The effects of Salinity, Depth, and Turbidity on Submerged Aquatic Vegetation (SAV) abundance in Eastern North Carolina

by

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Abstract

The state of North Carolina is concerned about the loss of submerged aquatic vegetation (SAV), which is critical fish and wildlife habitat in low-salinity estuaries. Sentinel sites have been established by the East Carolina University/Albemarle Pamlico National Estuarine Partnership (APNEP) SAV monitoring team at locations where SAV has been observed in historical surveys. Using monitoring data collected from low-salinity sentinel site locations in the Neuse River Estuary (NRE), Pamlico River Estuary (PRE), and Albemarle Sound (AS) from 2015 to 2019, I evaluated the effects of turbidity (measured by Secchi depth), salinity and water depth on SAV abundance (odds of occurrence, percent cover, percent frequency, and dry biomass). The maximum colonization depth of SAV was also analyzed. My goal was to understand what physical factors impact low-salinity SAV survival and growth in North Carolina estuaries by

focusing on the three dominant species found (*Ruppia maritima, Vallisneria americana*, and *Zannichellia palustris)*. Data came from inshore quadrat diver surveys that measured percent cover using 1 m^2 quadrats at depths of 0.25, 0.5, 0.75, and 1 m. Dry biomass abundance was determined by taking core samples along sampling transects. An ensemble data set from North Carolina Department of Environmental Quality (NCDEQ), Modmon, and North Carolina Department of Marine Fisheries (NCDMF) data bases) was used to create inverse distance weighted prediction models of Secchi depth and salinity for use in analysis of maximum colonization depth of SAV. SONAR methods included the use of Lowrance single beam 200khz echosounder to measure maximum colonization depth of SAV along 40 transects per sentinel site. Some sentinel sites were omitted from the analyses because they contained no SAV during the monitoring period. I observed that water transparency also called turbidity in this thesis (measured by Secchi depth), salinity, and water depth had significant effects on dominant SAV species with the direction of the association being species dependent. *Ruppia maritima* odds of occurrence increased with salinity, water depth, and turbidity. *Vallisneria americana* odds of occurrence showed a negative association with turbidity and salinity but a positive correlation with water depth out to 1 m. *Zannichellia palustris* odds of occurrence showed no significant effect with turbidity and salinity but was significantly associated with increased water depth. Differences in SAV species salinity and water transparency responses suggest these two factors contribute significantly to the distribution of dominant SAV species. Maximum colonization depth of SAV is greater in sentinel sites with higher Secchi depths, especially when a long-term average Secchi depth is used (ensemble data set). *Ruppia maritima* has been predicted by my logistic regression model and field observations to be favored in the competition among North Carolina SAV species.

The Effects of Salinity, Depth and Turbidity on Submerged Aquatic Vegetation (SAV) Abundance in Eastern North Carolina.

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LIST OF ABBREVIATIONS

AMS	Ambient Monitoring System	21
ANOVA	Analysis of Variance	25
APNEP	Albemarle-Pamlico National Estuary Partnership	1
AS	Albemarle Sound	3
BV	Biovolume	19
cm	Centimeter	17
CRFL	Coastal Recreational Fishing License	3
CTD	Conductivity Temperature and Depth	20
ECU	East Carolina University	3
GLM	Generalized Linear Model	24
GPS	Global Positioning System	11
Ha	Hectare	1
IDW	Inverse Distance Weighting	21
khz	Kilohertz	19
km	Kilometer	21
m	Meter	8
MCD	Maximum Colonization Depth	4
NCDMF	North Carolina Department of Marine Fisheries	1
NRE	Neuse-River Estuary	3
OR	Odds Ratio	25
ppt	Parts Per Thousand	6

PAR	Photosynthetically Active Radiation	4
PRE	Pamlico-River Estuary	3
PVC	Polyvinyl Chloride	17
SAV	Submerged Aquatic Vegetation	1
SONAR	Sound Navigation and Ranging	2
SS	Sentinel Site	3

Introduction

Submerged aquatic vegetation (SAV) is a term used to describe rooted vascular plants that grow underneath the water, including seagrasses, freshwater grasses, and macrophytes (Den Hartog and Kuo 2006). SAV plays an essential ecological role in estuarine ecosystems by oxygenating water and removing excess nutrients through filtering suspended sediments as well as acting as a wave-reducing buffer that helps control erosion and improve water clarity (Fonseca et al. 1992; Short and Wyllie-Echeverria 1996; de Boer 2007). It also provides a nursery ground habitat for many recreational and commercially important finfish, invertebrates, and shellfish (Beck et al. 2001; Unsworth et al. 2018). SAV can cycle nutrients and sequester a significant amount of carbon while only occupying 0.2% of the world's area (Fourqurean et al. 2012; Lefcheck et al. 2018). This environmentally vital resource has seen a global decrease in abundance due to many threats and stressors that stem from a broad spectrum of anthropogenic actions. Such stressors include eutrophication from excess nutrient runoff, sedimentation, physical disturbances such as boat scarring, diseases, and herbicides (Orth et al. 2006; Lefcheck et al. 2018; Orth et al. 2017). A global assessment of 215 studies conducted by Waycott et al. (2009) found that seagrass has been disappearing at a rate of 110 km^2 yr^{-1} since 1980 and that rates have accelerated from 0.9% yr^{-1} to 7% yr^{-1} in just 50 years. One hectare (Ha) of SAV can be estimated as providing $3,500-19,000 dollars in ecological services from supporting commercial fisheries, nutrient cycling, and sediment stabilization (Costanza et al. 1997; Waycott et al. 2009).

Limited research has been done on North Carolina SAV relative to other water bodies such as the Chesapeake Bay. Still, Albemarle Pamlico National Estuary Partnership (APNEP) and North Carolina Department of Marine Fisheries (NCDMF) are changing the narrative and supporting research and monitoring efforts in hopes of understanding SAV distribution in lower salinity environments (Davis and Brinson 1976; Kenworthy et al. 2012). It is estimated that 138,100 acres of SAV are located in the Albemarle-Pamlico Estuary, with 101,670 acres found in high salinity waters (NCDMF 2016). Low-salinity areas are not as well understood and easily quantified as they require on-the-water surveying methods such as acoustic sound navigation and ranging (SONAR) sampling, which is labor-intensive relative to aerial photography. The high-water turbidity in oligohaline regions of the estuary does not allow aerial photography surveying methods to be effective because SAV meadows cannot be seen from above remote sensing methods.

Ten different low-salinity SAV species can be observed in North Carolina, with *Ruppia maritima* (Linnaeus 1753*)*, *Vallisneria americana* (Michaux 1803), and *Zannichellia palustris* (Linnaeus 1753) being the most dominant in this study. The lack of quantitative monitoring data for North Carolina's low-salinity SAV species has created a gap in understanding species phenology and life history. This is because low-salinity species are more taxonomically diverse and exhibit greater temporal variations than high-salinity species (Kenworthy et al. 2012). Based on limited observations, it is believed that lower salinity SAV communities are more ephemeral than high-salinity species. It is also suggested that they share different maximum biomass periods. Observations from past studies in North Carolina show low salinity species have peak biomass in September, while high salinity species such as *Zostera marina* (Linnaeus 1753) and *Halodule wrightii* (Ascherson 1868) have peak biomass periods in April and August respectively (Davis and Brinson, 1990; Ferguson and Wood, 1994; Kenworthy et al. 2012).

Monitoring Protocol

This monitoring project first began in 2010 with funding from NC's Coastal Recreational Fishing License (CRFL) project to develop the Albemarle Pamlico National Estuary Partnership (APNEP) surveying protocol (Kenworthy et al. 2012). Two types of nondestructive boat-based acoustic SONAR surveying protocols were established through APNEP and SAV monitoring team members for low-salinity regions in North Carolina estuaries. 1) Rapid assessment (RA) method, the objective of which was to survey large amounts of an area quickly for presence and absence of SAV along a 1-m isobath using underwater acoustic SONAR. 2) Sentinel site (SS) method, which involved more intensive data collections by focusing on a smaller area extent (50 Ha). The RA objective was to create a baseline of SAV abundance along the shoreline of North Carolina's estuaries that previously had never been assessed with SONAR sampling. Sentinel sites were developed for repeated and intense sampling to detect variability in response to environmental stressors.

Work done in previous years through East Carolina University (ECU) research labs has shown a decline in SAV in each of North Carolina's estuaries based on the rapid assessment (RA) surveys compared to historical maps produced by NCDMF from 1981-2012. The RA method is a boat-based surveying method that runs parallel to shore around the entire estuary in the Albemarle Sound (AS), Pamlico River Estuary (PRE), and Neuse River Estuary (NRE). For the first time, this RA allowed us to quantify and estimate the linear extent of SAV with acoustic SONAR. A 52% loss was observed in the AS, a 97% loss in the PRE, and a 73% loss in the NRE when compared to historical aerial baselines (Speight 2020; Luczkovich et al. 2021). The RA was also used as one of the four criteria that established the 26 Sentinel Site (SS) locations. Sentinel sites were selected based on potential SAV habitat locations determined from RA surveys and historical SAV maps. The idea of a SS is that they are chosen to represent a fixed location in the estuary, which can be monitored to detect and understand changes in the ecosystems they represent (Jassby A.D. 1998). When done correctly, this approach allows researchers to extrapolate effects observed from sentinel subset locations to the large ecosystems that cannot be surveyed thoroughly.

Influencing Factors

Light is one of the most important factors regulating SAV survival and determines the maximum depth at which SAV can be found (Dennison 1987; Duarte 1991; Zimmerman et al.1994; Kenworthy and Fonseca 1996). The Maximum Colonization Depth (MCD) is a common and helpful way to evaluate water quality changes in SAV distribution because the deep edge of an SAV bed is influenced first by light limitations (Dennison et al. 1993; Kemp et al. 2004). Turbidity is a measure of water clarity and reflects the degree of light attenuation which can be made up of several components (suspended sediments, colored dissolved organic matter, and chlorophyll-a) (Kitchener et al. 2017). Secchi depth is the depth of water in which the high-contrast pattern on a submerged disk is no longer visible. This is a measure of water transparency which can be used to estimate light attenuation and relative water turbidity (Carruthers et al. 2001). Secchi depth is one standard measure used to determine light limitation for SAV because a Secchi disk, when lowered over the side of a vessel during the peak period of sunlight, disappears from view when light from the surface is attenuated below the level required for plant photosynthesis (10-11% surface irradiance) (Strickland 1958; Sheldon and Boylen 1977; Duarte 1991; Dennison et al. 1993). For all SAV species worldwide, there has been a strong positive relationship between water clarity and maximum water column depth in which SAV can grow

(Canfield et al. 1985; Chambers and Kalff 1985; Duarte 1991; Dennison et al. 1993; Caffrey et al. 2007). So, it is believed that Secchi depth is correlated with increased SAV abundance, and the maximum depth at which SAV can survive will increase with increased light penetration (Chambers and Kalff 1985; Duarte 1991; Dennison et al. 1993). Kemp et al. (1983) monitored stressors such as sediment, algal growth, herbicides, and turbidity in the Potomac River and modeled the SAV distribution. He observed that increased turbidity caused a significant reduction in the depth distribution of SAV in the Chesapeake Bay due to reduced photosynthetically active radiation (PAR) availability. Compared to land angiosperms, SAV requires some of the highest light levels of any plant group worldwide (Dennison et al. 1993). Human origin impacts on water clarity such as eutrophication and sedimentation events rank highest for pressures limiting SAV survival, leading to altered abundance and distribution (Duarte 2002; Orth et al. 2017). Eutrophication occurs when there is an increase in nitrogen and phosphorus runoff, which can stimulate an algal or phytoplankton bloom that severely limits the light availability and inhibits the photosynthetic process (Nixon 1995; Burkholder et al. 2007). Impacts on water clarity affect the amount of light available for photosynthesis, making it a hypothesized limiting factor for SAV growth and survival (Kemp et al. 2004).

Although light availability is the primary concern for SAV survival, some other environmental factors and stressors influence abundance, such as temperature, salinity, nutrients, wave energy, and sediment type (Kemp et al. 2004). Similar to the Chesapeake Bay, North Carolina is home to various SAV species whose distribution is often influenced by a salinity regime. In North Carolina, SAV community composition can be stratified into two large regions based on salinity (high and low). Ocean tides and marine conditions influence the high salinity zones, while the low-salinity areas are dominated by wind energy and freshwater discharge

(Kenworthy et al. 2012). High salinity species found in North Carolina include *Z. marina, H. wrightii and R. maritima.* There is more species diversity in the low salinity oligohaline regions due to lack of salinity stress, but they are mostly dominated by one or two species at each site. Ten low-salinity species have been found in previous North Carolina SAV studies by Ferguson and Wood (1994), Davis and Brinson (1990), and Quible and Associates (2011) as well as this study.

One of the most common species found in this monitoring effort was *Vallisneria americana* which has an optimal salinity tolerance that ranges from 0 to 5 parts per thousand (ppt) but has been found along the Atlantic coast in waters as high as 12 ppt (Bergstrom et al. 2006). Davis and Brinson (1990) observed *V. americana* in their 1973-1975 survey on the Pamlico River in average salinities ranging from 4.5 to 6.5 ppt, with peaking salinity found shortly at 11.5 ppt in July. Increased salinity can cause added stress to *V. americana* and has been shown to stunt growth and increase the light requirement for survival. French and Moore (2003) studied *V. americana* and found an interactive effect between light and salinity. Both high salinity and low light levels stunted plant growth and reproduction. French and Moore's work discovered that *V. americana* salinity tolerance is contingent on light availability and the effect of one factor was most significant when the other was not limiting (French and Moore 2003). Their study disagreed with previous laboratory experiments (Twilley and Barko 1990; Kramer et al. 1999) in that *V. americana* could not survive at salinities of 10 ppt or higher for long periods. Another widespread SAV species found in North Carolina estuaries is *Ruppia maritima*, which is known for surviving across a wide salinity range. The optimal salinity tolerance is from 5-15 ppt along the Atlantic coast but has been found growing in much higher salinities worldwide (>50ppt) (Kantrud 1991; Bergstrom et al. 2006). Although this SAV species can survive a range of salinity tolerances, pulses of freshwater and extreme salinities have been shown to negatively impact *R. maritima* growth (La Peyre and Rowe 2003). Also, *R. maritima* has been observed to have a high light demand for survival as wind-induced turbidity has been seen to govern the depth distribution (Kantrud 1991).

SAV can be considered "coastal canaries" and sentinel species that can be used as an early health indicator system for water quality, making it essential to monitor for management efforts (Orth et al. 2006; Moorman et al. 2017). Changes in SAV abundance allow resource managers to see advanced environmental degradation warnings through poor water quality (Orth et al. 2017). The establishment of minimal light requirements for SAV from water quality monitoring can set nutrient standards for agencies and prevent further declines in vegetation (Dennison et al. 1993; Kenworthy and Fonseca 1996).

Monitoring Approaches

SAV monitoring is categorized into three main methodology groups: physical (underwater diving), off-water remote (aerial photography, satellite imagery), and on the water remote (boat-based SONAR) (Sabol et al. 2002). Due to the study site locations in low-salinity turbid waters in North Carolina estuaries, my study was limited to the water boat-based acoustic SONAR survey methods and physical shallow water snorkeling. Hydroacoustic or SONAR is a growing technique used in various high and low-salinity studies to monitor SAV (Kenworthy et al. 2012). Aquatic plants are acoustically reflective due to the gas bubbles, allowing them to be identifiable with SONAR (Maceina and Shireman 1980). The limitations of SONAR are that species composition cannot be distinguished and must be paired with land-based monitoring approaches for a comprehensive study. Also, SONAR is known to be limited to depths greater than 0.76 meter (m) due to the heavy backscatter that can occur in shallower areas (Valley et al. 2015). These reasons are why the ECU/APNEP team paired boat-based SONAR transects with inshore diver surveys at depths from 0 to 1m to overlap and cover these shallow water depths.

Purpose of this Project

This study aims to understand physical limitations for dominant SAV species in North Carolina estuaries by observing the change in SAV abundance (odds of occurrence, cover, frequency, and biomass) with environmental factors, salinity, turbidity, and water depth. Accurately monitoring North Carolina's SAV abundance is the first step in identifying whether a change occurs by developing a baseline that allows us to understand SAV variability better. Shore survey data of sentinel site locations from 2015-2019 were used to understand what abiotic variables potentially limit SAV survival and abundance. Knowing limitations for North Carolina SAV species will allow for improved accuracy in SAV prediction maps and enable management agencies to be better able to explain variations of SAV abundance. Agencies could also use minimum light requirements for SAV to set nutrient loading standards for surrounding areas to reduce water turbidity and prevent further vegetation declines. Again, this research can be used to find suitable locations for restoration efforts of transplanting and reseeding based on North Carolina's SAV species tolerance ranges for light and salinity. SAV species composition is often determined by abiotic factors like salinity, turbidity, and water depth. Understanding the combined effects of environmental stressors on SAV is required to develop habitat requirements and restoration.

Objectives

I) Analyze how dominant SAV species *(Ruppia maritima*, *Vallisneria americana,* and *Zannichellia palustris*) change with water depth, salinity, and Secchi depth transparency in North Carolina estuaries. (Albemarle Sound, Pamlico River Estuary, and Neuse River Estuary)

II) Determine tolerance limits of water depth, salinity and Secchi depth for North Carolina's dominant SAV species based on biomass measures.

III) Evaluate North Carolina's SAV species diversity (*Ruppia maritima*, *Vallisneria americana*, *Zannichellia palustris*, and others) present at a sentinel site to determine whether it has changed over time based on 2015-2019 quadrat surveys.

Hypotheses

H1a: Turbidity (as measured by Secchi depth) and *Ruppia maritima* odds of occurrence will be negatively correlated at the ECU/APNEP sentinel sites.

H1b: Turbidity (as measured by Secchi depth) and *Vallisneria americana* odds of occurrence will be negatively correlated at the ECU/APNEP sentinel sites.

H1c: Turbidity (as measured by Secchi depth) and *Zannichellia palustris* odds of occurrence will be negatively correlated at the ECU/APNEP sentinel sites.

H2a: Turbidity (as measured by Secchi depth) will be negatively correlated with the maximum colonization depth (measured by SAV presence along the quadrat or SONAR transects) at the ECU/APNEP sentinel sites.

H2b: Salinity will be negatively correlated with the maximum colonization depth (measured by SAV presence along the quadrat or SONAR transects) at the ECU/APNEP sentinel sites.

H3a: Salinity will limit the distribution of *Ruppia maritima* to areas of low salinity at the ECU/APNEP sentinel sites.

H3b Salinity will limit the distribution of *Vallisneria americana* to areas of low salinity at the ECU/APNEP sentinel sites.

H3c: Salinity will limit the distribution of *Zannichellia palustris* to areas of low salinity at the ECU/APNEP sentinel sites.

