crane 1975, Montague 1980a, Daiber 1982, Bertness 1985). Most bioturbation studies have been conducted below the intertidal zone (Berner 1980). Beyond the intertidal zone, benthic sediments remain submerged continually; these sediments contain many marine animals that make and/or live in burrows, mainly bivalves, polychaetes, and crustaceans (ex. clams, worms, and shrimp and crabs) (Kristensen & Kostka 2005). These different animals make burrows with a wide variety of geometries that differ in their general shape, number of branches, number of openings, wall structure, tilt angle, diameter, length, and depth as well as density and distribution (Kristensen & Kostka 2005, Furukawa 2005). Most of the literature concerning bioturbation and sediment O<sub>2</sub> dynamics has been studied in benthic systems (Fenchel 1996), and not intertidal systems. Few studies have examined  $O_2$  dynamics in the intertidal zone in general (Brotas et al. 1990, Werner et al. 2006). The presence of burrows can cause many different changes within the sediment such as solute migration, particle transport, chemical reaction changes (including rate and type) (Berner 1980, Berner & Westrich 1985), and microbial and geochemical processes because they increase contact between the sediment and the overlying water (Kristensen & Kostka 2005). There has been much more work on sediment diagenesis where there is little or no bioturbation because the systems are much simpler (Berner 1980).

There have been studies that measure a variety of ion concentrations and reaction rates surrounding *Uca* spp. burrows (Aller 1984, Aller 1988, Nielsen et al. 2003, Gribsholt et al. 2003, Michaels 2004, Furukawa 2005). Gribsholt et al. (2003) made measurements radially surrounding highly drained *Uca pugnax* burrows at a depth of 5 cm. They made measurements from 0 - 40 mm from the burrow wall but did not measure O<sub>2</sub>. In a 2001 study, Furukawa found that calculated O<sub>2</sub> consumption rates were higher at the water-sediment interface than along a *Uca pugnax* burrow wall, which may be caused by a more active aerobic community at the water-sediment interface than at the burrow wall (Furukawa 2005). Many studies concerning *Uca* spp. ecological interactions do not examine individual burrows but compare areas with and without burrows (Bertness & Miller 1984, Bertness 1985, Kostka et al. 2002a, Kostka et al. 2002b, Michaels 2004, Holdredge et al. 2010, Thomas 2010).

Generally, studies have shown that macrofauna burrows affect solute fluxes and nutrient cycling (Aller 1988, Kristensen et al. 1991, Pelegri & Blackburn 1994) and some studies have looked at macro fauna burrows and  $O_2$  dynamics (Koike & Mukai 1983, Vetter & Hopkinson 1985, Aller 1988, Kristensen et al. 1991, Pelegri et al. 1994, Paterson & Thorn 1995, Pelegri & Blackburn 1995, Nielsen et al. 2004). Due to lack of sediment  $O_2$  dynamics studies within the intertidal zone or associated with *Uca* spp., the closest comparisons to be made are with benthic systems that are subtidal and the associated macrofauna. Studies of  $O_2$  dynamics associated with macrofaunal burrows in subtidal benthic systems is not an ideal comparison to fiddler crab burrows within the intertidal zone because many subtidal benthic animals irrigate their burrows for either feeding or respiration (Kristensen & Kostka 2005).

Due to the intuitive nature of the concept that the presence of a burrow in the sediment allows air and therefore  $O_2$  into the sediment, studies have only made visual observations or pure speculation on the topic. The burrowing activity of the soldier crab apparently oxygenated the lower sediments, but this change in  $O_2$  was not measured; instead it was only visually observed by color difference in the sediment (Webb & Eyre 2004b). The action of excavating a burrow, by bringing sediment from depth and depositing it on the sediment surface, may cause aeration of the excavated material (Montague 1980a).

Oxygen is important in these systems because it is the most favorable electron acceptor (Day et al. 1989) and it readily oxidizes reduced ions changing the overall sediment chemistry. Two things determine the amount of dissolved O<sub>2</sub> in the interstitial water: diffusion of O<sub>2</sub> into the sediment and O<sub>2</sub> consumption through reactions (Cai & Sayles 1996). Oxygen consumption can be of two different types: direct O<sub>2</sub> consumption by bacterially-mediated organic carbon degradation or oxidation of reduced inorganic ions (Hargrave 1969, Cai & Sayles 1996). Most sediment O<sub>2</sub> dynamics research has been conducted in benthic systems (Revsbech et al. 1981, Archer & Devol 1992, Munksby et al. 2002, Bryant et al. 2010, Glud et al. 2003) rather than in intertidal systems. Oxygen uptake is the most used measurement of total sediment mineralization for benthic systems (Thamdrup & Canfield 2000). Oxygen uptake in different aquatic systems is mainly controlled by temperature (Hargrave 1969) and the availability of organic carbon and its lability (Kristensen 2000), and flow rate for permeable sediments.

The number of studies on *in situ*  $O_2$  dynamics from natural undisturbed sediment is limited even for benthic systems (e.g. Archer & Devol 1992, Glud et al. 2003) Wenzhofer & Glud 2004), and studies in intertidal salt marsh systems are even more limited. Very little is known about biogeochemistry and macrofaunal burrows, which makes it important to do experiments and model the results (Furukawa 2005). Most studies that focus on sediment O<sub>2</sub> profile measurements are conducted in sediments which are continually submerged (Kim & Kim 2007). In a microcosm study, Holmer et al. (2002), found that O<sub>2</sub> penetration in a single sediment type collected from a mudflat did not differ significantly for waterlogged (drained 1 cm) and drained (drained 5 cm) treatments with O<sub>2</sub> penetration ranging from 2.2 to 2.5 mm.

In this study, the influence of *Uca pugnax* (mud fiddler crab) burrows on the dissolved  $O_2$  in interstitial water within associated sediments in a Virginia coastal salt marsh was examined. It was expected that higher pore water  $O_2$  concentrations would be measured at greater depths at close proximity to the burrow wall. Therefore the depth of maximum  $O_2$  penetration would be greater near the burrow and decrease with distance from the burrow. It was proposed that horizontal diffusion of  $O_2$  would occur at greater depths where the burrow drained and the burrow wall was exposed to the atmosphere instead of where the burrow remained flooded with oxygen-depleted sediment water. See conceptual model (Figure 2.1). In order to test these hypotheses, an *in situ* study was conducted within a temperate salt marsh system on the Atlantic Coast of the United States.

#### Methods

#### **Study Sites**

Three field sites consisting of intertidal salt marsh were selected on the coast of Virginia, USA. The two primary field sites were located on the Virginia portion of the





Figure 2.1. Conceptual Model. Showing oxygen penetration (arrows) surrounding a fiddler crab burrow. Left side: Oxygen penetrates farther vertically (V) into the sediment at the sediment surface than horizontally (R) away from the burrow (see inset) because of radial diffusion geometry (Fenchel 1996). Oxygen penetrates evenly for the entire area of the burrow wall that is exposed to air at low tide. Right side: Idealized schematic of oxygen concentrations; oxygen saturated pore water shown in red, anoxic pore water shown in blue.

Delmarva Peninsula. Salt marsh areas near tidal creeks were selected; the Lower Phillips Creek site (37.45 N, 75.83 W) and Machipongo River site (37.50 N, 75.78 W) were both located on the mainland and are part of the Virginia Coast Reserve - Long Term Ecological Research (VCR-LTER) site (Figure 2.2). A third site covered a larger area and was located on the southern end of Hog Island, a barrier island off the coast of the Eastern Shore of Virginia (37.3 N, 75.7 W), which is also a part of the VCR-LTER site. The two main field locations were chosen for their visual difference in sediment type; the Machipongo River site had a sandy mud compared to the finer mud of the Lower Phillips Creek site. The site on Hog Island had a range of sediment types from sandy mud to pure sand. All three sites were within the Hog Island Bay watershed and experience a semidiurnal tidal cycle. These marsh sites were all dominated by the macrophyte *Spartina alterniflora* (Loisel) (smooth cord grass).

#### Measurements

Burrows of *Uca pugnax* were selected within the three marsh sites. Selected burrows were not within 10 cm of other burrows or *S. alterniflora* stems. The O<sub>2</sub> concentrations were measured with a Clark-type O<sub>2</sub> microelectrode (Revsbech 1989) with an internal reference and a guard cathode. The microelectrode used was a Unisense® O<sub>2</sub> Minisensor with a 400 - 600  $\mu$ m tip (Unisense® OX500); it was connected to a 2 channel picoammeter (Unisense® PA2000) to determine O<sub>2</sub> concentrations within the top 10 mm of sediment. The microelectrode was calibrated with water bubbled with air in a calibration chamber for the atmospheric reading, and N<sub>2</sub> sparged water for the zero reading. The microelectrode was prepolarized in the lab daily and remained attached to the powered picoammeter while being transported to the field sites in order to ensure



Figure 2.2. Study Site. Left: Eastern United States. Virginia portion of the Delmarva Peninsula, 'The Eastern Shore of Virginia' indicated by arrow and circle. Middle: Detail of Virginia portion of the Delmarva Peninsula. The location of the Virginia Coast Reserve – Long Term Ecological Research study region. Circle indicates the Hog Island Bay watershed. Right, Hog Island Bay detail, arrows pointing to circles indicate the three study sites: 1. Lower Phillips Creek, 2. Machipongo River, and 3. South Hog Island.

polarization once field measurements began. To use this equipment *in situ*, the picoammeter was powered by a 12 volt battery. The O<sub>2</sub> microelectrode was held with a Micromanipulator (Unisense® MM33) which was attached to a modified In Situ Stand (Unisense® IS 19). The micro manipulator was leveled in all three dimensions to ensure that the microelectrode entered the sediment vertically.

Vertical O<sub>2</sub> concentration profiles were made from the sediment surface to a depth of 10 mm with measurement made at 500 µm depth intervals. The first profile was made as close to the burrow wall as possible without breaking through the burrow wall. Four subsequent profiles were made along a ray extending perpendicular to the burrow edge every 2 mm for a total of 5 profiles per 10 mm ray. Measurements were made along four subsequent rays in a similar manner (Figure 2.3) except when not possible (each burrow had at least three rays of measurements). The rays all extended from one side of the burrow because it was assumed that the position of the measurements would not affect the results and the *in situ* stand would therefore not have to be moved (Figure 2.3). The O<sub>2</sub> concentrations were measured adjacent to three different burrow types: Natural, Artificial, and a no burrow Control, for a total of three treatments (Table 2.1). The Artificial burrows were created the day before the measurements were taken. They were created using a masonry bit of diameter 1.27 cm, which was hand augered into the sediment to a depth of 20 cm (Montague 1982, Michaels 2004). The Artificial burrows were studied as a comparison for other studies that have utilized artificial burrows. Measurements were taken during low tide when the sediment surface was exposed to the atmosphere. The amount of time that the sediment was exposed to the atmosphere differed among burrows, but the measurements were taken from about three hours before



Figure 2.3. Sampling Scheme. Diagram showing location of burrow in comparison to vertical profiles. Small open circles represent vertical profile locations, 2 mm apart at 2 mm, 4 mm, 6 mm, 8 mm, and 10 mm from the burrow edge, creating a ray of five profiles. Each ray of profiles is considered a replicate.

low tide to three hours after low tide. Measurements were made on 16 days between July 27 and August 26, 2007. Temperature measurements were made coinciding with the time that  $O_2$  concentration measurements were being made for each burrow. The diameter of each Natural burrow was measured with calipers once the  $O_2$  concentration profiles for that burrow were measured.

	Lower Phillips Creek	Machipongo River	South Hog Island
Natural	7	6	6
Artificial	5	1	-
Control	5	1	-
Total	17	8	6

Table 2.1. Number of replicate burrows (n) for each treatment at each site.

Measured  $O_2$  concentrations were used to determine the depth of maximum  $O_2$ penetration. The rays from an individual burrow were used as replicates; therefore mean  $O_2$  profiles could be calculated for each of the five distances from a burrow. If within the  $O_2$  concentration profile  $O_2$  concentrations decreased to zero and then deeper within the profile the  $O_2$  concentration increased again to above zero, then the initial zero concentration was used as the depth for maximum  $O_2$  penetration.

#### Modeled O<sub>2</sub> Concentration Profiles, Consumption Profiles, and Fluxes

To determine the mean  $O_2$  consumption and total flux for each of the mean  $O_2$ profiles at the five distances from the burrow the PROFILE program (Berg et al. 1998) was used. This software models the diffusion-mediated  $O_2$  consumption rate within the sediment using the curvature of the measured  $O_2$  concentration profiles. The mean consumption profile was determined for each of the five distances from the burrow for each of the burrows (n = 25) at the two main field sites.

Following the manual for the PROFILE program, the basic input parameters for each run of the PROFILE program were the same for each run of the model (i.e. each distance from each burrow). Input values used are listed in Table 2.2. For each burrow, the measured porosity for that burrow was used in the model input. For each of the five distances from each individual burrow the mean measured  $O_2$  concentrations (nmol/ cm<sup>3</sup>) and corresponding depths (cm) were used in the model input. Biodiffusivity and irrigation were both zero for all input depths.

Line 2	Depth at top of calculation domain (cm)	0	
Line 3	Depth at bottom of calculation domain (cm)	0.1 cm below last non-zero concentration value*	
Line 4	Max number of equally spaced zones	number of non-zero data points minus 1, with a maximum number of 10	
Line 5	Type of boundary condition	3	
Line 6	First boundary condition: concentration at bottom (nmol/cm <sup>3</sup> )	0.0	
Line 7	Second boundary condition: flux at bottom (nmol/cm <sup>2</sup> /s)	0.0	
Line 8	Diffusivity in water (cm <sup>2</sup> /s)	1.17 E-5	
Line 9	Expression for sediment diffusivity	3	
Line 10	Concentration in water column	999.999 (dummy variable)	
Line 11	Minimum production rate (nmol/cm <sup>3</sup> /s)	-1.0 E+20	
Line 12	Maximum production rate (nmol/cm <sup>3</sup> /s)	1.0 E+20	
Line 13	Maximum deviation (in %) when accepting a calculated minimum	0.001	
Line 14	Level of significance in the F statistic	0.01	

 Table 2.2. Input Criteria for PROFILE Program

\*resulting in the input of only the first two zero concentration values (If concentration went to zero and back up the concentrations below the first zero were ignored.)

#### **Sediment Cores**

A sediment core with a diameter of 4.4 cm was taken near each burrow at the two main sites once all of the  $O_2$  measurements were complete. A slice of the top centimeter of each core was collected and oven dried at 60° until constant weight. The wet weight and dry weight of each sample was used to calculate porosity ((wet wt. – dry wt.)/ volume) and bulk density (dry wt./volume). The sediment samples were put in a muffle furnace at 500°C for 6 hrs. to determine organic content by loss on ignition.

#### **Statistical Analyses**

All statistical analyses were performed using SAS 9.2 (SAS Institute Inc., Cary, NC, USA). For the sediment samples, the differences in porosity, bulk density, and organic content between the two main sites were compared using a MANOVA. The differences in depth of maximum O<sub>2</sub> penetration and total flux were compared among the five distances from the burrow within each treatment (Natural, Artificial, no burrow Control), and among the three treatments within each of the five distances from the burrow. These comparisons were analyzed using the PROC MIXED command in SAS. Depth of maximum O<sub>2</sub> penetration, burrow, and ray were treated as random effects, and distance from the burrow (distance) was treated as a fixed effect; burrow was nested within treatment, and ray was nested within burrow within treatment; distance was a continuous variable. When a significant difference ( $p \le 0.05$ ) was determined for the interaction between treatment and distance the specific differences were determined by performing a post hoc test of all 45 possible contrast statements: pair-wise comparisons of the five distances within each of the three treatments, and pair-wise comparisons of the three treatments within each five distances. To make these tests conservative, a Dunn and Sidak correction was applied to the comparison-wise alpha:  $\alpha = 1 - (0.95^{1/45}) =$ 0.0011. Least squares means and standard error were calculated for depth of maximum O<sub>2</sub> penetration and total flux; these are the means and standard errors reported.

To determine how *Uca* spp. burrows affect surrounding sediment  $O_2$ , four different approaches were used to best understand the effects. First, the measured mean  $O_2$  profiles at each of the five distances from the burrows (n = 25) were determined. These mean  $O_2$  profiles were graphed concurrently with the  $O_2$  consumption profiles modeled with the PROFILE program. The result was a set of five depth profiles, one at each of the five distances from the burrow (2, 4, 6, 8, and 10 mm), for each of 25 burrows (burrows from the South Hog Island site were not included because porosity for those burrow location was not measured). Using the sets of depth profiles, the distance to which the presence of a burrow had an effect on the  $O_2$  concentration was interpreted. The second approach involved graphing all five mean  $O_2$  concentration profiles using a matrix to create 2-dimensional color contours (depth and distance from the burrow) for all of the burrows (n = 31). The third approach utilized statistical analysis to determine the depth at which the  $O_2$  concentration first reached zero (depth of maximum  $O_2$ penetration) for each individual  $O_2$  profile that was measured at the two main sites. The final approach also utilized statistical analysis by comparing the total  $O_2$  flux value calculated by the PROFILE program (Berg et al. 1998) for each distance from each burrow at the two main sites.

#### Results

The temperatures in which the measurements were made varied among burrows, resulting in different  $O_2$  saturation values for the measurements surrounding each burrow. The range in temperature in which the measurements were taken varied from  $21.5 - 34.5^{\circ}$  C. The solubility of  $O_2$  at these temperatures varied from  $172.2 - 212.2 \,\mu$ M. Burrow diameters for the two main sites ranged from  $8.8 - 15.7 \,\mu$ m with a mean of  $12.66 \,\mu$ m.

The sediment characteristics measured near each burrow at each of the two main sites consisted of porosity, bulk density, and percent organic content. Results of a MANOVA for these three sediment characteristics for the two sites was not significant (Wilks' Lambda=0.783; F(3,21)=1.94; p=0.1542). Porosity was slightly higher at Lower

	Lower Phillips Creek	Machipongo River
Porosity (ml/cm <sup>3</sup> )	$0.53 \pm 0.02$	$0.46 \pm 0.03$
Bulk Density (g/cm <sup>3</sup> )	$1.61 \pm 0.07$	$1.72 \pm 0.09$
Organic Content (%)	$3.32 \pm 0.34$	$1.95\pm0.49$

Table 2.3. Sediment Characteristics from the two main sites (mean  $\pm$  SE).

#### **Overall Concentration and Consumption**

In many of the mean profiles,  $O_2$  concentration increased from the first measurement at depth zero to the second measurement at 0.5 mm and then began to decrease steadily to zero. The  $O_2$  consumption rates modeled with the PROFILE program show this increase in  $O_2$  concentration as production (negative consumption) at the top of most of the profiles. Oxygen production is almost exclusively seen at the top of the profiles, but some of the profiles have a small amount of production occurring farther down in the profile. These rates of production found farther down in the profiles are much smaller than the initial production rates at the surface of the sediment.

#### **Concentration and Consumption Depth Profiles**

#### **Concentration**

At each distance from each burrow, one mean  $O_2$  depth profile was created (Figure 2.4). Each set of depth profiles consists of five graphs, one at each of the five distances from the burrow (Figures 2.5 – 2.8). The sets of depth profiles from Natural burrows from the two main sites (n = 13) showed a variety of  $O_2$  concentration profile



Figure 2.4. Oxygen Depth Profile Explanation. Oxygen depth profile for one distance from a single burrow. Solid squares (with error bars) connected by thin line show the mean ( $\pm$  SE) of measured O<sub>2</sub> concentrations for all profiles at that distance from a single burrow. Dotted line (appears as a thick line in smaller graphs) shows O<sub>2</sub> concentration modeled with PROFILE program for the same data and only sometimes differs from the measured values (ex. at 2.5 mm). Thin vertical line shows the O<sub>2</sub> consumption modeled with PROFILE program (negative values indicate production); consumption is equal to zero when the oxygen concentration profile. Figures 2.5 – 2.9 have the same axes, units and scales as this graph.

Figures 2.5 - 2.8 A - E. (Following Page) Measured oxygen concentration profiles with modeled oxygen concentrations and consumption profiles at five distances from different natural burrows A) 2 mm, B) 4 mm, C) 6 mm, D) 8 mm, E) 10 mm.

Figure 2.5. Graphs showing higher  $O_2$  concentration present at greater depth in the sediment close to the burrow wall compared to farther from the burrow wall (Type I). Shows expected effect of the presence of a burrow on  $O_2$  concentration.

Figure 2.6. Graphs showing higher  $O_2$  concentration present at slightly greater depth in the sediment close to the burrow wall compared to farther from the burrow (Type II). Shows a slight effect of the presence of the burrow on  $O_2$  concentration.

Figure 2.7. Graphs showing no difference in  $O_2$  depth penetration with distance from the burrow wall (Type III). Shows no effect from the presence of the burrow on  $O_2$  concentration.

Figure 2.8. Graphs showing a difference among the  $O_2$  depth penetration with distance from the burrow wall, but greater depth penetration is not close to the burrow (Type IV). Shows no effect from the presence of the burrow on  $O_2$  concentration.



patterns. Some of the sets showed higher  $O_2$  concentrations present at greater depth in the sediment close to the burrow wall, which was a clear effect of the presence of the burrow, where as others did not show higher  $O_2$  concentrations present at greater depth in the sediment near the burrow wall (Figures 2.5 - 2.8). For example, one of the sets from a burrow at the Machipongo River site showed higher  $O_2$  concentration at depth close to the burrow wall compared to farther from the burrow wall (Type I). This clear effect of the presence of the burrow could be seen at both the 2 mm and 4 mm distances from the burrow wall (Figures 2.5A & B). At the 2 mm distance from the burrow, the depth at which the mean  $O_2$  concentration reached zero ( $O_2$  penetration depth) was 6 mm (Figure 2.5A). This result was very different from the 6, 8, and 10 mm distances where the  $O_2$ penetration depth was much less at about 2 mm (Figures 2.5C - E). At the 4 mm distance, the  $O_2$  penetration depth was 4 mm (Figure 2.5B); this result was intermediate between the 2 mm and 6 mm distances.

