

EXPLORING POTENTIAL SITES FOR SALINITY GRADIENT RENEWABLE ENERGY ON THE
NORTH CAROLINA COAST AND EVALUATING THE POTENTIAL EFFECTS OF LOCAL
SALINITY REGIME VARIATION ON SAV COMMUNITIES DUE TO REVERSE
ELECTRODIALYSIS EFFLUENT

Hannah Catherine Palko

A thesis submitted to the faculty at the University of North Carolina at Chapel Hill in partial fulfillment of the requirements for the degree of Master of Science in the Curriculum for the Environment and Ecology in the Institute for the Environment.

Chapel Hill
2017

Approved by:

Lindsay Dubbs

Michael Piehler

W. Judson Kenworthy

© 2017
Hannah Catherine Palko
ALL RIGHTS RESERVED

ABSTRACT

Hannah Catherine Palko: Exploring Potential Sites for Salinity Gradient Renewable Energy on the North Carolina Coast and Evaluating the Potential Effects of Local Salinity Regime Variation on SAV Communities due to Reverse Electrodialysis Effluent
(Under the direction of Lindsay Dubbs)

North Carolina has many rivers, tributaries, and back barrier sounds that form complex estuarine systems, the largest of which is the Albemarle Pamlico Estuarine System. The extensive nature of North Carolina estuaries' vertical and horizontal salinity gradients makes this region a suitable subject for the exploration of salinity gradient energy as an alternative clean energy source. Geographic Information Systems was used to analyze North Carolina salinity data and explore potential natural and anthropogenic salinity gradient energy sites. Though salinity gradients exist within North Carolina estuaries, it was found that citing options were limited due to the proximity of high and low salinity water and the salinity variability at potential sites. The potential environmental impacts of salinity gradient energy and reverse electrodialysis technology, primarily installation effluent-induced salinity change effects on submerged aquatic vegetation growth, distribution, and abundance, were evaluated through literature review. Results were compiled in an annotated bibliography.

ACKNOWLEDGEMENTS

First, I would like to thank my advisor and mentor, Lindsay Dubbs, for supporting and encouraging me throughout the graduate research process. It has been an honor to work with her on this project, and I am grateful for the many skills she has helped me refine. Thank you to Jud Kenworthy and Mike Piehler for their guidance, wisdom, and expertise on complex topics. Data for this project were made available by Niels Lindquist from UNC Institute of Marine Science and Don Field from NOAA. Claire Johnson played an integral part in the geospatial analyses and fieldwork for this project, and I could not have completed my thesis without her. I would also like to thank Research Opportunities Initiative for funding the Salinity Gradient Energy project.

Thank you to Ella Wickliff for being a loving friend, roommate, and GIS expert. Thank you to the strong women from Meredith College that challenged me intellectually and encouraged me to continue my education. I am exceedingly grateful for my sister Rachel and my brothers Justin and Joseph because all of the love and support they have given me. Above all, I'd like to thank my parents for being invaluable teachers and for giving me all of the love, encouragement, and strength that I need to be successful.

TABLE OF CONTENTS

LIST OF FIGURES.....	ix
LIST OF TABLES.....	x
LIST OF ABBREVIATIONS.....	xi
1. INTRODUCTION	1
1.1 Estuarine Ecology	1
1.2 Salinity Gradient Energy	3
1.3 Reverse Electrodialysis (RED)	5
1.4 RED Operation: Influent and Effluent.....	6
1.5 Coastal NC and RED Siting.....	7
1.6 Research Questions and Hypotheses	8
2. MATERIALS AND METHODS.....	10
2.1 RED Installation Scenarios	10
2.2 Geospatial analysis for RED siting along the NC coast	11
2.2.1 Natural Salinity Gradient Geospatial Analysis using ArcGIS.....	12
2.2.2 Anthropogenic Salinity Gradient Geospatial Analysis using ArcGIS.....	14
2.3 Inflow and Outflow According to Power Generation.....	15
2.4 Ground Truth Data Collection at Anthropogenic Salinity Gradient Potential RED Sites	17
2.5 Submerged Aquatic Vegetation and Salinity Interaction Literature Review.....	17
3. RESULTS AND DISCUSSION.....	19

3.1 Geospatial Analysis for RED Siting Along the North Carolina Coast	19
3.1.1. Currituck Sound	25
3.1.2. Cape Fear River and New River	27
3.1.3 NPDES Permitted Discharger Locations—WWTP	35
3.2 Submerged Aquatic Vegetation and Salinity	40
3.2.1 Mechanisms of SAV Adaptation to Salinity Stress	45
3.2.2 Salinity and NC Saltwater SAV Species Annotated Bibliography	48
Plasmatic resistance and rate of respiration and photosynthesis of <i>Zostera marina</i> at different salinities and temperatures (Bieble and McRoy, 1971)	48
Salinity effects on <i>Ruppia maritima</i> L. cultured in vitro (Bird et al., 1993)	49
Reproduction of Coastal Submerged Macrophytes in Experimental Communities (Bonis et al., 1993)	50
An index to access and monitor the progression of wasting disease in eelgrass <i>Zostera marina</i> (Burdick et al., 1993)	51
Production ecology of <i>Ruppia maritima</i> L. s.l. and <i>Halodule wrightii</i> Aschers, in two subtropical estuaries (Dunton, 1990)	51
The influence of salinity on growth, morphology, leaf ultrastructure, and cell viability of the seagrass <i>Halodule wrightii</i> Ascherson (Ferreira et al., 2016)	53
Short-term impacts of salinity pulses on ionic ratios of the seagrasses <i>Thalassia testudinum</i> and <i>Halodule wrightii</i> (Garrote-Moreno et al., 2015)	53
Seed-bank development, germination and early seedling survival of two seagrass species from The Netherlands: <i>Zostera marina</i> L. and <i>Zostera noltii</i> hornem (Hootsmans et al., 1987)	54
Variation in leaf ultrastructure of <i>Ruppia maritima</i> L. along a salinity gradient. (Jagels and Barnabas, 1989)	55
The effect of salinity and ammonium on seed germination in <i>Ruppia maritima</i> from Florida Bay (Kahn and Durako, 2005)	56

Short Communication Influence of salinity and temperature on the germination of <i>Ruppia maritima</i> L . from the North Atlantic and Gulf of Mexico (Koch and Dawes, 1991)	57
Tropical seagrass species tolerance to hypersalinity stress (Koch et al., 2007).....	58
A study of the germination and viability of <i>Zostera marina</i> L. seeds (Lamounette, 1977)	59
Effects of salinity changes on growth of <i>Ruppia maritima</i> L. (La Peyre and Rowe, 2003).....	60
The influence of salinity on seagrass growth, survival and distribution within Biscayne Bay, Florida: Field, experimental and modeling studies (Lirman and Cropper, 2003).....	61
Salinity Tolerances of Five Marine Spermatophytes of Redfish Bay , Texas (McMillan and Moseley, 1967)	62
Seed germination for an annual form of <i>Zostera marina</i> from the sea of Cortez, Mexico (McMillan, 1983).....	62
Physiological effects of short-term salinity changes on <i>Ruppia maritima</i> (Murphy et al., 2003).....	63
Effects of salinity and water temperature on the ecological performance of <i>Zostera marina</i> (Nejrup and Pedersen, 2008).....	64
Seed germination and seedling growth of <i>Zostera marina</i> L. (eelgrass) in the chesapeake bay (Orth and Moore, 1983)	65
Influence of temperature and salinity on germination of eelgrass (<i>Zostera marina</i> L.) seeds (Pan et al., 2011)	66
Observations on the Ecology and Distribution of Florida Seagrasses. St. Petersburg, Florida (Phillips, 1960)	67
Reproductive strategies of eelgrass (<i>Zostera marina</i> L.) (Phillips et al., 1983).....	68
Synergistic effects of altered salinity and temperature on estuarine eelgrass (<i>Zostera marina</i>) seedlings and clonal shoots (Salo and Pedersen, 2014).....	69
Population specific salinity tolerance in eelgrass (<i>Zostera marina</i>) (Salo et al. 2014)	70

Life History Transitions in Response to Salinity Dynamics Along the Everglades-Florida Bay Ecotone (Strazisar et al., 2014)	71
The Autecology of <i>Zostera marina</i> in Relation to its Wasting Disease (Tutin, 1938)	72
Effects of salinity and nutrient load and their interaction on <i>Zostera marina</i> (Van Katwijk et al., 1999)	72
Salinity and temperature significantly influence seed germination, seedling establishment, and seedling growth of eelgrass <i>Zostera marina</i> L. (Xu et al., 2016)	73
3.2.3 Annotated Bibliography Summary and Conclusions in Relation to Shift in Salinity Regimes	74
3.3 Salinity, SAV, and Ecosystem Services	77
4. CONCLUSIONS	81
APPENDIX: WATER QUALITY DATA COLLECTED FROM POTENTIAL RED INSTALLATION SITES	87
REFERENCES	89

LIST OF FIGURES

Figure 1. Schematic representation of RED membrane stack	6
Figure 2. Potential environmental impacts of RED.....	7
Figure 3. Rivers, geographic features, and towns relevant to thesis	20
Figure 4. SalWise data low and high salinity points.....	21
Figure 5. Standard deviation at locations with low or high salinity in coastal NC	22
Figure 6. Potential RED sites and ambient salinity.....	24
Figure 7. Frequency distribution of salinity measurements in Currituck Sound at Duck, NC	26
Figure 8. Potential RED sites and mean ambient salinity in the Cape Fear River Estuary and river mouth.....	39
Figure 9. Frequency distribution of salinity measurements at the Cape Fear River mouth (A) and the mean salinity distribution at the Cape Fear River mouth (B).....	31
Figure 10. Potential RED sites and mean salinity in the New River Estuary and river mouth	32
Figure 11. Frequency distribution of salinity measurements at the New River mouth (A) and the mean salinity distribution at the New River mouth.....	34
Figure 12. NPDES permitted wastewater treatment plants in relation to high salinity.....	37
Figure 13. Frequency distribution of SalWise measurements at potential RED sites	40
Figure 14. SAV distribution in relation to wastewater treatment plants in the Morehead City, Beaufort, Atlantic Beach, and Pine Knoll Shores area	43
Figure 15. SAV distribution in relation to wastewater treatment plants in Sea Level, NC	44

LIST OF TABLES

Table 1. Scenarios of RED installation	11
Table 2. Complete mixing energy and volume influent needed per 1 kWh of energy generated.....	16
Table 3. NPDES Permitted wastewater treatment plants within 2000m of high salinity (30+ ppt) in coastal NC.....	37
Table 4. Ground truth data collected from three potential RED sites: Nelson Bay (A), Taylor Creek (B) and Bogue Sound at Indian Beach (C)	39
Table 5. Mean salinity tolerance and salinity ranges of native NC submerged aquatic vegetation species.....	42

LIST OF ABBREVIATIONS

APES	Albemarle Pamlico Estuarine System
CFRE	Cape Fear River Estuary
NPDES	National Pollutant Discharge Elimination System
NRE	Neuse River Estuary
ppt	parts per thousand (measurement of salinity)
RED	Reverse Electrodialysis
SAV	Submerged Aquatic Vegetation
SGE	Salinity Gradient Energy
WWTP	Wastewater Treatment Plant

1. INTRODUCTION

1.1 Estuarine Ecology

In broad terms, an estuary is a geographical region where a river or stream meets the ocean. Estuaries are characterized as partially enclosed bodies of water that have a free and open connection with the ocean, where ocean water is diluted by fresh water (Pritchard, 1967). This transition zone between freshwater and seawater acts as a natural filter; clays, humic acids, and metal oxides are removed from the water column via flocculation and coagulation before reaching the open ocean. After the estuarine geochemical filter removes these particulates, phytoplankton and submerged aquatic vegetation (SAV) utilize the available light and the abundance of nutrients from the river discharge to fix carbon as biomass and form the base of numerous food webs. High primary productivity supports a complex food web and a biodiverse ecosystem. SAV is ecologically important and provides many ecosystem services, including carbon sequestration and provision of nursery habitat for commercially important fish species.

Estuarine ecology is influenced by salinity, or the concentration of dissolved salts in water. Seawater contains ions such as sodium, chloride, magnesium, sulfate, calcium, potassium, bicarbonate, and bromine. Because estuaries are transitions zones between freshwater and saltwater, many estuarine organisms are euryhaline; they have evolved to tolerate a wide range of salinity. Tolerance ranges depend on the adaptations of osmotic processes within organisms on the cellular level. Specific ions are required for cellular

processes, and fluctuation of ion composition in the water column can be detrimental to biological processes.

Estuaries are primarily classified by their geomorphic structure, circulation, water balance, and salinity structure. Estuaries are classified by their geomorphology as being a drowned river valley, fjord, tectonic estuary, or bar-built. Ultimately, the geomorphic classification relies on knowledge of how the estuary was formed: either by flooded coastal plain river valleys due to sea level rise, glacial activity, tectonic activity, or formation of sandbars. Water balance describes the net flow of water in an estuary. An estuary with a positive water balance experiences a positive longitudinal density gradient that increases towards the sea. In contrast, an estuary with a negative water balance has a negative longitudinal density gradient that increases towards the land. Low inflow estuaries experience high evaporation rates and a salinity maximum zone due to little influence from the low river discharge. Based on the vertical salinity structure of the water column, an estuary can be classified as a salt wedge, strongly stratified, weakly stratified, or well mixed. Tidal forces and river discharge influence the vertical salinity profile. Strong river discharge and moderate to weak tidal influence results in a salt wedge estuary with a sharp pycnocline. A well-mixed estuary experiences the opposite effect—strong tidal effects and small freshwater discharge create a water column that is uniformly vertically mixed, though a longitudinal salinity gradient still exists (Valle-Levinson, 2010).

North Carolina has a very unique system of rivers, tributaries, and sounds that comprise the Albemarle Pamlico Estuarine System (APES). The largest estuary in North Carolina is the Albemarle-Pamlico Sound, which is a back barrier sound that lies between the mainland and the Outer Banks of North Carolina. The Albemarle and Pamlico Sounds,

along with the Roanoke and Croatan Sounds on either side of Roanoke Island make up the APES, which is not only the largest estuary in North Carolina, but also the second largest in the United States. Rivers that drain into this and all North Carolina estuarine systems are drowned river valleys, formed from rising sea level into coastal plain river valleys over a large geological time period. The Chowan and Roanoke Rivers drain into the Albemarle Sound, and the Pasquotank, Pamlico, and Neuse Rivers drain into the Pamlico Sound. The Neuse River Estuary is a salt-wedge estuary that frequently has a strong vertical salinity gradient, though the location of the salt-wedge fluctuates. Because the APES only has free connection with the Atlantic Ocean through few inlets in the Outer Banks, circulation patterns within much of the estuarine system is characterized by wind-driven tides, more so than lunar tides. Under the assumption that salinity gradients exist within these complex estuarine systems, North Carolina may be a suitable location for the exploration of harnessing salinity gradient energy.

1.2 Salinity Gradient Energy

Salinity gradient energy (SGE) is a form of alternative renewable energy that is generated from water with a specific salinity flowing into a water body of a different salinity. The difference in chemical potential between the two water sources drives the release of Gibbs free energy (Emami, Mehrangiz, Etemadi, Mostafazadeh, & Darvishi, 2013). The concept of SGE is not novel; it was proposed by Pattle (1954) and was described further by Weinstein and Leitz (1976), Clampitt and Kiviat (1976), and Jagur-Grodzinski and Kramer (1986). The 'hydroelectric pile', as it was first proposed, sought to capture the free energy obtainable from the mixing of river water and seawater, calculated to be equivalent to the free energy obtainable from a 680 ft. waterfall (Pattle, 1954). Further

studies were conducted to explore the potential use of SGE. In a study exploring the power output of a 'dialytic battery', which employed cation and anion exchange membrane alternation and fresh and salt water mixing, it was found that low salinity river water improved performance by increasing the potential difference across the ion exchange membranes. However, due to the increased resistance in the low salinity compartment, the lowest concentration of river water was not optimal (Weinstein & Leitz, 1976). A small amount of ions is necessary in the low salinity solution in order to minimize resistance and maximize power output (Clampitt & Kiviat, 1976). The combination of electrodialytic piles and fuel cells and electrochemical half-cells was also investigated.

Estuaries are interesting locations for the exploration of SGE because river water mixes with seawater, thus creating a salinity gradient where high and low salinity water coexists. Based on thermodynamic calculations, 1.4 MJ of power is generated from mixing 1 m³ of freshwater with 1 m³ of seawater (J W Post, 2009). The global SGE potential from river mouths is 1183 GW on average; however, not all river mouths are considered suitable for SGE generation (O. A. Alvarez-Silva, Osorio, & Winter, 2016). It is unknown how much of the free energy generated from freshwater and saltwater mixing can be converted to electricity. Practical power output is limited by technical obstacles such as membrane power density and stack resistance (Długołęcki, Gambier, Nijmeijer, & Wessling, 2009). Site-specific variability of river water and seawater mixing must be taken into account. At some locations, a large mixing zone of brackish water separates low and high salinity solutions. Large distances between low and high salinity solutions results in energy loss due to transport of water via pumping (O. Alvarez-Silva, Winter, & Osorio, 2014). However, vertical salinity gradients present in salt wedge estuaries are also considered for

exploitation. The two technologies that are currently employed in SGE generation are pressure retarded osmosis (PRO) and reverse electrodialysis (RED). This thesis will focus on the latter technology.

1.3 Reverse Electrodialysis (RED)

Reverse Electrodialysis (RED) is a membrane-based technology that utilizes two source waters with different salinities to produce electricity via the movement of electrons. In a RED unit, cation and anion exchange membranes alternate between the cathode and anode, and low salinity and high salinity solutions are fed alternating between the membrane stacks. The anion exchange membranes are permselective for anions like Cl^- and cation exchange membranes are permselective for cations such as Na^+ (Figure 1). The transport of ions from the high salinity solution to the low salinity solution by osmosis allows for electrons to be transferred from the anode, where oxidation occurs, to the cathode, where reduction occurs (Jan W. Post et al., 2007). The movement of ions, and the resulting flow of electrons, generates an electrical current that could be utilized to power an industrial or municipal facility, put into the power grid, or stored in a battery.

The initial pass of source waters through the RED system results in two waste streams—a low salinity solution that is slightly more saline than the original low salinity solution and a high salinity solution that is slightly less saline than the original high salinity solution. The two resulting waste streams can be fed back through the system, although the energy production on subsequent passes would be reduced due to the decrease in salinity gradient and chemical potential between the two source waters. Regardless, the probable fate of the waste streams is that they will be combined into one effluent stream to be discharged into the nearest body of water.

Based on suitability, sustainability, and variability of environmental conditions, it is estimated that the practical theoretical globally extractable salinity gradient energy (SGE) from river mouths meeting the ocean is 625 TWh per year. Although this is only equivalent to 3% of the global use of electricity, it could still decrease the dependence on fossil fuels and other forms of non-renewable energy.

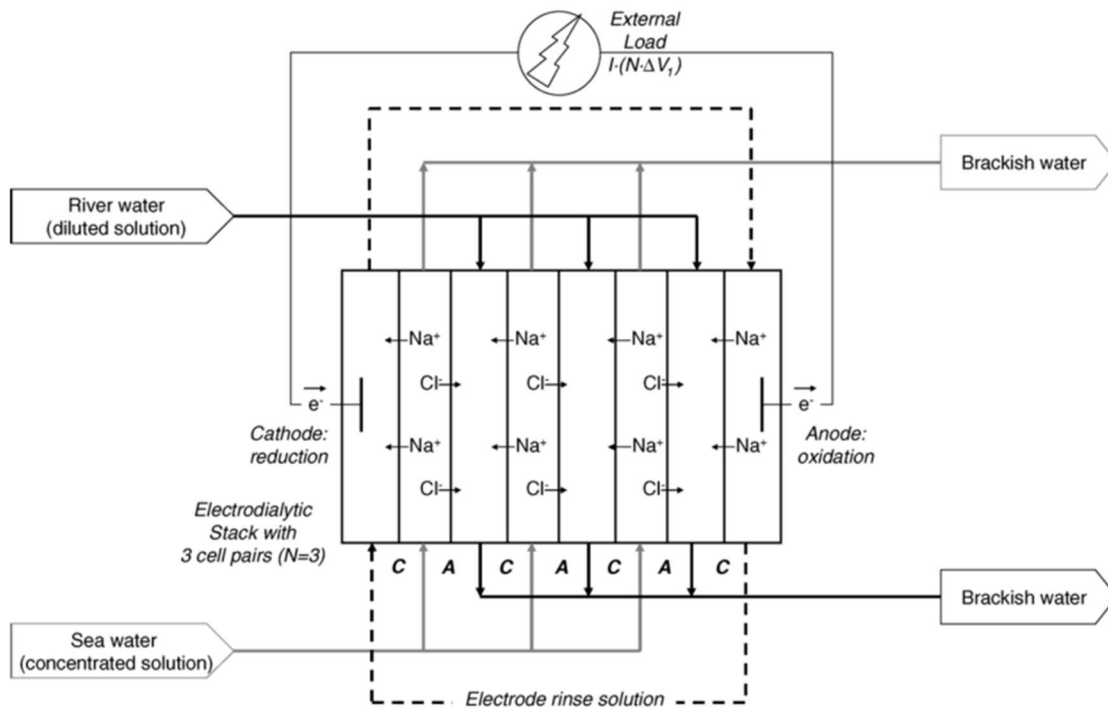


Figure 1: Schematic representation of reverse electrodialysis. Low salinity and high salinity solutions are alternatingly fed between anion and cation exchange membranes. The movement of ions from high salinity to low salinity facilitates oxidation in the anode and reduction at the cathode and generates an external flow of electrons (Post et al. 2007).

1.4 RED Operation: Influent and Effluent

As with any coastal construction project, there are potential environmental impacts in the construction, operation, and decommissioning phases of installment (Gill, 2005). We can anticipate that the environmental impacts of construction and decommissioning will be similar, if not identical, to the construction and decommissioning of any coastal

construction project such as a power plant or water treatment plant. In the operation phase, we can expect that there are potential environmental impacts on both the inflow and outflow sides of operation (Figure 2).

This thesis will primarily focus on the salinity regime of North Carolina with regard to siting a RED facility on the North Carolina coast and the potential environmental and ecological changes that might occur as a result of RED effluent-induced salinity changes.

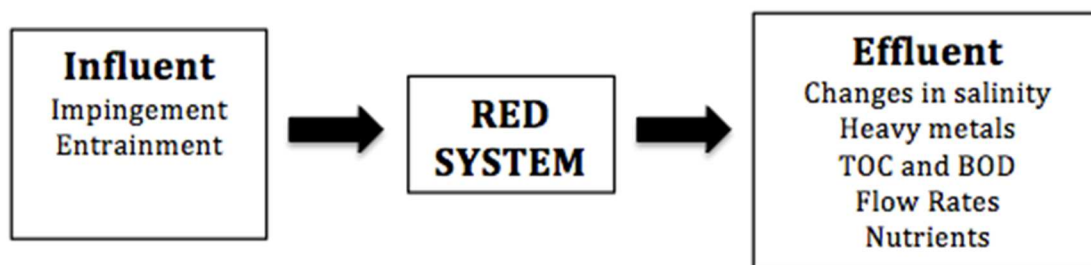


Figure 2: Potential environmental impacts due to reverse electrodialysis operation, considering the intake of source water as influent and the discharge of effluent once high and low salinity source waters pass through a RED membrane stack and are combined as effluent.