H4a: SAV abundance (percent cover and frequency measured with quadrats; odds of occurrence and biomass measured in SAV cores taken along transects) will be inversely related to water depth, with the greatest abundance found at shallow depths and lowest abundance at deepest depths for *Ruppia maritima.*

H4b: SAV abundance (percent cover and frequency measured with quadrats; odds of occurrence and biomass measured in SAV cores taken along transects) will be inversely related to water depth, with the greatest abundance being found at shallow depths and lowest abundance at deepest depths for *Vallisneria americana.*

H4c: SAV abundance (percent cover and frequency measured with quadrats; odds of occurrence and biomass measured in SAV cores taken along transects) will be inversely related to water depth, with the greatest abundance being found at shallow depths and lowest abundance at deepest water depths for *Zannichellia palustris.*

Methods

Study sites

Monitoring was conducted in the Albemarle Sound (AS), Pamlico River Estuary (PRE), and Neuse River Estuary (NRE) May through October during peak SAV growth times (Quible and Associates 2011). The AS is a large oligohaline estuary in northeast North Carolina. This estuary has a wind-driven tidal system from the small number of inlets connecting it with the ocean due to protection from the barrier island system. The freshwater inflow comes from the major tributaries draining into the sound including the Roanoke, Chowan, Perquimans, and Pasquotank rivers (Giese et al. 1985). The PRE is a drowned river valley estuary with an extreme salinity range of 0-18 ppt which is often influenced by the degree of freshwater runoff from the Tar River (Davis and Brinson 1990). The Tar River is the same watercourse as the Pamlico River, and the name changes at Washington, NC. This body of water is relatively shallow, averaging 3.3 m deep (Giese et al. 1985). Similar to the PRE is the NRE, which has a similar salinity range but slightly higher on average of 2-4 ppt at the river's mouth (Davis and Brinson 1990). The NRE has an average depth of 5 m at the mouth to approximately 2.4 m upriver at New Bern (Giese et al. 1985).

Sampling design

Sentinel sites were established in the AS, PRE, and NRE as fixed locations to be yearly sampled to allow statistical comparisons and evaluate SAV abundance changes. These sentinel site locations are samples of the entire AS, PRE, and NRE as it is not feasible to monitor the entire estuary annually intensively. Each sentinel site is 50 hectares in size and comprises 40 transects that run perpendicular to the shore. Each transect is spaced 25 m apart, extending roughly 500 m. The method is derived from APNEP protocols for measuring seagrass abundance at Sentinel Sites (Kenworthy et al. 2012). Transect lines were created in ExpertGPS and transferred to a Lowrance global positioning system (GPS), allowing boat operators to follow the exact course needed. Adhering to these transect lines allowed a grid to be created for the exact location over multiple years. Survey assessments of the Albemarle Sound (AS) started in 2015, and the locations are shown below (Figure 1). The Pamlico River Estuary (PRE) monitoring began in 2016 (Figure 2), and the Neuse River Estuary (NRE) monitoring began in 2017 (Figure 3). Sentinel sites are surveyed annually with acoustic SONAR, inshore diver quadrats, and video waypoints. Each sentinel site is surveyed as close to the calendar date of the previous to limit SAV differences coming from interannual variation. The exception to this is in the AS (2015-2016), as it was surveyed twice every year during spring and fall. Tables 1-3 include the location and dates of when sentinel sites were surveyed in each of the monitored North Carolina estuaries.

Sentinel site selection

Sentinel site monitoring locations were previously determined based on four criteria described in Speight (2020). Briefly, there are: 1) locations were selected that could be readily accessible for long term monitoring; 2) SAV had been previously present historically (based on NCDMF historical SAV Map); 3) SAV was present in SONAR and video samples from rapid assessment (RA) surveys; and 4) sites were distributed randomly to provide comprehensive coverage and be representative of the estuary. In ArcGIS, the estuary shoreline was divided into 1000 m by 500 m bins representing potential sentinel sites based on size. The bins that met the above four criteria in each estuary were then randomly selected. Only one site was not randomly selected but was chosen because the AS south shore was underestimated. Sites that contained all four criteria listed above were made into 50-hectare sentinel sites (1000 m x 500 m) with ten sites in the Albemarle Sound (AS) (Figure 1), six in the Pamlico River Estuary (PRE) (Figure 2), and ten in the Neuse River Estuary (NRE) (Figure 3).

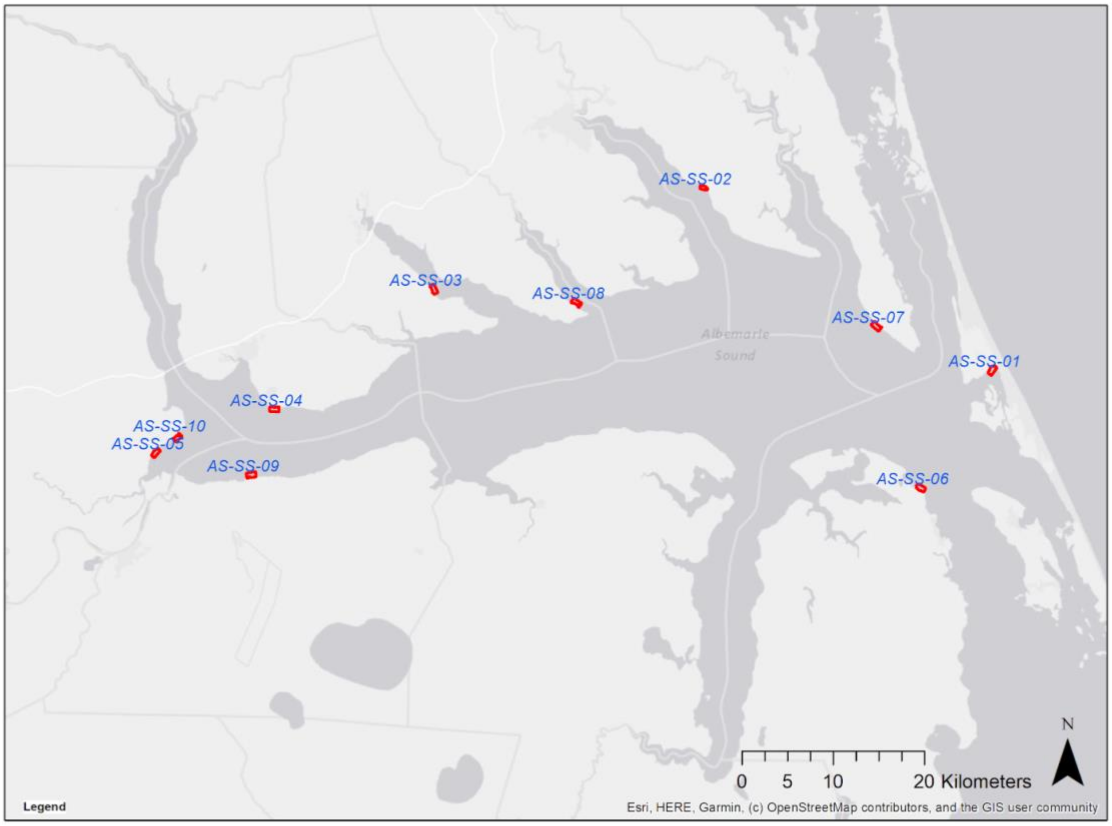

Figure 1. Sentinel site locations in the Albemarle Sound (AS)

Table 1. Albemarle Sound SAV Sentinel Site Surveys.

Sentinel Site	**Location**	**Latitude: Longitude**	**2015 Spr/Su**	**2015 Su/Fa**	**2016 Spr/Su**	**2016 Su/Fa**	**2017 Su/Fa**	**2018 Su/Fa**	**2019 Su/Fa**
AS-SS-01	Kitty Hawk Bay	36 03’31.98” N 75 42’12.89” W	10 Jun	16 Sep	20 Jun	8 Sep	29 Sep	21 Sep	04 Oct
AS-SS-02	Pasquotank River	36 13’57.44” N 76 03’26.45” W	25 Jun	9 Oct	29 Jun	21 Oct			
AS-SS-03	Perquimans River	36 07’35.04” N 76 22’57.16” W	16 Jun	8 Oct	25 May	16 Sep		15 Aug	13 Sep

AS-SS-04	Edenton, Midway	36 00’29.21” N 76 34’25.13” W	15 Jun	28 Sep	9 May	26 Sep		7 Aug	14 Jun
AS-SS-05	Batchelor Bay 1	35 57’46.29” N 76 43’04.02” W	20 May	11 Sep	24 May	18 Oct	8 Sep	9 Aug	2 Jul
AS-SS-06	Mann’s Harbor	35 56’12.77” N 75 47’19.65” W	2 Jun	26 Oct	9 Jun	19 Oct		29 Aug	
AS-SS-07	North River	36 05’48.06” N 75 50’35.52” W	11 Jun	17 Sep	27 Jun	3 Oct		22 Sep	6 Oct
AS-SS-08	Little River	36 06’59.25” N 76 12’31.68” W	24 Jun	28 Sep	6 Jul	23 Sep		24 Aug	14 Sep
AS-SS-09	Roanoke Mouth	35 56’ 31.18” N 76 35’59.76” W	28 May	16 Sep	12 May	30 Sep		14 Aug	15 Jul
AS-SS-10	Batchelor Bay 2	35 58’41.91” N 76 41’33.09” W	26 May	23 Sep	16 May	24 Oct	17 Aug	1 Aug	1 Jul

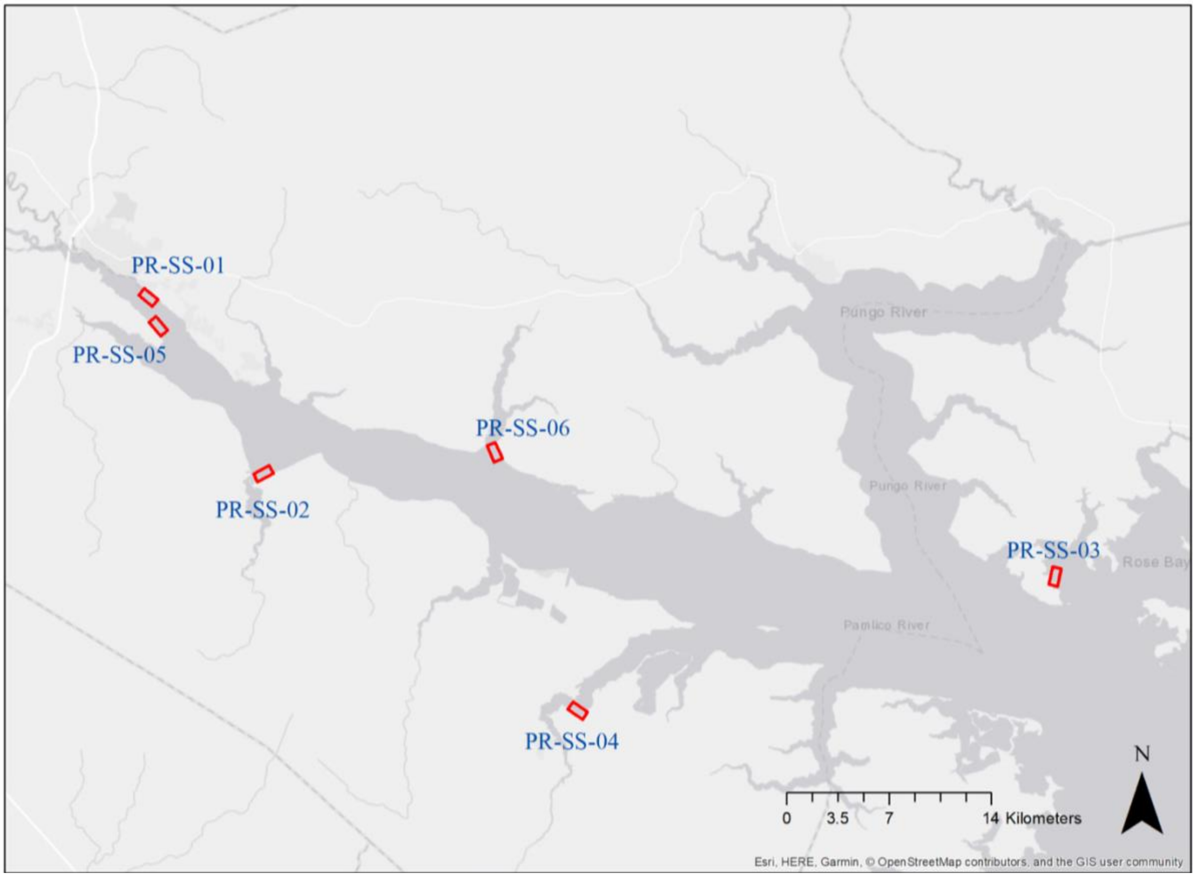

Figure 2. Sentinel site location in the Pamlico River Estuary (PRE)

Table 2. Pamlico River Estuary SAV Sentinel Site Surveys

Sentinel Site	**Location**	**Latitude: Longitude**	**2016**	**2017**	**2018**	**2019**
PR-SS-01	Riverside	35 31’36.75’’ N 77 01’55.18’’ W	9 Aug	14 Jun		7 Aug
PR-SS-02	Blount’s Bay	35 26’12.37’’ N 76 57’37.57’’ W	17 Aug	9 Jun		31 Jul
PR-SS-03	Rose Bay	35 23’12.75’’ N 76 28’28.11” W	19 Aug	26 Jun		16 Jul
PR-SS-04	South Creek	35 19’07.52” N 76 46’06.24” W	18 Aug	19 Jun		29 Jul
PR-SS-05	Whichard’s Beach	35 30’43.79” N 77 01’34.22’ W	11 Aug	7 Jun		6 Aug
PR-SS-06	Bath Creek	35 27’01.04” N 76 49’04.45” W	30 Aug	13 Jun		30 Jul

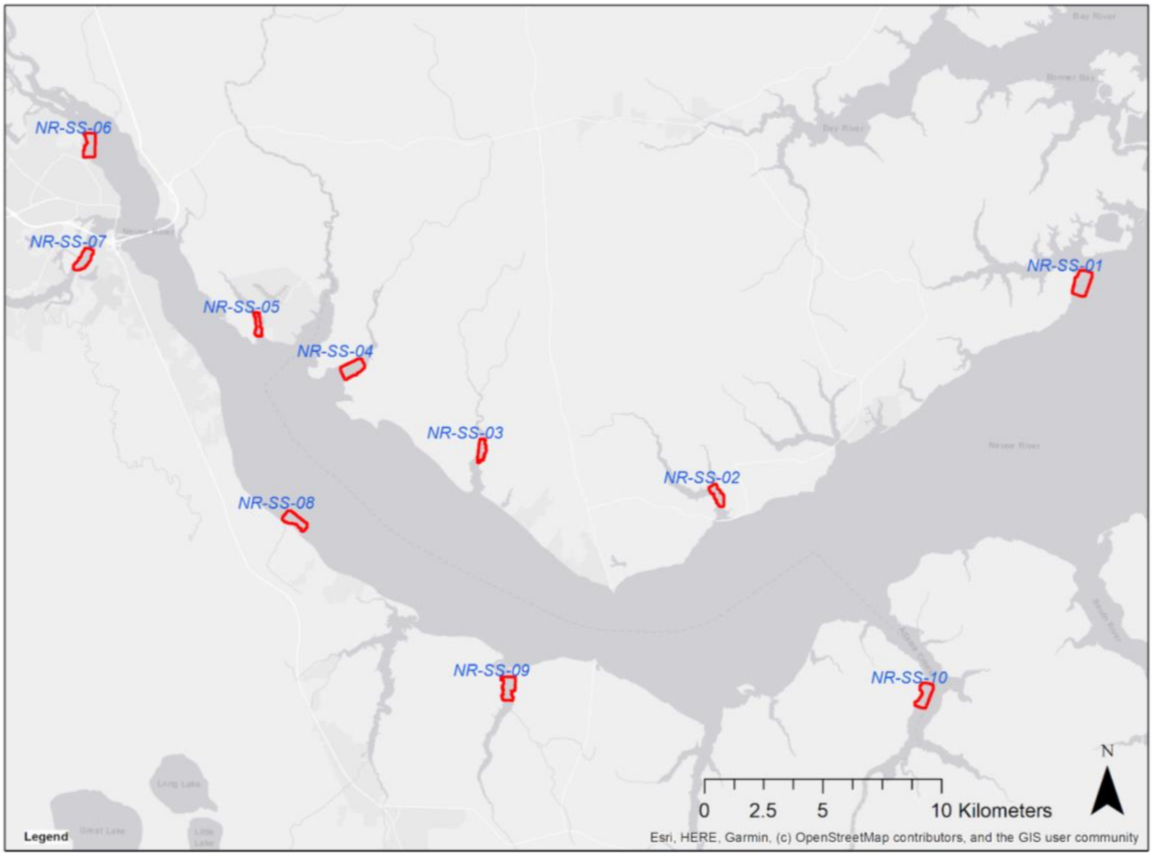

Figure 3. Sentinel site locations in the Neuse River Estuary (NRE)

Table 3. Neuse River Estuary SAV Sentinel Site Surveys

Sentinel Site	Location	Latitude: Longitude	2015	2016	2017	2018	2019
NR-SS-01	Pamlico Sound at Mouth of Neuse	35 05'07.09" N 76 35'26.56" W			28 Jun	2 Jul	18 Jun
NR-SS-02	Dawson's Creek	35 00'08.83" N 76 45'20.65" W			3 Jul	28 Jun	7 Jun
NR-SS-03	Beard Creek	35 01'07.27" N 76 51'55.45" W			28 Oct	15 Jun	22 May
NR-SS-04	Goose Creek	35 02'51.17" N 76 55'29.92" W			26 Jul	11 Jul	19 Jun
NR-SS-05	Fairfield Harbor	35 03'53.01" N 76 58'14.49" W			19 Jul	12 Jul	4 Jun
NR-SS-06	New Bern	35 07'50.45" N 77 03'00.52" W			13 Oct	6 Jun	21 May

NR-SS-07	Trent River	35 05’16.76” N 77 03’03.02” W	18 Jul	17 Jul	29 May
NR-SS-08	Flanner’s Beach	34 59’19.29” N 76 57’07.51” W	27 Jul	19 Jul	26 Jun
NR-SS-09	Hancock Creek	34 55’40.32” N 76 51’10.10” W	1 Aug	26 Jul	21 Jun
NR-SS-10	Adam’s Creek	34 55’42.56” N 76 39’33.68” W	4 Aug	27 Jul	28 Jun

Inshore Diver Quadrats

Inshore quadrat diver surveys were developed to sample in shallow waters (<1.0 m) where boat-based SONAR methods were unable to reach. The quadrat sampling protocol was developed based on the methodology described in Duarte and Kirkman (2001). Diver surveys were done using 1 m^2 quadrats made from PVC (polyvinyl chloride) sectioned into one hundred 10 x 10-centimeter (cm) squares with string. The quadrat was placed on the SAV canopy to determine percent cover at depths of 0.25 m, 0.50 m, 0.75 m, and 1 m. Ten transects were randomly selected out of the 40 transects from a randomly numbered sheet. A handheld Garmin 78Sc GPS receiver allowed for the precise location of transect lines while in the field. At the desired transect, the snorkeler dropped the 1 m^2 quadrat and counted the SAV percent cover at each depth (0.25,0.5,0.75, and 1 m) for each of the ten shore transects. If one blade of SAV was present in one of the 100 10-cm squares, then that 10-cm square was counted as full. Each depth was sampled in triplicate, perpendicular to the transect, to calculate the average percent cover at those depths on that transect. One hundred twenty quadrats were taken at each sentinel site (10 transects x 4 depths x 3 replicates at each depth) unless transect lines were in deeper areas or could not be sampled due to rocks, stumps, and other obstructions. Another SAV metric based on quadrats was percent frequency SAV. Frequency is often used to detect vegetation changes at a site and measure the patchiness in an SAV bed. To calculate percent frequency, take the number of positive SAV quadrats and divide by the total number of quadrats taken, as shown in Equation 1. Frequency can be measured at the sentinel site level and determined for each of the sampled quadrat depths (0.25-1.0 m). The total percent cover found at a sentinel site was multiplied by the species percent composition based on biomass from cores (see below) to compute the cover and frequency of each species to determine individual SAV species percent cover and frequency for each water depth.