Another set of depth profiles from a burrow at the same site showed higher  $O_2$  concentrations present at only slightly greater depths in the sediment near the burrow wall compared to farther from the burrow wall (Type II) (Figure 2.6). This set had a greater  $O_2$  penetration depth for the 2 mm distance only; the  $O_2$  penetration depth was 3.5 mm (Figure 2.6A). Farther from the burrow the  $O_2$  penetration depth was about 2 mm; this result was similar to the  $O_2$  penetration depth at greater distances from the burrow seen in Figure 2.5. Other sets of depth profiles showed no discernible  $O_2$  concentration pattern as the distance from the burrow wall increased. Some sets of depth profiles showed no difference in  $O_2$  depth penetration among the five distances from the burrow wall (Type III); indicating that there was no effect from the presence of the burrow, such
as a set from a burrow at the Lower Phillips Creek site (Figure 2.7). Each depth profile in this set had an  $O_2$  penetration depth of about 2 mm.

Other sets of depth profiles had differences between profiles, but they did not have higher  $O_2$  concentrations present at greater depths near the burrow, but at a greater distance (Type IV), indicating that there was no effect from the presence of the burrow. For example, a set of profiles from the Machipongo River site (Figure 2.8) had the greatest  $O_2$  penetration depth at the 6 mm distance (Figure 2.8C). Some of the sets of depth profiles from these Natural burrows show a very different pattern compared to the sets from the Control burrows (Figure 2.9). Most of the sets of profiles from the Control burrows did not have any  $O_2$  below a depth of 3 mm. As seen in Figure 2.9, the five profiles do not show a difference in  $O_2$  penetration depth, as would be expected when there is no burrow present. The sets of profiles from Artificial burrows had a similar pattern to those of the Natural burrows, which can be seen in Appendix 1.

#### Consumption

Depending on the curvature of the line depicting the modeled  $O_2$  concentration, the consumption is either positive or negative (production). If the  $O_2$  concentration profile line is a parabolic-type curve opening to the right, then consumption is occurring; if the  $O_2$  concentration profile line is a parabolic-type curve opening to the left, then production is occurring (see Figure 2.4). At the inflection point of the line, production switches to consumption or vice versa. At the top of many of the profiles there is an obvious spike in  $O_2$  concentration making an obvious parabolic-type curve opening left indicating production. These  $O_2$  concentration spikes show extremely negative consumption values (i.e. production) (ex. Figure 2.6D) compared to  $O_2$  concentration



Figure 2.9 A - E. Measured oxygen concentration profiles with modeled oxygen concentration and consumption profiles at five distances from a no burrow Control: A) 2 mm, B) 4 mm, C) 6 mm, D) 8 mm, E) 10 mm. Graphs showing no difference in  $O_2$  depth penetration with greater distance, as would be expected with no burrow present.

profiles that gradually decrease from an initial maximum (ex. Figure 2.5E). Only four profiles (Figures 2.6B, 2.7C, 2.7E, and 2.8B) from the twenty shown in Figures 2.5 - 2.8show no production at the top of the profile. There is no obvious pattern in the consumption rates as the distance from the burrow wall increases for the categories of burrow (Type I – IV) described in the above Concentration section (Figures 2.5 - 2.9). At a certain depth within the sediment,  $O_2$  is no longer present and therefore the rate of  $O_2$  consumption becomes zero. For the sediments at the two main sites, this depth would occur close to the depth of maximum  $O_2$  penetration, which is reported further in this section.

### **2D Color Contour Plots**

Color contours were used to visualize the change in  $O_2$  concentration with depth and distance from the burrow simultaneously. Using the color contours, the extent to which the presence of the burrows affected the sediment  $O_2$  concentrations could be determined more clearly. Color contours were created for all burrows measured (including those from Hog Island) (n = 31). The color contours from Natural burrows from the two main sites (n = 13) showed a variety of patterns (Figures 2.10 – 2.13). Some of the color contours appear exactly as hypothesized, showing greater depth of  $O_2$ penetration closer to the burrow wall (Type I), indicating an effect of the presence of the burrow, but this was only true for three of these Natural burrows (Table 2.4). The color contour of a burrow from the Machipongo River site shows higher  $O_2$  concentration present at greater depth near the burrow a clear effect of the presence of the burrow (Figure 2.10). At the 2 mm distance, the depth at which the mean  $O_2$  concentration reached the 0 - 13  $\mu$ M range ( $O_2$  penetration depth) was nearly 5 mm. For the 4 mm



Figures 2.10 – 2.13. Oxygen profile color contours for four different natural burrows.

Figure 2.10. Graph showing greater  $O_2$  penetration depth at close proximity to the burrow and less  $O_2$  depth penetration with distance from the burrow wall (Type I). Shows a clear effect of burrow presence on  $O_2$  concentration.

Figure 2.11. Graph showing slightly greater  $O_2$  penetration depth at close proximity to the burrow compared to farther from the burrow wall (Type II). Shows a slight effect of burrow presence on  $O_2$  concentration.

Figure 2.12. Graph showing no difference in  $O_2$  depth penetration with distance from the burrow wall (Type III). Shows no effect from the presence of the burrow on  $O_2$  concentration.

Figure 2.13. Graph showing a difference among the  $O_2$  depth penetration with distance from the burrow wall, but greater depth penetration is not close to the burrow (Type IV). Shows no effect from the presence of the burrow on  $O_2$  concentration.



distance the O<sub>2</sub> penetration depth was nearly 3 mm. Moving away from the burrow (6 -10 mm) the  $O_2$  penetration depths were between 2 – 2.5 mm, showing no effect from the burrow at these greater distances. Another color contour from a burrow from the same site showed a less dramatic effect on O<sub>2</sub> depth penetration near the burrow (Type II) (Figure 2.11). At the 2 mm distance the  $O_2$  penetration depth was just over 3 mm, but at the 4 -10 mm distances the  $O_2$  penetration depth was from less than 2 mm to just over 2.5 mm. The color contours of some burrows showed no evidence of the presence of the burrow. They had almost horizontal bands of equal O2 concentration across all distances (Type III), as shown in the color contour from a burrow from the Lower Phillips Creek site (Figure 2.12). This color contour had a  $O_2$  penetration depth of 1 - 2 mm that was almost constant across all distances with the slightly deeper values closer to the burrow. Color contours from some burrows showed no explainable pattern of O<sub>2</sub> concentration with distance from the burrow (Type IV). A color contour from a burrow at the Machipongo River site had the greatest O<sub>2</sub> penetration depth at the median distance from the burrow, indicating no effect from the presence of the burrow (Figure 2.13). Many of the color contours from these Natural burrows show a very different pattern compared to the sets from the Control burrows (Figure 2.14). The color contours from the Control burrows did not show any  $O_2$  below about 2.5 mm. As seen in Figure 2.14, there was no difference in O<sub>2</sub> penetration with distance from the burrow, this is the expected pattern when there is no burrow present. The color contours from Artificial burrows had a similar pattern to those of the Natural burrows, some showed a distinct effect from the burrow with higher O<sub>2</sub> concentrations at greater depths near the burrow wall, and others showed no pattern at all. The same four Natural burrows and Control burrow were used







as examples for both the set of depth profiles (Figures 2.5 - 2.8 and Figure 2.9) and the color contours (Figures 2.10 - 2.13 and Figure 2.14) so that comparisons could be made between them.

The color contours from the six burrows at the Hog Island site show three different patterns. Two burrows located in muddier sediment showed no effect on  $O_2$ penetration depth with distance from the burrow. The  $O_2$  concentrations varied little with distance from the burrow (Appendix 1; Figures A41 & A42). Three of the burrows were located in vary sandy sediment that was well-drained. These burrows had color contours that show high  $O_2$  concentration for all measured depths and distances (Appendix 1; Figures A43 – A45). The final burrow from the Hog Island site had a color contour with higher  $O_2$  concentrations at greater depth close to the burrow within the entire 10 mm depth profile (Appendix 1; Figure A46).

The category (Type I – IV) of  $O_2$  concentration pattern was determined for each of the color contours (excluding the three fully aerated ones from South Hog Island) by burrow treatment: Natural, Artificial, and no burrow Control (Table 2.4).

	Natural	Artificial	Control
Type I	10, A21, A25 (3 Total)	A30 (1 Total)	-
Type II	11, A27, A29 (3 Total)	A31, A33, A35 (3 Total)	-
Type III	12, A22 (2 Total)	A34 (1 Total)	14, A36, A38, A40 (4 Total)
Type IV	13, A23, A24, A26, A28, A29 (6 Total)	A32 (1 Total)	A37, A39 (2 Total)
Total	14	6	6

Table 2.4. Categories for O<sub>2</sub> Concentration Patters by Treatment. Figure Numbers for Corresponding Burrows Listed and Total Number of Burrows for Each Type.

Sets of depth profiles and color contours from the remaining 20 burrows from the two main sites and the color contours from the 6 South Hog Island Natural Burrows are in Appendix 1.

# Depth of Maximum O<sub>2</sub> Penetration and Total Flux Statistics

There was no significant treatment effect for depth of maximum  $O_2$  penetration, but there were significant differences found for distance (p<0.0001) and for the treatment x distance interaction (p=0.0002). Because there was a significant effect for the interaction between treatment and distance, post hoc tests consisting of all possible pairwise contrast statements were run. There was a significant difference (p<0.0001) in depth of maximum  $O_2$  penetration between distance 2 mm and the other four distances (4, 6, 8, 10 mm) within both the Natural and Artificial treatments (Figures 2.15A & 2.15B). Within the Control treatment, there were no differences detected for the depth of maximum  $O_2$  penetration among any of the five distances (Figure 2.15C). Within



Figure 2.15. Graphs of depth of maximum  $O_2$  penetration for each of the five distances from the burrow: 2 mm, 4 mm, 6 mm, 8 mm, and 10 mm within the three treatments A) Natural, B) Artificial, and C) no burrow Control. Means ± SE. Significant differences noted with different letters.



Figure 2.16. Graphs of depth of maximum  $O_2$  penetration for each of the three treatments Natural, Artificial, and no burrow Control within each of the five Distances A) 2 mm, B) 4 mm, C) 6 mm, D) 8 mm, and E) 10mm. Means ± SE. Significant differences noted with different letters.

distance 2 mm, there was a significant difference (p=0.0005) between the Artificial and Control treatments (Figure 2.16A). The next two most significant pair-wise contrasts were also within distance 2 mm, the difference between Artificial and Natural were nearly significant (p=0.006). Within distance 2 mm, the Artificial treatment had the greatest depth of maximum  $O_2$  penetration with a mean of  $3.3 \pm 0.2$  mm, the Natural treatment had the next largest depth of maximum  $O_2$  penetration with a mean of  $2.5 \pm 0.2$ mm and the Control treatment had the smallest depth of maximum  $O_2$  penetration with a mean of  $2.0 \pm 0.2$  mm (Figure 2.16A).

Table 2.5. Maximum  $O_2$  penetration depth (mm) for each of the three treatments at each of the five distances from the burrow. LS Means ( $\pm$  SE) reported.

		Distance (mm)					
		2	4	6	8	10	
Treatment	Natural	2.5 (± 0.2)	1.9 (± 0.2)	1.8 (± 0.2)	1.7 (± 0.2)	1.8 (± 0.2)	
	Artificial	3.3 (± 0.2)	2.1 (± 0.2)	1.9 (± 0.2)	2.0 (± 0.2)	2.0 (± 0.2)	
	Control	2.0 (± 0.2)	2.1 (± 0.2)	2.0 (± 0.2)	2.0 (± 0.2)	2.0 (± 0.2)	

There were no significant treatment, distance, or treatment\*distance interaction effects for total flux for the two sites. Total flux ranged from - 14.114 to 21.325 nmol/ cm<sup>2</sup>/s.

		Distance (mm)					
		2	4	6	8	10	
Treatment	Natural	21.325 (±7.403)	16.178 (±7.403)	10.845 (±7.403)	14.910 (±7.403)	9.061 (±7.403)	
	Artificial	3.754 (±10.8972)	-6.385 (±10.8972)	-5.118 (±10.8972)	-0.996 (±10.8972)	-7.354 (±10.8972)	
	Control	-1.991 (±10.897)	-6.880 (±10.897)	6.212 (±10.897)	-7.027 (±10.897)	-14.114 (±10.897)	

Table 2.6. Total Flux (nmol/cm<sup>2</sup>/s) for each of the three treatments at each of the five distances from the burrow. LS Means ( $\pm$  SE) reported.

### Discussion

These results show that *Uca pugnax* burrows in poorly drained sediments only significantly affect the concentration of O<sub>2</sub> in the surrounding sediment to a distance of 2 mm. At 2 mm distance from the burrow, the O<sub>2</sub> depth of the Natural burrow only differed from the Control by 0.5 mm. This extends the oxic zone very little in terms of depth, but it extends it by 25% compared to there being no burrow present. The increased volume of oxic sediment per burrow assuming an average diameter of 12.7 mm is 44.4 mm<sup>3</sup>. The number of crab burrows along the western Atlantic in marshes where crabs are present range from approximately 20 to 700 per m<sup>2</sup> (Allen & Curran 1974, Basan & Frey 1977, Aspey 1978, Krebs & Valiela 1978, Ringold 1979, Katz 1980, Montague 1982, Bertness & Miller 1984, Bertness 1985, Nomann & Pennings 1998, McCraith et al. 2003, Holdredge et al. 2010); approximately 42-50% of burrows have diameters equal to or greater than 12.7 mm (Bertness 1985), therefore, crab burrows could have an effect on under 900 mm<sup>3</sup> to over 13,000 mm<sup>3</sup> of sediment per m<sup>2</sup> of sediment surface.

Salt marsh sediments are typified by reduction reactions within the upper portion of the sediment profile (Day et al. 1989, Howarth 1993, Mitsch & Gosselink 1993): oxygen reduction, nitrate reduction, manganese reduction, iron reduction, sulfate reduction, methane production and others (Berner 1980, Fenchel et al. 1998, Howarth 1993). In this study, O<sub>2</sub> was not present below a certain depth for any given profile because it was consumed in reactions with reduced ions and by mineralization within the sediment (Furukawa 2005, Jorgensen & Revsbech 1985). The oxidation reactions extend the oxidized zone (Kristensen 2000) in the sediment profile. Therefore, in this study, at a distance 2 mm from the burrow, the oxidized zone also extends deeper into the sediment, but redox potential was not measured. The focus of this study was not redox potential and given the methods for this study, it was not possible to measure redox potential.

It is not surprising that the burrow only had an effect on depth of maximum  $O_2$ penetration to a distance of 2 mm because the vertical diffusion of  $O_2$  at the sediment surface was not quite 2 mm deep. The burrow likely had a greater effect at distances less than 2 mm distance from the burrow, but this distance was not measured in this study.

Total depth integrated  $O_2$  flux within the top 10 mm of the sediment was not different for the three treatments nor for the five distances from the burrow. There are many factors that affect pore water  $O_2$  flux including: diffusivity,  $O_2$  concentrations in overlying water,  $O_2$  penetration depth, sediment porosity, and organic content (Cai & Sayles 1996). Water quality data are available for a location near the Lower Phillips Creek site for the months of July and August during the study period. The data are from the mouth of Phillips Creek located about 2 km down stream from the field site. The July and August  $O_2$  concentrations were 121.2  $\mu$ M (60.2% saturation) and 105.6  $\mu$ M (50.9% saturation) respectively (McGlathery et al. 2000). This indicates that when the marsh surface is inundated the incoming water is not at  $O_2$  saturation (100%). Seasonally the  $O_2$ concentration in the inundating creek water changes from a low value in August of 105.6  $\mu$ M to a high value in February of 314.4  $\mu$ M. This seasonal fluctuation in  $O_2$  content of the incoming tidal water would also likely affect the seasonal pattern of  $O_2$ concentrations within the sediment. Oxygen penetration depth has been shown to vary seasonally (Cai & Sayles 1996). This study did not investigate the seasonal effect on  $O_2$ concentrations but rather focused on comparing the three treatments and having sufficient replication.

Oxygen is only measurable within the first few millimeters of shallow benthic and intertidal sediment because O<sub>2</sub> is the preferential and energetically the most efficient electron acceptor (Fenchel et al. 1998), and is also consumed in the oxidation of reduced compounds. The depth of O<sub>2</sub> penetration determines the depth distribution of numerous redox reactions in the sediment (Cai & Sayles 1996). Sediment with low amounts of organic carbon has less O<sub>2</sub> consumption and O<sub>2</sub> can penetrate deeper into the sediments (Cai & Sayles 1996). If the flux of a non-reactive solute (e.g. silica) was measured radially from a crab burrow, the effect of the presence of a burrow on diffusion exclusively could be determined and could be more easily modeled (Berner 1980).

Many burrowing dwelling macro/ meio fauna found in marine sediments e.g. shrimp (Webb & Eyre 2004a, Koike & Mukai 1983), bivalves (Berner 1980), and polychaetes (Berner 1980, Fenchel 1996, Aller 1988, Kristensen et al. 1991, Kristensen & Hansen 1999), irrigate their burrows for feeding or respiration (Furukawa 2005, Kristensen & Kostka 2005). This alters the O<sub>2</sub> diffusion and consumption rates within the surrounding sediment (Wenzhofer & Glud 2004). Also, the activity of respiration by these animals within their burrows increases O<sub>2</sub> uptake. Uca spp. do not irrigate their burrows (Pearse 1914, Dembowski 1926, Teal 1959, Crane 1975, de la Iglesia et al. 1994, Koretsky et al. 2002). Therefore, it is only the physical presence of the burrows themselves that affects O<sub>2</sub> diffusion. Some burrow dwelling macrofauna line their burrows with tubes or mucus (Kristensen 2000, Kristensen & Kostka 2005) which alters the organic carbon and the movement of water along the burrow wall (Kristensen & Kostka 2005). Uca spp. do not line their burrows with any material (Bertness & Miller 1984). Thalassinidean shrimp create burrows in intertidal and subtidal sediments and increase sediment  $O_2$  consumption by as much as 81% (Webb & Eyre 2004a). The increase in O<sub>2</sub> consumption was mainly caused by oxidation reactions and microbial respiration in the burrow (85%), but only 15% was attributed to respiration of the shrimp. Like the Uca spp., this shrimp is a detritivore, the shrimp decreased surface chlorophyll-a concentrations by 50%, affecting  $O_2$  production and consumption and depth penetration (Webb & Eyre 2004a).

Factors affecting  $O_2$  in surface sediments include temperature, salinity, sediment drainage, sediment type, and turbulence in certain cases. These are site specific, as are  $O_2$  consumption rate and the rate constant for organic carbon degradation, so there is no good general predictor for  $O_2$  penetration depth at different sites (Cai & Sayles 1996). Other environmental variables are also site specific: the concentration of terminal electron acceptors, toxic metabolites, microbial activities, and net solute transport (Furukawa 2005). Salt marshes are heterogeneous in almost every aspect both within and between marshes. This ecosystem does not only have dramatic changes within space, but these systems change over time, whether it be a tidal cycle or the length of time a burrow exists in the marsh. In this study, the burrow residence time (length of time that the burrows existed) is unknown. Burrows (especially if being maintained) affect  $O_2$ dynamics on different spatial and temporal scales, especially when coupled with tidal effects, which are pulsing perturbations within the system.

The pore space between the sediment particles did not drain in either of the two main field sites. Pore water advection is not important in these systems, therefore, oxygen was only able to enter the sediment pore water by diffusion from the surface water or the atmosphere. Many of the burrows in this study drained more than a centimeter, creating large amounts of surface area exposed to the atmosphere at low tide. But at depths of 10 mm even though the burrow wall was exposed to the atmosphere, the horizontal penetration of  $O_2$  was undetectable. It was surprising that  $O_2$  was rarely found more than a few millimeters from the sediment surface.

At the top of the  $O_2$  profiles the spike in  $O_2$  concentration (as well as the negative consumption rates) was most certainly due to  $O_2$  production from surficial microalgae or microphytobenthos. Microphytobenthos consist of microscopic algae and cyanobacteria that live on the sediment surface and produce  $O_2$  during photosynthesis (Revsbech et al. 1981, Baillie 1986, Revsbech & Jorgensen 1983, Miller et al. 1996). During the daytime,  $O_2$  production of these organisms can be high and the sediment can become saturated or supersaturated with  $O_2$  (Revsbech et al. 1988). Because this study was *in situ*, it was logistically impractical to make these measurements in the dark, therefore a portion of the measured surficial  $O_2$  concentration was affected by microphytobenthos photosynthesis. The spike of  $O_2$  near the sediment surface can be seen in many of the depth profiles (ex.

Figures 2.5B, 2.6D, and 2.6E). This spike in  $O_2$  concentration affected the  $O_2$  consumption as seen in the highly negative consumption values associated with dramatic spikes in  $O_2$  concentration at the surface. The total  $O_2$  flux calculations were likely greatly affected by the high production at the top of the profiles.

Different species groups of microphytobenthos may have different effects on O<sub>2</sub> penetration, but in this study the types of microphytobenthos present were not determined. The O<sub>2</sub> production on the sediment surface enhances the 3-layer model of a sediment profile: oxic zone, oxidized zone (suboxic), reduced zone (Kristensen 2000). The O<sub>2</sub> production on the sediment surface was not evident consistently. Other studies show these microphytobenthos are patchy in their presence spatially and are affected temporally by the diurnal cycle and the amount of light (Glud et al. 2002), and a similar patchy spatial pattern would be expected at this study location. The nonuniform presence of microphytobenthos on the sediment surface is likely partially due to the feeding habits of the Uca spp., because they are detritivores and obtain a portion of their nutrients from microalgae (Peterson et al. 1986, Peterson & Howarth 1987, Sullivan & Moncreiff 1990, Currin et al. 1995). With similar feeding habits to Uca spp., Webb & Eyre (2004b) found that the grazing habits of the soldier crab (*Mictyris longicarpus*) caused a reduction in the amount of microalgae on the sediment surface; this caused a reduction in benthic O<sub>2</sub> production and sediment O<sub>2</sub> consumption. In a light vs. dark experiment, there was considerably greater O<sub>2</sub> concentration at the surface in the light treatment where there were no crabs to consume the surficial microalgae. The presence of the crabs increased the depth of O<sub>2</sub> within the sediment because there was less organic carbon in the sediment to consume O<sub>2</sub> as shown by the net 24 hour consumption, therefore the O<sub>2</sub> was

able to penetrate deeper into the sediment (Webb & Eyre 2004b). Microphytobenthos can also colonize the walls of burrows altering the  $O_2$  flux at the vertical sediment surface (Furukawa 2005).

