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FACTORS INFLUENCING SPARTINA ALTERNIFLORA PRODUCTIVITY IN RELATIONSHIP TO ESTUARY INLET OPENING ELLISVILLE MARSH, PLYMOUTH, MA

A Dissertation Presented

by

ELLEN K. RUSSELL

Submitted to the Graduate School of the University of Massachusetts Amherst in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

September 2019 Plant and Soil Sciences

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FACTORS INFLUENCING SPARTINA ALTERNIFLORA PRODUCTIVITY IN RELATIONSHIP TO ESTUARY INLET OPENING ELLISVILLE MARSH, PLYMOUTH, MA

A Dissertation Presented

by

ELLEN K. RUSSELL

Approved as to style and content by:

Baoshan Xing, Chair

Michelle DaCosta, Member

Kevin McGarigal, Member

Wesley R. Autio Director, Stockbridge School of Agriculture

DEDICATION

"In the end we will conserve only what we love. We love only what we understand. We will understand only what we are taught" (Baba Dioum, 1968). This work is dedicated to all the strong women in my life, and to my Dad, who have loved, taught and conserved. Throughout this project, each of you influenced the outcome in many inspiring ways.

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V

ABSTRACT

FACTORS INFLUENCING SPARTINA ALTERNIFLORA PRODUCTIVITY IN RELATIONSHIP TO ESTUARY INLET OPENING ELLISVILLE MARSH, PLYMOUTH, MA

SEPTEMBER 2019

ELLEN K. RUSSELL, B.S., UNIVERSITY OF NEW HAMPSHIRE M.S., PURDUE UNIVERSITY

Ph.D., UNIVERSITY OF MASSACHUSETTS AMHERST

Directed by: Professor Baoshan Xing

A scientific basis for understanding the health of salt marsh vegetation, primarily Spartina alterniflora, in apposition to dredging of the tidal inlet to Ellisville Marsh in Plymouth, Massachusetts was determined. A three-hectare S. alterniflora loss, and coincident coastal bank erosion occurred; ostensibly due to inlet blockage from sand deposition, prolonged inundation, and meandering of inlet channel into coastal bank. This warranted dredging the tidal inlet, whereby tidal flushing would restore growth or discontinue die-off, and deter coastal bank erosion. A pre- and post dredge study was conducted assessing environmental factors related to plant productivity. Following dredging, an increase in tidal range (0.2 m, p<0.05) was observed, while measured hydroperiod lagged behind tidal range improvements by at least seven months. Inundation frequency ultimately increased for lower and decreased for higher elevation plots. Hydraulic connection between ocean and marsh was increased following opening. Percent cover of tall S. alterniflora increased from pre-dredge conditions in both landscape and plot level assessments (p<0.1), while short S. alterniflora and Spartina *patens* coverage decreased (p<0.001). Mean S. alterniflora stem density, above and

below ground biomass decreased (p < 0.001) with the onset of decline corresponding to the hydroperiod lag. S. alterniflora height increased linearly with hydroperiod (p<0.001, $r^2 = 0.12$). Root alcohol dehydrogenase activity and redox were inversely correlated, and mean redox shifted from -100 mv to +100 mv (p<0.001), the switch corresponding to the hydroperiod lag. Porewater salinity and ammonia did not vary, although orthophosphate declined (p<0.001). Haliaspis spartinae, an insect pest, was found to be associated with hydroperiod. The Marsh Equilibrium Model (MEM v. 5.4) demonstrated vertical resilience, whereas the Unvegetated to Vegetated Index (UVVI) approach did not show horizontal resilience. Hydroperiod substitution for depth in the MEM yielded lower biomass estimates and less vertical resilience. A significant (p<0.05) plant height dependency on below ground biomass density and weak Allee effect was demonstrated in mudflat fringe plots; possibly explaining why mudflat re-colonization has not occurred 10 years post dredge. Plant height is recommended as a sentinel indicator and potential replacement for continued permit monitoring rather than percent cover because of its positive correlation with environmental variables.

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CHAPTER 1

INTRODUCTION

Salt marshes are critical components of the estuary ecosystem. They serve as important migratory and resident bird feeding ground and breeding habitat (Shriver et al. 2004; Teal 1986; Keer and Zedler 2002; Bayard and Elphick 2011); fish and shellfish nursery habitat (Boesch and Turner 1984; Heck et al. 1995; Bertness 1984; Williams and Zelder 1999); and storm erosion and flooding buffer (Olff et al.1988; Warren and Neiring 1993; Chung et al. 2004). They provide essential wastewater treatment functions by removing nutrients and pollutants from direct discharges and non-point sources (Giblin et al. 1980; Teal et al. 1982; Silliman et al. 2009;) and they act as carbon sinks/sources (Morris et al. 1986, 1990, 2002, 2016; Kirwan and Mudd 2012; Howes and Goehringer 1994; Kludze and DeLaune 1994; Langley et al. 2009).

While it has been clearly established that coastal salt marshes are a valuable, as well as vulnerable natural resource (Teal 1969; Callaway 2004; Valeila 2008), the necessity for, and timing of, the approach to human protection of this resource is by no means standardized. Different schools of regulatory thought and practice remain as to the best way to intervene, if at all. Preventable losses often do not become recognizable until the resource has been substantially damaged reaching a "tipping point" from which it may not recover (Roman and Burdick 2012). Individual species and wetland protection laws, "no net loss of wetlands" provisions, and case-by-case restoration/coastal resiliency projects are some of the current tools available for coastal wetland protection, but are not always successful (Neckels et al. 2002; Vernberg 1993; Brown and Veneman 2001; Zedler 2001, 2013). In the United States, impacts from destructive storms, such as

Hurricanes Katrina and Sandy, and the global threat of climate induced sea level rise, have elevated interest in the protection of valuable coastal shoreline, including coastal salt marsh (Best et al. 2018; Cahoon 2006; Ganju et al. 2017, 2019; Kearney and Turner 2016; Norwacki and Ganju 2018; Schuerch et al. 2012; Weston 2014; Valeila et al. 2018;).

Increasingly the salt marsh resource is lost or damaged at a rate greater than its creation (Bradley and Morris 1990; Langley et al. 2009, Alizad et al. 2016). In addition to sea level rise, stressors threatening salt marsh survival include drought, prolonged inundation, subsidence or intentional diking, storm erosion and wrack deposition, herbivory, agricultural practices, draining and/or filling for construction or mosquito control, pollution, disease, and eutrophication (Deegan et al. 2007, 2013; Donnelly 2001; McKee et al. 2004; Mendelssohn et al. 1988; Morris et al. 2002; Olff et al. 1988; Twilley et al. 2005; Portnoy and Giblin 1997; Valeila 2008; Roman et al. 1997; Nyman et al. 1993, 1995; Reed 1995; Schneider and Useman 2005; Smith 2007; Teal 1996; Warren and Neiring 1993; White and Howes 1994; Wigand et al. 2018).

Some estimates put global losses of salt marsh at thousands of hectares annually (Langley 2009; Stedman and Dahl 2008) and predict a 5 to 20 % global loss of coastal wetlands by 2080 (from 2004 starting values) due to sea level rise alone (Nicholls 2004). A predicted sea level rise of 1 meter by 2100 may displace all but the most protected (or managed) of salt marshes (Laffoley 2010) and SLR along the North Atlantic coast has been reported to have been 3-4 times higher that the rate of global SLR (Sallenger et al. 2012, Parris et al. 2012, Jay et al. 2018). In the United States, relatively recent losses are more defined, at 50% of original acreages or 279,000 hectares lost from the 1950's to

2004 (Stedman and Dahl 2008; Kennish 2001). In the last decade, conditions known as "brown marsh syndrome" or "sudden marsh and/or vegetation die-off" (partially explained by instances of drought and/or invertebrate herbivory, respectively), as well as "waffle" appearance due to interior ponding, have had negative impacts on thousands of hectares of salt marsh along the Gulf of Mexico and Atlantic shorelines (McKee et al. 2004; Twilley et al. 2005; Teal et al. 1996; Valeila 2008; Smith et al. 2007; Schneider et al. 2005).

It is estimated from analysis of historical maps that Massachusetts (MA) has lost 41% of its salt marsh since 1777 (Bromberg and Bertness 2005, Bromberg et al. 2009) and, in the Boston Harbor region alone, only 19% of pre-colonial salt marshes remain. One such marsh, Ellisville Marsh, located in Plymouth, MA 40 miles south of Boston, is the subject of this dissertation. The productivity of its predominant primary producer, *Spartina alterniflora*, along with a broad suite of environmental variables, was followed in 2010 (one year before inlet dredging and presumably representing water logged conditions) and three years after inlet dredging in 2011-2013 (presumably representing better drained circumstances). The ultimate goal of this research was to inform future adaptive management efforts to protect the health of the salt marsh to the extent possible.

1.1 Study Area

Ellisville Marsh, located in Plymouth, MA (41° 50'25''N, 70° 32'08''W) was selected as a study area because from 1988 to 2003, it exhibited an approximate 3 ha loss of one of its principal salt marsh vegetation species, *Spartina alterniflora* as determined by comparison of 1985 and 2008 aerial photographs. This loss was ostensibly due to tidal

inlet narrowing (constriction of the main inlet channel providing inflow and outflow of tidal water) and subsequent presumed deleterious effects of periods of prolonged tidal inundation or water logging.

The 28 hectare salt marsh (Figure 1.1) experiences a semi-diurnal tidal cycle and is located interior to a low dune, coastal beach system, and to a continually changing barrier sand spit formed by the action of long shore, north to south coastal sand migration and storm induces changes. The marsh and adjacent harbor/embayment have historically been protected as a salt pond, also known as a coastal lagoon, under the Wetlands Protection Act 310 CMR 10.33, because of historic tidal restrictions blocking its function as an estuary. It sits adjacent to the Ellisville Moraine formed by a complex series of retreats and advances of the Cape Cod Bay and Buzzards Bay ice sheet lobes that occurred during the Wisconsinan glacial stage of the Pleistocene Epoch (Oldale and O'Hara 1984). Since 1991, the MA Department of Conservation and Recreation (DCR) has managed the northern half of the marsh as Ellisville State Park. The southern half has been owned by the Wildlands Trust, Inc. since 2003, and managed as the Shifting Lots Preserve. To the east is Cape Cod Bay and to the west is Ellisville Road. In addition to beachfront, the marsh directly abuts steep forested upland, roadway, and residential property. Several small freshwater creeks also enter the marsh along its margins.



Figure 1.1. Ellisville Harbor and Marsh, Plymouth, MA (2011). Note straight channel path exiting marsh to Cape Cod Bay in foreground and area of bare mudflat in background.

The marsh is of strategic interest because it is the largest salt marsh in Plymouth, MA. In 2002, the State of Massachusetts Executive Office of Energy and Environmental Affairs (EEA) tasked the Bureau of Coastal Management (CZM), Department of Fish and Game (Natural Heritage and Endangered Species Program (NHESP)), Division of Marine Fisheries, DCR, and Department of Environmental Protection (DEP) managers "to discover a long-term, sustainable solution to repeated obstruction of this marsh's tidal connection and protection of this critical resource" (Webber 2002). Despite, Ellisville Marsh being described in a survey of potential dieback sites as having "major" waterlogging (Smith and Carullo 2007), it was not until 2010 that federal and state permits were able to be obtained by the non-profit organization, Friends of Ellisville Marsh, Inc., to resume periodic maintenance dredging. These permits allowed dredging of approximately 2750 m³ of material from the historic entrance channel to Ellisville Marsh. The excavated material was used as beach nourishment or for dune construction over roughly a 4000 m² area south of the channel jetty in order to avert tidal restriction and subsequent waterlogging, while at the same time enhancing piping plover habitat (US Department of the Army, File No. NAE2009; MADEP File No. SE57-2380). Table 1-1 lists subsequent openings and associated excavation volumes.

It was in anticipation of this activity that this dissertation was conceived in order to study conditions pre and post-dredge in a scientific manner in order to provide a better understanding of how the system responds to the imposed changes in hydrology. Thus, future management decisions might be more likely to be based on scientific evidence and not merely anecdotal observations.

Excavation	
Date	Approximate Volume
	(Cubic Meters)
Jan-11	2750
Jan-12	810
Jan-13	766
Mar-13	1080
Mar-15	900
2016-2018	0
Mar-19	1870

Table 1.1. Ellisville Marsh inlet openings and associated excavation volumes

1.2 Historical Synopsis

A historical perspective is necessary to fully understand the changes taking place in this ecosystem. Throughout the last 300 years, the barrier spit and beachfront have altered in configuration, along with tidal inlet location (Figure 1.1 through 1.3).



Figure 1.2. (1985 aerial) Ellisville Harbor and Marsh, Plymouth, MA. Straight channel path exiting marsh to Cape Cod Bay and vegetated foreground.



Figure 1.3. (2001 aerial) Ellisville Harbor and Marsh, Plymouth, MA without dredging and prior to 2003 emergency opening. Note 90-degree bends taken by channel due to the 450 m long barrier spit and jetty structures.

As far back as 1770, local citizenry, lobster fishermen, Town of Plymouth, State of Massachusetts, and private individuals have endeavored to keep this channel mouth open for maintaining fishing industry, recreational boat access, and more recently, for protection against coastal bank erosion. In 1791, a surveyor scouting for the location of a waterway across Cape Cod discussed in his journal the hand dredging of the channel leading to this marsh performed by local inhabitants (Winthrop 1791). It states that the 'Ellisville' harbor and marsh is "artificial, made by opening a pond. The roots of trees found there prove it to be artificial".

Throughout the 1800's, the marsh functioned as a shifting agricultural lot for salt hay (*Spartina patens* and *S. alterniflora*) production (Ellis 1982). Several different private property owners owned the marsh and had rights to prime hay areas which "shifted" from year to year so that each owner would have the opportunity to harvest the most productive areas; thus, the current name of the southern half, Shifting Lots Preserve. Several structures (now in various states of decline) attest to the active fishing industry in the area (Curtis Journal 1927). These include a boathouse and two fish houses (operational from 1890-1925) that were used for catch weighing and bait/pot storage for the lobster fishermen, Irish moss gatherers, and the cod and mackerel weir fishermen.

In 1917, 1939 and 1951 project plans were created by the Massachusetts Department of Public Works to perform channel stabilization projects and/or channel dredging. In 1961, the Army Corps of Engineers constructed a permanent rock jetty along the northern side of the tidal inlet. This jetty, (as with others constructed at intervals along the entire coast of Cape Cod Bay), was meant to protect the channel from filling

due to littoral drift and sand deposition. The result of this installation appeared to be additional accumulation of sand directly north of the groin/channel inlet and subsequent wash over of sands into the inlet mouth during storms. At some point pre-1960 mosquito ditches were excavated throughout the marsh, but the timing of their installation is not documented.

Fishing from this harbor continued until 1987 when previously permitted channel maintenance dredging performed by lobstermen and the Town of Plymouth was ceased by a court order issued by the State of Massachusetts related to the prohibition of dredging in an Area of Critical Environmental Concern (ACEC). This effectively ended commercial fishing from this harbor. With yearly maintenance halted, on October 30, 1991, the "unnamed perfect storm", succeeded in almost completely blocking the already constricted channel mouth to Ellisville Marsh. The State of MA acquired the northern half of Ellisville Harbor and marsh (1991) and in early 2003, ownership of the southern half of Ellisville Marsh (renamed the Shifting Lots Preserve) switched from private to non-profit status. Boat mooring was thereafter prohibited.

During the 15-year time span (1988 - 2003) when no dredging took place, visible marsh vegetation loss occurred, amounting to about 3.2 ha, or roughly 10% of its 1985 vegetative status (Figures 1.3 through 1.6). The loss appeared to progress with time and in conjunction with lengthening periods of standing water. However, there was no specific data that indicated waterlogging was the cause of vegetation loss and only limited photographic evidence of the apparently eutrophic nature of the decline. In late 2003, the channel mouth was opened by dredging through the barrier spit using a "one-time emergency opening" permit obtained by a private property owner and issued

by the DEP under the salt pond regulations (310 CMR 10.33). This was issued in order to prevent ongoing coastal bank erosion caused by channel meandering (Figure 1.4).



Figure 1.4. (2003 aerial, text modifications) Ellisville Harbor and Marsh, Plymouth, MA following emergency opening. Note area of coastal bank erosion at upper left corner, remnant channel, and vegetation loss in upper right.

Prior to this reopening, the barrier spit was 450 m long and the mean semi-diurnal tidal range in the marsh/harbor area ranged from less than 1.0 m (prior to breaching barrier spit in 2003) to 1.5 m (post-breach condition) (Ramsey et al. 2006). Eventually winter storms accelerated barrier-spit formation and by 2008 the channel was again constricted (Figure 1.6). Channel dredging was reinitiated in 2010, and annual maintenance occurred thereafter in winters 2012 and 2013, as described in Table 1.1 (Applied Coastal Research and Engineering, 2017).



Figure 1.5. (1985 aerial, text modifications) Ellisville Harbor and Marsh, Plymouth, MA during pre-restriction tidal conditions. Note vegetated appearance of back potion of marsh (upper left).



Figure 1.6. (2008 aerial, text modifications) Ellisville Marsh, Plymouth, MA. Note area of blue exposed mudflat in marsh interior at upper left corner and channel in foreground curving towards coastline.

1.3 Research Justification

Since 1980, the harbor and marsh have been protected as part of a larger ACEC by the State of Massachusetts (Bewick 1980). In addition, three non-profit organizations (Wildlands Trust, Friends of Ellisville Marsh, Inc. (FoEM), and the Massachusetts Audubon) are today legally committed to the stewardship of Ellisville Marsh, and/or protection of its inhabitants and salt marsh habitat. Along with the opinion that this marsh is part of an important cultural heritage for the area, is the knowledge that it represents critical nearby habitat for the wider ecosystem, including offshore, open water eel grass bed, soft-shell and surf clam populations; and nesting piping plovers and least tern populations. It provides critical storm surge dampening, coastal protection, and aesthetic benefits that have inherent value that is difficult to quantify.

S. alterniflora was the primary vegetation lost during the time period from 1988 through 2003. The claim that marsh vegetation re-colonized 14 acres in a 2-year time span following the 2003 emergency opening (Ramsey et al. 2006) are not substantiated. Because *S. alterniflora* is generally the first species to be affected by changes in tidal conditions due to its growth preference at lower elevations, it was chosen as the primary focus of this study. As has been the case with other New England salt marshes, (Belliard et al. 2016; Buchsbaum et al. 2006; Naidoo and Kift 2006; Nearing and Warren 1980; Pennings et al. 2005; Spicer 2007; Racehorse 1997), it was surmised that prolonged inundation of the Ellisville Marsh due to restriction of tidal exchange (inlet channel restriction), was the cause for vegetation loss.

1.3.1 Conceptual Framework of Study

This field study examined an exhaustive list of variables that have been shown in other salt marshes to influence, alone or in combination, *S. alterniflora*'s distribution and productivity (Figure 1.7). Interdependencies under both pre and post-dredge field conditions were evaluated by first looking at each environmental factor and its relationship to *S. alterniflora* productivity separately (Chapters 2 through 5) and then by examining interactions using multivariate statistics and modeling to provide a synthesis of understanding (Chapter 6). The impact of dredging across the entire marsh, not just in areas covered by *S. alterniflora*, was evaluated.



Figure 1.7. Ellisville Marsh conceptual framework for study of *Spartina alterniflora* productivity and interacting variables.

The conceptual framework was formulated a priori to help describe productivity response of the *Spartina alterniflora* population in Ellisville Marsh to hydroperiod fluctuations, as well as to other environmental and climatological covariates. The conceptual framework for marsh function describes the interaction and influences on the ultimate productivity of *S. alterniflora*. It addresses three state variables (pore water, soil/sediment, and the *S. alterniflora* plant itself) undergoing change, two forcing functions (large arrows) of climate and tidal exchange, four process functions (small numbered boxes), and twenty measured variables (boxed variables adjacent to each bold-boxed state variable) that were assessed.

1.3.2 Overall Study Hypothesis

The overarching hypothesis for this study is that decreases in hydroperiod (length of time that standing water remains on marsh surface) and increased tidal range and excursion (due to removal of blockages of tidal flow) will enhance the productivity of *S. alterniflora*. Documentation of the conditions within Ellisville Marsh that may be responsible for changes in *S. alterniflora* abundance will help support the mandate from the EEA and may help identify a balanced, sustainable solution and define if, or when, there ought to be human intervention. Sustainable solution, in this context, meaning to provide hydraulic connection to the marsh for adequate tidal exchange, within reasonable expense and effort, and to provide minimal to no detrimental impact on this marsh and closely tied resources, including the coastal bank and dune system. In addition, because a large component of holding the dredging permits involves the ongoing expense of monitoring, early discernment of conditions that may predict vegetation stress responses to changes in marsh hydrology may prove useful.

CHAPTER 2

MARSH HYDROLOGY

2.1 Introduction

The hydrologic conditions within a salt marsh govern most, if not all, attributes of the salt marsh. It influences the plants that grow, the benthic invertebrate populations, nekton occurrence, and the microbial induced geochemistry of the sediments. Without the proper hydrological conditions, the salt marsh would cease to function as such (Teal 1986).

Both tidal (saline) and freshwater inputs were assessed. The system is fed principally from tidal input, with stream and groundwater inputs considered small relative to the daily volumetric contribution from seawater (USGS Report SIR 2009-5063; USGS Circular 1338; SMAST 2005). For this reason, the primary focus was on three tidal factors critical to establishing and maintaining growth conditions appropriate for *S. alterniflora*. These were the length of time seawater remains on the marsh platform (hydroperiod or length of inundation), its vertical excursion influenced by tidal range and marsh elevation, and the frequency of inundation across the marsh platform. These were assessed under both pre- and post dredge conditions. Monitoring changes in the barrier spit formation near the estuary inlet was also relevant because of its influence on channel and marsh hydrology, and associated roles in marsh formation (Roman and Burdick 2012).

All three tidal factors are known to have impact on the productivity of *S*. *alterniflora* as they may influence oxygen supply to the plant and cause changes in

sediment and porewater chemistry that affect growth, reproduction and senescence (Bradley and Morris 1990; Burdick and Mendelssohn 1990; Buchsbaum et al. 2006; Cahoon and Reed 1995; D'Alpaos et al. 2006; Kearney and Turner 2016; King 1982; Morris et al. 1990; Mudd et al. 2009; Olff et al. 1988; Weigert et al.1983). They may also be responsible for forming or altering physical barriers to growth and influencing competition and herbivory from resident or invasive species (Bertness and Ellison 1987; Burke et al. 2003).

Research addressing salt marsh hydrology has focused on the resilience of salt marsh ecosystems to adapt to and withstand changes in sea level rise (SLR) (Cahoon et al. 2002; Crosby et al. 2016; Ganju et al. 2017; Morgensen and Rogers 2018; Morris et al. 2002, 2005, 2013; Mudd et al. 2009; Kirwan et al. 2009, 2010, 2012, 2013; Faghherazzi et al. 2010; Roman and Burdick 2012). The elevation of the marsh platform must increase to keep pace with increased water level in order that the marsh does not submerge and convert to open water (Morris et al. 2002; Mudd et al. 2009; Roman et al. 1997). Not withstanding any continental lift in the area, increases in elevation of the marsh platform occur principally by deposition of both organic matter and mineral sediment that outpace subsidence.

In its current dredged situation, Ellisville Marsh undergoes flood dominance (Ramsey et al. 2006; Applied Coastal Engineering, Inc. 2017) meaning stronger inward, flooding flows as compared to ocean-ward ebb flows. In this flood dominant situation, factors that alter ocean tidal regime, such as SLR, or astronomical and storm events can be the primary contributors to changes in hydrology and sediment transport. Anthropogenic changes such as dredging of the inlet also cause large swings in hydraulic
conditions. While the manipulation of water level during this study was not in this investigator's control, it was possible to document hydraulic conditions one summer prior to (2010) and three summers following inlet dredging (2011-2013).

2.1.1 Objectives

This data collected provided information regarding the types of changes, their impact on *S. alterniflora*, and ultimately a marsh-wide estimation of the relationships between hydrology and vegetation productivity under pre- and post dredge circumstances. Specifically, tidal amplitude and inundation periods calculated for each year of study either supported or refuted the hypothesis that dredging will increase tidal range and flushing and, that these increases in turn positively relate to *S. alterniflora* productivity.

2.2 Materials and Methods

2.2.1 Tidal Range

Tidal height was monitored using three semi-permanent tidal gauges (or loggers) and a reference gauge for shore-based barometric pressure corrections (HOBO® Water Level Loggers, Model U20-001-01). Loggers were factory calibrated prior to purchase on July 13, 2009 and found to be accurate within +/- 0.03 psi for a range of applied pressures (0 to 30 psi). These conditions bracketed the range of pressures encountered with very little drift through the life of the study.