$$SAV\ \%\ Frequency = \frac{\#\ of\ Quadrats\ with\ SAV}{Total\ \#\ of\ Quadrats} x100 \qquad \text{Equation (1)}$$

Sediment Cores

If SAV was present, then a 30-cm diameter sediment core was taken (one core per depth) and brought back to the lab to be identified, dried, and weighed to calculate the corresponding biomass. Samples taken from the core were placed into labeled plastic bags stored on ice until being relocated to a refrigerator. Once in the lab, SAV species were identified using field guides and separated into aluminum containers to be dried in a furnace at 60° C for 48 hours to ensure all moisture was removed. Once dried, samples were weighed to calculate total biomass (above and below ground) for each core taken that corresponded to a sentinel site location, transect, and depth based on the waypoint latitude and longitude from the GPS. The percent composition of SAV species was determined by dividing the species biomass by the total biomass found at each sentinel site. This formula can be seen below in equation 2. The percent frequency or occurrence of SAV species can be found in a similar manner shown in equation 3. Since it was possible to have multiple species present in a core sample, the denominator for the number of species identified (494) when determining species percent occurrence was larger than the number of cores taken (418).

$$Species\ \%\ Composition = \frac{Biomass\ of\ one\ SAV\ species}{Total\ SAV\ Biomass} x100 \qquad \text{Equation (2)}$$

$$Species\ \%\ Occurrence = \frac{\#\ of\ times\ species\ was\ observed}{Total\ \#\ of\ all\ species\ observed} x100 \qquad \text{Equation (3)}$$

Boat-Based Acoustic SONAR

At each sentinel site, 40 transects perpendicular to shore transects (spaced 25 m apart) were conducted by boat with two echo sounders capable of single-beam and side-scan SONAR. SONAR uses acoustic pings in the water and records the time it takes to detect the return echo to determine depth and ground type. Underwater acoustic SONAR sampling has been used in various studies to monitor and detect SAV (Lefebvre et al. 2000; Foster et al. 2011; Luczkovich et al. 2013; Aleksandra et al. 2015; Greene et al. 2018). Multiple SONAR transducers were used jointly during sampling (Lowrance Elite-9 ti^2 and BioSonics DTX). A Lowrance Elite-9 ti^2 SONAR unit ($999.99) equipped with a Total scan skimmer ($299.99) 455/800 kilohertz (kHz) transducer were used in conjunction with BioBase cmaps (https://www.biobasemaps.com/), a cloud-based data processing algorithm, to create SAV maps of vegetation, bathymetry, and ground hardness. Lowrance's transducer allows for side-scan imaging to be done simultaneously with traditional (single-beam) sonar pings. SL2 files were then saved and sent to BioBase cmaps to be processed automatically using their SAV algorithm subscription service. BioBase measures vegetation as biovolume (bv), calculated as the plant height divided by the water depth. The algorithm counts only vegetation taller than 0.05% of the water column. For example, in 1 m of water, the grass must be at least 5 cm in height to be counted to avoid false-positive detections. BioSonic's DT-X Extreme echosounder single-beam 420-kHz transducer and Panasonic Toughbook laptop computer were also used to collect SONAR data during the sentinel site surveys. Visual Accusation software DT4 files were automatically synced to the Panasonic Toughbook laptop, which allowed us to create data acoustic reports manually. DT4 files were played back in the lab using BioSonics Visual Habitat software to calculate percent cover measurements. This was done by counting the number of SAV positive ping points from the total number of SONAR pings on a transect. Both Lowrance and BioSonics DTX transducers were corrected for a 0.28 m bottom transducer offset.

Water Quality

All sentinel site monitoring locations began with water quality measurements of salinity (ppt), Secchi depth (m), water temperature (°C), conductivity (umhos), and dissolved oxygen (mg/L and %). A Secchi disk 20 cm in diameter was used to measure Secchi depth as a relative measure of turbidity at the start of each survey between 9 am and 11 am. All measurements were performed approximately at the 20^{th} transect in the middle of the sentinel site. The water quality instruments used included the YSI pro-2030 multi-meter and Castaway conductivity temperature and depth (CTD). They were deployed in conjunction to measure water quality variables. Castaway CTD provided salinity and temperature profiles through the water column to visualize thermoclines and haloclines. The YSI pro-2030 measured salinity, dissolved oxygen, conductivity, and temperature for both surface and bottom depths to compute an average.

Because only one water quality measurement was collected for each sentinel site per year, other water monitoring programs in the area were used to compute a yearly average of

Secchi depth and salinity. An ensemble of water quality data sets from North Carolina monitoring programs was used to model the Secchi depth and salinity throughout the interest estuaries, and predictions were interpolated for sentinel site locations that were nearby. The programs included the North Carolina Department of Marine Fisheries (NCDMF) trawl survey (Program 120), NCDMF mesh gillnet survey (Program 915), North Carolina ambient monitoring system (AMS), and ModMon sampling stations. Water quality monitoring data collected at mid-channel stations may not represent conditions at adjacent shallow water depths where sentinel sites were located. However, it has been shown through parallel measurements in the Chesapeake Bay that mid-channel stations were statistically indistinguishable 90% of the time when the distance was less than 2 kilometers (km) (Karrh 2000). Monitoring programs used in this study that were mid-river sampling stations included ModMon and AMS, while P120 and P915 were stratified throughout the rivers and used in conjunction to compute averages of Secchi depth and salinity for the years ECU/APNEP surveyed SAV from 2015-2019 at each sentinel site. The AS only contained a few monitoring stations from the North Carolina ensemble monitoring programs. Due to this limitation, a yearly average of Secchi depth and salinity could not be determined for the AS sites based on the ensemble data.

ArcMap Interpolation

To compute a yearly average of Secchi depth and salinity for each of the sentinel sites in the PRE and NRE, an inverse distance weight (IDW) interpolation was performed using the data collected from the ensemble water quality programs in those bodies of water (See APPENDIX H). The IDW technique computes an average value across unsampled locations using values from nearby sampled weighted locations. The ArcGIS program ArcMap (10.5.1) allowed me to make a prediction surface of Secchi depth and salinity for the PRE and NRE for each of the surveyed years (2016-2019). This could not be done for the AS because it had limited water monitoring stations and not enough data to support an interpolation. Before creating the interpolation map for Secchi depth and salinity, only the NRE and PRE monitoring stations were selected leaving only 245 samples for 2019, 272 for 2018, 405 for 2017, and 164 samples in 2016. In 2018 the PRE was not surveyed, and sentinel surveys did not begin in the NRE until 2017, so the sample numbers for those years (2016 and 2018) only represent one estuary and do not combine both PRE and NRE monitoring samples.

Maximum Colonization Depth

To determine the deep edge of an SAV grass bed, often referred to as the maximum colonization depth (MCD), SONAR data had to be used in conjunction with quadrat diver surveys. The inshore quadrat surveys went to a maximum depth of 1 m, which did not represent the deep edge for most sentinel sites. The MCD of SAV was calculated at each sentinel site that contained SAV by looking through the SONAR echogram and side-scan imagery using a program called Reefmaster. Reefmaster allowed SONAR files to be played back in the lab to determine the MCD for each sentinel site by averaging the maximum depth observed along the ten random transects used in the quadrat survey for each sentinel site. The ten quadrat transects were used for averaging the MCD to avoid potential false-positive readings as these transects had been physically verified as having SAV presence or absence by inshore divers. A linear regression approach was used to analyze the relationship between turbidity (measured by Secchi depth) and the MCD of SAV. A regression model was created using ECU/APNEP Secchi depth measurements and the ensemble data set of interpolated Secchi depth measurements.

Species Accumulation Curve

In order to estimate species diversity for the study sites based on sampling effort, species accumulation curves were created in R studio with the "vegan" package for every year monitored (2015-2019) and estuary (AS, PRE, and NRE). The sampled effort was not equal for every year and estuary location, which made a direct comparison of species diversity not possible. The number of SAV positive surveys totaled 52 for the entire monitored project. Species accumulation curves showed the rate at which new species are found within a community and show the expected number of detected species as a function of sampling effort to provide information on species richness. First, the data were put into a matrix of species absence (0) and presence (1) for each SAV positive sentinel site survey to create the accumulation curves. Next, the R function "specaccum" was used within the "vegan" package to create an average of the randomly generated species accumulation curves. Two species accumulation plots were created: one with the species accumulation curves separated by year (2015-2019) and the other created by subsetting the data by estuary location (AS, NRE and PRE).

Statistical Analyses

Statistical analyses were created in R studio using version 3.6.2. The experimental unit of analysis for each sentinel site survey was the four measured water depths (0.25, 0.5, 0.75, and 1 m) since only one water quality measurement was taken for each sentinel survey. So, one value per depth was taken from each sentinel site that went into the regression model. A logistic regression binomial approach was used for predicting the odds of SAV occurrence with continuous predictors of salinity, Secchi depth, and water depth. This approach was used because logistic regression allows for predicting a binary event occurring like SAV absence/presence at a sentinel site. To perform the binary logistic regression in R, each sentinel survey was given a one or zero to represent SAV presence or absence. If any SAV was found during the sentinel site survey for each measured water depth, it was given the binary number 1. In R studio, the base function "glm" was used with the binomial family to initiate a logistic regression. A binary logistic regression model was created for SAV species *R. maritima*, *V. americana* and *Z. palustris* based on the sentinel site survey presence/absence identified in core samples. Three columns were added to the data file to record each species presence/absence for the four measured water depths at each sentinel site. For example, if *R. maritima* was absent at a sentinel site survey, it would receive a 0 for every water depth for that site (0.25, 0.5, 0.75, and 1 m) even if another SAV species was present at that sentinel site. R studio put the output for beta (β) estimates in logit form, which must be exponentiated to obtain the odds ratio to allow for a more straightforward interpretation of effect size. A Wald test and Hosmer and Lemeshow goodness of fit test was used for model evaluation to determine if the full model outperformed the null model in explaining variability and if the predicted model fit the observed data. A Hosmer and Lemeshow goodness of fit test that has a small P-value (<0.05) indicates that the model is a poor fit while a large P-value (>0.05) means there is not enough evidence to suggest the model is a poor fit (Peng et al. 2002; Hosmer et al. 2013).

Violin plots were created in R studio to visualize dominant species salinity tolerances by measuring the SAV species dry biomass across the observed salinity gradient. Violin plots allow researchers to depict numeric data distributions with a combination of a kernel density curve and boxplot. Optimal salinity tolerance was determined by the plot of the highest species biomass and the associated salinity range. Species dry biomass comes from core samples, and they were transformed with a $\log_{10}$ approach due to a small number of samples containing high biomass, skewing the data towards larger values. The salinity (ppt) measurements used in the violin plots came from ECU/APNEP point measurements at sentinel site locations.

To analyze SAV abundance (frequency, cover, and biomass) against the four measured water depths (0.25, 0.5, 0.75, and 1 m) one-way analysis of variance (ANOVA) was performed for each of the SAV abundance metrics for all three dominant SAV species. Arcsine square-root transformations were performed on percent frequency and cover data (proportions) before running one-way ANOVAs to improve normality assumptions. For SAV dry biomass, a $\log_{10}$ approach was used to meet normality test assumptions. If water depth showed a statistically significant difference, then a Tukey honestly significant difference (HSD) post-hoc test was performed to determine those depths statistically different from one another. A statistical effect size of how much variance in the response variable was accounted for by the explanatory variable was measured with omega squared (ω^2). Lakens (2013) suggested benchmark values for interpreting omega squared values: 0.01 represents a small effect, 0.06 a medium effect, and 0.14 a large effect. Omega squared was selected because it is believed to be less biased than other effect size measurements when sample sizes are small (Lakens 2013). Water depths were limited to 1 m sampling because of the monitoring design. Inshore quadrat sampling was a way of measuring the shallow depths where boat-based acoustic SONAR could not accurately measure.

Odds Ratios

Odds ratios (ORs) are commonly used to reveal the strength of the independent variable's contribution to the outcome in logistic regressions. They are defined as the odds of the outcome occurring versus not occurring for each independent variable (Stoltzfus 2011). Odds ratios greater than one mean that the event is more likely to occur as the predictor variable increases, while odds ratios less than one mean that the event is less likely to occur as the predictor variable increases. In logistic regression, the β output is given in logit units, and this needs to be exponentiated to obtain the odds ratio, which is useful for assessing the effect size. For continuous independent variables used in the model, it is important to have meaningful measurements to express the degree of changes associated with predictor variables (Stoltzfus 2011; Hosmer et al. 2013). This is why beta estimates for Secchi depth and water depth which were originally expressed as 1 m units of change, were multiplied by 0.1 before being expressed as odd ratios. Now the odds ratio represents the 0.1 m change with predictor variables Secchi depth and water depth [OR (0.1) = exp $(0.1*\beta_1)$]. The measured water depth in this study only went out to 1 m, so the measurement unit should be smaller to have more meaningful interpretations of the predictor variables Secchi depth and water depth.

Results

Overview

In total, 108 surveys were conducted by the ECU/APNEP team at sentinel sites located in the AS, PRE, and NRE. The survey years ranged from 2015-2019 in the AS, 2016, 2017, and 2019 for the PRE and 2017-2019 for the NRE. Out of the total 108 sentinel site surveys, ECU/APNEP team members only found SAV present in 52 surveys. The total number of sentinel sites surveyed was 60 (10 sites in 5 years, however in 2015 and 2016 these SS were surveyed twice a year) in the AS, 18 (6 sites in 3 years) in the PRE, and 30 (10 sites in 3 years) in the NRE. In the AS, two out of the ten sentinel sites never contained SAV during any point of the sentinel site surveys from 2015-2019 (sites 5 and 9). Also, four NRE sentinel sites (1, 4, 8, and

10) did not have SAV present within the survey period. With these sentinel sites not having any SAV during the multi-year study, they were removed from the analysis, reducing the overall number of surveys at sentinel sites from 108 to 83.

Species Composition

Ten SAV species were identified in the 418 core samples taken from 2015-2019 in the AS, PRE, and NRE sentinel sites. The three most abundant species by dry biomass were *V. americana, R. maritima,* and *Z. palustris,* making up a combined 80% of the total biomass (Table 4). A detailed list of species presence for every sentinel site survey can be found in APPENDIX A. The most frequent species found in the core samples was *R. maritima.* It was observed to be co-occurring with all other nine SAV species during the 2015-2019 monitoring period. There was a total of 201 observations of *R. maritima* in the 418 cores (48%) and 40.7% occurrence of all SAV species observations (Table 4.) *Vallisneria americana* was next with 115 species observations (23.3% occurrence), followed by *Zannichellia palustris* identified in 73 cores (14.78% occurrence). The NRE and PRE had *V. americana* as the dominant species, while the AS had *R. maritima* as the dominant SAV species based on the dry biomass found in cores. Nine SAV species were found in the AS from 2015-2019, nine species in the PRE in 2016, 2017, and 2019 and eight species in the NRE from 2017-2019. Composition maps for each estuary can be found in APPENDIX B for years 2015-2019, while a summary table based on cores and quadrats for each sentinel site can be found in APPENDIX C.

Twenty out of the 52 SAV positive sentinel site surveys contained only one SAV species (38.46%). Two SAV species were found co-occurring in 19 sentinel site surveys (36.54%), and 13 sentinel site surveys contained three or more SAV species (25%). *Ruppia maritima* was observed in 37 out of the 52 SAV positive surveys, while *Vallisneria americana* was present in 16 surveys and *Zannichellia palustris* 11. *Ruppia maritima* was found occurring with another SAV species in more surveys than found alone (25 to 12 sentinel site surveys, respectively).

Table 4. SAV species found in 2015-2019 Sentinel Surveys. Percent composition is based on dry biomass weight, while species occurrence is how many times the species was identified in a core sample—computed with equations 2 and 3.

Scientific Name	Common Name	% Composition	% Occurrence
Ruppia maritima	Widgeon grass	37.75%	40.69%
Vallisneria americana	Wild celery	34.99%	23.28%
Myriophyllum spicatum	Eurasian watermilfoil	9.10%	8.30%
Zannichellia palustris	Horned pondweed	7.16%	14.78%
Naja guadalupensis	Bushy pondweed	5.16%	3.04%
Zostera marina	Eel grass	1.89%	1.21%
Stuckenia pectinata	Sago pondweed	1.48%	2.83%
Halodule wrightii	Shoal grass	0.91%	2.22%
Potamogeton crispus	Curly pondweed	0.14%	0.61%
Potamogeton pusillus	Slender pondweed	0.13%	0.61%

Change Over Time

Changes in SAV percent cover at each sentinel site overtime were apparent in monitoring years (see APPENDIX D). *Ruppia maritima* was found to have increased percent cover at most sentinel sites in all estuaries (AS, PRE, and NRE), but a decrease was observed at AS-SS-08. *Vallisneria americana* cover was observed to decline in the AS and NRE, but two sentinel sites in the PRE furthest upstream showed percent cover increases through the monitoring period (PR-SS-01 and PR-SS-05). *Zannichellia palustris* increased in percent cover at locations in the PRE and NRE but showed decreased cover at one sentinel site in the AS (AS-SS-03). Results of the analyses indicate salinity played a role in these observed changes in the AS and NRE.

Ruppia maritima

A multiple logistic regression analysis was used to investigate the influence of salinity, turbidity (measured by Secchi depth), and water depth on *R. maritima* occurrence. No significant interaction effect between any combination of water depth, salinity, or Secchi depth was observed for the dominant SAV species resulting in the interactions being dropped from the model. All three environmental variables were statistically significant in the model, with water depth and salinity having the largest effect on the odds of *R. maritima* occurrence. Salinity significantly affected the odds of *R. maritima* occurrence (Wald χ^2=14.845, P<0.001; Table 5). The fitted odds ratio for salinity was 1.11 [95% CI (1.036, 1.309)]. The logistic regression model suggested that for every 1 part per thousand (ppt) increase in salinity, the odds of *R. maritima* being present (versus not being present) are expected to increase by a factor of 1.11, holding all other variables constant. The result of an OR > 1.0 indicated, a positive association between *R. maritima* occurrence and salinity. A helpful way of interpreting this is to say that the odds of *R. maritima* occurrence increase by 11% for each one ppt unit increase in salinity. Our model is limited to predictions in the 0-20 ppt range; the highest salinity recorded for *R. maritima* was 18 ppt. This result makes sense for *R. maritima* because it has a wide salinity tolerance. Secchi depth was another significant predictor in the logistic regression model for *R. maritima* (Wald χ^2=10.843, P<0.001; Table 5). The estimated odds ratio predicted that for every 0.10-m increase in Secchi depth the odds of *R. maritima* being present (versus not being present) change by a factor of 0.795 [OR=0.795, 95% CI (0.69, 0.91)]. An odds ratio < 1.0 indicates the event (*R. maritima* being present) is less likely to occur as the predictor variable (Secchi depth) increases. Secchi depth predictions were limited to the observed range of 0.35-1.6 m. This can be interpreted in terms of percentage change as the odds of *R. maritima* occurrence decreases by

20.5% for every 0.10 m increase in Secchi depth. Water depth was also statistically significant in the model (Wald χ^2= 5.026, P=0.024; Table 5). The fitted odds ratio for water depth was 1.115 [95% CI (1.101,1.23)]. These results suggested that for every 0.10-m increase in water depth, the odds of *R. maritima* being present (versus not being present) are expected to increase by a factor of 1.115, holding all other variables constant. An odds ratio of 1.115 equates to a percent change in the odds of *R. maritima* occurrence of 11.5% for every 0.10 m increase in water depth. My logistic regression model was limited to predictions in water depth from 0-1 m. Model evaluations using Hosmer and Lemeshow goodness of fit test examine whether the observed proportions of events are similar to the predicted outcomes in subgroups of the data using a Pearson chi-square test. A P-value > 0.05 indicated that our model was a good fit to the data (P=0.159). A Wald test showed that our full model of predictor variables (salinity, Secchi depth and water depth) was more effective at explaining variability than the (null) intercept only model.