1.5 Coastal NC and RED Siting

According to NOAA, in partnership with the US Census Bureau, 39% of US citizens live within coastal shoreline counties, and 52% live in coastal watershed counties (NOAA, 2013). With a growing coastal population and the accompanying energy demand, it is beneficial to locate energy sources near demand to minimize the transmission loss and provide energy where it is most needed. Fortunately, there are many potential renewable energy sources in coastal regions, including wave energy, tidal energy, wind energy, and salinity gradient energy such as RED.

Coastal North Carolina is especially promising for SGE production because of the natural and anthropogenic salinity gradients due to APES and the Outer Banks barrier

islands that separate freshwater flow from rivers and mesohaline sound water, respectively, from the Atlantic Ocean. Along with the natural salinity gradient, there are also places with anthropogenic salinity gradients, where industrial or municipal effluent is discharged into the ocean or sound. There is a potential for using treated wastewater as the dilute source water, paired with seawater, to employ RED for electricity production. The number of sites for source water intake and effluent discharge is justification for the investigation of RED implementation in coastal North Carolina.

Although RED is a promising source of renewable energy, it has the potential to contribute to the existing pressures and impacts that humans have caused in coastal ecosystems. While coastal ecosystems provide goods and services to humans, human impact on marine ecosystems is prevalent in coastal regions. A meta-analysis of global data sets on anthropogenic drivers of ecological change showed that there are virtually no marine ecosystems that experience zero human impact, and a large portion of marine ecosystems are affected by multiple anthropogenic drivers (Halpern et al., 2008). One ecological community of elevated importance to coastal economies and ecosystem health are submerged aquatic vegetation (SAV) communities.

1.6 Research Questions and Hypotheses

This research is being conducted as a component of a multi-disciplinary project whose goal is to explore the technological, environmental, and economic implications of a RED installment on the coast of North Carolina. This thesis on the local environmental implications of RED aims to address the following specific research questions:

- 1) Where on the North Carolina coast can RED technology be utilized to generate electricity, based on the natural and anthropogenic salinity gradients?

- 2) How does salinity –absolute concentrations and variations over time - affect SAV growth, primary productivity, and reproduction and how might these impacts affect SAV distribution and abundance at effluent discharge sites?
- 3) How will changes in SAV species distribution change the efficacy of SAV ecosystem services?

I use geospatial analysis of a compiled salinity dataset of North Carolina estuaries and back barrier sounds (SalWise) in ArcGIS to address question #1; identifying locations where high and low salinity source waters are co- located. Several of these locations were chosen for more in-depth scenario analyses of salinity variability to evaluate RED site suitability and estimate how RED effluent could possibly disrupt or change the local salinity regime. To address question #3, I conduct a comprehensive literature review of the impacts of salinity on SAV processes, community composition, distribution, and abundance and the effect on ecosystem service provision. I use this literature review, SAV geospatial data (APNEP 2008), and SAV sample collection to estimate the potential for RED effluent to impact SAV for the above-mentioned scenarios. Using the theoretical energy generated per cubic meter of influent observed by the technology team of this salinity gradient energy project, I estimate the volume of water necessary to generate energy comparable to commercial-sized power plants.

2. MATERIALS AND METHODS

2.1 RED Installation Scenarios

The installation of a RED facility and its power generation capacity is determined by its size and the source of its water. There are several plausible scenarios of source water pairings on the North Carolina coast, given natural and anthropogenic salinity gradients and the size of the RED installation (Table1). Assuming that the smallest commercial size power plant has a nameplate capacity of 100 MW, any nameplate capacity below this threshold would be considered a small package plant.

My goal was to explore potential source water pairs that represent each of the possible RED installation size and pairing scenarios. Anthropogenic water sources include industrial waste, treated municipal or industrial wastewater, and storm water runoff, each requiring National Pollutant Discharge Elimination Systems (NPDES) permits. Collected rainwater is a possible source of low salinity water, though the seasonal variability in rainwater availability is not explored in this thesis. Therefore, the three scenario pairings that were addressed in this thesis were: 1) river water and sound water, 2) river water and seawater, 3) WWTP (small and large) and sound water. Complete mixing energy and necessary volumes of influent for each of these pair were compared to the flow rates from the following freshwater sources: Pamlico River, New River, Cape Fear River, Beaufort WWTP, Snug Harbor WWTP, and PruittHealth WWTP. This provides an indication of the

feasibility of energy production using natural and anthropogenic salinity gradients in North Carolina.

Table 1: Scenarios of Reverse Electrodialysis Installation

	Natural Salinity Gradient	Anthropogenic Salinity Gradient
Small package plant	<ul style="list-style-type: none"> • Seawater/rainwater • Seawater/sound water 	<ul style="list-style-type: none"> • Seawater/ small WWTP (Snug Harbor and PruittHealth WWTPs) • Seawater/ storm water • Industrial waste/ storm water
Commercial Size	<ul style="list-style-type: none"> • Seawater/Sound water • Seawater/ river water (Pamlico River, New River, Cape Fear River) 	<ul style="list-style-type: none"> • Seawater/ large WWTP (Beaufort WWTP)

2.2 Geospatial analysis for RED siting along the NC coast

According to the Coronell Lab at UNC Chapel Hill, collaborators on the salinity gradient energy technology project, a salinity ratio of 30:1 is the optimal ratio for energy production using RED. However, there are other factors contributing to power output, such as presence/absence of multivalent ions and the conductivity of the low salinity water source.

Both natural and anthropogenic gradients were considered as potential RED source waters. Natural surface water salinity data used in this analysis was acquired from Niels Lindquist (UNC Institute of Marine Science), who compiled the Shellfish and Sanitation data and ferry monitoring data in coastal North Carolina. However, because there was no proof of consistent methodology between sources, there are many limitations to using this data

set in a GIS analysis. Likewise, mapping errors may be the cause of unusual outliers found in the dataset.

Anthropogenic source water locations and salinity data were acquired from the EPA Enforcement and Compliance History Online (ECHO) website. National Pollutant Discharge Elimination System (NPDES) permit data were found through the EPA website and NC OneMap.

SAV coverage data was acquired from Don Field at NOAA. Data represent digitization of visible SAV and imagery collected with digital mapping cameras. SAV coverage is classified as dense, patchy, or unvegetated. Dense seagrass meadows are areas in which the substrate is 70% covered or greater. Patchy seagrass meadows are discontinuous communities covering more than 10% but less than 70% of the substrate. Initial mapping efforts by the Albemarle-Pamlico National Estuary Partnership, NC Division of Marine Fisheries, US Fish and Wildlife Service, and NOAA were complete in 2008 and were updated in 2011.

2.2.1 Natural Salinity Gradient Geospatial Analysis using ArcGIS

A geospatial analysis of the natural salinity gradients in coastal APES waters was conducted to identify locations where fresh and saline waters naturally exist in close proximity, potentially only separated by a narrow barrier island, only three miles wide at its widest point. Although an economic and engineering analysis would reveal exact feasible pumping distances, a 2000 m radius is assumed to be a maximum pumping distance for a RED installation.

Because of the large size of the SalWise data set, the data had to be constrained and summarized before analysis could take place. The data were first constrained based on date; measurements taken before January 1st, 2000 were removed from the data set, making the study period for the salinity between the years 2000 and 2013. Next, significant figures of the latitude and longitude were reduced to 4 decimal places in order to reduce the number of unique locations. This reduces location precision to within 10m of the geographical location. Lastly, summary statistics were generated for each unique location where multiple measurements were taken over the course of the study period. Latitude and longitude fields were concatenated into one field labeled "location," and the attribute tables for each region (Northern Region, Albemarle Sound, Pamlico Sound, Pamlico River, Neuse River, New River, and Southern Region) were exported from ArcGIS into excel, where PivotTable was used to generate summary statistics for each unique location. PivotTables were then converted into CSV files and reloaded into ArcGIS. Regional data were merged into one SalWise Summary dataset, where each feature represented summary statistics such as the number of measurements, mean, maximum, minimum, range, standard deviation, and variance of salinity measurements taken at that geographical location.

The mean salinity was used to identify locations of low salinity (less than or equal to 1 ppt) and high salinity (greater than or equal to 30 ppt) source waters. Under the assumption that the salinity measurements had a normal distribution, the mean was determined as an appropriate summary statistic. Locations that fell within either the low or high salinity range were extracted from the dataset, and the standard deviations were used to represent variation at those locations. The buffer tool in ArcGIS 10.4 was used to create

polygons with a radius of 2000m around high salinity locations, and the intersect tool was used to clip the low salinity locations that fall within the buffer zone. The geospatial analysis of the natural salinity gradient identified estuaries that possibly have a steep salinity gradient to be utilized by RED technology. The locations identified are Currituck Sound near Duck, NC, the New River Estuary, and the Cape Fear River Estuary. The salinity regimes at these locations were examined at a closer level.

2.2.2 Anthropogenic Salinity Gradient Geospatial Analysis using ArcGIS

Along with natural salinity gradients, anthropogenic salinity gradients were considered in the RED siting geospatial analysis. Potential RED sites were selected based on the proximity of anthropogenic freshwater sources to natural concentrate locations using ArcGIS 10.4. Using the buffer and intersect tools previously used in the natural salinity gradient geospatial analysis (Section 2.2.1), NPDES permitted wastewater treatment plant (WWTP) dischargers that were within 2000m of a high salinity point were highlighted. Previous research has explored the use of treated industrial or municipal wastewater for RED (Brauns, 2008, 2009), and it is believed that treated wastewater effluent could combat the problem of membrane resistance in RED stacks; increased membrane resistance decreases power output.

Wastewater treatment plants (WWTP) are the most probable source of reliable anthropogenic freshwater, as treated wastewater must comply with strict discharge regulations. WWTP also have fairly consistent flow rates, compared to the seasonal variation in storm water collection and discharge. Because much of the Outer Banks of North Carolina uses individual septic systems to treat wastewater, there are few large

municipal WWTP facilities north of Ocracoke Island and Cape Hatteras. Therefore, efforts were focused on locations south of Cape Lookout to identify NPDES discharge sites in proximity of the ocean or a consistently mixohaline body of water. This analysis identified sites to ground truth the SalWise dataset and explore the potential RED pairings. The sites chosen for this study, based on this analysis, were 1) Bogue Sound near the Pine Knoll Shores WWTP, 2) Nelson Bay near the Snug Harbor retirement facility and PruittHealth WWTPs, and 3) Taylor's creek near Beaufort WWTP (Table 5).

2.3 Inflow and Outflow According to Power Generation

In a RED system, membrane technology is utilized to harness the potential energy, therefore the energy production is contingent upon the power density of the membranes themselves, which could be affected by the conductivity ratio, conductivity of the dilute solution, and other factors such as DOC (Kingsbury, personal communication). A baseline study conducted by the Coronell Lab at UNC Chapel Hill approximated membrane power density based on the pairing of potential sources waters in coastal North Carolina. These pairings include: seawater/freshwater (rainwater), seawater/ treated wastewater, and pickle plant brine/ storm water. The power densities for these pairings were 0.19 W/m², 0.18 W/m², and 0.6 W/m², respectively (Kingsbury et al., unpublished data).

Using these power densities, a flow velocity within reasonable and appropriate limits of a RED facility (Tedesco et al., 2016), and the baseline measurement of a kilowatt hour (kWh), the volume of necessary influent can be estimated. A potential RED facility will aim to maximize the power output while minimizing economic burden. Given the complete mixing energy of the RED stack prototype in kWh/m³ of mixed solution, the complete mixing energy per m³ of influent would be two times the energy per m³ of mixed solution,

or effluent. To find the volume of water needed to generate 1 kWh of energy, one would simply take the inverse of the complete mixing energy (Table 2).

To be considered a commercial size power plant with a 100 MW capacity the RED installation will have to be capable of producing 876,000 MWh of energy, being that there are 8760 hours in one year. Considering the values in column 4 of Table 2, a proportion can be used to determine the volume of influent needed to have a small package plant nameplate capacity of 1 MW (Equation 1). A similar proportion can be constructed to find the volume of influent needed to have a nameplate capacity of 100 MW. The volume of influent for each of the five scenarios described in section 2.1 is calculated and compared to volume of water available, according to the flow rates of each river or WWTP. Assuming that the RED stack would be a flow-through system where the volume of influent equals the volume of effluent, predictions about the change in salinity due to completely mixed RED effluent can be made.

Table 2: Complete mixing energy and volume influent needed per 1 kWh of energy generated. The volume of influent needed per 1 kWh of energy generated is found by taking the inverse of the complete mixing energy (kWh/m³ of influent).

Water Pair	Complete Mixing Energy, kWh/m ³ mixed solution	Complete Mixing Energy, kWh/m ³ influent	Volume influent (m ³) needed per 1 kWh energy generated, based on theoretical energy
Seawater/ Brackish River or sound water (less than 1 ppt salinity)	0.153	0.306	3.27
Seawater/ WWTP Effluent	0.141	0.282	3.55

(1) Equation
$$\frac{X \text{ m}^3}{1 \text{ kWh}} = \frac{Y \text{ m}^3}{1000 \text{ kWh}}$$

Where: X = volume of influent needed per 1 kWh of theoretical energy
Y = volume of influent needed per 1000 kWh (1 MWh) of theoretical energy

2.4 Ground Truth Data Collection at Anthropogenic Salinity Gradient Potential RED Sites

The potential RED sites identified for the anthropogenic salinity gradient GIS analysis (Section 2.2.2) were sites chosen as ground truth data collection points for the anthropogenic salinity gradient sites. Data were collected at Bogue Sound, Nelson Bay, and Taylor's Creek in July, September, and November 2016 and February 2017. SAV species presence, salinity, conductivity, temperature, dissolved oxygen (DO), turbidity, and pH were recorded at each site (Appendix 1). SAV species presence was determined by taking three random SAV cores in areas of patchy SAV coverage. The individual SAV shoots in each core were described to the lowest taxonomy possible. Salinity, temperature, conductivity, DO, turbidity, and pH, were measured at the water surface and near bottom using a YSI. These data were compared to the SalWise data set to assess the reliability of the SalWise dataset in representing the salinity variability at the potential RED sites.

2.5 Submerged Aquatic Vegetation and Salinity Interaction Literature Review

The following databases were used to identify past research pertaining to SAV salinity tolerance: Web of Science, JSTOR, BioOne, WorldCat, and Google Scholar. No date constraint was used, though many key publications are between 1980 and present. Search terms were initially kept broad. These included "Seagrass" and "Submerged Aquatic

Vegetation” and “Salinity.” Including more specific terms such as “Eelgrass”, “*Zostera marina*”, “Widgeon grass”, “*Ruppia maritima*”, “Shoal grass”, “*Halodule wrightii*”, “Atlantic coast”, “North Carolina”, “Pamlico Sound”, “Metabolism”, “Primary production”, “Growth,” “Interactions”, “Osmotic”, “Salt tolerance”, and “Salinity variability” then narrowed the search. Publications were considered if research was conducted either *in situ* or *ex situ*. Laboratory mesocosm experiments, field experiments, and field observations were all considered in this literature review.

Relevant publications were then thoroughly examined. Species, experimental design, salinity ranges tested or observed, response variables measured, and salinity preference in each publication were recorded in an Excel spreadsheet. There is limited research on NC native saltwater SAV and salinity interactions, though 32 publications were included in this literature review. Within each species, similarities and differences between methodology and salinity preferences were noted.

3. RESULTS AND DISCUSSION

3.1 Geospatial Analysis for RED Siting Along the North Carolina Coast

Salinity gradients are present in coastal North Carolina, although most occur over a large spatial scale as mixing and dilution occurs. The goal of the geospatial analysis of this thesis was to determine locations where water of high and low salinity points coexist within 2000m of each other, a distance at which the water could be utilized for RED energy generation. Locations where the mean salinity is low, i.e. less than or equal to 1 ppt (blue points) or high, i.e. greater than or equal to 30 ppt (red points) were highlighted in the dataset (Figure 4). Considering the 30:1 optimal salinity ratio for RED energy generation, these locations represent water sources that could be utilized in a RED stack. Many low points exist upriver and many high points congregate around inlets; however, there are some locations of nearby high and low salinity. NPDES permitted wastewater treatment plants were also considered potential source waters.

Although there are few low points within the Pamlico Sound, Bogue Sound, and within the sound landward of the barrier islands south of Cape Lookout, the Albemarle and Currituck Sounds show several points of low salinity due to the flow of the Albemarle, Perquimans, Pasquotank and North Rivers and the absence of an inlet north of Oregon Inlet, separating Bodie Island and Pea Island.

At these locations of low and high salinity, the standard deviation reflects the variability of the salinity regime within the APES and southern region of the NC coast. This

variability is due to the freshwater flow of rivers that are fed by rainwater, and the intrusion of seawater via inlets. Wind-driven and lunar tides also influence salinity regimes in many of the sample locations; therefore time of day and prevailing weather conditions influence the salinity regime in a way that may not be captured in this analysis due to limited data of time of day and measurement depth. The standard deviation at points of mean low and high salinities was also calculated (Figure 5). Most points have a standard deviation between 0 and 1 ppt, however points along the Intracoastal Waterway south of Cape Lookout are more variable.

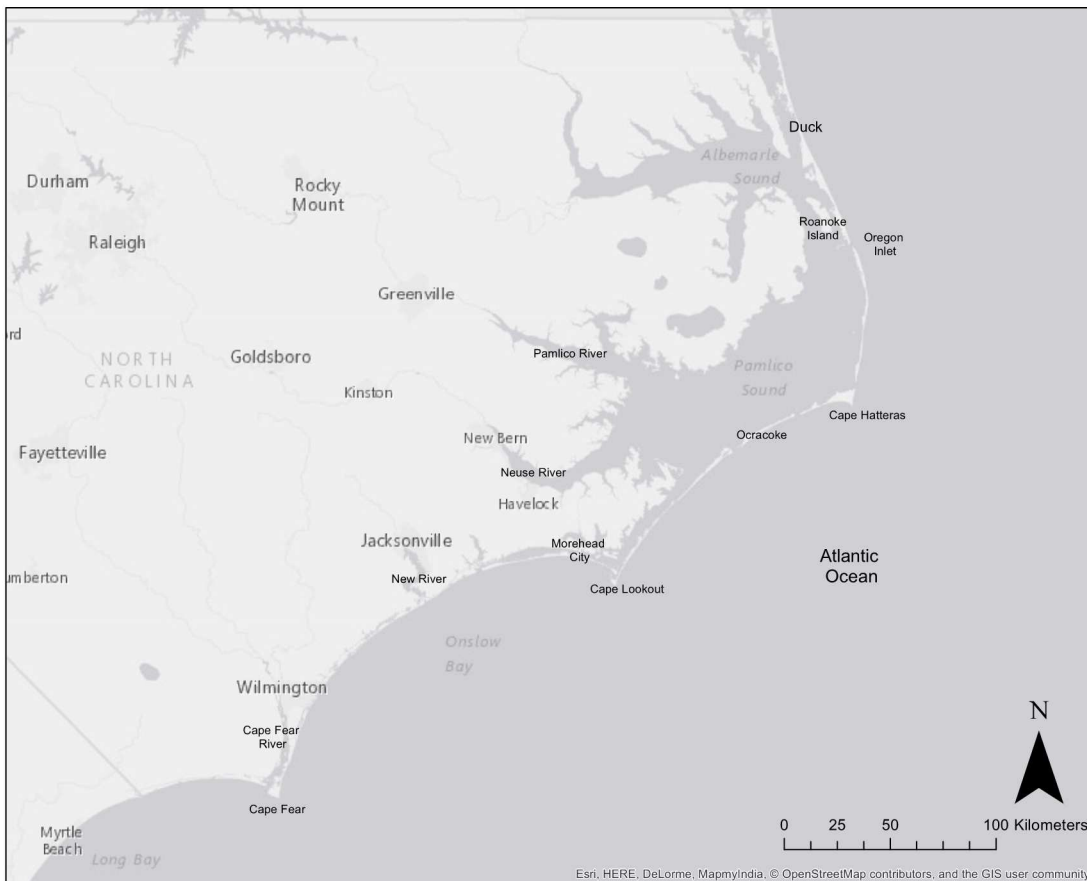


Figure 3: Map of coastal North Carolina, including prominent features, bodies of water, and towns relevant to this research.

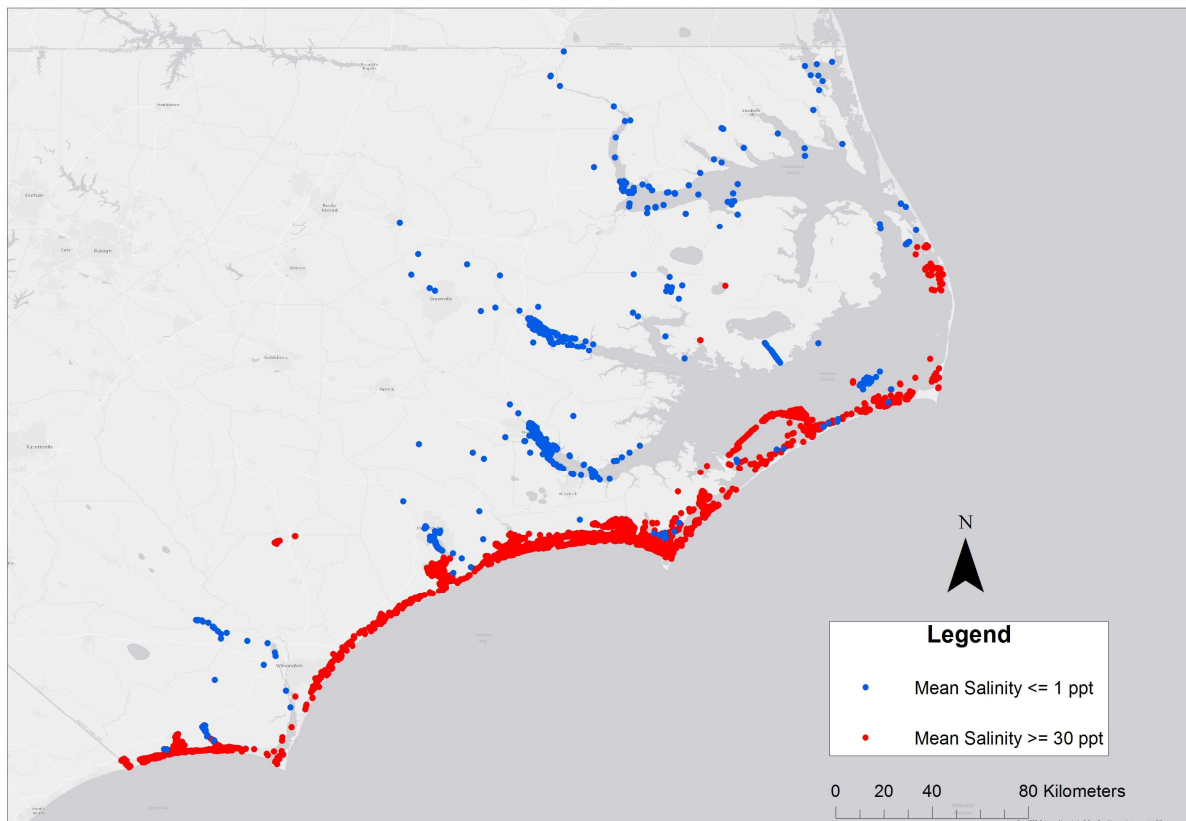


Figure 4: SalWise data points with mean low salinity (blue; less than or equal to 1 ppt) and mean high salinity (red; greater than or equal to 30 ppt)