Tidal loggers were placed inside a stilling well (7.6 cm OD, 91.4 cm long perforated PVC pipe) and then affixed to a buoy and anchoring system in order to remain

fixed in horizontal space and submerged during all tidal fluctuations to avoid vandalism during the summer monitoring period. All water levels were considered relative to one another and were not directly referenced to a vertical datum except in 2010. Horizontal locations were recorded using a hand held Garmin 76 GPS unit (with wide area augmentation system (WAAS) 84 enabled). Loggers were placed to be representative of inner marsh, channel, and ocean front conditions (Figure 2.1). In 2011, 2012, and 2013, the channel logger had to be moved to compensate for changes in channel path due to sand sedimentation, yet it remained in the predominant channel flow path. The nearby vertical datum used in 2010 also became buried.



Figure 2.1. Locations of pressure loggers (PG) and water quality monitoring locations (EVH), Ellisville Marsh, Plymouth, MA

Duration of logging was approximately 30-35 days spanning the months of July and August (with pressure readings recorded every 10 minutes) and covering one complete spring to neap cycle each summer. Slight variations in length of deployment between years were due to storm events necessitating early removal and differing timing of deployment and retrieval due to tide height and allowable access.

Tidal range was determined by downloading logger data using a HOBO® Base Station Coupler and HOBOware® Pro software. Pressure readings were converted to depth of submergence for each sensor after correction for barometric pressure changes, salinity and temperature. Mean high water (MHW) and mean low water (MLW) depths were subtracted for a 21-day period (inclusive of spring and neap tides) to reflect the mean excursion in tidal height, i.e. mean tidal range/amplitude.

All channel and marsh readings were normalized for astronomical differences in tides between years using changes observed in the ocean front logger located down coast from the inlet. The ocean front logger was considered to represent tidal conditions unaffected by dredging.

2.2.2 Hydroperiod

Hydroperiod was measured using button-sized temperature sensors (Maxim Thermochron iButton sensors, Model DS1921G-F5#) to register temperatures of soil surface and ambient air. Factory determined accuracy of button loggers was +/- 1° C. Steep declines in soil surface temperatures (after negating any similar dips in ambient temperatures or precipitation events) were considered to represent contact with cold tidal waters and onset of tidal inundation, while more gradual increases in temperature reflected water subsidence (Figure 2.2). Because water subsidence was not as clearly demarcated as to when water had completely retreated from the surface, a modification was used to indicate when water had receded. The inner marsh HOBO® pressure reading

that corresponded (+/- 5 min) to the timing of the observed dip in soil surface temperature measured with the I-button was used as the indicator for the height of water that caused each specific plot to be submerged and conversely, uncovered. At the outset, loggers were not intentionally placed in pannes, or places that appeared to be permanently wet. Training data across a complete six hours of tidal fluctuation was collected for six locations on two different occasions in order to verify the timing of temperature dips indicating submergence and nearby pressure logger indicating slack to ebb tidal retreat.



Figure 2.2. Example temperature and corresponding pressure measurements used to determine hydroperiod interval (HP) at PZ10 location.

Temperature loggers were placed inside tin enclosures (Specialty Bottle Supply, 1/2 oz. flat tin screw top), sealed closed with boat caulk, and then affixed using zip-ties to

the base of each permanently installed piezometer located in the center of each observation plot (Figure 2.3). Because of the highly conductive nature of the tin enclosure, temperatures within the cases were not always representative of ambient temperatures because during very hot, clear days the cases warmed considerably from solar heating. This situation was preferable because it served to make temperature drops due to tidal inundation even more dramatic.

Ninety-seven loggers were placed (Figure 2.3) and GPS locations recorded. Three mud flat loggers 92, 93 and 94, were later abandoned in 2011 because of piezometer losses due to the unstable muds, however, GPS locations allowed return for vegetation measurements. Two additional loggers were placed, each on the tops of two adjacent piezometers; one representative of a non-canopy situation (only low growing plants present) and one representative of a canopy covered situation (*S. alterniflora* plot coverage) to assess differences related to ambient temperature.

Data were collected for 21-days at 20 minute sampling intervals. As with the pressure loggers, data were collected to encompass a complete spring to neap tidal cycle in the 21-day monitoring period. Hydroperiod for each plot was determined by downloading temperature-logging information into comma-separated files using OneWireViewer[™] software. Data were then screened against any similar drops in ambient temperature/precipitation events and then hydroperiod was determined using the steep decline and corresponding marsh pressure reading to calculate for each tide the length of time that each logger was under water. For each plot location, this time period was summed across the 21-day logging period (total inundation/hydroperiod) and then divided by the frequency of inundation (corresponding to number of individual tides) to

determine the mean tidal hydroperiod in minutes for each summer's 21-day logging period. Total hydroperiod was considered the sum of all spring to neap tide inundations in the summer 21-day period each year.

2.2.3 Barrier Spit, Inlet and Channel Morphology

Changes in barrier spit length or height, and relative elevations were collected at least twice per year at seven transects across the beachfront and barrier spit (Figure 2.4). Elevations were measured using Emory Rod techniques for observing and comparing dynamic coastal environments (O'Connell 2000). The base of each permanent monument was also surveyed to the 1929 National Geodetic Vertical Datum subsequently covered by sand. Due to Hurricane Sandy (October 2012) and the Nemo Blizzard (February 2013), transect origin posts for BT06 were lost and could not be reestablished in 2013 due to eroding dune. Because the decision was made by FoEM to maintain the inlet opening every winter regardless of length of barrier spit, the barrier spit was not observed to increase until 2013. This increase was due to affects of a spring storm that occurred following that year's winter dredging.

The length of barrier spit was measured from a fixed, surveyed benchmark at its northernmost end to its southern-most tip at peak low tide height using a compass bearing of 205° SW. The topographic measurements of spit surface, along with width and length distances, were used to track barrier spit formation.



Figure 2.3. Temperature logger and piezometer locations (blue and pink dots with various identifiers shown) within Ellisville Marsh, Plymouth, MA. Graphic courtesy Town of Plymouth, MA/Kim Tower.



Figure 2.4. Beachfront transect (BT) locations used to determine topography. Graphic courtesy of Jason Burtner/MA CZM.

Channel and creek morphology and the dendritic pattern of the channel configuration, was followed by classifying first through fourth order creeks in the marsh. Creeks that maintain substantial volume (and > 3 meters in width) during low tide are said to provide refuge for fin fish and other nekton (Williams et al. 1999) and were classified as 4th order, while 3rd order creeks were generally less than 3m wide yet still maintained substantial volume at low tide (Zedler 2001). Second order creeks were those with tributaries, but with little to no creek volume during low tide, while first order creeks/ditches were classified as those with no tributaries and dry at low tide. The number of each class was tallied using both aerial photography and in-field verification and compared across years. Finally, the distance from each plot's central piezometer was measured to the closest ditch or creek edge (referred to as distance to ditch (DTD)) in 2011 and 2013. This measurement was helpful in understanding any erosion that might be occurring and the connectivity of creeks that might influence drainage.

2.2.4 Freshwater Inputs

Flow rates of surface water input were estimated during the summers of 2010 to 2013, using either instantaneous measurements of cross sectional area and timed-travel along stream dimensions, or direct collection of pipe/culvert flow. Data were collected once per summer season (when possible) with the understanding that this would provide only a limited representation of freshwater input to the system because of the lack of seasonal representation and groundwater input estimation. In Fall 2012, an additional freshwater source was uncovered that was previously obscured by dense *Phragmites australis* growth along Ellisville Road, and its flow contribution was measured by direct collection only in year 2013. No measurement of groundwater input was made, other than

to measure water levels within the piezometers during ebb tide conditions in August of 2010 in an attempt to discern a typical water level water level at a known tidal condition.

2.2.5 Climate Factors

Three sources of precipitation and temperature data were evaluated; the Plymouth Municipal Airport, the UMass Cranberry Experimental Station located in Wareham, MA, and a nearby Ellisville barometric pressure/rain gage location. As previously described, temperature data obtained from the ambient loggers was not considered the true temperature because of the heating effect caused by the tin capsules, however, it was useful when comparing relative increases/decreases. Water temperatures reflected real time and true values because these were obtained from the HOBO® pressure loggers that were designed for this type of data collection. Snow pack and freeze data were obtained from on line weather information sources (cli-MATE MRCC Application Tools and U.S. Department of Commerce, NOAA National Climatic Data Center 2010-2014).

2.2.6 Statistical Analyses

Hydroperiod and tidal range data and their residuals were normally distributed (Shapiro-Wilk, p > 0.01) and considered independent. They also met the classical statistical testing assumption for homogeneity of variance between years (Bartlett, K^2 =2.295, p=0.5262, with outlier locations PZ02 and PZ16 eliminated). In this case, a one-way analysis of variance (ANOVA) was used followed by a post-hoc Tukey Honest Significant Difference (HSD) test for mean separation (α =0.05). With outliers included, there was not homogeneity of variance across years and therefore, a Welch's ANOVA unequal variance test was used followed by a Bonferroni correction for mean separation

($\alpha = 0.05$). Spatial semi-variograms describing the expected difference in value of hydroperiod between plots in relationship to a given relative orientation (northing distance in seconds) were produced using techniques described by Clark (1979) and the geoR package available in R (Ribeiro and Diggle 2001). During each summer monitoring period there were no storm surges that occurred during the 21-day periods of monitoring compared between years. Further modeling of hydrology data and its relationship to other ecosystem covariates is addressed in subsequent chapters. All statistical calculations and testing were performed using packages available from R software (2016 R Core Development Team, R 3.3.0).

2.2.7 Study Limitations

In 2010 the base elevation for the channel logger was approximately 2 feet NVGD29 as determined from engineering plans for channel construction. In order to keep the logger submerged under all tidal situations and not vandalized, the channel logger had to be moved in 2011 to an alternate location within the revised channel flow path. In 2012 and 2013 the channel flow path altered again due to sand deposition and the logger was moved and remained in the 2013 location in 2014. These changes make data from this study difficult to compare against other marsh systems where elevation measurements relative to mean sea level are presented. In addition, modeled hydroperiod and tidal range determinations were only determined for a summer spring to neap tidal cycle each year. There was a 2 to 4% failure rate across years of button loggers due to leakage of salt water into protective tin enclosures and subsequent corrosion, meaning that in some cases, temperature, and thereby, hydroperiod data was missing for certain locations. Missing data was replaced with the mean of the closest two surrounding plots.

2.3 Results

2.3.1 Tidal Range

Corrections for astronomical differences between pre (2010) and post-dredge (2011-2013) years were made to normalize and remove tidal water level differences unrelated to influence of dredging. Pre-dredge tidal range (2010) in the marsh was significantly smaller (ANOVA, Tukey HSD p<0.05) than post- dredge (2011-2013) and ranged from 1.02 m to 1.20 m (Table 2.1, Figure 2.5). These results are similar to previous findings (Ramsey et al. 2006) where the mean tidal range increased from 1 to 1.5 m immediately following the 2003 emergency opening. Tidal ranges in years post-dredge (2011-2013) were not significantly different from one another. A slight downward trend in mean tidal range for the marsh was observed from 2011 to 2013.

2.3.2 Tidal Hydroperiod

Mean tidal hydroperiod (across 21-day observation period) for the marsh platform declined by 25 minutes from 2010 (145 min) to 2013 (120 min) (Table 2.1 and Figure 2.6). Mean hydroperiod for high elevation plots (classified as such because their percent cover was =/<25% *S. alterniflora*) ranged from 98 to 129 minutes, and low elevation plots (>25% cover *S. alterniflora*) ranged from 132 to 167 min (Figure 2.6). Hydroperiod was not corrected for yearly astronomical variations in tidal height as was done for tidal range values (whose intent was to evaluate the result of dredging on hydrology only). Tidal hydroperiod in 2010 and 2011 was considered significantly different (ANOVA, Tukey's HSD, p<0.05) from those in 2012 and 2013 for both high and low elevation plot groupings.



Figure 2.5. Mean tidal range for marsh and channel across years. ANOVA (mean +/- standard deviation) Tukey HSD mean separation, bars shown with shared letters are not statistically different (p<0.05). Data represent a 21-day spring to neap tidal cycle.



Figure 2.6. Mean tidal hydroperiod per tide for marsh and channel across years. ANOVA (mean +/- standard deviation) Tukey HSD mean separation, bars shown with shared letters are not statistically different (p<0.05). Outliers PZ02 and PZ16 were removed from data set. Data represent a 21-day spring to neap tidal cycle.

Hydrologic Variable	Location	2010	2011	2012	2013
Mean Tidal Range (m)	Marsh	1.02 +/- 0.25	1.29+/- 0.23	1.27+/- 0.22	1.2 +/- 0.26
Mean hydroperiod					
(minutes per inundation)	All plots	145 +/-39	151+/-35	122 +/-30	120 +/-30
	Low plots (n=50)	162	167	136	137
	High plots (n=42)	125	129	109	98
Mean 21-day total					
hydroperiod		3282 +/-	5560 +/-	3270 +/-	3375 +/-
(minutes)	All Plots Low plots	1789	1864	1594	1801
	(n=50)	4087	6392	3935	4295
	High plots		1016		1 = = 2
	(n = 42)	3027	4016	2055	1773
Mean frequency of inundation	All plots	23	37	27	28
days)	Low plots	25	39	29	32
	High plots	24	31	18	18
Freshwater Discharges to marsh (m ³ /day, instantaneous flow)	<u>Ellisville</u> <u>Road</u> Source 1- creek	1210	3629	933	NA
2004 mean annual flow	Source 2-		274	27.4	0 7 0
rate Source 1 (creek = 801	spring Source 3-	NA	NA	NA	25.8
$m^{3}/day - SMAST 2005)$	culvert	NA	NA	NA	45.9
	<u>Lane</u>	4060	4406	3974	NA
Total Precipitation (cm) (June-August)	Plymouth Airport	27	30.5	29.7	38.3
Mean Air Temperature (°C) (June-August)	Plymouth Airport	22.1	21.6	21.2	21.7

Table 2.1. Hydrologic variables for Ellisville Marsh, Plymouth, MA

Superimposed spring tidal cycles for the marsh, channel and ocean front loggers are shown in Figures 2.7 and 2.8. The cycling reveals a change in period or timing between the inner marsh responses to ocean front tides. In 2010, complete marsh emptying occurred 220 minutes (3 hrs. and 40 min) after low tide. In effect, as shown in Figure 2.7, the marsh was not entirely drained before the channel began filling, meaning that the emptying waters were met by the incoming tide well before the marsh drained maximally. In 2011 through 2013 August months, the marsh was better drained (flatter curve than in 2010) prior to the ocean tide beginning to rise and the channel filling. The time difference between marsh emptying and ocean tidal turn in 2013 was 170 minutes (2 hrs. and 50 minutes) translating to a 50 min (+/-10 min) decline from conditions in 2010.



Figure 2.7. 2010 Pre-dredge superimposed tidal cycles for spring August tide. Note out-of-phase troughs for channel and marsh as compared to ocean tides.



Figure 2.8. 2011 Post-dredge superimposed tidal cycles for spring August tide. Note synchronicity of crests and troughs due to unrestricted inlet.

Channel order tallies are shown in Table 2.2. The total number of creeks increased slightly in 2012 when the primary influent channel (4th order) split in two 3rd order creeks due to sand deposition. By 2013, however, the number of second and first order creeks had decreased by 3 from 2010 levels. The *S. alterniflora* along the edges of lost creeks disappeared and did not re-establish. In addition, a deep relict pool abutting Ellisville Road filled with sediment and remained as mudflat for much of the tidal cycle following dredging.

2.3.3 Freshwater Inputs

Certain plots routinely exhibited reduced pore water salinity (Chapter 4) and nearby creeks exhibit stratification of freshwater above saltwater. Table 2.1 lists the contributions from fresh, non-groundwater sources to Ellisville Marsh. Despite some data

Year	2008	2010	2011	2012	2013	2014			
<u>Creek Order</u>									
Fourth	1	1	1	1	1	1			
Third	4	4	4	6	3	3			
Second	10	10	9	9	8	8			
First Total	<u>18</u> 33	<u>18</u> 33	<u>19</u> 33	<u>19</u> 35	<u>19</u> 31	<u>19</u> 31			
<u>Barrier Spit Length</u> (<u>m)</u>									
	183	365	~ 0	~ 0	128	NA			

Table 2.2. Creek order tallies and barrier spit lengths for Ellisville Marsh and inlet, Plymouth MA (2008 through 2014).

gaps, the creek traveling beneath Salt Marsh Lane appears to represent the largest above ground contribution to the marsh system. In addition, analyzing water budgets for the area, USGS modeling has shown a decrease in groundwater inflow (16% from 1985 to 2205) to Savery Pond (upstream of Ellisville Marsh and creek source no.1 along Ellisville Road) due to Town of Plymouth pumping increases (Masterson et al. 2009). This creek volume was also reduced when pumping of Savery Pond for cranberry irrigation, harvest and frost protection lowers the pond level by as much as 0.5 feet (according to 2016 staff gauge readings) where stream flow was prohibited from exiting (personal communication with P. Marcoux 2016).

2.3.4 Barrier Spit, Inlet and Channel Morphology

Figures 2.9 and 2.10 depict the changes in beach topography that have occurred across years for transects closest to the estuary inlet. Some of the changes are man-made

(such as the creation of a southern berm along the channel during winter 2011-2012 dredging operations at Transect 5), while some are storm/wind driven. The beach on the state park side of the inlet (Transects 6 and 7) experienced substantial erosion in 2012 as seen by the dune scarp having moved westward (Figure 2.10, Transect 6) and loss of BT06 and BT07 for measurement in 2013. The length of the barrier spit (Table 2.2) changed in 2013, as compared to the two years prior, due to storm damage that could not be repaired within the allowable winter dredging time period (November through early February). Therefore, the barrier spit was allowed to reform somewhat in 2013 and its length increased from the prior two years (Table 2.2).



Figure 2.9. Beachfront topography showing erosion filling of secondary channel bed South of channel.



Figure 2.10. Beachfront topography along transect 6 showing erosion of dune Fall 2012 and sand movement North of channel.

2.3.5 Climate Factors

Precipitation and ambient temperature data were obtained from an onsite temperature data logger in a "no-canopy" location. Because the marsh surface is essentially flat, no specific data on aspect was necessary. PAR data was not collected. Upon further inspection, monthly precipitation, temperature, growing degree-days, and hard freeze data were considered to be important for *S. alterniflora* growth and the Plymouth County Municipal Airport Station (41.9097, -70.7294 degrees, USW00054769 (GHCN) provided this data (SC-ACIS Version 2/NOAA 2019). The Wareham data source was not used due to drastic differences in weather patterns between it and Ellisville Marsh (even though the two locations are only 20 miles distant). Local rainfall gauge data was compared with the airport data and found to be similar. The fall and winter of 2012 saw four storms, two that caused beachfront erosion. Precipitation was highest in 2013 due to a wetter than normal June, while mean June through August temperatures for all years remained within two degrees of one another.

2.4 Discussion

At the time of study implementation there were relatively few documented studies that had used the iButton technique for heat tracing and submergence monitoring within a salt marsh (Tully 2007; McKenna 2007; Bayard et al. 2011). A recent mathematical approach has been posited as one method to determining hydroperiod and its relationship to tidal range (Kefelegn 2019), but would not have accurately represented the true lag following the 2011 dredge event. As compared to more expensive loggers and elaborate sediment elevation tables (SETs) used to determine accretion/subsidence or real time kinematic (RTK) GPS measurement of elevation (Cahoon et al. 2002), this study's technique proved to be relatively inexpensive, although its implementation and interpretation is probably a comparable level of effort.

The covering of temperature buttons with cold ocean water, colder than ambient air/surface soil temperature, provided a detailed analysis of the spatial and temporal variability in inundation, and allowed increased replication for statistical purposes. Others have extrapolated near inlet pressure readings from a single logger across entire reaches of marsh in order to determine marsh wide elevation relative to mean sea level (Ramsey et al. 2006, DeLaune et al. 1983, McKenna 2007). This neglects barriers to flow within the marsh's interior morphology and changes in pore space-flushing dynamics, and may miss subtle spatial and temporal variability within the marsh interior locations.

The empirical relationship of spatial variability in plot hydroperiod across the marsh and across the four years of study changed from one of relative independence (HP10) to a more linear gradient (HP13) representing a trend in gradient in hydroperiod from east to west. This reflects the increase in tidal excursion across the marsh and possibly more uniform drainage (Figure 2.11).

Mean tidal range increased by 0.2 m from pre-dredge conditions as of 2011 and remained elevated through 2013. In addition, the marsh drained more efficiently as evident from tidal cycles that had less delay and greater communication between high tide conditions occurring in the ocean and those in the marsh. However, the hypothesized decrease in mean hydroperiod following dredging lagged behind the increase in tidal range and was not observed until the summer of 2012. The reason for the lag in hydroperiod response is unclear. Mean lunar tidal differences between 2010 and subsequent years were +4.9 cm (2010 vs. 2011), -16.5 cm (2010 vs. 2012), and +7.9 cm (2010 vs. 2013). Lunar induced tidal height differences were 24 cm of each other between 2012 and 2013, yet the mean hydroperiods for 2012 and 2013 were essentially identical (Table 2.1). Therefore, there was no identifiable contribution from lunar variation to changes in observed hydroperiod for the observed 21-day cycles. Increases in constructed berm height observed in the Spring 2012 as compared to Spring 2011 (Figure 2-9) were observed possibly meaning that more materials were removed from the channel inlet area during dredging in winter of 2012 than in the prior year, and that this accounted for increases in drainage and tidal range. Another possible explanation is that more efficient drainage initiated by winter dredging prior to 2011, began flushing of pore spaces, removing finer materials and that this change may have taken time to be evident

and allow better drainage. It is also possible that a significant decline in below ground biomass in 2012 (Chapter 3) contributed to more open pore space and less resistance to flow.



Figure 2.11. Spatial semi-variograms for hydroperiod data. Distance in longitudinal seconds and semi-variance in latitudinal min².

Plots with greater than 25% *S. alterniflora* cover experienced more frequent inundation than they had prior to dredging because of the sheer increase in volume of unrestricted tidal flow that covered the marsh surface (as shown by an increase in total 21-day hydroperiod for the plots (Table 2.1). By 2012, higher elevation plots (</= 25% *S. alterniflora*) were inundated less frequently and for shorter time periods.

Creek order changes (Table 2.2) lagged even further behind the change in mean hydroperiod. Creek dimensions are known to respond to increased flows where typically additional flow and thereby, concurrent erosion, increases their width (D'Alpaos et al. 2006) and reduces the overall complexity of the marsh interior channels. This data was not evaluated in a statistical sense, although it may suggest future trends towards loss of creek structure and conversion to more open water conditions. The documented changes in morphology may be useful for future analyses.

Precipitation and freshwater inputs to the marsh hydrological situation remain a small component as compared to the semi-diurnal tidal cycles. Furthermore, although 2013 had higher precipitation amounts than other years, this rainfall did not affect hydroperiod determination for that year's summer season as there was only one instance where the loggers sensed what was apparently rainfall inundation and not tidal influx. Average monthly growing season (June through August) temperatures remained similar across years and therefore, this factor was unlikely to have contributed substantially to differences in vegetation (Chapter 3).

In summary, the primary findings pertaining to hydrology are as follows:

- The tidal range and efficiency of marsh emptying increased as predicted in response to dredging, whereby an increase in tidal range of 0.2 m was observed and the delay between marsh emptying and ocean rising was reduced by approximately 1 hour as measured the summer of 2011 following dredging.
- Tidal hydroperiod, lagged for at least 7 months behind changes in tidal range following dredging, and possibly as much as 18 months, since the switch to a more drained hydroperiod was not actually measured until August of 2012.

Hydroperiod was reduced by 2012 for both the high and low portions of the marsh platform, and to a greater degree for the higher elevation plots. Mean tidal hydroperiods across all plots ranged from 120 (in 2013) to 151 minutes per tide (in 2011). Mean hydroperiod summed across all tidal cycles of the spring to neap cycle for all plots ranged from 3270 (in 2012) to 5560 minutes per cycle (in 2011).

- Inundation frequency increased for lower elevation plots and declined for higher elevation plots once hydroperiod shifted. The mean frequency of inundation for all plots ranged from 23 (in 2010) to 37 (in 2011).
- Beach topography changes near the channel inlet (whether storm included or anthropogenic) included increased erosion to the north of the channel, particularly observed in Spring of 2013, and filling of remnant channel to the south across all years.