Table 5. Logistic regression output for *Ruppia maritima* odds of occurrence with salinity, Secchi depth, and water depth as environmental predictors. Model evaluations show that the predictive model is better than the null model and adequately fits the data. Secchi depth and water depth units are scaled to show 0.10m units of change instead of 1m.

Ruppia maritima							
Predictors	β Estimate	Std. error	Wald's χ^2	df	p	Odds ratio	CI (2.5- 97.5%)
(Intercept)	-0.5051	0.614	0.676	1	0.410	0.603	0.18 - 2.02
Salinity	0.1066	0.028	14.845	1	**<0.001**	1.113	1.05 - 1.18
Secchi depth	-0.2285	0.069	10.843	1	**<0.001**	0.795	0.69 - 0.91
Water depth	0.1088	0.049	5.026	1	**0.024**	1.115	1.01 – 1.23
Test			χ^2	df	p		
Overall model evaluation							
Wald test			27.65	2	<0.001		
Goodness-of-fit test							
Hosmer & Lemeshow			11.83	8	0.159		

Vallisneria americana

Vallisneria americana odds of occurrence were statistically significant with all predictor variables (salinity, Secchi depth, and water depth) (Table 6). Water depth was the most statistically significant of the variables tested (Wald χ^2=13.271, P<0.001; Table 6). The fitted odds ratio for water depth was [OR=1.35, 95% CI (1.15, 1.60)]. This suggests that for every 0.10-m increase in water depth, the odds of *V. americana* being present (versus not being present) are expected to increase by a factor of 1.35, holding all other variables constant. Another way of saying this is that the odds of *V. americana* occurrence increase by 35% for each 0.10-m increase in water depth (depth range limited to 1 m). Secchi depth also showed a significant positive association with *V. americana* occurrence (Wald χ^2=5.433, P=0.019; Table 6). The fitted odds ratio of *V. americana* being present are 1.18 times as likely to occur than to not occur with every 0.10 m increase in Secchi depth [OR=1.18, 95% CI (1.03, 1.37)]. This means for every 0.10 m increase in Secchi depth, we can expect an increase of 18% in the odds of *V. americana* occurrence (the largest Secchi depth recorded was 1.6 m). Salinity was found to significantly affect the odds of *V. americana* occurrence (Wald χ^2=4.334, P=0.037; Table 6). For every 1 part per thousand (ppt) increase in salinity, the odds ratio of *V. americana* occurrence at a site was 0.87 as likely as not being present, holding all other variables constant [OR=0.87, 95% CI (0.75, 0.98)]. This OR is <1.0, which indicates the odds of *V. americana* are lower as the predictor (salinity) increases. This result can also be expressed as a 13% decrease in the odds of *V. americana* occurrence with every 1 ppt increase of salinity (i.e., there is a negative association between *V. americana* presence and salinity). A Hosmer and Lemeshow goodness of fit test indicated that our model is a good fit to the data (P=0.823).

Table 6. Logistic regression output for *Vallisneria americana* odds of occurrence with salinity and Secchi depth as predictors. Salinity, Secchi depth, and water depth all show a statistically significant relationship with the odds of occurrence of *V. americana*. Model evaluations show that the predictive model is better than the null model and adequately fits the data. Secchi depth and water depth units have been scaled to 0.10 units of change instead of 1m.

Vallisneria americana							
Predictors	β Estimate	Std. error	Wald's χ^2	df	p	Odds ratio	CI (2.5 -97.5%)
(Intercept)	-5.4913	0.985	31.081	1	<0.001	0.0041	0.00 – 0.03
Salinity	-0.1393	0.067	4.334	1	**0.037**	0.870	0.75 – 0.98
Secchi depth	0.1710	0.073	5.433	1	**0.019**	1.186	1.03 – 1.37
Water depth	0.3003	0.082	13.271	1	**<0.001**	1.350	1.15 – 1.60
Test			χ^2	df	p		
Overall model evaluation							
Wald test			22.27	3	**<0.001**		
Goodness-of-fit test							
Hosmer & Lemeshow			4.360	8	0.823		

Zannichellia palustris

The multiple regression logistic model for *Z. palustris* was only statistically significant for the environmental predictor variable water depth (Wald χ^2= 9.132, P=0.0025; Table 7). The model predicted that for a 0.10 m increase in water depth, the odds of *Z. palustris* being present (versus not being present) are expected to increase by a factor of 1.32, holding all other variables constant [OR=1.32, 95% CI (1.11, 1.60) Table 7]. This is to say that the odds of Z. *palustris* occurrence increase by 32% for each 0.10-m increase in water depth. These results were limited to water depths between zero and one meter as we did collect core and quadrat data deeper than 1 m depth. Model evaluations of Hosmer and Lemeshow goodness of fit test indicate that the model was a good fit to the data (P=0.815).

Table 7. Logistic regression output for *Zannichellia palustris* odds of occurrence with salinity, Secchi depth, and Water depth as environmental predictors. Model evaluations show that the predictive model is better than the null model and adequately fits the data. The units of measure were scaled to 0.10m for Secchi depth and water depth.

Zannichellia palustris							
Predictors	β Estimate	Std. error	Wald's χ^2	df	p	Odds ratio	CI (2.5- 97.5%)
(Intercept)	-4.8293	1.106	19.044	1	<0.001	0.0089	0.00 - 0.06
Salinity	0.0544	0.044	1.535	1	0.215	1.0558	0.96 – 1.15
Secchi depth	0.0055	0.096	0.003	1	0.955	1.0055	0.82 – 1.20
Water depth	0.2765	0.091	9.132	1	**0.002**	1.3185	1.11 – 1.60
Test			χ^2	df	p		
Overall model evaluation							
Wald test			10.47	3	0.0149		
Goodness-of-fit test							
Hosmer & Lemeshow			4.444	8	0.815		

Cover

To further evaluate water depth's influence on dominant SAV species abundance (percent cover, frequency, and biomass), I examined plots of abundance along the transects depth gradients at each sentinel site. Percent cover reflects how abundant a plant is by providing a measurement of how much space a species occupies on a 1 m^2 quadrat at each depth. The percent cover metric combining all SAV species found in this study increased with increasing water depths of 0.25, 0.5, 0.75, and 1 m (Figure 4). As water depth increased there was on average more SAV cover found along the transects. An arcsine square-root transformation was performed on the data to help normality and improve heteroscedastic assumptions prior to an ANOVA test. Water depth as a factor was statistically significant after running a one-way ANOVA ($F_{3,185}$) = 8.1659, P=<.001 ω^2=0.10). A Tukey honestly significant difference (HSD) post-hoc test revealed that mean percent cover of combined SAV was statistically greater at water depths 0.50, 0.75, and 1 m than at the 0.25 m water depth. ANOVA results are provided in APPENDIX E.

A similar one-way ANOVA was performed for each dominant SAV species. The mean percent cover for *V. americana* differed significantly with water depth ($F_{3, 185}$) = 2.71, P=0.0465 ω^2 = 0.03 (see APPENDIX E). A Tukey HSD post-hoc comparison showed that mean percent cover of *V. americana* was statistically greater at 1.0 m than 0.25 m water depth (Figure 4). Although statistically significant, the omega squared can be interpreted that water depth has a negligible effect size on *V. americana* cover (ω^2=0.03).

For the other two dominant SAV species found during the study (*R. maritima and Z. palustris),* no statistically significant difference in mean cover with water depth was observed (APPENDIX E). Although *R. maritima* results were not statistically significant, it was detected in the shallowest depths (0.25 m and 0.50 m) more than the other two dominant SAV species.

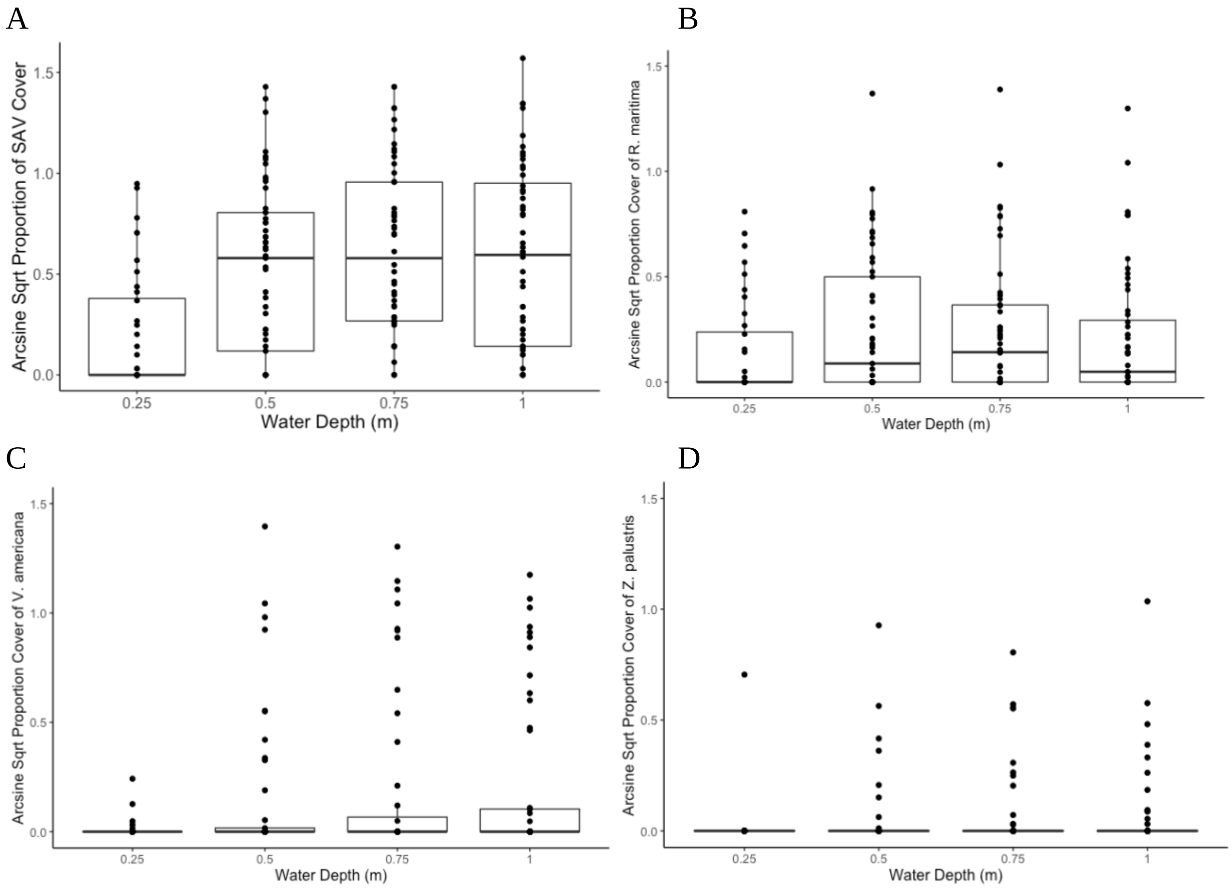

Figure 4. Arcsine square-root transformed cover data for (A) all SAV species combined; (B) *R. maritima*; (C) *V. americana*; and (D) *Z. palustris* across the four measured water depths (0.25, 0.5, 0.75, and 1 m). Only statistically significant results in water depth and percent cover were observed for plots A and C. Mean arcsine square-root transformed cover at 0.5, 0.75, and 1.0 water depths were statistically greater than mean cover at 0.25 m depths for plot A, while plot C shows the mean cover for *V. americana* is significantly greater at the 1 m water depth than 0.25 m.

Frequency

Percent frequency is another way to measure the abundance of SAV, which can be used to determine patchiness of an SAV bed. The mean percent frequency for all SAV species combined in this study increased with the quadrat measured water depths (0.25, 0.5, 0.75, and 1.0 m) with the highest mean frequency of occurrence observed at the 0.75 m water depth (Figure 5). The mean percent frequency of all SAV species combined was statistically different among water depths (ANOVA ($F_{3,181}$) = 10.294, P=<0.001 ω^2 = 0.13 APPENDIX E). A Tukey post-hoc comparison test revealed that the mean percent frequency of SAV was statistically greater at measured water depths 0.5, 0.75, and 1 m compared to the mean frequency at the 0.25 m water depth.

Vallisneria americana was the only dominant SAV species with a statistically significant difference in mean quadrat frequency among water depths. A one-way ANOVA determined that *V. americana* mean frequency statistically differed over water depth ($F_{3, 181}$) = 2.724, P= 0.0457 ω^2=0.03 (APPENDIX E). A Tukey HSD post-hoc test revealed that the mean frequency of *V. americana* was statistically greater at 1 m water depth compared to 0.25 m. Although, the difference was statistically significant, the low value of the omega squared can be interpreted as water depth having a small effect on *V. americana* frequency (ω^2=0.03).

Ruppia maritima and *Zannichellia palustris* mean percent frequency did not show statistically significant differences with the measured water depths of 0.25, 0.5, 0.75, and 1 m (APPENDIX E).

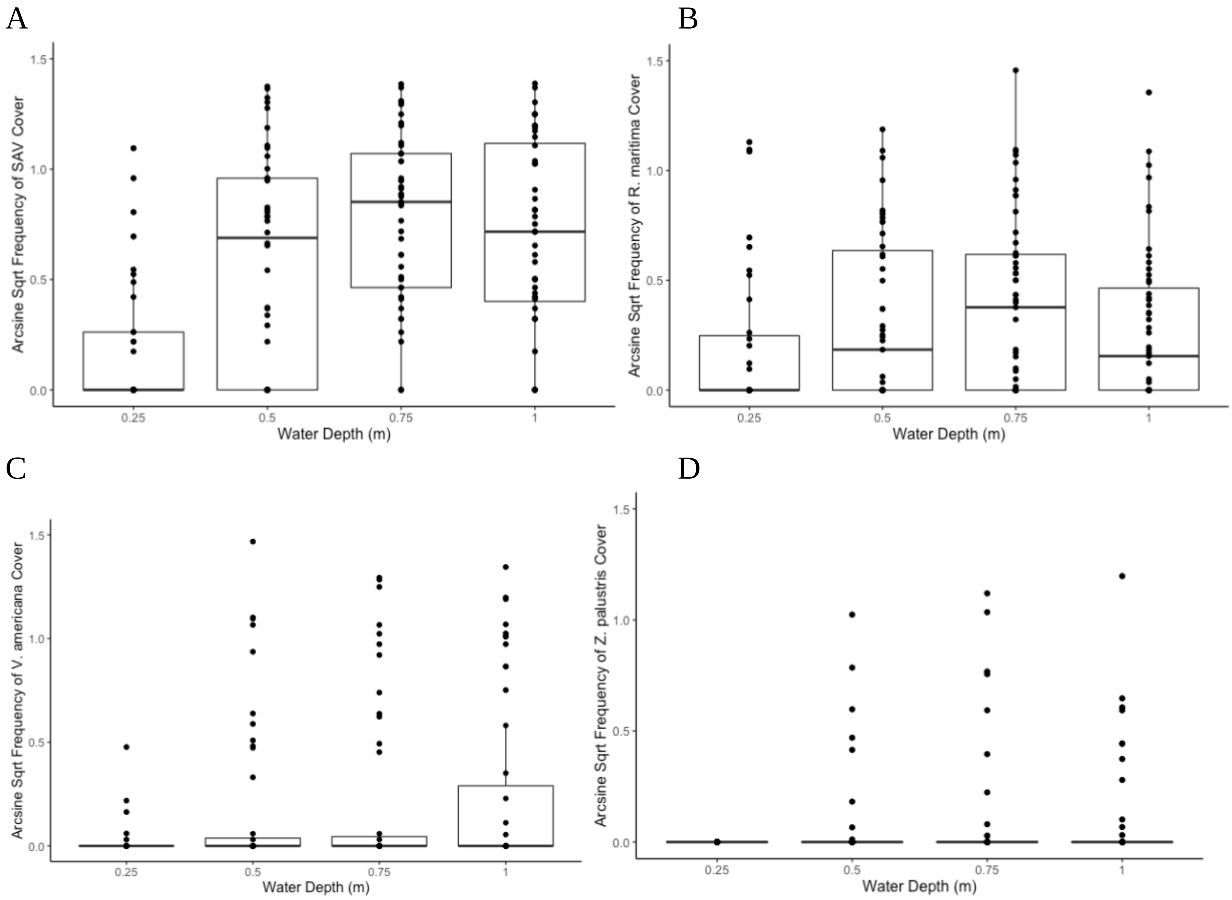

Figure 5. Arcsine square-root transformed frequency for (A) all SAV species combined; (B) *R. maritima*, (C) *V. americana*, and (D) *Z. palustris* across the four measured water depths (0.25, 0.5, 0.75, and 1 m). Plot A and C show frequency being statistically different with water depth. Mean arcsine square-root transformed frequency at 0.5, 0.75, and 1.0 m water depths were statistically greater than mean cover at 0.25 m depths for plot A, while plot C shows the mean frequency for *V. americana* is significantly greater at the 1 m water depth than 0.25 m.

Biomass

Biomass is another metric that measures plant abundance, because it is the dry mass of a plant in a given area, which reflects the amount of energy stored in the vegetation indicating productivity at a location. The total biomass found during this study for all SAV species significantly increased with water depths ($F_{3,176}$) = 3.6756, P=0.01331 ω^2=0.04 (APPENDIX E).

A Tukey HSD post-hoc test determined that the two water depths of 0.75 and 1 m had statistically greater mean biomass than that observed at 0.25 m water depth (Figure 6).

To determine what effect water depth has on the mean biomass for individual dominant SAV species, a one-way ANOVA was performed for each. None of the ANOVA showed any statistically significant results, although *V. americana* mean biomass was the closest to being significant $(F_{3,176}) = 2.322$, $P=0.076$, $\omega^2=0.02$ (APPENDIX E).