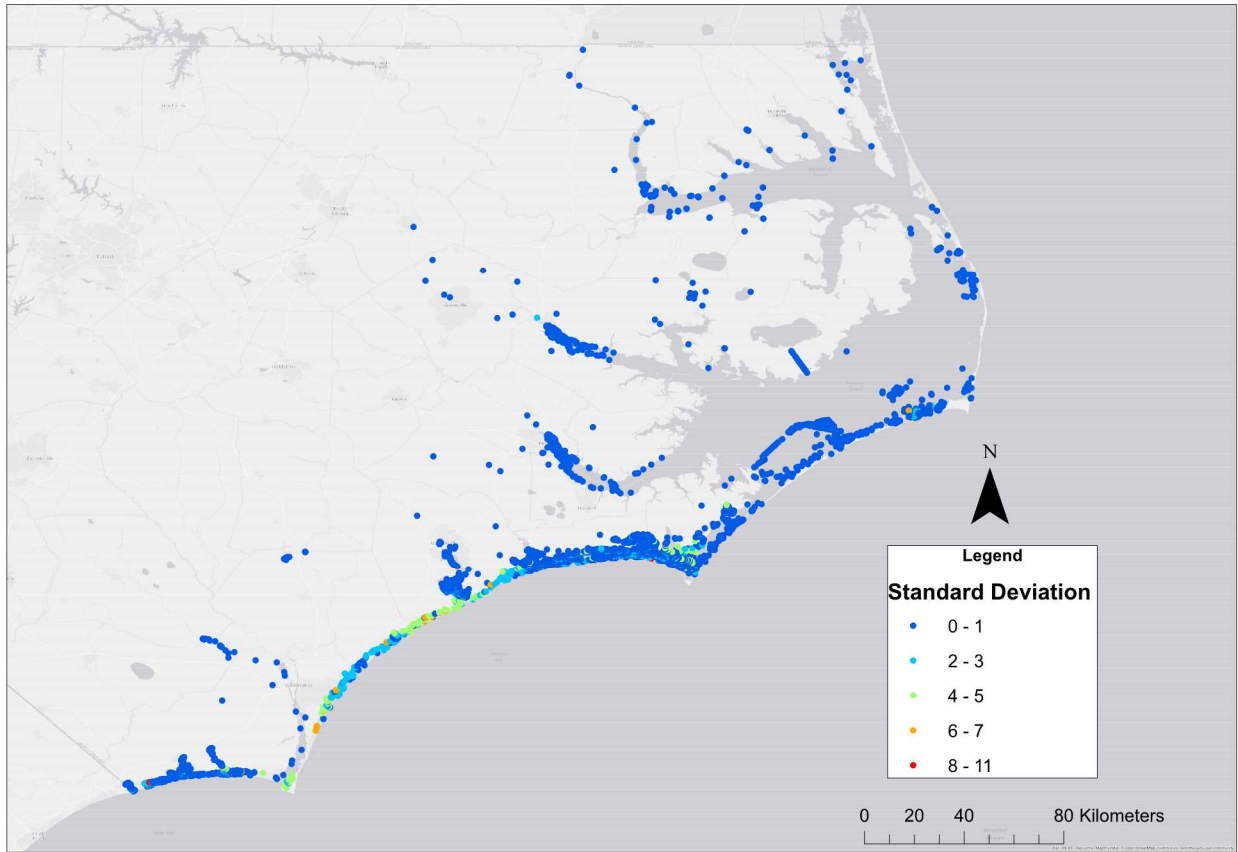


Figure 5: Standard deviations of SalWise data points with mean low and high salinity in Eastern NC

From the geospatial analysis in ArcGIS, potential RED sites were identified based on proximity of naturally low mean salinity to high mean salinity and proximity of NPDES permitted dischargers (low salinity) to naturally high mean salinity (Figure 6). Low salinity points and NPDES permit locations that were 2000m from either a high salinity point or the approximate 2012 shoreline were selected as potential RED sites. Many sites can be disregarded because the ambient mean salinity is variable and/or predominantly mesohaline. These sites include those in the Pamlico Sound near Buxton, Frisco, and Ocracoke. Although high and low salinity measurements have been taken at some point during the study period, the salinity is highly variable due to saltwater flow through inlets and freshwater input via rivers and rainfall. The highest standard deviation in this region was 20 and the highest range was 31. This is also the case in the Croatan Sound, Stumpy Point, and among some of the small rivers and tributaries that flow into the APES. The Neuse River Estuary (NRE) also initially appears to be an appropriate RED site, however, data from the Neuse River Estuary Modeling and Monitoring Project (ModMon), an ongoing collaborative project between the University of North Carolina and the North Carolina Department of Environmental Quality, indicates that the salt wedge location is highly variable, depending on tides, wind, rainfall, and storm events. Therefore the vertical salinity gradient in the NRE would be difficult to utilize for RED.

NPDES permitted discharger effluent is variable in composition, so therefore some NPDES sites that are expected to have a brine effluent. Reverse osmosis water treatment plants were disregarded as potential RED sites because the small difference in salinity between reverse osmosis effluent and sound water yielded low power density outputs when fed through a RED prototype. Nevertheless, there are several locations where there is

an apparent salinity gradient suitable for RED. I will expand upon those potentially suitable locations further in the following sections.

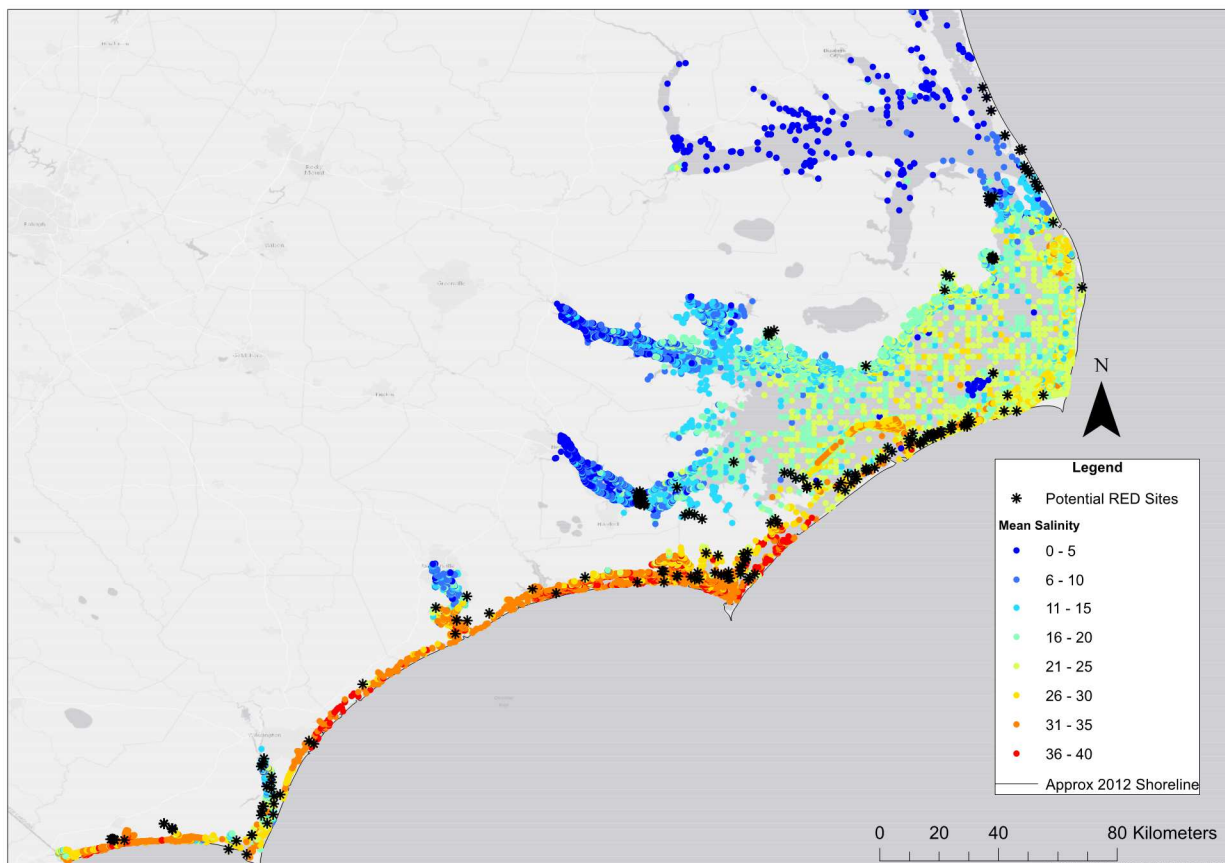


Figure 6: Potential RED sites and the ambient mean salinity along the NC coast. Potential RED sites (black) are points of low salinity (less than or equal to 1 ppt) that are within 2000 m of a high salinity (greater than or equal to 30 ppt) point or the approximate 2012 shoreline. Potential RED sites are shown in relation to the ambient mean salinity.

3.1.1. Currituck Sound

The first potential location is the Currituck Sound, near Duck, NC, where there are three points within 2000m of the shoreline that have a minimum salinity of 0 ppt. The mean salinity at these three locations ranges from 4.43 to 4.5, but the maximum salinity at all three sites is 16 ppt. These three locations each have 80 salinity measurements, and the maximum salinity of 16 was recorded on October 21, 2008 at all three sites. This recording was taken after Hurricane Hanna, which made landfall in North Carolina on September 5th, 2008. Hurricane force winds, or south/southeast winds, could explain the unusually saline water in the Currituck Sound at this time. A frequency distribution of salinity measurements taken at the three Duck sites highlighted in the geospatial analysis as potential RED locations was constructed (Figure 7). The majority of measurements for all three sites were between 0 and 5 ppt. The geographic coordinates of these sites are:

Duck Site 1 36.198° N -75.772° W

Duck Site 2 36.1686° N -75.7599° W

Duck Site 3 36.1281° N -75.7461° W

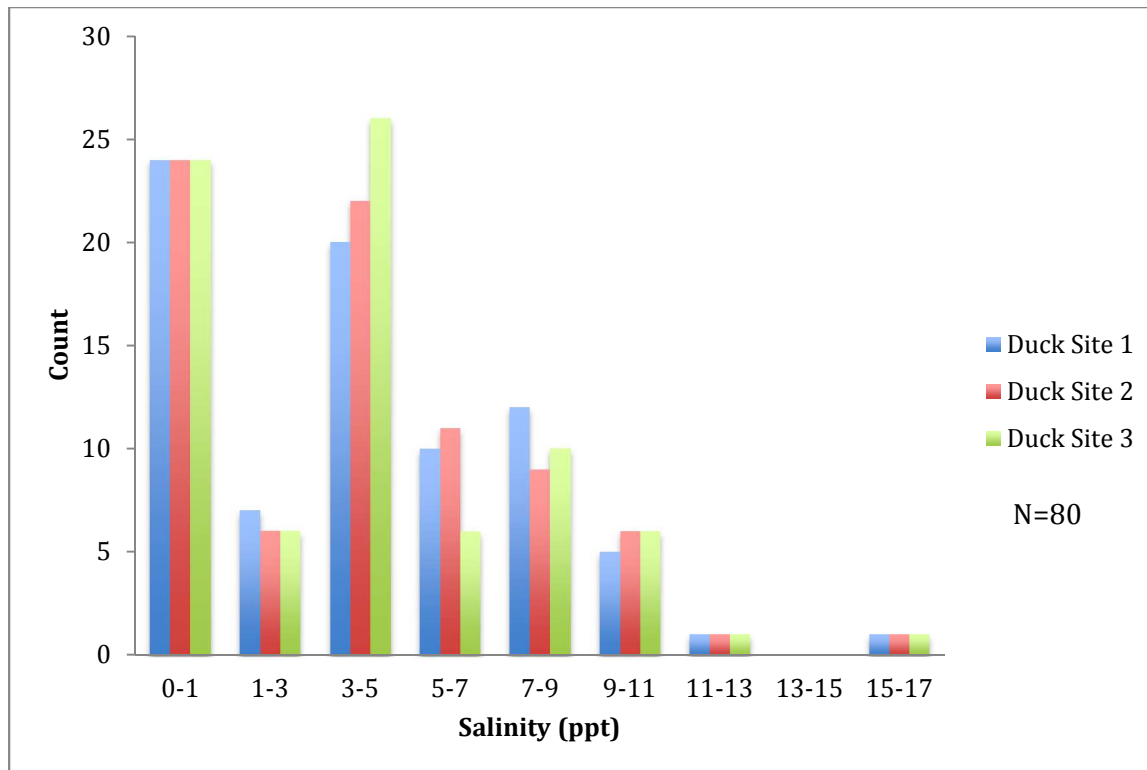


Figure 7: Frequency distribution of salinity measurements in Currituck Sound at three sites near Duck, NC.

Also in the Currituck Sound, several points in Beasley Bay have a mean and minimum salinity of 1.1 ppt and were therefore not included in the possible RED locations because they are higher than the 1 ppt cutoff used to designate low salinity source water. However, this water could be suitable for RED energy generation if paired with source water with salinity above 30 ppt to maintain the 30:1 salinity ratio. These points are approximately 936 m from the shoreline of the Atlantic Ocean, well within the distance constraint for RED water sources. The lack of data points in the Currituck Sound limited the results of the geospatial analysis in this region. Currituck Sound predominantly has a low salinity, as it is fed by the Northwest River, North Landing River, Roanoke River, and Chowan River (Harned & Davenport, 1990). The closest inlet is Oregon Inlet, located approximately 40 km to the south, and the tidal exchange with the Atlantic Ocean is

therefore limited (Robinson & McBride, 2006). Currituck Sound is susceptible to estuarine evolution as climate change induces sea level rise. Storm frequency influences sediment flux and inlet formation, which could drastically alter the salinity regime in the Currituck Sound in the future (Moran, Mallinson, David, Culver, Leorri, & Mulligan, 2015). RED power capacity is affected by the salinity of water passed through stacks, and therefore estuarine evolution of the Currituck Sound should be taken into account for long-term siting of a RED installment. Seawater intrusion into the freshwater Currituck Sound due to sea level rise or storm over-wash would make these sites less than optimal for future energy generation via RED.

3.1.2. Cape Fear River and New River

At the mouths of the Cape Fear and New Rivers, there are several points that are highlighted as potential RED locations due to the proximity of high and low salinity. This points to the possibility of these river mouths having a small mixing zone and a steep salinity gradient. However, closer examination shows that both estuaries and river mouths have highly variable salinity throughout. Though there are points of low salinity upriver, ambient salinity points have means of 6 to 25 ppt at the mouth of the Cape Fear River and the New River, respectively (Figure 9 and Figure 11). Even if water is pumped from upriver to the river mouth via gravity, water upriver of the estuary is not consistently fresh. Water from further upriver would possibly be more reliably fresh, though the distance to the high salinity source water would increase greatly.

There are several potential RED locations identified throughout the Cape Fear River Estuary (Figure 8). However, the mean salinity at locations throughout the estuary

indicates that salinity is highly variable. Locations of natural salinity gradient that are identified as potential RED are actually located in areas where ambient mean can range from 10 to 30 ppt without a clear mixing zone.

The Cape Fear River Estuary (CFRE) is fed by the largest and most industrialized watershed in North Carolina, including two black water streams that flow into the CFRE: the Black and Northeast Cape Fear Rivers. Transport of salt and other constituents in the CFRE, as in all estuaries, depends on hydrodynamic characteristics such as tides, river discharge, wind stress, and local geomorphology (Becker, Luettich, & Mallin, 2010) . While the NRE flows into the Pamlico Sound and has a nearby inlet, the CFRE mouth has an open connection with the Atlantic Ocean. Therefore, tidal currents are influential in transport and mixing. Salinity intrusion varies from about 29 km to 53 km upstream from the mouth of the river, depending on low- or high-flow conditions and flood tide post storm events. The CFRE is classified as a partially-mixed estuary, where appreciable stratification exists. Bottom water in the CFRE typically has a higher salinity than surface water, regardless of tidal period. However, the salinity of the bottom water is higher during low-tidal range period (Becker, Luettich, & Seim, 2009). Likewise, the horizontal transport of salt and the resulting density gradient is driven by tides (Becker et al., 2009).

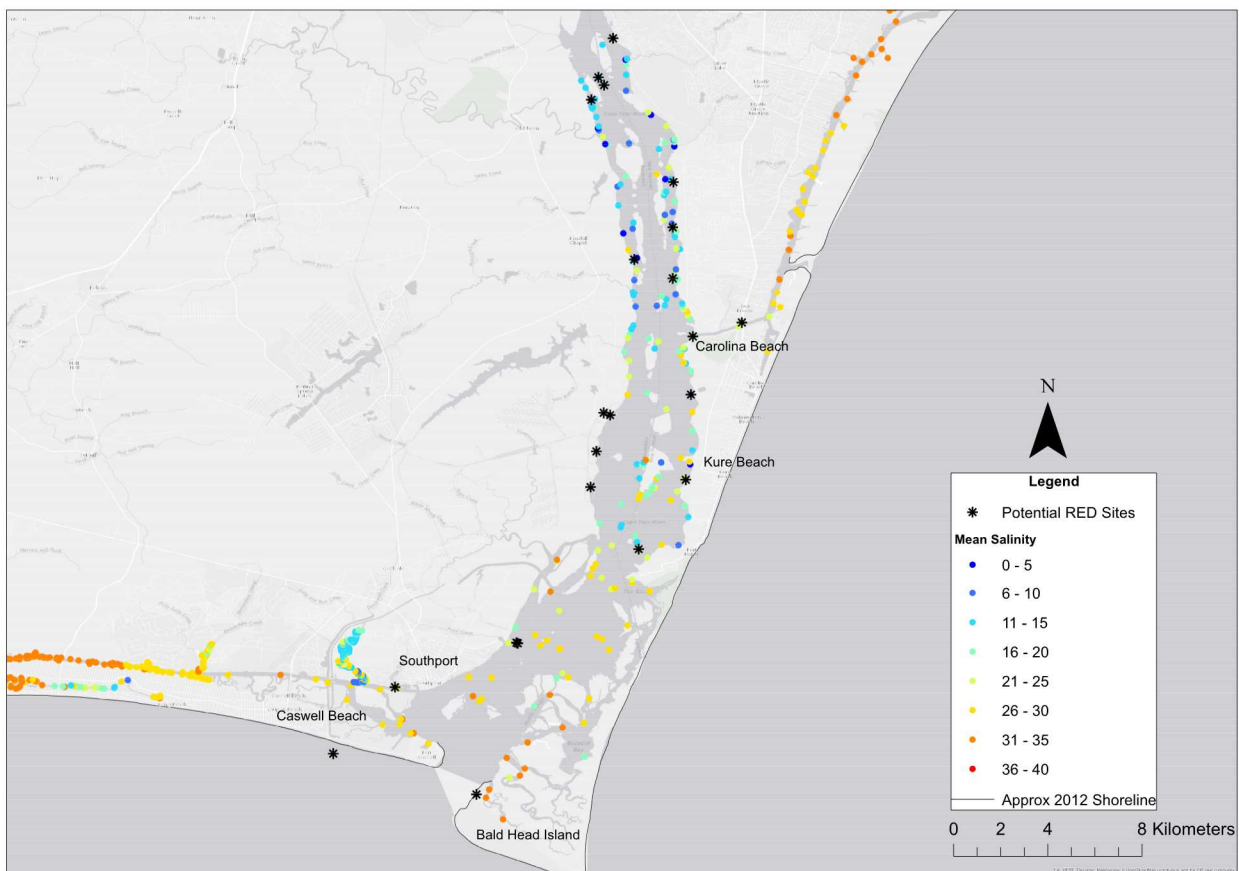


Figure 8: Potential reverse electrodesalination sites and mean salinity in the Cape Fear River estuary Potential RED sites in the Cape Fear River (black) are locations of mean low salinity (less than or equal to 1 ppt) that are within 2000m of a mean high salinity (greater than or equal to 30 ppt) point or the approximate 2012 shoreline.

The mean discharge of the Cape Fear River is $160 \text{ m}^3/\text{s}$ or $5.05 \times 10^9 \text{ m}^3/\text{year}$. Based on the theoretical complete mixing energy of the prototype RED stack membranes created in the Coronell Lab at UNC Chapel Hill, $2.94 \times 10^7 \text{ m}^3$ of influent is needed for a RED installment to have a yearly capacity of 1 MW, if seawater and freshwater are paired. For a yearly capacity of 100 MW, a RED installment utilizing seawater and freshwater would require $2.94 \times 10^9 \text{ m}^3$ of influent. Though the Cape Fear River discharge is within the required influent volume, a RED installment would need to be engineered to utilize a majority of the water discharged from Cape Fear River into the Atlantic Ocean.

Therefore, the challenges in utilizing the natural salinity gradient of the CFRE include pumping water from the lower river where the water is consistently low salinity and pumping a large enough volume water to generate power based on the desirable size and use.

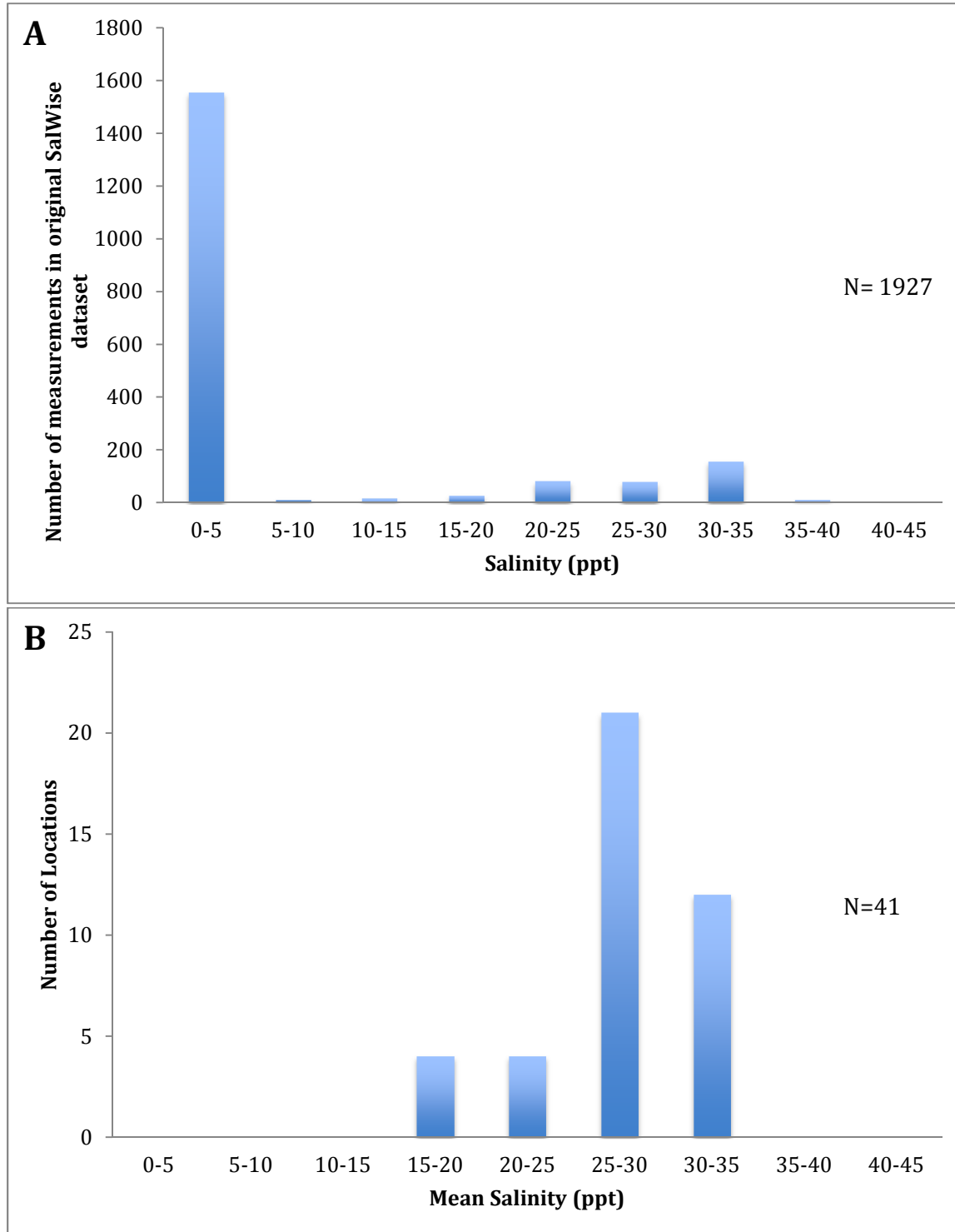


Figure 9: Frequency distribution of salinity measurements at the Cape Fear River Mouth (A) and the mean salinity distribution at the Cape Fear River Mouth (B).