Because *Uca* spp. only inhabit their burrows during high tide during their active season and do not irrigate their burrows, it is the physical presence of the burrow in the sediment and not the activity of the crab that alters sediment  $O_2$  dynamics. In benthic sediments containing the polychaete *Hediste diversicolor*, Wenzhofer & Glud (2004) were able to determine the amount of  $O_2$  uptake caused by the presence of the infauna burrow versus diffusive  $O_2$  uptake. Most of the  $O_2$  consumption was attributed to faunal activity, but this was mostly due to respiration surrounding the burrows due to irrigation of the burrow by the polychaete, and not respiration by the polychaete, showing that the presence of a burrow alone (if irrigated) has a large effect on  $O_2$  uptake without faunal respiration being involved.

Irrigation of burrows by polychaetes brings  $O_2$  to greater depths (Cai & Sayles 1996), but because *Uca* spp. do not irrigate their burrows this is not a mechanism by which  $O_2$  would have a greater penetration depth surrounding their burrows. From the geometry and density of macrofaunal burrows, the surface area of sediment exposed to the atmosphere can be compared to the surface area of sediments not containing burrows; the presence of burrows can account for the major portion of the volume of oxic sediment compared to that from the sediment surface (Wenzhofer & Glud 2004, Fenchel 1996, Kristensen 2000). Burrow densities and diameters were measured in other areas of the two marshes in this study. At these nearby locations, there was an overall mean burrow density of 112 /m<sup>2</sup> for the two sites. In this study, the natural burrows had a 0.484 mm

increase in the depth of oxic sediment compared to sediment without burrows, therefore each burrow essentially adds additional surface area through which  $O_2$  can diffuse. The increased area equals the burrow opening circumference multiplied by the  $O_2$  penetration depth increase. Using the burrow density of the nearby area, there is an effective 0.2 % increase in surface area of the marsh caused by the presence of the burrows. Marshes that have well-drained sediments will have a greater increase in effective surface area from the presence of burrows.

Burrow characteristics change water movement and O2 diffusion into and around burrows. The tidal action of waves and currents can affect water flow through burrows (Berner 1980) and therefore the flushing of the sediment; if there are two openings there can be tidal irrigation especially if the openings differ in elevation (Stieglitz et al. 2000, Munksby et al. 2002). If the sediment is not flat at the sediment-water interface, water can be forced into or out of the sediment depending on the topography (Huettel et al. 1998). This passive irrigation by tides is an important process because Uca spp. do not irrigate their burrows (Kristensen & Kostka 2005). The 3-D topographic structure of the sediment surface can affect diffusion by increasing the surface area and also the angle at which the microelectrode is inserted into the sediment (Glud et al. 2003). In the process of excavating burrows, Uca spp. can change the surface topography near the burrow entrance by depositing the fill from the burrow (Kristensen & Kostka 2005). Some Uca spp. also build chimneys surrounding the opening of the burrow (Wada & Murata 2000, Shin et al. 2005, Slatyer et al. 2008), this would also change the movement of water near the burrow when they are submerged by the tide. None of the burrows in this study had chimneys or signs of sediment mounding near the burrow opening. At low tide Uca spp. also sometimes plug their burrows (Pearse 1914, Dembowski 1926, Teal 1959, Crane 1975, de la Iglesia et al. 1994, Koretsky et al. 2002). This would affect water movement surrounding the burrow as well as  $O_2$  diffusion.

Sediment characteristics including texture, porosity, and percent organic carbon can alter interactions between the burrow and surrounding sediment and sediment pore water. In this study, these sediment characteristics were not statistically different between the two main sites, but at Lower Phillips Creek the porosity was slightly higher than at Machipongo River with values of  $0.53 \pm 0.02$  ml/cm<sup>3</sup> and  $0.46 \pm 0.03$  ml/cm<sup>3</sup> respectively. Bulk density was slightly lower at Lower Phillips Creek  $1.61 \pm 0.07$  g/cm<sup>3</sup> compared to  $1.72 \pm 0.09$  g/cm<sup>3</sup> at Machipongo River. The percent organic carbon at Lower Phillips Creek was 1.7 times higher than at Machipongo River; at Lower Phillips Creek the percent organic carbon was  $3.32\% \pm 0.34$  compared to  $1.95\% \pm 0.49$  at Machipongo River. The site on South Hog Island had very different sediment from the two main sites. This sandy site had high porosity and low organic content because that area was affected by an overwash and some of the sediments were almost pure sand and had little marsh vegetation present; therefore, little organic carbon had been produced. Some of the burrows on South Hog Island were in sediments that drained fully, allowing air to penetrate the sediment surface. In sediments such as these, the time since the tide ebbed leaving the sediment surface exposed to the atmosphere likely makes a large difference in the concentration of O<sub>2</sub> within the sediments surrounding the burrows. If the water has had enough time to drain, the sediment can be fully aerated to a depth of greater than 10 mm (see Appendix 1; Figures A43 - A45). In the two main sites that have muddy sediments, diffusion is the sole mechanism of  $O_2$  transport, but in sandy

sites, like South Hog Island, pore water advection can dominate as the mechanism of  $O_2$  transport instead (Kristensen & Hansen 1999). In these sandy sediments, the sediment is able to drain completely allowing  $O_2$  to penetrate to great depths. Because the presence of a burrow aids in preferential drainage in these sediments one can make  $O_2$  depth profile measurements before the sediment completely drains (soon after the start of ebb tide) so that the effect of the burrow on the  $O_2$  in the surrounding sediment can be observed more clearly (see Appendix 1; Figure A46). These measurements are time dependant and not steady-state over extended periods (a tidal cycle) (Berner 1980).

Microprofiles enable calculations of diffusive  $O_2$  exchange and volume specific flux (consumption/production) (Rasmussen & Jorgensen 1992, Berg et al. 1998), but it is one dimensional and assumes a flat sediment surface (Roy et al. 2002, Glud et al. 2003). From this study, the modeled consumption results from the PROFILE program utilize 1-D diffusion models and might not fully describe the 3-D diffusion flux near the burrow wall, therefore, underestimating or overestimating the  $O_2$  flux close to the burrow wall where there is a likely effect from the horizontal dimension not just the vertical dimension (Aller 1980, Cai & Sayles 1996).

*S. alterniflora* is the dominant macrophyte in the salt marshes in this region (Valiela et al. 1978). Oxygen penetration at the sediment surface and surrounding *Uca pugnax* burrows does not extend into the root zone of *S. alterniflora* at a depth of 1 to 20 cm (Blum 1993), which is below the 8 mm maximum depth of  $O_2$  penetration in this study. *S. alterniflora* has aerenchyma tissue to move  $O_2$  into roots. The amount of  $O_2$  within the roots is dependent on time of day, tidal level, and season (Gleason & Zieman 1981). Passive  $O_2$  release causes oxidation of the sediment around its roots and rhizomes

(Howes et al. 1981). Holmer et al. (2002) found an oxic microzone extending up to 2.5 mm from the root surface of *Spartina anglica* plants, indicating that diffusion of  $O_2$  from roots within the sediment also extends only a few millimeters from the  $O_2$  source.

## Conclusions

Based on the results, it is concluded that this study was able to reject the null hypothesis for the effects of  $O_2$  penetration close to the burrow wall. At a distance of 2 mm there was significantly greater  $O_2$  penetration than farther from the burrow wall. However, it is also concluded that this study failed to reject the null hypothesis for the depth to which this effect extended. It was originally hypothesized that the increased depth of O<sub>2</sub> penetration would extend to greater depths within the sediment if the burrow did not contain stagnant water. For this reason, a revised conceptual model was created to show that  $O_2$  is not present along the entire extent of the burrow wall exposed to the atmosphere (Figure 2.17). Additionally,  $O_2$  is not present to a constant distance out from the burrow wall (radial penetration) along depth profiles. The presence of Uca spp. burrows likely aids in oxidizing rather than aerating the surrounding sediments. A future study could examine redox potential and other reduced ions surrounding Uca spp. burrows following a similar methodology. In these salt marsh sediments, surface layer  $O_2$  is only present in the top few millimeters of the sediment column. Dissolved  $O_2$  in the sediment is likely consumed through oxidation of reduced ions and oxidation of organic matter. There is no direct effect of O<sub>2</sub> surrounding Uca spp. burrows on S. alterniflora production because the sediment volume affected by the burrows is a small percentage of the total sediment volume, and contact with roots in these areas is minor. Oxygen concentration profiles and penetration depth surrounding Uca spp. burrows depend



Figure 2.17. Revised Conceptual Model. Showing that  $O_2$  is not present along the entire exposed burrow wall. Oxygen is not present evenly along depth profiles close to the burrow wall. In the initial conceptual model,  $O_2$  had even distances of radial penetration away from the burrow wall to the depth of the water level. In the revised conceptual model,  $O_2$  does not have even distances of radial penetration away from the burrow wall. The distance of radial penetration decreases with depth, making  $O_2$  concentrations taper out as they reach greater depths.

greatly on the characteristics of the sediment in which the burrows are located. The  $O_2$  depth penetration is highly dependent on whether or not the sediment is able to drain, allowing air to enter the pore space, therefore the  $O_2$  depth penetration is dependent on the sediment characteristics including sediment texture and organic matter content.

# Chapter 3. Interactions among flood regime, sediment type, and fiddler crab burrows within a salt marsh mesocosm experiment

#### Background

The level of the world's oceans have changed throughout geologic time depending on the global temperature and global extent of ice (Miller et al. 2005). Following the last glacial maximum 21,000 years before present, sea-level rise (SLR) was rapid. In the Holocene (8,000 years before present), SLR began to slow down; SLR has been more stable for the last 2000 years (Fleming et al. 1998, Fleming 2000, Milne et al. 2005, Morhange et al. 2001). Recent rates of SLR derived from the instrument record shows that since 1870 sea-level has been increasing at faster rates (Bindoff et al. 2007 IPCC). Global eustatic SLR is due to the change in volume of water in the worlds oceans. The majority of the increase in the volume of water in the oceans is due to the thermal expansion of ocean water caused by warmer ocean temperature. A smaller portion of the increase in the volume of water in the olacial melt on land surfaces (e.g. mountain glaciers and small ice caps) (Church et al. 1991, IPCC 2001, Rahmstorf et al. 2007). Relative sea-level is the combined effect of the changes in the elevation of the Earth's crust in addition to changes in sea-level.

The Virginia coast is experiencing high rates of relative sea-level rise (RSLR). This is due to subsidence of the Earth's crust in addition to increases in sea-level. The rate of subsidence in this area is largely due to the local isostatic movement of the Earth's crust. Gravitational equilibrium of the Earth's crust during the last glaciation caused the land under the glacier to subside, causing a glacial forebulge beyond the subsidence area in the region of Virginia. When the glacier receded, the land where the glacier once was experienced isostatic rebound, and the glacial forebulge began to subside (isostatic adjustment). The subsidence of the glacial forebulge in Virginia is the cause of the high rate of RSLR (Miller & Douglas 2006, Engelhart et al. 2009, Hobbs et al. submitted). The east coast of the southern part of Virginia is experiencing one of the highest rates of RSLR along the east coast of the US (Stevenson et al. 1986) (Figure 3.1). Mid-Atlantic SLR has been determined from various tide gage records and other methods. Areas along the Virginia (USA) coast have been experiencing RSLR with rates with an average of 3 mm per year (Larsen 1998). Larsen (1998) determined a rate of 3.0 mm/yr for the mid-Atlantic from 1937 to 1997. The rate of RSLR in the study area is high, estimates include 3.5 - 3.9 mm/yr (Erwin et al. 2004), 2.8 -4.2 mm/yr (Ortel et al. 1989) 3.1 - 4.3 mm/yr (Emory & Aubry 1991). Recent predictions for future SLR include: 18 - 60 cm by 2100 (IPCC-Fourth Assessment 2007), 50 - 140 cm above the 1990 level by 2100 (Rahmstorf 2007), and 80 - 200 cm by 2100 (Pfeffer et al. 2008).

Sea-level rise is a potential threat to coastal salt marshes around the world, especially in areas where subsidence is occurring concurrently (Cahoon 1997). As sealevel rises, coastal marshes must either expand landward at a matched rate, or build up the sediment surface elevation in order to counter the rise in sea-level. Salt marshes are positioned on the landscape in relation to mean low and high tides (Bertness & Miller 1984) (Figure 3.2). If marshes are not able to expand landward nor increase their surface elevation, then the extent of coastal marsh will decrease (Kana et al. 1988, Moorhead & Brinson 1995) (Figure 3.3). In order for marshes to expand landward, the slope of the marsh is an important factor; steep slopes can prevent marsh transgression (Brinson et al. 1995). Sediment supply is also important for expansion at the marsh edge; deposition on



Figure 3.1. Relative sea-level rise rates for the East Coast of US. Showing high rate of RSLS in region of Easter shore of Virginia. Adapted from Stevenson et al. 1986.



Figure 3.2. Profile of salt marsh from tidal creek or open water to high, with marsh types indicated.



Figure 3.3. Changes in extent of marsh type with an increase in mean sea-level (Adapted from Moorhead & Brinson 1995).

the edge of the marsh can cause marsh expansion (prograding marsh) (Brinson et al. 1995); also marsh encroachment onto mudflats is another mechanism for marsh expansion (Erwin et al. 2004). In many developed areas, landward migration of marshes is not possible due to the presence of permanent structures; therefore vertical accretion becomes the sole mechanism for persistence of coastal marshes. If the vertical accretion of a marsh through sediment deposition and/or organic matter buildup cannot keep pace with an increase in relative sea-level, a shift in tidal inundation will occur. This will likely result in well-drained marsh sediments gradually becoming more poorly drained as the duration and vertical extent of sediment saturation increases. This research will provide new information on how one member of these complex communities, the fiddler crab (*Uca* spp), may influence the effects of sea-level rise on salt marsh processes.

As marshes become water-logged, sediments become increasingly anoxic and the chemical pathway of organic matter oxidation shifts to primarily sulfate reduction (Howarth 1993). This reduction pathway produces sulfide (Berner 1977), which is toxic to *Spartina alterniflora* (smooth cordgrass) (Bradley & Morris 1990a, Koch et al. 1990), the dominant macrophyte in salt marshes along the east coast of North America (Valiela et al. 1978). *S. alterniflora* has two growth-forms, tall and short, which are dependent on tidal inundation and edaphic characteristics; the two different forms grow in the low and high intertidal zone respectively (Valiela et al. 1978). Sulfide concentrations as low as 250 µM are able to decrease nitrogen uptake by *S. alterniflora* (Bradley & Morris 1990a), and concentrations of 2000 µM can reduce leaf elongation (Koch et al. 1990). *S. alterniflora* contends with many edaphic stressors (Mitsch & Gosselink 1993). Salt marshes are often considered nitrogen limited systems (Sullivan & Daiber 1974, Valiela

& Teal 1974, Patrick & DuLaune 1976, Mendelssohn 1979), this can limit production of plants growing within these environments. Production of *S. alterniflora* can also be limited by salt stress because salt must be excluded or secreted which is an energetic expense (Bradley & Morris 1990a). The sediment in which *S. alterniflora* grows is often anoxic which puts greater stress on the plants because they must pump oxygen into their roots through their aerenchyma (Maricle & Lee 2002).

Fiddler crabs (*Uca* spp.) are ubiquitous in marshes along the east coast of North America and other coastlines around the world (Teal 1958, Crane 1975). These decapods are detritivores and consume sediment from the marsh surface (Genoni 1991). Uca spp. excavate burrows that they use for mating, protection from prey, and hibernation in the winter (Montague 1980a). It is commonly considered that their burrowing activity facilitates aeration of the sediment (Genoni 1991, Nomann & Pennings 1998), release of sulfide (Katz 1980, Bertness & Miller 1984), increase sediment drainage (Bertness 1985, Hughes et al. 1998) and impact the energy and nutrient transfer within the marsh (Montague 1980b). A conceptual model for fiddler crab burrow interactions within well drained sediments are shown in Figure 3.4. Any changes that occur in the chemistry of the pore water have the potential to affect the growth and productivity of salt marsh plants in the vicinity. Changes in burrow density likely affect aboveground plant production through changes in the sediment and/or pore water characteristics (Bertness 1985). Instead of directly grazing on plants (a top-down control), fiddler crabs can affect plant productivity indirectly: their burrowing activity can alter the sediment and/or pore water (a bottom-up effect), which in turn affects the production of S. alterniflora, the dominant macrophyte with which they are associated.



Figure 3.4. Fiddler crab burrow interactions in well-drained sediment (adapted from Michaels 2004).

Many researchers have speculated about the ecological role that fiddler crabs play (Katz 1980, Montague 1980a, Bertness & Miller 1984, Bertness 1985, Genoni 1991, Nomann & Pennings 1998, Walsh 1998), but only a few studies have specifically investigated the changes in sediment pore water chemistry caused by their burrowing activity (Montague 1982, Bertness 1985, Nielsen et al. 2003, Michaels 2004, Thomas 2004, Kristensen & Kostka 2005, Koska et al. 2002a, Kostka et al. 2002b, Thomas & Blum 2010). To date, the few studies that have investigated the effect of changes in burrow density on aboveground plant production have not had consistent results. Montague (1982) and Bertness (1985) found that increased burrow density caused significantly increased S. alterniflora production; Bertness (1985) also found that decreased burrow density caused significantly decreased production, but Nomann and Pennings (1998) found that decreased burrow density had no effect on plant production in hypersaline sediments. Michaels (2004) found that there was no significant difference in aboveground S. alterniflora productivity with increased or decreased burrow density. Holdredge et al. (2010) found that with crab removal from sandy marsh environments there were significant decreases in above- and belowground biomass and stem height.

These conflicting results are most likely due to the location or position in the marsh in which the experiments were conducted: low marsh, mid-marsh, or high marsh. Different areas of a single marsh can be quite different depending on the sediment type (texture), organic matter content, distance to open water, elevation, and frequency/duration of tidal inundation. This study provides a more rigorous framework for examining the role that crab burrows play in marsh dynamics by controlling for the

effects of certain marsh characteristics, such as duration of tidal inundation, sediment type, and extent of drainage.

# Introduction

By aerating sediment, fiddler crab burrows can help maintain or increase S. alterniflora productivity (Montague 1982, Bertness 1985, Walsh 1998) and thereby aid the long-term persistence and resilience of the marsh. By maintaining higher productivity, the resilience and stability of a marsh is increased, allowing it to recover faster after a disturbance and to withstand chronic stressors in terms of maintaining its structure and function. The positive feedback of sediment aeration by fiddler crab burrows maintaining or increasing S. alterniflora productivity can occur when marshes are relatively well-drained (Bertness 1985). However, the presence of fiddler crab burrows affects sediment chemistry differently in poorly drained sediments. Instead of aerating the sediment and releasing toxic sulfide, the presence of the burrows can cause an increase in pore water sulfide concentration (Michaels 2004), thereby exacerbating the effects of RSLR instead of promoting marsh persistence. Such an increase in sulfide concentration can cause a decrease in S. alterniflora productivity, and lead to a negative feedback where RSLR increases and a greater area of marsh is affected by increased inundation, potentially leading to increased sulfide concentrations. Such a negative feedback would further decreases the persistence of the marsh.

There has been recent discussion of how fiddler crabs in their role as ecosystem engineers (*sensu* Jones et al. 1994) alter pore water biogeochemistry of salt marshes. It is typically thought that an increase in crab burrow density in marshes increases aeration and redox potential of the marsh sediment and would also help to release toxic sulfide from the sediments (Katz 1980, Bertness & Miller 1984, Bertness 1985, Nomann & Pennings 1998). An *in situ* study found that increased fiddler crab burrow density could significantly increase pore water sulfide concentrations in marshes that had poorly drained sediment (Michaels 2004). This increase in sulfide concentration is likely a result of either increased sulfate reduction, or decreased sulfide oxidation. These results are interesting because similar studies have not been conducted in water-logged sediments and the results were opposite those found in studies conducted in well-drained sediments.

# **Conceptual Model**

The relative sea-level associated with a coastal salt marsh has a direct effect on the drainage and aeration of the sediment due to the amount of time the marsh is inundated (Figure 3.5). The drainage and aeration of the sediment, in turn, has a direct effect on the pore water within the sediment. Sediment pore water, in turn, has a direct effect on the productivity and production of *S. alterniflora*. The productivity and production of *S. alterniflora*. The productivity and production of *S. alterniflora* within the marsh has an effect on the persistence of the salt marsh. Salt marsh persistence, in turn, has a direct effect on relative sea-level.

If this conceptual model is considered with an increase in relative sea-level, there would be a subsequent decrease in sediment drainage and aeration (Figure 3.6). A decrease in sediment drainage and aeration, in turn, would increase sediment pore water sulfide concentrations. An increase in pore water sulfide in turn decrease *S. alterniflora* productivity and production. A decrease in *S. alterniflora* productivity and production would lead to a decrease in salt marsh persistence. A decrease in salt marsh persistence






Figure 3.6. Conceptual model with increase in relative sea-level, showing the relationship among sea-level, sediment, pore water, and *Spartina alterniflora* growth. As well as relationship of fiddler crab burrows to sediment drainage, and hypothesized relationship of fiddler crab burrows and pore water sulfide (in red).

leads to a decrease in sediment buildup. Finally a decrease in sediment buildup leads to an increase in relative sea-level rise, creating a positive feedback loop.

If this conceptual model is considered with the presence of fiddler crab burrows, then following the accepted earlier paradigm the burrows would increase sediment drainage and aeration, then following through the conceptual model, this would ameliorate the effects of relative sea-level rise (Figure 3.6 [Red line]). However, in poorly drained sediments, if the presence of fiddler crab burrows increased pore water sulfide concentration as was reported by Michaels (2004), then the presence of fiddler crab burrows could strengthen the positive feedback loop and exacerbate the effects of relative sea-level rise (Figure 3.6 [Red dashed line]).

# **Research Question**

Within a salt marsh system, how do flood regime, sediment type, and fiddler crab burrows interact to affect *S. alterniflora* productivity and production, and what role does pore water sulfide play?

# **General Hypotheses**

It was hypothesized that: 1.) increased flooding (increased water depth at low tide) would decrease primary production. 2.) Sediments which remain water-logged at low tide would have lower production. 3.) Sandy sediments would have higher production. 4.) Fiddler crab burrows would have different effects on production depending on sediment drainage.