CHAPTER 3

MARSH VEGETATION

3.1 Introduction

As a predominant primary producer within the salt marsh, Spartina alterniflora var. Loisel (Smooth or Saltwater Cordgrass), Family Poaceae, experiences habitat conditions that are considered extreme for other plants. Spartina alterniflora is a flood tolerant halophyte capable of salt excretion, exclusion, and accumulation (Bradley 1991). It is an obligate wetland species growing in erect; monospecific stands (Dai and Wiegert 1996; Crow 2000; Tiner 1987). As a perennial grass it has two possible genotypic and/or phenotypic expressions, reportedly (short (\leq 46 cm) and tall (> 46 cm) (Tiner 1987) growing to heights as tall as 1.5 m (Davis et al. 2004), both having been shown to relate to the extent of competition, salinity and waterlogging experienced (Gallagher 1988; Bertness et al. 1987, 2009; Shea 1975; Deng 2007). Pollen is windborne although some plants have been known to self-pollinate (Davis et al. 2004). Seeds are dispersed long distances by travel with ocean currents and wrack deposition. Plants can be vulnerable to pollen loss as it settles on water. Seed set has been demonstrated to show an Allee effect (Davis et al. 2004) whereby isolated plants produce limited to no viable pollen until rhizome growth enables further colonization. The plant uses a C-4 metabolic pathway and in northern latitudes experiences dormancy and some die-off of underground biomass (Morris 1990; Dai and Wiegert 1996). It has 5-8 principal, flat leaves, ranging from 5-20 mm wide and 20-60 cm long with hollow stems and rhizomes ranging from 5 to 25 mm at the stem base (Tiner 1987). Rooting depth may extend to 60 cm in some cases. Within

the roots, stems and rhizomes, aerenchyma function as pathways for gas conduction and cooling (Maricle 2002; Teal 1966; Arenovski and Howes 1992). It is considered an invasive species on the west coast of the United States and in other parts of the world, including China (Davis et al. 2004; Buckley and Metcalf 2006; AN SQ et al. 2007).

Abiotic (bottom-up) factors, such as the resource gradients of salinity, nutrients, sulfide, oxygen (examined in Chapter 4) and hydrology (Chapter 2), modify salt marsh architecture, function, and productivity and are some of the more well studied components of the estuary system influencing S. alterniflora (DeLaune et al. 1983; Howard and Mendelssohn 1999; Howes et al. 1984, 1986; Valiela and Teal 1974; Mendelssohn and McKee 1981). Challenges include; osmotic regulation and growth under excessive salt conditions (Cavalieri 1983, Smart 1980); waterlogging (due to sea level rise or other anthropogenic causes) and adaptation to coincident oxygen stress and sulfide toxicity (Mendelssohn et al. 1981; Morris and Dacey 1984); heat (the plant's temperature optimum is between 30 to 44°C) and drought related stresses (Twilley et al. 2005; Morris 2002; Schneider and Useman 2005); and finally, cold stress (Idaszkin and Bortolus 2010). In addition, anthropogenic influences such as draining, dredging, filling, and pollution, have negatively impacted S. alterniflora productivity (White and Howes 1994; Portnoy and Giblin 1997; Donnelly and Bertness 2001; Morris et al. 2002; Olff et al.1988; Ramsey 2006; Roman et al. 1997; Warren and Neiring 1993).

The additive effects or synergisms between abiotic factors and biotic (top-down or predator driven) agents can be just as influential and the interactions more complex (Alberti et al. 2009; Bertness et al. 2009; Boyer and Zelder 1996; Deegan et al. 2007; Denno 1979; Fleeger et al. 2008; Emery et al. 2001). Top down herbivore control of *S*.

alterniflora, has been attributed to fungi, nematodes, snails, crabs, geese, and to a lesser extent, insects (Abraham et al. 2005; Bertness et al. 2009; Burke et al. 2003; Lamondia and Elmer 2008; Japoshvili and Russell 2012; Silliman 2002; Silliman and Zieman 2001). Mutualistic affiliation between the ribbed mussel, *Guekensia demissa*, and *S. alterniflora* (and in Ellisville Marsh *S. patens)* confers stability to underground plant structure as well as sheltering the mussel (Bertness 1984).

Deegan et al. (2007, 2013) and Wigand et al. (2018) performed large-scale alterations of two salt marsh creeks, where ecosystem-wide nutrient manipulation and predator removals pointed to a possible synergistic impact contrary to expectations (i.e. removal of fish and addition of nutrients negatively impacted benthic microalgae and destabilized soil strength). Recent work in Atlantic coastal salt marshes has examined the lower intertidal limit of distribution of *Spartina sp.* in relation to the abiotic nutrient, salinity, and oxygen gradients and the biotic influence of crab herbivory (Alberti et al. 2009, Bertness et al 2009). In these cases, herbivory effects outweighed bottom-up abiotic factors in establishing the lower elevation limit of *S. alterniflora* distribution.

Control by insect herbivory has received relatively less evaluation than other forms of herbivory and certainly less than studies of abiotic controls (Hacker and Bertness 1995; Moran and Goolsby 2010; Boyer and Zelder 1996; Denno 1979). In San Diego salt marshes, scale insect herbivory on *S. alterniflora* was unresponsive to bottomup nutrient supplementation of its host plant (Boyer and Zelder 1996). Still others have found that aphids actually prefer less productive plants due to decreased abundance of predators on less robust hosts (Hacker and Bertness 1995). Because there was evident herbivory by scale insects in Ellisville Marsh, and no other obvious evidence of damage

due to other herbivores (including the infamous marsh crab *Sesarma reticulatum* (Coverdale et al. 2010) which was not detected in Ellisville Marsh in the four years of study), the scale insect was selected as a biotic factor of interest in this research.

3.1.1 Objectives

Percent cover, species richness, above and below ground biomass, plant height, stem density, and scale density measurements helped determine for Ellisville Marsh a productivity status for each year's population of *S. alterniflora* and other marsh vegetation, including *Spartina patens*. Analysis of the fermentative enzyme, alcohol dehydrogenase (ADH) in *S. alterniflora* roots was performed in order to point to possible metabolic differences that exist during the pre- and post dredge differences in inundation that might be responsible for any decreased productivity. This enzyme has been known to increase in *S. alterniflora* roots subject to excessive inundation and represents a possible adaptation to plant inundation stress (Mendelssohn et al. 1981, 1988; Burdick et al. 1987,1990; Maricle and Lee 2002).

3.2 Methods and Materials

3.2.1 Percent Cover and Species Richness

Percent cover estimates at the plot level were made for each of ninety-four vegetation plots placed at 60-ft intervals along five transects during the first two weeks of August for each study monitoring year (2010 through 2013) and during following years as part of permit monitoring conditions. Transects were perpendicular to the main channel flow and their origins were randomly assigned. Transects were located 92 m

apart from one another and their lengths varied according to accessibility and other marsh features.

A 1.0 m² PVC quadrat frame with central ring was overlain on each permanently installed piezometer and then a compass heading of 50° NE was used to align quadrat frame edges accordingly. Visual estimates of the mid-point of a range of percent cover classes was used to estimate coverage according to techniques previously outlined by Carlisle et al. (2002) for salt marsh monitoring. Tiner (1987) was used as the taxonomic authority with the exception of *Salicornia europaea* being also classified as *Salicornia depressa* due to a taxonomic change. Estimates of percent wrack, mud, and dead, but rooted, plant coverage was also made for each plot. Photographic record of each plot was also kept. Relative abundance and species richness for the marsh platform as represented by the plots was tallied and compared across years.

An additional inventory of plant species within and around the marsh margin was performed on three occasions (April 2, July 1, and September 15, 2011) with the expert assistance of Irena Kadis/Arnold Arboretum. Species were photographed and categorized into an online database for future use. As part of this effort, invasive species were identified and a measurement made of the invasive form of *Phragmites australis* patch along Ellisville Road. This patch was measured in successive years to determine its aerial extent. Equally important, was identification of the invasive Spotted Knapweed (*Centaurea maculosa*) amongst dune grasses immediately adjacent to salt marsh.

A land cover change assessment was performed to discern plant cover on a marsh wide scale. Three high resolution (0.15 m) aerial infrared orthophotographs of Ellisville Marsh were commissioned by the Friends of Ellisville Marsh, Inc. and flown by Col East,

Inc., N. Adams, MA: September 13, 2008, 8:25 am, September 9, 2011, 14:55 pm, and August 16, 2018, 10:30 am. All three photographs were taken within 1 hour of low tide and flown using the same photo id control points. A digital terrain model was created using a triangular irregular network and the images were orthorectified and then projected to North American Datum (NAD) 1983 Massachusetts State Plane Mainland FIPS 2001 Feet.

Each aerial was assessed independently from one another and a supervised classification analysis conducted using ArcGIS Info v10. Supervised classification used the photos' red/blue/green wavelengths to differentiate between the features within the image. Following classification, the classes were compared to field notes and plot cover makeup to determine the identity of plant species or the geological feature being observed. Each category was confirmed by zooming in to the original aerial photo to confirm the designation determined by the classification model and where there were uncertainties as to classification, field reconnaissance was used to confirm the cover class. Resulting classifications were combined where appropriate, and turned into maps of the land cover for each pixel. Pixel numbers were then translated into area estimates of each land cover classification.

3.2.2 Above- and Below Ground Biomass

Above ground biomass (AGB) samples were harvested from across the marsh platform during the first two weeks of August in each monitoring year and considered to represent peak standing biomass (Milner and Hughes 1968). Below ground biomass (BGB) was collected from September through October and represent low, end of the year amounts where turnover is occurring.

For AGB harvests, a 17.8 cm diameter hoop (0.025 m^2) was haphazardly thrown into one of each successive plot quadrants within the larger 1 m² plot (one clockwise quadrant rotation each year), the enclosed stem and leaf material was cut to ground surface, placed in labeled paper bags and oven dried at 60° C (ABGBD) before weighing to constant weight (+/- 0.1 g). Dead and live stems were counted to obtain the total stem density in the hoop area, but dead stems were removed from each harvest before drying and weighing.

BGB was collected using a 7.6 cm wide (at top opening) by 20 cm long (contained 830 cm³ water volume when measured) tapered cylindrical corer which was placed just outside of the plot frame on the edge closest to the AGB harvest circle, pushed into the soil until the top of the opening was at ground surface, retracted and the biomass core pushed out into a labeled, perforated plastic baggie. The cores were then washed within the baggie in a continuous stream of saltwater to remove adherent nonroot organic matter, sands, clays and silts, yet trap root material prior to freezing. Frozen cores were transported to the laboratory, thawed, air dried followed by oven drying at 70° C (BGBD) and weighed until constant weight (+/- 1 g) was obtained. No attempt was made to distinguish live versus dead root biomass, or to separate rhizome material, because of the interwoven and complex nature of the cored material.

3.2.3 Plant Height and Stem Density

Spartina alterniflora height and stem density measurements were made at the time of each plot's above ground biomass harvest and in 2014 as part of continued monitoring. The height of the entire plant (PH) from culm/stem emergence from the soil to the tip of the inflorescence (or lacking flowers, to the tip of the tallest leaf) was

measured for the three plants judged to be the tallest in each plot. As stated previously, stem density (SD) counts recorded the number of live and dead stems in each 17.8 cm diameter (0.025 m^2) hoop harvest.

3.2.4 Herbivore Counts

The scale herbivore, *Haliaspis spartinae*, was visibly present in high number on many *S. alterniflora* plants throughout the salt marsh. Their greatest visibility was during the time period when below ground biomass samples were collected (September through October). During this time, three plants were haphazardly selected from each plot and the lowest leaf blade removed, placed into plastic containers and transferred to the field laboratory. Using a dissecting scope, the density of the scale was estimated by counting the number of white tests (scale coverings) per 1 cm² of leaf surface area in the area of most dense appearance. During this analysis, a parasitic wasp, *Encarsia ellisvillensis*, was identified for the first time and its occurrence quantified (Japoshvili and Russell 2012).

To learn if the scale insect was affecting macro and micronutrient concentrations within the leaves, a limited analysis (n=3) of un-infested and infested leaf tissue was performed by the UMass Agricultural Extension Plant Tissue Laboratory, Amherst, MA following 2013 tissue analysis methods.

3.2.5 Root Alcohol Dehydrogenase (ADH) Enzyme and Protein Analysis

Alcohol dehydrogenase is known to play a role in anaerobic metabolism in *S. alterniflora* and *S. patens* (Mendelssohn et al. 1981, Burdick and Mendelssohn 1987, 1990; Maricle et al. 2006). It catalyzes the reduction of acetaldehyde in the presence of its coenzyme nicotinamide adenine dinucleotide (NADH) during fermentation to form

ethanol. Because ADH has greater affinity for ethanol, as opposed to acetaldehyde, the reverse reaction of oxidation of ethanol was followed in this assay (Crawford 1966).

Two buffer systems have been described in the literature (sodium pyrophosphate and TRIS-HCl) (Bergenmeyer 1963; Crawford 1966; Benz et al. 2007; Wang et. al. 2009). However, because the TRIS-HCl buffer (pH 8.5) appears in the more recent literature (Benz 2007; Jiang and Whang 2006) and was shown when comparing different buffer systems to yield the more active preparation (Crawford 1966), it was used for this experimental protocol (Appendix A). The pH optimum for the oxidation of ethanol is in the alkaline region (pH 8.5), and for its reduction, near neutrality. The reaction velocity was determined by the method of Vallee and Hoch (1955) in which the rate of absorbance change at 340 nm resulting from reduction of NAD⁺ to NADH was measured using a temperature controlled UV spectrophotometer (Thermo Electron Corp., Spectronic Genesys 2). One unit ADH activity reduces one µmol NAD⁺ to NADH per minute at pH 8.5 and 25°C.

Triplicate root samples for ADH analysis were collected randomly as sub-samples from washed below ground biomass root cores. Root material selected for analysis was white and turgid, as opposed to black and flaccid appearing. Care was taken to avoid rhizomes being incorporated into the sample. Selected sub-samples were weighed and immediately placed into pre-labeled aluminum foil packets, frozen on dry ice for 2 to 3 days prior to transfer to a -80° C freezer to await analysis. Not all root sub-samples were analyzed, instead, a random selection was chosen across the spectrum of hydroperiod. Thirty-seven locations were analyzed in triplicate (111 analyses) for both ADH and soluble protein.

Standard curves were prepared fresh for each set of analyses (generally weekly) and standard addition was performed to determine any possible enzyme inhibition. ADH activity was normalized against soluble protein content of the tissue and fresh weight in order to allow comparison to literature. The Bradford assay (Appendix A), using bovine serum albumin as a standard, was used for protein quantification at 595 nm.

3.2.6 Statistical Analysis

Apriori power estimates were performed in 2009 using training surveys performed by this investigator and individuals from the MA CZM to ascertain an appropriate sample/plot number. Regional standards (Neckels and Dionne 1999, 2002) were also consulted for determining appropriate plot number. Apriori power estimates were 87% for percent cover data and species abundance using 90 plots. Data residuals were from a normal distribution for ABGD, BGD, PH, and SD (Pearson's chi-square test, α =0.05), however, ADH data and herbivore counts required log transformation. Percent cover data were converted to proportions and arc sin square root transformed (with 0's replaced by ¹/₄ divided by n where n was the total number of possible species) to try to meet normality, but was unsuccessful. Untransformed relative abundance data were therefore used for the ANOVA across years. Data did not meet strict requirements for homogeneity of variance across years in every case so a Welch correction for nonhomogeneity in one-way testing was used. Due to the plants' perennial nature, samples were considered repeated measures of possibly the same clonal plant material over time. Spatially, plots were considered independent of one another. Data for PH were normally distributed and met requirements for homogeneity of variance across years in 4/5 univariate tests

3.2.7 Study Limitations

Because Ellisville Marsh is essentially the only salt marsh in Plymouth County of any appreciable area, and because of its complex history as a salt pond or coastal lagoon and a salt marsh, it was difficult to identify any nearby reference location with a similar history for simultaneous study of vegetation. As determined when evaluating temperature and precipitation records between Plymouth and Wareham (located 20 miles away from Plymouth) there was substantial variation in these two variables making use of vegetation data from distant marshes not entirely relevant. Therefore, a restoration index, weighing change at "control" versus "restoration" sites as has been used to identify success of restoration (Roman and Burdick 2012; Zedler 2001), could not be calculated. Finally, due to freezer malfunction, 2010 ADH samples were prematurely thawed and enzyme activity not analyzed and only a comparison of 2011 versus 2012 samples was performed.

3.3 Results

3.3.1 Percent Cover Comparisons

Percent cover summary statistics are shown in Table 3.1. Species with less than 5 occurrences each year were considered rare and removed from the comparisons (11 out of 17 classifications) (Table 3.2). The percent cover data were converted to relative abundance proportions (essentially very similar values due to no canopy overlaps) to test differences across years using ANOVA (Table 3.3). Short *S. alterniflora* declined across all years from 2010 levels (ANOVA, Tukey's HSD, p= 0.0342), whereas *Salicornia depressa* increased in 2011 (p=0.005) from 2010 levels, but then reverted to 2010 levels

in 2012 and 2013. Finally, the relative abundance of bare (p<0.1) and wrack areas (p<0.05) increased from 2010 levels in 2012 and 2013 (Table 3.3).

In the early spring 2012 (prior to that year's growing season), it became obvious that grasses from the prior year (2011) remained dead and standing (Figures 3.1 and 3.2) throughout the salt marsh. This occurrence was not typical for the marsh (based on visual observations since 2003) and dead vegetation remained attached throughout the summer and fall. While there was a dip in mean percent tall *S. alterniflora* (live) cover in 2012 from the prior two years, it was not considered statistically significant. Similarly, although there appeared to be a steady decline across all years in mean percent cover of *S. patens*, it was not considered significant. However, landscape and photographic data appear to radically demonstrate this change due to large die-offs of *S. patens* on the north side of the channel (Figure 3.3).



Figure 3.1. Appearance of dead standing marsh vegetation during Spring 2012. Plot 85 and plot frame in foreground.



Figure 3.2. Appearance of *Spartina alterniflora* in Plot 69 - August 2011 (left) and August 2012 (right). Prior year's vegetation appears dead and still standing affecting 2012 season's growth.



Figure 3.3. Area of *Spartina patens* die-off the summer of 2014 on the north side of the channel on Ellisville Harbor state park property.
Statistic			YEAR		
	2010	2011	2012	2013	2014
Mean	5.41	2.99	0.71	0.96	0
Sd	17.95	14.2	3.22	7.87	0
Se	1.85	1.46	0.33	0.81	0
	10.00	45.40	26.06	<u> </u>	46.00
Mean	40.23	45.43	36.06	51.55	46.82
Sd	42.73	41.35	3.14	39.3	34.19
Se	4.41	4.26	3.73	4.05	3.53
Mean	31.11	28.17	27.69	21.95	15.83
Sd	40.73	38.12	39.58	35.93	27.65
Se	4.2	3.93	4.08	3.7	2.85
Maan	51	4 1 1	2 5 1	2 02	2 10
Mean	3.1 10.19	4.11	2.31	2.83	2.18
Sa	19.18	10.75	12.91	14.3/	12.04
Se	1.98	1./3	1.33	1.48	1.3
Mean	3.57	5.26	5.73	5.37	7.69
Sd	13.8	18.03	18.68	18.28	20.52
Se	1.42	1.86	1.93	1.88	2.11
Mean	0.05	2 66	0.68	0.52	0
Sd	0.03	10.61	2.51	2.26	0
Se	0.23	1 09	0.26	0.23	0
50	0.02	1.07	0.20	0.25	U
Mean	8.77	8.93	12.14	10.21	17.06
Sd	17.77	16.46	21.78	18.3	20.17
Se	1.83	1.7	2.25	1.89	2.08
Mean	1 1 5	15	2.82	2.18	2.83
Sd	7.63	7.87	12 52	14 01	14 37
Ju	1.05	1.07	14.04	17.01	17.57
	Statistic Mean Sd Se Mean Sd Se Mean Sd Se Mean Sd Se Mean Sd Se Mean Sd Se Mean Sd Se Mean Sd Se	StatisticMean 5.41 Sd 17.95 Se 1.85 Mean 40.23 Sd 42.73 Se 4.41 Mean 31.11 Sd 40.73 Se 4.2 Mean 5.1 Sd 19.18 Se 1.98 Mean 3.57 Sd 13.8 Se 1.42 Mean 3.57 Sd 13.8 Se 1.42 Mean 0.05 Sd 0.23 Se 0.02 Mean 8.77 Sd 17.77 Se 1.83 Mean 1.15 Sd 7.63	StatisticMean 5.41 2.99 Sd 17.95 14.2 Se 1.85 1.46 Mean 40.23 45.43 Sd 42.73 41.35 Se 4.41 4.26 Mean 31.11 28.17 Sd 40.73 38.12 Se 4.2 3.93 Mean 5.1 4.11 Sd 19.18 16.75 Se 1.98 1.73 Mean 3.57 5.26 Sd 13.8 18.03 Se 1.42 1.86 Mean 0.05 2.66 Sd 0.23 10.61 Se 1.09 1.675 Se 1.777 16.46 Se 1.15 1.5 Sd 1.15 1.5 Mean 1.15 1.5	StatisticYEAR201020112012Mean 5.41 2.99 0.71 Sd 17.95 14.2 3.22 Se 1.85 1.46 0.33 Mean 40.23 45.43 36.06 Sd 42.73 41.35 3.14 Se 4.41 4.26 3.73 Mean 31.11 28.17 27.69 Sd 40.73 38.12 39.58 Se 4.2 3.93 4.08 Mean 5.1 4.11 2.51 Sd 19.18 16.75 12.91 Se 1.98 1.73 1.33 Mean 3.57 5.26 5.73 Sd 13.8 18.03 18.68 Se 1.42 1.86 1.93 Mean 0.05 2.66 0.68 Sd 0.23 10.61 2.51 Se 1.09 0.26 Mean 8.77 8.93 12.14 Sd 17.77 16.46 21.78 Se 1.83 1.7 2.25 Mean 1.15 1.5 2.82 Sd 7.63 7.87 12.52	StatisticYEAR2010201120122013Mean 5.41 2.99 0.71 0.96 Sd 17.95 14.2 3.22 7.87 Se 1.85 1.46 0.33 0.81 Mean 40.23 45.43 36.06 51.55 Sd 42.73 41.35 3.14 39.3 Se 4.41 4.26 3.73 4.05 Mean 31.11 28.17 27.69 21.95 Sd 40.73 38.12 39.58 35.93 Se 4.2 3.93 4.08 3.7 Mean 5.1 4.11 2.51 2.83 Sd 19.18 16.75 12.91 14.37 Se 1.98 1.73 1.33 1.48 Mean 3.57 5.26 5.73 5.37 Sd 13.8 18.03 18.68 18.28 Se 1.42 1.86 1.93 1.88 Mean 0.05 2.66 0.68 0.52 Sd 0.23 10.61 2.51 2.26 Se 0.02 1.09 0.26 0.23 Mean 8.77 8.93 12.14 10.21 Sd 17.77 16.46 21.78 18.3 Se 1.83 1.7 2.25 1.89

Table 3.1. Percent cover summary (n=94), SAS short and SAT tall *S. alterniflora*, SP *S. patens*, SD *Salicornia depressa*, JG *Juncus gerardii*, DS *Distichlis spicata*

Table 3.2. Genus species detected and species richness in Ellisville Marsh, MA. Asterisk indicates species considered rare (detected < 5% frequency of occurrence).

Genus species	Species Richness	
(*rare)	(year - #)	
Spartina alterniflora (tall)	2010 - 14 species	
Spartina alterniflora (short)	2011 - 14 species	
Spartina patens	2012 - 15 species	
Distichlis spicata	2013 - 14 species	
Juncus gerardii	2014 - 14 species	
Salicornia depressa		
Salicornia virginica *		
Limnomium nashii*		
Iva fructescens*		
Scirpus robustus*		
Agrostis stolonifera*		
Plantago maritima*		
Triglochin maritinum*		
Spergularia marina*		
Solidago sempervirens*		
Atriplex patula*		

Table 3.3. Means and ANOVA results for marsh wide ARCSINSQRT transformed abundance (percent cover) and *Haliaspis spartinae* counts across years. (ANOVA, Tukey HSD separation of means, $\alpha = 0.05$.)

		Year				
	2010	2011	2012	2013	2014	
Species						p-value
Tall S.						
alterniflora	а	а	а	а	а	0.066
Mean	40.2	45.4	36.1	51.6	46.8	
Short <i>S</i> .						
alterniflora	а	а	b	b	b	0.0014
Mean	5.4	2.9	0.7	1	0	
Spartina						
patens	a	a	a	a	b	0.0041
Mean	31.1	28.2	27.7	21.9	15.8	
Salicornia						
depressa	а	b	b	ab	b	0.0014
Mean	0.05	2.7	2.5	0.52	1.7	
BARE	а	а	а	а	b	0.0007
Mean	8.8	8.9	12.1	10.2	17.1	
WRACK	а	а	а	а	b	0.0285
Mean	1.1	1.5	2.8	2.2	3.8	
Haliaspis						
spartinae	ab	bc	bc	с	na	0.0274
#/cm ² Mean	66	25	86	50	NA	

Results of the landscape scale cover classification are shown in Figures 3.4 and 3.5, and Table 3.4. The infrared aerial photos were taken September 3, 2008 at 8:25 am and September 9, 2011 at 14:55 pm, and August 16, 2018, 10:30 am (all three within 1 hour of low tide). When comparing the 2008 (2 years prior to dredge) and 2011 (one year following dredge) orthophotographs, differences in shadow and color intensity were noticeable. This was primarily due to upland features creating shadow, as well as the time of day variability in transpiration as indicated by different intensity of pinks and reds. Also, because the pre-dredge photo was taken in 2008, 2 years before the dredging in winter 2010-2011, the changes in appearance represented in 2011 cannot be entirely attributed to dredging, nor can the 2011 photo be considered as a reduce hydroperiod condition as discussed in Chapter 2. The 2018 photo was shot digitally, while the 2008 and 2011 were taken using film. Because of this a wider spectrum was available for analysis with the 2018 photograph.