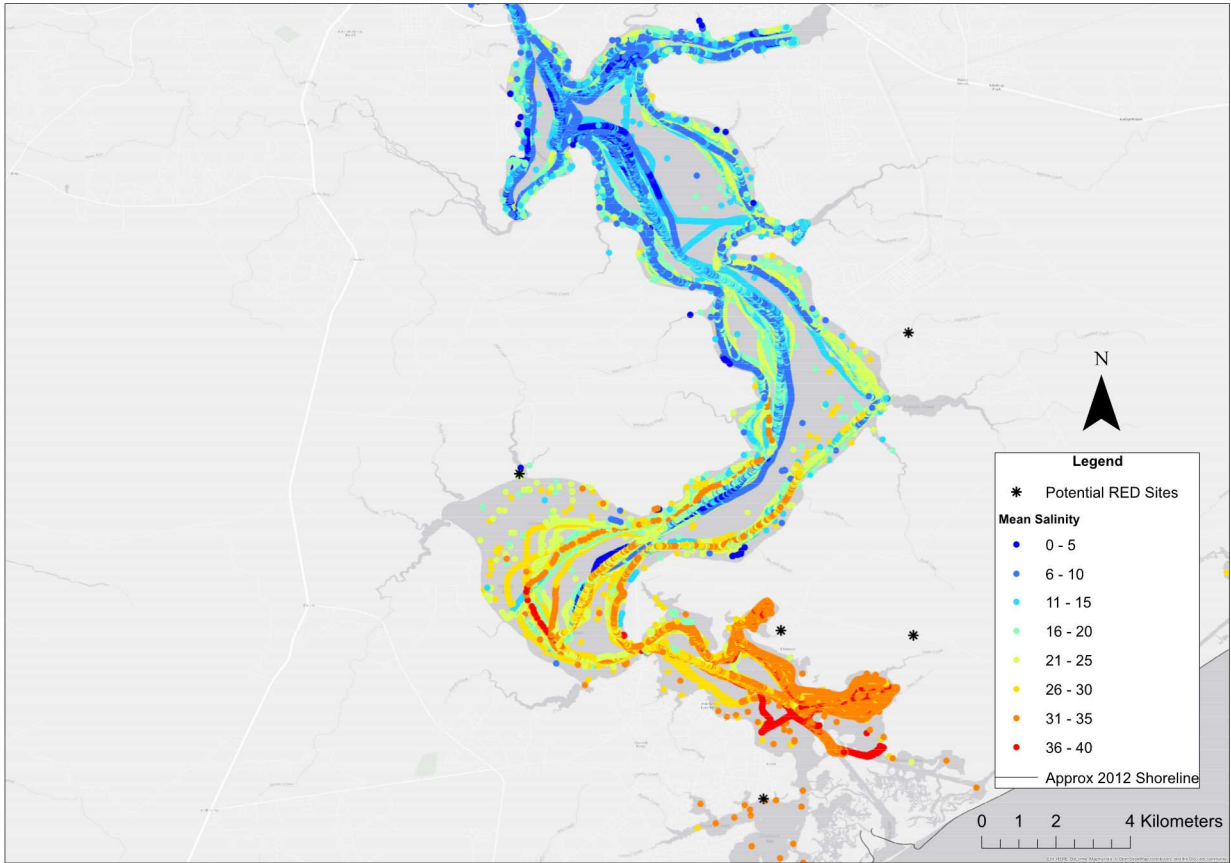


Figure 10: Potential reverse electrodialysis sites and mean salinity in the New River estuary and river mouth. Potential RED sites in the New River (black) are locations of mean low salinity (less than or equal to 1 ppt) that are within 2000m of a mean high salinity (greater than or equal to 30 ppt) point or the approximate 2012 shoreline.

Four out of the five potential RED sites in the New River Estuary are points of natural salinity gradient. The only NPDES site is the Bayshore Marina & Racket Club, which is a minor industrial process and commercial facility that discharges into Chadwick Bay. Although there is distinguishably more low salinity points at the head of the estuary and high points at the mouth of the estuary, there is not a clear line denoting the mixing zone of fresh and saline water in the estuary. In fact, mesohaline and polyhaline water can be found just south of Jacksonville. Likewise, measurements indicating oligohaline points are found at the mouth of the New River as well. I compared the frequency distribution of salinity measurements at various salinities and the frequency of mean salinity at the same locations at the mouth of the New River (Figure 11). Similar to the Cape Fear River, most locations at the mouth of the New River have a mean of 25 to 35 ppt. However, there are many measurements of salinity less than 5 ppt. This indicates that the salinity at the mouth of the river may be too variable to be utilized for RED. However, there are several small tributaries that flow into the New River for which there are no salinity data.

According to the USGS (2017), the New River has a mean discharge of 6.23 m³/s or 1.96x10⁸ m³/year. As previously mentioned, a commercial size RED installment with a yearly capacity of 100 MW would require 2.94x10⁹ m³ of influent per year if freshwater and seawater were paired in the RED stacks. A small package-size plant with a yearly capacity of 1 MW would require 2.94x10⁷ m³ of influent.

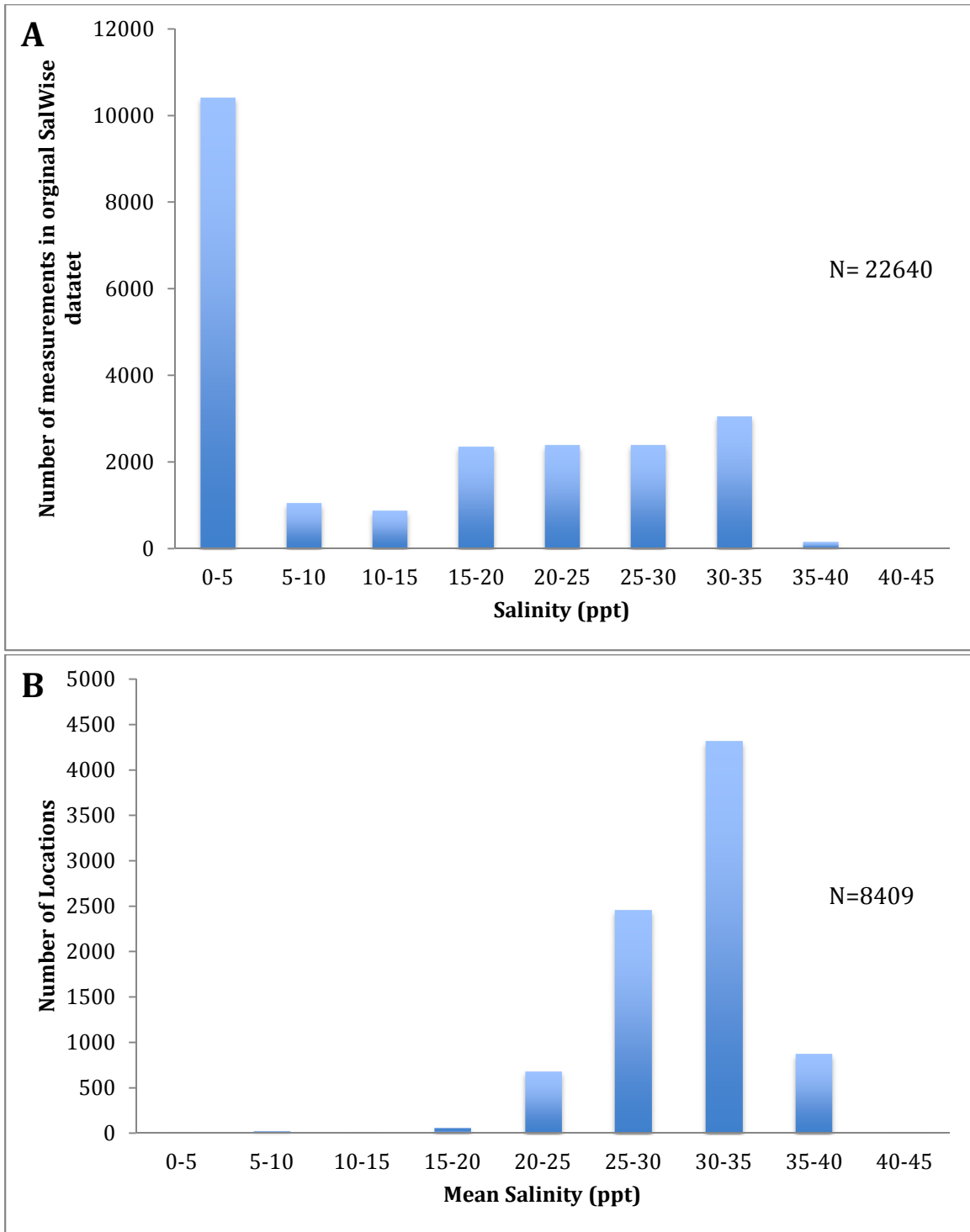


Figure 11: Frequency distribution of salinity measurements at the New River Mouth (A) and the mean salinity distribution at the New River Mouth (B).

3.1.3 NPDES Permitted Discharger Locations—WWTP

Treated wastewater is an attractive source water source for RED because of the removal of organic matter that could potentially cause biofouling in the membranes. Of the NPDES permitted dischargers that are located 2000m from a point of high salinity, I focused on wastewater treatment plants (WWTP). Depending on the level of treatment (primary, secondary, or tertiary), solid waste is settled and dissolved and suspended organic solids are removed. A degree of effluent quality must be achieved before discharging it into the receiving water. The United States Clean Water Act requires that municipal WWTP implement at least secondary treatment; however, over 30% of facilities have at least tertiary treatment. Secondary treatment can remove up to 90% of organic matter in the wastewater via aerobic bacteria metabolism. The microorganisms take advantage of the aerobic environment as wastewater is passed over a trickle filter media or passed through an aeration tank, and organic matter is broken down. Advanced methods of wastewater treatment, or tertiary treatment, include disinfection with chlorine, ozone, or ultraviolet radiation.

There are several WWTP in the coastal region of North Carolina (Table 3), with the closest high salinity body of water and the facility flow rate. This research focused on the Morehead City area, and the mean salinity at the discharge locations (Figure 12). Though there are a few instances of low salinity, potentially due to storm events or large amounts of rainfall, many WWTP discharge locations in the Morehead City have a mean salinity between 30 and 40 ppt. The consistency of high

salinity makes the area suitable for a RED installation if seawater or sound water and WWTP effluent were paired.

According to the baseline study of source water pairing and membrane power density conducted by the Coronell Lab at UNC Chapel Hill, if saltwater from the ocean or the sound is paired with treated wastewater in the RED stacks, $3.11 \times 10^7 \text{ m}^3$ of influent is required for a RED installment to have a yearly capacity of 1 MW. The flow rates of the WWTP fall well below what is required for a commercial-size power plant. For comparison, North Carolina's primary source of energy in 2015 was from nuclear power plants. The largest power plant by generation in North Carolina is the McGuire nuclear power plant operated by Duke Energy Carolinas with a generation of 19,536,002 MWh of energy in 2015. However, the largest plant by capacity is the Roxboro coal-fire power plant operated by Duke Energy Progress with a net summer capacity of 2,439 MW (US EIA 2017).

Table 3: National Pollutant Elimination Systems (NPDES) permitted wastewater treatment plants within 2000m of high salinity (30+ ppt) in Carteret County

Permit Number	Facility Name	Closest High Salinity	Approx. Distance to High Salinity (m)	Design Flow Rate (m ³ /year)
NC0021831	Beaufort WWTP	Taylor Creek	218	2.072x10 ⁶
NC0028827	Snug Harbor WWTP	Nelson Bay	396	2.763x10 ⁴
NC0047759	Taylor Extended Care Facility	Nelson Bay	156	1.934x10 ⁴
NCG530134	NC Aquarium at Pine Knoll Shores	Bogue Sound or Atlantic Ocean	433	0 (Recycled gray water system, flow not reported)

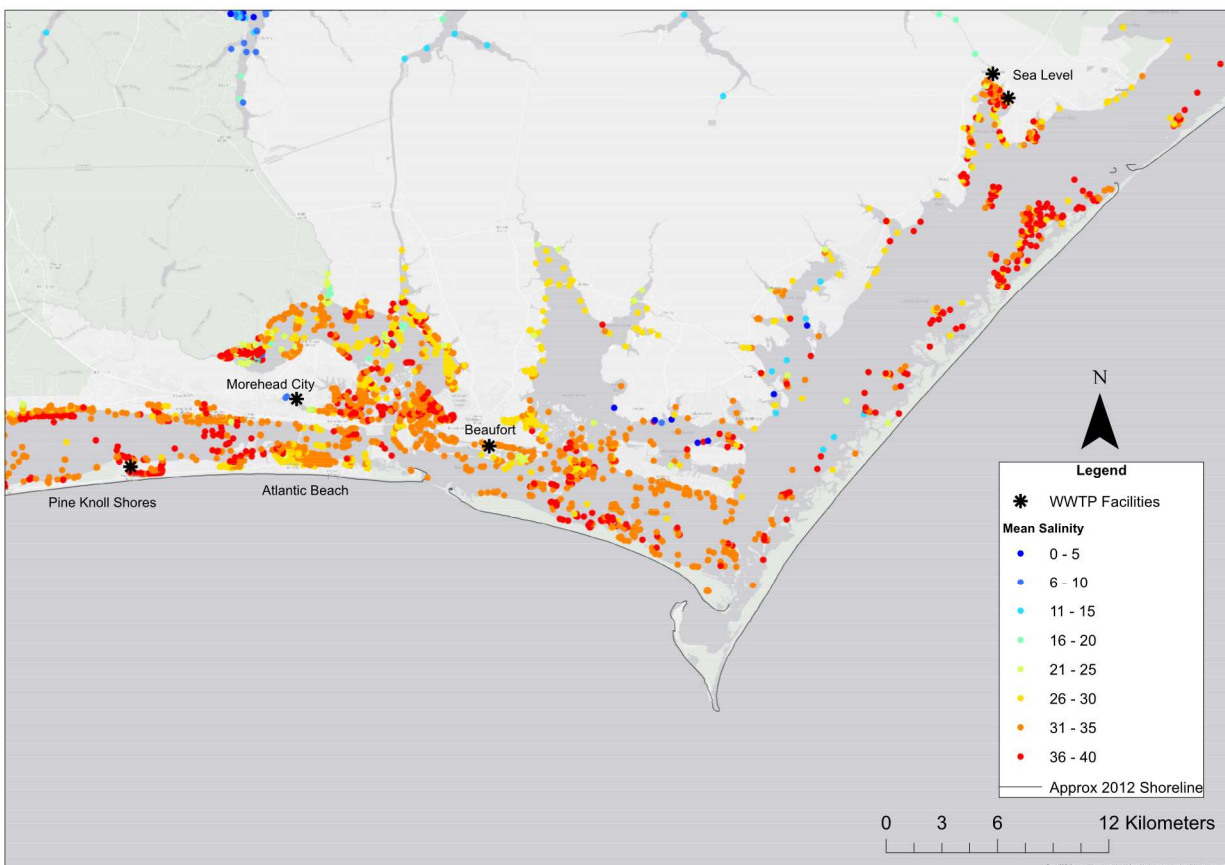


Figure 12: National Pollutant Discharge Elimination System (NPDES) permitted wastewater treatment facilities (black) in relation to mean salinity in Carteret County, North Carolina.

In the scenario of utilizing WWTP effluent as the low salinity water in a RED installment, it would be paired with the nearest high salinity water source, which consequently may be the receiving body of water into which WWTP effluent is discharged. The three high salinity bodies of water that were investigated as potential were: Nelson Bay (near Snug Harbor and Taylor Extended Care Health Facility), Taylor Creek (near Beaufort WWTP), and Bogue Sound (near the NC Aquarium at Pine Knoll Shores)(Figure 12). Bogue Sound is also within 2000m of the Morehead City WWTP, though measurements were taken on the barrier island side of the sound, rather than the mainland side where the Morehead City WWTP is located.

Near-surface and near-bottom salinity, dissolved oxygen, temperature, conductivity, pH, chlorophyll-a, and turbidity measurements were taken at all three sites in September and November 2016, and February 2017. In July 2016, the measurements were taken at mid-water column, so an accurate representation of stratification is not illustrated. Based on this data, Taylor Creek is the only high salinity water that was consistently above 30 ppt at both depths. Measurements in September were taken just after tropical storm Hermine. Bogue Sound experienced slightly lower salinity in November, though the water column was well mixed and the salinity was only slightly below 30 ppt.

Table 4: Ground truth data for salinity and SAV species composition from three potential RED sites on three dates: Nelson Bay (A), Taylor Creek (B), and Bogue Sound at Indian Beach (C). n/a = no data collected.

A		Nelson Bay			
		Jul-16	Sep-16	Nov-16	Feb-17
Salinity (ppt)	Surface	24.54	3.5	31.69	33.93
	Bottom	n/a	25.71	31.78	33.68
SAV		<i>H. wrightii</i>	<i>H. wrightii</i>	n/a	n/a
B		Taylor Creek			
		Jul-16	Sep-16	Nov-16	Feb-17
Salinity (ppt)	Surface	36.97	35.28	31.98	36.44
	Bottom	n/a	35.37	31.88	36.44
SAV		n/a	n/a	n/a	n/a
C		Bogue Sound at Indian Beach			
		Jul-16	Sep-16	Nov-16	Feb-17
Salinity (ppt)	Surface	38.06	33.03	29.01	32.54
	Bottom	n/a	33.02	29.02	32.52
SAV		<i>H. wrightii</i>	<i>H. wrightii</i>	n/a	n/a

A frequency distribution of SalWise data points at Bogue Sound, Nelson Bay, and Taylor Creek was created (Figure 13). Nelson Bay has the widest distribution of salinity measurements, though most measurements are between 15 and 35. Nelson Bay is fed by Salters Creek, a freshwater stream, and is modified by the construction of a canal connecting Nelson Bay with Long Bay. The bay experiences stratification after heavy rainfall, as we saw in the September ground truth data taken a few days after tropical storm Hermine. Therefore, it's not unusual that the SalWise data in Nelson Bay contained salinity measurements below 15 ppt. Bogue Sound has a slightly more narrow range of salinity measurements, though, as seen in the ground truth data, there are instances of salinity less than 30 ppt. Taylor Creek is the most consistent in having a salinity of 30 ppt or higher, making it a more reliable high salinity source water for RED. Though there are a few

SalWise measurements that are below 25 ppt, a majority of measurements are between 25 and 35 ppt. All of the ground truth measurements at Taylor Creek were between 31 and 37 ppt. The ground truth data confirms that the SalWise dataset is reasonably accurate in representing the salinity regime at the potential RED sites for all water depths.

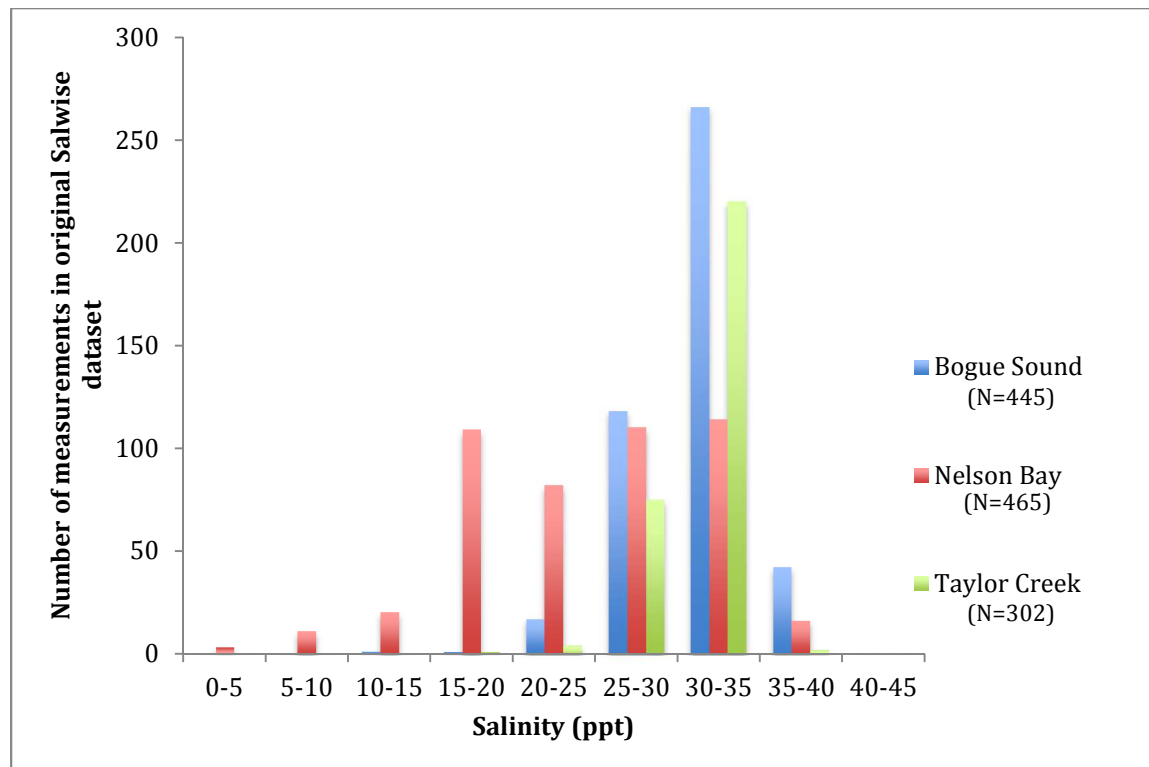


Figure 13: Frequency distribution of SalWise salinity measurements at potential reverse electrodialysis sites along the North Carolina coast.

3.2 Submerged Aquatic Vegetation and Salinity

Submerged aquatic vegetation (SAV) has been used in the past as a bioindicator of the effects of effluent from desalination plants because of their high sensitivity to changes in salinity (Fernández-torquemada & Sánchez-lizaso, 2007). In the Mediterranean, *Posidonia oceanica* was shown to have high epiphyte load and nitrogen content in leaves, along with high necrosis marks in areas affected by the desalination effluent (Gacia, Invers,

Manzanera, Ballesteros, & Romero, 2007). Changes in salinity can also modify the carbon and oxygen balance in the plant, potentially leading to long term changes in growth and proliferation (M. S. Koch, Schopmeyer, Kyhn-Hansen, Madden, & Peters, 2006).

Due to the mixing of low salinity water and high salinity water, RED effluent will have a salinity that is equal to the average of the two source waters. This effluent has the potential to have a salinity that is different than that of the receiving water.

Within North Carolina, the dominant SAV species found growing in relatively higher salinity environments are *Zostera marina* (eelgrass), *Ruppia maritima* (widgeon grass), and *Halodule wrightii* (shoal grass). There are also several low salinity tolerant species such as *Vallisneria americana*, *Potamogeton pectinatus*, *Myriophyllum spicatum*, *Ruppia maritima*, and *Naja guadalupensis*. Table 5, from Ferguson and Wood (1994) and the NC Coastal Habitat Protection Plan show the range and average environmental conditions for each of the SAV species found in coastal North Carolina waters (NCDEQ, 2016). While each species has a particular life history and subsequent requirements, the primary habitat requirements for NC SAV species are well understood. The factors that limit SAV distribution, growth, and abundance are light, salinity, wave energy, temperature, sediments and nutrients (NC Department of the Environment and Natural Resources, 2005). Previous research has shown that reduction of growth and primary production, reduced meadow coverage, necrosis, and even shoot death can occur in SAV species when exposed to water more saline or less saline than the average ambient water in which it grows (Gacia et al., 2007; Sánchez-Lizaso et al., 2008).