### Methods

#### **Experimental Framework**

In this experiment, I am trying to represent marshes under conditions common to the mid-Atlantic coast. This region consists of sediment starved barrier island systems because sediment entering from the mainland is deposited in lagoons and the continental shelf does not supply sediment to the system (Letherman et al. 1982, Hobbs et al. submitted). Because of coastal development there is no area for marsh transgression. This area is experiencing a current rate of sea-level rise of 3 mm per year as a modest estimate. Projecting this rate of relative sea-level rise, conservatively assuming it remains steady, forward 50 years would lead to a15 cm increase in relative sea-level. The hypotheses set forth were tested by executing a large mesocosm experiment that mimicked salt marshes.

### **Flood Regime Manipulation Experiment**

#### **Treatments**

This experiment consisted of three treatments, flood regime, sediment type, and burrows, with two levels for each treatment. The <u>flood regime treatment</u> consisted of 'current sea-level' or 'increased flooding' (increased duration and vertical extent of sediment saturation); for this treatment the difference in flood regime only occurred at low tide. The current sea-level was allowed to drain 17 cm from the sediment surface at low tide, where as the increased flooding treatment was only allowed to drain 2 cm from the sediment surface at low tide; this created a difference in drainage depth of 15 cm at low tide. The <u>sediment treatment</u> consisted of either sand or mud. Sediment cores with dimensions of 15 cm (6") diameter and 25 - 30 cm depth containing *S. alterniflora* rhizomes and emergent shoots were transplanted into mesocosms. The sand treatment consisted of sandy sediment collected from an over wash site on the southern tip of Hog Island (Figure 3.7) and represented well-drained sediment. The mud treatment consisted of muddy sediment collected near the creek bank at Lower Phillips Creek (Figure 3.7) and represented sediment.

The <u>burrow treatment</u> consisted of sediment in which burrow additions were made or no burrow additions were made. The burrow additions were produced using a 1.27 (1/2") masonry bit. Burrows were hand augered to a depth of 20 cm at a density of 64 burrows per mesocosm or 114 burrows/m<sup>2</sup>. Sediment removed during burrow creation was deposited on the sediment surface.

#### Mesocosm Design with Semi-diurnal, Tidally-inundated, Bottom-up Flooding

Water was pumped using submersible pumps (DAYTON, 1P809) with pump filter bags (AQUATIC ECO-SYSTEMS Pump Bag®, PFB102) housed in floating baskets so the pumps were at the surface of the water no matter the tidal stage. The pumps were located in a dredged spur off of the main channel of a large tidal creek in Wachapreague, Virginia on the VIMS ESL campus. The pumped water flowed through 50 µm bag filters (COLE-PARMER, EW-01519-56) to remove larva from the water. The pumps ran continuously filling 2 large fiberglass tanks (gondolas) measuring 1.90 m x 5.61 m, which each contained 12 mesocosms (smaller fiberglass tanks) measuring 75 cm in each dimension (Figure 3.8). As the gondolas filled, water flowed into the 12 mesocosms through standpipes (2" PVC) attached to the bottom of one of the sides of the



Figure 3.7. Locations from which sediment cores were collected.

mesocosm (Figure 3.9). Each standpipe entered the mesocosm through a 2" PVC coupler and then connected to 2" PVC well screen (0.008" slots spaced every 3/16 inches) connected in the shape of a 48 x 52 cm (19 x 21 inch) rectangle on the bottom of the tank (Figure 3.9). First, the well screen was covered with screen cloth, which was overlaid with 25.4 cm (10 inches) of gravel. Next, the gravel was covered with screen cloth and then overlaid with 12.7 cm (5 inches) of sand. Finally, 25.4 cm (10 inches) of treatment sediment (either sand or mud) was added as the top layer, leaving 10.2 cm (4 inches) of open space in the top of each tank. The sand for the treatment layer was obtained from a local sand and gravel retailer, the mud for the treatment layer was a mix (sand:mud ratio of 5:1 by volume) of sand from the retailer and mud from a spur off of a tidal creek near the VIMS dock in Wachapreague, VA. The mesocosms were constructed and filled with sediment during summer 2005. The matrix sediment into which the S. alterniflora cores were transplanted remained in the drained mesocosms from the end of the summer in 2005 until April 2006. In the spring of 2006, five sediment cores were transplanted into each mesocosm in the pattern of an X with even spacing between them.

Once the water in the gondolas reached a depth of 68.6 cm (27 inches), it flowed out through an overflow pipe in the wall of the gondola. The gondolas remained filled (high tide) for approximately 6 hours; then the gondolas drained through a solenoid valve (MAGNATROL, 33G27-W), which was connected to a repeat cycle timer (OMRON, H3CR-F8-300AC100240). The valves remained open for approximately 6.5 hours (6 hrs 37 min) to allow the mesocosms to drain (low tide) and the valves remained close for approximately 6 hours (5 hrs 48 min). The mesocosms only drain to the height of the attached standpipes, because the pressure head in the mesocosm equaled that of the



Figure 3.9. Mesocosm diagram showing how mesocosms were constructed. Drainage amount determines flood regime treatment. For Current sea-level drainage amount is 17 cm, for increased flooding treatment drainage amount is 2 cm.

pressure head of the standpipe. As a further precaution, a standpipe was connected to the inside of the gondola so that when the water drained out of the gondolas it only drained to the level of the mesocosm standpipes. The timer was set so that the time for the valves to open (low tide to start) moved ahead one hour each day (making a 25 hour day). The beginning of each successive low tide progresses forward by half an hour. *Experiment* 

All 120 S. alterniflora sediment cores were transplanted into mesocosms by April 27 (Figure 3.10A). After being transplanted, the mesocosm remained at the high tide water depth for 13 days giving them time to acclimate before the experiment began. The experiment began on May 10, 2006 and ran until September 15, 2006. Root in-growth bags and pore water equilibrators were installed in each mesocosm. The root in-growth bags were made from 1/16" delta 35 netting (Nylon Net Company, Memphis), and were 6.5 x 20 cm in size and filled with matrix sediment (Blum 1993) (Figure 3.10B). The root bags were buried vertically next to three of the five S. alterniflora cores. Pore water equilibrators were installed in the mesocosms to collect water from five depths (2, 6.5, 12.5, 20, 25 cm). The pore water equilibrators were made from 20 cm glass scintillation vials (Wheaton, GPI Thread 24-400) that were fitted with a 0.2 µm HT Tuffryn membrane (Pall, Gelman) held by an open-top screw cap (Wheaton, open-top black phenolic screw cap, GPI Thread 24-400). A 40 cm section of 2" schedule 40 PVC was used to position the equilibrators at the proper depth, the top 10 cm of which remained above the sediment surface. Holes were added to the 2" PVC at the 5 desired depths and 5 cm sections of thin wall 1" PVC were inserted into the holes in order to kept the scintillation vial in place and flush with the sediment surface (Mozdzer 2009).



Figure 3.10. Experimental design showing A) cores of *Spartina alterniflora* planted in mesocosm, B) pore water equilibrators (arrows top), *Spartina alterniflora* with bird bands for productivity measurements (circles bottom), and root bags (arrows bottom), C) artificial crab burrows at a density of 114/ m<sup>2</sup> or 64/ mesocosm.

The water equilibrators were installed within the remaining two S. alterniflora cores on August 4, and allowed to equilibrate for 32 days (Figure 3.10C). Burrows were artificially added to half of the mesocosms of each treatment using standard methods (Montague 1982, Michaels 2004) consisting of hand augering holes to a depth of 20 cm using a 0.5 inch masonry bit with an attached handle. Burrows were added at a density that was comparable to natural salt marsh systems on the Eastern Shore of Virginia; 64 1.27 cm burrows per mesocosm (114, 0.5 inch burrows/ $m^2$ ) (Figure 3.10C). Burrows were added monthly throughout the growing season. S. alterniflora productivity was monitored monthly throughout the growing season using the bird banding technique (Morris & Haskins 1990) and a production value per core was determined monthly. Endof-season biomass was collected from the three cores from which productivity was measured. S. alterniflora stems were cut at the sediment surface and frozen. Plant material was divided between live and dead, then dried at 60°C, only the live weigh fraction of end-of-season biomass was used in calculation of end-of-season biomass because some of the dead material was removed during Tropical Storm Ernesto. Three sediment cores were taken from each mesocosm within three of the S. alterniflora cores after aboveground biomass was collected. Syringe cores (10 cc) were taken at depths of 3, 10, and 15 cm in order to measure sulfate reduction rates.

# **Chemical Analyses**

Pore water from the equilibrators was analyzed for concentrations of sulfide, reduced iron (Fe(II)), and sulfate using standard methods. Pore water sulfide concentrations of an unfiltered subsample from equilibrators was analyzed immediately after collection following the method of Cline (1969). The remaining pore water from the equilibrators was filtered to sterilize using a syringe filter (Acrodisc, GHP 0.2  $\mu$ m). Pore water Fe(II) concentrations were analyzed following the Ferrozine method of Gibbs (1979). A 1.0 ml pore water subsample was preserved with 0.02 ml of 5 N HCl and frozen until analyzed colorimetrically for Fe(II) concentration. Pore water from the equilibrators was analyzed for sulfate concentration at three depths 2, 6.5, and 12.5 cm using a Dionex Ion Chromatography System (Model ICS-3000) with an AG18 column and suppressed conductivity detection.

Sulfate Reduction Rate (SRR) were measured within two of the Spartina alterniflora cores. PVC sediment cores (5 cm diameter) were collected and 10 cc detipped syringe cores were remove through holes within the PVC core at depths of 3, 10, and 15 cm. The subcores were immediately injected with 50  $\mu$ l of H<sub>2</sub><sup>35</sup>SO<sub>4</sub> (1  $\mu$ Ci) (Herlihy 1987 after Jorgensen 1978) and capped with serum stoppers. The injected subcores were left to incubate for approximately 1 h, and then frozen in an ice and ethanol bath to stop the reaction. The total reduced inorganic sulfur (free sulfide, S0, FeS, and FeS2) within the subcores was extracted using the chromium reducible sulfide method following Thomas 2004 (after Fossing & Jorgensen 1989). Once extracted and trapped with 0.5 N NaOH, duplicate 1 ml aliquots were used for analysis. Each aliquot was pipetted into a 20 ml scintillation vial to which 10 ml of Beckman Coulter Ready Safe Liquid Scintillation Cocktail was added and subsequently read on a Beckman LS 6500 Multipurpose Scintillation Counter with quench correction. To determine sulfate reduction rates, the fraction of injected  ${}^{35}SO_4{}^2$  converted to  ${}^{35}S^2$  was calculated (Thomas 2004).

From a sediment core collected from one *Spartina alterniflora* core in each mesocosm, sediment iron sulfide was measured at a depth of 12.5 cm. Sediment iron sulfide was extracted following the chromium reducible sulfide method (Fossing & Jorgensen 1989, Thomas 2004) and trapped with 40 ml of 4% ZnAc with a few drops of isoamyl alcohol added to prevent foaming. This method actually determines the total reduced inorganic sulfur (free sulfide, S°, FeS, and FeS<sub>2</sub>), but because the sediment samples were dried at 60°C for 24 hours before analysis it is being considered that concentrations of only FeS and FeS<sub>2</sub> were measured.

# **Statistical Design**

The experiment followed a modified split plot design (Figure 3.8). Each gondola was a plot ('current sea-level' treatment or 'increased flooding' treatment) and within each plot there were subplots ('sand' treatment or 'mud' treatment). Instead of having 6 'current sea-level' plots and 6 'increased flooding' plots each with one of each subplot level treatments (sand or mud), the design was modified so that each plot contained all of the 6 subplot level replicates. The positions of the 12 mesocosms (subplots) within each gondola (plot) were randomized.

# **Statistical Analyses**

All statistical analyses were performed using SAS 9.2 (SAS Institute Inc., Cary, NC, USA). Data for all variables collected from the mesocosms were analyzed using Proc Mixed in SAS. Each variable (at each depth where applicable) was analyzed using Proc Mixed to determine overall significant ( $\alpha$ =0.05) treatment effects for flood regime, sediment type, burrow, and all possible interactions. The data were then analyzed for significant differences ( $\alpha$ =0.05) among the eight unique 'treatment combinations' also

using Proc Mixed in SAS. A correlation analysis was then performed on a subset of each variable: sulfide concentration, Fe(II) concentration, sulfate concentration, and iron sulfide at a depth of 12.5 cm, as well as SRR at a depth of 15 cm, production from the month of August, and end-of-season biomass. For the correlation analysis, a higher probability ( $\alpha$ =0.10) was used because more variability was expected because the analysis was performed using all treatments together, instead of for the unique treatment combinations.

#### **Expected Results**

Expected results for the main points of interest from the general research question (pore water sulfide concentration and *S. alterniflora* production) are set forth for the experiment as follows.

**Sulfide:** It was expected that for the:

<u>flood regime treatment</u>, the current sea-level treatment would have lower sulfide concentrations than the increased flooding treatment,

<u>sediment type treatment</u>, the sand treatment would have lower sulfide concentrations than the mud treatment

<u>burrow treatment</u>, treatments with burrows where drainage was possible would have lower sulfide than treatments with burrows where drainage was not possible.

S. alterniflora production: It was expected that for the:

<u>flood regime treatment</u>, the current sea-level treatment would have greater production than the increased flooding treatment,

<u>sediment type treatment</u>, the sand treatment would have higher production than the mud treatment,

burrow treatment, treatments with burrows where drainage was possible would have

greater production than treatments with burrows where drainage was not possible.

More specific expected results by treatment are listed in the following tables.

Table 3.1. Expected results for pore water sulfide concentrations within mesocosm experiment with two flood regime and two sediment scenarios with and without fiddler crab burrows.

		Flood Regime Treatment		
		Current Sea-level	Increased Flooding	
Sediment Treatment	Sand	Lowest sulfide concentration	• Intermediate sulfide concentration	
	Mud	• High sulfide concentration	• Highest sulfide concentration	
Burrow Treatment	No Burrow	<ul> <li>Low sulfide concentration in sand</li> <li>Intermediate sulfide concentration in mud</li> </ul>	<ul> <li>High sulfide concentration in mud</li> <li>Low – intermediate sulfide concentration in sand</li> </ul>	
	Burrow	<ul> <li>Decreased sulfide concentration in sand</li> <li>Increases sulfide concentration in mud</li> </ul>	•Increased sulfide concentration in mud and sand	

Table 3.2. Expected results for *Spartina alterniflora* production within mesocosm experiment with two flood regime and two sediment scenarios with and without fiddler crab burrows.

		Flood Regime Treatment		
		Current Sea-level	Increased Flooding	
Sediment Treatment	Sand	• Highest production	• Low production	
	Mud	• High production	• Lowest production	
Burrow Treatment	No Burrow	• High production	• Low production	
	Burrow	Highest production	Lowest production	

# Results

#### Pore water sulfide

Sulfide concentration had a significant ( $p \le 0.05$ ) treatment effect with depth for all three treatments individually (flood regime, sediment type, and burrows), and interactions between flood regime x sediment and flood regime x burrow (Table 3.3). The flood regime treatment had significantly higher sulfide concentration for increased flooding compared to current sea-level for the top four depths (2, 6.5, 12.5, 20 cm). The sediment type treatment had significantly higher sulfide concentrations for sand compared to mud for the top three depths (2, 6.5, 12.5 cm). The burrow treatment had significantly higher sulfide concentration for burrow compared to no burrow for the top two depths (2, 6.5 cm). For sulfide concentration the interaction between flood regime x sediment type was significant at the top two depths (2, 6.5 cm), where as the interaction between flood regime x burrow was only significant at a depth of 2 cm. The interaction between sediment type x burrow was not significant, nor was the interaction among flood regime x sediment type x burrow at any depth.

Depth profiles of sulfide concentration differ between the two flood regime treatments, between the two sediment type treatments and between the two burrow treatments (Figure 3.11).

# Within the no burrow treatment

Within the <u>sand</u> sediment, the increased flooding treatment had higher sulfide concentrations than current sea level along the entire depth profile (Figure 3.11A & C). Within the <u>mud</u> sediment the increased flooding treatment had less variable sulfide concentrations. The sulfide concentrations for increased flooding were higher at the top

Table 3.3. Treatment effects by depth for pore water sulfide, reduced iron, and sulfate, sediment iron sulfide, and sulfate reduction rate. Significant treatment effects  $(p \le 0.05)$  are noted as follows: Flood Regime (F), Sediment Type (S), Burrow (B), as well as interactions. No significant treatment effect is noted with X.





Figure 3.11. Depth profiles for sulfide concentrations for the four flood regime-sediment combinations A) current sea-level & sand, B) current sea-level & mud, C) increased flooding & sand, and D) increased flooding & mud comparing concentration for treatments with burrows and without burrows. Means ± SE.

three depths (2, 6.5, 12.5 cm), but did not differ lower in the profile. In the current sealevel treatment there was no sulfide within the top 6.5 cm (Figure 3.11B & D). Within <u>current sea-level</u>, both sand and mud had low or no sulfide to a depth of 6.5 cm (Figure 3.11A & B). At the middle depth, 12.5 cm, sand had higher sulfide concentration than mud. At the lowest two depths, the mean sulfide concentration was higher for mud, but with the high variability the two sediment types did not have different sulfide concentrations at this depth. Within <u>increased flooding</u>, the sand had higher sulfide concentrations along most of the depth profile except for the lowest point where the concentrations do not differ (Figure 3.11C &D).

# Within the burrow treatment

Within <u>sand</u>, increased flooding had higher sulfide concentration than current sealevel for all depths (Figure 3.11A & C). Within <u>mud</u>, increased flooding had higher sulfide concentrations than current sea-level for all depths except at 25 cm (Figure 3.11B & D). Within <u>current sea-level</u>, mud had a higher sulfide concentration than sand at 2 cm. At the intermediate depths (6.5, 12.5, 20 cm) mud had lower sulfide concentrations than sand. At the lowest point on the profile, mud and sand did not differ in sulfide concentration (Figure 3.11A & B). Within <u>increased flooding</u>, sand had higher mean sulfide concentrations for the extent of the depth profile than mud (Figure 3.11C &D). <u>Comparison of no burrow & burrow treatments for each of the remaining four treatment</u> <u>combinations</u>

Within <u>current sea-level and sand</u>, sulfide concentrations for burrow vs. no burrow were similar at all five depths. Slight differences were observed at the intermediate depths, at 6.5 cm, burrows slightly increased sulfide concentration; at 12.5 and 20 cm burrows slightly decreased sulfide concentration (Figure 3.11A). Within <u>current sea-level and mud</u>, burrows decreased mean sulfide concentrations a the three lowest points in the profile (Figure 3.11B). Within <u>increased flooding and sand</u>, burrows increased sulfide concentration at all depths along the profile (Figure 3.11C). Within increased flooding and mud, burrows increased sulfide concentrations at all depths along the profile (Figure 3.11D).

### Sulfide concentrations for the eight treatment combinations at 5 depths

Sulfide concentration at 2 cm depth was significantly higher within three of the treatment combinations, all within increased flooding (Figure 3.12A). Both of the sand treatments (burrow and no burrow) and the mud–burrow treatment had higher sulfide concentrations than all four of the current sea-level treatments and the mud–no burrow within increased flooding. Within the increased flooding–mud treatment there was a significant increase in sulfide concentration in the burrow treatment compared to no burrow.

Sulfide concentration at 6.5 cm depth was highest within the increased flooding treatment combinations (Figure 3.12B). The sand–burrow treatment had the highest concentration, followed by the sand–no burrow, and then the mud–burrow and finally the mud–no burrow which did not differ significantly from the current sea-level treatments, but was marginally higher. Similar to the 2 cm depth, within the increased flooding–sand treatment there was a significant increase in sulfide concentration in the burrow treatment compared to no burrow. There was also a trend of increased sulfide concentration in the burrow treatments.



Figure 3.12 . Sulfide concentrations for the eight treatments at depths of A) 2 cm, B) 6.5 cm, C) 12.5 cm, D) 20 cm, and E) 25 cm. Means  $\pm$  SE. Significant differences noted with different letters (p  $\leq$  0.05).





Figure 3.12 . Sulfide concentrations for the eight treatments at depths of A) 2 cm, B) 6.5 cm, C) 12.5 cm, D) 20 cm, and E) 25 cm. Means  $\pm$  SE. Significant differences noted with different letters (p  $\leq$  0.05).

Sulfide concentration at 12.5 cm depth was higher for the current sea-level treatments at this depth than they were for the 2 and 6.5 cm depths (Figure 3.12C). In the increased flooding treatment, burrows marginally increased sulfide concentration within both sand and mud. In the current sea-level treatment, burrows marginally decreased sulfide concentration. Sulfide concentration was not significantly different for the treatment combinations at 20 and 25 cm depths (Figure 3.12D & E). Similar patters as ones noted at higher depths were distinguishable.

# Pore water reduced iron (Fe(II))

Fe(II) concentration had a significant ( $p \le 0.05$ ) treatment effect only for the sediment treatment; this significant effect occurred at all five depths (2, 6.5, 12.5, 20, 25 cm) (Table 3.3). The sediment type treatment had significantly higher Fe(II) concentrations for mud compared to sand; mud had two to three orders of magnitude higher Fe(II) concentration than sand at each depth.

Depth profiles for Fe(II) show a distinct pattern. Treatments with sand all had very low Fe(II) concentrations of less than 20  $\mu$ M with little variability (Figure 3.13A & C). Treatments with mud had high Fe(II) concentrations, means varied from ~200  $\mu$ M to ~ 2000  $\mu$ M (Figure 3.13B & D). Both of the mud treatments at both of the flood regime treatments (current sea-level and increased flooding) without burrows had lower Fe(II) concentrations at the top of the profile and it increased until the 12.5 cm depth. The mud–current sea-level treatment with burrows did not have a consistent Fe(II) concentration pattern with depth. The mud–increased flooding treatment with burrows had higher Fe(II) at the top of the profile and it decreased slightly with increased depth.



Figure 3.13. Depth profiles for reduced iron concentrations for the four flood regime-sediment combinations A) current sea-level & sand, B) current sea-level & mud, C) increased flooding & sand, and D) increased flooding & mud. comparing concentration for treatments with burrows and without burrows. Means ± SE.

In all of the treatments with sand, the treatments with and without burrows did not differ (Figure 3.13A & C). In the current sea-level–mud treatment burrows slightly increased Fe(II) concentrations at the top four depths (Figure 3.13B). In the increased flooding–mud treatment burrows decreased Fe(II) concentration along most of the depth profile (Figure 3.13D).