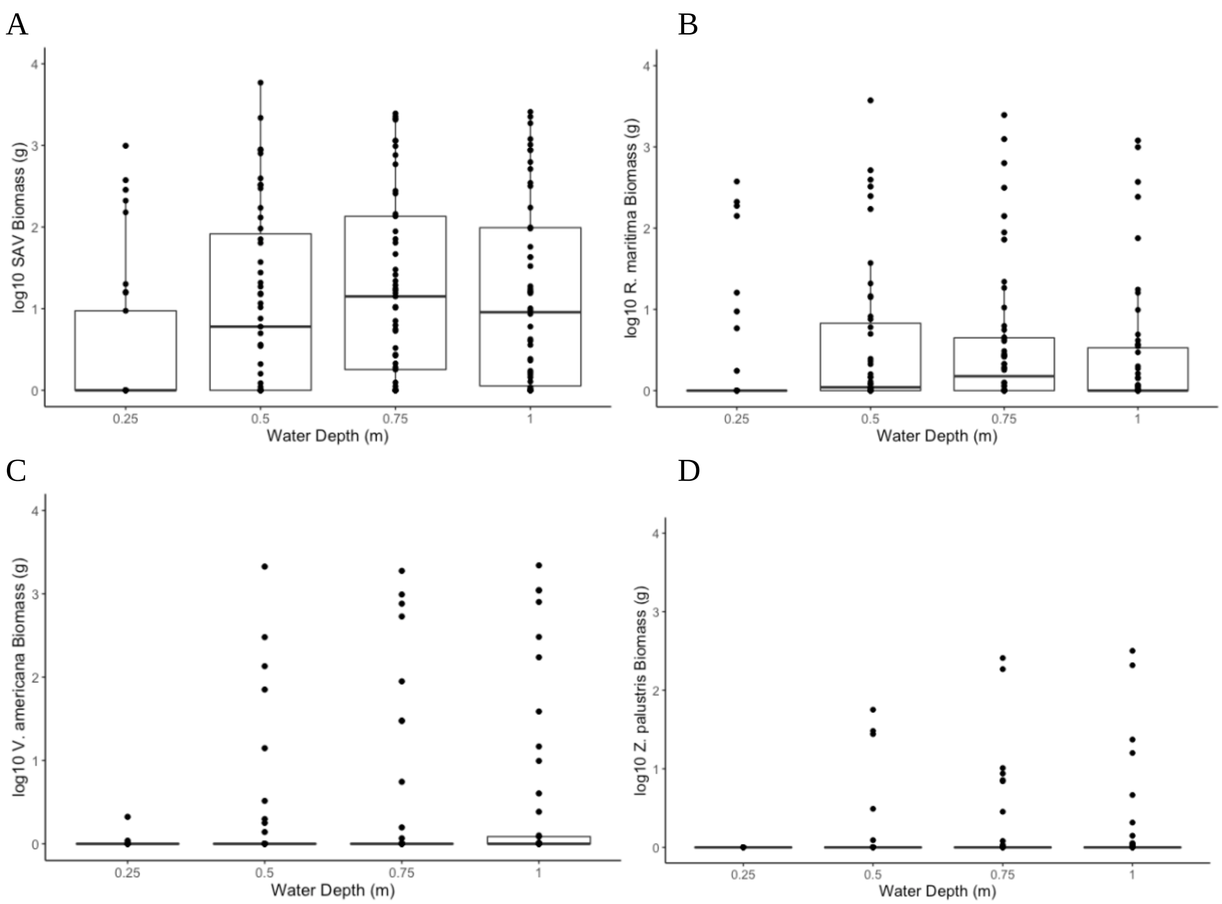

Figure 6. Log_{10} transformed dry biomass for (A) all SAV species combined; (B) *R. maritima;* (C) *V. americana*, and (D) *Z. palustris* across the four measured water depths (0.25, 0.5, 0.75, and 1 m). A statistical difference in mean dry biomass was observed in plot (A) 0.75- and 1 m depths were significantly greater than the mean biomass measured at the 0.25 m water depth. Dominant SAV species biomass showed no statistically significant difference in mean dry biomass with *R. maritima*, *V. americana* or *Z. palustris* across any water depths.

Maximum Colonization Depth

Maximum colonization depth (MCD) was determined for all 52 SAV-positive sentinel site surveys using single-beam SONAR and plotted against the point measurements of turbidity measured by Secchi depth. The MCD of all SAV-positive sentinel site surveys Secchi depth ranged from 0.63 to 1.99 m (Table 8). A positive relationship was observed between Secchi depth and MCD of SAV based on the linear regression analysis. Still, it was not shown to be statistically significant (y=1.09+0.175(x), ($F_{1,50}$) = 0.865, P= 0.356) (Figure 7). These results do not support my hypothesis (H2a) that turbidity is negatively correlated with MCD. Instead, based on the ECU/APNEP Secchi depth measurements, I reject the hypothesis that there is a relationship between MCD, and turbidity.

However, the maximum colonization depth was statistically associated with Secchi depth using the ensemble data set for just the PRE and NRE (Figure 8). Secchi depth was significantly related to MCD (y=0.299+0.8969(x), ($F_{1,24}$) = 9.511, P=0.00508, adj R^2=0.25). This model predicted that for every 1 m increase in Secchi depth, the MCD was expected to increase by 0.896m. Using this longer-term ensemble data set, I can conclude that the MCD is affected by turbidity for all SAV species in the PRE and NRE during the 2016 – 2019 period.

Salinity as measured by the ECU/APNEP team was not a good predictor of MCD of SAV (y=1.21+0.006(x), ($F_{1,50}$) = 0.44, P= 0.509) (Figure 9). Salinity was also not significant with MCD of SAV when measurements from the ensemble data set were used (y=0.961+0.009(x), ($F_{1,24}$) =0.35, P=0556). These findings do not support my hypothesis (H2b) that salinity will be negatively correlated with the maximum colonization depth of SAV. I reject the hypothesis that there is an association between salinity and the maximum colonization depth of SAV at our sentinel site locations.

Table 8. Sentinel site characteristics based on SONAR surveys and ECU/APNEP water quality measurements of Secchi depth and salinity. Peak year abundance is based on the quadrat percent cover found at a sentinel site. NA represents sites that do not contain SAV through any point of the yearly sentinel survey.

Sentinel Site	Peak Abundance Year	AVG MCD range (m)	Salinity range (ppt)	Secchi depth range (m)
AS_SS_01	2015	1.32 – 1.99	0 - 18.87	0.60 - 0.86
AS_SS_02	2015	0.65 – 1.0	0 – 4.23	0.35 – 0.76
AS_SS_03	2016	0.87 – 1.0	0 – 0.94	0.65 – 1.47
AS_SS_04	2015	1.04 – 1.43	0 – 0.41	0.68 – 1.20
AS_SS_05	NA	0	0 – 0.10	0.77 – 1.49
AS_SS_06	2015	1.01	0 – 4.75	0.57 – 0.81
AS_SS_07	2019	0.77 – 1.04	0 – 3.89	0.60 – 1.30
AS_SS_08	2016	1.03 – 1.23	0 – 1.99	0.49 – 0.77
AS_SS_09	NA	0	0 – 0.51	0.65 – 1.30
AS_SS_10	2015	1.0	0 – 0.10	0.43 – 1.57
PR_SS_01	2019	1.02 – 1.34	0 – 4.87	0.70 – 0.93
PR_SS_02	2017	0.67 – 1.0	3.30 – 10.56	0.35 – 0.95
PR_SS_03	2017	0.63 – 1.21	10.55 – 12.0	0.53 – 0.85
PR_SS_04	2017	0.67 – 0.86	0 – 14.93	0.67 – 0.93
PR_SS_05	2019	1.14 – 1.58	0 – 6.41	0.63 – 0.90
PR_SS_06	2019	0.82 – 1.13	0 – 13.1	0.64 – 0.66
NR_SS_01	NA	0	14.19 – 17.01	0.64 – 0.80
NR_SS_02	2019	1.33	5.75 – 12.70	0.59 – 0.80
NR_SS_03	2019	0 – 1.42	7.35 – 9.60	0.47 – 1.10
NR_SS_04	NA	0	3.34 - 15.50	0.70 – 1.13
NR_SS_05	2019	1.12	6.20 – 6.80	0.84 – 1.0
NR_SS_06	2019	1.08 – 1.47	0.12 – 6.40	0.99 – 1.40
NR_SS_07	2018	1.07 – 1.75	1.33 – 2.70	0.75 – 1.30
NR_SS_08	NA	0	6.19 – 12.60	0.74 – 1.06
NR_SS_09	2019	0.81 – 1.48	5.89 – 15.70	0.68 – 1.0
NR_SS_10	NA	0	11.84 -20.60	0.80 – 1.22

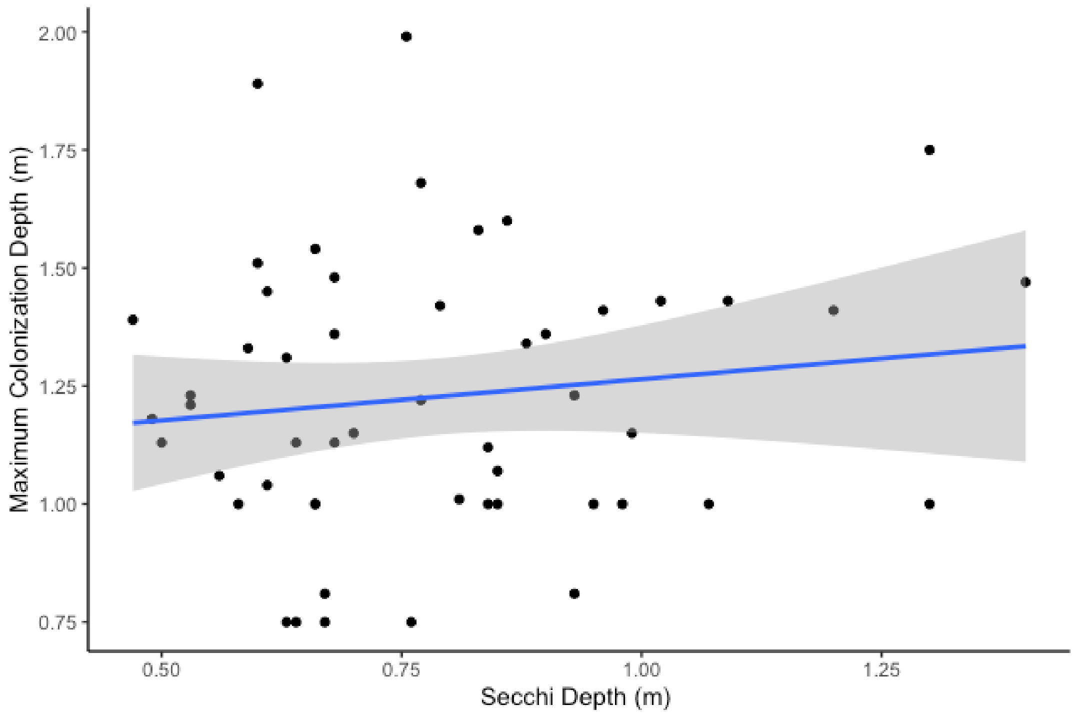

Figure 7. Linear model of the relationship between Secchi Depth (m) from ECU/APNEP point measurements and the maximum colonization depth (m) of SAV for 52 SAV-positive sentinel site surveys.

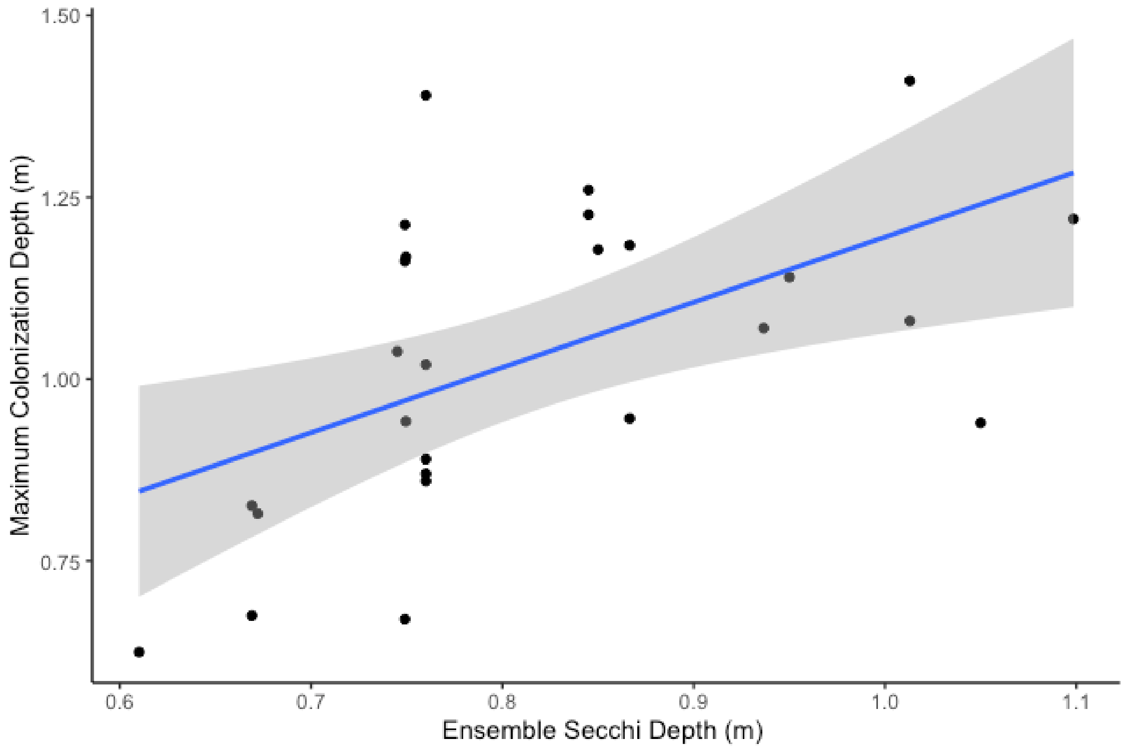

Figure 8. Linear model of the maximum colonization depth (MCD) average for sentinel site surveys containing SAV (n=26) in the PRE and NRE from 2016-2019 using the ensemble water quality data. The AS was omitted as it did not have enough water quality stations to compute a yearlong average Secchi depth. The MCD average was calculated by playing back SONAR transects and averaging the maximum colonization depth in meters based on the same ten transects used for inshore quadrat surveys.

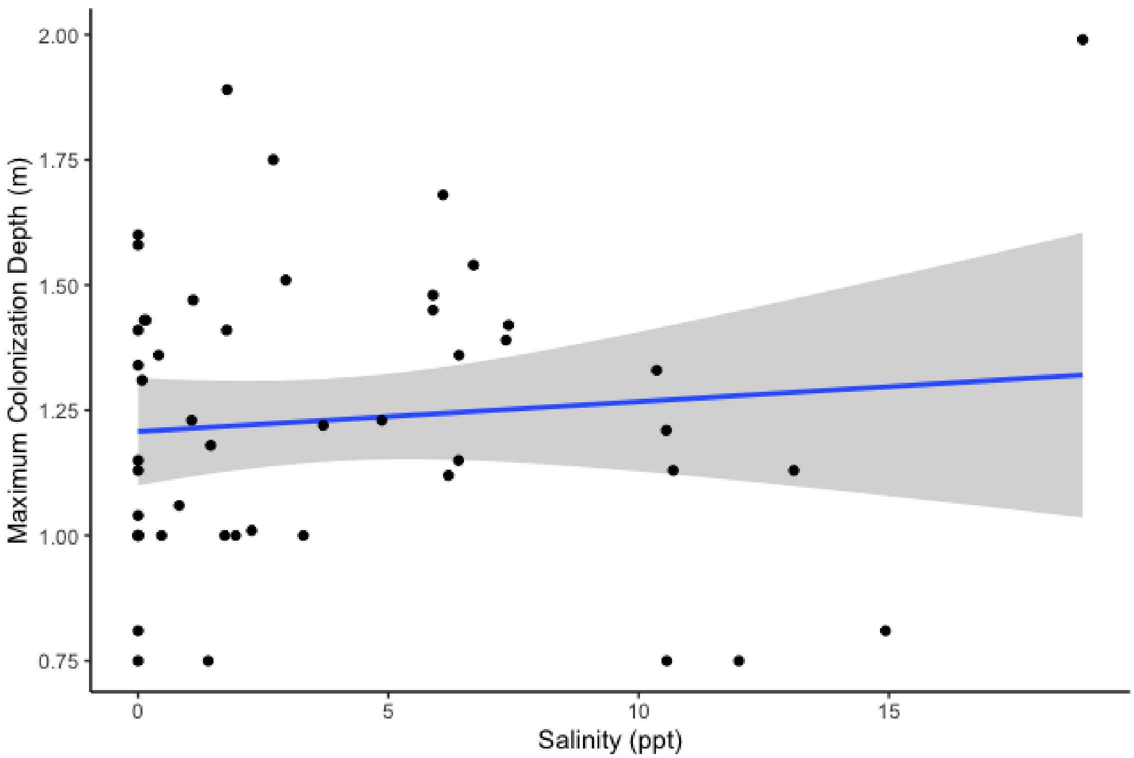

Figure 9. Linear model of the relationship between salinity (ppt) from ECU/APNEP point measurements and the maximum colonization depth (m) of SAV for 52 SAV-positive sentinel site surveys.

Species Salinity Range

The three most common SAV species identified during this study were *Ruppia maritima, Vallisneria americana,* and *Zannichellia palustris* (Table 4). A violin plot was chosen to display the salinity range for these common SAV species, as it is a combination of the boxplot and kernel density plot. Advantages of violin plots are they show the entire data distribution. Figures 10-12 display the optimal salinity range for each dominant species of SAV (*R. maritima, V. americana and Z. palustris)*. Optimal salinity ranges can be observed where the green shade "violins" (is narrowest). For *R maritima,* this was the salinity range of nine ppt which, agrees with other Mid-Atlantic Coast and Chesapeake Bay observations (Bergstrom et al. 2006) (Figure 10). The optimal salinity observed in this study for *V. americana* was around 2-4 ppt, as this salinity range had the narrowest green-shaded region (Figure 11). This observation also corresponds to other studies, which find *V. americana* growing optimally between 0 and 5 ppt (Bergstrom et al. 2006). Figure 12 displays the violin plot for *Z. palustris,* which shows an optimal salinity range of 5 ppt. This salinity result is consistent with other Atlantic coast findings with an optimal salinity range of 0 to 11 ppt (Bergstrom et al. 2006). The salinity range in which each species was observed growing is also apparent, representing the salinity tolerance for each species. For *V. americana* this range was 0-6 ppt, while *R. maritima* was found at a wide salinity range of 0 to 18 ppt, and for *Z. palustris* a salinity range of 0 to 11 ppt. Salinity and Secchi depth ranges for all observed species are presented in Table 9 and Figure 13.

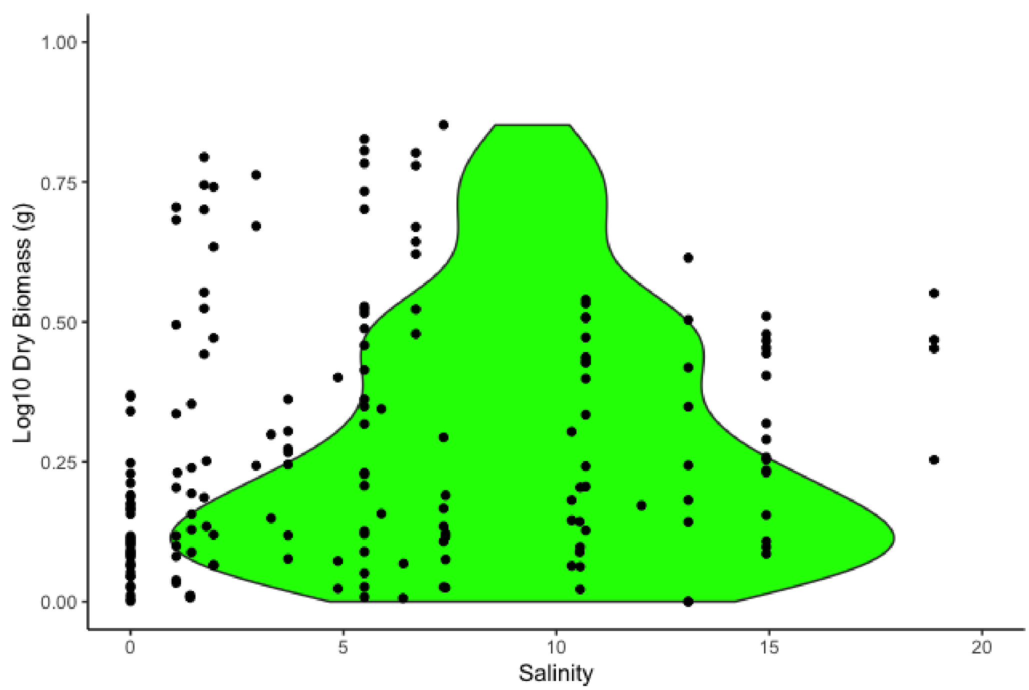

Figure 10. Log_{10}-transformed dry biomass (g) in core samples of widgeon grass (*Ruppia maritima*) at sentinel sites (AS, PRE, and NRE) in 2015-2019 as a function of salinity. Points indicate biomass for each core taken, and violin plots show the habitat range occupied of this species in both salinity and biomass.