Table 5: Submerged aquatic vegetation species native to North Carolina and the range and average environmental conditions necessary for growth. (Ferguson and Wood, 1994)

SAV species	Environmental parameter					
	Salinity (ppt)		Secchi depth m (ft)		Water depth m (ft)	
	Range	Average	Range	Average	Range	Average
<i>HIGH SALINITY SEAGRASS</i>						
Eel Grass	10 - >36	26	0.3 - 2.0 (1.0 - 6.6)	1.0 (3.3)	0.4 - 1.7 (1.3 - 5.6)	1.2 (3.9)
Shoal Grass	8 - >36	25	0.4 - 2.0 (1.3 - 6.6)	1.0 (3.3)	0.1 - 2.1 (0.3 - 6.9)	0.8 (2.6)
Widgeon Grass	0-36	15	0.2 - 1.8 (0.7 - 5.9)	0.7 (2.3)	0.1 - 2.5 (0.3 - 8.2)	0.8 (2.6)
<i>LOW SALINITY SEAGRASS</i>						
Redhead Grass	0-20	1	0.4 - 1.4 (1.3 - 4.6)	0.9 (3.0)	0.4 - 2.4 (1.3 - 7.9)	0.9 (3.0)
Wild Celery	0-10	2	0.2 - 2.0 (0.7 - 6.6)	0.6 (2.0)	0.2 - 2.3 (0.7 - 7.6)	1.0 (3.3)
Eurasian Watermilfoil	0-10	2	0.2 - 1.4 (0.7 - 4.6)	0.6 (2.0)	0.5 - 2.4 (1.6 - 7.9)	1.1 (3.6)
Bushy Pondweed	0-10	1	0.2 - 2.0 (0.7 - 6.6)	0.7 (2.3)	0.5 - 1.7 (1.6 - 5.6)	1.0 (3.3)
Sago Pondweed	0-9	2	0.2 - 0.4 (0.7 - 1.3)	0.3 (1.0)	0.6 - 0.9 (2.0 - 3.0)	0.8 (2.6)

SAV is widely distributed over the coast of North Carolina, and is present in close proximity to potential RED sites utilizing WWTP effluent and ambient water (Figure 14 and 15). SAV is ephemeral and changes from year to year, but it is clear that SAV is present near the WWTP effluent discharge in Pine Knoll Shores and Sea Level. Historically, there has not been SAV in Taylor's Creek, which is consistent with what is shown in this SAV distribution data.

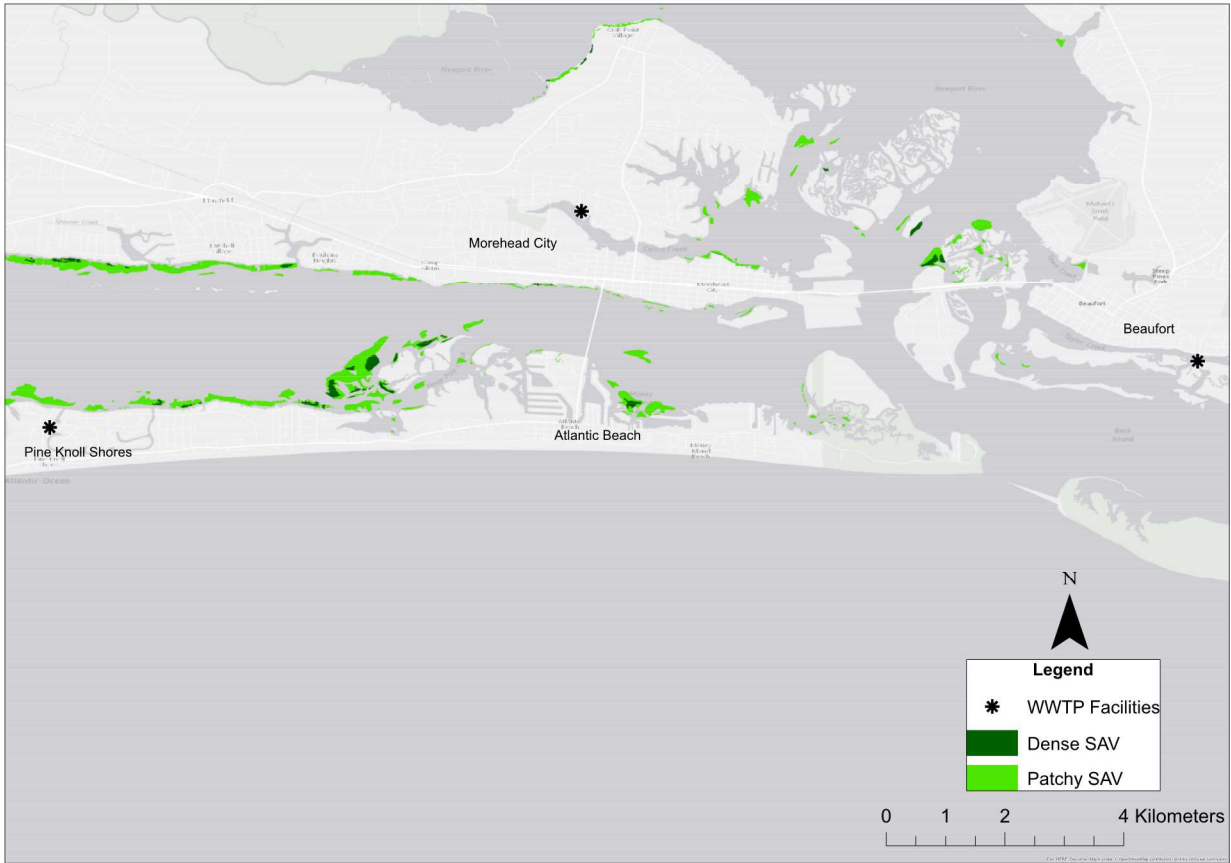


Figure 14: Submerged aquatic vegetation (SAV) distribution in relation to wastewater treatment plant facilities in the vicinity of Morehead City, Beaufort, Atlantic Beach, and Pine Knoll Shores, North Carolina

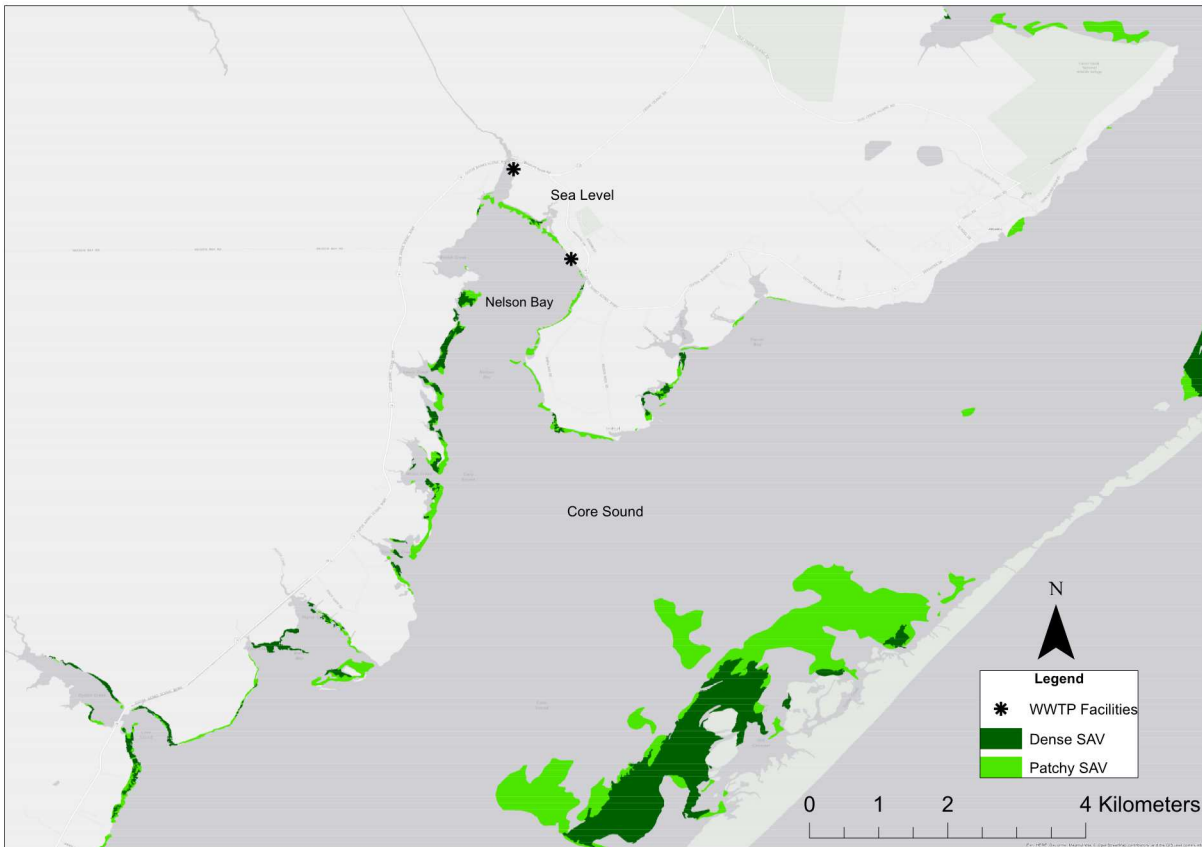


Figure 15: Submerged aquatic vegetation (SAV) distribution in relation to wastewater treatment plant facilities in Sea Level, NC

3.2.1 Mechanisms of SAV Adaptation to Salinity Stress

As summarized by Touchette (2007), salt damage can occur due to four physiological processes in plants. These are (i) loss of ionic equilibrium due to an influx and accumulation of Na^+ and Cl^- , (ii) the disruption of cellular metabolism due to Na^+ disturbance of vital enzymes, (iii) loss of osmotic balance following ion accumulation, and (iv) decreased photosynthetic efficiency, coupled with the increased production of reactive oxygen species (Touchette, 2007). Seagrass species employ various morphological and physiological mechanisms in response to salinity stress.

Mechanisms employed to prevent salt damage must permit necessary K^+ uptake while minimizing harmful Na^+ accumulation. Previous research suggests that Na^+ ions have the ability to use the transport systems developed for K^+ to cross the cell membrane (Fernández, Garcia-Sanchez, & Felle, 1999). Therefore, plants could have a problem maintaining ion potentials across the cell membranes. Some species such as *Z. marina* have high selectivity that strongly favors K^+ over Na^+ and may have sodium exclusion mechanisms such as proton pumps that permit plants to live in environments with elevated Na^+ concentrations (Fernández et al., 1999). It is likely that selective ion flux and ion partitioning between cytoplasm and vacuoles play a role in the maintenance of ion concentrations within plant cells, although more research is needed to fully understand the mechanisms behind these processes (Touchette, 2007).

Previous research found that most plants increase ion concentrations within cells under hyperosmotic stress. Once an osmotic equilibrium is reached, the plants replace the metabolically disruptive ions with solutes (Murphy, Kinsey, & Durako, 2003; Touchette,

2007). It is predicted that plants within their optimal range of salinity tolerance are able to acclimate and achieve equilibrium fairly quickly, but may take several days or weeks to acclimate when exposed to water outside of their typical salinity distribution (Berns & Muller, 2003; Touchette, 2007). There are many organic osmolytes used to achieve osmotic balance; sucrose and proline may be the principle osmolytes involved in *Z. marina* and *R. maritima* (Murphy et al., 2003; Touchette, 2007; Ye & Zhao, 2003). Although organic osmolytes production involves extra energy expenditure, research has shown that high concentrations of these solutes only minimally inhibit enzymes, compared to the detrimental inhibition due to unwanted ions. Osmolytes also help in stabilizing macromolecules (Kirst, 1990; Touchette, 2007; Yancey, Clark, Hand, Bowlus, & Somero, 1982). These organic osmolytes may also exhibit an array of protective properties for plant cells (Touchette, 2007).

Other physiological adaptations to salinity stress include plant tissue and cell ultrastructure manipulation. In response to increased salinity, previous research shows an increased epidermis and mesophyll thickness (Parida & Das, 2005), increased exodermis thickness, and increased cell layers of the cortex (Hose, Clarkson, Steudle, Schreiber, & Hartung, 2001). An increase in the cell layers of the cortex could also serve as a barrier to prevent the flow of ions to the aerial parts of the plant (Ferreira et al., 2016). Previous research also reveals ultrastructure manipulations under saline stress, such as an increase in the number of invaginations of the plasma membrane and cell wall (Ferreira et al., 2016; Iyer & Barnabas, 1993; Jagels & Barnabas, 1989). The invaginated membrane structure increases the membrane surface area, therefore increasing the cellular capacity for solute influx and efflux. Likewise, the electrochemical gradients can aid in the removal of harmful

ions from the cytosol by transporting them to the vacuole or out of the cell. These mechanisms are useful to seagrasses as they attempt to maintain a favorable membrane potential.

Another mechanism for adaptation and acclimation to salinity stress is cell wall elasticity. Turgor pressure within the cell may change rapidly due to water fluxes into or out of the cell, based on the environmental salinity that the cell is facing, although the cell wall elasticity varies among plant species (Touchette, 2007). Plants with lower cell wall elasticity have more flexible cell walls and therefore are more tolerant to short term salinity fluctuations. Conversely, plants with higher cell wall elasticity are more rigid and small fluctuations in cell volume can cause substantial changes in turgor pressure (Touchette, 2007), however more studies are needed to fully understand the roll of cell wall elasticity in withstanding salt stress.

Photosynthesis and respiration are two processes that can be influenced by salinity changes or salt stress. Photosynthesis is inhibited due to declining chloroplast content, inhibition of electron flow, decreased photosystem function, diminished rubisco abundance and activity, and changes in chloroplast ultrastructure (Touchette, 2007). Previous research has shown that such hypersaline conditions actually increases respiration rate, while respiration rates in distilled water was found to below normal (Berns & Muller, 2003; Biebl & McRoy, 1971; Fernández-Torquemada & Sánchez-Lizaso, 2005; Amanda E. Kahn & Durako, 2006). Salinity could also affect seed germination and survival.

3.2.2 Salinity and NC Saltwater SAV Species Annotated Bibliography

The following section is an annotated bibliography of literature that explores the effects of salinity on SAV function. I opted to use a narrative summary structure so that I could more thoroughly elaborate on methods and results in a way that a summary table of optimal ranges would not allow. In many cases, literature did not come to a clear conclusion about the salinity tolerance ranges. A summary narrative for each publication was the most efficient and thorough way to present how salinity affected SAV plants at the experimental or observed salinity levels. The annotated bibliography includes a citation for each publication that was reviewed and a short narrative summary.

Plasmatic resistance and rate of respiration and photosynthesis of *Zostera marina* at different salinities and temperatures (Bieble and McRoy, 1971)

The purpose of this study is to explore the salinity tolerance of two forms of *Zostera marina* found in Izembek Lagoon, Alaska Peninsula: tidepool and subtidal. Samples from each of these locations were placed in various concentrations of seawater to test the osmotic resistance and rates of photosynthesis and respiration. The treatment levels for this experiment are: 0 (distilled water), 1.0 (average salinity of seawater), 2.0 times seawater, 3.0 times seawater, and 4.0 times seawater. Dilutions of 0.1, 0.2 and 0.3 times seawater are also tested. No significant difference was observed between populations of the subtidal and tidepool eelgrass. In both populations, 4 x concentration of seawater resulted in partial death or death. There was indication of increased heat resistance with increased salinity. Respiration was significantly suppressed when exposed to distilled water. Respiration decreased only slightly in water that was 2.0 times the concentration of seawater. Respiration in 3.0 times seawater was slightly higher than respiration in 1.0

times seawater. However, photosynthesis was significantly suppressed by any concentration other than seawater. The authors concluded that although *Z. marina* is known to be a euryhaline species that can tolerate a wide range of salinity, more thorough studies must be conducted to understand how different populations adapt and survive in their environments in relation to seasonal and diurnal variations (Biebl & McRoy, 1971).

Salinity effects on *Ruppia maritima* L. cultured in vitro (Bird et al., 1993)

In this *in vitro* experiment, the effects of salinity on rhizome growth and rooting are explored. *Ruppia maritima* plants were collected from Beaufort, North Carolina and transported to the laboratory. Five experimental levels were created using artificial seawater: 0,5,10,15 and 20 ppt, with 7 replicates per salinity level.

The growth and rooting experiments used the same experimental salinity levels. The number of new nodes was highest in plants cultivated in 0 ppt or 5ppt salinity, and intermediate growth of new nodes occurred in 10 ppt artificial seawater. The lowest growth occurred in 15 to 20 ppt artificial seawater. Salinity and carbon source had significant effects on root length-- plants growing in media of biocarbonate and 5, 10, and 15 ppt salinity had significantly longer roots. The presence of root hairs was also observed throughout the growth and rooting experiments, and visible root hairs were only found on roots that were 12 mm or longer. Overall growth rate was at least twice as high at lower salinity than the growth rate at 15 or 20 ppt. This experiment gives insight into the optimal growing conditions for *in vitro* cultivation. *R. maritima* grows rapidly in lower salinity that enhances root growth. This information is important in restoration projects—it is important to know ideal cultivation conditions as well as the salinity tolerance levels for

transplanting individuals into an ideal bay, sound, or estuary (Bird, Cody, Jewett-Smith, & Kane, 1993).

Reproduction of Coastal Submerged Macrophytes in Experimental Communities (Bonis et al.,1993)

This study explored the effects of salinity on reproduction of coastal submerged macrophytes, including *Ruppia maritima*. The results were interpreted in terms of species isolation or competitions based on the spatio-temporal structure and gradient of environmental conditions. The authors hypothesized that composition of vegetation is correlated with composition of the seed bank, and that biomass does not accurately predict the number of seeds produced. The purpose of this study was to explore whether all species respond in similar ways to salinity stress via reproductive responses such as seed production. Total reproduction, reproductive effort, and investment in seed production were the measurements of reproduction in this experiment. The five salinity treatments used are 0,1,2,4, and 6 g/L of Cl⁻. In the spring, reproductive efforts increased with salinity (highest in 2 g/l Cl⁻, experiment tested up to 6 g/l Cl⁻). Reproduction was strongly correlated with biomass production in the spring. Lowered salinity may result in reduced reproductive effort. The effects of salinity variation can be cumulative, as seed bank formation might be disrupted. Patterns of seed production based on environmental parameters that exist in gradients, such as salinity, could give indication of species dominance in an area. The results indicate that salinity may affect community composition. If there is a decrease in growth and reproductive effort in certain species, there may be

fewer individuals of that species or fewer seed germinations the next season (Bonis, Grillas, van Wijck, & Lepart, 1993).

An index to access and monitor the progression of wasting disease in eelgrass *Zostera marina* (Burdick et al., 1993)

The purpose of this study was to explore the effects of environmental parameters on the persistence of wasting disease in *Zostera marina* meadows. Mesocosms were used to examine the presence of disease in various controlled conditions that were subsequently compared with field conditions. A “wasting index method” was utilized to classify the degree to which shoots were affected by the disease. This index was used to classify the persistence of the disease in *Z. marina* meadows in Great Bay Estuary in New Hampshire. Because salinity has been proposed to affect the die-off of *Z. marina*, and the authors hypothesized that there was a correlation between increased salinity and increase in wasting index. Likewise, a decrease in salinity below a threshold of 20 to 25 ppt reduced the spread of the disease and allowed for plant recovery and growth. Although salinity is not the sole determinant of disease spread, it is an influential environmental parameter (Burdick, Short, & Wolf, 1993).

Production ecology of *Ruppia maritima* L. s.l. and *Halodule wrightii* Aschers, in two subtropical estuaries (Dunton, 1990)

This was an observational study conducted in two south Texas estuaries: the Guadalupe Estuary that has a salinity range of 0 to 25 ppt and the Nueces Estuary that has a salinity range of 32 to 38 ppt. The authors were primarily interested in the growth rates,

seasonality of growth, time of flowering, and persistence of over-wintering populations of *Ruppia maritima* and *Halodule wrightii*. The two estuaries in question are characterized by differences in freshwater and nutrient input. Two study sites in each estuary were established, for a total of four study sites. Depth and sediment characteristics were recorded for all sites. The Nueces Estuary was is protected from waves and adverse sea conditions, while the Guadalupe Estuary was more exposed. Growth rate was measured in terms of lead elongation rates, shoot production, plant density, and biomass. A leaf-clipping technique was used to estimate leaf elongation, and biomass was measured as dry weight in the laboratory. Estimates of epiphyte biomass were also made. Temperature, salinity, photosynthetically active radiation (PAR), and dissolved inorganic nitrogen (DIN) measurements were taken within the water column at each site. In terms of seasonality of growth, it was found that *R. maritima* completed its life cycle over a period of three to five months. Overwintering populations of *R. maritima* occurred at low nutrient/high salinity site, but not at the high nutrient/low salinity site. However, shoot production of *R. maritima* did not differ between the two sites of differing salinity and DIN regime and no significant correlation was found between *R. maritima* shoot production and salinity. These results indicate that salinity and DIN have little direct effect on *R. maritima* growth dynamics, however increased nutrients due to freshwater input might indirectly affect growth by promoting rapid growth of algae that can significantly attenuate light. *Halodule* was absent in the low salinity estuary, but had a large overwintering population in the high salinity estuary (Dunton, 1990).

The influence of salinity on growth, morphology, leaf ultrastructure, and cell viability of the seagrass *Halodule wrightii* Ascherson (Ferreira et al., 2016)

Because little is known about how *Halodule wrightii* adapts to alterations in salinity, the purpose of this study was to determine how salinity changes effect growth, morphology, leaf ultrastructure, and cell viability. Specimens were collected in Lagoa da Coneição Channel in Florianopolis, Santa Catarina, Brazil. Samples were collected from meadows that were at a depth of approximately 1m. Samples were places in aquaria with a salinity of 35, which is approximately the salinity at the collection site. The three salinity treatment levels were 25, 35 (control), and 45. These salinities were chosen based on the natural variation at the sample sites. Overall, the results show that *H. wrightii* has the ability to grow even under hypersaline conditions. Plants had higher cell viability at salinity 35, cell viability decreased at 25 and 45. Greater number of mitochondria in samples in salinity 25, retraction of plasma membrane, more hydropotens, greater number of invaginations of plasma membrane in plants in salinity 45. Samples from the salinity 45 treatment also showed the least amount of chloroplasts. Maximum growth occurred in salinity 35, however there was no statistically significant difference between the three treatment levels. The authors concluded that the results demonstrated that salinity of 25 and 45 reduced cell viability. However, *H. wrightii* can tolerate salinity below but not above 35 (Ferreira et al., 2016).