#### Fe(II) concentrations for the eight treatment combinations at 5 depths

At all five depths, all treatments with sand had consistently very low Fe(II) concentrations (Figure 3.14). At a depth of 2 cm, there was no significant difference among treatments (Figure 3.14A). At a depth of 6.5 cm the increased flooding–mud–no burrow and current sea-level–mud–burrow treatments had the highest Fe(II) concentration (Figure 3.14B). The current sea-level–mud–no burrow and increased flooding–mud–burrow treatments had intermediate Fe(II) concentrations. Although all of the sand treatments were consistently low, they did not differ significantly from the treatments with intermediate concentrations. The 12.5, 20, and 25 cm depths showed a very similar pattern to that of the 6.5 cm depth, although there was no significant difference among treatments at the 25 cm depth.

### **Sediment iron sulfide**

Sediment iron sulfide had a significant ( $p \le 0.05$ ) treatment effect only for the sediment treatment; sediment iron sulfide was measured only at a depth of 12.5 cm (Table 3.3). The sediment type treatment had significantly higher sediment iron sulfide for mud compared to sand. The sediment iron sulfide concentration was 20 times greater in treatments with mud than those with sand (Figure 3.15).



Figure 3.14 . Reduced iron concentrations for the eight treatments at depths of A) 2 cm, B) 6.5 cm, C) 12.5 cm, D) 20 cm, and E) 25 cm. Means  $\pm$  SE. Significant differences noted with different letters (p  $\leq$  0.05).



Figure 3.14. Reduced iron concentrations for the eight treatments at depths of A) 2 cm, B) 6.5 cm, C) 12.5 cm, D) 20 cm, and E) 25 cm. Means  $\pm$  SE. Significant differences noted with different letters ( $\alpha = 0.05$ ).



Figure 3.15. Sediment iron sulfide concentrations for the eight treatments at a depth of 12.5 cm. Means  $\pm$  SE. Significant differences noted with different letters (p  $\leq$  0.05).

# Pore water sulfate

Sulfate concentration had a significant ( $p \le 0.05$ ) treatment effect only for the sediment treatment; this significant effect occurred at all three depths measured (2, 6.5, 12.5 cm) (Table 3.3). The sediment type treatment had significantly higher sulfate concentrations for mud compared to sand; mud had greater than double the Fe(II) concentration than sand at each depth.

Depth profiles of sulfate concentrations show that the sand treatments had low concentrations compared to the mud and only varied slightly with depth (Figure 3.16A & C). Sulfate concentrations in the mud treatments were much higher and generally increased with depth (Figure 3.16B & D). In all cases, except current sea-level–sand in which concentrations varied very little, burrows increased mean sulfate concentrations (Figure 3.16B, C, D).

Sulfate concentration was highest within the mud treatments for all depths (2, 6.5, 12.5 cm). At a depth of 2 cm, three of the mud treatments had significantly higher sulfate concentrations than all of the sand treatments (Figure 3.17A). The increased flooding–mud–no burrow, and both current sea-level–sand treatments had intermediate sulfate concentrations; both of the increased flooding–sand treatments had the lowest sulfate concentrations. At a depth of 6.5 cm, all of the mud treatments were significantly higher than all of the sand treatments (Figure 3.17B). The increased flooding–mud–burrow treatment had the highest concentration, followed by the current sea-level–mud–burrow and increased flooding–mud–no burrow treatments which were both intermediate; the remaining mud treatment had the lowest sulfate concentration of the



Figure 3.16. Depth profiles for sulfate concentrations for the four flood regime-sediment combinations A) current sea-level & sand, B) current sea-level & mud, C) increased flooding & sand, and D) increased flooding & mud comparing concentration for treatments with burrows and without burrows. Means ± SE.



Figure 3.17. Sulfate concentrations for the eight treatments at depths of A) 2 cm, B) 6.5 cm, and C) 12.5cm. Means  $\pm$  SE. Significant differences noted with different letters (p  $\leq$  0.05).

mud treatments. At a depth of 12.5 cm, all of the mud treatments had significantly higher sulfate concentrations than all of the sand treatments (Figure 3.17C).

# Sediment sulfate reduction rate

Sulfate reduction rates (SRR) were generally lower in treatments with sand (Figure 3.18). For both the current sea-level–sand and increased flooding–sand treatments there was no difference in SRR between treatments with and without burrows, and there was no obvious trend with depth (Figure 3.18A & C). The current sea-level– mud treatment had increased SRR with depth for treatments with and without burrows, and treatments with burrows had higher SRR than those without burrows (Figure 3.18B). The increased flooding–mud treatment had higher SRR with burrows for the 3 and 15 cm depths than without burrows (Figure 3.18D).

### Sediment sulfate reduction rate for the eight treatment combinations at 3 depths

At a depth of 3 cm, the increased flooding– mud–burrow treatment had significantly higher SRR than all other treatments (Figure 3.19A). The increased flooding–mud–no burrow, current sea-level–mud–burrow, and current sea-level–sand–burrow treatments all had intermediate SRRs. The remaining four treatments had the lowest SRRs. At a depth of 10 cm, only the current sea-level–mud–burrow treatment was significantly higher than the other treatments (Figure 3.19B). The four treatments with sand had the lowest SRRs. At a depth of 15 cm, the current sea-level–mud–burrow and increased flooding–mud–burrow had the highest SRRs (Figure 3.19C). The four treatments with sand had the lowest SRRs, and the remaining two treatments had intermediate SRRs.



Figure 3.18. Depth profiles for sulfate reduction rates for the four flood regime-sediment combinations A) current sea-level & sand, B) current sea-level & mud, C) increased flooding & sand, and D) increased flooding & mud comparing concentration for treatments with burrows and without burrows. Means ± SE.



Figure 3.19. Sulfate reduction rate for the eight treatments at a depths of A) 3 cm, B) 10 cm, and C) 15 cm. Means  $\pm$  SE. Significant differences noted with different letters (p  $\leq$  0.05).

Spartina alterniflora production and end-of-season biomass

There were significant ( $p \le 0.05$ ) treatment effects for each month of production (June, July, August). For June, the sediment treatment and flood regime x sediment x burrow interaction were both had significant effects. Production was negative in June; the sand sediment had significantly greater production (less negative) than the mud. For July, only the sediment treatment had a significant effect; mud had significantly higher production and was over double that of sand. For August, flood regime, sediment, and flood regime x burrow interaction were all significant. The current sea-level treatment had significantly higher production than the increased flooding treatment. The mud treatment had significantly higher production than the sand treatment.

Among all treatments, production increased monthly (Figure 3.20A), from negative values in June, to positive values in July, and higher values in August. Between the flood regime treatments, increased flooding had lower production, but this decrease was only significant for the month of August (Figure 3.20B). Between the sediment treatments, there was a significant difference ( $p \le 0.05$ ) in production for each month (figure 3.20C). Mud had a higher production in both July and August. There was no significant difference in production between treatments with and without burrows (Figure 3.20D).

Among the eight treatment combinations there was a significant difference in production for July and August (Figure 3.21). In August, within current sea-level, both the mud and sand treatments had slightly higher production with burrows (Figure 3.21C). Within increased flooding, burrows within the mud treatment significantly decreased production compared to without burrows. Between the two flood regimes, comparing



Figure 3.20. Overall monthly production for A) all treatments, B) Flood regime treatments, C) Sediment type treatments, D) Burrow treatments. \* indicate significant difference between treatments ( $p \le 0.05$ ).



Figure 3.21. Production for the eight treatment combinations during A) June, B) July, and C) August. Means  $\pm$  SE. Significant differences noted with different letters (p  $\leq$  0.05).

mud treatments with burrows, the current sea-level treatment had the highest overall production and significantly higher production than the increased flooding treatment which had the lowest overall production. The remaining treatments did not differ from each other.

End-of-season biomass had a significant treatment effect for sediment only. The mud sediment had significantly higher biomass and was double that of sand. There was a significant difference for the end-of-season biomass for the eight treatment combinations (Figure 3.22). The sand treatments all had the lowest biomass. The mud treatments within current sea-level had the highest biomass, and the mud treatments within increased flooding were intermediate. There was no difference in biomass between each pair of burrow and no burrow treatments within the same flood regime and sediment treatment.

Belowground production had no significant treatment effects. There was no significant difference in belowground production among the eight treatment combinations (Figure 3.23).

### **Correlation analysis**

The correlation analysis yielded many highly correlated variables (Table 3.4). Out of the 21 possible correlations, all but four were significant at a probability of  $\alpha$ =0.10, these correlations show an either positive or negative effect between the variables within this experiment. The positive and negative relationships between chemical variables within this experiment can be visualized from the results of the correlation analysis (Figure 3.24). The positive and negative relationships between each chemical variable and the aboveground *S. alterniflora* variables can be visualized as well (Figure 3.25).


Figure 3.22. End-of-season biomass from 3 cores in each mesocosm. Means  $\pm$  SE. Significant differences noted with different letters (p  $\leq$  0.05).



Figure 3.23. Belowground production from root bags adjacent to 3 cores in each mesocosm. Means  $\pm$  SE. Significant differences noted with different letters (p  $\leq$  0.05).

Table 3.4. Pearson Correlation Coefficients for 7 variables over all treatments (n = 24 mesocosms). Top number is Pearson Correlation Coefficient, bottom number is the probability. Significant probabilities ( $p \le 0.10$ ) are highlighted in yellow.

	Reduce	ed Iron	Sulf	ide	Aug Produ	∣ust cti∨ity	End of S Bion	Season nass	Sulf	ate	Iron S	ulfide	Sulfate Reduction Rate
Reduced Iron													
Sulfide	-0.44243												
		0.0304											
August Productivity	0.35587		-0.15817										
		0.0879		0.4604									
End of Season Biomass	0.61816		-0.43607		0.73529								
		0.0013		0.0332	<.0001								
Sulfate	0.59171		-0.35512		0.58807		0.86857						
		0.0023		0.0886		0.0025	<.0001						
Iron Sulfide	0.42544		-0.38626		0.3801		0.67472		0.69692				
		0.0382		0.0623		0.0669		0.0003		0.0002			
Sulfate Reduction Rate	0.30673		-0.3027		0.28245		0.54139		0.43918		0.56162		
		0.1449		0.1505		0.1811		0.0063		0.0318		0.0043	



Figure 3.24. Diagram depicting significant ( $p \le 0.10$ ) correlations between chemical variables within mesocosm experiment including all treatments (n = 24).



Figure 3.25. Diagram depicting significant correlations ( $p \le 0.10$ ) between chemical variables and *Spartina alterniflora* variables within mesocosm experiment including all treatments (n = 24).

## Discussion

In this mesocosm experiment, the depths to which the two flood regime treatments drained for the current sea-level and increased flooding treatments were 17 cm and 2 cm respectively. Therefore, regardless of the flood regime there was no difference in drainage below 17 cm for either flood regime treatment. For this reason, it is understandable why there were fewer significant treatment effects with greater depth as seen in Table 3.3. Sediment type had a significant treatment effect for all variables at most depths (Table 3.3). The reason for this is that the sediment types used in the experiment were very different, and the differences caused by the sediment types alone overwhelmed the other treatment effects. For the pore water variables, the iron inadvertently added to the system became reduced within the mud sediments and altered the overall sediment chemistry. For the S. alterniflora parameters, the aboveground and belowground biomass of the two marshes where the S. alterniflora sediment cores were collected from differed. Besides these two reasons, the ability of the sand treatments to drain in comparison with the mud treatments was the intended difference between the two sediment types. When each treatment was analyzed individually for significant effects, for instance flood regime, each of the two levels of flood regime (current sealevel and increased flooding) contained treatments with both sand and mud sediment and treatments with and without burrows. The large differences in some of the variables (reduced iron, and iron sulfide concentrations, end-of-season biomass, and production) between the two sediment types likely masked other treatment effects. Therefore, the more important differences in which I was interested were the differences among the

eight treatment combinations, for instance, differences between the same flood regime and sediment type with and without burrows.

# Sulfide

The treatment effect results of sediment type for pore water sulfide were unexpected. There was a significant difference between sediment types at the top three depths, but the sand treatment had significantly higher sulfide concentration compared to mud. This unexpected result will be discussed later in the iron sulfide section. The treatment effects for flood regime, in which increased flooding had higher sulfide concentration than current sea-level and treatments with burrows had higher concentrations than those without, concurred with the hypotheses. The depth profiles for the treatments with and without burrows showed that, as expected, in treatments at depths that did not drain, either because 1) they had mud sediment that physically could not drain no matter the flood regime or 2) the flood regime was increased flooding, the presence of burrows increased sulfide concentrations. The current sea-level treatments had low sulfide concentrations at the tops of the depth profiles which would be expected because these treatment were not water-logged near the top of the sediment profile. As expected, increased flooding treatments had greater sulfide concentrations than current sea-level. The expected effect of the presence of burrows in sediments that were waterlogged was clearly evident at a depth of 2 cm (Figure 3.12A); there was significantly higher sulfide concentration in the increased flooding-mud treatment with burrows compared to without burrows. This effect was similar for the increased flooding-sand treatment, but there was only marginally higher sulfide concentration in the treatment with burrows compared to without burrows. By a depth of 6.5 cm, the presence of

burrows in the increased flooding-sand treatment yielded significantly higher sulfide concentration compared to the treatment without burrows. The trend of higher sulfide concentration with burrows compared to without was consistent in the increased flooding-mud treatment at depths of 6.5 and 12.5 cm, but the differences were not significant at those depths. The expected result that the presence of burrows would decrease sulfide concentration within sediments that were well-drained was observed as a trend at a depth of 12.5 cm, but this result was not significant. In the current sea-levelsand treatments the presence of burrows did marginally decrease pore water sulfide concentration. Because all of the mesocosms never drained below a depth of 17 cm, it is understandable that there were no significant differences in pore water sulfide concentration at depths of 20 and 25 cm. Also, burrows were added to a depth of 20 cm and the lower portions of the burrows likely filled in with sediment. A study by Gribsholt et al. (2003) found results similar to those hypothesized for this study; namely that for sediments that were highly drained, the SRR was very low adjacent to burrow walls at a depth of 5 cm compared to SRR at a distance of 3.5 cm from the burrow. This shows that while it is possible for burrows in well-drained sediments to reduce sulfide production, the effect of the burrows is highly localized and may not have an effect on the overall bulk sediment chemistry.

The increase in sulfide concentration in treatments that contained burrows was likely a result of either increased sulfate reduction or decreased sulfide oxidation. These burrows contained stagnant water when the surrounding sediment was unable to drain. The proportions in which the five main reduction reactions occurred in the treatments with burrows likely shifted, resulting in an increase in the proportion of organic matter oxidized through sulfate reduction (Howarth 1993). Sulfate-reducing bacteria (SRB), which are inhibited by oxygen (Boulegue et al. 1982), would be less inhibited in areas containing macropores with stagnant anoxic water because there would be no oxygen present. Also, there could have been an increase in the rate of organic matter oxidation due to organic-rich sediments from the surface entering the burrows (Hines & Jones 1985), which would stimulate SRB (Westrich & Berner 1984, Howarth 1993).

Sulfide concentrations within this experiment concurred with the expected results for two of the main treatment effects (flood regime and burrow), but not for sediment type. Sulfide concentrations were significantly higher in the increased flooding treatments compared to current sea-level treatments at all but the deepest sampling point where there was no effect. Within the top two sampling points ( 2 and 6.5 cm), treatments with burrows had significantly higher sulfide concentrations than treatments without burrows. The treatment effect for sediment type showed significantly higher sulfide concentrations within sand sediment compared to mud, and was opposite that of the expected results, which will be discussed in the iron sulfide section.

#### **Reduced iron (Fe(II))**

The main result of the experimental effect on pore water Fe(II) concentration was that treatments with sand sediments contained very low concentrations of Fe(II) compared to those with mud. The matrix sediment of the mesocosms consisted of sand that was purchased from a sand and gravel supplier. This sand likely had a large amount of oxidized iron dust associate with it. Within the mud treatments, the iron present on the sand grains was reduced, where as, in the sand treatments, much of the iron remained oxidized and was flushed from the top portion of mesocosms through the more porous sediment. Large amounts of iron oxide were observed within the lower section of the mesocosms when the mesocosms were emptied. Low Fe(III) was evident from the low total iron concentrations within many of the sand treatments from a pore water analysis from water samples collected in earlier in the summer (unpublished data). The concentration of reactive Fe(III) has a strong relationship to iron (III) reduction (Thamdrup 2000). Therefore, the rate of Fe(III) reduction would have been high until Fe(III) was depleted. In the sand treatments Fe(III) concentrations limited Fe(III) reduction, therefore lower Fe(II) concentrations were present within these sediments. Bioturbation from macrophytes and macro fauna has been shown to increase the dominance of Fe-cycling in salt marsh sediments compared to sulfate reduction (Gribsholt et al. 2003). The reduction of Fe(III) was considered an abiotic reaction with sulfide (Howarth 1993), but other studies have shown that microbial Fe(III) reduction is also important in these sediments (Luther et al. 1992, Jacobsen 1994, Kostka & Luther 1995). Within this study, the process that produced Fe(II) was likely microbial reduction. Different microbial communities in the locations from which the sediment cores were originally collected may also account for the differences in reduced iron between sand and mud treatments, but this was not determined.

### Sediment iron sulfide

The pore water sulfide and Fe(II) have an important interaction in this experiment; this is evident from sediment iron sulfide concentrations. At the depth in which iron sulfide was measured, 12.5 cm, iron sulfide concentrations were about 12 times greater in treatments with mud compared to those with sand. This result is caused by the reaction of and different concentrations of sulfide and Fe(II) in the pore water of

different treatments. The treatments with mud had very high Fe(II) concentrations which reacted with sulfide to produce high concentrations of iron sulfide. This high concentration of iron sulfide is the reason for lower than expected concentrations of sulfide within the pore water of treatments with mud. The treatments with sand had low Fe(II) concentrations, so there was little Fe(II) present to react with the sulfide to produce iron sulfide in large amounts. Therefore, instead of the treatments with mud having high sulfide concentrations as expected, they instead had high iron sulfide concentrations. This explains the unexpected results for pore water sulfide concentrations within the mud treatments. In the increased flooding–mud treatment, the addition of burrows decreased the Fe(II) concentration. This may have been because the Fe(II) in this treatment was being removed by the production of iron sulfide.

# Sulfate

Sulfate concentrations were consistently lower and less variable in the sand treatments compared to treatments with mud. This could have been due to greater flushing through the sand sediment compared to the mud. Sulfate concentrations were never limiting because they were at least 11.6 mM, which is above the concentration at which sulfate becomes limiting (4 mM) (Pallud & Van Cappellen 2006). Within the mesocosm pore water, there was a 3-fold difference in sulfate concentration between the sand (11.6 -19.8 mM) and mud (33.6 - 59.8 mM) sediment treatments. Sulfate concentration in another mesocosm study (Gribsholt & Kristensen 2002a), was also highly variable. Within the September sampling of their mesocosm pore water, sulfate concentrations ranged from below 7.5 mM to above 17.5 mM, a 3-fold difference. Although the sulfate concentrations in the mesocosm mud treatments in this study were high, the large range in sulfate found (a 3-fold difference between sand and mud treatments) is not abnormal.

### Sulfate Reduction Rate (SRR)

Sulfate reduction rates were 1 or 2 orders of magnitude lower than expected; especially with the high concentrations of sulfide in the pore water and the large amounts of iron sulfide produced in the mud sediments. Typical values of SRR within salt marsh sediments range from less than 100 to 2500 nmol/cm<sup>3</sup>/day (Gribsholt & Kristensen 2002b, Gribsholt & Kristensen 2003, Gribsholt et al. 2003, Thomas 2004, Hyun et al. 2007). Although the SRR values in this experiment were certainly incorrect, I believe the relative SRR among treatments was valid. I think that during the procedures to measure the decays per minute (dpm) the aliquot samples were not well mixed with the liquid scintillation cocktail. But because this was true for all samples, the relative differences among the treatments are likely still valid. Greater organic content from S. alterniflora roots and rhizomes in the mud sediment would support a greater amount of sulfate reduction (Hines et al. 1989, 1999). Although measured new root growth did not differ among treatments, there were greater amounts of S. alterniflora belowground root/rhizome biomass (personal observation) within the mud treatment S. alterniflora sediment cores. High concentrations of dissolved organic compounds are associated with high root biomass and in turn can cause high SRR (Gribsholt & Kristensen 2003). In treatments that had low sulfate concentrations there were also low SRRs, although SRR is independent of dissolved sulfate concentrations unless they are very low (Boudreau & Westrich 1984). Sulfate reduction in salt marshes is often the dominant pathway for organic matter oxidation (Howes et al. 1984, Kostka et al. 2002a, b). The amount of SRB may have also differed between the two marshes where the *S. alterniflora* sediment cores were collected but the size of sulfate reducing microbe communities is not directly correlated with SRR (Pallud & Van Cappellen 2006). SRR is stimulated from organic carbon leaching from damaged roots in the coring process (Gribsholt & Kristensen 2002b); therefore, the greater root mat within the mud sediment could have also stimulated the SRR compared to that of the sand sediment which had a less dense root arrangement.

### Spartina alterniflora production and end-of-season biomass

It is likely that the June production was negative because the plants in the S. alterniflora sediment cores were still acclimating to the mesocosms after they were transplanted. The experiment started on May 10, which was 13 days after the last core was transplanted into the system. The May measurements for S. alterniflora production were made May 22 - 24, less than a month after the S. alterniflora cores were transplanted into the mesocosms; the June production measurements were taken one month later. The negative June production reflects the die back of the tops of the tallest leaves between the end of May and the end of June. The main treatment effect for production for the flood regime treatment did not have a significant effect until August, when the current sea-level treatment had significantly higher production than the increased flooding treatment. This finding was in line with the expected results for the flood regime treatment. The overall treatment effect for the sediment type treatment was opposite that of the expected results for the months of July and August when the production was positive. In July and August, the mud treatment had significantly higher production this is certainly due to the belowground structure of the plants at the two sites from which the cores were collected. The greater *S. alterniflora* belowground biomass within the mud sediments (personal observation) likely allowed for greater aboveground production. There was no overall treatment effect for production within the burrow treatments, this is likely because burrows had different effects on production depending on both the flood regime and the sediment type. By August, the expected effects of burrows on production could be seen within the increased flooding–mud treatments, where the presence of burrows significantly decreased production, and within the current sea-level–mud treatment, where the presence of burrows marginally increased production. The end-of-season biomass seems to have been dictated by the sediment type, and therefore the locations from which the cores were obtained. As expected, increased flooding treatments did have lower biomass, but this was only true for the increased flooding–mud treatments compared to the current sea-level–mud treatments and the trend was not significant.