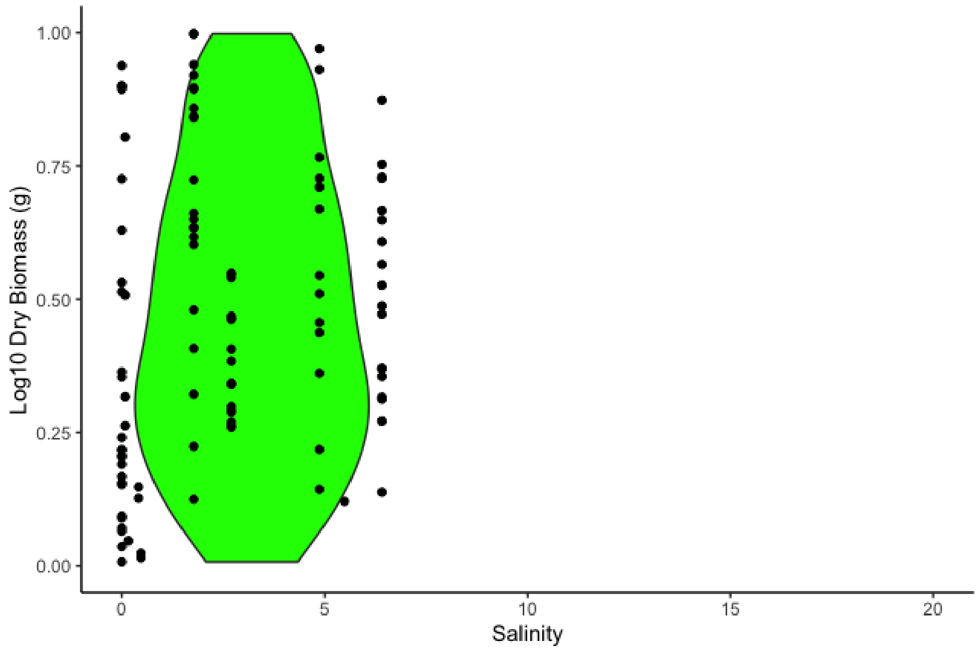

Figure 11. Log_{10} transformed dry biomass (g) in core samples of *Vallisneria americana* at sentinel sites (AS, PRE, and NRE) in 2015 and 2019 as a function of salinity. Violin plots show habitat range occupied by species for both salinity and biomass.

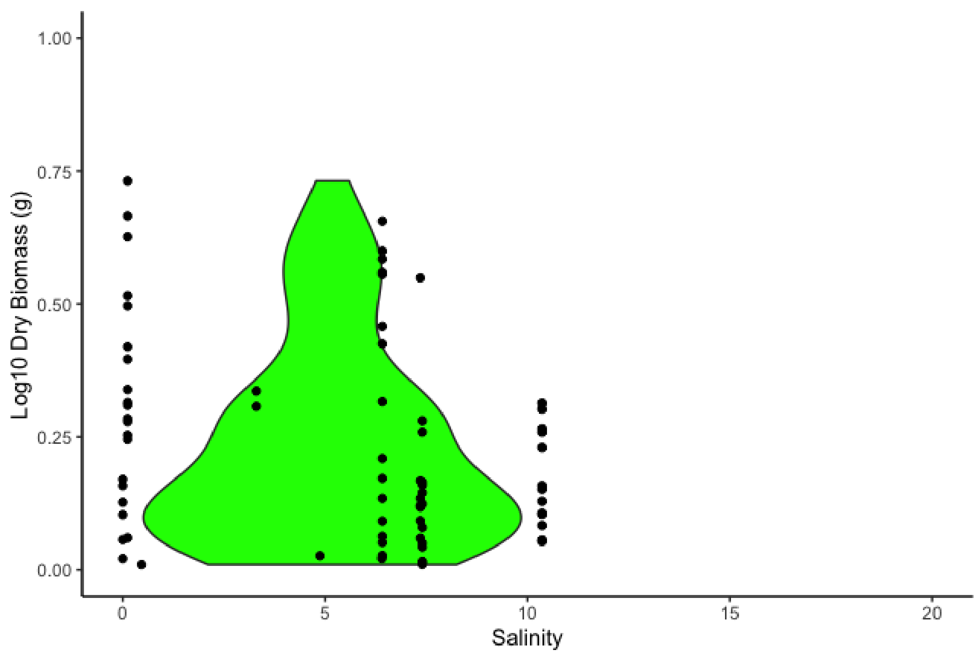

Figure 12. Log_{10}-transformed dry biomass (g) in core samples of widgeon grass *Zannichellia palustris* sentinel sites (AS, PRE, and NRE) in 2015 and 2019 as a function of salinity. Points indicate biomass for each core taken, and violin plots show the habitat range occupied of this species in both salinity and biomass.

Table 9. The occurrence of SAV species according to the environmental variables of salinity and Secchi depth (m) in the Albemarle Sound, Pamlico River Estuary, and Neuse River Estuary from 2015-2019. N equals the number of sentinel site surveys in which each species was found.

	Environmental Variables				
		Salinity (ppt)		Secchi Depth (m)	
SAV Species	N	Range	Average	Range	Average
Ruppia maritima	37	0 - 18	4.8	0.5 - 1.4	0.7
Vallisneria americana	16	0 - 6.4	1.4	0.6 - 1.3	0.9
Zannichellia palustris	11	0 - 10	4.2	0.5 - 1.1	0.8
Myriophyllum spicatum	10	0 - 18	4.3	0.5 - 1.2	0.8
Naja guadalupensis	7	0 - 6.4	1.3	0.5 - 1.0	0.9
Charaphytes	6	0 - 12	5.0	0.6 - 1.4	0.9
Stuckenia pectinata	5	0 – 3.0	1.4	0.6 - 1.0	0.7
Halodule wrightii	4	1.7 – 18	10	0.5 - 1.0	0.7
Zostera marina	3	5.8 - 12	8.4	0.5 - 0.7	0.6
Potamogeton pusillus	3	0 - 7.4	4.6	0.8 - 1.2	1.0
Potamogeton crispus	2	0 - 6.4	3.2	0.9 - 1.2	1.1

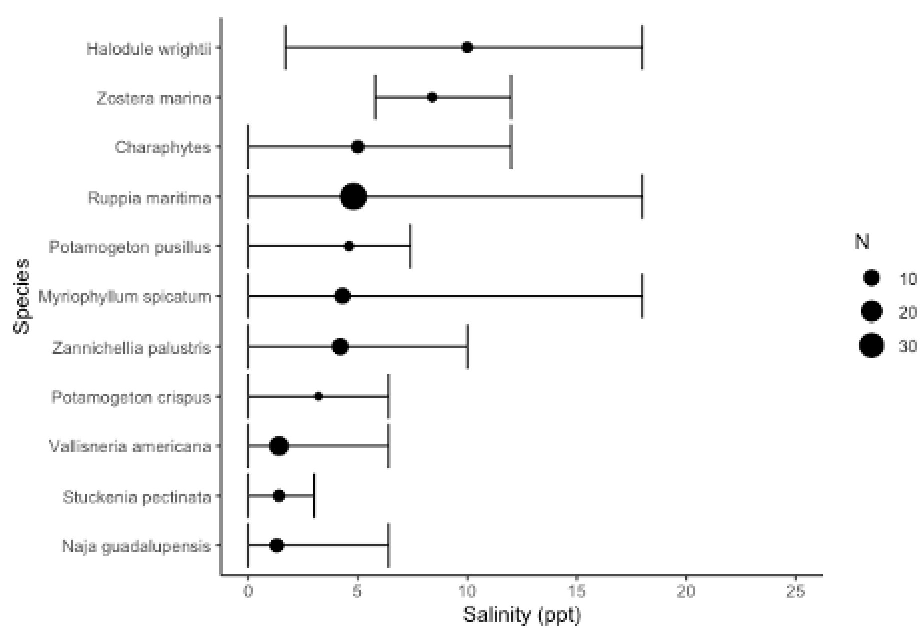

Figure 13. The average salinity (ppt) with ranges (min and max salinities) for individual SAV species growing at different sentinel sites across all estuaries. The N is the number of sentinel site surveys at which each species was found.

Species Diversity

Species accumulation curves were created to analyze how species diversity has changed at sentinel site locations over time. Species accumulation curves were plotted for each survey year (2015-2019) for all estuaries combined as well as separated by estuary (AS, PRE, and NRE). Ecologists often want to measure species diversity, but unbiased estimates of species richness are challenging to analyze, as it is often influenced heavily by sampling effort (Deng et al. 2015). Species accumulation curves can make a fair comparison of the expected number of species for a fixed number of surveys.

When looking at the species accumulation curves of SAV at the combined estuaries for each year, 2018 appears to have the greatest species diversity, followed by 2019, 2015, 2016, and 2017, standardized by sampling effort (Figure 14). When analyzing the species accumulation curve by estuary location, NRE appears to have the highest species richness when sampling effort is equal for each estuary at 10 sentinel sites closely followed by PRE and AS (Figure 15).

Appendix B provides a map of the species present at each sentinel site and the percent composition based on core dry biomass. Twenty sentinel site surveys only contained one SAV species (38% of total SAV positive surveys). Two SAV species co-occurring were found in 19 out of the 52 SAV positive sentinel site surveys (36%), and three or more SAV species were found co-occurring in 25% of the SAV positive surveys. The most SAV species found during a single sentinel site survey is five, which was at the AS sentinel site 4 in the spring of 2015. The SAV species present in order of biomass composition were *N. guadalupensis, V. americana, M. spicatum, P. crispus, and P. pusillus.*

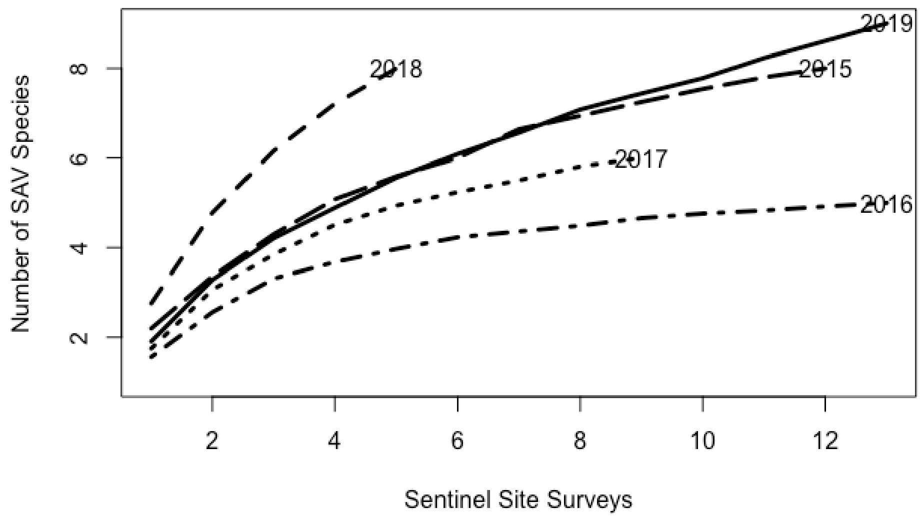

Figure 14. Species accumulation curves for all three estuaries combined based on 52 SAV positive sentinel site surveys (2015-2019). In 2015, 12 sentinel site surveys had SAV present, 2016 had 13, 2017 had 9, 2018 had 5, and 2019 had 13.

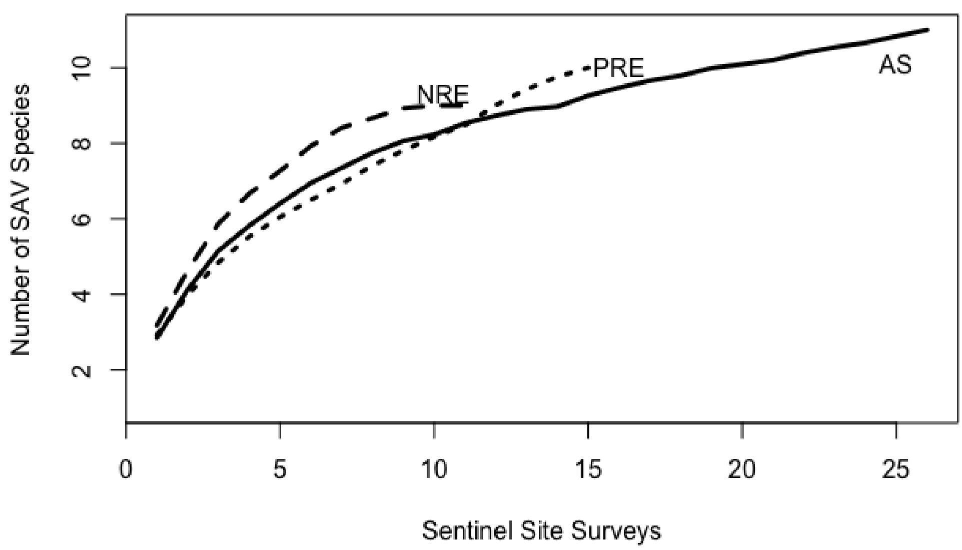

Figure 15. Species accumulation curves for the Albemarle Sound (AS), Neuse River Estuary (NRE), and Pamlico River Estuary (PRE) based on 2015-2019 SAV positive sentinel site surveys (N=52). The number of 52 SAV positive sentinel site surveys divided by estuary location are 26 in the AS, 15 in the PRE, and 11 in the NRE.

Discussion

My goal was to determine what abiotic factors affect SAV abundance in North Carolina estuaries. With this project, I looked at the influence of abiotic factors including water depth, salinity, and turbidity measured with Secchi depth on dominant SAV species (*R. maritima, V. americana, and Z. palustris*) found during the monitoring period (2015-2019). My results suggest that turbidity (measured by Secchi depth), salinity, and water depth have significant effects on SAV abundance, but the direction of influence is dependent on the SAV species (Table 10).

The odds of finding *Ruppia maritima* and *Vallisneria americana* present were significantly influenced by all three environmental predictors (salinity, Secchi depth, and water depth) in the multiple logistic regression model. For the three dominant SAV species, the odds of SAV occurrence increased with water depth out to 1 m. *Vallisneria americana* was the only SAV species that showed a significant change with water depth in abundance (measured by percent cover and frequency metrics). Mean cover and frequency of SAV abundance metrics showed a significant difference with water depth, in that the highest percent cover and frequency were found at the deepest measured water depth of 1 m. Dry biomass of SAV did not show any significant differences at the four water depths for any individual dominant SAV species.

Table 10. Results summary table from hypotheses tested.

SAV Species	Metric	Sampling method	Conclusion
R. maritima	Odds of occurrence	Cores and Quadrats	Turbidity positively associated with SAV

V. americana	Odds of occurrence	Cores and Quadrats	Turbidity negatively associated with SAV
Z. palustris	Odds of occurrence	Cores and Quadrats	Turbidity not associated with SAV
All SAV Species	Maximum Colonization Depth	SONAR	Turbidity negatively associated with SAV
All SAV Species	Maximum Colonization Depth	SONAR	Salinity does not affect the MCD of SAV
R. maritima	Odds of occurrence	Cores and Quadrats	Salinity positively associated with SAV
V. americana	Odds of occurrence	Cores and Quadrats	Salinity negatively associated with SAV
Z. palustris	Odds of occurrence	Cores and Quadrats	Salinity not associated with SAV
R. maritima	Odds of occurrence, Cover, Frequency, Biomass	Cores and Quadrats	Increased water depth positively associated with SAV occurrence
V. americana	Odds of occurrence, Cover, Frequency, Biomass	Cores and Quadrats	Increased water depth positively associated with SAV occurrence, cover, and frequency
Z. palustris	Odds of occurrence, Cover, Frequency, Biomass	Cores and Quadrats	Increased water depth positively associated with SAV occurrence

Secchi Depth

Past research on SAV has identified light as being one of the dominant limiting factors for SAV occurrence (Dennison 1987; Duarte 1991; Zimmerman et al.1994; Kenworthy and Fonseca 1996; Kemp et al. 2004). Secchi depth was used to estimate the light levels through water turbidity during each sentinel site survey. High Secchi depths values indicate low turbidity (clear water), while low Secchi depth values indicate high turbidity (stained water). Water turbidity measured with Secchi depth significantly affected the odds of occurrence for SAV species *R. maritima* and *V. americana* but in opposing directions. Secchi depth showed a surprisingly negative relationship for the odds of *R. maritima* occurrence. The logistic regression model predicted that for every 0.10 m increase in Secchi depth, the odds of *R. maritima* being present (versus not being present) decrease by 20.5%. This indicates that *R. maritima* is less likely to occur as Secchi depth increases (more transparent water). This finding does not support my hypothesis (H1a) that turbidity would be negatively associated with the odds of *R. maritima* occurrence (Table 10). Although not tested in this study, but a possible cause of this association between increased *R. maritima* and turbidity is the impact of interspecific competition with *V. americana*. Orth and Moore (1998) research found a zonation difference with *Ruppia maritima* and *Zostera marina* in the lower Chesapeake Bay. In their study, *R. maritima* was found to be more abundant in the shallowest zones, while *Z. marina* inhabited the deeper zones. They proposed the spatial difference was regulated by light and temperature. They concluded *Ruppia maritima* is a high light-demanding species and the reason it was found growing in only shallow water locations is because that is where light levels were sufficient. In contrast, they stated that *Z. marina* was a low light-demanding species that was able to withstand lower light levels in the deeper water (Orth and Moore 1998). This study was not identical to mine in that it was performed at high salinity locations in the Chesapeake Bay, but I propose a zonation pattern is occurring in North Carolina's estuaries between *R. maritima* and *V. americana* due to light, salinity and interspecific competition. *Ruppia maritima* is growing in shallow waters, similar to what Orth and Moore (1998) found, but it can also grow in deeper waters and at low Secchi depths when *V. americana* is not present due to high salinity. The reason the logistic regression model predicted the odds of *R. maritima* occurrence to increase with decreasing Secchi depth (more turbid waters) was because of light limitation as well as competition with *V. americana* in low-salinity and high Secchi depth light areas. Hillmann and La Peyre (2019) have also shown interspecific interactions for *Ruppia maritima* and *Myriophyllum spicatum* with their mesocosm work. They observed that *R. maritima* when in mixture with *M. spicatum* under yielded in abundance when compared to monoculture groups under low-salinity and high light level conditions. Although, they concluded their work that interspecific competition does not have as strong as an effect as salinity and light when ultimately controlling species distribution it still should be taken into account.