Short-term impacts of salinity pulses on ionic ratios of the seagrasses *Thalassia testudinum* and *Halodule wrightii* (Garrote-Moreno et al., 2015)

This study explored the effects of pulse salinity events on the ion accumulation in *Halodule wrightii* and *Thalassia testudinum*. Rhizome fragments were collected from

shallow beds at Big Lagoon, Florida. These fragments were placed in aquaria and were exposed to different salinity treatments: 10, 23 (ambient salinity and control), 30, 40, 50, and 70 psu. The plant fragments were not allowed time for acclimation; all salinity changes were abrupt. The response parameter measured was ion accumulation. Results showed that Cl^- ions increased with increased salinity. Na^+ also increased with increasing salinity. K^+ concentrations were lower in the highest salinity compared to the other treatments. Ca^{2+} concentrations also decreased with increased salinity in both species. Ion accumulation in seagrass leaves could aid in osmotic adjustment, however large accumulations could be harmful and therefore some seagrass species store ions in vacuoles. *H. wrightii* had higher ratios (K^+/Na^+ and $\text{Ca}^{2+}/\text{Na}^+$) at 23 psu, which was the ambient salinity of the study site. Therefore, it may be that *H. wrightii* is physiologically better suited to withstand high salinity compared to *T. testudinum*. The authors concluded that 50 psu is the salinity that leads to reduced growth and decreased photosynthesis. This could be because of the loss of osmotic balance and the depletion of integral ions. Both species in this study had high tolerance for salinity increases, but short-term pulses of extremely high salinity were detrimental to both SAV species (Garrote-Moreno et al., 2015).

Seed-bank development, germination and early seedling survival of two seagrass species from The Netherlands: *Zostera marina* L. and *Zostera noltii* Hornem (Hootsmans et al., 1987)

This study utilized field data collection and laboratory experiments to explore seed banks and flowering of *Zostera marina* in the Netherlands. Germination of *Zostera* seeds was investigated in relation to environmental parameters such as salinity, temperature, stratification, desiccation, and anaerobiosis. Samples were collected biweekly from field sites,

and seeds and flowering shoots were enumerated in the laboratory. Germination experiments were carried out in the laboratory; the salinities tested were 1, 10, 20, 30, and 40 ppt. Experiments were also designed to test the effect of desiccation and anaerobic conditions. Seedling survival was calculated and reported in relation to the initial amount of seeds and the amount of seeds that germinated. From the field observations, the authors found that *Z. marina* flowered between June and October. It was calculated that a plant with 10 shoots per m² produces about 200 seeds per m². Germination percentage was significantly effected by temperature, salinity, and stratification. Optimum germination occurred at 30 °C and 1.0 ppt, and germination was lower at higher salinity and lower temperatures. *Z. marina* seedlings experienced optimal survival at 10°C and 20 ppt. This corresponds with other research that has found that *Z. marina* requires low salinity for germination, but grows best in mesohaline water. Stratification was found to increase germination in *Z. marina*, showing that stratification can overcome germination inhibition due to high salinity. There was a detrimental effect of desiccation on germination of *Z. marina* seeds. The authors conclude that ecotypic variation could explain the enormous plasticity of the *Z. marina* species across the different habitats (Hootsmans, Vermaat, & Van Vierssen, 1987).

Variation in leaf ultrastructure of *Ruppia maritima* L. along a salinity gradient. (Jagels and Barnabas, 1989)

In this study, leaf ultrastructure of *R. maritima* individuals growing in high and low salinity was compared. Individuals of this species were collected from three sites along a salinity gradient in a tidal bay near East Franklin, Maine. The bay is fed by a freshwater

stream and is connected to the open ocean in Frenchman's Bay. The three sites have a mean salinity of 8, 12, and 24 ppt, respectively. Collected plants were transported to the laboratory, where light and electron microscopy were used to determine differences in ultrastructure between plants from three locations along the salinity gradient. The leaf epidermal of low-salinity (5 ppt) plants compared to high salinity (28 ppt) plants had larger central vacuoles, fewer chloroplasts, reduced plasmalemma area, fewer mitochondria, thinner walls, reduced extracytoplasmic volume, and symplastic connections with mesophyll cells. The authors concluded that the relationship between leaf ultrastructure and salinity might be an indication of the ability to adapt to changing salinity within its limited geographical area. With the ability to regulate cytoplasmic turgor pressure and alter the ultrastructure of the cell wall, SAV may be able to adapt to salinity variability. This is especially pertinent in estuarine locations where saltwater and freshwater mix, and it could point to resilience in areas susceptible to anthropogenic salinity alteration (Jagels & Barnabas, 1989).

The effect of salinity and ammonium on seed germination in *Ruppia maritima* from Florida Bay (Kahn and Durako, 2005)

This is an experimental study that investigated the germination of *Ruppia maritima* seeds from Florida Bay in response to salinity manipulation, different light regimes, and varying ammonium treatments. Seeds were collected in early August and kept in ambient seawater as they were transported to the laboratory in Wilmington, NC. Eight treatment salinities were used: 0-70 in increments of 10. Germination was monitored throughout the experimental period. For the direct salinity treatment experiment, salinities were not

changed throughout the experimental period. In the salinity adjustment and ammonium experiment, salinity was increased every three days. At every salinity increment of 10, ten seedlings were maintained at that salinity for the duration of the experiment. Overall germination was low in the direct salinity treatment, and in the light:dark treatment, germination occurred in the 0, 10, and 20 salinity treatments. Only one seed germinated in the dark treatment, with the salinity treatment of 10 ppt. In the salinity adjustment experiment, the media salinity at the time of seed germination was recorded. Germination only occurred at salinities between 0 and 28, with the majority (70%) germinating between 0 and 10 ppt. There was no germination above 28 ppt. The authors conclude that this research supports the prediction that freshening events in Florida Bay could result in the expansion of this species of SAV; however, the influence of other water chemistry parameters such as temperature and nutrients must be considered as well (A. E. Kahn & Durako, 2005),

Short Communication Influence of salinity and temperature on the germination of *Ruppia maritima* L . from the North Atlantic and Gulf of Mexico (Koch and Dawes, 1991)

This was an experimental study with the purpose of investigating the influence of salinity and temperature on seed germination in the species *Ruppia maritima*. This species can have an annual or perennial life cycle based on environmental conditions. Therefore, it is hypothesized that seed germination is also controlled by environmental conditions. Plants of this species were sampled from two latitudinally isolated populations—one from the Gulf of Mexico on the west coast of Florida and the other from Pamlico Sound in North Carolina. Three different salinities were tested: 0, 15, and 30 ppt, using diluted seawater. At

23°C. Different temperatures were also tested: 17, 23, and 29 °C at 0 ppt salinity. *R. maritima* seeds from NC had an earlier germination time and a significantly higher germination rate than seeds from FL. However, germination rate of the NC seeds was significantly lower at 30 ppt compared to 0 and 15 ppt (which had no sig. difference). The study suggests that there was an ecotypic difference in seed germination patterns due to salinity but not temperature (E. W. Koch & Dawes, 1991).

Tropical seagrass species tolerance to hypersalinity stress (Koch et al., 2007)

The goal of this study was to explore the upper salinity tolerance of two species of seagrass using a laboratory mesocosm experimental design. The authors tested seagrass hypersalinity tolerance under two scenarios: pulsed rapid increase that mimics exposure to brine effluent and gradual increase, such that seagrass might experience in field conditions due to sea level rise or show evaporative basins. Eight salinity treatment levels were used: 35, 40, 45, 50, 55, 60, 65, and 70 psu, and samples collected from north-central Florida Bay were immediately placed in the mesocosm tanks. The parameters observed over the course of this study are leaf osmolality, mortality, growth rate (defined by new shoot production), and quantum efficiency of photosystem II and chlorophyll fluorescence. In *R. maritima*, leaf osmolality increased with salinity from 50 to 60 psu, then dropped at 70 psu. At ambient temperature, there was a greater relationship between salinity and osmolality from 37 to 60 psu. None of the treatments resulted in total shoot mortality, as *R. maritima* still flowered at 70 psu. This study presented evidence that *H. wrightii* was the most salt tolerant of the species tested, with maintaining or increasing shoot numbers in all hypersaline treatments. There was only a significant reduction in number of new shoots at

70 psu. In *R. maritima*, shoot reduction was greatest at 55 psu and higher salinity. *H. wrightii* only experienced reduced quantum efficiency in the highest salinity treatments. There was little change in *R. maritima* quantum efficiency with increasing salinity, and only a slight decrease at 70 psu. The authors concluded that the adult form of seagrasses show thresholds of hypersalinity tolerance based on the rate at which salinity increases. In pulsed events, such as discharge from a reverse osmosis plant, the threshold may be lower than in conditions where salinity gradually increases (M. S. Koch et al., 2006).

A study of the germination and viability of *Zostera marina* L. seeds (Lamounette, 1977)

This thesis used laboratory experiments to determine the environmental factors that control seed germination in *Zostera marina* plants from the Great South Bay, NY. Flowering plants were harvested during June and July, and seeds from collected and sterilized, then placed in petri dishes with 30 mL of 10 ppt water. Three salinity treatment levels were used: 10 ppt, 19 ppt, and 28 ppt. Seeds were observed over the three week study period and development was classified as 1) No development, 2) splitting of seed coat, 3) erection of plumule, 4) plumule growth, 5) appearance of chlorophyll, and 6) development of root hairs. Seed viability was also explored in this study. Field observations were used to look for the presence of seedlings in the determined study area. Maximum germination occurred between November and February. Germination also varied with salinity; germination doubled as salinity was decreased from one treatment level to the next lowest. This research also tested the effects of temperature on salinity. Low strength seawater (10 ppt) was found to significantly increase germination rates. This research may

be evidence that although *Z. marina* is able to tolerate high salinity, salinity variation may actually be beneficial to this species as germination occur at greater rates during freshening events (Lamounette, 1977).

Effects of salinity changes on growth of *Ruppia maritima* L. (La Peyre and Rowe, 2003)

Although *Ruppia maritima* is known to be able to withstand a wide range of salinity averages, the authors of this study suggest that salinity variation may be detrimental. This study utilizes a greenhouse experimental design to compare the growth rates of individuals of the *R. maritima* species after pulsed salinity changes. Individual ramets were collected from Lake Pontchartrain, LA. In this experiment, there were five treatment levels that began at 10 g/L. This initially salinity was created using artificial seawater. Two of the treatments had one salinity change and three treatments had two salinity changes. Salinity levels were maintained for three weeks and salinity changes occurred gradually over 48 hour periods in order to mimic salinity changes that frequently occur on the northern coast of the Gulf of Mexico. This includes both freshening events and increased salinity from storm events. Relative growth rate was significantly lowered with a short freshwater pulse and pulsed salinity increase (when salinity was raised twice). Pulsed changes in salinity (10 g/L over 48 hours) either fresher or more saline, may negatively affect growth in the short term. This study indicates that both freshening and high salinity events can negatively affect *R. maritima* short-term growth rates. However, with increased storm events, rising sea level, and overall changing salinity regime of the sound due to climate change, short-term effects on growth rate may translate to widespread changes in species growth rates

and distribution. However, the results of this study do coincide with the previously suggested optimal salinity range for *R. maritima* at 10 to 20 g/L (La Peyre & Rowe, 2003).

The influence of salinity on seagrass growth, survivorship and distribution within Biscayne Bay, Florida: Field, experimental and modeling studies (Lirman and Cropper, 2003)

This study utilizes field surveys, salinity exposure experiments, and seagrass simulation models to evaluate the distribution and abundance of several SAV species in Biscayne Bay, Florida. Blade density of the three main seagrass species was determined by surveying random sampling locations within the bay. The sampling locations were divided into two salinity regions, one with a mean salinity of 23 ppt and one with a mean salinity of 33.1 ppt. Salinity exposure experiments were conducted by placing seagrass rhizomes into a microcosm. The salinity treatments used were 5, 10, 15, 20, 25, 30, 35 (ambient), 40 and 45 ppt. Production in *Halodule wrightii* was estimated using the clipping method and after two weeks extension rates were determined. In *H. wrightii*, the highest mean blade extension occurred at 35 ppt; the lowest mean blade extension rate occurred at 45 and 5 ppt treatments. However, the blade extension rate did not fall beyond 76% of maximum blade extension rate in any of the treatment levels. This study gave evidence for the widely accepted classification of *H. wrightii* as a pioneer species that is able to utilize space that other species have been removed from. *H. wrightii* was dominant in areas of Biscayne Bay that had high nutrients and fluctuating salinity (Lirman & Cropper, 2003).

Salinity Tolerances of Five Marine Spermatophytes of Redfish Bay , Texas (McMillan and Moseley, 1967)

This was an experimental study carried out by using indoor growth rooms and outdoor ponds to control salinity in *R. maritima* and other marine species that are native to Redfish Bay in Texas. Plants were collected from the Redfish Bay in 1965 and were transported to the laboratory. Individuals were placed in trays in tanks with salinity of 28 ppt. Light and water levels were kept constant throughout the study. In the indoor tank, salinity was increased by 0.75 ppt per day while the outdoor tank was increased at 1.0 ppt per day. Therefore, individuals were exposed to salinity ranging from 28 to 75 ppt. The response parameters that were measured were growth rates and chlorophyll content. Growth rate terminated at 46 ppt in outdoor tank and 70 ppt in control tank. This study suggests that although it is believed that salinity affects the distribution of *R. maritima*, it may have a higher tolerance than that what is suggested by its habitat. At high salinities, chlorophyll content dropped to a level below that of the plants in seawater. This study provides evidence that *R. maritima* is resilient in its ability to withstand salinity fluctuations. Therefore, the range and distribution of *R. maritima* in Texas or North Carolina may be attributable to environmental parameters other than salinity. This could include light availability, nutrients, temperature, and predation, as this study did not account for any of these variables (McMillan & Moseley, 1967).

Seed germination for an annual form of *Zostera marina* from the sea of Cortez, Mexico (McMillan, 1983)

The purpose of this study was to examine various environmental parameters that influence the germination of *Zostera marina* seeds in the Sea of Cortez, Mexico. Seeds were

collected and a laboratory growth chambers were used to test the effects of various salinities on seed germination. Artificial seawater was prepared using deionized water. Half of the seeds were kept at 15 ppt and half were kept at 35 ppt. The temperature was maintained at 18 to 20°C. Seeds that were exposed to higher temperatures had lower germination rates. In 15 ppt, there was 12-26% germination and in 35 ppt, there was 15-23% germination. Most of the germination occurred in the summer months following the collection, however there were some germinations in the winter. Although other studies show *that Z. marina* experiences higher germination rates at lower salinity, this study saw similar germination rates at 15 and 35 ppt.

Physiological effects of short-term salinity changes on *Ruppia maritima* (Murphy et al., 2003)

Although *Ruppia maritima* may tolerate and grow in a wide range of salinities, the effects of short-term salinity changes on plant physiology are in question in the present study. Using laboratory mesocosm experimental design, the authors explored how short-term salinity changes effects leaf-tissue osmolality, leaf tissue solutes, and maximum quantum yield. Samples of *R. maritima* were collected from Madeira Bay in Florida. Four treatment levels were used: 0, 10, 20, and 40 ppt. All changes were compared to the ambient salinity of 30 ppt. Both salinity and time had significant affects on leaf tissue osmolality. Significant interaction between time and salinity was also observed. Osmolality increased with salinity from 1 day post treatment to 2 days post treatment for all treatment levels except 0 ppt. Salinity also had a significant affect on non-vascular osmolality, but the changes over time were not significant. Proline levels in 0 and 10 ppt treatments

decreased, while proline levels in 40 ppt treatment increased. Proline levels in 20 ppt treatment stayed relatively constant. There was a decrease in the total amount of carbohydrates with increased salinity, but the remaining carbohydrates were more than likely used for osmoregulation in the cytoplasm. Quantum yields were the lowest in the 0 and 40 ppt treatments; the 10 and 20 ppt treatments had significantly higher quantum yields. In 10 ppt salinity there was little change in the parameters measured, which indicates that there was little stress at this treatment level. However, significant changes were seen in all parameters at the 0 and 40 ppt treatment levels. The authors concluded that this species could disappear from environments that shift from estuarine to marine. This could have major implications in areas that are susceptible to changing salinity regime due to sea level rise and increased storm events (Murphy et al., 2003).

Effects of salinity and water temperature on the ecological performance of *Zostera marina* (Nejrup and Pedersen, 2008)

In this study, *Zostera marina* ecological performance was investigated using aquaria with various salinity and water temperature treatments. Ramets were collected from Odense Fjord in Denmark and transported to the laboratory for experimentation. Twenty-one aquaria were exposed to seven water temperatures: 5, 10, 15, 20, 25, 27.5 and 30°C. Twenty Four aquaria were exposed to eight levels of salinity: 2.5, 5, 10, 15, 20, 25, 30, and 35 ppt. Both of these experiments lasted six weeks and the parameters measured were shoot morphology, photosynthetic capacity, growth (leaf production, elongation, and formation of side shoots), and mortality. Formation of new side-shoots per shoot was highest at 10 ppt, and then decreased with increasing salinity. There was a slight increase

in new side-shoot formation at 35 ppt. Shoot biomass was greatest at 15-20 ppt. Maximum photosynthetic rate increased with salinity to 20 ppt, and then decreased with increasing salinity beyond 20 ppt. Number of new leaves per shoot increased with salinity and was highest at 15 ppt. At constant temp (15 C), mortality decreased with increasing salinity. Mortality was lowest at 25 ppt, increased slightly at 30 and 35 ppt. Therefore, the authors concluded that these results suggest that the optimal salinity and temperature for *Z. marina* growth are above 15 ppt and between 15 and 20°C. the authors recognize that this study was focused on the adult form of this species, and salinity and temperature optimums may differ at different life stages (Nejrup & Pedersen, 2008).

Seed germination and seedling growth of *Zostera marina* L. (eelgrass) in the chesapeake bay (Orth and Moore, 1983)

This study explored the effects of sites with varying salinity regimes and *Zostera marina* seed germination and seedling growth in the Chesapeake Bay. Eight sites were established for this study, and the salinity range for each site was recorded. The lowest salinity range was 8-15 ppt and the highest salinity range was 25 to 32 ppt. Most sites had a salinity range of 15-20 ppt. Seeds were collected from each site and tested for viability. An in situ experimental design was used to test seed germination in the field. Seedling growth was estimated by taking monthly samples, measuring the length of the primary leaf, counting the number of shoots per seedling and the total number of leaves per seedling. No significant correlation was found between germination and salinity; however, *Z. marina* growth was slightly higher at locations with a lower average salinity. Seed germination occurred every month except June through August. The highest percentage of seeds

germinated between December and March. The authors hypothesize that high water temperatures induce dormancy. Though there was no correlation between salinity and *Z. marina* seed germination, salinity may act in combination with a number of other environmental parameters to effect seed germination (Orth & Moore, 1983).

Influence of temperature and salinity on germination of eelgrass (*Zostera marina* L.) seeds (Pan et al., 2011)

Because seagrass restoration is being used in many parts of the world, the authors' goal in this study is to explore the environmental parameters that should be considered in restoration efforts. The two prominent environmental parameters are temperature and salinity. *Z. marina* seeds were collected from Sanggou Bay in China. This study utilized a bench-top incubator in the experimental design. Four temperature treatment levels were established: 4, 9, 14, and 24°C. The nine salinity treatment levels were 5 to 45 in increments of 5. Seed germination was the primary response variable that was observed in this study. Statistical analyses showed that there was a significant interaction between temperature and salinity on germination. Salinity seemed to have a stronger effect than temperature on germination. At low temperature, germination was low across all salinities. Generally, germination was higher at 5 to 10 psu across all temperatures, but was highest at 5 to 10 psu and 14°C. The results of this research are comparable to the findings of past research. The authors suggest that because low salinity is rarely encountered in estuarine and marine seagrass beds, *Z. marina* seeds can be artificially germinated *in vitro* and then transplanted in restoration projects. The authors also conclude that freshwater input could

facilitate *Z. marina* growth and aid in the maintenance of the population in Sanggou Bay (Pan et al., 2011).

Observations on the Ecology and Distribution of Florida Seagrasses. St. Petersburg, Florida (Phillips, 1960)

This report is an observational study that was conducted in Florida by the Florida Board of Conservation. The species studied are *R. maritima* and *H. wrightii*, along with several other saltwater species that are native to Florida. The response parameters that were recorded are flowering and presence/absence of individuals of each species at each location. Monthly visits to stations in Tampa Bay, Boca Ciega Bay, and Tarpon Springs were made to observe changes in flora and water quality. Observations of freshwater inflow, tidal range, and currents were made, all of which could influence the salinity regime. Temperature and salinity measurements were taken over the course of this observational study. *Ruppia maritima* was found throughout the year, but was more abundant in the winter and spring at the Tampa Bay site. In Boca Ciega Bay, *R. maritima* was rare and restricted to areas of low salinity. *R. maritima* was not present in Tarpon Springs. No correlation between flowering and salinity was apparent. All sites had a salinity of 28 ppt or less during flowering, except for the Cats Point Bank site, which had a salinity of 31.4 ppt before, during, and after flowering and fruiting, *Ruppia* was found in water ranging from fresh to 33.2 ppt salinity, but it seemed to be restricted to areas at or below 25 ppt, and was seen to thrive in salinities much lower. *R. maritima* was never a dominant species at any of the sites, though it was subdominant in Tampa Bay (Phillips, 1960).

Reproductive strategies of eelgrass (*Zostera marina* L.) (Phillips et al., 1983)

This study utilizes a laboratory experimental design to analyze the effects of temperature and salinity variation on seed germination. Samples of *Zostera marina* were collected from a wide range of geographical locations, including Alaska, Gulf of California, North Carolina and Rhode Island. Mature seeds were collected at these locations and were shipped to the laboratory in Seattle. The parameters measured and observed in this study are reproductive strategies, frequency of flowering, seed germination. It is suggested that reproductive strategies are influenced by salinity; in subtidal areas of minimal fluctuation, perennial plants reproduce vegetatively. Conversely, intertidal samples tended to have a larger proportion of flowering shoots. However, in sites that are not estuarine and have consistently high salinities, there is not an observable difference in frequency of flowering. Germination rate was highest at 10 ppt, which is consistent with findings from similar studies on *Z. marina*. Higher salinity seemed to inhibit seed germination. The authors concluded that the results correlated with the geographic variation in life history patterns, and life history within the central geographic range develops in response to salinity regime. In subtidal areas where perennial plants flourish and do not experience physical or biological disturbance, asexual growth is the primary strategy of expansion. Intertidal individuals experience wider fluctuations in all environmental parameters, including salinity, so the incidence of sexual reproduction via flowering is higher. The higher germination rate at lower salinities mediates this life history pattern (Phillips, Stewart Grant, & Peter McRoy, 1983).

Synergistic effects of altered salinity and temperature on estuarine eelgrass (*Zostera marina*) seedlings and clonal shoots (Salo and Pedersen, 2014)

As previous research suggests, salinity and temperature are two important factors affecting the performance and distribution of *Zostera marina*. The goal of this study was to examine the combined effects of salinity and temperature on adult and seedlings, using a five-week aquarium experiment. Plants were collected from Isefjorden, Denmark and transported to a laboratory where a three-factorial experiment was conducted. Salinity, temperature, and the interaction of salinity and temperature were examined. Three levels of salinity were used: 5, 12.5, and 20. Likewise, three levels of temperature were used: 15, 20, and 25 °C. Therefore there were nine treatment levels and three replicates for each treatment. The response variables measured were mortality rate, leaf elongation rate, number of new leaves produced and stress (or degree of necrosis and tissue concentrations of starch and sucrose). Leaf elongation rates were lower at the lowest salinity (5 ppt) than at the medium or high salinity (12.5-20 ppt). Leaf production was significantly affected by salinity and temperature, but not their interaction. Low salinity had negative effect on leaf production. The combination of high temperature and low salinity yielded higher mortality rates. Plants from the low salinity treatment were more necrotic than plants from the medium treatments. The authors concluded that high temperature and low salinity are two common stressors in an estuarine environment and are associated with negative synergistic effects on the survival of *Z. marina*. This has implications for climate change due to frequent heat waves and increases in precipitation (Salo & Pedersen, 2014).