The result that measured belowground production was not significantly different among treatment combinations is understandable. The method in which the belowground production was measured was not ideal. Because the mesocosms were not natural systems, the typical method of measuring root production was not suitable. When root production is measured *in situ*, root bags of native sediment devoid of live roots are buried vertically within the sediment, and the plants in the surrounding area grow roots in all directions, so roots grow into the root bag from plants from all sides (Blum 1993). In this experiment, root bags could not be buried within the six inch diameter *S. alterniflora* sediment cores because this would have caused too much disturbance to the cores. Instead, root bags were buried vertically adjacent to three of the five sediment cores. This meant that as roots were growing out from the initial *S. alterniflora* sediment core only a small proportion of the roots would end up growing into the adjacent root bag. Much of the root growth likely occurred within the original sediment core area, and this could not be measured. Therefore, even though there were no significant differences among treatments the way root production was measured, there may have been differences which were not detected by this method. Plants often use more energy for root production instead of shoot production when they are growing in water-logged conditions (Gribsholt & Kristensen 2003). High sulfide concentrations, over 1000  $\mu$ M, have been shown to decrease root biomass and change the morphology of belowground structures (Koch & Mendelssohn 1989).

Belowground production is one factor that can aid in sediment build up. Thomas & Blum (2010) found that where there were burrows within an infrequently inundated marsh there was decreased belowground production and higher belowground decomposition. This infers that in their study site the presence of burrows could hinder the ability of the marsh to buildup sediment enough to over come RSLR. With the ability to measure belowground production properly within the mesocosms, an interaction between burrow presence or absence and belowground production may have been detected. In some marshes, biogenic accretion (belowground biologic processes) buildup the sediment elevation enough to overcome the rate of RSLR they are experiencing (Blum & Christian 2004).

The fact that within this experiment burrows were artificially created and not made by actual crabs may have also played a role in the results for above- and belowground production particularly within the sand treatments. Holdredge et al. (2010) found that with crab removal in sandy salt marshes, above- and belowground production decreased and nitrogen uptake by *S. alterniflora* also decreased. They suggest that lack of deposition of nitrogen by fiddler crabs within their crab removal plots was the likely the cause for this effect. From this suggestion, it could be inferred that the mesocosm treatments with sand sediment and burrows may have had higher above- and belowground production had they contained actual fiddler crabs.

#### **Correlation analysis**

The correlations between chemical variables within the this study fit with the data presented in the results section (Figure 3.24). Generally, where there were high sulfate concentrations there were high sulfate reduction rates, and where sulfate reduction rates were high there were high iron sulfide concentrations (instead of high sulfide). Where sulfide concentration was low, there were high iron sulfide concentrations because the sulfide was converted to iron sulfide. Where sulfide concentration was low, there were high Fe(II) concentrations and in turn high iron sulfide concentrations. Finally where sulfate was high, instead of sulfide being high (it was low), iron sulfide was high. The above relationships follow the connections between the variables for treatments with mud sediment, and the opposite example starting with low sulfate concentrations would follow the connections between variables for treatments with sand sediment. The relationships between chemical variables were mainly driven by the differences between the mud and sand sediment treatments, and not by the flood regime or burrow treatments. The flood regime and burrow treatments had important effects in specific cases when specific treatment combinations were compared at specific depths.

The correlations between chemical variables and S. alterniflora aboveground variables within this study concur with the data presented in the results section (Figure 3.25). Because high sulfide concentrations negatively affect the growth of S. alterniflora (King et al. 1982, Bradley & Morris 1990a, Koch et al. 1990) the result that pore water sulfide concentration is negatively correlated with S. alterniflora end-of-season biomass agrees with the conceptual model. Therefore, high sulfide concentrations led to low endof-season biomass and low sulfide concentrations led to high end-of-season biomass. The low sulfide concentrations were a result of the production of sediment iron sulfide; iron sulfide was positively correlated with both end-of-season biomass and August production. The remaining chemical variables were all positively correlated with end-ofseason biomass, August production, or both variables. These results again relate back to the sediment type in the treatments. The initial amount of S. alterniflora within the sediment cores differed, and although the treatments were exposed to different flood regimes and the presence or absence of burrows, the initial difference between the two sediment types persisted throughout the experiment.

#### Interpretation of results in the context of RSLR

Although it is an important part of the conceptual model (Figure 3.5), sediment buildup was not addressed within this study because it was not a natural system. Belowground production did not result in a significant treatment effect, but if the true belowground production could have been assessed there may have been a significant treatment effect. Treatment effects for belowground production could have led to conclusions about how flood regime, sediment type, and the presence or absence of burrows would affect biogenic sediment buildup, and therefore further elucidate ability of marshes to keep pace with RSLR. Aboveground production also directly affects sediment buildup, therefore conditions of flood regime, sediment type, and whether burrows are present can have another impact on sediment buildup, and therefore the ability to keep pace with RSLR.

The production response of another species, *Schoenoplectus americanus*, to changes in flood regime were determined by growing sediment plugs at different elevations within a tidal creek. Production was low at both the highest and lowest elevations, therefore lowest and highest inundation levels. There was a mid-elevation range in which *S. americanus* responded with high aboveground production (Kirwan & Guntenspergen 2012).

# Conclusions

The interactions among the pore water variables and *S. alterniflora* aboveground production determined from this study match the conceptual models (Figures 3.5 & 3.6) set forth in the introduction. Through this experiment, it has been shown that increased flooding (low tide water depth) can significantly decrease primary production (Figures 3.20B & 3.21B), and increased flooding in the presence of burrows can also significantly decrease primary production in poorly drained sediments (mud treatment) (Figure 3.21C). This research also determined that within poorly drained sediments (the increased flooding–sand treatment) the presence of burrows can significantly increase pore water sulfide concentrations (Figure 3.12B). An unexpected result from this experiment was the demonstration of the clear reaction between high concentrations of pore water reduced iron within water-logged salt marsh sediments and pore water sulfide to form iron sulfide. The removal of high concentrations of sulfide from the pore water had a

positive effect on the production of *S. alterniflora* as determined by the correlation analysis (Table 3.4). Salt marsh persistence depends on the presence of *S. alterniflora*, using end-of-season biomass as an indicator of salt marsh persistence, sulfide concentration was the only variable measured that negatively affected the persistence of the marsh.

#### **Application and Relevance**

These results increase the understanding of the interactions among flood regime, fiddler crab burrows, and sediment, which are all important components of salt marsh ecosystems. The degree of sediment drainage and therefore the amount of time inundated, surface elevation, and sediment characteristics play a key role in the persistence of salt marsh systems. Because accelerated RSLR is a growing threat to remaining salt marsh habitats, it is necessary to investigate and understand its possible effects before these systems are permanently altered. Coastal development considerably increases the potential for salt marsh loss with relative sea-level rise (Titus 1986). In many areas, landward expansion of marshes is not possible such that once salt marshes can no longer transgress landward, restoration and re-creation are the only options for continued existence of these habitats.

Restoration is a key to promoting the persistence of salt marshes; this research will aid in making well-informed decisions concerning the development of restoration sites that can overcome the effects of relative sea-level rise. Sediment type, organic matter content, and the degree of drainage within marsh sediments are critical when planning the restoration of coastal marshes, but often the least expensive and most readily available sediment source is used. Sediment type and degree of drainage will become even more important in future restoration projects because future restored or created systems will have continued threat from RSLR.

## **Chapter 4: Intertidal marsh field survey**

#### Introduction

The region of coastal Virginia (USA) is experiencing high rates of relative sealevel rise (RSLR), as describe in Chapter 3. Marshes in this region will experience an increase in the frequency, duration, and depth of flooding (hydroperiod) with the current and especially predicted increases in RSLR if these marshes are not able to keep pace with the increase sea level. Marshes are potentially capable of keeping pace with RSLR through sediment buildup (i.e. sediment deposition on the surface of the marsh and/or sediment surface elevation increase due to belowground processes). Marshes also have the ability to transgress across the landscape by shifting the transition zones between marsh type and also encroaching on adjacent upland ecosystems (Kana et al. 1988, Moorhead & Brinson 1995, Brinson et al. 1995).

Sediment elevation change is variable within different zones of single marshes as well as between marshes in different areas of a region. Some marshes have been able to keep pace or increase sediment elevation at a rate that is greater than RSLR, where as others have not. Blum & Christian (2004) found that, in a mainland marsh in Virginia, vertical accretion from belowground processes (biogenic accretion) differed according to marsh zone with the low and high marsh unable to keep pace with RSLR without depositional inputs to the sediment surface, but the mid-marsh had high vertical accretion well over the amount necessary to keep pace with RSLR. Deposition on the marsh surface occurs at a greater rate close to creek banks (Christiansen et al. 2000), therefore the low marsh zone may receive enough sediment input to keep pace with RSLR, but this is not true for the high marsh. Erwin et al. (2006) found that lagoonal marshes in Virginia are not keeping pace with RSLR and are only increasing in elevation 0.7 -1.4 mm/yr where as RSLR is 3.9 mm/yr in those locations. Due to differences in RSLR, the rate of accretion necessary to keep pace with RSLR can differ by location (Cahoon & Reed 1995).

Transgression across landscape is not necessarily possible for marshes; it is dependent on the slope of the terrestrial transition and sediment supply (Brinson et al. 1995). Marshes can also expand by prograding at the low marsh open water transition, and while this is rare in mainland marshes (Brinson et al. 1995), it has occurred within a lagoonal marsh within the VCR-LTER site (Erwin et al. 2004). Due to erosion, loss of marsh area along the marsh edge at creek banks and lagoonal margins is a common occurrence within this region (Brinson et al. 1995, Erwin et al. 2004, McLaughlin 2010). These erosional areas provide sediment to the system, which would increase the sediment supply available for deposition on the surface of the marsh. In this case although the marsh would be losing area by marshward erosion, the supply of sediment could increase the likelihood of the marsh to have great enough accretion rates to keep pace with RSLR. Within the Virginia coast system, back barrier island marshes have an additional potential sediment source in certain locations. The barrier islands experience overwash events during extremely large storms, as occurred on the southern end of Hog Island during the Ash Wednesday Nor'easter of 1962. The deposition of large amounts of sand on the fringing marsh of the island and onto the adjacent mudflat immediately changes the sediment elevation, and new marsh begins to colonize the intertidal zone (Walsh 1998).

Many factors determine the ability of a specific marsh area to increase sediment surface elevation sufficiently to keep pace with RSLR. Mineral and organic accretion on

the sediment surface, and belowground processes including root growth, organic matter decomposition and pore water retention all play a role in how the surface elevation of a marsh can change. Accretion on the sediment surface is dependent on the presence and density of plants to decrease the strength of currents thereby acting as sediment traps (Gleason et al. 1979, Leonard & Luther 1995, Christiansen et al. 2000, Leonard et al. 2002), on the amount and type of suspended sediments in the water column, and on the duration and frequency of flooding (Cahoon & Reed 1995). Biogenic accretion is affected by many factors including belowground production, decomposition, sediment drainage (dictated by organic matter content and sediment type), and bioturbation. Because the production of plants (Spartina alterniflora) is important for sediment deposition in the low marsh, understanding the chemistry of the pore water that the plants are in contact with is also important (see Chapter 3 Figure 3.5). Therefore, in order to understand how RSLR may affect natural salt marshes within the Virginia Coast Reserve - LTER site, interactions among a variety of variables within marshes of different geomorphic settings need to be understood.

### Objective

The objective of this study is to explore the relationships among chemical, biological, and physical characteristics of coastal salt marshes along the Atlantic Coast of Virginia (USA) that differ in regional geomorphic setting, topographic location, and sediment type.

### **General hypotheses**

It was hypothesized that differences is sediment (between mud and sand) would drive many differences in the parameters measured among different salt marshes. Variation in elevation among salt marshes was also hypothesized to affect the characteristics measured, because differences in elevation would create differences in flood regime. Mud sediments were expected to have higher reduced ion concentrations (e.g. sulfide, reduced iron, iron sulfide), higher sulfate reduction rates, lower sulfate concentrations, higher porosity and organic content, and lower bulk density than sand sediments. It was expected that *S. alterniflora* would have more aboveground production and less belowground production in sand sediments than in mud sediments.

# Methods

As a compliment to the mesocosm experiment described in Chapter 3, similar variables were studied within natural marshes within the VCR-LTER site. Salt marsh field sites within the Machipongo Box Transect (VCRLTER IV & V Proposals 2000, 2006) of the VCR-LTER site on the Eastern Shore of Virginia (Figure 4.1) were chosen to represent a variety of geomorphic settings (mainland and back barrier island marshes), topographic location (tidal creek and bayside), and sediment types. Within the Machipongo Box Transect, three mainland marshes and three back barrier island marshes were chosen. The two types of geomorphology chosen were creek bank and bayside marshes (marshes adjacent to open water, not tidal creeks). The two sediment types chosen are common within the VCR-LTER site and were mud sediments and sandy sediments. The areas of marsh selected for this study consist of two mainland tidal creek marshes, a mainland bayside marsh, two back barrier island bayside marshes, and one back barrier island tidal creek marsh (Table 4.1).



Figure 4.1. Map showing Delmarva Peninsula; square indicates study location. Inset: Study area, stars indicate locations of the six salt marsh field sites surrounding Hog Island Bay.

Table 4.1. Matrix of geomorphic settings (mainland vs. island) and topographic locations (creek vs. bayside) for 6 sites within the Machipongo Box Transect. Sediment type is also noted.

		Geomorphic Setting							
		Mainland	Island						
ttion	ek	Lower Phillips Creek (LPC) Mud	George's Stake (GEOR) <b>Mud</b>						
ic Loca	Cre	Machipongo River (MACH) <b>Mud</b>							
Topograph	Bayside		South Hog Island (SHMUD) <b>Mud</b> (no overwash)						
		Upshur Neck (UPSH) Sand	South Hog Island (SHSAND) Sand (overwash)						

These marshes were studied in order to determine the interactions among elevation, hydroperiod, sediment characteristics, presence or absence of fiddler crab burrows, *Spartina alterniflora* production, and pore water ion concentrations in natural salt marsh systems.

Study plots were set up in the low marsh area of each site and each contained a monotypic uniform stand of *S. alterniflora*. Each study plot was approximately equal in size (36 m<sup>2</sup>). In these marshes, the water level (subsurface and surface), sediment surface elevation, fiddler crab burrow density, *Spartina alterniflora* stem density and end-of-season aboveground biomass, and root growth were determined. The pore water ion concentrations, sediment iron sulfide, and sulfate reduction rates that were measured within these sites were the same ones measured in the mesocosm experiment (Chapter 3).

In order to collect the above data, a 1 meter water level recorder (Remote Data Systems, Ecotone WM Water Level Monitor, WM16k1015) was installed at each field site between June 19 - 20, 2006 and remained at the sites until August 30 – Sept 7, 2006. The surface of the marsh at each water level monitor was surveyed using a Trimble 4000 GPS unit with sub-centimeter accuracy, except for LPC where a laser theodolite was used. Elevation dictates flooding frequency (Cahoon & Reed 1995), therefore the elevation at each site was used to determine flooding frequency by comparison with the NOAA tide gauge in Wachapreague, VA.

The locations for the water level recorder were the center of each plot and ranged in distance from the nearest open water from 10 - 29 m. Four pore water equilibrators were installed in each study site to collect pore water at five depths (2, 6.5, 12.5, 20, 25 cm) within the sediment. The four pore water equilibrators were located in a line with the water level monitor parallel to the nearest open water; two water equilibrators were located on either side of the water level monitor, 1 m apart from each other. The water equilibrators were installed at each field site between June 19 - 20, 2006 and were collected between August 1 - 2, 2006.

The pore water equilibrators were made from 20 cm glass scintillation vials (Wheaton, GPI Thread 24-400) that were fitted with a 0.2  $\mu$ m HT Tuffryn membrane (Pall, Gelman) held by an open-top screw cap (Wheaton, open-top black phenolic screw cap, GPI Thread 24-400). A 40 cm section of 2" schedule 40 PVC was used to position the equilibrators at the proper depth, the top 10 cm of which remained above the sediment surface. Holes were added to the 2" PVC at the 5 desired depths and 5 cm sections of thin wall 1" PVC were inserted into the holes in order to kept the scintillation vial in place and flush with the sediment surface (Mozdzer 2009).

When the pore water equilibrators were collected they were sealed with parafilm and kept on ice until they were returned to the lab. Initial unfiltered subsamples were analyzed colorimetrically immediately after collection for determination of sulfide concentration following the method of Cline (1969). The remaining sample from each equilibrator was filtered to sterilize using a syringe filter (Acrodisc, GHP 0.2 μm).

Pore water from the equilibrators was analyzed for sulfate concentration at three depths 2, 6.5, and 12.5 cm using a Dionex Ion Chromatography System (Model ICS-3000) with an AG18 column and suppressed conductivity detection. After filtration, a 1.0 ml subsample was preserved with 0.02 ml of 5 N HCl and frozen until analyzed for reduced iron (Fe(II)) concentration following the Ferrozine method (Gibbs 1979).

Spartina alterniflora stem density was counted and aboveground biomass collected within a 25 cm x 25 cm quadrat in three areas of the study plot between August 30 – September 7, 2006. Root in-growth bags were buried at the six field sites when the water level recorders were installed and they were collected when aboveground biomass was collected. The root in-growth bags were made from 1/16" delta 35 netting (Nylon Net Company, Memphis) and were 6.5 x 20 cm in size and filled with sediment that had previously been collected from the six sites (Blum 1993). The root bags were buried vertically in the sediment near the locations of the water level recorders.

PVC sediment cores with an inner diameter of 5 cm were collected to analyze sediment at the five equilibrator depths. Four acrylic sediment cores with an inner diameter of 4.4 cm were collected from the study sites to complete additional sediment analyses. Sediment slices at the five equilibrator depths were analyzed for porosity, bulk density, and organic matter content. Sediment slices were dried at 60°C for 24 hrs. The wet weight and dry weight of each sample was used to calculate porosity (wet wt. – dry

wt.)/volume) and bulk density (dry wt./volume). Sediment samples were put in a muffle furnace at 500°C for 6 hrs to determine organic content by loss on ignition.

Sediment cores were collected at the six field sites August 16 -17 in order to determine sulfate reduction rates. PVC sediment cores (5 cm diameter) were collected and 10 cc de-tipped syringe cores were remove through holes within the PVC core at depths of 3, 10, and 15 cm. The subcores were immediately injected with 50 µl of  $H_2^{35}SO_4$  (1 µCi) (Herlihy 1987 after Jorgensen 1978) and capped with serum stoppers. The injected subcores were left to incubate for approximately 1 hour and then frozen in an ice and ethanol bath to stop the reaction. The total reduced inorganic sulfur (free sulfide, S0, FeS, and FeS2) within the subcores was extracted using the chromium reducible sulfide method following Thomas 2004 (after Fossing & Jorgensen 1989). Once extracted and trapped with 0.5 N NaOH, duplicate 1 ml aliquots were used for analysis. Each aliquot was pipetted into a 20 ml scintillation vial to which 10 ml of Beckman Coulter Ready Safe Liquid Scintillation Cocktail was added and subsequently read on a Beckman LS 6500 Multipurpose Scintillation Counter with quench correction. To determine sulfate reduction rates, the fraction of injected  ${}^{35}SO_4{}^{2-}$  converted to  ${}^{35}S^{2-}$ was calculated (Thomas 2004).

From one of the 4.4 cm sediment cores collected at each field site a dried sediment slice (9-10 cm depth) was used to determine sediment iron sulfide concentration. Sediment iron sulfide was extracted following the chromium reducible sulfide method (Fossing & Jorgensen 1989, Thomas 2004) and trapped with 40 ml of 4% ZnAc with a few drops of isoamyl alcohol added to prevent foaming. This method actually determines the total reduced inorganic sulfur (free sulfide, S°, FeS, and FeS<sub>2</sub>), but because the sediment samples were dried at 60°C for 24 hours before analysis it is being considered that concentrations of only FeS and FeS<sub>2</sub> were measured.

The number and diameters of fiddler crab burrows was measured within a 25 cm x 25 cm quadrat in three areas of the study plot in the middle of the summer.

# Statistical analyzes

Data for six variables collected at five depths (sulfide, sulfate, Fe(II), porosity, bulk density, and organic matter content) and burrow density and diameter were condensed for analysis; there were a few reasons for this. At some locations, sediment cores obtained were not deep enough to allow sample collection at a depth of 25 cm. In the well-drained sediment at SHSAND the pore water equilibrators at 2 cm depth were empty when collected. These missing values at depths of both 2 and 25 cm also necessitated removal of data from these two depths from the analysis. For the six variables with depth profiles, the pattern of differences among sites was largely consistent along the depth profiles once below 2 cm (Appendix 3.1, 3.2). In order to reduce the number of variables, these data were condensed by integrating each of them across the 6.5, 12.5 and 20 cm depths. Burrow density and diameter were condensed by creating a new variable, burrow area. This was mainly due to the fact that replicates for some sites, including all replicates at UPSH, did not have any burrows and therefore burrow diameter was unknowable (missing, but not zero). The use of a principle component analysis (PCA) to reduce the dimensionality of these data was not applicable to this analysis due to the differing number of replicates per variable. At the six sites, for each parameter (38) there were 2 - 4 (mean of 3.6) replicates per site for a total of 12 - 24 (mean of 21.8) observations per variable. In order to have enough degrees of freedom to

run the multiple tests necessary, the data needed to be condensed. Related sets of parameters (pore water variables, sediment variables, *Spartina alterniflora* variables) that were collected from the same sampling devise (water equilibrator, sediment core, quadrat) and had the same number of replicates could be analyzed using MANOVAs once the data were condensed.