Despite most shadow being subtracted from each image, residual border shadows were likely counted as water. Differences in photo time of day, early morning versus mid afternoon, meant different spectrums used for classification. Therefore, the ability to discern *Juncus gerardii, Iva fructescens* and by default, sparsely interspersed *S. alterniflora*, as completely separate species was compromised (particularly in the 2018 photo). The estimated area occupied by the combination of the two high marsh plants, *S. patens* and *Distichlis spicata* is appropriate, as were the mudflat and sand areas because their color classifications did not change between years. Only 4 out of 94 plots (4.3%) identified to be *S. alterniflora* by interpretation were considered incorrectly classified when 2008 and 2011 results were ground-truthed in 2013. The unvegetated (UV) to

vegetated (V) indices (I) for each year were calculated by summing open water, sand and mud areas as representative of "unvegetated" and summing all other classification to represent "vegetated" so that the UVVI could be calculated (Table 3.4).

Table 3.4. Ellisville Marsh supervised land cover classification comparisons usingIR orthophotographs - September 3, 2008, September 9, 2011, and August 16, 2018.

	2008			2011				
2008 Land Cover	Pixel Count	Square Meters	2011 Land Cover	Pixel Count	Square Meters	2018 Land Cover	Pixel Count	Square meters
Water	3216034	72361	Water	3012914	67791	Water	3217473	72393
Spartina			Spartina			Spartina		
patens/			patens/			patens/		
Distichlis			Distichlis			Distichlis		
spicata	3530284	79431	spicata	1956175	44014	spicata	3838106	86357
Spartina			Spartina			Spartina		
alterniflora/			alterniflora/			alterniflora/		
Juncus			Juncus			Juncus		
gerardii	2249424	50612	gerardii	3694762	83132	gerardii	4197129	94435
Iva			Iva			Iva		
fructescens/			fructescens/			fructescens/		
Juncus			Juncus			Juncus		
gerardii	2003084	45069	gerardii	2486129	55938	gerardii	104563	2353
Mud	1992337	44828	Mud	1561795	35590	Mud	1363595	30681
Sand	231197	5202	Sand	490582	11038	Sand	546825	12304
Total	13222360	297503		13202357	297503		13267691	298523
UVVI		0.70			0.62			0.63

3.3.2 Spartina alterniflora Productivity Metrics

Summary productivity statistics were calculated for all plots with greater than 25% *S. alterniflora* percent cover present in any year (minus the outlier locations PZ2 and PZ16 determined during the hydrology analysis). Descriptive statistics (mean, range and one standard deviation) for each year's ABG dry (ABGD), BGB dry (BGBD), plant height (PH), and stem density (SD) are presented in Tables 3.5 and 3.6. Plots that had intermittent *S. alterniflora* present were kept in consideration and when the plant was not

present, their measurements were reported as zero %. Results of repeated measures ANOVAs are presented in Figures 3.6 and 3.7.



Figure 3.4. Marsh vegetation cover comparisons 2008 and 2011. Red sample dots within channel centers represent water quality/pressure logger locations, all others are vegetation plots. Graphic courtesy of Lori Pelech/UMass Department of Ecological Conservation.



Figure 3.5. Marsh vegetation cover comparisons 2018. Graphic courtesy of Kate Fickas/UMass Department of Ecological Conservation.

Table 3.5. *Spartina alterniflora* biomass summary statistics. ABGD and BGBD refer to above and below ground biomass dry weight, respectively. Numerical designation in column heading refers to abbreviated year of harvest (e.g. 10 = 2010).

units	ABGD10 g dry wt./ 0.025m ²	ABGD11 g dry wt./ 0.025m ²	ABGD12 g dry wt./ 0.025m ²	ABGD13 g dry wt./ 0.025m ²	BGBD10 g dry wt./ 830 cc	BGBD11 g dry wt./ 830 cc	BGBD12 g dry wt./ 830 cc	BGBD13 g dry wt./ 830 cc
nobs	56	56	56	56	56	56	56	56
min	0	4.9	0	10.1	5.1	6.2	0	4
max	51.8	37.2	36.2	42.4	144.1	83.3	54.8	92.4
mean	19	16.4	12.8	22.8	46.6	35.5	23.3	37.7
med	18.4	15.1	12.9	21.3	44.1	33.4	24.6	34.6
sd	10	8	7.9	7.8	25.1	16	13	21.2
se	1.3	1.1	1.1	1	3.4	2.1	1.7	2.8

Table 3.6. *Spartina alterniflora* plant height (PH) and stem density (SD). Numerical designation in column heading refers to abbreviated year of harvest (e.g. 10 = 2010).

	PH10	PH11	PH12	PH13	SD10	SD11	SD12	SD13
units	cm	cm	cm	cm	# per	# per	# per	# per
					$0.025m^2$	$0.025m^2$	$0.025m^2$	$0.025m^2$
nobs	56	56	56	56	56	56	56	56
min	17.8	17.8	49	41.4	0	0	0	0
max	144.8	173.5	133.9	165.1	21	29	13	25
mean	76.8	87.5	83.2	97.4	9.9	9.6	5.6	10.1
median	71.1	89.3	84.6	95.5	10	8.5	5	9
sd	28.5	25.5	16.5	22.8	5.2	5.8	3	5.2
se	3.8	3.4	2.2	3	0.7	0.8	0.4	0.7

There was a significant effect of year for all productivity measures (two-way repeated measures ANOVA, p<0.001) (Figures 3.6 through 3.7). SD, ABGD, and BGBD were lower in the year 2012 as compared to the other years. The 21-day total hydroperiod had an effect on both PH (p <0.001) and BGBD (p < 0.1). Below ground biomass for just *S. alterniflora* containing plots was related to hydroperiod, although the relationship was not as strong as with plant height.

Closer examination of the plant height (PH) relationship to hydroperiod revealed a positive, statistically significant, linear relationship with increases in 21-day total hydroperiod (Figure 3.8). However, there are likely to be other influential factors as the relationship was weak ($R^2 = 0.12$). Use of PH as a sentinel variable indicating marsh status may have merit if a stronger relationship to an increased list of multiple covariates is developed (Chapter 6).



Figure 3.6. Mean above and below ground biomass interaction with total 21-day hydroperiod for just plots containing >/= 7% cover *Spartina alterniflora*. Repeated measures ANOVA both ABGD and BGBD ***year (p<0.001), BGBD *hydro (p=0.0788).



Figure 3.7. Mean stem density and plant height interaction with total 21-day hydroperiod for just plots containing >/= 7% cover *Spartina alterniflora*. Repeated measures ANOVA both SD and PH *** year (p<0.001), PH *** hydro (p<0.001) and year: hydro * (p=0.029).

During 2011, the mean plant height for S. alterniflora plots was significantly higher than pre-dredge conditions, and this factor was positively correlated with hydroperiod, perhaps representing the wetland plant's ability to elongate to reach light during higher water conditions or its response to short-term fertilization by suspended sediment as a result of dredging. The additional plant height possibly contributes to an increased ABGB and a decreased root to shoot ratio although fertilization effects were not specifically studied. In other frequently flooded plants (such as rice and creeping bent grass) the elongation that occurs in response to long periods of flooding does not increase biomass, rather stems become less robust structurally and cannot support the plant's height gain resulting in lodging (Kende et al. 1998, Jiang and Wang 2006). In 2011, the obvious lodging of S. alterniflora that occurred was in certain areas immediately alongside ditches. Here plants were observed to grow very tall and by the end of the growing season were prostrate. This necessitated that certain locations (PZ10 and PZ16) be removed as outliers from analysis. Even with outliers removed, in 2011 the maximum heights were the highest as compared to all other years (Table 3.6).



Figure 3.8. Linear regression of plant height to 21-day summed hydroperiod for all four years of observation, n=224 ($y\sim4.65x10^{-3}x+65.6$, R² = 0.12, p=1.19x10⁻⁷), 95% confidence intervals.

Under 2010 pre-dredge conditions, if both low and high elevation plots are considered, root/rhizome to shoot ratios calculated are higher than post-dredge, possibly meaning that there is relatively more root "stock" available from previous years' growth and that less of the plant's energy was put into aboveground stem and leaf growth in the waterlogged condition. Due to the plant's perennial nature, the AGBD formed in one year might be unrelated to the hydroperiod of the current year, and more likely related to conditions imposed by the prior year's hydroperiod and the prior year's BGBD. Therefore, it was reasoned that comparing the ABGD to the prior years hydroperiod might improve the linear relationships. This was the case for 2010 to 2011 ($R^2 = 0.19$, p=0.00071), but not for any other pairing (Figure 3.9).



Figure 3.9. Linear regression with 95% confidence intervals of hydroperiod against above ground biomass (ABGD) lagged one year for *Spartina alterniflora* containing plots. Only the lagged relationship between 2010 and 2011 was considered significant (p=0.0007, $R^2=0.19$).

3.3.3 Herbivore Analysis

A difference in mean *H. spartinae* count across years was present (ANOVA,

Tukey's HSD, p<0.05) with 2013 abundance less than 2010 (Table 3.3). Figure 3.10

shows the apparently chlorotic and damaged appearance of leaves with *Haliaspis* present. Tissue analysis of leaves (n=3) with and without *H. spartinae* present showed no statistical difference (α =0.05) in nitrogen content (1.65% +/- 0.2) or other measured macronutrient, except for copper. The mean concentration of copper (Cu) in infested plants (279 +/- 20 ppm) was roughly 30 times higher than in plants without the insect test (9 +/- 5 ppm). All leaves analyzed were +/- 1.8 cm in height difference from one another.



Figure 3.10. *Haliaspis spartinae* (white scale coverings) on surface of *Spartina alterniflora* leaves. Plot No. 36 - September 2010.

3.3.4 Root Alcohol Dehydrogenase (ADH) and Soluble Protein

Because pre-dredge ADH results were not available for 2010 due to freezer failure and inadvertent sample thawing, a comparison between pre-and post dredge values was not made. However, root ADH from plots in 2011 and 2012 were assessed to try to determine any relationships between hydroperiod and ADH activity. ADH concentrations required log transformation for residuals to meet the assumption of normality and sample populations had homogeneity of variance for the two years. Mean ADH concentrations for all locations sampled ranged from 9 to 188 µmole NAD+ reduced (ADH activity units)/µg soluble protein-hr., also expressed as 116 to 6241µmole ADH activity/g fresh wt-hr. Addition of ADH (0.75 ADH units/ml) to extracts with little to no ADH activity yielded 100% to > 100% recovery in all cases (n=3) meaning enzyme inhibition did not occur in those assays.

There were no significant linear relationships between hydroperiod (21-day total or mean) and root AHD level; however, there was a significant difference in mean root ADH concentration (ANOVA p=0.0449) when compared across years (Figure 3.11) with 2011 samples having significantly higher ADH activity than 2012 samples.



Figure 3.11. *Spartina alterniflora* root alcohol dehydrogenase activity. ANOVA (mean +/- sd), bars shown with shared letters are not statistically different (p<0.05).

3.4 Discussion

Both above and below ground biomass ranges were within those typical for New England salt marshes (200-1300 g dry wt./m²/yr. for ABGB and 3620-7547 g dry wt./m²/yr. for BGB) (Steever 1976; Ellison et al. 1986) (Table 3.5). The range of root and rhizome (BGB) to shoot (ABGB) ratios (RRTS) after adjusting to a meter squared basis were on the low side, ranging from means of 1.75 (post dredge) to 3.15 (pre-dredge) as compared to those reported for marsh organ experiments (2 to 12) (Morris and Currin 2013; Morris 2002). Plant heights were also typical of northern salt marsh *S. alterniflora* vegetation, (30-200 cm) (Steever 1976), as were stem densities with higher stem densities in plots with short *S. alterniflora* (Morris 2002) (Table 3.6).

The productivity per unit area of *S. alterniflora* appears to have declined in 2012 as indicated by the statistical decrease in BGB and PH (Repeated Measures ANOVA, p < 0.001). In the summer of 2011, BGB and PH had the highest correlation coefficient (Person's r =0.51) of all variables. The dip in plot percent cover in 2012 also mimics declines in these and other productivity indicators. There was a declining trend in the other productivity responses (ABGD and SD) across years as shown on the repeated measures interaction plots (Figure 3.6 and 3.7).

The increased drainage, as shown by shorter hydroperiod beginning in 2012 (Figure 2.4) and delay in response to increased tidal range, may have contributed to these changes. Because the plant is perennial and the prior year's rootstock determines, in part, the efficacy of the following year's growth, if the plants did not senesce or go through dormancy properly in the fall of 2011 this may have caused poorer growth in 2012. Hull et al. (1976) have documented in *S. alterniflora* substantial transfer of photosynthate to

the below ground biomass component upon the onset of aboveground senescence in the fall. If this process was interrupted due to dredging effects, this could account for the poorer growth of below ground biomass in 2012. It could also be a possible reason why average hydroperiod did not decline until 2012 as the reduced below ground biomass component in 2012 allowed more open pore space for flushing and faster drainage.

It is notable that the total percent cover of the short ecophenotype of *S*. *alterniflora* declined significantly since dredging. The short form is generally associated with more waterlogged conditions (Maricle 2006; Burdick and Mendelssohn 1987). High elevation marsh plots dominated by *S. patens, Juncus gerardii,* and *Distichlis spicata* maintained their same species assemblages, although landscape cover comparisons did show a decline in the *S. patens/D. spicata* populations from 2008 to 2011; the length and frequency of inundation for high marsh plots has been reduced since 2011-2012. Plot percent cover estimates did not show a significant decrease in *S. patens* until 2014. Statistical increases in percent cover of bare and *Salicornia depressa* could be interpreted to mean that the elevation of these plots may be becoming lower across time. *S. depressa* is also an early colonizer of bare areas (Tiner 1987).

Re-colonization with *S. alterniflora* of the mudflat along Ellisville Road that suffered vegetation losses has not been evident, nor was there any large acreage regrowth of this species as alluded to after the 2003 opening (Ramsey 2006). In addition, although not part of the monitored plots, the bordering *Phragmites australis* patch along Ellisville Road has increased in area since 2010, likely due to stagnation of freshwater along the roadside related to road subsidence and a culvert in need of repair. Re-growth of herbicide treated *P. australis* has also occurred along the state park border. The total number of species encountered within the marsh remained essentially the same across years (14 or 15). Overall, the UVVI for all three years, when compared to other northeastern salt marshes undergoing varying stages of horizontal losses, are considered to represent non-resilient platforms where UVVI > 0.1 (Ganju et al. 2017).

The apparent inverse relationship between *H. spartinae* abundance and *S. alterniflora* productivity metrics, while not significant between all years, was of interest. Recalling the work of Boyer and Zelder (1994, 1996) this insect's preference is for less healthy plant materials, which may have been the case in Ellisville Marsh. In the San Diego marshes, *H. spartinae* was observed to decline in number when the plant received nitrogen additions (considered a stress factor) contrary to the increase in numbers that had been expected (Boyer and Zedler 1996) and contrary to Larssen (1989) and Louda (1988) who determined that an already stressed plant is less likely to withstand insect herbivore onslaught.

Burke et al. (2003) has reported a Cu concentration of 6.11 ppm for healthy *S*. *alterniflora* leaf tissue similar to that found in the non-infested plants studied from Ellisville Marsh (9 ppm). It could be that the fibrous appearing scale insect test structures adsorbed copper particulate preferentially from surrounding sediment/seawater, or that the plant itself, because of damage caused by insect sucking may have had increased copper uptake. No other element, however, was found to deviate in this manner (including B, Fe, Zn, Mn, Mg, Na, Ca, K, P, N) making adsorption from seawater a less likely mechanism. Xu et al. (2018) report Cu and Zn plant accumulation increasing with waterlogging duration under field conditions.

The mean ADH level found in 2011 (1659 µmole ADH activity/g fresh wt./hr.) was higher than those reported elsewhere for *S. alterniflora* under waterlogged conditions. Mendelssohn and McKee (1988) described levels ranging from 150 to 600 µmol ADH activity/g fresh wt-hr for flooded *S. alterniflora*. A laboratory experiment by Maricle et al. (2006) found up to 300µmol ADH activity/g fresh wt-hr under flooded conditions compared to values as high as 6241µmole ADH activity/g fresh wt-hr in Ellisville Marsh. The significant decrease in mean hydroperiod evident in 2012 may have had a favorable impact on metabolism by increasing drainage and unsaturated pore space resulting in more O₂ being available to the plant accounting for the significant decrease in requisite ADH activity. The decrease in fermentative ADH activity, and therefore, increase in additional ATP available through aerobic respiration, however, may not have been enough to positively counteract *S. alterniflora* productivity declines in this year.

In summary, *S. alterniflora* plant height showed a statistically significant positive relationship with increasing 21-day hydroperiod, while other productivity indicators (aside from percent cover) appeared to decline to their lowest points in 2012 and then rebound close to pre-dredge circumstances by 2013. The relationship between water level/length of inundation and plant height is a documented phenomenon with more intensely studied grasses such as rice and creeping bentgrass (Jiang and Wang 2006; Kende 1998).

Finally, there was an inverse relationship between plant biomass and mean *H*. *spartinae* density, but not enough information to ascertain whether the scale was a contributor to plant stress or whether its preference was for already stressed plant material. The increase in Cu in plants with tests present deserves further research to

determine if this is the insect's direct affect on the plant tissue and what metabolic role this might play in plant productivity in widely varying hydraulic conditions.

CHAPTER 4

MARSH WATER AND SOIL CHEMISTRY

4.1 Introduction

Chemical constituents, including nutrients, enter the marsh system in the semidiurnal flux of tidal water. They travel through the soil pore spaces, and as sheet flow, over the soils/sediments in the salt marsh. Inputs from upstream and groundwater sources also contribute to the chemical makeup of the pore water (water in the pore spaces of the soil/sediment). Microorganisms in turn alter these constituents, and other abiotic and biotic factors, as do the salt marsh plants themselves. A large body of literature exists on nutrients in salt marshes because of the interest in eutrophication and the marsh's ability to assimilate excess nutrient (Darby 2006; Fox et al. 2012; Howes 1986; Morris et al. 1982, 2002, 2006; Smart 1980; Valeila et al. 1974, 1976, Deegan et al. 2007, Wigand et al. 2018). More recently, research has focused on determining the affects of nutrients on productivity (or lack thereof) and on marsh platform building in order to keep pace with the rise in sea level (Kirwan et al. 2010; Morris et al. 2013; Deegan et al. 2013).

Typically, nitrogen is considered limiting for salt marsh above ground vegetation, despite it being readily prevalent in influent sources as nitrate (NO₃⁻) and being rapidly converted by microorganisms to available ammonium (NH₄⁺) for plant uptake (Morris et al. 2013, 2002; Deegan et al. 2013). Because divalent Mg, Mn, and Ca are predominant in seawater, they compete for cation exchange sites on silts and clays, thereby excluding monovalent NH₄⁺ and making it less available for uptake by the root. These same cations compete with NH₄⁺ for uptake mechanisms at the root membrane level and impede NH₄⁺

uptake (Morris and Dacey 1984). Uptake of NH_4^+ as opposed to NO_3^- comes at a lower energy cost for the plant overall because it can be directly synthesized into glutamate, and then into nitrogen containing amino acids without the larger ATP requirement of $NO_3^$ assimilation (Cronk 2001). Furthermore, the prevalence of sulfides (S^{2-}_x) in the soils interferes with NH_4^+ uptake by plants because sulfides interact with cytochrome c oxidase and ADH respiratory/fermentation enzymes (Maricle et al. 2006; Bagarinao 1992) effectively poisoning metabolism.

Most information on *S. alterniflora* growth has been developed by nitrogen fertilization studies in situ or via greenhouse/lab experiments where control over just the nitrogen variable was possible (Daleo 2008; Smart 1980; Howes et al. 1986; Morris et al. 1982, 1984, 2013; Valeila 2008). In general, as nitrogen supplies are increased, so too are *S. alterniflora* above and below ground productivities, although there have been studies showing a decline in below ground biomass with increasing fertilization (Deegan et al. 2013; Wigand et al. 2018; Darby 2006). Morris et al. (2002) describe a quadratic function as a phenomenological fit to increases in above ground biomass on fertilized plots as compared to unfertilized, control plots.

The biogeochemical cycles of Fe, P and S in salt marsh soils/sediments are also interwoven. Microorganisms, pH and degree of oxygenation combine to create complex cycling between concentrations of various forms of the three inorganic elements in the liquid, solid, and gaseous phases of the salt marsh. In unsaturated conditions, bacterial and fungal respirations compete for oxygen with the roots' process of oxygen consumption (Burke et al. 2003). In saturated conditions, such as extended periods of water logging, oxygen in the root zone declines even further. Even without

microorganisms' respiration competing for oxygen, in inundated circumstances, less oxygen enters roots due to the poorer diffusion of oxygen in water as opposed to air (approximately 10,000 fold slower) (Thompson and Greenway 1991; Lennard 2003). Under these circumstances the impact of accumulating sulfides becomes important. If conditions in the sediment are anaerobic then the sulfur forms present may be either soluble (S²⁻, HS⁻, and H₂S) or insoluble (FeS₂), and possibly include sulfur containing organic compounds (Luther et al. 1985). If conditions in the sediment become oxidized, liberated sulfur and iron can form unfavorable physical and chemical soil/sediment and porewater conditions (Portnoy and Giblin 1997a, 1997b) including acidification.

Phosphorous can become a limiting nutrient for plant production in the marine environment (Smith 1984). If ferric iron concentrations are high then it can combine with available PO_4^{3-} to form FePO₄ and limit P availability for the plant. In addition, high concentrations of Fe³⁺ present in oxidized conditions can precipitate and form plaques of ferrihydrite along root zones that prevent the physical exchange of necessary plant nutrients causing iron toxicity and bronzing of plants (Cronk and Fennessy 2001). In a salt marsh, chroma values (</= 2) for the sediment indicate that the form of iron present is likely to be the reduced ferrous (Fe²⁺) form (Brady and Weil 1996). Plants have been shown to have higher growing season phosphorus levels in short height phenotype populations compared to those in tall stand leaf material as reported by Gallagher (1980), while Darby (2006) determined that below ground biomass decreased by 40 to 60% with P/Fe additions. Valeila (1974) showed no phosphorus limitation in stands of low marsh *S. alterniflora* using fertilization experiments.

Salt marsh sediments are classified as Sulfaquents and Sulfihemists because of their high concentrations of sulfidic materials and organic matter (Brady and Weil 1996; Richardson and Vepraskas 2001). The typical average concentration of dissolved sulfate in seawater is 2,700 mg/l (Burton 1976). Its presence in pore water means it can be used as an alternative electron acceptor in anaerobic conditions by sulfate reducing bacteria to produce sulfide (Giblin and Howarth 1984). One mole of reduced sulfate accounts for mineralization of 2 moles of organic carbon-to-carbon dioxide (Jorgensen 1977, Howarth and Teal 1979). One study found sulfate reduction rates to be higher in short S. *alterniflora* stands (90 mmoles $SO_4^{2-}/m^2/day$) versus tall *S. alterniflora* stands (30 mmoles $SO_4^{2}/m^2/day$), although the authors recommended further study of tall grass sites. Giblin and Gaines (1990) report that in salt marshes, 62-99% of reduced sulfur from sulfate reduction ends up as insoluble FeS_2 (pyrite), with only a minor component resulting in soluble or acid-volatile sulfides. The pH of the soils and pore water determine the predominant sulfur form, with S^{2-} found at pH values near 14, neutral to alkaline pH values prescribe primarily the presence of HS^{-} , and pH values less than 7 indicate H_2S will be the dominant form. Sulfate is found across the entire spectrum of pH, while S° is found in higher concentrations only at low pH values. Pore water levels of sulfide in salt marsh with S. alterniflora have been reported to range from 1 to 8 mM with highest levels at depths of 10 to 18 cm bgs (Carlson and Forrest 1982, Stribling and Cornwell 2001).