The logistic regression model supported hypothesis (H1b) for *Vallisneria americana* as it predicted increased odds of occurrence for *V. americana* with increased Secchi depths. Secchi depth did not significantly affect *Zannichellia palustris* odds of occurrence based on the logistic regression results. A larger sample size for this species would be needed to detect changes in occurrence with physical variables of salinity and Secchi depth. Based on these results, I reject the hypothesis (H1c) that Secchi depth affects the odds of *Z. palustris* occurrence.

Salinity

It has also been shown that salinity can limit oligohaline SAV communities (Patrick et al. 2017). Our findings demonstrate that salinity has a significant effect on specific SAV species odds of occurrence and that the degree of influence depends on the SAV species salinity tolerance range. *Ruppia maritima* showed a significant positive relationship with salinity. The logistic regression model predicted that for every 1-ppt increase in salinity, the odds of *R. maritima* occurrence increased by a factor of 1.11. This result supports my hypothesis (H3a) that salinity will not limit the distribution of *R. maritima*. These results also build on existing literature showing *R. maritima* can grow over a range of salinities (Kantrud 1991; Bergstrom et al. 2006). In contrast, *Vallisneria americana,* a freshwater grass, showed opposite results when salinity was analyzed with the logistic regression model. It was observed for *V. americana* that as salinity increased, the presence of this species was associated with decreased odds of occurrence. This result supports the hypothesis (H3b) that salinity will limit the distribution of *V. americana* to low salinities. This is consistent with what can be found in the literature for this SAV species (French and Moore 2003; Bergstrom et al. 2006; Boustany et al. 2009). Salinity and light mesocosm studies have been performed by Boustany et al. (2009) and French and Moore (2003), which provide consistent findings for *V. americana* that salinity limits this species distribution to low-salinity locations. Their studies also provided information on the interaction of salinity and light for *V. americana*. They showed that the salinity tolerance is contingent on light availability, as higher light gives additional energy used for osmotic regulation (French and Moore 2003). The best growth for *V. americana* was observed at one ppt and decreased with salinity (Boustany et al. 2009).

Salinity did not have a statistically significant effect on *Z. palustris* in my logistic regression model. I reject hypothesis H3c that *Z. palustris* was salinity limited. I did not show a significant change in the odds of occurrence over the salinity range found in this study (0-20 ppt). Since *Z. palustris* has an optimal salinity range observed on the Atlantic coast from 0-11 ppt (Bergstrom et al. 2006), it is not surprising that an increase or decrease in the odds of *Z. palustris* occurrence was not predicted with my model for the salinity range present in these estuaries. During the monitoring period, the average salinity found for *Z. palustris* was 4.33 ppt, right in the middle of its optimal salinity range. Also, as previously mentioned, the small sample size for this SAV species may not allow for the model to accurately predict any significant differences with salinity.

Water Depth

Water depth was analyzed as it considers a combination of factors, such as light and wave energy. The multiple logistic regression model showed that the odds of *R. maritima* occurrence increased with increasing water depth out to 1 m. Still, the one-way ANOVA showed that mean cover, frequency, and biomass measures of abundance for *R. maritima* were not significantly different among water depths. This means that odds of occurrence of *R. maritima* increased in deeper waters out to 1 m depth, but that there was no difference in the other SAV metrics analyzed for *R. maritima* with depth. Where *R. maritima* occurred, there was extreme variations in mean abundance metrics that preclude a statistical result that showed increasing or decreasing trend as water depth increased. So, there is not, on average, more *R. maritima* as measured by percent cover in quadrats, biomass in core samples, or frequency of *R. maritima* along transects with depth. The logistic regression is a better statistical technique for determining depth-related trends in occurrence than ANOVA, because it does not assume a normal distribution and logistic regression modeled the presence and absence of *R. maritima* well. Analysis of variance, however, does assume a normal distribution, and that assumption was violated even after the data were transformed through arcsine square-root (for proportions) and $\log_{10}$ (for biomass). Also, since each metric provides a different SAV abundance measurement, one cannot assume they should agree, even if the predictor variable water depth is the same. I can conclude that the logistic regression analysis gives the best estimate of how *R. maritima* varies with water depth as logistic regression assumptions are met. In terms of my hypothesis (H4a), this result does not support my prediction that SAV abundance will be inversely correlated with water depth for *R. maritima*, as I showed the opposite trend with the logistic regression model.

Similar results for water depth and SAV abundance can be observed for *Zannichellia palustris*. Water depth was only statistically significant in the logistic regression model, while the mean cover, frequency, and biomass for *Z. palustris* did not differ significantly with water depth. The logistic regression model predicted the odds of *Z. palustris* occurrence increased as water depth increases out to 1 m. Contrary to the hypothesized association of SAV abundance inversely related to water depth, the logistic regression model for *Z. palustris* does not support my hypothesis (H4c) and shows the opposite is found (more *Z. palustris* in deeper water depths).

Vallisneria americana was the only SAV species that differed significantly with water depth for the mean cover, frequency, and odds of SAV occurrence abundance measurements. When looking at the mean cover versus water depth for *V. americana*, a statistically significant difference was detected between the measured water depths 0.25 m and 1 m (P= 0.045). More *V. americana* mean cover was found at the deepest measured water depth (1 m) compared to 0.25 m. A statistically significant difference in the mean frequency of *V. americana* in quadrats was also detected between the 0.25 m and 1 m with greater frequency being found at 1 m. These results agree with the estimated odds of *V. americana* occurrence increasing with water depth, as analyzed by the logistic regression model, which predicted that more *V. americana* would be present (versus not present) at increased water depths out to 1 m. Although all of these SAV metric results (cover, frequency, and odds of occurrence) differed significantly with water depth for *V. americana*, it does not support my hypothesis (H4b) that an inverse relationship would be observed with SAV abundance and water depth. I predicted that more SAV abundance would be found at the shallowest depths (0.25 m and 0.50 m) since they experience higher light levels, but this prediction was not supported with statistical findings.

Water depths for dominant SAV species were significant in the logistic regression models because they were compared to the 0.25 m water depth (intercept), which rarely contained SAV. The low abundance of SAV at 0.25 m could be due to the influence of wave action or fluctuating water levels. Wave energy has been proposed as a limiting factor in other research studies on SAV. It has been shown that high wave energy in shallow water can prevent SAV from being established even when light requirements are met (Koch 2001). This can occur as wave energy exerts drag on the plants itself and through indirect impacts of sediment resuspension (Koch 2001). Also, extreme variation in water levels in the PRE (PR-SS-02 Blount’s Bay) have been observed, which exposed SAV to long periods (days to weeks) of air desiccation and sun exposure (Pleva and Luczkovich 2013 unpublished). These two factors need to be examined in the future as possible explanations for the absence of SAV and low SAV metrics at 0.25 m.

Maximum Colonization Depth (MCD)

The maximum colonization depth (MCD) of SAV is a standard metric researchers use to assess if light is limiting the survival of SAV. In many studies, it has been reported that MCD was observed to increase with increasing Secchi depth using light availability models (Chambers and Kalff 1985; Dennison et al. 1993; Kemp et al. 2004). The MCD of SAV in this study showed varying results with turbidity (measured by Secchi depth) for the two different water quality data sets. For the ensemble data set, created from North Carolina water monitoring station programs using an inverse distance weighting (IDW) interpolation method (in the surrounding area for the PRE and NRE stations), a significant positive relationship with Secchi depth and MCD of SAV was observed (P=0.005). However, when using the ECU/APNEP water quality Secchi measurements taken at the start of the sentinel site surveys at the AS, PRE and NRE, the relationship between Secchi depth and MCD was not significant (P= 0.357). These findings contradict light availability models, (Chambers and Kalff 1985; Dennison et al. 1993; Kemp et al. 2004) which propose a clear positive association with Secchi depth and MCD. Reasons for this difference can come from the fact that most of the light model research has been done in clear lakes which do not have factors of tides, algal blooms, and river discharge like estuaries (Batuik et al. 2000). Also, unlike the light availability model research that use large data sets from multiple studies over multiple periods of time, Secchi depths were only measured once during our ECU/APNEP surveys. If the measurement was taken after heavy rain, a windy day or a particular clear day (no wind) this could introduce great variability in Secchi depth measurements taken on a single day, and it might not indicate the seasonal turbidity experienced at that sentinel site.

The discrepancy in MCD from the two analyses could be due to several factors, including the longer-term nature of the Secchi depth measurements in the ensemble data from the PRE and NRE rather than the ECU/APNEP surveys' point measurements. The correlation of ECU/APNEP point measurements and ensemble Secchi depth measurements is analyzed in APPENDIX F but show no correlation with one another (r=0.0035). It is not clear if either data set truly represents the Secchi depth average for each year. Some of the water monitoring stations used in the ensemble data to create the interpolated IDW average are located in the middle of the river, where turbidity conditions may differ significantly from near-shore areas where sentinel sites are located. However, the ensemble data set did integrate measurements over a longer period, so may be more accurate than ECU/APNEP point measurements. Continuous data monitoring for turbidity in future studies should be implemented at each sentinel site to understand better the full seasonal environmental influence of salinity and turbidity measurements. The MCD for specific SAV species could not be determined as acoustic SONAR cannot distinguish one SAV species from another.

Species Tolerance Range

The extreme salinity range in the PRE and NRE can make it a stressful environment for low-salinity species. Davis and Brinson (1990) reported a considerable loss of SAV abundance in the PRE and attributed it to increased turbidity and salinity stress. They observed two years of high turbidity (1979, 1980) and one year of salinity stress (1981) that combined eliminated almost all SAV in the PRE, which had been documented previously as *V. americana* abundance (Davis and Brinson 1990). For this study, SAV species tolerance ranges are expressed in (Table 9) and reflect the minimum and maximum salinity and turbidity for each SAV species observed during this study. The range of salinity can best be displayed in figure 13 for all SAV species observed in this study. The highest salinity recorded was 20 ppt at the mouth of the NRE at SS 10, which never contained any SAV during the monitoring period. For many sentinel sites an extreme variation in salinity between years was observed from ECU/APNEP point salinity measurements (AS-SS-01 ranged from 0-18 ppt). Such large fluctuations in salinity must also be looked at further with continuous data loggers when monitoring at ECU/APNEP sentinel sites. Long periods of high or low salinity can dramatically change and impact the species distribution and be the cause of major SAV abundance changes at sites.

Light can limit the depth ranges and geographic distribution of SAV species. Different SAV species have tolerances for low-light limits, for *V. americana* the critical percent of light needed is at least 5%, while *R. maritima* needs 28% (Batuik et al. 2000). The critical percent of light is the light percentage, which plants are highly impacted at and can die. These have been determined from shading and light manipulation experiments. Light availability might have been an overall controlling factor for SAV presence, but the community composition at ECU/APNEP sentinel sites was primarily determined by salinity (see APPENDIX G). ECU/APNEP team members detected *Ruppia maritima* growing at sentinel sites of high and low Secchi depths and high and low salinities. *Vallisneria americana* was commonly found at sentinel sites with high Secchi depths (>0.77 m) and low salinities (<6 ppt). *Zannichellia palustris* was mainly found at sentinel sites with high Secchi depths (>0.77 m) and intermediate salinities (>5 ppt <11 ppt). With future increases of salinity and turbidity expected in North Carolina estuaries, *R. maritima* has been predicted by my logistic regression model and field observations to be favored in the competition among these SAV species and may become dominant.

Species Diversity

Species accumulation curves were created to capture species richness changes at sentinel site locations through the monitored years (2015-2019). This analysis was used because the sampling effort was not equal for every year and estuary location, making a direct comparison of species diversity impossible. Species accumulation curves estimate the expected number of observed species as a function of sampling effort. This can make for a fair comparison of species richness at any level of sampling effort, because the expected number of species is computed for a fixed number of sentinel site surveys, using a randomization procedure.

The species accumulation curve showed 2018 predicted the highest species richness at the observed level of sampling effort (only five sentinel sites included SAV during this year, estuaries AS and NRE were only surveyed). The sampling year 2016 had the lowest species richness predicted at any sampling effort (based on sentinel site data in the AS and PRE with 13 surveys). For some of the survey years (2018 and 2019), the accumulation curve did not reach a clear asymptote, meaning that more sampling may have been required to find additional SAV species.

The AS, PRE, and NRE were also evaluated for SAV species richness differences by species accumulation curves. The NRE had the steepest accumulation curve, which means that there was more species richness located in this estuary on average predicted at any level of sampling effort (based on 11 sentinel site surveys containing SAV). The PRE had the subsequent highest species richness expected at any sampling effort based on 15 SAV positive sentinel site surveys. The AS had the lowest species richness indicated by the accumulation curve at any level of sampling effort and does not show a clear asymptote meaning that more species could be found with increased sampling effort. The difference in species richness observed by the species accumulation curves is likely not biologically significant. The species richness between estuaries for 2015-2019 have only one species difference (the AS has nine SAV species, while PRE and NRE have nine and eight, total species respectively). This small change in species richness could be due to sampling error as multiple student researchers have helped with fieldwork, and misidentification of species can not be ruled out.

Limitations

A drawback in this study design was that water depth measured with cores and quadrats stopped at 1 m, although for some sentinel sites, SAV was observed at deeper depths based on acoustic SONAR. Analyzing deeper water depths would likely show a decrease in SAV abundance at a certain depth where photosynthetic light levels are not sufficient but cannot be determined with the same methods used in this study since water depth measurements of cores and quadrats stopped at 1 m. Initially, the ECU/APNEP monitoring design did not intend quadrat estimates to be a sole indicator of SAV change, but instead was used to supplement other sampling methods of SONAR and remote surveying (Kenworthy et al. 2012). Methodology for cores and quadrats were developed to survey shallow waters where boat-based acoustic SONAR could not reach.

Another major limitation to this study was that continuous environmental data loggers were not used in conjunction with SAV surveys. This would allow for constant turbidity and salinity measurements that could have provided more accurate measurements than the single water quality measurement taken at each sentinel site every year. I recommend future monitoring in the AS, PRE, and NRE to include continuous water quality and wave energy monitors at sentinel site locations to better measure the relationship of SAV abundance with water quality variables.

The change of species diversity was also challenging to capture through our study design, because not every sentinel site was surveyed every year. As this project expanded from ten sites in the AS (2015) to six more in the PRE (2016) and then ten more in the NRE (2017), the workload became challenging to survey each sentinel site every summer intensively. Also, the 26 sentinel sites could not all be surveyed by our team in a single month but took a whole growing season to complete. Thus, year to year comparisons at a given sentinel site may reflect growing season effects as well as interannual effects.

Recommendations and Future Outlook

The observational design of this study allows us to have great external validity with our research findings but needs experimental tests to achieve internal validity confidence. I recommend that for future studies, test *R. maritima*, *V. americana,* and *Z. palustris* in growing tanks with ranging salinities and light levels to understand SAV changes in abundance measurements (similar to Hillman and La Peyre 2019). Other suggestions include adding an interaction effect of SAV species on one another, specifically between *R. maritima* and *V. americana* in a laboratory setting. I would also like to see other factors besides light and salinity be examined, such as wave energy, surrounding land use (herbicides from agriculture), substrate type and exposure times due to low water. To predict the occurrence of SAV accurately through statistical modeling, it is necessary to include all environmental variables that can affect SAV abundance, which may include biological factors such as herbivory from ducks, crabs, fish, turtles and seed dispersal limitations. This is easier said than done, as factors limiting SAV could be site-specific and not universal throughout the estuaries.

SAV loss is often a symptom of a larger problem and an indication of poor water quality (Orth et al. 2006). Since the first records of seagrass in 1879 there has been a gradual global decline reported by Waycott et al. (2009). Worldwide seagrasses are experiencing multiple environmental stressors with anthropogenic actions being the most prevalent, leading to increased nutrient and sedimentation runoff which impacts water clarity (Orth et al. 2006). Future work in my lab should evaluate the surrounding land use of North Carolina estuaries using GIS maps to better understand pollution runoff of herbicides and other chemicals. Threats from climate change on SAV are expected to increase (sea-surface temperature, sea level rise and intensity of storms) which will have further undesirable effects on SAV species (Orth et al. 2006). Increased temperature may extend the growing season for some species, but warming waters also can decrease photosynthesis and increase respiration impacting reproduction and germination. Changes in precipitation patterns will likely increase phosphorus and nitrogen loading leading to more eutrophic conditions reducing water clarity (Short et al. 2016). How well SAV species adapt to these stressors is unknown but recent reports of decline are not optimistic. Further SAV loss will have tremendous impacts on coastal ecosystems as SAV provides many ecological services and supports invertebrates, finfishes and waterfowl. SAV is critical primary and secondary nursery habitat for commercial and recreational fisheries, and without these habitats then decreases in fish stocks can be assumed (Beck et al. 2001). Public awareness is needed to help effectively spread the importance of SAV knowledge to make this a prominent issue. With global conservation efforts to reduce anthropogenic climate change it is believed that this SAV crisis can be adverted (Orth et al. 2006). With coastal impacts from climate change expected to increase environmental variability, a shift in community composition for disturbance tolerant species such as *Ruppia maritma* are expected to be favored.

Conclusion

This thesis aimed to understand and document the effects of salinity, turbidity (measured by Secchi depth), and water depth on dominant SAV species in North Carolina estuaries by observing the change in SAV abundance (odds of occurrence, cover, frequency, and biomass). This study intended to provide resource managers insight into dominant SAV species tolerance for salinity, turbidity, water depth, and information on species diversity changes through this monitoring period at ECU/APNEP sentinel sites.

With multiple logistic regression models for dominant SAV species, I confirmed salinity, turbidity (measured by Secchi depth), and water depth significantly impact SAV species occurrence. I can conclude the odds of *R. maritima* occurrence are predicted to increase with increasing salinity, turbidity, and water depth. These findings are limited to only the observed measurements of salinity (0-20 ppt), turbidity (Secchi depth 0.37-1.57 m), and water depth (0-1 m) measured in this study. My research shows *Ruppia maritima* can be found inhabiting a range of salinities (0-18 ppt) and documented in Secchi depths of 0.5 m to 1.4 m (0.7 m average). *Ruppia maritima* was not limited by water depth in depths examined, and odds of SAV occurrence increased with depth (limited to 1 m). *Ruppia maritima* may be a generalist and opportunistic species that can live with a large range of varying stressors, but may not dominate in constant conditions when competing with *V. americana*

For dominant SAV species *Vallisneria americana,* I can conclude that increased turbidity (low Secchi depths) and higher salinity reduce the odds of *V. americana* occurrence. This study has shown that *V. americana* prefers low salinities (0-6.4 ppt) with an average salinity of 1.4 ppt found at our ECU/APNEP sentinel sites. The average Secchi depth was 0.9 m when *V. americana* was found present. SAV metrics of percent cover and frequency also demonstrated a significant difference among measured water depths (0.25 m differs from 1 m). *Vallisneria americana* is likely to be the competitive dominant SAV species in low salinity with high Secchi depths.

Zannichellia palustris did not show significant changes of occurrence with salinity or turbidity but can be concluded to have higher odds of occurrence with increased water depths out to 1 m. The average Secchi depth at a sentinel site when *Z. palustris* was detected was 0.8 m, and salinity ranged from 0-10 ppt (4.2 ppt on average). The small sample size associated with this species is likely the cause for the lack of statistical significance with salinity and turbidity (only 11 sentinel site surveys *Z. palustris* was observed).