Population specific salinity tolerance in eelgrass (*Zostera marina*) (Salo et al. 2014)

The purpose of this study was to explore the performance of *Zostera marina* from two regions with different average ambient salinity when exposed to a series of fixed salinity treatments. Plants were collected from geographically isolated locations: a low salinity region from the northern Baltic Sea, Finland, and a high salinity and highly variable region in the Southwestern Baltic Sea, Denmark. The authors hypothesized that the two populations would react differently have had different tolerance for hyposalinity. The salinity treatments used in the aquaria experimental design were: 2, 4, 6, 9, 12.5, 15, 20, and 25. The parameters measured were primary production, mortality, and leaf elongation rate/ growth rate. Both populations showed a decrease in F_v/F_m values, indicating an increase in stress, with lower salinity. The low salinity population showed stress at lowest salinity (2 ppt) and the high salinity population showed stress at <9 ppt. The high salinity population had a higher mortality rate at lower salinities and the low salinity population had an overall low mortality rate, but mortality was not significantly correlated with salinity. Leaf elongation rates significantly differed between populations, with faster growth rates occurring in the low salinity population. There was a general trend of slower growth rates at lower salinity. The number of leaves per shoot was affected by the interaction between salinity and population. The results indicated that salinity and population origin significantly influenced *Z. marina* performance. However, even in high salinity, the low salinity population did not experience severe decline in performance. There was an observed horizontal shift in performance curves, which is indication that prior adaptation can affect salinity tolerance and subsequent stress. The authors concluded

that this species has the capability for plasticity in salinity tolerance, though their origin does effect the stress experienced in new salinity treatments (Salo, Pedersen, & Bostrom, 2014).

Life History Transitions in Response to Salinity Dynamics Along the Everglades-Florida Bay Ecotone (Strazisar et al., 2014)

As previous research suggest, *Ruppia maritima* is able to tolerate a wide range of salinity. However, rapid salinity changes could limit *R. maritima* growth and distribution. Life history transitions such as seed germination, seedling and adult survival, and sexual reproduction were examined at three sites across the Everglades ecotone. The tree sites had mean salinities of 11, 14, and 21 psu. There was no statistically significant difference in seed germination rates between sites. The upper site (lowest salinity) had twice as many seeds transition into seedlings. There was a five-fold increase in live shoots at the upper site compared to the central and lower sites. There was an indication that individuals at the upper site had a higher growth rate, as well as a higher survival rate. The numbers of ungerminated seeds within the seed bank and germination rates were not significantly different as a function of salinity across the ecotone. Seedling and adult survival were negatively correlated with site-specific salinity variance, therefore it's suggested that rapid changes in salinity could create a "bottleneck" for successful transitions between seedling and adult. The authors suggest that salinity levels that promote early life stage survival should be targeted in SAV restoration projects (Strazisar, Koch, & Madden, 2014).

The Autecology of *Zostera marina* in Relation to its Wasting Disease (Tutin, 1938)

In this report, the author elucidates the autecology of *Zostera marina*. This includes the physiological description and the habitat. The habitat parameters that are examined include the bottom sediment that is suitable for growing, amount of light necessary, with relation to wave action, depth, and turbidity, temperature, and chemical compositions of the sea, specifically salinity. Tutin makes the observation that *Z. marina* can withstand salinities ranging from 10 to 40 g/L in laboratory mesocosm experiments without harm. The plants also tolerated complete immersion in freshwater for two days before there was any noticable harm or visible signs of mortality in the plant. All of these factors are related to the wasting disease, though the relationship between salinity variation and persistence of the disease was not explored (Tutin, 1938).

Effects of salinity and nutrient load and their interaction on *Zostera marina* (Van Katwijk et al., 1999)

The purpose of this study was to explore the separate and interactive effects of nutrients and salinity on *Zostera marina* in the Netherlands. Two populations were sampled: an estuarine population and a marine population. The salinities tested are 23, 26, and 30 ppt, at various ratios of nitrate:ammonium:phosphate. Vitality of plants after being exposed to experimental salinity and nutrient treatments was calculated based on the number of shoots, size of shoots, presence of necrosis, and the number of missing leaves. These measurements were taken 2 and 5 weeks after the treatment commenced. Vitality of individuals from an estuarine population decreased at salinity levels of 26 and 30 ppt. However, plants from the marine population only saw a decrease in number of shoots in

the 30 ppt treatment. At low salinity (23-26 ppt), plants from both populations benefited from increased nutrients. When plants were stressed by salinity, they responded indifferently or negatively to nutrient input. The authors conclude that an increase in nutrient input into estuarine or marine environment will restrict *Z. marina* to areas of low salinity. The treatment levels were designed in a way that reflects the nutrient and salinity regime of the Wadden Sea and the eastern shores of the United States, so the authors conclude that these results are descriptive of *Z. marina* over large geographic areas. The dynamics between salinity and nutrients should be explored more, especially in relation to climate change, anthropogenic eutrophication, and sea level rise (Van Katwijk, Schmitz, Gasseling, & Van Avesaath, 1999).

Salinity and temperature significantly influence seed germination, seedling establishment, and seedling growth of eelgrass *Zostera marina* L. (Xu et al., 2016)

Much research has been focused on optimal conditions for seagrass restoration efforts. Because many seedlings will be established in vitro and then transplanted into the field, the primary effort of this study is to explore the optimal growth conditions for *Zostera marina* seedlings. Salinity treatments (0, 5, 10, 15, 20, 25, 30, 35, and 40 ppt) and temperature treatments (5, 10, 15, and 20 °C) were used to create treatments to test the effects on seed germination. Separate salinity treatments (10, 20, and 30 ppt) were created to explore the effects on seedling establishment and growth. Salinity had a greater effect on seed germination than temperature. Seed germination significantly increased at low salinity and high temperature, which is contradictory to adult *Z. marina* preferences. Seedling establishment differed significantly between salinity treatments. Seedlings from a

treatment of 15 ppt had a higher establishment rate compared to seedlings of lower treatment levels. The establishment rate decreased with salinity. The authors concluded that freshwater input is beneficial for seed germination. This information is beneficial for restoration projects, but it could also give insight into how variable salinity regimes influence *Z. marina* distribution (Xu et al., 2016).

3.2.3 Annotated Bibliography Summary and Conclusions in Relation to Shift in Salinity Regimes

This literature review has indicated that there is a great deal of research on salinity tolerance and SAV species. Many studies utilize a mesocosm design to test ranges of salinity on SAV ramets that were collected from the field at various locations. Many studies observe the main effects on salinity on various SAV growth parameters, including leaf elongation rate, seed germination, seedling growth, mortality, indications of stress and photosynthetic capability. In many cases, there was no significant statistical correlation between salinity and response variable; however, general trends were seen across studies on the same species. The reviewed literature gives an adequate overview of the potential impacts that salinity variation can ensue, though replication with consistent methods would more accurately illuminate patterns and processes that salinity variation induces in SAV species.

Many studies reported interesting findings regarding the large threshold of salinity tolerance in aquaria or mesocosms, but there was little research investigating the relationship between salinity tolerance in a laboratory experiment and the ambient salinity at which *R. maritima*, *H. wrightii*, and *Z. marina* are actually found *in situ*. A few studies examined the effects of temperature and salinity on SAV performance, with mixed results. In some cases salinity and temperature have significant interactive effects (Pan et al., 2011;

Salo & Pedersen, 2014), while other results indicate that temperature and salinity independently affected SAV performance. Few studies have explored the interactive effects of salinity and nutrients, photosynthetically active radiation (PAR), and stratification (Dunton, 1990; Hootsmans et al., 1987; A. E. Kahn & Durako, 2005; Van Katwijk et al., 1999). Mesocosms and aquaria are valuable in testing controlled levels of salinity and temperature to determine tolerance thresholds. However, the nuances of natural variation in other environmental parameters are not captured with this method. It has been suggested that plant origin and ambient salinity has an effect on salinity tolerance in mesocosm experiments (Salo et al., 2014). Because SAV samples for these studies were taken from a large geographic range, further research and meta-analyses are needed to explore the relationship between salinity tolerance ranges and population adaptation to ambient salinity.

In regard to predicting effects of salinity manipulation on existing SAV populations, the results from these studies provide a preliminary guide to determining whether salinity is outside of the observed tolerance range of a species and to what degree response variables are affected. However, site-specific variations in plant adaptation to salinity, nutrients, and other environmental parameters prevent us from knowing exactly how plant performance, distribution, and abundance would change with changing salinity.

When considering a RED installation, it is possible to predict the effluent salinity, as it will most likely be an average of the salinity of the two influent streams. Therefore, if the low salinity stream is 1 ppt and the high salinity stream is 30 ppt, we would expect the effluent stream to have a salinity of 15.5 ppt. According to past research, this salinity is within the known tolerance range of all three species, though it may be on the low end of

the range for *H. wrightii* and *Z. marina*. In regards to germination, 15 ppt is actually slightly higher than the optimal range for *Z. marina* seed germination. Whereas plants may not experience negative effects due to a shift in salinity regime to that within this range, the interactive effects between salinity and nutrients or salinity and temperature may render unexpected negative outcomes.

RED may manipulate the salinity regime near an effluent discharge location, though salinity manipulation may also occur via climate change—large storm events with unusually high precipitation could lead to greater river discharge into sounds, thus decreasing salinity, or instigate inlet formation, thus increasing salinity and resulting in other physical changes such as shoaling and burial. Global sea level rise is a persistent trend that will ultimately affect coastal communities around the world. Models have shown the complexity of barrier island migration in response to sea level rise, and it's plausible that sea level rise will affect the salinity regime in the APES, most likely making a larger portion of estuary more saline (Moore, List, Williams, & Stolper, 2010). With the evidence from this literature review, we can make reasonable predictions that could be empirically tested in further research employing mesocosms where effects of RED effluent on SAV could be directly measured. Any definitive conjectures about how SAV meadows will respond will also depend on the presence of ecotypic variance.

Based on this literature review and analysis, I predict that *H. wrightii* may be the most vulnerable to the effects of RED technology, though research and understanding of low salinity effects on this species is lacking.

3.3 Salinity, SAV, and Ecosystem Services

The term “ecosystem services” is used to describe ecological processes that benefit human communities and economies, although there is no standardized definition or unit of measurement to describe these services (Boyd & Banzhaf, 2007). Daily’s list of ecosystem services includes processes such as purification of air and water, mitigation of droughts and floods, generation and preservation of soils, detoxification and decomposition of wastes, dispersal of seeds, and cycling and movement of nutrients, among other things (Daily, Gretchen, 1997). The Millennium Ecosystem Assessment classifies services as supporting (nutrient cycling, soil formation, and primary production), provisioning (food, fresh water, wood and fiber, fuel, etc.), regulating (climate, flood, disease, water, etc.), or cultural (aesthetic, spiritual, educational, recreational, etc.). These processes are seen as ecosystem services because of their relationship to human well-being, such as security, basic material for good life, health, good social relations, and freedom of choice and action (Duraiappah et al., 2005). Being that the classification system and language surrounding ecosystem services is not well-defined or accepted across all disciplines (Hattam et al., 2015; Wallace, 2007), the economic value of these services is highly disputed.

SAV ecosystems are particularly beneficial in North Carolina, as they are one of the most productive ecosystems on Earth (Dennison et al., 1993; Fourqurean et al., 2012; Orth et al., 2006). It is estimated that SAV biomass and underlying soils of SAV meadows could store as much as 19.9 Pg of organic carbon (C_{org}) (Fourqurean et al., 2012). This large reservoir of carbon results from a combination of high primary production, filtration of particles out of the water column to store within the soil, and low decomposition rates due to low oxygen levels within the soil (Fourqurean et al., 2012). Carbon is sequestered and

fixed as C_{org} via primary production. The facilitation of sedimentation and reduction of resuspension of particles by SAV contributes to the accumulation of C_{org} (Kennedy et al., 2010). Unlike terrestrial ecosystems where the dominant location of stored organic carbon is within tissues of living trees, the C_{org} in coastal ecosystems is primarily stored in the organic-rich soils from which the vegetation is growing. Because these soils are anaerobic, C_{org} can be preserved on very large time scales, in the order of thousands to millions of years (C M Duarte, 2000; Carlos M. Duarte et al., 2010; Carlos M. Duarte et al. , 2013)

Along with providing the ecosystem service of being a carbon sink, SAV acts as an ecosystem engineer and promotes biodiversity (Duffy, 2006). SAV communities provide nursery areas for juvenile fish that are commercially and recreationally important. A meta-analysis by Heck et al. (2003) concluded that abundance, growth, and survival were greater in SAV ecosystems, compared to unstructured and unvegetated environments (Heck, Hays, & Orth, 2003). The physical structure of SAV meadows creates heterogeneity in an otherwise featureless benthic habitat. Structural diversity increases community, biomass, and primary and secondary production in both the benthic and pelagic (Duffy, 2006). The relationship between structural diversity and biodiversity within ecosystems makes SAV an important indicator of ecosystem health and quality.

Previous research has shown that aggregate SAV biomass and species richness of organisms that use SAV as habitat are positively correlated (C M Duarte, 2000). Therefore, if a change in ambient salinity were to affect primary production rates, growth rates, or reproduction within an SAV ecosystem, the species richness may also be negatively affected. This is detrimental because of the large amount of C_{org} that is stored in the biomass and sediments of SAV meadows. Because biodiversity is hypothesized to enhance

the stability of ecosystem properties such as total plant biomass, salinity-altering effluent could detrimentally impact SAV communities where SAV have a narrow range of tolerance for salinity fluctuations. High diversity is hypothesized to act as a buffer against disturbance in many ecosystems, not just in SAV ecosystems (Duffy, 2006).

It is hypothesized that ecosystem services vary by seagrass genera and geographical location. Because seagrass species vary in size and root depth, it is expected that each species provides a unique set of ecosystem services. The challenge is that most research on seagrass ecology is not investigated through the lens of ecosystem services. Twenty-seven seagrass ecosystem services have been identified by previous research and literature review. These services are: compost fertilizer, fish habitat, food for humans (seagrass plants or associated species), invertebrate habitat, nursery, pharmaceuticals, raw materials, vertebrate habitat, carbon sequestration, coastal protection, geomorphology, sediment accretion, sediment stabilization, animal food from associated species, mariculture, seagrass as food for animals, water purification, bequest value, cultural artifacts, education, recreation, research, spiritual and religious value, tourism, source of information, and genetic resources (Nordlund, Koch, Barbier, & Creed, 2016). Provision of research is an ecosystem services that is heavily evident due to the large number of publications on the topic of seagrass ecology. Other ecosystem services are less evident. There is an information disparity in regards to many of these ecosystem services, such as pharmaceutical, geomorphology, and raw material use.

Based on literature review and collaboration with seagrass experts, previous research concluded that ecosystem services vary among global bioregions and among seagrass genera, and more genera present is correlated with the provision of more

ecosystem services per bioregion (Nordlund et al., 2016). Number of roots, size and shape of leaves, and number of vegetative shoots per square meter differ between SAV species in North Carolina (Larkum, Orth, & Duarte, 2006). It was also concluded that seagrass size is positively correlated with frequency of ecosystem service provision (Nordlund et al., 2016). Therefore, it can be expected that a change in species composition due to salinity regime alteration would affect ecosystem service provision, though the degree to which those changes occur is a subject for further research.

4. CONCLUSIONS

Based on the geospatial analysis of North Carolina's salinity regime using SalWise data and the exploration of the necessary volumes of water required for RED energy production, coastal North Carolina is not an optimal region for this form of salinity gradient energy. The only location where consistently low salinity (approximately 1 ppt) and high salinity (greater than or equal to 30 ppt) coexist within a proximity that is feasible for RED energy generation is the Currituck Sound, near Corolla, NC (northeastern region of the Albemarle Sound). There are few data points from this region, though it is known that the Currituck Sound is predominately freshwater due to the large volume of river flow into the sound and the lack of exchange with the ocean via an inlet. Although the geospatial analysis revealed several low salinity points that intersect with high salinity along the North Carolina coast, there are no other locations where low salinity and high salinity bodies of water reliably coexist in a proximity suitable for energy production via RED. For example, there are several low and high salinity points in the Neuse River Estuary where the salt wedge is presumed to be. The data in the SalWise dataset was not collected with profiles. Therefore, it is unknown in most cases if dense high salinity water is present underneath low salinity freshwater. Regardless, the salinity regime in the Neuse River Estuary, Cape Fear River Estuary, and the New River Estuary is highly variable and may not be suitable for a RED installation.

There are several anthropogenic salinity gradients on the North Carolina coast. Eleven wastewater treatment plants are within 2000m of a high salinity body of water. However, the flow rates of these facilities would yield less than 1 kWh of energy if used as influent in a RED installation. This is far below a typical commercial sized power plant, though it could be useful in self-sustaining the wastewater treatment plant. The calculated energy production is based on a theoretical mixing energy that does not take into account factors that affect membrane resistance. Biofouling and ion concentrations, particularly concentrations of multi-valent ions, can increase membrane resistance by reducing the flow of ions across the membrane, subsequently reducing the external flow of electrons (Długołęcki et al., 2010; Veerman, Saakes, Metz, & Harmsen, 2009). At the ground truth potential RED locations, the ambient salinity ranged from 3.5 ppt to 36.97 ppt, though the lowest salinity was only recorded at Nelson Bay after tropical storm Hermine in September 2016.

It is expected that RED effluent would have a salinity that is an average of the two influent streams. In a hypothetical scenario of mixing river water and sound water in a RED membrane stack, the effluent would have salinity equal to the average of the two source water salinities. Using 30 ppt and 1 ppt water for high and low salinity RED source waters respectively, would yield an effluent stream of 15.5 ppt salinity. In the case of the Cape Fear and New Rivers, a RED facility located at the mouth of the river would pump freshwater from the head of the estuary to mouth using gravity. The effluent would be fresher (less saline) than the sound into which it is discharged. The extent to which an effluent stream would alter ambient salinity regime would be calculated as a dilution factor based on the capacity of the RED facility, the size of the discharge pipe, flow rate of the

effluent, and the hydrology of the receiving water. A review of previous research on salinity interactions with native NC saltwater SAV species reveals that some SAV species, particularly *Z. marina*, may benefit from salinity variation at different life stages. A freshening event may facilitate seed germination in *Z. marina*, but may reduce the growth rate of other saltwater SAV species, thus potentially changing the species composition within the ecosystem. In the hypothetical scenario of a RED installation in Duck, where water from the Currituck Sound and the Atlantic Ocean are mixed, discharging the effluent into the sound would increase the salinity at the discharge location. This may facilitate the growth of SAV species that grow in mesohaline water such as *R. maritima* and *H. wrightii*. However, an introduction of mesohaline water into a freshwater system could be detrimental to low salinity species, as they are sensitive to increased salinity.

In a hypothetical scenario of RED installation utilizing anthropogenic salinity gradient, mixing WWTP effluent and ambient water in a RED stack would essentially dilute the initial WWTP effluent before discharging it into the closet body of water that is presumably high salinity. This process could lower the salinity variability at a WWTP effluent discharge location and make it more suitable for SAV growth. An increase in habitat suitability for SAV species would increase the provision of ecosystem services in that area. SAV is an ecosystem that provides valuable services to humans, including sequestering large amounts of carbon as biomass and in sediments. This is especially pertinent now due to anthropogenic contributions to the increase in atmospheric CO₂.

According to NOAA and the observations at the Mauna Loa Observatory in Hawaii, atmospheric CO₂ has increased significantly since industrialization in the twentieth century. A major source of CO₂ to the atmosphere is the combustion of fossil fuels for

energy, transportation, and industrial and residential uses (EPA 2016). According to NOAA, CO₂ emissions to the atmosphere due to burning fossil fuels have increased from 6.8 Pg C yr⁻¹ to 9.8 Pg C yr⁻¹ from 2001 to 2014 (CarbonTracker CT2015 NOAA, Peters et al., 2007). Due to the role of CO₂ as a major greenhouse gas in Earth's atmosphere, we see the effects of rising atmospheric CO₂ in the form of global climate change, inducing changes in the cryosphere, sea level, ocean acidity, precipitation, and global mean temperatures (IPCC Report 2013). For example, Solomon et al. (2007) estimated that the global mean temperature has risen 0.74° C since pre industrial times. Previous research has shown that global climate change has had an effect on ecosystems, including phenology, species range and distribution, invasion of species, extinctions, and disruption of ecosystem structure and function (Márquez, Real, Olivero, & Estrada, 2011; Sekercioglu, Schneider, Fay, & Loarie, 2008; Walther et al., 2002; Warren, Price, Fischlin, de la Nava Santos, & Midgley, 2011). Specifically in marine ecosystems, the impacts of anthropogenic climate change can induce detrimental ecological transformations such as altered food web dynamics, shifting species distributions, and decreased productivity, among other things (Hoegh-Guldberg & Bruno, 2010).

Sea level rise is a pertinent global issue for coastal communities' infrastructure and ecology. Glacial melting, land ice melting, and thermal expansion of water due to increased mean global temperatures contribute to rising global sea levels. The primary physical impacts of sea level rise on coastal zones are increased erosion, saltwater intrusion into ground water, and a decline in coastal wetlands, saltmarshes and mangroves due to insufficient sediment supply (Nicholls & Cazenave, 2010). Previous research has indicated that within North Carolina, the spatial patterns and rate of sea level rise have historically

varied, though the 20th century rate of sea level rise was likely the fastest rate in at least 2,900 years. (Kopp, Horton, Kemp, & Tebaldi, 2015). This is particularly concerning on the Outer Banks of NC if sea level rise induces major erosion and inlet formation where seawater would then flow into primarily fresh sounds such as the Currituck Sound. Sea level rise presents a multitude of issues in coastal communities, though for the scope of this thesis, the issue of sea level rise is twofold; first, inlet formation due to sea level rise would alter the natural salinity regime in the back barrier sounds in coastal NC, thus affecting natural salinity gradients and rendering potential RED installation sites unsuitable. Second, sea level rise could influence habitat suitability of SAV species in coastal NC, though not necessarily in a negative way. *Z. maritima* and *H. wrightii* tolerate higher levels of salinity than *R. maritima*, so an inlet formation and seawater flow into a freshwater sound could influence SAV species distribution. However, sediment dynamics involved in barrier island migration and inlet formation (Moore et al., 2010) could affect turbidity and therefore light availability to limit SAV photosynthesis and growth. Anthropogenic sea level rise could affect the utilization of alternative energy and distribution of key species that provide important ecosystem services such as carbon sequestration and nursery habitat for commercially important fish.

Anthropogenic sea level rise and consequent affects on coastal ecosystems are fueled by the growing world population and energy consumption. World population has increased from 3 billion to 6 billion from 1959 to 1999, and the current global population is estimated at 7.8 billion. The Census Bureau estimates that by 2042, Earth will be home to 9 billion people (U.S. Census Bureau 2016). Although global growth rates have slowed, the United States alone has a population of over 324 million people and is continuing to grow.

With population increase comes an increase in energy demand, and the U.S. Energy Information Administration reports that total energy use per capita in the US is significantly greater than the global energy use per capita. Three main fossil fuels, petroleum, coal, and natural gas, have provided for 80% of the energy consumption of the United States for the past 100 years (U.S. EIA 2017). While fossil fuel combustion is useful in energy production, it is not inexhaustible and therefore is not a reliable or sustainable option for the energy consumption of future generations. Much research is being conducted to explore possible renewable energy sources to relieve the dependence on fossil fuels that contribute to greenhouse gas emissions and anthropogenic climate change (Dincer, 2000; Kabir, Lemongo-Tchamba, & Fernandez, 2015; Lund, 2007; Omer, 2008; Panwar, Kaushik, & Kothari, 2011).