Sulfide can eventually enter the plant without metabolic control causing respiratory poisoning in extreme conditions (Bradley and Morris 1990; Taiz and Zeiger 2006). A total soil sulfide concentration of 250 µg S/g soil (0.025%) may limit *S*.

alterniflora growth (Krairapanond et al. 1991, Lamers et al. 2013). As discussed previously (Chapter 3), soluble sulfide may also limit fermentative processes by inhibiting alcohol dehydrogenase activity (necessary to continue ATP formation) (Bradley and Morris 1990; Cronk and Fennessy 2001; Koch et al. 1990; Maricle and Lee 2006).

4.1.1 Objectives

This study presents data on nutrients and sulfide in pore waters, surface water monitoring results, and concentrations of sulfur in soils, along with other typical parameters such as pH and salinity, in order to try to determine their relationship to *S*. *alterniflora* productivity and the marsh response to dredging as a whole.

4.2 Methods and Materials

4.2.1 Sample Collection and Handling

4.2.1.1 Marsh Water Quality and Upstream Surface Water Contributions

The Massachusetts Estuaries Project (MEP) planned, established and coordinated a citizens' baseline water-quality monitoring program for Ellisville Harbor. This work was required for incorporation of the embayment into the MA Estuaries Project – a collaborative effort between MADEP and School for Marine Science and Technology (SMAST) established to develop nitrogen-loading limits for 89 embayments in southeastern Massachusetts. Four years of water quality (2003 through 2007) monitoring were collected for validation of the linked watershed embayment model implemented under the Estuaries Project.

Volunteer citizens collected water samples at pre-determined stations on a schedule that was established by SMAST. Dissolved oxygen, salinity, pH, conductivity, turbidity, total dissolved solids, total suspended solids (2013 & 2014 only), Secchi disk depth, and chlorophyll measurements were collected from two upstream freshwater feeder creek locations (EVH01, EVH02), three inner marsh locations (EVH03-EVH05), channel (EVH06) and ocean front (EVH07) reference locations on at least two occasions each summer (Figure 2.1). These samples were analyzed for a suite of analytes by SMAST, including: NO₂⁻, NO₃⁻, NH₄⁺, dissolved organic nitrogen (DON), particulate organic nitrogen (PON), chlorophyll *a*, pheophytin *a*, ortho-phosphate, salinity, and dissolved oxygen (DO). No sulfides were analyzed.

Volunteer training, equipment needed to perform water quality sampling, a QAPP, and necessary data management were provided by SMAST. Project records indicated that no prior water quality data existed for Ellisville Harbor. Methods used by the UMass Dartmouth SMAST are documented elsewhere ("Summary of Water Quality Monitoring Program for the Plymouth, Kingston and Duxbury Harbor Embayment System (2003 to 2004)", Draft Final July 20, 2005.). The sampling program was conducted on a summer seasonal basis from 2003 to 2005, 2007, by the author and volunteers, and then reinitiated, with slight method modifications, as part of permit monitoring requirements by the FEM in 2010 through 2013. Separate study of Savery Pond, a principal upstream water source for the marsh, was conducted in 2012 due to a history of observed and documented blue-green algae blooms and known cranberry bog discharge into the pond. Methods used by Aquatic Control Technology, Inc. are

documented elsewhere in the report entitled "2012 Water and Sediment Quality Survey of Savery Pond-Plymouth, MA", Final Report, November 2012.

4.2.1.2 Pore Water Samples

In order to collect marsh pore water, ninety-seven (3 later abandoned in 2011), 5.08 cm OD diameter, 0.91 m deep PVC piezometers were installed in the spring of 2010 across the five pre-designated transects at 60-ft intervals. The lower 46 cm of each PVC pipe was perforated by drilling 1 cm holes every 2 cm and was wrapped in landscape cloth before installation, similar to the methods outlined in Carlisle (2002) for larger diameter groundwater wells. The piezometers were packed only with the original marsh materials dug from the installation hole. Marsh groundwater/pore water samples were collected 1 to 2 hours after the high tide using a hand-held bailing pump (Cole Palmer). Each piezometer was pumped to dryness and allowed to recover for approximately 30 min before sampling. Samples were pumped into 118 ml (4 oz.) volume, polyethylene, Standup Whirl pack bags, placed on ice and analyzed within 1 to 3 hours of collection. No sample preservation, other than being maintained at 4 °C, was used. Samples were allowed to equilibrate to room temperature (25°C) prior to chemical analysis. During this time period suspended sediment was allowed to settle and care was taken not to resuspend material when taking sample aliquots for analysis or when inserting the pH probe.

4.2.1.3 Soil Samples

Sub-samples of soil samples collected for physical properties analysis (Chapter 5) were air and oven dried (100° C for 24 hr.), and then ground to fine particles in a mortar

and pestle. Visible organic material was removed using a 2mm sieve and were stored in plastic Baggies to await pH and sulfur analysis.

4.2.2 Salinity, pH, Temperature and REDOX Analyses

Salinity of the pore water was measured using a hand-held refractometer (VISTA Model A366ATC) with automatic temperature compensation. Results were reported in ppth (%). Calibration of the optics was checked using distilled water on a routine basis. Soil salinities were not measured.

Pore water pH measurements were made with a multipurpose meter with KCl and calomel electrodes. Sample pH was measured using a 3-point pH buffer calibrated Oakton pH meter with glass electrode. To provide a comparison between in-situ soil pH measurements and lab pH measurements, in situ soil pH values were obtained by placing a hand held pH electrode (Forestry Suppliers Co.) directly into the subsurface sediment. This field-tested soil was then collected and transferred to the laboratory for drying prior to measuring pH using laboratory methods. Soil pH was determined in the laboratory by rewetting previously air-dried soil in a ratio of 3 parts distilled water to 1 part soil. A Fisher Accumet pH meter (Model 805 MP) and combination Ag/AgCl probe were then placed into the paste/slurry and the pH measured +/- 0.2 pH units. Water temperature was measured by the multimeter system used for pH measurement and soil temperatures were measured with a direct temperature probe inserted to a depth of 30 cm bgs.

Oxidation-reduction potential (ORP)/REDOX probes were constructed as in Vepraskas and Bouma (1976) by soldering 1.25 cm platinum wire (20 gauge) to copper wire (12 gauge). The copper wire was sealed inside a 0.67 cm PVC pipe using epoxy (Lucite Corp.) to make the probes watertight and to seal the platinum to copper junction in epoxy. REDOX potentials were measured using a digital multi-meter (Craftsman Model 82082).

A standard ferrous-ferric solution for ORP measurements referred to as a "poised" solution (Light 1972) was prepared by dissolving 39.21g/L ferrous ammonium sulfate, 48.22g/L ferric ammonium sulfate and 56.2 ml/L concentrated H₂SO₄. When measuring the potential of the platinum electrode versus the Ag, AgCl reference electrode with 4.0 M KCl filling solution, the expected EMF for this solution was +475 mV at 25 °C. Probes that did not come within +/- 30 mv of this value, or within +/- 10 mv of each other, were not used. Probes were checked prior to each summers' measurements of ORP.

Field measurements were made generally at a depth of 30 cm corresponding to the mid-depth of marsh plant root zone. Several measurements within and around each plot border were collected during initial phases of collection (pre-dredge conditions) to verify that there were generally consistent readings between small spatial distances. This effort was not repeated during successive sampling rounds because of the difficulty in accomplishing coverage of all 94 plots during similar tidal conditions and within a reasonable time frame.

Marsh redox conditions were measured every year when below ground biomass was harvested during September or October in 2010 through 2013. In addition, in August 2012, a second round of measurements was collected. Because tidal conditions could potentially damage the probes, they were not left in place, rather each measurement was made at least 1 hr. into an ebb tide following insertion to the appropriate depth (30 cm) and after a 5-minute stabilization period. Results were recorded and corrected to Standard Hydrogen Equivalents by adding a +200 mv correction factor as in Light

(1972). Output of the electrodes was measured using a saturated Ag, AgCl electrode. Corrections for temperature and use of the Ag, AgCl electrode versus a standard hydrogen electrode were made by adding +244 mV to meter readings (this value was adjusted depending upon sediment temperatures) (Rabenhorst 2009).

4.2.3 Chemical Analyses

4.2.3.1 Soluble Sulfides and Total Sulfur

Total dissolved sulfide in pore water was analyzed using Hach Co. Method 8131 (Methylene Blue Method) with a range of detection from 0.01to 0.6 mg/l as S²⁻. This test works on the principal that hydrogen sulfide and acid-soluble metal sulfides react with N, N-dimethyl-p-phenyldiamine oxalate to form methylene blue under acidic conditions (Cline 1969). Methylene blue absorbance was measured at a wavelength of 665nm (1 cm light path) using a Hach spectrophotometer (Model DR2010).

If necessary, dilutions using distilled water were performed to obtain concentrations within the measurable range. Some unaccounted sulfide loss may have occurred during dilution and storage. Samples were allowed to settle for a 30-minute period prior to analysis and aliquots were taken from the top 5 cm of each sample bag. No separation for dissolved or insoluble sulfide determination was performed because of the difficulty presented by manual filtration. In deference to these laboratory constraints, the samples may not represent absolute sulfide concentration, but were intended for relative comparisons across piezometer locations as all samples were treated alike. Field duplicates were analyzed at a frequency of 1 duplicate per 20 samples and relative percent difference was calculated for each duplicate pair. Blank samples consisting of

distilled water were also analyzed at a 1 to 20 frequency. Unfortunately, standard addition to determine accuracy of the laboratory technique was not performed due to the difficulty in preparing a stable sulfide standard for titration in the field. The Hach Company documentation claims a standard deviation of ± -0.003 mg/l S²⁻ in their test laboratory.

Thirty-three soil samples from randomly selected locations and depths ranging from 15 to 46 cm (from all years) were analyzed for total sulfur at the Marine Biological Laboratory, Ecosystems Laboratory in Woods Hole, Massachusetts. Sample depths corresponded to the root zone and the depth of redox probe insertion. Samples were ground to fine particle size, weighed; combusted at 1350°C in a LECO S632 total sulfur analyzer, and the total amounts of SO₂ quantified using coulometry. Anthracite standards (ranging from 0.38 to 3.1% S) were used as calibration samples and run at a frequency of 1 per 10 samples. Field duplicate samples were run at a frequency of 1 per 20. Results were reported as percent total sulfur.

4.2.3.2 Ammonia

A modification of the salicylate method (Bower and Holm-Hansen 1980) was used to determine both high and low pore water concentration ranges of NH₄⁺. The high range ammonia test (Hach Co. Method 10031) measured concentrations up to 50.0 mg/l NH₃-N, while Hach Co. Method 8155 was used for low concentration samples up to 0.5 mg/l. Any necessary dilutions were prepared using nanopure water. Both methods are appropriate for a seawater matrix and work on the principle that ammonia compounds will combine with chlorine to form monochloramine. This in turn reacts with salicylate to form 5-aminosalicylate. In the presence of nitroprusside catalyst, the 5-aminosalicylate is

oxidized to form a blue color that is masked by excess reagent to give a green colored solution measured at a wavelength of 655 nm. Field duplicates, standard addition and blank samples were measured at a frequency of 1 per every 20 samples analyzed. Standard addition to determine accuracy of the technique was performed using 200 μ l additions of a 10 mg/L NH₃-N standard solution purchased from the Hach Company.

4.2.3.3 Orthophosphate

Hach Co. Method 8048 PhosVer 3 was used to analyze for reactive phosphorus in a range from 0.01 to 5.0 mg/l PO_4^{3-} . Sample pH values were within the neutral range (6 to 8 pH), therefore, no pH adjustment was necessary prior to analysis of orthophosphate (reactive phosphorus). Phosphate-free detergents were used when cleaning glassware, and sample Whirl pack bags were certified as phosphate-free. The method used involved the reaction of orthophosphate with molybdate in acid medium to produce a phosphomolybdate complex that was subsequently reduced by ascorbic acid yielding a blue color due to molybdenum. Absorbance was measured at 890 nm. Field duplicates, standard addition (1.0 mg/L PO_4^{3-} standard Hach Co.), and blank samples were measured at a frequency of 1 per every 20 samples analyzed for quality control.

4.2.4 Statistical Analyses

As in prior chapters, summary statistics are presented for each analyte and residuals were evaluated for normalcy and sample populations for homogeneity of variance across years in order to use classical statistical testing (ANOVA) and mean separation (Tukey's HSD) across years. Temperature and pH data were merely presented as ranges and not subject to statistical testing. Where appropriate, pore water results from

plots containing >25% cover *S. alterniflora* plots were considered across years, and results from higher elevation plots were evaluated in comparison to lower elevation plots.

4.2.5 Study Limitations

The relative percent difference (RPD) of field duplicate samples (grand mean, n=16) for pore water sulfide duplicate samples obtained in the field was 38%. The sulfide field duplication results do not reflect a high degree of reproducibility, and this likely indicates possible entrainment of differing amounts of suspended solids containing insoluble sulfur or changes in indicator status due to warming and oxygen shifts within the sample. It was not appropriate to compare specific sulfide concentrations with other study results or spatially across the marsh, yet it was useful to look at marsh wide trends across years as all samples were treated similarly. Laboratory reproducibility was acceptable at +/- 8% for sulfide analysis. RPDs for ammonium and orthophosphate are considered adequate for comparison to other studies, as well as across the marsh platform and between years (+/- 5% laboratory RPDs).

4.3 Results

4.3.1 Surface Water

Results from sampling surface waters from 2004 through 2013 are tabulated in Table 4.1. Surface feeder streams had little to no influent nutrient, chlorophyll or solids content and are not represented. To provide a more meaningful summary of the eight years and 20 rounds of data, means were calculated across all three inner marsh locations

EVH03, EVH04, and EVH05 (Figure 2.1) (as most representative of marsh water quality) and compared across years.

In general, the salinity, pH, total dissolved solids and conductivity measurements increased from 2010 to 2013, most likely reflecting greater flushing with seawater. Chlorophyll (a) also increased by 2 to 3 orders of magnitude in the most recent 4 years as compared to the first 4 years of monitoring. However, this may be due to differences between laboratory measurements (2003-2007) versus in situ YSI probe measurements (2010-2013). Dissolved oxygen appears to have been relatively stable across years, with the exception of a marked increase following winter 2003 dredging. Differences in weather conditions (wind/wave, light/temperature and precipitation) during sampling may have influenced measurements, however, because canoe/kayak access was required, sampling was not performed during adverse weather or Beaufort conditions greater than 2 Force (light breeze with no visible breaking wave crests).

Nitrogen and phosphorous were analyzed by SMAST from 2003-2005, and in 2007 in order to classify the eutrophication status of the marsh which was considered to have a poor to moderate Bay Health Index (School of Marine Science and Technology-SMAST 2005) depending upon the spatial position of the sampling. Due to expense, these analyses were not included for the ongoing FEM monitoring program because the nutrient related water quality parameters had not changed over the 4 year time period. However, from 2010 to 2012 it was observed that the southern most second order creek had visible opaqueness (possibly algal bloom), and it was learned that a failing septic system (proximate to PZ76 location) and located approximately 30m from Salt Marsh Lane creek was replaced in either late 2012 or early 2013.

Table 4.1. Water quality summary for inner Ellisville Marsh locations EVH03,EVH04, and EVH05 collected from 2003 through 2013.

		2003	2004**	2005	2007	2010	2011**	2012	2013
Parameter	(units)								
Dissolved Oxygen	mg/L	6	8.2	8.9	7.2	6.6	6.2	6.4	7.5
Salinity	psu	28.1	28.3	26.2	23.6	29.8	24.8	28.8	30.7
рН	Range	NA	NA	NA	NA	7.4-7.6	6.1-7.4	5.7-7.8	7.8-8.0
Conductivity	uS/cm	NA	NA	NA	NA	33581	36606	44408	47576
Turbidity	NTU	NA	NA	NA	NA	2	3.5	2.6	1.9
Total Dissolved Solids	mg/L	NA	NA	NA	NA	21.81	23.73	28.79	30.91
Total Suspended Soilds	mg/L	NA	NA	NA	NA	NA	NA	7106	9852
Secchi Disk Depth	m	DND	DND	DND	DND	DND	DND	DND	DND
Chlorophyll	mg/L	0.0153	0.0022	0.05	0.074	0.76	0.15	3.99	2.13

* All values are Means calculated across all summer monitoring events in a year & combined EVH03-05 loc **Years in bold are summer immediatley following inlet opening NA-Not Available, DND - Did Not Disappear

4.3.2 Pore Water

Unlike the surface water measurements, pore water salinity and pH did not vary significantly across years and ranged from 4 to 41 ppth (or practical salinity units – psu) and 5.3 to 8.1 pH units, respectively. Tables 4.2 and 4.3 present summary statistics for ammonia and orthophosphate, as well as pore water sulfide concentrations and redox potential (all collected from depths ranging from 20 to 46 cm bgs). Pore water ammonia concentrations did not vary significantly across years. Contrastingly, orthophosphate concentrations declined considerably (ANOVA, Tukey HSD, p<0.05) the first summer following the dredging event. Pore water sulfide results were positively correlated with NH_4^+ levels in 2011 (r=0.52), although the field reproducibility of the sulfide results was not acceptable for all sample duplicates as previously stated (Study Limitations).

Table 4.2. Pore water NH_4^+ (NH4) and PO_4^{3-} (PO43) summary statistics for plots containing >25% percent cover *Spartina alterniflora*. Numerical designation in column heading refers to year of collection (e.g. 10 = 2010). Columns with the same letters are not significantly different (ANOVA, Tukey's HSD, p<0.05).

units	NH410 (mg/L)	NH411 (mg/L)	NH412 (mg/L)	NH413 (mg/L)	PO4310 (mg/L)	PO4311 (mg/L)	PO4312 (mg/L)	PO4313 (mg/L)
sig. diff.	а	а	а	а	а	b	b	b
nobs	56	56	56	56	56	56	56	56
min	0.03	0	0	0	0.1	0.02	0.02	0.65
max	22.2	23.2	27.2	13.13	127	4	8	11.28
mean	3.135	1.825	2.778	2.363	8.916	1.061	1.426	2.629
median	0.425	0.6	0.9	1.55	1.085	1.04	1.085	1.995
sd	5.124	3.36	4.62	2.543	22.227	0.728	1.396	1.796
se	0.685	0.449	0.617	0.34	2.97	0.097	0.187	0.24
cv	163.432	184.145	166.279	107.628	249.28	68.609	97.909	68.295

Table 4.3. Pore water sulfide (S) and oxidation-reduction potential (REDOX) summary statistics for plots containing > 25% percent cover *Spartina alterniflora*. Columns with the same letters are not significantly different (ANOVA, Tukey's HSD, p<0.001). Sulfide porewater results failed QA/QC for duplicate replication.

units	S10 mg/L	S11 mg/L	S12 mg/L	S13 mg/L	REDOX10 Eh (mv)	REDOX11 Eh (mv)	REDOX12 Eh (mv)	REDOX13 Eh (mv)
sig.								
diff.	NA	NA	NA	NA	а	а	b	b
nobs	56	56	56	56	56	56	56	56
min	0.01	0.02	0.01	0.05	-202.00	-270.00	-60.00	-182.00
max	58.50	47.50	23.00	16.42	67.00	174.00	341.00	396.00
mean	4.35	6.05	2.11	1.65	-110.80	-61.52	128.38	147.57
median	0.23	0.37	0.14	0.45	-119.00	-101.00	113.00	130.50
sd	10.79	11.27	5.36	2.99	65.81	132.12	88.27	138.28
se	1.44	1.51	0.72	0.40	8.79	17.66	11.80	18.48
cv	247.81	186.37	253.61	180.93	-59.39	-214.77	68.76	93.70

4.3.3 Soil Chemistry

Mean oxidation-reduction potential (ORP) measurements (REDOX), collected

from depths 30 cm beneath ground surface, showed a dramatic reversal in charge (ANOVA, Tukey's HSD, $p = 2.26 \times 10^{-11}$) as measured the summer of 2012 and remained positive in 2013 (Table 4.3 and Figure 4.1). This redox finding corroborates the observed decline in sulfide pore water concentrations; at least the decreasing trend in soluble porewater sulfide appears consistent with what would be expected with more positive Eh readings. Most importantly, this finding coincides with the decline in mean hydroperiod observed the summer of 2012.



Figure 4.1. Oxidation-reduction potential (ORP) for marsh sediment, Ellisville Marsh, Plymouth, MA. Columns with the same letters are not significantly different (ANOVA, Tukey's HSD, p<0.001).

Percent sulfur levels in the soil samples ranged from 0.15 to 1.5%, with means from 0.47 to 1.1% across years. There was a significant increase (ANOVA, Tukey's HSD, p= 0.0092) in total sulfur concentration in samples from 2013 as compared to 2010, 2011, and 2012 levels.
In-situ soil pH values were slightly acidic ranging from 5.2 to 6.4 (+/- 0.2) pH units). Soil pH values determined after drying in the laboratory were very acidic to slightly acidic ranging from 2.3 to 5.7 (+/- 0.2) pH units. The increased acidity in laboratory samples was due to the re-hydration of dried soils containing high concentrations of SO_4^{2-} to form a soil paste that likely caused the formation of H₂SO₄ (Brady and Weil 1996).

4.4 Discussion

Both dredging events (winter 2003 and 2011) had the effect of re-suspending sediment from the marsh and mudflat surfaces. While not measured directly, observers of dredging activities noted visibly entrained black sediment leaving marsh with the newly released flow of water (author's personal communications with Paul Martino 2003, and Eric Cody 2011). Because this material might act in a short-term fashion to fertilize the marsh, an increase in NH_4^+ and PO_4^{3-} concentration within the pore water might be a reasonable expectation, as has been speculated by Valiela et al. (1974) as an explanation for height and biomass increases. Either this increase did not occur, was not detectable, or plant uptake/geochemical processes altered concentrations by the first summer following dredging. Pore water NH_4^+ levels across all years remained similar, while contrastingly PO_4^{3-} levels declined following dredging (ANOVA, p<0.001).

Phosphate is not typically a limiting nutrient for *S. alterniflora* (Valeila 1974; Gallagher et al. 1980). Howes et al. (1986) report that well drained sediments enhance uptake of phosphate by *S. alterniflora* roots, which in turn reduces concentrations in pore water. This might be the explanation for the substantial decrease in porewater PO_4^{3-} in

2011 following dredging. By 2013 however, pore water concentrations of PO_4^{3-} (2.6 mg/L, +/- 1.8) were higher than values reported for mean annual pore water concentrations from a well drained marsh (1.4 mg/L) analyzed using a similar ascorbic acid method (Stribling and Cornwell 2001). *S. alterniflora* plant tissue concentrations of phosphorus were slightly lower; 0.2% (+/- 0.02) from the well drained marsh discussed above and 0.3% (+/-0.05) for Ellisville Marsh.

Overall, the oxygenation status of the marsh appeared changed by the summer of 2012. The change was delayed by at most 18 months and at least 7 months behind the initial dredging event. The ORP showed a reversal in 2012 to positive conditions that relates well to the decrease in mean hydroperiod and the decrease in ADH activity between 2100 and 2012 conditions (Figure 4.1). ADH levels show a weak, although significant (p< 0.05) negative linear response to increasing REDOX values (Figures 4.2 and 4.3).



Figure 4.2. Comparison of ADH activity and REDOX conditions Ellisville Marsh, Plymouth, MA. ANOVA (mean +/- sd), bars shown with shared letters are not statistically different (p< 0.05).

This shift in ORP from negative to positive likely accompanies shifts in microbial communities and oxidation states of iron and sulfur, as well as other elements. Mineralization of carbon under anaerobic conditions is depressed as compared to aerobic conditions (Keiluweit et al. 2017, Thomas et al. 2019) and rapid reversal of redox conditions could shift respiration to aerobic processes increasing labile CO_2 and converting the salt marsh to a carbon source as opposed to sink.



Figure 4.3. Linear regression (p = 0.057, $r^2=0.13$) of *Spartina alterniflora* ADH activity to REDOX condition showing 95% confidence interval, Ellisville Marsh, Plymouth, MA.

Organisms that mediate the oxidation/reduction of sulfur containing compounds include the dissimilatory sulfate reducing bacteria and assimilatory sulfide oxidizers. Because dissimilatory sulfate reduction is strictly an anaerobic process, and since sediment conditions had become more aerobic in 2012, it was reasoned that the amount of H_2S and FeS_2 (less soluble than H_2S) might have declined in the more oxidized situation. It was also assumed that sulfate would be flushed from the soils with increased drainage. Mean concentrations of soil S did decline from 2010 to 2012, but then became higher than 2010 levels (0.67%) by 2013 (1.06%) (p = 0.0092). Assuming that contributions of sulfate to sediment from overlying seawater have remained the same across years, an increase in total sulfur in 2013 soils may indicate in essence a "recovery" to higher sulfur conditions; recovery in this sense meaning a sulfur concentration similar to a lower elevation marsh circumstance. It may also be that the $SO_4^{2^2}$ form of sulfur is becoming more concentrated in the sediment under more aerobic conditions because flushing time has reduced as compared to 2011 values. Unfortunately, the analysis of sulfur in soils did not provide further sulfur speciation.