Means and standard errors for each variable were calculated for each site. ANOVAs or MANOVAs where appropriate were used to determine differences in independent variables among sites. Because of the number of staticstical test being run (8), a bonferroni adjustment 1/8 \* 0.05 = 0.00625 was used to determine the  $\alpha$  level needed for each test (MANOVA or ANOVA) to be significant. For the MANOVAs, if the Wilks' Lambda was significant (p<0.00625), then univariate tests were performed on each variable within the MANOVA, these ANOVAs were also determined to be significant only if the p<0.00625. Once a univariate test was determined to be significant, post hoc Tukey tests were run to determine difference among individual sites, the  $\alpha$  value for these tests could be set at 0.05, because overall significance had already been determined for the individual variable with the lower bonferroni adjusted  $\alpha$  value.

Pore water ion concentrations (sulfide, sulfate, and Fe(II)) that were measured from the same water equilibrators were initially analyzed for significant differences with a MANOVA. *Spartina alterniflora* density (stem and new shoot) and end-of-season biomass were all measured within the same quadrat therefore a MANOVA was initially used to determine if there were significant differences in these variables among sites. Differences in belowground production among sites were analyzed using an ANOVA. Sediment characteristics (porosity, bulk density) that were all collected from the sediment cores and had an equal number of replicates were initially analyzed for significant differences with a MANOVA. Differences in organic content were analyzed using an ANOVA, because the sample size differed from those porosity and bulk density. Differences in iron sulfide concentration were analyzed using an ANOVA. SRR at three different depths (3, 10, 15 cm) were initially analyzed for significant differences with a MANOVA. Differences in burrow area were analyzed using an ANOVA.

A correlation analysis was performed on a the condensed dataset to determine if and how these variables relate to each other within these natural marshes.

### Results

# Elevation, drainage, and flood regime

The 6 field sites ranged in elevation from -0.4587 m at SHSAND to 0.5205 m at LPC (Table 4.2). From the water level recorder data it was determined that the water table did not drop below the surface of the sediment at most of the sites (Appendix 3.3). One site, SHSAND, did drain somewhat when the recorder was initially installed at the site, but it did not drain to depths below 12.5 cm and did not drain below the sediment surface a few weeks after installation. Using the water level data from June 20 to July 31 it was determined that this site was drained to a depth of 2 cm less than 6 % of the time.

The percentage of time that each site was flooded was determined using elevation data and ranged from 24.60 % at LPC to 93.14 % at SHSAND (Table 4.2). All graphs presented list field sites from high elevation (on the left or top) to low elevation (on the right or bottom).

Table 4.2. Site names and abbreviations with elevation above mean sea-level (MSL) and % of time flooded from June 1 to Sept 30, 2006 as calculated from elevation comparison to NOAA tide gauge in Wachapreague, VA.

Site	Elevation above MSL (m)	% of Time Flooded
Lower Phillips Creek (LPC)	0.5205	24.60
Machipongo River (MACH)	0.3500	38.74
Upshur Neck (UPSH)	0.0658	58.83
George's Stake (GEOR)	-0.1314	71.88
South Hog Mud (SHMUD)	-0.1571	73.65
South Hog Sand (SHSAND)	-0.4587	93.14

#### **Pore water**

Results of a MANOVA for the depth integrated (6.5, 12.5, 20 cm) sulfide, Fe(II) and sulfate concentrations among the 6 sites was statistically significant (Wilks' Lambda = 0.077; F(15,39.049) = 3.98; p=0.0003). Given the significance of the overall test, the univariate main effects were examined. Pore water sulfide concentration was significantly different among the 6 sites (p<0.0001) and ranged from 141.7  $\mu$ M ± 8.9 at SHSAND to 3283.6  $\mu$ M ± 64.3 at LPC. LPC and GEOR had the highest sulfide concentrations and were significantly different from SHMUD and UPSH which had intermediate concentration from SHMUD, UPSH, or SHSAND; SHSAND had the lowest sulfide concentration, and was not significantly different from MACH. Pore water sulfate and Fe(II) concentrations were both not significantly different among the 6 sites (Figure 4.2B & C). Sulfate concentrations ranged from 14.4 mM ± 0.9 at LPC to 28.2



Figure 4.2. Depth integrated (6.5, 12.5, 20 cm) concentrations for A) sulfide, B) sulfate, C) reduced iron for the six field sites. Means  $\pm$  SE. Significant differences determined with post hoc Tukey tests and are noted with different letters (p  $\leq$  0.05).

mM  $\pm$  1.6 at SHSAND. Fe(II) concentrations ranged from 6.3  $\mu$ M  $\pm$  2.0 at SHSAND to 16.8  $\mu$ M  $\pm$  7.2 at LPC.

#### Spartina alterniflora density, end-of-season biomass, and belowground production

For the three variables related to Spartina alterniflora (stem density, new shoot density, end-of-season biomass) a one-way MANOVA test of differences among the sites was statistically significant (Wilks' Lambda = 0.012; F(15, 22.486) = 5.90; p< 0.0001). Given the significance of the overall test, the univariate main effects were examined. Stem density was significantly different among the 6 sites (p<0.0001) and ranged from  $133/m^2 \pm 19$  at LPC to  $736/m^2 \pm 66$  at UPSH (Figure 4.3A). UPSH had significantly higher stem density than all other sites, SHMUD had the next highest stem density and was not significantly different from GEOR, MACH, or SHSAND. LPC had the lowest stem density and was also not significantly different from GEOR, MACH, or SHSAND. New shoot density was significantly different among the 6 sites (p<0.0001) and ranged from  $136/m^2 \pm 8$  at LPC to  $741/m^2 \pm 71$  at UPSH (Figure 4.3A). New shoot density generally followed the same pattern as stem density, but UPSH was significantly higher than all other sites, and there was no significant difference among the remaining 5 sites. End-of-season biomass was not significantly different among the six sites, and ranged from 324.3 g/m<sup>2</sup>  $\pm$  84.4 at LPC to 600.3 g/m<sup>2</sup>  $\pm$  153.9 at GEOR (Figure 4.3B). The results of an ANOVA determined that there was a significant difference in belowground production among the 6 sites (p=0.0010) with a range of 0.208 g/bag  $\pm$  0.067 at SHSAND to 0.755 g/bag  $\pm$  0.144 at LPC (Figure 4.3C). LPC had the highest belowground production, but was not significantly higher than SHMUD, which had an



Figure 4.3. *Spartina alterniflora* A) stem and new shoot density, B) end-of-season biomass, and C) belowground production for the six field sites. Means  $\pm$  SE. Significant differences determined with post hoc Tukey tests and are noted with different letters (p  $\leq$  0.05).

intermediate amount of belowground production. The remaining 4 sites had significantly lower belowground production than LPC, but not significantly different than SHMUD.

# Sediment characteristics: porosity, bulk density, and organic content

Results of a MANOVA for porosity and bulk density for the 6 sites was significant (Wilks' Lambda = 0.006; F(10,34) = 41.26; p<0.0001). Given the significance of the overall test, the univariate main effects were examined. Porosity was significantly different among the 6 sites (p<0.0001) and ranged from 0.46 ml/cm<sup>3</sup>  $\pm$  0.01 at SHSAND to 0.88 ml/cm<sup>3</sup>  $\pm$  0.01 at SHMUD (Figure 4.4A). The four sites, SHMUD, GEOR, UPSH, and SHSAND were all significantly different from each other with porosity decreasing from SHMUD to SHSAND; LPC and MACH had intermediate porosity values between those of SHMUD and GEORG and were not significantly different from either. Bulk density was significantly different among the 6 sites (p < 0.0001) and ranged from 0.72 g/cm<sup>3</sup> ± 0.03 at LPC to 1.67 g/cm<sup>3</sup> ± 0.02 at SHSAND (Figure 4.4B). Bulk density for SHSAND and UPSH were not significantly different from each other. GEOR, MACH, and LPC had significantly different bulk density from each other and from SHSAND and UPSH. SHMUD had an intermediate bulk density with a value between that of MACH and LPC. The results of an ANOVA determined that there was a significant difference in organic content among the 6 sites (p=0.0001) with a range of  $1.00\% \pm 0.15$  at SHSAND to  $10.15\% \pm 0.76$  at SHMUD (Figure 4.4C). SHMUD, with the highest organic content did not differ significantly from MACH or LPC; the organic content for GEOR was lower than, but did not differ significantly from


Figure 4.4. Depth integrated (6.5, 12.5, 20 cm) sediment characteristics for A) porosity, B) bulk density, C) organic content for the six field sites. Means  $\pm$  SE. Significant differences determined with post hoc Tukey tests and are noted with different letters (p  $\leq$  0.05).

MACH or LPC. UPSH and SHSAND had the lowest organic content and did not differ significantly from each other.

# Sediment iron sulfide

The results of an ANOVA determined that sediment iron sulfide concentration was significantly different among the 6 sites (p<0.0001), and ranged from 8.38 umol/cm<sup>3</sup>  $\pm$  1.04 at SHSAND to 64.33 umol/cm<sup>3</sup>  $\pm$ 6.20 at MACH (Figure 4.5A). MACH had significantly higher iron sulfide than the other sites; LPC and GEOR differed significantly from MACH, but not from each other. SHMUD was intermediate between and did not differ significantly from either LPC and GEOR or UPSH. SHSAND had the lowest iron sulfide concentration and did not differ significantly from UPSH.

# Sediment sulfate reduction rate (SRR)

Differences in sulfate reduction rates (measured at three depths) among the 6 sites were analyzed with a one-way MANOVA. Results of a MANOVA for these three variables (three depths) for the 6 sites was not significant (Wilks' Lambda = 0.210; F(15,44.57) = 2.26; p=0.0181). The overall range in SRR for all three depths was from 4.57 nmol/cm<sup>3</sup>/day ± 0.17 at LPC at a depth of 10 cm to 21.20 nmol/cm<sup>3</sup>/day ± 11.32 at LPC at a depth of 15 cm (Figure 4.5B).

At a depth of 3 cm, SHMUD and LPC had the highest SRR, where as UPSH had the lowest; the remaining sites had intermediate SRRs. At a depth of 10 cm, there was a completely different pattern in SRR among sites. SHMUD had the highest SRR and LPC had the lowest; the 4 remaining sites were all intermediate. At a depth of 15 cm, a third pattern was detected, LPC had the highest SRR with high variability, where as MACH had the lowest, and the remaining sites had intermediate SRRs.



Figure 4.5. A) Sediment iron sulfide concentration at a depth of 10 cm, B) depth profile of sulfate reduction rates, and C) burrow area for the six field sites. Means  $\pm$  SE. Significant differences determined with post hoc Tukey tests and are noted with different letters (p  $\leq$  0.05).

#### Burrow area, density, and diameter

The results of an ANOVA revealed that burrow area was not significantly different among the 6 sites. Burrow area was zero at UPSH and ranged from 32.9 cm<sup>2</sup>/m<sup>2</sup>  $\pm$  32.9 at SHMUD to 178.9 cm<sup>2</sup>/m<sup>2</sup>  $\pm$  53.1 at MACH (Figure 4.5C). Burrow density at sites that had fiddler crab burrows ranged from 8/m<sup>2</sup>  $\pm$  8 at SHMUD to 192/m<sup>2</sup>  $\pm$  50 at MACH and burrow diameters ranged from 5.73 mm  $\pm$  5.73 at SHMUD to 13.27mm  $\pm$  4.46 at LPC.

# **Correlation analysis**

A correlation analysis of the data from all 6 sites using the 15 variables described above yielded 10 significant pairs of correlated variables ( $\alpha$ =0.05) (Table 4.3). Because many of the significant correlations had significance at much less than p=0.05, it can be assumed that the number of significant correlations is greater than would have been from chance alone as calculated using a binomial distribution. Sulfide was significantly positively correlated (p=0.0011) with reduced iron and significantly negatively correlated (p=0.044) with sulfate. Reduced iron and root biomass were significantly positively correlated (p=0.0257). Reduced iron and SRR at a depth of 15 cm were significantly positively correlated (p=0.0493). SRR at a depth of 15 cm and belowground production were significantly positively correlated (p=0.0091). Stem density and new shoot density were significantly positively correlated (p=0.0139). Bulk density was significantly negatively correlated with both porosity (p=0.0021) and organic content (p=0.0012). Porosity was significantly (p=0.0019) positively correlated with organic content. Sediment iron sulfide and burrow area were significantly positively correlated (p=0.0169). All of these significant correlations had Pearson correlation coefficients over

	Stem Density	New Shoot Density	EOS Biomass	Belowground Production	Burrow Area	Sulfide	Reduced Iron	Sulfate	Porosity	Bulk Density	OM Content	FeS	SRR 3cm	SRR 10cm	SRR 15cm
Stem Density															
New Shoot Density	0.90211 0.0139														
EOS Biomass	0.15019	0.44477													
	0.7764	0.3768													
Belowground	-0.19779	-0.22017	-0.43321	_											
Production	0.7072	0.6751	0.3908												
Burrow Area	-0.48723	-0.75619	-0.56058	-0.25332											
	0.327	0.0819	0.2472	0.6281											
Sulfide	-0.159	-0.2812	-0.30511	0.80263	-0.05567										
	0.7635	0.5893	0.5565	0.0546	0.9166										
Reduced Iron	-0.281	-0.32389	-0.29697	0.8662	-0.11398	0.97247									
	0.5896	0.5312	0.5676	0.0257	0.8298	0.0011									
Sulfate	0.15934	0.45933	0.76328	-0.67388	-0.385	-0.8234	-0.76434								
	0.763	0.3595	0.0774	0.1422	0.451	0.044	0.0768								
Porosity	-0.26571	-0.5562	-0.496	0.57216	0.38701	0.68853	0.58862	-0.78434	-						
	0.6108	0.2517	0.317	0.2354	0.4485	0.1304	0.219	0.0647							
Bulk Density	0.5098	0.75484	0.53656	-0.5598	-0.51292	-0.63431	-0.58013	0.75705	-0.96224						
	0.3015	0.0828	0.2724	0.248	0.2981	0.1761	0.2274	0.0814	0.0021						
OM Content	-0.38993	-0.66215	-0.50237	0.46513	0.4975	0.50157	0.41981	-0.66167	0.96433	-0.97188					
	0.4448	0.1519	0.3098	0.3526	0.3153	0.3107	0.4073	0.1523	0.0019	0.0012					
FeS	-0.44864	-0.78441	-0.63201	0.09952	0.89179	0.36767	0.2732	-0.70543	0.73478	-0.79841	0.76183				
	0.3722	0.0647	0.1782	0.8512	0.0169	0.4733	0.6004	0.1174	0.0962	0.0569	0.0783				
SRR 3cm	-0.53498	-0.49266	-0.07332	0.4367	0.04366	0.12531	0.17882	-0.06567	0.53515	-0.61817	0.67156	0.16323			
	0.2741	0.3208	0.8902	0.3866	0.9346	0.813	0.7346	0.9016	0.2739	0.1909	0.1441	0.7573			
SRR 10cm	0.35853	0.40247	0.6344	0.0288	-0.53751	0.12077	0.01834	0.25207	0.21056	-0.03974	0.15545	-0.31733	0.3062		
	0.4852	0.4289	0.1761	0.9568	0.2714	0.8197	0.9725	0.6299	0.6888	0.9404	0.7687	0.54	0.5551		
SRR 15cm	-0.48082	-0.41368	-0.43937	0.92117	-0.12751	0.67846	0.81285	-0.57282	0.3987	-0.48869	0.34354	0.10762	0.43559	-0.24645	
	0.3343	0.4149	0.3834	0.0091	0.8098	0.1385	0.0493	0.2348	0.4336	0.3253	0.505	0.8392	0.3879	0.6378	

# Table 4.3. Pearson Correlation Coefficients for 15 variables collected in the six field sites. Top number is Pearson correlation coefficient, bottom number is the probability. Significant probabilities ( $p \le 0.05$ ) are highlighted in yellow.

 $\pm 0.80$  and six out of ten of them were over  $\pm 0.90$ ; therefore not only were the correlations significant, they were also highly correlated.

### Discussion

These results suggest that the low marsh areas chosen for this study are representative of the range of marshes in this region with respect to geomorphic setting, topographic location, and sediment type (Table 4.1). All but two of the mainland sites (LPC and MACH) were unique with regards to their combination of geomorphic setting, topographic location, and sediment type. It is possible that all of the factors in the  $2 \times 2 \times 2$  design could be important, but the lack of replicate marshes within each  $2 \times 2 \times 2$  combination prevents any unpooled examination of these factors.

# Elevation, drainage, and flood regime

The large range in elevation among the six sites shows that low marsh (*sensu* Bertness 1991, Mitsch & Gosselink 1993) areas in this region exist within at least a 90 cm range of elevation. The South Hog Sand (SHSAND) site might be considered marsh edge more so than low marsh. The three island sites had the lowest elevation. This could have been just by happenstance or partly because of the route of access to the marshes; island sites via water and mainland sites via upland. As seen in the water level recorder data (Appendix 3.3), each site was inundated daily, and for the most part the sediments at these sites did not drain, as is common in many salt marsh areas (Howes et al. 1981). It was expected that the sediment in the mud sites would not drain and would remain water-logged while the sediment surface was exposed at low tide. However, the converse was also expected; that the sediment in the sand sites would drain at low tide when the sediment surface was exposed. The two sand sites (SHSAND and UPSH) did not drain

very much. This might have been because they were at the mid to low end of the elevation gradient. Therefore, they did not have as much time to drain between the time that the tide receded exposing the sediment surface and the time that the marsh surface was flooded again. This would have been especially true for SHSAND because it was the marsh at the lowest elevation in this study.

The percentage of time that each site was flooded as calculated from site elevation and the NOAA tide gauge at Wachapreague, VA was somewhat surprising, and might not have been quite accurate. The Wachapreague tide gauge is not in the same watershed as the six sites and is 15.1 to 21.6 km from field sites on the mainland and 19.1 to 24.6 km from sites on Hog Island, this could affect the calculations of the percentage of time flooded. For LPC, the result of the marsh being flooded less than 25% of the time seems a little low, as this would generally mean the site was only flooded for 1.5 hours on either side of the two high tides each day. In the other extreme, for SHSAND, the result of the marsh being flooded over 90% of the time seems quite high, as this would mean that the marsh surface was only exposed for just over an hour during each low tide. Unfortunately, tide gauges operated by the VCR-LTER closer to the field sites at Red Bank and on northern Hog Island were not operational during the study period.

# **Pore water**

Sulfide concentration varied greatly among sites and was over 20 times higher at LPC than SHSAND (Figure 4.2A). The expectation that sites with sand sediment would have lower sulfide concentrations was partially met; SHAND had the lowest concentration, but the other sand site, UPSH, had intermediate sulfide concentration and did not differ significantly from some of the mud sites (Figure 4.2A). There were no

trends in sulfide concentration in terms of differences between sites on the mainland versus island, or creek side versus bayside.

Although mean sulfate concentrations were twice as high at SHSAND compared to LPC, the variability in sulfate concentration within sites likely cause the sulfate concentrations at the different sites to not be significantly different from each other. It looks as though there is a negative trend between sulfate concentration and elevation, where the higher elevation sites had lower sulfate concentrations. Because sulfate enter the via incoming sea water and lower sites are inundated more of the time, this trend makes sense. There were no trends in sulfate concentration with regards to mainland versus island sites, creek side versus bayside sites, nor mud versus sand sites.

Reduced iron concentration did not differ significantly among the six sites (Figure 4.2B). As with sulfide concentration, the expectation that reduced iron concentration would be lower at the sites with sand sediments was partially met; SHSAND did have the lowest reduced iron concentration, but the other sand site, UPSH, had an intermediate Fe(II) concentration among the six sites. Although there were no significant differences in Fe(II) concentration among the six sites, the pattern of highest to lowest concentration exactly matched that of sulfide concentration, which did have significant differences among the sites. Again, there were no trends in Fe(II) concentration between mainland versus island sites or creek side versus bayside sites.

#### Spartina alterniflora density, end-of-season biomass, and belowground production

The results that stem density and new shoot density nearly mimicked each other was interesting. In four of the six sites the means for stem density and new shoot density were very close, in the other two sites (MACH & SHAND) they were quite different,

MACH had lower new shoot density than stem density and SHAND had higher new shoot density than stem density. New shoots replace senescing older shoots; in this region these new shoots overwinter and produce the new growth in the spring. The timing of the stem density measurements, at the end of the summer after florescence, was such that the plants had put energy into new shoot growth. The result that there was no significant difference in EOSB among sites was likely due to the variability of EOSB within each site. Interestingly, stem density was not an indication of EOSB. Morris & Haskin (1990) found that although two sites had the same aboveground production, one site had double the stem density of the other. Sites with the highest EOSB (GEOR, SHSAND, SHMUD) had moderate stem densities, indicating that these plants were taller and/or more stout. UPSH had the highest stem density by double, but end-of-season biomass was moderate. The result of belowground production having little difference among sites follows the results of EOSB. The only notable trend was that the site with the highest belowground production had the highest sulfide concentration and the two sites with the lowest belowground production had the lowest sulfide concentrations. This is contrary to the finding of Koch & Mendelssohn (1989) who found that high sulfide concentrations, over 1000 µM, can decrease root biomass and change the morphology of belowground structures. Water-logged sediments are likely to have greater sulfide concentrations and plants often put more energy into belowground production when they are growing in water-logged conditions (Gribsholt & Kristensen 2003). There were no obvious trends in *S. alterniflora* parameters for the pairs of geomorphic settings, topographic location, or sediment type.

#### Sediment characteristics: porosity, bulk density, and organic content

The results of the sediment parameters porosity, bulk density, and organic matter content were as expected. Within the low marsh, these three sediment parameters have a predictable relationship to each other. The silt, clays, and organic matter of mud sediments have the capacity to contain a high volume, and because they have little sand they therefore have lower bulk density. Sandy marsh sediments generally have less organic matter and generally hold less water than muddier sediments.

#### **Sediment iron sulfide**

The results for iron sulfide concentration within the marsh sediments were as expected. The two sand sites (SHSAND and UPSH) had the lowest iron sulfide concentrations, and the mud sites had at least double the concentration of those of the sand sites. Iron sulfide concentration did not follow the same pattern from high concentration to low as either sulfide or Fe(II). There were no trends in iron sulfide concentration between mainland versus island sites or creek side versus bay side sites.

#### Sediment sulfate reduction rate (SRR)

Sulfate reduction rates were 1 or 2 orders of magnitude lower than expected; especially with the high concentrations of pore water sulfide and iron sulfide measured at some of the sites. I think that during the procedures to measure decays per minute (dpm) the aliquot samples were not well mixed with the liquid scintillation cocktail. Because SRRs are highly variable within sites at all depths and there are no trends in SRR among sites at the three depths, it is difficult to determine if the lack of trends is due to the laboratory procedure or if the rates are truly this variable.