With coal and natural gas being the leading NC electric power industries responsible for emissions of sulfur dioxide, nitrogen oxide, and carbon dioxide, it is imperative that research and innovation be aimed towards finding energy sources that reduce environmental impact while providing the energy needed due to growing demands. RED is a promising form of clean alternative energy, though siting options and power outputs are limited in North Carolina. Further research is needed to develop technology for reliable clean alternative energy that utilizes our natural resources in ways that do not contribute to the existing anthropogenic pressures on coastal ecosystems.

**APPENDIX: WATER QUALITY DATA COLLECTED FROM POTENTIAL RED
INSTALLATION SITES**

A		Nelson Bay			
		Jul-16	Sep-16	Nov-16	Feb-17
Temp (deg. C)	Surface	33.55	28.75	16.2	11.06
	Bottom		27.9	16.26	11.47
Salinity (ppt)	Surface	24.54	3.5	31.69	33.93
	Bottom		25.71	31.78	33.68
pH	Surface	8.2	8.02	8.35	8.95
	Bottom		7.92	8.46	8.57
Chl a	Surface	15.4	17.1	9.4	2.1
	Bottom		18.8	10.5	3.3
Dissolved Oxygen	Surface	105.4	60.7	104.1	97.3
	Bottom		75.2	101.9	98.4
SAV		H. wrightii	H. wrightii	n/a	n/a

B		Taylor Creek			
		Jul-16	Sep-16	Nov-16	Feb-17
Temp (deg. C)	Surface	30.16	28.1	14.23	11.76
	Bottom		27.73	14.11	11.82
Salinity (ppt)	Surface	36.97	35.28	31.98	36.44
	Bottom		35.37	31.88	36.44
pH	Surface	8.28	7.89	8.47	8.36
	Bottom		8.04	8.56	8.4
Chl a	Surface	5.3	3.2	3.6	2.7
	Bottom		5.9	4.7	3.2
Dissolved Oxygen	Surface	88.5	97.2	99.7	100.1
	Bottom		96.2	100.9	100.2
SAV		n/a	n/a	n/a	n/a

C		Bogue Sound at Indian Beach			
		Jul-16	Sep-16	Nov-16	Feb-17
Temp (deg. C)	Surface	33.25	26.66	13.66	10.27
	Bottom		26.68	13.56	10.27
Salinity (ppt)	Surface	38.06	33.03	29.01	32.54
	Bottom		33.02	29.02	32.52
pH	Surface	8.14	38.46	8.38	8.48
	Bottom		38.44	8.46	8.48
Chl a	Surface	16.2	20.8	4.5	3.7

	Bottom		18.1	5.7	3.7
Dissolved Oxygen	Surface	133.9	97.2	98.9	98.8
	Bottom		92	99.7	98.9
SAV		H. wrightii	H. wrightii	n/a	n/a

REFERENCES

- Alvarez-Silva, O. A., Osorio, A. F., & Winter, C. (2016). Practical global salinity gradient energy potential. *Renewable and Sustainable Energy Reviews*, 60, 1387–1395.
- Alvarez-Silva, O., Winter, C., & Osorio, A. F. (2014). Salinity Gradient Energy at River Mouths. *Environmental Science and Technology Letters*, 1(10), 410–415.
- Becker, M. L., Luettich, R. A., & Mallin, M. A. (2010). Hydrodynamic behavior of the Cape Fear River and estuarine system: A synthesis and observational investigation of discharge-salinity intrusion relationships. *Estuarine, Coastal and Shelf Science*, 88(3), 407–418.
- Becker, M. L., Luettich, R. A., & Seim, H. (2009). Effects of intratidal and tidal range variability on circulation and salinity structure in the Cape Fear River Estuary, North Carolina. *Journal of Geophysical Research: Oceans*, 114(4), 1–20.
- Berns, D. M., & Muller, P. H. (2003). Physiological Responses of *Thalassia testudinum* and *Ruppia maritima* to Experimental Salinity Levels . *College of Marine Science, Master of*.
- Biebl, R., & McRoy, C. P. (1971). Plasmatic resistance and rate of respiration and photosynthesis of *Zostera marina* at different salinities and temperatures. *Marine Biology*, 8(1), 48–56.
- Bird, K. T., Cody, B. R., Jewett-Smith, J., & Kane, M. E. (1993). Salinity effects on *Ruppia maritima* L. cultured in vitro. *Botanica Marina*, 36(1), 23–28.
- Bonis, A., Grillas, P., van Wijck, C., & Lepart, J. (1993). The Effect of Salinity on the Reproduction of Coastal Submerged Macrophytes in Experimental Communities. *Journal of Vegetation Science*, 4, 461–468.
- Boyd, J., & Banzhaf, S. (2007). What are ecosystem services? The need for standardized environmental accounting units. *Ecological Economics*, 63(2–3), 616–626.
- Brauns, E. (2008). Towards a worldwide sustainable and simultaneous large-scale production of renewable energy and potable water through salinity gradient power by combining reversed electrodialysis and solar power? *Desalination*, 219(1–3), 312–323.
- Brauns, E. (2009). Salinity gradient power by reverse electrodialysis: effect of model parameters on electrical power output. *Desalination*, 237(1–3), 378–391.
- Burdick, D. ., Short, F., & Wolf, J. (1993). An index to access and monitor the progression of wasting disease in eelgrass *Zostera marina*. *Marine Ecology Progress Series*, 94, 83–90.
- Clampitt, B., & Kiviat, F. (1976). Energy Recovery from Saline Water by Means of Electrochemical Cells. *American Association for the Advancement of Science*, 194(4266),

719–720.

- Daily, Gretchen, C. (1997). Introduction: What are Ecosystem Services? In *Nature's Services: Societal Dependence on Natural Ecosystems* (pp. 1–10).
- Dennison, W. C., Orth, R. J., Moore, K. a, Stevenson, J. C., Carter, V., Kollar, S., ... Batiuk, R. a. (1993). Assessing Water Quality with Submersed Aquatic Vegetation Habitat requirements as barometers of Chesapeake Bay health. *Bioscience*, 43(2), 86–94.
- Dincer, I. (2000). Renewable energy and sustainable development: a crucial review. *Renewable and Sustainable Energy Reviews*, 4(2), 157–175.
- Długolecki, P., Gambier, A., Nijmeijer, K., & Wessling, M. (2009). Practical potential of reverse electrodialysis as process for sustainable energy generation. *Environmental Science and Technology*, 43(17), 6888–6894.
- Długolecki, P., Ogonowski, P., Metz, S. J., Saakes, M., Nijmeijer, K., & Wessling, M. (2010). On the resistances of membrane, diffusion boundary layer and double layer in ion exchange membrane transport. *Journal of Membrane Science*, 349(1–2), 369–379.
- Duarte, C. M. (2000). Marine biodiversity and ecosystem services: an elusive link. *Journal of Experimental Marine Biology and Ecology*, 250, 117–131.
- Duarte, C. M., Kennedy, H., Marba, N., & Hendriks, I. (2013). Assessing the capacity of seagrass meadows for carbon burial: Current limitations and future strategies. *Ocean and Coastal Management*, 83, 32–38.
- Duarte, C. M., Marbà, N., Gacia, E., Fourqurean, J. W., Beggins, J., Barrón, C., & Apostolaki, E. T. (2010). Seagrass community metabolism: Assessing the carbon sink capacity of seagrass meadows. *Global Biogeochemical Cycles*, 24(4), 1–9.
- Duffy, J. E. (2006). Biodiversity and the functioning of seagrass ecosystems, 233, 233–250.
- Dunton, K. H. (1990). Production ecology of *Ruppia maritima* L. s.l. and *Halodule wrightii* Aschers, in two subtropical estuaries. *Journal of Experimental Marine Biology and Ecology*, 143(3), 147–164.
- Duraiappah, A. K., Naeem, S., Agardy, T., Ash, N. J., Cooper, H. D., Díaz, S., ... Assessment, M. E. (2005). *Ecosystems and human well-being: Synthesis*. (J. Sarukhan & A. Whyte, Eds.) *Ecosystems* (Vol. 5). Washington, DC: World Resources Institute.
- Emami, Y., Mehrangiz, S., Etemadi, A., Mostafazadeh, A., & Darvishi, S. (2013). A Brief Review about Salinity Gradient Energy. *International Journal of Smart Grid and Clean Energy*, 2(2), 295–300.
- Fernández-torquemada, Y., & Sánchez-lizaso, J. L. (2007). Monitoring of brine discharges

from seawater desalination plants in the Mediterranean. *International Journal of ...*, 1(3), 449–461.

- Fernández-Torquemada, Y., & Sánchez-Lizaso, J. L. (2005). Effects of salinity on leaf growth and survival of the Mediterranean seagrass *Posidonia oceanica* (L.) Delile. *Journal of Experimental Marine Biology and Ecology*, 320(1), 57–63.
- Fernández, J. A., Garcia-Sanchez, M. J., & Felle, H. H. (1999). Physiological evidence for a proton pump and sodium exclusion mechanisms at the plasma membrane of the marine angiosperm *Zostera marina* L. *Plant Physiology*, 50(341), 1763–1768.
- Ferreira, C., Simioni, C., Schmidt, É. C., Ramlov, F., Maraschin, M., & Bouzon, Z. L. (2016). The influence of salinity on growth, morphology, leaf ultrastructure, and cell viability of the seagrass *Halodule wrightii* Ascherson. *Protoplasma*. <http://doi.org/10.1007/s00709-016-1041-4>
- Fourqurean, J. W., Duarte, C. M., Kennedy, H., Marbà, N., Holmer, M., Mateo, M. A., ... Serrano, O. (2012). Seagrass ecosystems as a globally significant carbon stock. *Nature Geoscience*, 5(7), 505–509.
- Gacia, E., Invers, O., Manzanera, M., Ballesteros, E., & Romero, J. (2007). Impact of the brine from a desalination plant on a shallow seagrass (*Posidonia oceanica*) meadow. *Estuarine, Coastal and Shelf Science*, 72(4), 579–590.
- Garrote-Moreno, A., McDonald, A., Sherman, T. D., Sánchez-Lizaso, J. L., Heck, K. L., & Cebrian, J. (2015). Short-term impacts of salinity pulses on ionic ratios of the seagrasses *Thalassia testudinum* and *Halodule wrightii*. *Aquatic Botany*, 120, 315–321.
- Gill, A. B. (2005). Offshore Renewable Energy : Ecological Implications of Generating Electricity in the Coastal Zone. *Journal of Applied Ecology*, 42(4), 605–615.
- Halpern, B. S., Walbridge, S., Selkoe, K. A., Kappel, C. V, Micheli, F., D'Agrosa, C., ... Watson, R. (2008). A global map of human impact on marine ecosystems. *Science*, 319(5865), 948–952.
- Harned, D. A., & Davenport, M. S. (1990). *Water-Quality Trends and Basin Activities and Characteristics for the Albemarle-Pamlico Estuarine System, North Carolina and Virginia*.
- Hattam, C., Atkins, J. P., Beaumont, N., Börger, T., Böhnke-Henrichs, A., Burdon, D., ... Austen, M. C. (2015). Marine ecosystem services: Linking indicators to their classification. *Ecological Indicators*, 49, 61–75.
- Heck, K. L. J., Hays, G., & Orth, R. J. (2003). Critical evaluation of nursery hypothesis for seagrasses. *Marine Ecology Progress Series*, 253, 123–136.

- Hoegh-Guldberg, O., & Bruno, J. (2010). The Impact of Climate Change on the World's Marine Ecosystems. *Science*, 328(5985), 1523–1528.
- Hootsmans, M. J. M., Vermaat, J. E., & Van Vierssen, W. (1987). Seed-bank development, germination and early seedling survival of two seagrass species from The Netherlands: *Zostera marina* L. and *Zostera noltii* hornem. *Aquatic Botany*, 28(3–4), 275–285.
- Hose, E., Clarkson, D. T., Steudle, E., Schreiber, L., & Hartung, W. (2001). The exodermis: a variable apoplastic barrier. *Journal of Experimental Botany*, 52(365), 2245–2264.
- Iyer, V., & Barnabas, A. D. (1993). Effects of varying salinity on leaves of *Zostera capensis* Setchell. I. Ultrastructural changes. *Aquatic Botany*, 46(2), 141–153.
- Jagels, R., & Barnabas, A. (1989). Variation in leaf ultrastructure of *Ruppia maritima* L. along a salinity gradient. *Aquatic Botany*, 33(1317), 207–221.
- Kabir, A., Lemongo-Tchamba, I., & Fernandez, A. (2015). An assessment of available ocean current hydrokinetic energy near the North Carolina shore. *Renewable Energy*, 80, 301–307.
- Kahn, A. E., & Durako, M. J. (2005). The effect of salinity and ammonium on seed germination in *Ruppia maritima* from Florida Bay. *Bulletin of Marine Science*, 77(3), 453–458.
- Kahn, A. E., & Durako, M. J. (2006). *Thalassia testudinum* seedling responses to changes in salinity and nitrogen levels. *Journal of Experimental Marine Biology and Ecology*, 335(1), 1–12.
- Kennedy, H., Beggins, J., Duarte, C. M., Fourqurean, J. W., Holmer, M., Marbá, N., & Middelburg, J. J. (2010). Seagrass sediments as a global carbon sink: Isotopic constraints. *Global Biogeochemical Cycles*, 24(4), 1–9.
- Kirst, G. O. (1990). Salinity Tolerance of Eukaryotic Marine Algae. *Annual Review of Plant Physiology and Plant Molecular Biology*, 41, 21–53.
- Koch, E. W., & Dawes, C. J. (1991). Short Communication Influence of salinity and temperature on the germination of *Ruppia maritima* L. from the North Atlantic and Gulf of Mexico, 40, 387–391.
- Koch, M. S., Schopmeyer, S. A., Kyhn-Hansen, C., Madden, C. J., & Peters, J. S. (2006). Tropical seagrass species tolerance to hypersalinity stress. *Aquatic Botany*, 86(1), 14–24.
- Kopp, R. E., Horton, B. P., Kemp, A. C., & Tebaldi, C. (2015). Past and future sea-level rise along the coast of North Carolina, USA. *Climatic Change*, 132(4), 693–707.
- La Peyre, M. K., & Rowe, S. (2003). Effects of salinity changes on growth of *Ruppia maritima*

- L. *Aquatic Botany*, 77(3), 235–241. [http://doi.org/10.1016/S0304-3770\(03\)00109-8](http://doi.org/10.1016/S0304-3770(03)00109-8)
- Lamounette, R. G. (1977). *A study of the germination and viability of Zostera marina L. seeds*. Adelphi University.
- Larkum, A. W. D. D., Orth, R. J., & Duarte, C. M. (2006). *Seagrasses : Biology, Ecology, and Conservation. Library*.
- Lirman, D., & Cropper, W. P. (2003). The influence of salinity on seagrass growth, survivorship and distribution within Biscayne Bay, Florida: Field, experimental and modeling studies. *Estuaries*, 26(1), 131–141.
- Lund, H. (2007). Renewable energy strategies for sustainable development. *Energy*, 32(6), 912–919.
- Márquez, A. L., Real, R., Olivero, J., & Estrada, A. (2011). Combining climate with other influential factors for modelling the impact of climate change on species distribution. *Climatic Change*, 108(1), 135–157.
- McMillan, C., & Moseley, F. (1967). Salinity Tolerances of Five Marine Spermatophytes of Redfish Bay , Texas. *Ecology*, 48(3), 503–506.
- Moore, L. J., List, J. H., Williams, S. J., & Stolper, D. (2010). Complexities in barrier island response to sea level rise: Insights from numerical model experiments, North Carolina Outer Banks. *Journal of Geophysical Research*, 115(F3), F03004 1-27.
- Moran, K., Mallinson, David, J., Culver, S. J., Leorri, E., & Mulligan, R. P. (2015). Late Holocene evolution of Currituck Sound, North Carolina, USA : environmental change driven by sea-level rise, storms and barrier island morphology. *Journal of Coastal Research*, 31(4), 827–841.
- Murphy, L. R., Kinsey, S. T., & Durako, M. J. (2003). Physiological effects of short-term salinity changes on *Ruppia maritima*. *Aquatic Botany*, 75(4), 293–309.
- NC Department of the Environment and Natural Resources. (2005). Coastal Habitat Protection Plan: Chapter 4. Submerged Aquatic Vegetation, 253–309.
- NCDEQ. (2016). *North Carolina Coastal Habitat Protection Plan*.
- Nejrup, L. B., & Pedersen, M. F. (2008). Effects of salinity and water temperature on the ecological performance of *Zostera marina*. *Aquatic Botany*, 88(3), 239–246.
- Nicholls, R. J., & Cazenave, A. (2010). Sea Level Rise and Its Impact on Coastal Zones. *Science*, 328(2010), 1517–1520.
- NOAA. (2013). National Coastal Population Report: Population Trends from 1970 to 2020,

- Nordlund, L. M., Koch, E. W., Barbier, E. B., & Creed, J. C. (2016). Seagrass ecosystem services and their variability across genera and geographical regions. *PLoS ONE*, *11*(10), 1–23.
- Omer, A. M. (2008). Green energies and the environment. *Renewable and Sustainable Energy Reviews*, *12*(7), 1789–1821.
- Orth, R. J., Carruthers, T. I. M. J. B., Dennison, W. C., Duarte, C. M., James, W., Jr, K. L. H., ... Williams, S. L. (2006). A Global Crisis for Seagrass Ecosystems, *56*(12), 987–996.
- Orth, R. J., & Moore, K. A. (1983). Seed germination and seedling growth of *Zostera marina* L. (eelgrass) in the chesapeake bay. *Aquatic Botany*, *15*(2), 117–131.
- Pan, J., Jiang, X., Li, X., Cong, Y., Zhang, Z., Li, Z., ... Yang, G. (2011). Influence of temperature and salinity on germination of eelgrass (*Zostera marina* L.) seeds. *Journal of Ocean University of China*, *10*(2), 147–152.
- Panwar, N. L., Kaushik, S. C., & Kothari, S. (2011). Role of renewable energy sources in environmental protection: A review. *Renewable and Sustainable Energy Reviews*, *15*(3), 1513–1524.
- Parida, A. K., & Das, A. B. (2005). Salt tolerance and salinity effects on plants: A review. *Ecotoxicology and Environmental Safety*, *60*(3), 324–349.
- Pattle, R. E. (1954). Production of Electric Power by mixing Fresh and Salt Water in the Hydroelectric Pile. *Nature*.
- Peters, W., Jacobson, A. R., Sweeney, C., Andrews, A. E., Conway, T. J., Masarie, K., ... Tans, P. P. (2007). An atmospheric perspective on North American carbon dioxide exchange: CarbonTracker. *Proceedings of the National Academy of Sciences of the United States of America*, *104*(48), 18925–18930.
- Phillips, R. C. (1960). *Observations on the Ecology and Distribution of Florida Seagrasses*. St. Petersburg, Florida: Florida State Board of Conservation Marine Laboratory.
- Phillips, R. C., Stewart Grant, W., & Peter McRoy, C. (1983). Reproductive strategies of eelgrass (*Zostera marina* L.). *Aquatic Botany*, *16*(1), 1–20.
[http://doi.org/10.1016/0304-3770\(83\)90047-5](http://doi.org/10.1016/0304-3770(83)90047-5)
- Post, J. W. (2009). Blue Energy: electricity production from salinity gradients by reverse electrodialysis. Wageningen University, Wageningen, NL
- Post, J. W., Veerman, J., Hamelers, H. V. M., Euverink, G. J. W., Metz, S. J., Nymeyer, K., & Buisman, C. J. N. (2007). Salinity-gradient power: Evaluation of pressure-retarded

- osmosis and reverse electrodialysis. *Journal of Membrane Science*, 288(1–2), 218–230.
- Robinson, M. M., & McBride, R. a. (2006). Benthic foraminifera from a relict flood tidal delta along the Virginia/North Carolina Outer Banks. *Micropaleontology*, 52(1), 67–80.
- Salo, T., & Pedersen, M. F. (2014). Synergistic effects of altered salinity and temperature on estuarine eelgrass (*Zostera marina*) seedlings and clonal shoots. *Journal of Experimental Marine Biology and Ecology*, 457, 143–150.
- Salo, T., Pedersen, M. F., & Bostrom, C. (2014). Population specific salinity tolerance in eelgrass (*Zostera marina*). *Journal of Experimental Marine Biology and Ecology*, 461, 425–429.
- Sánchez-Lizaso, J. L., Romero, J., Ruiz, J., Gacia, E., Buceta, J. L., Invers, O., ... Manzanera, M. (2008). Salinity tolerance of the Mediterranean seagrass *Posidonia oceanica*: recommendations to minimize the impact of brine discharges from desalination plants. *Desalination*, 221(1–3), 602–607.
- Sekercioglu, C. H., Schneider, S. H., Fay, J. P., & Loarie, S. R. (2008). Climate change, elevational range shifts, and bird extinctions. *Conservation Biology*, 22(1), 140–150.
- Strazisar, T., Koch, M. S., & Madden, C. J. (2014). Seagrass (*Ruppia maritima* L.) Life History Transitions in Response to Salinity Dynamics Along the Everglades-Florida Bay Ecotone. *Estuaries and Coasts*, 38, 337–352.
- Tedesco, M., Scalici, C., Vaccari, D., Cipollina, A., Tamburini, A., & Micale, G. (2016). Performance of the first reverse electrodialysis pilot plant for power production from saline waters and concentrated brines. *Journal of Membrane Science*, 500, 33–45.
- Touchette, B. W. (2007). Seagrass-salinity interactions: Physiological mechanisms used by submersed marine angiosperms for a life at sea. *Journal of Experimental Marine Biology and Ecology*, 350(1–2), 194–215.
- Tutin, T. G. (1938). The Autecology of *Zostera marina* in Relation to its Wasting Disease. *New Phytologist*, 37(1), 50–71.
- Valle-Levinson, A. (2010). Definition and Classification of Estuaries. In A. Valle-Levinson (Ed.), *Contemporary Issues in Estuarine Physics* (pp. 1–11). New York: Cambridge University Press.
- Van Katwijk, M. M., Schmitz, G. H. W., Gasseling, A. P., & Van Avesaath, P. H. (1999). Effects of salinity and nutrient load and their interaction on *Zostera marina*. *Marine Ecology Progress Series*, 190, 155–165.
- Veerman, J., Saakes, M., Metz, S. J., & Harmsen, G. J. (2009). Reverse electrodialysis: Performance of a stack with 50 cells on the mixing of sea and river water. *Journal of*

Membrane Science, 327(1–2), 136–144.

Wallace, K. J. (2007). Classification of ecosystem services: Problems and solutions. *Biological Conservation*, 139(3–4), 235–246.

Walther, G., Post, E., Convey, P., Menzel, A., Parmesan, C., Beebee, T. J. C., ... Bairlein, F. (2002). Ecological responses to recent climate change. *Nature*, 389–396.

Warren, R., Price, J., Fischlin, A., de la Nava Santos, S., & Midgley, G. (2011). Increasing impacts of climate change upon ecosystems with increasing global mean temperature rise. *Climatic Change*, 106(2), 141–177.

Xu, S., Zhou, Y., Wang, P., Wang, F., Zhang, X., & Gu, R. (2016). Salinity and temperature significantly influence seed germination, seedling establishment, and seedling growth of eelgrass *Zostera marina* L. *PeerJ*, 4, e2697.

Yancey, P. H., Clark, M. E., Hand, S. C., Bowlus, R. D., & Somero, G. N. (1982). Living with Water Stress : Evolution of Osmolyte Systems. *Science*, 217(4566), 1214–1222.

Ye, C. J., & Zhao, K. F. (2003). Osmotically active compounds and their localization in the marine halophyte eelgrass. *Biologia Plantarum*, 46(1), 137–140.