In summary, the marsh surface water quality indicated a general shift to increasing levels of dissolved solids (reflecting dissolved solids in seawater), increasing dissolved oxygen following dredging, yet essentially no overall change in salinity within pore water. Orthophosphate within the pore water significantly declined with decreased hydroperiod, while sulfur content of soils were significantly higher in 2013 as compared to 2010. Dissolved sulfide levels in the porewater may show a declining trend across years (although this data did not meet strict quality assurance requirements) possibly meaning a decrease in sulfide toxicity. Increasing ORP was significantly correlated with decreasing activity of ADH in the *S. alterniflora* root samples and coincided with observed decreases in hydroperiod in 2012.

It appears that certain circumstances prior to summer of 2012 may have been critical to *S. alterniflora* productivity and to alteration of environmental conditions within Ellisville Marsh. This includes the statistically significant decrease in hydroperiod, below ground biomass and plant height productivity indicators, the shift to more positive oxidation-reduction state of the soils, and coincident lowered ADH activity. It is unlikely that the lack of hard freeze and snow pack in the fall and winter of 2011-2012 accounted for all of these coincident and varied types of changes. Further multivariate analysis (Chapter 6) of the relationships between the measured productivity and environmental factors may help to elucidate changes.

CHAPTER 5

MARSH SOIL PHYSICAL CHARACTERISTICS

5.1 Introduction

If allowed landward transgression, salt marshes are habitats continually undergoing succession. In their seminal paper on community structure, Hairston et al. (1960) describe how bogs and ponds accelerate their own demise because of slow rates of detritus decomposition leading to filling in of the resource. In a similar fashion, slow rates of decomposition within anaerobic salt marsh soil/sediment and/or increased deposition of organic and/or mineral materials, can lead to progressive accretion and conversion to upland; especially if landward expansion is possible (Redfield 1965). In essence, salt marshes are always maintaining a balance amongst forces that regulate the accumulation and loss of organic matter/inorganic sediment. Inadequate tidal flushing accelerates succession by failing to remove detritus/accumulated wrack debris, limiting oxygen, slowing rates of decomposition, and in the case of Ellisville Marsh, by shifting salinities to those preferred by more brackish or freshwater, and often more invasive species such as *Phragmites australis*.

In Ellisville Marsh mineral sediment deposition is ongoing. Sandbars are visible in the marsh interior in aerial photographs (Figures 1.4 and 1.5) and sand has been observed to routinely "float" on the quiescent water surface into the marsh (Figure 5.1). The percent cover by the sand classification increased in 2018 by 42% from 2008 levels (Chapter 3). Storms also contribute large amounts of sand to the marsh inlet while eroding beachfront (Chapter 1). These sands have potential for interior migration. The

sandbars form at bends in the tidal channel, and could cause some interior obstruction to flow in secondary channels. Ultimately, this might limit finer sediment contributions to interior portions of the marsh. As the primary tidal channel becomes more constricted by sand and hydraulic inefficiencies result, interior obstructions will likely increase, causing less overall sediment to be delivered to the interior sections of the marsh, slowing accretion, and decreasing elevation gains that would help combat water level rise.



Figure 5.1. Sand flotillas entering Ellisville Marsh, Plymouth, MA, July 17, 2012.

5.1.1 Objectives

To understand the spatial distribution of the sediment/soil materials present and ongoing surficial deposits within the marsh, soil profiles were logged and samples collected for analysis of organic matter and particle size (percent clay, silt and sand fractions). Accretion plates were also used to collect materials across seasons in order to determine one year's worth of accumulation. Because change in soil makeup was assumed to be a slow process, the intent was not to document changes in soil profiles over time or enable pre- and post-dredge comparisons. Rather, the question posed was how do soil properties relate to environmental variables that were previously shown to be important to *S. alterniflora* growth, such as hydroperiod. In addition, the accretion rate could be used to provide input to a simulation model used to determine the resilience of the marsh system under hypothetical/real sea level increases.

5.2 Methods and Materials

5.2.1 Sample Collection and Handling

Soil borings were conducted in a random fashion by selection of 8 to 10 plot locations per year using a random number generator, without substitution, so that by the end of the 4-year study/permit period, 37 plot/piezometer locations were sampled. The number of soil samples taken per location depended upon whether there were obvious defining vertical differences in the soil profile. On average, two samples per borehole location were collected for 68 soil samples overall. Boreholes were dug with a corer and spade and soil was laid out in progression so that the profile could be viewed properly without caving/water intrusion interference. Approximately 100 to 500g of soil from each location were placed into separately labeled plastic bags and brought to the field laboratory within 1 hour for texturing and color characterization. This was followed by freezing each sample to prevent biological decomposition prior to drying and analysis for physical properties in the University of Massachusetts Amherst Soils Laboratory.

5.2.2 Soils Classification

Abbreviated soil profiles (ending 61 cm deep into the marsh sediment) were prepared using methods as outlined in the Soil Survey Manual, Soil Survey Division Staff (1993). A maximum depth of 61 cm was used because this was generally the maximum rooting depth or the depth where groundwater infiltration prevented soil column exposure. Immediate appearance and wet soil color was determined using ranges for hue, value, and chroma, as supplied by the Munsell Color Soil Color Charts (2000). Soil texture, structure, consistency and redox features were described afterwards on soil profile forms. Infrequently the qualitative presence of reduced iron was also ascertained by assessing the soil sample with α , α '- dipiridyl and looking for the characteristic pink color indicating reduced iron. Because of the dark nature of the marsh soils, a paper filter and soil smear was used in order to visualize the color. Examination of the exterior of rhizomes and roots for areas of iron oxide staining also provide a qualitative indication of iron form present.

5.2.3 Organic Matter Analysis

Organic matter was measured using the loss on ignition technique as outlined in Methods for Soil Analysis, Part 2 (Black et al. 1965) and as described further by Nelson and Sommers (1982). Previously ground and oven dried (103° C, until constant weight achieved) soil was combusted in a muffle furnace at 500° C for 16 hrs., allowed to cool, and then reweighed. The weight lost during ignition was then considered to be equal to the mass of organic matter. Results were then reported as percent by weight organic matter after taking initial sample weights into consideration. As discussed by Nelson and Sommers (1982) subjecting soils to this temperature results in overestimation of organic

matter because hydrated aluminosilicates lose structural water and carbonate minerals can be decomposed.

Another technique used for determining the contribution of refractory organic material (such as peat) to underlying salt marsh soils involved the determination of stable plant fragment content (SPFC) as described by Twohig and Stolt (2011). Samples for the SPFC were air dried and then passed through a 2 mm sieve by shaking. Plant materials that remained on top of the sieve screen and did not pass through were weighed after oven drying at 60 °C for 24 hrs. Results were reported as a percent of the initial air-dried sample and corresponded to the % of refractory material.

5.2.4 Soil Bulk Density

Soil bulk density was determined in 2014 in the top 20 to 30 cm at six locations using the core method (Blake 1965). A 60 ml syringe with its top conical section removed was pushed into the soil, rocked slightly once inserted to its full extent and removed with soil core intact. Using the volume gradation along the syringe barrel, the volume of the core was estimated by gently pushing the soil core to the end of the syringe using the plunger, trimming the end until flush, and then making note of the cylinder volume occupied by the soil. Material was then pushed into tared weigh boats and dried for 24 hours at 105 °C. The weight of the soil after drying divided by the volume yielded the bulk density in g/cm³.

5.2.5 Particle Size Analysis

Particle size determination for each soil sample began by grinding in a mortar and separation of the gravel using a 2 mm (No.10) sieve. Those samples that had been

identified by the loss on ignition procedure as having organic matter percentage greater than or equal to 8% were first treated by removal of organic matter with 30% H₂O₂ digestion and then heating at 50-60° C for 1 hr. prior to undergoing particle size analysis. This was necessary because of known interferences of organic matter with clay and silt separation (Day 1965). Following organic matter removal, procedures included slight modifications of those described by Day (1965). A 25 g sub-sample of the ground material was used for analysis and placed into a 250 ml Erlenmeyer flask. Twenty-five ml 5% sodium hexametaphosphate (Calgon detergent) was added to the flask containing the sample; the flask was stoppered and then shaken on a shaker table at low setting for 1 hr. After shaking, the sample was washed out of the flask through a 270µm sieve to remove the sand fraction. This material was reserved for later separation by washing the material remaining on top of the sieve into a tared 100 ml beaker. The materials passing through the sieve were collected in a 1200 ml graduated cylinder/settling column, the volume brought to the 1130 ml mark with distilled water.

For the silt fraction determination, a metal plunger was pushed into the column in order to suspend all materials and after a 10 second time period, a 25 ml aliquot was withdrawn from the cylinder from a depth corresponding to 10 cm along the pipette length. This aliquot was placed into previously tared 50 ml beaker labeled T1 and oven dried at 100° C overnight. The remaining water column was allowed to settle for 7.5 hours, then a second 25 ml aliquot for the 10 cm depth was placed into a tared 50 ml beaker (labeled T2) and dried overnight at 100° C. This material constituted the clay fraction of the sample. The previously collected sand fraction was then sieved through a

series of sieves representing sand sizes ranging from 0.002 mm to 2 mm and the material retained and/or passing through each sieve were weighed (+/-0.01 g).

5.2.6 Sediment Accretion Plates and Marker Horizons

In 2012, 21 accretion plates (rough-surface, ceramic floor tiles) ranging in size from 117 to 420 cm² were placed randomly on the surface of 21 plots across the marsh platform. Tiles were nestled under the vegetation when present and remained in place where deposited materials accumulated, decomposed, compacted or eroded over the span of approximately one year. At the end of the elapsed time, plates were collected and materials adhering to each surface were measured for thickness, scraped into a weigh boat, dried at 100° C and weighed (+/- 0.1 g). Following this, organic matter and particle size were determined according to methods described in Sections 5.2.3, with one exception. The combined silt and clay fraction contribution was calculated by subtracting the percent dry weight of sand plus organic matter from 100.

Surface accretion using a different technique was also attempted by spreading fine white feldspar across four 0.1 m² locations across the marsh and waiting 2 years before measuring accretion. Locations represented one high marsh marker horizon (MH) (13), two intermediate height locations (MH9 and MH26), and one low region (MH72). Marker horizon plots were 1 m north of each corresponding piezometer (PZ9, PZ13, PZ26, and PZ72). In July 2016, the marker horizons could not be located despite painstaking location having been used, so no estimates of accretion were available using this technique.

5.2.7 Statistical Analyses

Data were interpreted using a number of different approaches. As stated previously, the intent was not to show changes across time. Instead, the goal was to examine spatial difference in soil properties with depth and across the marsh and possibly relate these differences to those amongst and between other root-zone and pore water variables. The statistical packages "aqp" 1.6.0 and "geoR" through the 2013 R Core Development Team, R 3.1.0 (Beaudette et al. 2012) were used to obtain a spatial representation of the physical properties of the soil samples. Median values with depth were calculated by aggregating across multiple slices of all profiles. This required normalization for differing horizon depths and number of contributing samples. Samples representing the upper 30 cm of the root zone were carried forward for use in a constrained ordination (Chapter 6) evaluation of soil properties in relationship to other below ground environmental variables for the subset of soil sampling locations (McCune and Grace, 2002). This allowed further tailoring of parameters for use in simulation modeling (Chapter 6).

5.2.8 Study Limitations

Soil samples for organic matter and particle size analysis were collected at a frequency of approximately 10 locations per year and provided only a subset of the total plot number across the marsh. They also represent both high and low marsh elevations and therefore are not all relatable to the *S. alterniflora* population, but can be used to describe situations representing the whole marsh. Because locations were selected randomly some areas of the marsh were not equally represented. Accretion rates reflect

2012 - 2013 conditions only and bulk density measurements represent 2014 conditions only.

5.3 Results

5.3.1 Spatial Representation of Soil Properties

The depth profiles of median percent organic matter, sand, silt and clay are shown in Figures 5.2 through 5.4 for high elevation plots, low elevation plots, and all sampled plots, respectively.







Figure 5.3. Median percent concentrations sand, silt, clay and organic matter (om) with depth for low elevation plots (>25% *S. alterniflora* percent cover) bounded by 25th and 75th percentiles.



Figure 5.4. Median percent concentrations sand, silt, clay and organic matter (om) with depth for all sampled plots bounded by 25th and 75th percentiles.

The median sand concentration for all plots appears highest (30 to 50 %) in the

rooting zone mid-depth (20 to 60 cm) interval, while organic matter (15 to 20%) and silt

(40 to 70%) are highest in the upper rooting zone (20 cm). The median clay distribution was generally near 15 % throughout the profile intervals, except for a slight increase to 20 % observed in high elevation plots at approximately 10 cm. Contrary to expectations, the low elevation median levels of organic matter (15-20%), and by extension organic carbon (using a 1.72 conversion factor), do not quite classify the median soil as a histosol (12-18% as organic carbon with clay content between 0 to 60%), but rather as a mucky mineral soil (Richardson and Vepraskas 2001). There were however several instances where individual samples had organic matter content greater than 25% with fibrous root matter and visible peat greater than 16 inches in thickness which defines an organic soil (Tiner 1999).

Visual representations of soil boring results give the vertical and horizontal distributions of soil organic matter; sand, clay and silt across transect and plot locations in a north to south direction are provided in Appendix B. All locations (with the exception of PZ01-03, PZ05, PZ14, PZ24-25, PZ44, PZ49, PZ76) are considered low elevation locations and are classified as tidal soils because they are either flooded daily or have sulfidic materials within 5 inches of the surface as characterized by the rotten egg odor of hydrogen sulfide (NE Hydric Soils Technical Committee 2004). The master horizons, O and A, are represented most frequently, with a B-horizon found in only one location at PZ76 (close to marsh margin). The A horizon was characterized by a mixture of partially degraded organic matter, roots and intermixed mineral soils. The O horizon was assigned if the predominant material present was judged to be solely organic matter during textural classification.

Transects most landward (T04 and T05) (furthest perpendicular distance from the shoreline) were under represented by the random selection process as compared to other transects and so were combined for visual presentation. The reader is cautioned to refer to legend for each separate figure because the divergent spectrum shown is counterintuitive when interpreting organic matter. The darker blue/green colors represent low concentrations, the darker brown colors represent highest concentrations, and the thicknesses without color represent the midrange concentrations.

Initial visual impressions are that the highest silt and clay concentrations were found in the most landward locations, while the highest sand and lowest organic matter concentrations were found in locations most seaward or nearest to ditches, creeks, or channels. The organic matter within the marsh appears to be lowest in the locations where sand concentrations are highest. Figures 5.5 and 5.6 depict the spatial distribution of particle sizes across the marsh for depths inclusive of the rooting zone (15 to 30 cm).

It was reasoned that the sandier the sediment material in the rooting zone depth, the more likely that redox conditions would be high due to the larger pore space and that the closer distance to a water conveyance structure, the larger the amount of sand deposited. In order to examine these assumptions, a Pearson's test for correlation assessed average redox conditions across all four years of measurement and found them inversely correlated, with both rooting zone organic matter and clay concentrations (p=0.056, r = -0.32 and p=0.031, r= -0.36, respectively). However, the percent sand and silt in the rooting zone did not correlate with the distance to nearest flow conveyance structure (ditch, creek, or channel abbreviated as DTD) nor did they correlate with average redox measurements.

5.3.2 Marsh Accretion and Bulk Density

On average, 16 % of the material accumulated (by weight) on accretion plates was organic, 64 % was sand and 21 % were silts and/or clays (by weight using subtraction) amounting to an 85% fraction being inorganic. This compares well to the 80% fraction found by Vogel et al. (1996). Distribution of deposition varied, with lower elevation plates accreting on average 0.471 g/cm²/year and higher elevation plates accreting less at 0.025 g/cm²/year on average. Average thickness of the sediment layer (in wet state) on low elevation plates ranged from 8 to 16.4 mm/year (+/- 1 mm), while high elevation plates did not have measurable thicknesses (< 1.0 mm) even after the year period. As stated previously, marker horizons were either not present two years after placement or could not be found. Refractory organic material, as represented by the analysis of SPFC, had mean results of 0.23%, 0.19% and 0.08% for 2010, 2011, and 2012, respectively. The mean dry bulk density for Ellisville Marsh was determined to be 0.5 g/cm³ reflecting the lower organic matter content in the upper 20-30 cm.



GPSW



Figure 5.5. Horizontal distribution of percent organic matter (top panel) and percent sand (bottom panel) for sampled plots as identified by GPS location in decimals north (N) and west (W). Results are inclusive of 15 to 30 cm depth in rooting zone only.





Figure 5.6. Horizontal distribution of percent silt (top panel) and percent clay (bottom panel) for sampled plots as identified by GPS location in decimals north (N) and west (W). Results are inclusive of 15 to 30 cm depth in rooting zone only.

5.4 Discussion

Others have reported bulk densities in the top 50 cm of depth ranging from 0.2 to 1.0 g/cm³ for northern salt marshes (Chmura and Hung 2004; Bricker-Urso et. al. 1989; Ganju et al. 2017, Llewellyn 2008). This range encompasses the bulk density (0.5 g/cm³) obtained for Ellisville Marsh. The higher bulk density implies that compaction of sediment due to ice loading or head of water may not have large effects on subsidence due to pressure when void spaces are full of water, and furthermore, rebound following drainage of any compressed peat materials is likely in New England marshes (Argow et al. 2006).

Llewellyn (2008) used a dry bulk density 0.33 g/cm³ representative of reference marsh sediment materials; this report of 15-mm/yr. thickness of accumulation is similar to the maximum value measured directly for Ellisville Marsh (16.4 mm for a nearly one year period). Others have reported mean low marsh accumulation rates of 2.7 to 4.44 mm/yr. (Bricker-Urso et al.1989, Vogel et al. 1996). Using Surface Elevation Tables (SETs), micro-tidal marshes in New England have surface elevation mean increases reported to be 2.3 mm/yr. (Burdick and Peter 2015). These were collected over the past 20 years representing a more inclusive estimation accounting for marsh subsidence as well as elevation gain.

Ellisville Marsh's mineral sediment supply is primarily from tidal and not upstream sources, as evident by slow feeder stream flow rates (Chapter 2) with low solids content (Chapter 4). Ocean events that enhance sediment transport such as tidal surge, and wind and wave action, must be the primary contributors to the sediment in the marsh. Even during quiescent periods, suspended solids (mean concentration 6.65 g/L (+/- 0.01

g) of coarse, medium and fine sands per hour) were measured floating in with incoming tides (Figure 5.1), the sand likely held up on the surface by salt bonding between fresh and saline layers (Brady and Weil 1996). If just a small portion of this sand actually is intercepted by creek walls or plant structures and allowed to settle on the marsh platform, it alone could account for the high percentage of sand accretion, acting in effect as thinlayer sand placement/deposition. Because Ellisville Marsh has little to no area to migrate landward, this propensity towards accretion will become increasingly more important in maintaining this resource in a balance between conversion to open water/lower elevation circumstances. Drastic increases in flow, like those brought about by dredging or storm breaches of the barrier spit and associated instantaneous increases in water level, can accelerate creek bank erosion and channel widening (Figure 5.7) that counteract the positive effects of sediment deposition (Fagherazzi et al. 2004, 2012, Ganju et al. 2017, 2019, Morris and Currin 2013). It is likely that a balance between the two situations has existed over time, and at least partially, accounting for the sustainability of this platform to date.

The median percent by weight concentration of sand within the top layers of the fixed soil profile locations and that accumulated on the accretion plates as expected are not equivalent. Because the accretion plates were fewer in number than the number of soil profiles and there were extremes in accumulation even across low elevation plates, the concentrations of sand were compared to the medians in the profiles. It may be that the accretion plates registered such high sand concentrations given the phenomenon of sand flotation during quiescent periods and considering the increased storm activity during the yearlong accretion period (Applied Coastal Engineering 2017).



Figure 5.7. Calving of channel banks following increased winter flow events (Photographed July 2012) looking west from channel inlet in Ellisville Marsh, Plymouth, MA.

Surface layers of aggregated soil profiles showed a median sand percentage in the top 30 cm peaking at approximately 50% in high elevation plots, as opposed to the higher mean accretion residual sand content of 64% in the top few centimeters of deposition. These percentages, when supplemented with the inorganic silt and clay fraction, are similar to those described by Vogel et al. (1996), where 80% was the total inorganic component.

CHAPTER 6

SYNTHESIS AND MODELING

6.1 Introduction

There are many numeric models available for the study of saltmarsh processes and platforms (Fagherazzi et al. 2012), particularly as regards to these systems' resilience in the face of ongoing sea level rise, storm surge and other hydrology changes. Models range from empirical and physical models of a single point, a single transect of elevation, two dimensions across a marsh platform, and landscape scale coastline models. The more intensive models cover results of complex biological and geomorphic processes (UVVI, DELFT3d), while others simply consider basin fill (salt marsh species biomass keeping pace with increasing water elevation) dynamics, also known as "bathtub" type models (e.g. SLAMM) with some modifications made for accretion.

Criticisms of current mechanistic models include, infrequent temporal sampling, single point measurements of elevation underestimating spatial variability, and neglecting open water conversion and the impact this has on cascading lateral marsh erosion (Fagherazzi et al. 2012; Alizad et al. 2016; Mudd et al. 2009; Ganju et al. 2017; Smith et al. 2017). The time frame of this Ellisville Marsh study (10 years overall, with 4 years of intense data collection), and its spatial coverage for inundation measurement and percent cover at both plot and landscape scales, allows consideration of marsh resilience by taking advantage of the physical, chemical and biological processes observed over the longer-term.

6.2 Conceptual Model

A conceptual framework for modeling approaches was formulated a priori (Figure 1.7) to help describe productivity response relationships of the *Spartina alterniflora* population in Ellisville Marsh to hydroperiod fluctuations, as well as to other environmental and climatological variables (Figure 1.7). The ultimate goal being to help answer the question as to whether it is healthier for the salt marsh grasses to have the inlet periodically opened by dredging (post dredge condition), or to leave the grasses to undergo the periodic oscillations of a natural coastal pond with limited communication with Cape Cod Bay (pre-dredge condition). Both endpoints of the pendulum arc have their own unique implications.

The conceptual framework for marsh function describes the interacting forces and processes that influence the ultimate productivity of *S. alterniflora*. It addresses three state variables undergoing change (pore water, soil/sediment, and the *S. alterniflora* plant itself), two forcing functions (large arrows) of climate and tidal exchange, four process functions (small numbered boxes 1- 4), and twenty measured variables (boxed variables adjacent to each bold-boxed state variable) that were assessed. Functions 1 through 4 are process functions prescribed by variation in tidal amplitude (and by default hydroperiod) and climate fluctuations in precipitation, temperature, growing degree-days and light conditions necessary for growth of the plant. These in turn influence nutrient and oxygen content in pore water and sediment available for plant root uptake and microbial degradation, the amount of sediment available for deposition, and rates of photosynthesis.

6.3 Objectives

Using the data from this study, four modeling approaches were used in an attempt to understand the influences of dredging on marsh resilience, with emphasis on how resilience relates to the productivity of *S. alterniflora*. The approaches are:

1). To determine vertical accretion, the use of the numerical Morris (©2010) Ver. 5.4 Marsh Equilibrium Model was employed under pre and post dredge sea level and tidal range conditions using Ellisville Marsh derived data as inputs. This vertical resilience information was then combined with the previous UVVI assessment for horizontal resilience (Chapter 3) to obtain an overall two-dimensional estimate of Ellisville Marsh resilience. This model addresses the "Soil/Sediment" and "Plant" state variables and uses several of the Ellisville Marsh measured variables (e.g. BGB, ABGB, TSS, Stable Plant Fragment Content, bulk density and accretion rate) in relation to tidal range depth (D).

2.) Fluctuations in hydroperiod result from episodic dredge operations or from events like storm surge (as described in Chapter 1), as well as those induced less dramatically from progressive sea level rise (currently reported as 0.282 +/- 0.0.016 cm/yr. per Boston, Massachusetts buoy # 8443970, April 2019). Because it was shown that the measured increase in hydroperiod within Ellisville Marsh did not correspond directly to the changes in tidal range brought about by dredging (Chapter 2), reliance on using tidal range (D) as the principal driver of salt marsh restoration and/or *S. alterniflora* growth limits may be remiss, particularly in situations where the interior marsh microtopography, creek/channel architecture or soil pore space structure/soil particle sizes prohibit immediate and complete communication with tidal flow. Consideration of any

lag in hydroperiod duration over the marsh's surface may be the more influential to plant growth and is warranted. Therefore, it was used as the variable replacing typical tidal range or depth of water (D) estimates to evaluate this study's data (after discernment that units would be interchangeable).