# **Burrow area**

The total burrow area for fiddler crab burrows did not differ significantly among the six marshes and was highly variable within sites. Although the range in burrow area was very high, from UPSH having no crab burrows at all, to MACH having  $178.9 \text{ cm}^2/\text{m}^2 \pm 53.1$ , the other four sites had more moderate burrow areas. There were no trends in burrow area between mainland versus island sites, creek side versus bayside sites, nor mud versus sand sites.

#### **Correlation analysis**

All of the correlations that were significant were also highly correlated (i.e. Pearson correlation coefficients were all over 0.80 and six out of ten were over 0.90) (Table 4.3). Many of the significant correlations were noted earlier because they were visually obvious from the bar graphs of means and standard errors. The positive and negative correlations among sediment characteristics (porosity, bulk density, and organic matter content) were expected because they are generally related to each other as discussed earlier. The relationships among these three sediment characteristics were highly correlated above  $\pm 0.96$ , which definitively shows how strongly they are related to each other within intertidal salt marsh sediments in this region. This region does not have the peat accumulation of the salt mashes of New England, and so these relationships would likely only hold true for sediments that are mostly mineral (Bradley & Morris 1990b). The relationship between stem density and new shoot density was also noted earlier and was highly correlated. Sulfide concentration and reduced iron concentration were also highly correlated. These ions are both formed in reducing environments; therefore, it follows that they would be positively correlated. Sulfate concentration and

sulfide concentration were highly negatively correlated because sulfate is converted to sulfide (among other ions and compounds) in reducing conditions (Fenchel et al. 1998).

Reduced iron and SRR (at 15 cm) were correlated but with a lower correlation coefficient (0.81). Reduced iron is produced in reducing environments and higher SRRs would therefore be expected in more highly reducing environments. However, the fact that the other two depths of SRR were not correlated with reduced iron or sulfide and that SRR (at 15 cm) was also not correlated with sulfide indicates that this significant correlation could just be by chance alone. The positive correlation of reduced iron and SRR (at 15 cm) with belowground production is understandable; higher reduced iron concentrations and SRRs would be present in more water-logged sediments, and plants often produce more roots when they are growing in water-logged conditions (Gribsholt & Kristensen 2003). One of the correlations was less explainable or understandable; sediment iron sulfide concentration was highly correlated with burrow area. One would think that, as burrows were being excavated, iron sulfide within the sediment would be brought to the surface, and therefore there would be less iron sulfide at 10 cm depth (the depth of measurement) in sediments that had greater burrow area per m<sup>2</sup>.

#### Interpretation of results in the context of RSLR

Within this study, marshes that were located on tidal creeks would likely have more sediment deposition due to the flocculation of sediment within the water column and result in greater deposition near the creek banks (Christiansen et al. 2000). Marshes that were located along the open bay (especially where the sediment was sandy) would have a greater likelihood of sediment deposition during storm events when increased turbulence would be able to carry sand and deposit it on the marsh surface (Cahoon & Reed 1995). Surface accretion is not a linear process over time, but has seasonal and inter annual variation partially due to tides and storms. For this reason, accretion measurement can be variable over short and long-term measurements. Flooding frequency and duration affect the amount of allochthonous material deposited within a marsh and this material can be composed of both mineral and organic matter (Cahoon & Reed 1995). Cahoon & Reed (1995) also showed that accretion was significantly related to duration of flooding.

The low intertidal marshes in this study had high flooding frequency, but without monitoring marker horizons at the field locations it is impossible to know how sediment deposition during the study period varied among the sites and between the geomorphic settings, topographic locations and the sediment types. Monitoring sediment deposition on different time scales is an important factor in understanding the potential for these marshes overcome RSLR. It is important to have long-term measurements not only of sediment deposition, but the internal changes in elevation of the marsh surface (Cahoon et al. 2002a, b)

*S. alterniflora* aboveground production is thought to have a parabolic shaped relationship to tidal level, if it is growing at a depth too low in relationship to mean high tide, then *S. alterniflora* production becomes unstable (Morris et al. 2002). This relationship is likely unique to different coastal areas and settings. If the rate of sediment accretion within a marsh matches the rate of SLR, *S. alterniflora* can maintain its optimal depth relative to mean high tide. In marshes in the same region as this study, Kirwan et al. (2011) found that *S. alterniflora* production, in marshes that were high in the intertidal

zone, was unresponsive to interannual sea-level over the decade-long study period. This is an indication that the position within the marsh can also dictate how *S*. *alterniflora* production and therefore marsh persistence will be affected by RSLR.

# Conclusions

Interactions within salt marsh systems that determine salt marsh persistence are complicated. These interactions differ with differing position in the tidal range, sediment type, distance to open water, sediment drainage, edaphic characteristics, sediment supply, and many other parameters many of which are interrelated. Although the results of this study do not suggest how well the salt marsh sites studied may be able to keep pace with the high RSLR in this region, they do make evident the necessity to have long-term data on many of the parameters measured in this study, as well as other measurements that were beyond the scope of this short-term study.

It would be beneficial to measure the pore water ion concentrations as well as *Spartina alterniflora* characteristics measured in this study at locations where sediment elevation tables (SET), root-SETs, and marker horizons are located. Monitoring these parameters over time in concert with elevation changes within these marshes would allow determination of the response of the marsh to changes in localized relative sea-level. This information would aid in understanding the future persistence of marshes as they either gain elevation to keep pace with RSLR, transgress across the landscape, or decline in extent. Having as much information as possible about individual marshes that are threatened by RSLR will help to inform the management of these systems in order for them to persist while experiencing RSLR. Insights gained from understanding the

### **Chapter 5. Conclusions and Synthesis**

Salt marshes have a high degree of heterogeneity; therefore, it is important to study these systems on multiple scales. The previous three chapters have investigated interactions within salt marshes on the micro-, meso-, and macro-scales. The combination of both *in situ* measurements and experiments occurring both in the field and within constructed marsh mesocosms acted in a complimentary fashion to further our understanding of the complex interactions between salt marsh sediments, pore water, and biota and how differences in inundation and drainage due to sea-level rise may affect these interactions.

The *in situ* experiment measuring  $O_2$  concentration surrounding individual burrows (Chapter 2) elucidated the extent to which a fiddler crab burrow can oxygenate the bulk sediment in typical poorly drained intertidal marshes of coastal Virginia. In sediments that remain water-logged during low tide, the presence of an individual crab burrow can increase oxygen concentration in the surrounding sediments but only out to a distance of 2 mm. The depth to which this  $O_2$  increase was measurable was 2.5 mm and did not extend into the root zone of *Spartina alterniflora*. The presence of the burrow may aid in oxidizing the sediment, but not in aerating or oxygenating it. This is because oxygen penetration into the sediment allows for aerobic organisms to facilitate oxidation of reduced ions. This would change the redox potential of the sediment, but not oxygenate it. The additional amount of oxygenated sediment is small and is comparable with an effective increase in marsh surface area of 0.2% within these water-logged marshes. In contrast, crab burrows may lead to greater oxidation or even oxygenation or aeration of nearby bulk sediments, but only when sediments are well-drained. Because fiddler crabs are found throughout a range of well- and poorly-drained marsh conditions, it is inappropriate to always attribute their burrowing activities to leading to greater sediment aeration.

The mesocosm experiment (Chapter 3) demonstrated that fiddler crab burrows can have opposite effects on sediment chemistry and primary production depending upon the flood regime and the potential extent of drainage of the marsh sediments. The presence of crab burrows can significantly increase pore water sulfide concentrations in water-logged sediments, but may have little noticeable effect in more well-drained sediments. *S. alterniflora* had significantly lower production when growing under poorly drained conditions compared to when growing under more well-drained conditions. The presence of burrows within the same sediment type (mud) had completely opposite effects on *S. alterniflora* production depending on the flood regime. Where muddy sediments could not drain at low tide crab burrows significantly lowered production compared to where muddy sediments were not water-logged and crab burrows increased production.

The mesocosm experiment was successful in that it controlled for the natural variations within marshes and allowed for examination of specific interactions that are not possible within natural systems. Within this experiment, sediment type drove many of the results. If the mesocosms could have been entirely filled with native (and not purchased) sediments, then the complicating results caused by the great amount of iron added to the system could have been avoided. Although this effect was not intended, it did elucidate the potential for iron additions in highly reduced sediments to bind with sulfide and remove it from the pore water leading to more favorable growing conditions

for *S. alterniflora*. Not all of the results from the mesocosm experiment are able to be scaled up to marsh-wide generalizations, but the information gained from this experiment would not have been able to have been obtained from an *in situ* experiment. The narrow framework provided by this mesocosm experiment enabled specific hypotheses to be tested while controlling for the natural variation within salt marsh systems.

As seen from the field survey chapter (Chapter 4), natural salt marshes within the Virginia Atlantic Coast are located in a diverse range of landscape settings. This increases the overall heterogeneity of this marsh system. Predictable interactions among pore water, *S. alterniflora* and sediment parameters within these diverse field sites were not detected due to the interacting confounding influences of geomorphic setting, topographic location, duration of inundation, and highly variable sediment properties. This finding emphasizes the usefulness of the flood regime manipulation experiment.

Position and location in the marsh as well as sediment characteristics dictate drainage; therefore, different results might have been obtained if field sites were located in the same marshes, but in different areas. Salt marshes have notoriously high spatial heterogeneity due to interactions among macrophytes, macro fauna, hydrology, topography and many other characteristics. Although this chapter did not focus on fiddler crab burrows, the fact that there were sites with and without burrows (which was not determined *a priori*) and that the sites with burrows had a large range in burrow densities from 8 to 192/m<sup>2</sup> shows that on a larger scale, different marshes could be affected by crab burrows to different degrees (or not at all if not present).

Studying similar parameters on different scales in both natural and constructed systems was beneficial because the interactions observed in one setting helped to make

clear the interactions in the other. Similar correlation analyses were performed on data in both Chapters 3 and 4. The results of these analyses helped to show that in the system where the flood regime and sediment type were controlled (the mesocosm experiment) many more of the variables that were expected to be correlated had significant correlations that ranged from being weakly (0.38) to strongly (0.87) correlated. Conversely, in the field survey, few variables were significantly correlated in comparison because these were natural systems with a typical degree of heterogeneity.

Synthesizing the results from the micro-, meso-, and macro-scales studies, it becomes apparent that bioturbation caused by fiddler crabs has the ability to alternately facilitate or inhibit salt marsh persistence depending on flood regime and sediment drainage within the marshes they inhabit. Where vertical marsh accretion is able to keep up with the rate of relative sea-level rise (RSLR) and the rooting zone of *S. alterniflora* is well-drained, fiddler crab burrows may somewhat lower pore water sulfide concentrations and enhance primary production, possibly leading to a positive feedback loop resulting in prolonged salt marsh persistence. In contrast, where vertical marsh accretion falls behind the rate of RSLR and the sediments experience a greater hydroperiod and become progressively more poorly drained, fiddler crab burrows may exacerbate the stresses experience by the marsh vegetation, contributing to a different feedback loop whereby higher pore water sulfide concentrations and reduced primary production lead to an accelerated rate of marsh loss.

# Appendices

# **Appendix 1**

# **Oxygen Profiles for the following Burrows:**

<u>Natural Burrows</u> Figures A1 –A4 Figures A5 – A8 Figure A9

Artificial Burrows Figures A10 – A13 Figures A14 – A15

<u>Control Burrows</u> Figures A16 – Al9 Figure A20

# **Oxygen Color Contours for the following Burrows:**

<u>Natural Burrows</u> Figures A21 – A25 Figures A26 – A29

Artificial Burrows Figures A30 – A35

<u>Control Burrows</u> Figures A36 – A40

# Natural Burrows located on South Hog Island:

Figure A41 – A46





















Figure A25.















od Regime ediment		Burrow			Sulfide (uM)			Reduced Iron (uM)				
E S	S		2 cm	6.5 cm	12.5 cm	20 cm	25 cm	2 cm	6.5 cm	12.5 cm	20 cm	25 cm
Current Sea-level Sand Mud	pn	No Burrow	3.2 (±0.0)	4.1 (±0.5)	176.7 (±60.2)	401.2 (±377.4)	598.8 (±468.4)	120.0 (±55.1)	288.7 (±83.5)	1,048.4 (±451.3)	1,080.8 (±326.2)	970.6 (±438.7)
	W	Burrow	50.1 (±43.6)	4.2 (±0.5)	128.1 (±105.3)	151.4 (±126.2)	362.6 (±201.1)	934.4 (±540.2)	640.9 (±166.1)	1,334.5 (±459.9)	1,545.1 (±545.6)	554.9 (±329.6)
	pu	No Burrow	3.7 (±0.3)	24.4 (±9.5)	464.7 (±108.9)	386.1 (±27.8)	351.9 (±65.8)	6.2 (±5.0)	12.1 (±5.2)	3.3 (±0.5)	2.9 (±0.7)	3.2 (±0.7)
	eS	Burrow	4.5 (±0.5)	99.2 (±57.5)	375.7 (±38.0)	365.4 (±18.6)	351.1 (±29.2)	6.3 (±4.9)	6.9 (±2.2)	3.3 (±0.5)	3.5 (±0.8)	2.8 (±0.4)
Increased Flooding Sand Mud	pn	No Burrow	106.1 (±35.4)	251.0 (±50.3)	316.9 (±112.9)	327.8 (±99.8)	513.4 (±138.0)	505.6 (±181.6)	800.8 (±414.1)	2,042.4 (±988.3)	1,188.6 (±500.1)	1,288.3 (±891.4)
	W	Burrow	319.9 ( <del>±6</del> 5.1)	555.3 (±212.0)	665.9 (±267.9)	528.8 (±163.2)	575.9 (±223.0)	524.1 (±358.2)	313.1 (±190.1)	193.5 (±106.9)	309.6 (±209.4)	362.7 (±172.6)
	nd	No Burrow	312.9 ( <del>±</del> 62.8)	698.1 (±198.4)	658.7 (±135.3)	641.7 (±78.6)	639.3 (±67.0)	4.9 (±1.4)	2.6 (±0.3)	3.3 (±0.4)	2.3 (±0.4)	3.2 (±0.4)
	Sa	Burrow	506.5 (±63.2)	1,087.6 (±197.6)	822.2 (±145.4)	785.4 (±85.2)	835.1 (±99.0)	5.8 (±1.6)	3.7 (±1.1)	2.4 (±0.3)	2.5 (±0.3)	2.7 (±0.3)

# Appendix 2. Table of summary statistics for Chapter 3. Means (± SE).

od Regime	Sediment	Burrow	FeS		:	Sulfate (mM)	)	SRR (nmol/cm³/day)			
Flo	0,		12.5		2 cm	6.5 cm	12.5 cm	3 cm	10 cm	15 cm	
Current Sea-level Sand Mud	ud	No Burrow	404.03	(±23.66)	37.6 (±11.9)	33.9 (±7.4)	53.7 (±7.2)	4.86 (±0.59)	11.87 (±1.75)	15.56 (±4.52)	
	M	Burrow	281.70	(±57.42)	45.1 (±11.3)	40.1 (±7.5)	59.8 (±8.9)	18.12 (±5.29)	52.39 (±22.01)	54.28 (±18.77)	
	nd	No Burrow	23.30	(±5.40)	19.8 (±0.5)	16.6 (±1.1)	19.0 (±1.0)	12.57 (±5.43)	8.19 (±3.02)	7.12 (±3.20)	
	Sa	Burrow	31.47	(±9.59)	17.6 (±1.0)	17.9 (±0.6)	18.5 (±1.0)	17.00 (±3.59)	7.34 (±2.13)	5.72 (±0.74)	
Increased Flooding Sand Mud	pn	No Burrow	365.27	(±62.85)	33.6 (±3.9)	39.1 (±5.7)	46.1 (±8.3)	26.07 (±3.00)	18.63 (±0.58)	19.97 (±3.50)	
	Μ	Burrow	393.47	(±19.63)	40.8 (±3.1)	51.6 (±7.3)	57.9 (±11.6)	46.54 (±3.03)	19.13 (±2.61)	36.14 (±8.51)	
	nd	No Burrow	28.77	(±8.44)	13.2 (±0.8)	16.6 (±0.8)	16.9 (±1.1)	4.64 (±1.09)	3.06 (±0.27)	3.49 (±0.16)	
	Sa	Burrow	36.77	(±5.07)	11.7 (±1.5)	16.6 (±1.0)	18.4 (±1.1)	3.38 (±0.48)	3.23 (±0.34)	3.77 (±0.26)	

Appendix 2. Table of summary statistics for Chapter 3. Means (± SE).

Flood Regime	Sediment	Burrow		Month	nly Production (	End-of-season Biomass (g/core)	Belowground Biomass (g/bag)	
	0		June		July	August		
Current Sea-level	pr	No Burrow	-1.273	(±0.392)	7.063 (±1.343)	10.569 (±2.181)	28.350 (±4.671)	0.208 (±0.044)
	W	Burrow	-1.523	(±0.267)	4.536 (±1.150)	15.383 (±2.762)	27.562 (±2.622)	0.218 (±0.052)
	nd	No Burrow	-1.060	(±0.207)	1.945 (±0.338)	5.417 (±1.001)	10.984 (±1.578)	0.151 (±0.031)
	Sa	Burrow	-0.590	(±0.208)	2.080 (±0.472)	8.881 (±3.436)	12.320 (±1.336)	0.170 (±0.085)
Increased Flooding	nd	No Burrow	-1.652	(±0.535)	2.678 (±0.924)	10.088 (±2.745)	19.439 (±4.839)	0.202 (±0.071)
	Μ	Burrow	-0.876	(±0.169)	6.104 (±2.603)	4.498 (±2.141)	19.981 (±5.908)	0.129 (±0.045)
	pu	No Burrow	-0.695	(±0.272)	2.170 (±0.349)	5.961 (±1.159)	11.560 (±1.018)	0.097 (±0.028)
	ő	Burrow	-1.183	(±0.276)	2.886 (±1.078)	5.880 (±1.426)	11.207 (±1.144)	0.271 (±0.060)

Appendix 2. Table of summary statistics for Chapter 3. Means ( $\pm$  SE).



Appendix 3.1. Pore water depth profiles for A) sulfide, B) sulfate, and C) reduced iron concentrations for the six field sites. Means  $\pm$  SE.


Appendix 3.2. Sediment characteristic depth profiles for A) porosity, B) bulk density, and C) organic content for the six field sites. Means  $\pm$  SE.

Appendix 3.3. Tide and Well data from June 20 to September 7, 2006 (Days on xaxis in SAS day format). A) NOAA observed Tidal heights (m) at Wachapreague, VA, and water heights (cm above sediment surface) from water level recorders at B) South Hog Sand, C) South Hog Mud, D) George's Stake, E) Lower Phillips Creek, F) Machipongo River, G) Upshur Neck.













Site	Sulfide (uM)	Sulfate (mM)	Reduced Iron (uM)		
LPC	3,283.6 (±64.3)	14.4 (±0.9)	16.8 (±7.2)		
MACH	1,205.6 (±165.5)	18.4 (±1.0)	7.7 (±2.3)		
UPSH	1,637.7 (±290.2)	21.2 (±1.9)	9.6 (±1.3)		
GEOR	3,003.3 (±236.6)	19.6 (±1.3)	14.2 (±0.8)		
SHMUD	1,720.4 (±318.3)	22.1 (±3.0)	10.0 (±2.4)		
SHSAND	141.7 (±8.9)	28.2 (±1.6)	6.3 (±2.0)		
Site	Stem Density	New Shoot Density	End-of-Season	Belowground Biomass	
	(#/m <sup>2</sup> )	(#/m <sup>2</sup> )	Biomass (g/m²)	(g/bag)	
LPC	133.3 (±19.2)	136.0 (±8.0)	324.320 (±84.425)	0.755 (±0.144)	
MACH	293.3 (±29.7)	160.0 (±33.3)	359.947 (±21.978)	0.220 (±0.037)	
UPSH	736.0 (±66.6)	741.3 (±70.6)	476.053 (±51.727)	0.394 (±0.070)	
GEOR	298.7 (±10.7)	325.3 (±5.3)	600.320 (±153.929)	0.402 (±0.056)	
SHMUD	362.7 (±59.4)	373.3 (±46.5)	521.973 (±121.753)	0.501 (±0.034)	
SHSAND	218.7 (±5.3)	384.0 (±80.0)	594.400 (±126.631)	0.208 (±0.067)	
Site	Porosity (ml/cm <sup>3</sup> )	Bulk Density (g/cm <sup>3</sup> )	Organic Content (%)		
LPC	0.865 (±0.010)	0.724 (±0.031)	8.720 (±0.580)		
MACH	0.828 (±0.035)	0.875 (±0.020)	9.003 (±0.947)		
UPSH	0.611 (±0.004)	1.615 (±0.011)	2.090 (±0.043)		
GEOR	0.797 (±0.018)	1.028 (±0.049)	6.342 (±0.302)		
SHMUD	0.882 (±0.009)	0.839 (±0.042)	10.145 (±0.762)		
SHSAND	0.463 (±0.010)	1.670 (±0.017)	1.003 (±0.153)		
Site	Sediment FeS	SRR 3cm	SRR 10cm	SRR 15cm	Burrow Area
	(umol/cm <sup>3</sup> )	(nmol/cm <sup>3</sup> /day)	(nmol/cm <sup>3</sup> /day)	(nmol/cm <sup>3</sup> /day)	(cm <sup>2</sup> /m <sup>2</sup> )
LPC	42.69 (±4.43)	14.50 (±8.73)	4.57 (±0.17)	21.20 (±11.32)	76.121 (±28.165)
MACH	64.33 (±6.20)	10.61 (±2.05)	5.45 (±0.93)	6.78 (±1.27)	178.891 (±53.129)
UPSH	9.75 (±1.69)	5.96 (±0.37)	9.93 (±1.72)	8.78 (±2.84)	0.000 (±0.000)
GEOR	36.61 (±1.68)	9.96 (±2.51)	13.13 (±1.50)	10.51 (±1.17)	53.582 (±25.117)
	) (				
SHMUD	26.55 (±3.57)	19.31 (±4.35)	15.88 (±3.67)	11.48 (±2.33)	32.950 (±32.950)

Appendix 4. Table of summary statistics for Chapter 4. Means  $(\pm SE)$ 

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