3.) Next, in an attempt to explain why re-colonization of bare mudflat (3 hectares in the marsh's western portion) by *S. alterniflora* did not occur from 2011-2013, or for that matter for 9 years post dredging, assessment of two fitness measure proxies, calculated photosynthesis (PN) and plant height (PH) for *S. alterniflora*, were considered along with the density measure of below ground biomass (BGB) in order to evaluate the possible existence of an Allee effect that might negatively influence re-establishment. An Allee effect is considered a positive relationship between individual fitness and any type of density measure (Stephens et al. 1999). This effectively is a consideration of the "Plant" state variable's productivity responses (Figure 1.7).

On the West coast of the United States and in China, *S. alterniflora* is considered an invasive plant (Davis et al.; 2004; Zhu et al.; 2019), yet it too has certain optimal growth rates and has been known to be demonstrate a weak density dependent Allee effect during colonization (Buckley and Metcalf 2006; Davis et al., 2004). In areas of low population density, plants producing seed (representing potential increase in fitness for the population) may effectively lose the seed until rhizomatous growth brings the plants into close enough contact that any produced seed will be trapped (Davis et al., 2004). This is the opposite of the Allee effect that describes an increase in fitness with increase in density. Because taller *S. alterniflora* plants are more likely to undergo flowering and possess inflorescences (Valiela et al. 1978; Ryan et al. 2007; and Crosby et

al. 2015), and because PH is significantly linearly related to inundation period (HP) (Chapter 3), it was reasoned that plant height might serve as a proxy for seed set and this would in turn, serve as a fitness component to be described as it relates to the density measure of below ground biomass (BGB). Here BGB is important for the plant's perennial outcome, and is a primary deterrent to erosion and critical to accretion. Without plant root stabilization, the plant may be uprooted, and surrounding sediment eroded during fast tidal flows and high wave energy.

4.) Finally, a comparison was made of the best fit non-parametric linear models of productivity as they relates to a suite of environmental covariates and certain climate variables (Figure 1.7), with the goal of using a non-destructive, sentinel parameter with minimal associated measurement/process error, that in turn is related to the hydroperiod status of the salt marsh.

6.4 Modeling Methods

6.4.1 Vertical Accretion Modeling

Modeling by Mudd et al. (2009) and Morris et al. (1986, 2002, 2016) uses an overall mass conservation approach with above ground biomass per unit area (B) as the changing dependent variable over time and depth (D). Both below and aboveground fractions are represented as live below ground (dry root/rhizome biomass) and live above ground (dry leaf and shoot biomass). The root and rhizome to shoot ratio (RRTS) along with belowground biomass turnover estimates and peak standing biomass and all are incorporated into a phenomenological quadratic determination of the dependent *S*. *alterniflora* response variable (B) optimum in relation to depth of water (D).

Increases and decreases in *S. alterniflora* biomass (growth and death) relate to increases and decreases in marsh surface elevation, wrack and sediment deposition from tidal flow, and below ground biomass turnover caused by microbial degradation. The greater the depth or longer a point or an area is under water per tidal inundation, the greater time allowed for materials to settle, meaning greater amounts of accretion with heavier particles deposited before lighter weight fines and organic material. If this deposition exceeds subsidence and erosion, then elevation builds. Longer periods of water pressure exerting downward force on the sediment surface can also cause greater amounts of compaction of peat/below ground biomass if minimal or no live root network is present. Increasing the nitrogen and oxygen available for microbial processes can accelerate decomposition of peat further contributing to subsidence (Deegan et al. 2013, Wigand et al. 2018, and Morris 2002). In these cases, despite sedimentation, there is less to resist subsidence and more chance of erosive tides causing scouring of any deposited materials.

Finally, the greater the density (and complexity of plant structure) of above ground vegetation, the greater chance that materials (wrack and sediment) transported by tides will become trapped and contribute to accretion and the larger the detritus/wrack load when the plants senesce and decay every year. Ultimately, if accretion does not keep pace or outpace any rise in water level within the marsh, over time the elevation of the marsh platform will decline to the point where it is submerged so often that vegetation will not grow and an intertidal mudflat is formed as likely the case for Ellisville Marsh from 1987 through 2010.

Process function [4] (Figure 1.7) is the result of accumulation minus subsidence. Accumulation is the result of deposition of both mineral and organic material accretion minus their erosion. Accretion in a salt marsh as described by Morris et al. (2002, 2009) is as follows: $dS/dt = m (q + k_s B_s) D^2/T + (k_r + B_r) (Eq.1)$,

where, $dS/dt = accretion rate (g/m^2/year)$

S = accretion per unit surface area (g/m^2)

t = time (year)

m = suspended solids (g/L)

q = surface loading rate/surface settling velocity, is treated as a constant $(L/m^2/year)$ or in more recent reports by Morris and Currin (2013) Q is units of $g/cm^3/yr$.

 k_s = efficiency of sediment trapping by vegetation, is treated as a constant and is species dependent (%) or in more recent descriptions of Morris and Currin (2013) Ks is in units of cm⁻¹yr⁻¹.

 B_s = annual end-of-season above ground standing biomass density

(g biomass/m²/year) response described as from previous study (Morris 2002);

 $B_s = aD+bD^2+c$ (Eq.2), where a, b, c = parabolic parameters describing the growth optimum vs. D. Note: the nomenclature for a and b is essentially reversed from the algebraic form of a parabolic equation where, y~ax²+bx+c

D = (unit less) = MHW - EL/MHW-MLW, where MHW equals mean high water, EL equals platform elevation of interest, and MLW equals mean low water T = tidal range (m) k_r = refractory fraction of annual root and rhizome production, treated as a constant (%) (collected from Ellisville Marsh and measured as stable plant fragment content (SPFC))

B_r = annual root and rhizome production (g/m³/yr.) as a function of root to shoot ratio (m) and below ground biomass turnover rate (some fraction of 1/year)
After solving for dS/dt it is then divided by the bulk density (g/m³) of the marsh soils, giving the expression units of elevation (m) per time (yr.) that can be compared against sea level, changes in tidal range and any episodic rise in elevation over time.

In this Ellisville Marsh study, all inputs (Table 6.1) were measured with the exception for the value of q, known as the surface-loading rate (or settling velocity). This variable is similar to the value used to design settling clarifiers in the wastewater treatment industry. Assuming the vegetated area (m^2) of *S. alterniflora* platform is what is loaded and has the opportunity to accrete solids via biomass interaction, then using the calculated area from the 2018 land cover assessment enabled determination of the loaded area to which the trapping and turnover estimates are relevant. Using open channel flow area estimation and velocity measurement using timed rate of travel of a floating object, a rough approximation of the flow rate was obtained (26,000,000 MG per tidal fill) and a consequent q value equal to 2.66 L/m²/yr. More recent literature (Morris and Currin 2013), reports the settling rate (large Q) in units of g/m³-yr and has altered the K_s value to 1/m-yr, units.

For the Ellisville assessment, the below ground biomass turnover rate was varied around a 0.4/yr. value derived from literature (Ellison et al. 1986). When positive oxidation-reduction potential conditions were present it was arbitrarily assigned a value

of 0.6/yr., reflecting the increased microbial degradation under aerobic conditions. Subsidence due to compaction/tectonic movements was considered to have been minimal in the study time period, so no subtraction from accumulation was performed. The estimated vertical land movement for the NOAA Boston long-term tidal gauge station would only have had a very small effect at -0.84 mm/yr. (Zervas et al. 2013).

Table 6.1. Input Variables to Marsh Equilibrium Model v. 5.4 for pre (2010) and post (2013) dredge conditions at Ellisville Marsh. Asterisk (*) indicates post dredge input same as pre-dredge condition.

		Pre-	Post
Input Variable	units	Dredge	Dredge
Sea Level Forecast	cm/100yr	100	*
Sea Level at t=0	cm	0	*
20th Century Sea Level Rise Rate	cm/yr.	0.3	*
Mean Tidal Range/Amplitude	m	1.02	1.2
Marsh Elevation at t=0	cm	74	*
Maximum Elevation Growth Limit	cm	120	*
Minimum Elevation Growth Limit	cm	-20	*
Optimum Growth Elevation	cm	50	*
Maximum Peak Biomass (ABGBD)	g/m ²	2072	1544
Below Ground Biomass to Shoot Ratio (RRTS)	$m^{-1}, g/g$	3.15	2.13
Refractory Fraction Below Ground Biomass			
(SPFC)	%	0.17	*
Below Ground Biomass Turnover Rate	yr. ⁻¹	0.4	0.6
Maximum (95%) Rooting Depth	cm	30	*
Trapping Coefficient (ks)	%	0.04	*
Surface Loading Rate (Q)	g/cm ³ /yr.	0.0018	*
Suspended Solids Mineral Concentration (m)	mg/L	66	*
Suspended Solids Organic Concentration (m)	mg/L	0	*
Accretion Rate (dS/dt)	$g/cm^2/yr$.	0.551	*

6.4.2 Hydroperiod Substitution Modeling

Since the water depth in the model above is proportionate to the length of time that any one point is under water (Kefelegn 2019), hydroperiod (rather than D) was selected as a more appropriate proxy as the independent explanatory variable given the observed lag following dredging. It too becomes unit less when substituted by dividing the spring to neap total hydroperiod for the corresponding entire tidal period length during the same spring to neap time frame (32,024 minutes). The unit less D variable is considered equal to elevation divided by tidal range where MHW-EL/MHW-MLW (Morris 2002). This was replaced with this study's measured spring to neap cycle HP variable, also made unit less using per 21 day tidal duration, in order to determine constants for import into the biomass density calculation to see if a difference in outcome of this altered model was evident. This approach is justified in that hydroperiod reflects a lagged response to dredging, while the unit less D variable, reliant on normalization by tidal range, did not. This is further warranted because changes in tidal range in this study did not correspond with the chemical (REDOX shift) and biological (ADH, biomass (AGB and BGB)) declines, as did HP. This step primarily addresses changes to the tidal forcing function (Figure 1.7) as it appears across the marsh platform and accounts for variation in micro-elevations across the marsh which digital terrain models or elevation surveying might not detect.

The same parabolic parameterization of the relationship between peak biomass (ABGBD) and unit less HP (rather than unit less depth) was conducted using the nonlinear regression (nls) function in R ver. 3.3.3. The coefficients were used to identify the

peak biomass estimate and the extent of inundation time in the MEM; in order to assess whether resilience claims from the tidal range induced model can compare to the hydroperiod dictated model.

6.4.3 Fitness versus Density Modeling

Fitness proxies of PH and plant photosynthesis (PN) were modeled using parametric techniques with theoretically representational phenomenological (exponential) and mechanistic (Ricker) deterministic functions and the normal error distribution. The dependent PH and independent BGB variables for the 56 *S. alterniflora* plots were measured outright each of four years. The measurement error around PH was likely small as the plant heights were not a complex measurement and the three tallest plants were measured from each plot across the 4-years by the same operator. The BGB measurement, however, was more likely to have contributed error given that it is virtually impossible to remove all entrained soil particles during washing without losing fine root material. The method for determining the net rate of dry matter production (PN) in g/plant/hour of *S. alterniflora* canopy using equations put forward by Morris (1982) probably contributes process error in that the constants (ψ , η , λ , ρ , α) used for calculation of Morris's Pn were generated using greenhouse study which might vary from direct field CO2 determination methods.

The equation used for calculation of photosynthesis was as follows (Morris 1982): $Pn = FBT \{\psi NL/[(N+\eta)(L+\lambda)]-\rho\}$ (Eq. 2) with slight modifications where,

F = fraction of canopy biomass that is photosynthetically active (g/g) (green vs. non-green biomass) - this data was collected during ABGB sampling as dead versus live fraction

B= dry biomass contained the top surface of the canopy (g/plant)

T = air temperature (°C)

 ψ = maximum weight specific rate of gross production per degree of air

temperature above 0°C and below the plant's temperature optimum (g/g-hr.-°C);

7.1 x 10 ⁻⁴ +/- 1.7 x 10 ⁻⁴ (g/g - °C- hr.)

N =concentration of nitrogen in leaves (%)

 $L = solar radiation (mW/cm^2)$

 $\dot{\eta}$ = % nitrogen of dry leaf weight that gives half saturation rate of response;

0.36 +/- 0.29(%)

 λ = the value of L that gives half saturation rate of response; 30 +/- 10 mW/cm²

 ρ = weight specific rate of dark respiration per degree of air temperature;

$$2.3 \times 10^{-5} + 6.0 \times 10^{-6} (g/g - C - hr)$$

The available solar radiation for photosynthesis (L) was taken from monthly records for the period June through August (Perez, SUNY/NREL/TP-581-41364, April 2007 Geospatial Data Science DNI Resource, Plymouth County) for a modeled time period from 1998 through 2009 and did not directly encompass the 2010 to 2013 study period. Note that this model assumes no interference with light transmission due to cloud cover as it was developed under greenhouse artificial lighting conditions. However, atmospheric moisture is compensated for in the incident light value and therefore, a separate cloud cover variable was not developed. Monthly measurements of temperature for the growing season from June through August were obtained through the Plymouth County Municipal Airport (41.9097, -70.7294 degrees, USW00054769 (GHCN) ACIS/NOAA 2019).

Stem density measurements were used to convert the biomass density (B) into a biomass/plant that are then used to calculate Pn. Nitrogen (N) was measured at the UMass Extension Service (Chapter 3). According to Morris (1982), this equation is an oversimplification of photosynthesis, but in the case of *S. alterniflora* in vitro experiments, this equation described most of the variation in *S. alterniflora* production. It does not consider precipitation as it is assumed that the soil is at field capacity.

When plant height and photosynthesis fitness measures are plotted against below ground biomass (Figures 6.1 and 6.2), for both plot interior and fringe (bordering mudflat or creek) locations, what could be conceived as an Allee effect response for the Loess line of PH versus BGB shows a brief increase in fitness with increasing density, followed by a decrease and then plateau. This functional shape suggests a possible weak Allee effect in the fringe population, but not in the interior population. Its presence in the fringe plots might account for why, even though tallest plants are known to have more inflorescences and therefore set seed, the seed is more prone to losses next to open water situations and therefore colonization occurs slowly, if at all. The mechanistic Ricker function (f (x)=a*x*exp (-b*x)) that emulates this shape was chosen to substitute for the raw BGBD data. A phenomenological fit of the exponential deterministic function represented as (f (x)=a*exp (b*x)) also may describe the data, so it too was tested.


Figure 6.1. LOESS graph of plant height (PH as cm) as a function of below ground biomass dry weight (BGBD as g/830 cc) density where *Spartina alterniflora* plots located along the fringe of the mud flat are shown in red and plots located in interior marsh are in blue-green.



Figure 6.2. LOESS graph of photosynthesis (PN as g/plant/day) as a function of below ground biomass (BGB as g/830 cc) density where *Spartina alterniflora* plots located along the fringe of the mud flat are shown in red and plots located in the marsh interior are in blue-green.

The null hypothesis (Ho) for the above relationships between fitness indicator (PN or PH) and the density measure of below ground biomass (BGB) is that the distribution will follow a random process given by the continuous normal probability distributions as follows: $y \sim Normal (\mu = 0, s_d = \sigma)$ for both fitness proxies and both fringe and interior *S. alterniflora* populations. The Normal error distribution is appropriate given that both plant height and photosynthesis data are continuous and have previously shown a Normal/log Normal distribution independently.

The alternate hypothesis (H1) is that the relationship between fitness indicator (PN or PH) and the density measure of below ground biomass (BGB) is a random process given by the Normal probability distribution for the residuals, with the fitness (y) varying as the Ricker function of x ($y \sim a * x * exp (-b * x)$) or as an exponential function of x, where $y \sim a * exp (b * x)$, for both PH and PN fitness proxies and both fringe and interior populations.

The panel package (plm) within R 3.3.3 was chosen using normal errors to model the data. It is appropriate for repeated measures component of this data set and provides a robust determination of the error component that would result from heteroskedasticity, particularly given that 2012 plant growth had such a different appearance (Chapter 3). Values for BGB (g/830 cc) were converted to a per plant basis based on nearby stem density measurements.

6.4.4 Modeling for Sentinel Indicator

Non-parametric redundancy analysis (a variant of constrained ordination that describes the linear relationships between two sets of continuous variables) was used to evaluate environmental and plant productivity indicators' and their relationship to the

single species, *S. alterniflora*. Because salt marsh grasses grow in response to elevation, inundation, and a variety of other factors (Silvestri et al. 2005), and because *S. alterniflora* grows at the lowest elevation and acts as a "first responder" to tidal influx (as compared to other species of salt marsh grasses), the productivity metrics for this species alone were chosen for constrained ordination by relevant environmental variables using redundancy analysis (RDA).

This provided an extension of the multiple regression analysis whereby only a single dependent variable was assessed and extraneous or redundant independent variables were first removed from analysis where appropriate. It was an appropriate test because it was applied to one species only where linear relationships (axes lengths < 2 standard deviations using Decorana test) between many of the environmental and productivity status indicators have been previously established. For example, growth has been shown to vary with salinity, as with tidal datum or range (McKee and Patrick 1988).

6.5 Results and Discussion

The results of the four modeling approaches are presented in below and are discussed in the following sections.

6.5.1 Multivariate Analysis of Metrics

6.5.1.1 Soil Types Constrained by Below Ground Variables

A redundancy analysis (RDA) of soil particle type (organic matter (OM), CLAY, SILT, and SAND) as constrained by total and average hydroperiod (HP), distance to closest drainage ditch (DTD), below ground biomass (BGB), percent sulfur (PERCS),

salinity (SAL), and redox conditions (REDOX) was performed to provide insight as to how these properties might relate to one another (Figure 6.3). This approach whereby biotic variables (rather than separate species) are constrained by abiotic variables across sites has been used successfully elsewhere (Klanderud et al. 2015, McCune and Grace 2002).

The RDA1 and RDA2 axes explain 85 and 54%, respectively, of the relationship between the soil types and constraining properties. Sand and organic matter (OM) are best explained by the RDA1 axis. Sand and silt have an inverse relationship to one another while clay appears relatively unrelated to sand and silt as indicated by their orthogonal vectors. The percent total sulfur is most closely related to the silt concentration, while redox, salinity, distance-to-ditch and belowground biomass variables are more closely tied to the OM and clay content of the sample. Total hydroperiod relates most directly to the finest (CLAY) particle size and co-varies with PERCS.

These findings make sense given the assumption that the longer the water sits above a location, the more likely that sediment will settle – the heaviest would settle in a shorter time period, followed by the smaller clay particles that take longer. Therefore, as hydroperiod increases there is more chance for slower settling clay particles to deposit. HYDRO and REDOX are inversely related corroborating their relationship discussed in Chapter 4. The overall constraining analysis explains 46% of the variance (p = 0.007) and indicates the strong impact that the environmental constraints have upon the SAND and OM components of this particular marsh system.



Figure 6.3. Redundancy analysis (RDA with scaling =2) of soil particle size fraction (red) and relevant environmental variables (blue) explaining 46% of variation with soil core locations as black, n=27, soil fractions = CLAY, SAND, SILT, and OM (Organic Matter) constrained by percent soil sulfur (PERCS), below ground biomass (BGB), salinity (SAL), redox potential (REDOX), distance-to-ditch (DTD), and total 21-day hydroperiod (HYDRO).

6.5.1.2 Spartina alterniflora Productivity Constrained by Environmental Variables

Each year's productivity data were constrained against environmental variables for all plot locations that contained greater than 25% cover *S. alterniflora* (Figure 6.4). The proportion of variation explained by the constrained analysis ranged from 30% in 2010 to 22% by 2013. Unexplained variation may be attributed to climate variables or other factors not evaluated in this RDA. Overall, the ordination was statistically significant when tested using ANOVA (p<0.005 for 2010-2011, p<0.02 for 2012-2013 data) and both axes were also considered significant.

When interpreting the angles between just the environmental vectors an inverse relationship was discovered between the *Haliaspis* abundance and the pore water salinity for 2010 and 2011 years' data. *Haliaspis* apparently had a preference for sites with lower porewater salinities. Similarly, but not surprisingly, an inverse relationship between NH4 porewater concentration and REDOX potential was observed possibly reflecting that as the REDOX potential declines, the NH4+ concentration in the porewater increases. This trend has been documented in many studies of marsh porewater nitrogen content and REDOX conditions (Howes et al. 1981, 1984, 1994; Morris et al. 1984).

The vectors for total hydroperiod (HYDRO) and PH were found to travel in essentially the same direction due to their obvious inherent covariance (also demonstrated in Chapter 3). Plant height was most closely related to the HYDRO vectors and BGB was most related to the porewater SAL vector. However, the occurrence of the PO43 vector emulating the hydroperiod direction, although at a lesser magnitude, is counter to the earlier made assumption that orthophosphate was flushed out of the pore spaces (Chapter 4) due to increased flushing or greater tidal range. This would have necessitated a

demonstrated inverse relationship where as HYDRO increases the concentration of PO43 should decrease.

Above ground biomass (ABGB) and plot percent cover (PCOVSA) were very closely related suggesting that continued harvesting of aboveground biomass is not necessary and that percent cover is closely related to ABGB. These relationships were determined by drawing a perpendicular from the point represented by the productivity indicator to the closest environmental vector.

Akaike Information Criteria (AIC) analysis was used to determine the best-fit model from the suite of environmental variables (Table 6.1). Because of the parameter numbers and relatively small sample size the AIC was corrected to AIC_c for determining model weights. The top weighted models between years had the variables HYDRO, SAL, HAL, and, unexpectedly NH4 given its similarity each year, as common components towards determining the distribution of productivity indicators.





Figure 6.4. Redundancy analysis (RDA with scaling = 2) of each year's *Spartina alterniflora* productivity indicators (red) versus environmental constraints (blue) in Ellisville Marsh plots (black), n=56. Above ground biomass dry (ABGD), below ground biomass dry (BGBD), plant height (PH), percent cover (PCOV), and stem density (SD) are the productivity variables, while salinity (SAL), redox potential (REDOX), distance-to-ditch (DTD), total 21-day hydroperiod (HYDRO), *Haliaspis* count (HAL), porewater sulfide concentration (S2), and porewater ammonium concentration (NH4) represent the environmental suite of constraints.

Table 6.2. Top weighted models (red) assessing *Spartina alterniflora* productivity variables ranked by each model's average Akaike's Information Criteria (AICc) value corrected for small sample size (n=56), relative likelihoods (L) and Akaike's weights (ω) across 100 iterations for k (number of factors).

Rank	Year	Model	k	AICc	ΔAICc	L	ωi(AICc)
1	2010	HYDRO + NH4 + SAL + HAL	4	424.31	1.29	0.75	0.29
2		HYDRO + NH4 + SAL	3	425.61	1.64	0.65	0.26
3		HYDRO + NH4	2	427.24	5.31	0.11	0.04
4		HYDRO	1	432.55	2.51	0.03	0.01
		HYDRO + HAL + REDOX + S2 + NH4 +					
5		PO43 + SAL + DTD	8	435.06	0	1.00	
1	2011	SAL + HYDRO + HAL + S2 + DTD	5	406.64	0.82	0.91	0.22
2		SAL + HYDRO + HAL + S2	4	407.46	0.94	0.89	0.21
3		SAL + HYDRO +HAL	3	408.41	1.00	0.90	0.21
4		SAL + HYDRO	2	409.40	3.23	0.31	0.07
5		SAL	1	412.63	-1.60	0.23	0.06
		HYDRO + HAL + REDOX + S2 + NH4 +					
6		PO43 + SAL + DTD	8	411.03	0	1.00	
1	2012	HAL + SAL + NH4	3	405.11	0.85	0.97	0.38
2		HAL + SAL	2	405.95	2.69	0.40	0.16
3		HAL	1	408.64	-0.95	0.17	0.07
		HYDRO + HAL + REDOX + S2 + NH4 +					
4		PO43 + SAL + DTD	8	407.69	0	1.00	
1	2013	HYDRO	1	404.84	0.62	0.48	0.29
2		HYDRO + SAL	2	405.46	0.21	0.15	0.09
		HYDRO + HAL + REDOX + S2 + NH4 +					
3		PO43 + SAL + DTD	8	405.67	0	1.00	

6.5.2 Plant Height as a Sentinel Indicator

Mean above and belowground biomass for all plots decreased the years following dredging and their measurements represent a destructive sampling technique, one that in times of threatened salt marsh conditions should not be routinely repeated. A positive linear relationship existed between plant height and hydroperiod (p<0.001, R^2 =0.12, pooled data across years), but the same could not be said for the SD or ABGB measurements (Figures 3.6 through 3.8). Plant height was related to HP, REDOX, SAL, and DTD using multiple linear regression (p=2.49 x 10⁻¹⁰, R²=0.21) and HP (HYDRO), SAL and DTD were also components of the highest weighted models describing

productivity in the RDA above. Mean plant height that was also found to positively vary with mean summer season rainfall totals (p<0.001, $R^2 = 0.95$).



Figure 6.5. Mean *Spartina alterniflora* above ground biomass (g/0.025m²) and mean plant height (mean +/- sd, n=56) in relationship to total precipitation for the summer growing season (June through August), Ellisville Marsh, Plymouth, MA for years 2010 through 2013. Precipitation data were obtained through the Plymouth County Municipal Airport (41.9097, -70.7294 degrees, USW00054769 (GHCN) ACIS/NOAA 2019).

Because PH has a consistent positive relationship with hydrology factors (HP, DTD,

precipitation, SAL and REDOX) as compared to above ground biomass and hence,

percent cover, it would serve as a more rapid indicator of hydrologic conditions than plot

percent cover estimates.

6.5.3 Fitness Versus Density Relationships

The distributions of raw PH data, assigned as from an interior or mudflat fringe locations (LOC), have different shapes when plotted against the density measure of below ground biomass (BGB) (Figures 6.1 and 6.2). They also have obvious differences in height. The results of the linear panel modeling using R ver. 3.3.3 were significant (p = 9.549 x 10^{-9} , $R^2 = 0.26$) and showed effects of LOC (p<0.001) and YEAR (varied significance depending upon year) and BGB (as a Ricker function of BGB as g/830 cc/plant, p<0.05) on the continuous plant height (PH) variable. For every unit change in the Ricker function of BGB, there was a 0.075 unit change in plant height. All other function modeling with PH or PN as the fitness measure as related to BGB was insignificant. Figure 6.6 shows the PH dependent variable with a deterministic Ricker function superimposed. As a check on the parametric panel model, bootstrapped confidence interval determinations (10,000 permutations with replacement) of the coefficient for the Ricker function of BGB as it relates to PH were 0.021 (2.5%) and 0.165 (97.5%) and the range did not include 0. The bootstrapped standard deviation around the coefficient (effectively the empirical standard error) was +/-0.037.

Plant heights are taller at the fringes of mudflat and creeks as compared to those in the interior of the marsh. The taller fringe plants' relationship to BGB density follows what appears to be a weak Allee effect, followed by a decline in fitness as density increases (Figure 6.1). This means that fitness is greater when densities are low which could be an example of resource inhibition/scarcity at higher BGB densities. Interior plots further away from flow conveyance structures with limited flow will not experience as frequent inundation when flows are restored, while plots next to open water in general

experience inundation regardless of flow restrictions. Being more frequently inundated possibly reduces salinity build up in the sediment and increases opportunities for exposure to suspended or dissolved nutrients allowing greater plant heights at the fringes.



Figure 6.6. Possible applicable deterministic reltionship for plant height fitness measure to below ground biomass density measure as Ricker function superimposed on raw data.

Coupled with the lack of horizontal resilience demonstrated by the UVVI comparison, this may explain why re-colonization does not occur, i.e. fringe plants exist where erosive forces are higher and despite exhibiting greater fitness at low BGB densities do not overcome. Possibly, the taller plants, putting more of their photosynthate energy into reproduction, have less to proportion to below ground biomass, and thereby erosion resistance. Their proximity to open water may ultimately mean greater erosive potential of BGB. In addition, losses of seed to stronger tidal flows that carry seed away from protected areas that might have provided germination success are greater at fringe locations.

6.5.4 Vertical and Horizontal Resilience to Sea Level Rise and Episodic Changes

Figure C.1 through C.3 outcomes from the MEM (ver. 5.4) using pre and postdredge, as well as episodic conditions, are provided in Appendix C (copyright permission granted 6/17/19 from J.T. Morris to allow presentation of input/output panels). In summary, the marsh platform surface survives the increase in sea level rate under both pre (102 cm) and post dredge (120 cm) tidal range conditions (Figures C.1 and C.2). Post-dredge marsh elevation converges with sea level rise more quickly than under predredge conditions most likely due to lower root/rhizome to shoot ratios. This response is facilitated by the model's use of maximum peak standing biomass (the highest growing season value encountered at harvest). If a higher suspended solids value were to be used, similar to the content present in Ellisville Marsh due to the ever-present disturbed nature of sands, whether due to dredging or storm events, this would contribute even further to marsh resilience in the face of current sea level rise rates (0.3 cm/yr.). Without further ocean or landward expansion of the marsh allowed, and if a series of 20 cm episodic rises occurs like that encountered following 2011 dredging (assuming the barrier spit has caused near blockage each year), the marsh submerges much more quickly (Figure C.3).

6.5.5 Hydroperiod Substitution

The result of the functional quadratic relationship $ABGBD \sim a (D) + b (D^2) + c$ (Morris (2007), Mudd et al. (2009), Fagherazzi (2012), Kirwan (2012)), where D is substituted with Ellisville Marsh hydroperiod data is shown in Figure 6.7 (A. and B.). This parabolic relationship (for just plots containing S. alterniflora, n=224) of the ABGBD to HP (made unit less relative to spring neap cycle duration) resulted in parameters a, b and c equal to -4017, 1998, and 519***, respectively with a Pearson's r equal to 0.13. Bootstrapped confidence intervals on c (which reflects the height of the parabola along the y-axis and was the only significant parameter) were 275.5 (2.5%) and 761.5 (97.5%). The lines drawn from the intersection of the red abscissa line to the xaxis (Figure 6.7 B.) indicates that all of Ellisville Marsh's plots are inundated less than 68.5% of the time and beyond this point standing biomass will be non-existent. This is a similar finding to the MEM inundation time output for pre- and post dredge conditions (Figures C.1 and C.2, Figure 6.7 C.) where marsh is inundated less than 60% of the time over the 100-yr. time frame. The bulk of the Ellisville Marsh plot data during the spring to neap cycle period studied depicts inundation occurring over approximately 25% of the total tide cycle time frame (Figure 6.7 B.) equating to a 3 hour hydroperiod.

Ellisville Marsh's measured standing biomass (as measured from 2010 to 2013 growing seasons) relationship to hydroperiod appears to rest at the flatter, optimum area of the curve Figure 6.7 A. and B. When taking the derivative of the curve, the rate of change in above ground biomass is positive until it becomes 0 as it approaches a relativized HP of 0.25 (inflection point) and then the rate of change in biomass becomes negative (Figure 6.7 D.). When linearizing the quadratic equation by taking the log of

both sides (log ABGBD = log (a*HP) + $2*\log(b*HP) + \log(c)$), the intercept of the linear model is highly significant, and the log (a*HP) coefficient is significant (p = 0.038), but the remaining variables are dropped because of co-linearity. Using the AICc function both an intercept only and a full nls model are considered to have equal weights, meaning that for the range of hydroperiod data measured there is no difference from a straight line, again possibly confirming that the marsh plots are functioning at or near the optimum of the hydroperiod range. Any further increase in HP beyond this optimum is likely to result in further declines in biomass.



Figure 6.7. Hydroperiod (HP) substitution for depth in non-linear parabolic function (A. and B.) estimation of parameters a, b, and c for equation, ABGBD ~ $a*HP^2+b*HP+c$, where a = -4017.118, b = 1998.186, and c = 518.498 with white oval representing predominance of measured Ellisville Marsh data. C. Marsh Equilibrium Model v. 5.4 output corresponding to hydroperiod substitution findings using lower functional biomass concentration equal to c (vertex). D. Derivative of HP vs. ABGBD data showing positive rate of change in relationship until xvec(HP) reaches 0.25 (equivalent to 3 hours of a 12 hour complete tidal cycle).

6.6 Study Limitations

The modeling ignores ice and pressure induced compaction and erosion, evapotranspiration rates, direct measurement of seed production, dispersal, and germination, storm-generated wrack effects, and groundwater input. It focuses on the Spartina alterniflora population and does not consider competitive forces exerted by other species (other than Haliaspis). The obvious impacts of reversal of oxidative conditions pre and post-dredge on benthic organisms and microbial populations were not measured, nor were the exchanges of macro and micronutrients, and oxygen by roots via active and passive pathways explored in their entirety (Figure 1.7 process functions 2 and 4). Because the sampling of above and below ground biomass were from two different areas to prevent degradation of the plot percent cover, and furthermore because the stem density measurements used to convert between below ground biomass on a volume basis to a per plant representation were from different areas, this means that the BGB can have different interpretations. Finally, caution must be used when assessing the deterministic functions where limited or zero data at the tails of the distributions may give a biased representation.

6.7 Discussion, Implications and Scope of Inference for Ellisville Marsh's Future Sustainability

The impact of inlet opening on Ellisville Marsh and the changes in hydrologic conditions provided favorable conditions for tall *S. alterniflora* expansion (as indicated by increases in percent cover) with the following exceptions:

- Previous die-off areas that had converted to mudflat did not show reestablishment, and, plots in near mud flat margins showed an overall decline in *S. alterniflora* fitness (as determined from calculations of photosynthesis and plant height) with increasing below ground biomass density; a slight increase in plant height as a fitness measure with low below ground biomass density was apparent in the fringe locations and followed a Ricker functional approximation and may represent a weak Allee effect at least initially preventing invasion and spread. Short *Spartina alterniflora* biomass (below and above ground) decreased following dredging and percent cover estimates showed only a small improvement for tall *S. alterniflora* between pre- and post-dredge conditions for plot scale estimates, however larger improvements were evident using the landscape cover estimates.
- Reversal of negative redox conditions to positive within the plant root zone did not meet the definition of characteristic salt marsh sediment by the second summer following dredging. This condition was corroborated by plant root enzyme activity findings for alcohol dehydrogenase that indicated decreased activity when redox conditions became positive possibly signifying more oxygenated conditions. These altered subsurface conditions are relevant to the unstudied benthic organisms and microbial populations, and ultimately, to the amount of carbon sequestered. These changed conditions can feed back to plant productivity by way of changes in mycorrhizal and bacterial populations surrounding the root and altered root exudates (Burke et al. 2003).

- This work used a unique approach for measuring hydroperiod directly rather than indirectly through use of elevation. It allowed detection of a delay in hydroperiod response to tidal range rectification that would not have been detected using water and land surface elevation only. Repeated lags and the coincident effects of increased drainage following future inlet openings would likely show similar negative effects on above and below ground biomass, and by extension, vertical resilience.
- Vertical elevation was deemed to keep pace and exceed sea level rise scenarios • under both pre dredge and post-dredge input conditions over an approximate 100year time frame using the MEM version 5.4, while horizontal resilience was poor (pre-dredge UVVI equal to 0.7) to slightly improved post dredge (UVVI of 0.66). High mineral suspended solids may have contributed to this improvement, as over the 10 year period from 2008 to 2018 the area covered by sand increased by 1.37 times. When hydroperiod substitutions were made for depth measurements, the parabolic relationship between above ground biomass and hydroperiod agreed in part with the MEM vertical resilience approximations, with the exception that the peak biomass as input should realistically be the vertex of the curve taken from the hydroperiod parabolic function instead of maximum peak standing biomass. Convergence of the marsh elevation and mean sea level elevation was faster when hydroperiod was used rather than depth of water. The shape of the relationship was a parabolic representation of above ground biomass and hydroperiod with a hydroperiod of approximately 3 hours being the optimum inundation period.

- Although plot percent cover is the current permit-monitoring requirement thought
 to represent marsh health, the data could not be effectively transformed to
 represent proper normality conditions for classical significance testing and so,
 ultimately monitoring using this type of analysis is inappropriate. Landscape
 cover estimates have their own inherent errors and are at the mercy of the tidal
 and climate conditions during which orthophotographic data is collected making
 comparisons across years at times difficult, if not impossible, for species that
 grow intermixed and require fine discernment. Therefore, the plant height
 correlation with hydroperiod was chosen as a more relevant relationship because
 of its linear variation with hydroperiod and other hydrologic parameters
 (precipitation, salinity and distance to ditch), and more importantly, its relevant
 modeled below ground biomass density dependency.
- A new species for science was discovered in Ellisville Marsh. The insect, a parasitic wasp, *Encarsia ellisvillensis*, preys upon the *Spartina alterniflora* plant pest, *Haliaspis spartinae*. *H. spartinae* and its inverse relationship to salinity was also a common factor (present in 3 out of the 4 top-weighted models) explaining productivity variation so that recording the presence/absence of the scale insect as at the same time as measuring plant heights, may be a signifier of freshening conditions due to decreased tidal flow (after accounting for the effects of annual precipitation differences).
- Recommendations for future dredging include keeping the frequency of dredging to less than every two years between events (more frequently following large storm depositions) in order to forestall sediment oxygenation changes and

associated lags behind tidal range and large swings in hydroperiod. This involves build up of the barrier spit that contributes to blockage by approximately a 500foot length (equivalent to two years of accumulation) that is still effective at directing channel meandering ocean-ward (Applied Coastal Engineering, Inc. 2017). Another approach might be to continue to allow ocean ward expansion of the salt marsh to the degree possible given increases in sea level and storm activity necessitating barrier spit evolution, while at the same time directing channel meandering away from coastal dune and bank by fixed, hard structures in the intertidal zone such as reef balls, or as demonstrated in the 2003 channel rerouting, discretely and functionally placed jersey barriers. The protection of the coastline afforded by the barrier spit would be an added benefit.

- As of this writing, there are plans to permit construction of a replacement rock jetty to the north side of the channel and to supplement this project with some level of dune replenishment. This may repair losses of dune that were observed during 2012 and may help to retain some volume of sands before they are washed over into the inlet. This may also starve to some degree the down coast sediment supply to the beachfront south of the channel. Continued dredging efforts will remain necessary so that the estuary inlet does not become occluded and so that channel meandering towards the coastal bank does not occur. Neglecting this activity and allowing the barrier spit to gain elevation and extent will only allow greater perturbations in the marsh system following the inevitable inlet opening.
- Ellisville Marsh has had somewhat of a unique management history and because of its relatively small size as compared to other salt marshes, it might be assumed

that the scope of this study's inference is unto itself or limited. In fact, the conditions that this marsh experiences when inlet constrictions are rectified are tantamount to representation of a marsh's response to the intermediate-range scenarios predicted for sea level rise by the year 2025 (MA CZM 2013). As such, the marsh response to these perturbations should be looked at as a demonstration of how a "hemmed-in" marsh will respond. Individuals responsible for the protection of this type of resource might use the results of this 4-year study and ongoing vegetation and tidal range assessment to forecast for their specific northeastern marshes.

APPENDIX A

ALCOHOL DEHYDROGENASE/SOLUBLE PROTEIN PROTOCOL

Extraction Procedure from *Spartina alterniflora* root sample for (Alcohol Dehydrogenase (ADH) and Soluble Protein determination):

- Under liquid nitrogen in an ice-chilled mortar, approximately 0.5 g root material was homogenized for several minutes by grinding to powder in 2 ml of cold TRIS-HCl buffer (0.1 M, pH 8.5) with a final concentration of 5 mM dithiothreitol. Dithiothreitol has been used to trap or deactivate an inactivator of ADH (Shimomura and Beevers 1983). An additional 2 ml of extraction buffer was used to complete grinding. Benz (2007) reported using a ratio of 100 μL TRIS-HCL extraction buffer/0.01g fresh weight root tissue from *Piriqueta carolina* or a ratio of 0.1 mg/µl extraction buffer. For maize, Thompson and Greenway (1991) used 0.3 g root tissue/1.5 ml extraction buffer~0.2 mg/µl. For creeping bentgrass, Jiang and Wang (2006) used 0.5 g fresh root sample in 4 ml extraction buffer or a ratio of 0.125 mg/µl. Studies using *Spartina patens* and *S. alterniflora* did not report the exact ratio of root tissue to extraction buffer (Burdick and Mendelssohn 1987; Mendelssohn et al. 1981; McKee and Patrick 1988).
- 2. An additional ml of extraction buffer was used to rinse pestle and mortar to help pour extract into tubes. A total of 5 ml per ~0.5 g sample was used to provide a 0.1 ratio of root tissue to µl extraction buffer. Liquid was poured into pre-labeled 15 ml centrifuge tubes and kept on ice.

- Properly balanced centrifuge tubes were spun at 20,000 g for 30 min at 4°C. The literature reports widely varying spin times.
- 4. Tubes with supernatant were kept on ice in the refrigerator.
- 300 µl of clear supernatant was used for each ADH analysis (see ADH assay procedure below).

Procedure: Soluble Protein Analysis

The Bradford assay, a colorimetric protein assay, was used to determine soluble protein. It is based on an absorbance shift of the dye Coomassie Brilliant Blue G-250 under acid conditions when a redder form of the dye is converted into a bluer form on binding to protein (Bradford, 1976). During the formation of this complex, two types of bond interaction take place: the red form of Coomassie dye first donates its free electron to the ionizable groups on any protein. This causes a disruption of the protein's native state, consequently exposing its hydrophobic pockets. These pockets on the protein's tertiary structure bind non-covalently to the non-polar region of the dye via Van der Waals forces, positioning the positive amine groups in proximity with the negative charge of the dye. The bond is further strengthened by the ionic interaction between the two. The binding of the protein stabilizes the blue form of the Coomassie dye; thus the amount of the complex present in solution is a measure for the protein concentration, and can be estimated by use of an absorbance reading. The (bound) form of the dye has an absorption spectrum maximum at 595 nm.

Standard Curve

1. Test tubes (15 ml) labeled 0 through 9,

- Corresponding to 0, 0.1, 0.2, 0.3, 0.4, 0.5, 1.0, 2.0, 3.0, and 4.0 ml of 1µg/µl BSA stock solution, and
- 3. Corresponding to 5.0, 4.9, 4.8, 4.7, 4.6, 4.5, 4.0, 3.0, 2.0, and 1.0 ml 0.1 M TRIS-HCl extraction buffer (with DTT).
- 4. 100 µl of each protein standard solution was then added into 3 ml of Coomassie protein stain reagent and absorbance read at λ=595 nm (light path 1 cm, final volume 3.1 ml). Absorbance was read 5 minutes after developing color was initiated. TRIS-HCl buffer as a blank.
- 5. The absorbance was converted to soluble protein concentration based on the standard curve.

Sample Assay

1. 100 μ l of sample extract was added into 3 ml of color reagent and absorbance was read at λ =595 nm (light path 1 cm, final volume 3.1 ml). Absorbance was read 5 minutes after developing color was initiated. TRIS-HCl buffer was used as a blank.

Procedure for Alcohol Dehydrogenase Assay

Standard curves were prepared with known concentrations of ADH. The specific activity of yeast ADH (EC 1.1.1.1) was \geq 300 IU/mg in its crystallized form when stored at 4° C.

ADH Yeast Protein Accession Number: P25377

Assay Mixture per Benz et al. (2007)

0.2 ml NAD+ (20 mg/ml dH2O)

0.2 ml 95% ethanol

0.6 ml 0.05 M TRIS-HCl with 5 mM DTT, pH 8.5

0.1 ml sample extract/ADH solution

Final reaction volume was 1.1 ml. Note that the reaction volumes of each component were tripled in order to read absorbance properly in the cuvette and had a final reaction volume of 3.3 ml.

Sample Assay

The first three components of the assay mixture were added and then the reaction begun by adding extract. The formation of NADH was followed at 340 nm (25°C) every 30 seconds following extract addition for a total of 7 minutes.

Reagent Preparation

a. TRIS-HCI Buffer: TRIS-HCl will react with Coomassie stain because it has an amino group, so therefore, one must subtract reagent blank for each protein assay. Prepare 0.05 M TRIS-HCl buffer containing 5mM DTT, by dissolving 6.05 g of tris (hydroxymethyl) aminomethane (MW 121.14 g/mole) and 77.125 mg dithiothreitol (MW 154.25 g/mole) into 500 ml deionized water and bring to pH 8.5 (Benz 2007) using hydrochloric acid (HCl)(used 12 N droplet addition). (Calculate the number of moles of TRIS that are required by multiplying the molar concentration of buffer by the volume of buffer being made (**moles of TRIS = mol/L x L).** Next, determine how many grams of TRIS this is by multiplying the number of moles by the molecular weight of TRIS (121.14 g/mol) **grams of TRIS = (moles) x (121.14 g/mol).**) Dilute the buffer with deionized water to reach the desired final volume (1.0 L) of solution. Check pH and store at 4°C.

b. Protein Stain: 0.01% Coomassie Brilliant Blue G-250, 4.7% ethanol, 8.5%

phosphoric acid (Bradford 1976).

- add 100 mg Coomassie Brilliant Blue G-250 to 50 ml 95% ethanol; dissolve on stir plate in beaker covered with watch glass to lessen evaporation
- added 100 ml 85% phosphoric acid (may need more like 105-110 ml in order to lessen blue color)
- diluted to 1 L with distilled water (DI) and filtered with funnel and Whatman No.
 1 filter paper directly into glass storage bottle (Note: took about 6 hrs. to filter with successive filter paper changes).
- Store at room temperature in dark or aluminum foil covered bottle.

c. Protein Stock: Prepared a 0.1% solution = $1\mu g/\mu l$ Bovine Serum Albumin (BSA) (dissolved 40 mg BSA into 40 g DI by stirring very slowly or else BSA would not go into solution completely and would foam). Stored at 4°C.

d. NAD⁺ (Cofactor): Make up fresh each assay day. Needed 20-mg/ml concentration NAD⁺ for use. Dissolve xx mg into xx ml of TRIS buffer (note: Benz says to use dH20) depending upon how much volume anticipated for use in one day. Since only had 1 g of NAD, dissolved 200 mg/10ml (enough for 50 assays per day). Pipetted 0.2 ml into each assay mixture to yield final molarity in assay mixture of 2.0 mM using NAD MW = 663.4 g/mole.

e. Ethanol (Substrate): Use 95% EtOH directly.

f. ADH (Enzyme): Make up fresh each assay day/for standard curve/spiking

purposes. (Bergenmeyer says to dissolve in 0.1% Bovine Serum Albumin, but this protocol used TRIS-HCl buffer as in Sigma and Benz procedures.) For a functional assay, needed approximately 0.75 unit/ml of ADH activity to add to assay solution per Sigma procedure (final volume in assay 3.0 ml). Prepared 5 ml of 1 mg/ml ADH solution, dissolved 5 mg ADH into 5 ml 0.05 M, pH 8.5 TRIS – HCl buffer. Assuming the activity of ADH is ~ 300 IU per mg, this solution must be diluted 400x to achieve a 0.75 unit/ml ADH activity solution, so 0.5 ml of 1 mg/ml ADH was brought to 200 ml in volumetric flask. Prepared standard solutions to encompass a range of activity around 0.75 units/ml when standard curve was necessary. Stored at 25° C/4°C depending on its intended use i.e. needed temperature acclimated to 25° C for spectrophotometer to avoid fogging. Pipetted 0.3 ml and inverted cuvette to begin assay.

Pre-testing and General Considerations

Prepared a standard curve at outset of each assay, if necessary. Because ADH is not stable in suspension, assays had to be conducted rapidly. Care was taken to make sure that testing with one batch of reagents represents complete spectrum of variable conditions in hydroperiod and that triplicate samples per location were performed using same reagent batches. For example, wanted to avoid all "short" hydroperiod samples from being analyzed under the same conditions, and "long" hydroperiod samples being tested with entirely different reagent batch or considered grouping the dates of sampling.

APPENDIX B



ELLISVILLE MARSH SOIL BORING LOGS

Figure B.1. Vertical distribution of soil organic matter (loss on ignition) for transect 1 PZ01 through PZ19 (top) and transect 2 PZ22 through PZ44 (bottom). Varying widths of columns are irrelevant to interpretation.



Figure B.2. Vertical distribution of soil organic matter (loss on ignition) for transect 3 PZ49 through PZ74 (top) and transects 4/5 PZ76 through PZ91 (bottom). Varying widths of columns are irrelevant to interpretation.



Figure B.3. Vertical distribution of sand for transect 1 PZ01 through PZ19 (top) and transect 2 PZ22 through PZ44 (bottom). Varying widths of columns are irrelevant to interpretation.



Figure B.4. Vertical distributions of sand for transect 3 PZ49 through PZ74 (top) and transects 4/5 PZ76 through PZ91 (bottom). Varying widths of columns are irrelevant to interpretation.



Figure B.5. Vertical distribution of silt for transects 1, PZ01 through PZ 19 (top) and transect 2, PZ22 through PZ44 (bottom). Varying widths of columns are irrelevant to interpretation.



Figure B.6. Vertical distributions of silt for transect 3 PZ49 through PZ74 (top) and transects 4/5 PZ76 through PZ91 (bottom). Varying widths of columns are irrelevant to interpretation.



Figure B.7. Vertical distribution of clay for transect 1 PZ01 through PZ19 (top) and transect 2 PZ22 through PZ44 (bottom). Varying widths of columns are irrelevant to interpretation.



Figure B.8. Vertical distribution of clay for transect 3 PZ49 through PZ74 (top) and transects 4/5 PZ76 through PZ91 (bottom). Varying widths of columns are irrelevant to interpretation.

APPENDIX C



MARSH EQUILIBRIUM MODEL VER. 5.4 OUTPUTS (©MORRIS 2010)

Figure C.1. Pre-dredge conditions as input to Marsh Equilibrium Model Ver. 5.4


Figure C.2. Post dredge conditions as input to Marsh Equilibrium Model Ver.5.4.



Figure C.3. Repeated episodic increases as input to Marsh Equilibrium Model Ver.5.4.

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