A general decrease of SAV at ECU/APNEP sentinel sites for the AS has been observed when looking at the quadrat percent cover change through the monitoring period. Simultaneously, the PRE and NRE show a gradual increase in percent cover (APPENDIX D). When looking at dominant SAV species percent cover, *V. americana* appears to be decreasing in the AS and NRE but increasing or staying the same at PRE sentinel sites. *Ruppia maritima* has an overall increase in cover within all estuaries from 2015-2019 but decreases at AS-SS-08. *Zannichellia palustris* is primarily found in the NRE and shows an increase in percent cover through the monitoring period. Salinity plays a role in these observed changes in the AS and NRE. Increases in salinity may be causing *V. americana* to decline and *R. maritima* and *Z. palustris* to increase at ECU/APNEP sentinel site locations.

This study’s objectives were completed as the occurrence and abundance of dominant SAV species (R. *maritima*, *V. americana*, and *Z. palustris)* at ECU/APNEP sentinel sites were analyzed by examining the effect of environmental factors to determine their influence on SAV abundance. Species tolerance ranges were established from this study by documenting salinity, Secchi depth, and water depth associated with each species (Table 9). Species diversity was also evaluated using species accumulation plots to assess SAV diversity change through unequal sampling measurements.

In summary, dominant SAV species found at ECU/APNEP sentinel sites do not grow well in shallow waters (0.25 m). This is likely due to wave action, sediment resuspension and air exposure that occur in that depth zone. *Ruppia maritima* occurred at locations with higher salinity and surprisingly low Secchi depths but was found present at a range of salinity and turbidity levels. It is an opportunistic SAV species that can establish itself over a wide range of conditions. *Vallisneria americana* occurred at ECU/APNEP sentinel sites with low salinities (0-6.4 ppt) and high Secchi depths (>0.77 m) but was also found present at low Secchi depth sites that also had low (<5 ppt) salinities. *Zannichellia palustris* odds of occurrence did not show or were not associated with salinity and Secchi depth changes, but odds of occurrence increased with water depth relative to 1 m. *Zannichellia palustris* may be dominant in some intermediate salinities.

SAV has many important ecological functions from stabilizing sediments, which reduce erosion, acting as a carbon sink, filtering suspended sediments and providing nursery ground habitat for commercially important finfish, invertebrates and shellfish (Fonseca et al. 1992; Short and Wyllie- Echeverria 1996; Beck et al. 2001; de Boer 2007). With such great ecological importance in North Carolina’s estuaries, it is crucial for management agencies to understand what factors are limiting growth and survival of individual SAV species to better estimate species abundance and develop habitat requirements. Management plans for individual SAV species and community types may be more effective than a single estuary plan. Impacts of turbidity, salinity and water depth on dominant SAV species are provided in this study to improve SAV prediction maps and allow for management agencies to better explain variations of SAV abundance for specific SAV species in North Carolina. Understanding the combined effects of environmental stressors on SAV is required to develop habitat requirements and the first step to implement restoration efforts.

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APPENDIX A: SENTINEL SITE WATER QUALITY

APPENDIX A-1**.** 2019 Albemarle Sound sentinel site survey date, Secchi depth, salinity and species present. NA represents no species found at the sentinel site.

Albemarle Sound 2019				
Sentinel Site	Date	Secchi Depth (m)	Salinity	Species present
AS_SS_01	4-Oct-19	0.77	6.09	*R. maritima, M. spicatum, V. americana*
AS_SS_03	13-Sep-19	0.65	.2	NA
AS_SS_04	14-Jun-19	0.84	.06	NA
AS_SS_05	2-Jul-19	0.81	.05	NA
AS_SS_07	6-Oct-19	0.77	3.7	*R. maritima, Chara sp.*
AS_SS_08	14-Sep-19	0.77	.5	NA
AS_SS_09	15-Jul-19	0.76	.06	NA
AS_SS_10	1-Jul-19	0.80	.05	NA

APPENDIX A-2**.** 2018 Albemarle Sound sentinel site survey date, Secchi depth, salinity and species present. NA represents no species found at the sentinel site.

Albemarle Sound 2018				
Sentinel Site	Date	Secchi Depth (m)	Salinity	Species present
AS_SS_01	21-Sep-18	0.755	18.87	*R. maritima, M. spicatum, H. wrightii*
AS_SS_03	15-Aug-18	1.09	0.94	NA
AS_SS_04	7-Aug-18	0.93	0.3	NA
AS_SS_05	9-Aug-18	1.28	0.7	NA
AS_SS_06	29-Aug-18	0.66	3.16	NA
AS_SS_07	22-Sep-18	0.60	3.89	NA
AS_SS_08	24-Aug-18	0.70	1.99	NA
AS_SS_09	14-Aug-18	0.82	0.42	NA
AS_SS_10	1-Aug-18	0.97	0.05	NA

APPENDIX A-3**.** 2017 Albemarle Sound sentinel site survey date, Secchi depth, salinity and species present. NA represents no species found at the sentinel site.

Albemarle Sound 2017				
Sentinel Site	Date	Secchi Depth (m)	Salinity	Species present
AS_SS_01	29-Sep-17	0.66	6.7	*R. maritima, M. spicatum*
AS_SS_05	8-Sep-17	1.0	0.10	NA
AS_SS_10	17-Aug-17	0.74	0.10	NA

APPENDIX A-4**.** Summer 2016 Albemarle Sound sentinel site survey date, Secchi depth, salinity and species present. NA represents no species found at the sentinel site.

Albemarle Sound 2016 (Summer)				
Sentinel Site	Date	Secchi Depth (m)	Salinity	Species present
AS_SS_01	20-Jun-16	0.86	0	*R. maritima, M. spicatum*
AS_SS_02	29-Jun-16	0.76	1.4	*R. maritima*
AS_SS_03	24-May-16	1.07	0.47	*V. americana, Z. palustris*
AS_SS_04	10-May-16	1.09	0.16	*V. americana*
AS_SS_05	25-May-16	1.5	0.05	NA
AS_SS_06	9-June-16	0.73	1.16	NA
AS_SS_07	27-Jun-16	1.3	1.73	*R. maritima*
AS_SS_08	6-Jul-16	0.53	1.07	*R. maritima, M. spicatum, N. guadalupensis*
AS_SS_09	12-May-16	0.76	0.05	NA
AS_SS_10	16-May-16	1.57	0.05	NA

APPENDIX A-5**.** Fall 2016 Albemarle Sound sentinel site survey date, Secchi depth, salinity and species present. NA represents no species found at the sentinel site.

Albemarle Sound 2016 (Fall)				
Sentinel Site	Date	Secchi Depth (m)	Salinity	Species present
AS_SS_01	8-Sep-16	0.60	1.78	*R. maritima, M. spicatum*
AS_SS_02	21-Oct-16	0.35	0	NA
AS_SS_03	16-Sep-16	0.85	0.48	NA
AS_SS_04	26-Sep-16	0.85	0	*V. americana, N. guadalupensis*
AS_SS_05	18-Oct-16	0.90	0	NA
AS_SS_06	19-Oct-16	0.78	0	NA
AS_SS_07	3-Oct-16	0.61	0	*R. maritima*
AS_SS_08	23-Sep-16	0.56	0.82	*R. maritima*
AS_SS_09	30-Sep-16	0.88	0	NA
AS_SS_10	24-Sep-16	1.09	0.04	NA

APPENDIX A-6**.** Summer 2015 Albemarle Sound sentinel site survey date, Secchi depth, salinity and species present. NA represents no species found at the sentinel site.

Albemarle Sound 2015 (Summer)				
Sentinel Site	Date	Secchi Depth (m)	Salinity	Species present
AS_SS_01	10-Jun-15	0.60	2.95	*R. maritima, M. spicatum, S. pectinata*
AS_SS_02	25-Jun-15	0.58	0	*V. americana, R. maritima*
AS_SS_03	16-Jun-15	0.98	0	*V. americana, S. pectinate*

AS_SS_04	15-Jun-15	1.20	0	*V. americana, M. spicatum, N. guadalupensis, P. perfoliatus, P. crispus*
AS_SS_05	20-May-15	0.77	0.06	NA
AS_SS_06	2-Jun-15	0.81	2.27	*R. maritima, S. pectinate*
AS_SS_07	11-Jun-15	0.66	1.95	*R. maritima, S. pectinate*
AS_SS_08	24-Jun-15	0.50	0	*R, maritima*
AS_SS_09	28-May-15	1.30	0.06	NA
AS_SS_10	26-May-15	0.84	0	*Chara*

APPENDIX A-7**.** Fall 2015 Albemarle Sound sentinel site survey date, Secchi depth, salinity and species present. NA represents no species found at the sentinel site.

Albemarle Sound 2015 (Fall)				
Sentinel Site	Date	Secchi Depth (m)	Salinity	Species present
AS_SS_01	16-Sep-15	0.61	5.89	*R. maritima, M. spicatum*
AS_SS_02	9-Oct-15	0.58	4.23	NA
AS_SS_03	8-Oct-15	1.47	0	NA
AS_SS_04	28-Sep-15	0.68	0.41	*V. americana, N. guadalupensis*
AS_SS_05	11-Sep-15	1.49	0.07	NA
AS_SS_06	15-Sep-15	0.57	4.75	NA
AS_SS_07	17-Sep-15	0.63	0	*R. maritima, S. pectinate*
AS_SS_08	28-Sep-15	0.49	1.45	*R. maritima*
AS_SS_09	16-Sep-15	0.65	0.51	NA
AS_SS_10	23-Sep-15	0.43	0	NA

APPENDIX A-8**.** 2019 Neuse River Estuary sentinel site survey date, Secchi depth, salinity and species present. NA represents no species found at the sentinel site.

Neuse River Estuary 2019				
Sentinel Site	Date	Secchi Depth (m)	Salinity	Species present
NR_SS_01	18-Jun-19	0.74	14.19	NA
NR_SS_02	7-Jun-19	0.59	10.36	*R. maritima, Z. palustris*
NR_SS_03	22-May-19	0.79	7.4	*R. maritima, Z. palustris, P. pusillus, Chara sp.*
NR_SS_04	19-Jun-19	0.70	3.32	NA
NR_SS_05	4-Jun-19	0.84	6.2	*Chara sp.*
NR_SS_06	21-May-19	1.02	0.12	*Z. palustris*
NR_SS_07	29-May-19	0.75	1.33	NA
NR_SS_08	26-Jun-19	0.93	6.19	NA
NR_SS_09	21-Jun-19	0.68	10.69	*R. maritima*
NR_SS_10	28-Jun-19	1.22	14.99	NA

APPENDIX A-9**.** 2018 Neuse River Estuary sentinel site survey date, Secchi depth, salinity and species present. NA represents no species found at the sentinel site.

Neuse River Estuary 2018				
Sentinel Site	Date	Secchi Depth (m)	Salinity	Species present
NR_SS_01	2-Jul-18	0.64	14.34	NA
NR_SS_02	28-Jun-18	0.65	5.75	NA
NR_SS_03	15-Jun-18	0.47	7.35	*R. maritima, Z. palustris, H. wrightii, Z. marina*
NR_SS_04	11-Jul-18	1.13	10.80	NA
NR_SS_05	12-Jul-18	0.87	6.3	NA
NR_SS_06	6-Jun-18	0.99	6.4	*R. maritima, Z. palustris, N. guadalupensis, P. pusillus*
NR_SS_07	18-Jul-18	0.96	1.77	*V. americana, H. wrightii*
NR_SS_08	19-Jul-18	1.04	8.82	NA
NR_SS_09	26-Jul-18	0.68	5.89	*Z. marina*
NR_SS_10	27-Jul-18	0.82	11.84	NA

APPENDIX A-10**.** 2017 Neuse River Estuary sentinel site survey date, Secchi depth, salinity and species present. NA represents no species found at the sentinel site.

Neuse River Estuary 2017				
Sentinel Site	Date	Secchi Depth (m)	Salinity	Species present
NR_SS_01	28-Jun-17	0.80	17.01	NA
NR_SS_02	3-Jul-17	0.80	12.7	NA
NR_SS_03	28-Oct-17	1.1	9.6	NA
NR_SS_04	26-Jul-17	0.90	15.5	NA
NR_SS_05	19-Jul-17	1.0	6.80	NA
NR_SS_06	13-Oct-17	1.40	1.1	*R. maritima, N. guadalupensis, Chara sp.*
NR_SS_07	18-Jul-17	1.30	2.7	*V. americana*
NR_SS_08	27-Jul-17	0.90	12.6	NA
NR_SS_09	1-Aug-17	1.0	15.7	NA
NR_SS_10	4-Aug-17	0.80	20.6	NA

APPENDIX A-11**.** 2019 Pamlico River Estuary sentinel site survey date, Secchi depth, salinity and species present. NA represents no species found at the sentinel site.

Pamlico River Estuary 2019

Sentinel Site	Date	Secchi Depth (m)	Salinity	Species present
PR_SS_01	7-Aug-19	0.93	4.87	*R. maritima, V. americana, Z. palustris*
PR_SS_02	31-Jul-19	0.67	10.56	*R. maritima*
PR_SS_03	16-Jul-19	0.64	12	*R. maritima, H. wrightii, Z. marina, Chara sp.*
PR_SS_04	29-Jul-19	0.93	14.93	*R. maritima*
PR_SS_05	6-Aug-19	0.90	6.41	*R. maritima, V. americana, Z. palustris, P. crispus*
PR_SS_06	30-Jul-19	0.64	13.1	*R. maritima*

APPENDIX A-12**.** 2017 Pamlico River Estuary sentinel site survey date, Secchi depth, salinity and species present. NA represents no species found at the sentinel site.

Pamlico River Estuary 2017				
Sentinel Site	Date	Secchi Depth (m)	Salinity	Species present
PR_SS_01	14-Jun-17	0.70	0	*R. maritima, V. americana*
PR_SS_02	9-Jun-17	0.95	3.3	*R. maritima, Z. palustris*
PR_SS_03	26-Jun-17	0.85	NA	*R. maritima*
PR_SS_04	19-Jun-17	0.67	0	*R. maritima*
PR_SS_05	7-Jun-17	0.83	0	*V. americana, Z. palustris, N. guadalupensis*
PR_SS_06	13-Jun-17	0.66	0	*R. maritima, Z. palustris*

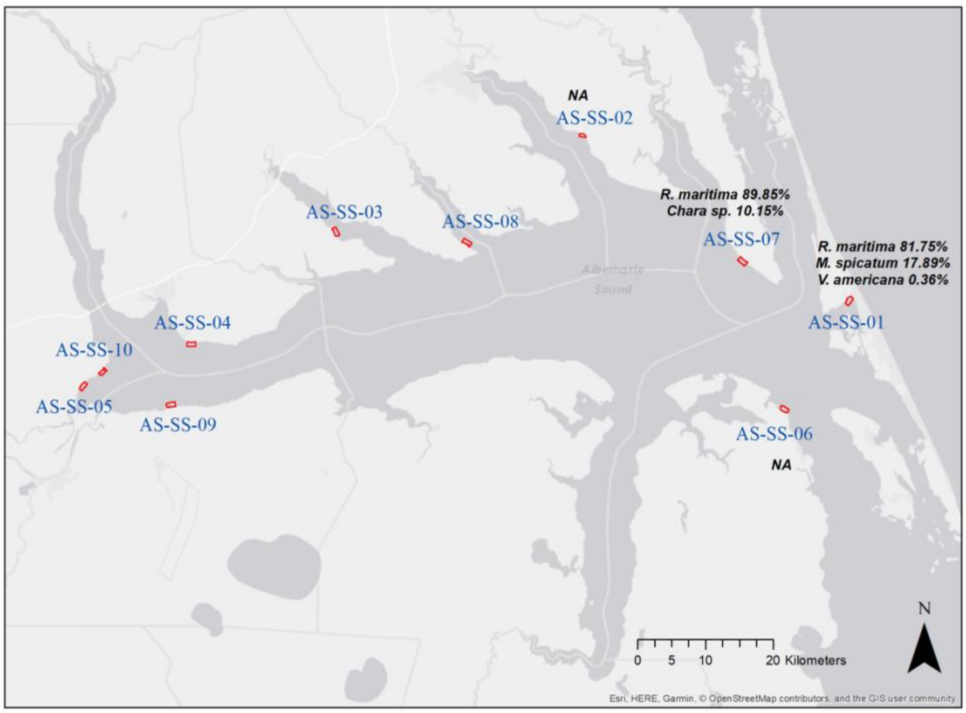
APPENDIX A-13**.** 2016 Pamlico River Estuary sentinel site survey date, Secchi depth, salinity and species present. NA represents no species found at the sentinel site.

Pamlico River Estuary 2016				
Sentinel Site	Date	Secchi Depth (m)	Salinity	Species present
PR_SS_01	9-Aug-16	0.88	0	*V. americana, M. spicatum*
PR_SS_02	17-Aug-16	0.35	4.16	NA
PR_SS_03	10-Aug-16	0.53	10.55	*R. maritima*
PR_SS_04	18-Aug-16	0.67	0	NA
PR_SS_05	11-Aug-16	0.63	0.08	*V. americana*
PR_SS_06	30-Aug-16	0.64	0	NA

APPENDIX B: SPECIES COMPOSITION MAPS

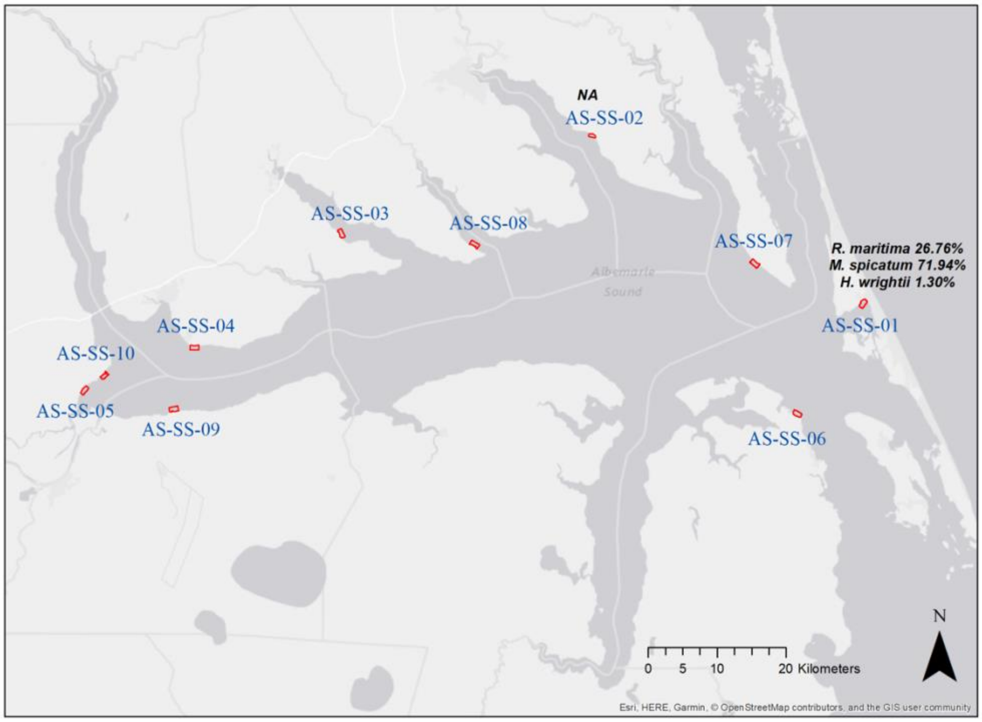
Percent composition of SAV species based on core dry biomass. NA represents that the sentinel site was not surveyed that year. Sentinel sites with no NA or species are sites that were surveyed but did not contain SAV.

Albemarle Sound 2019



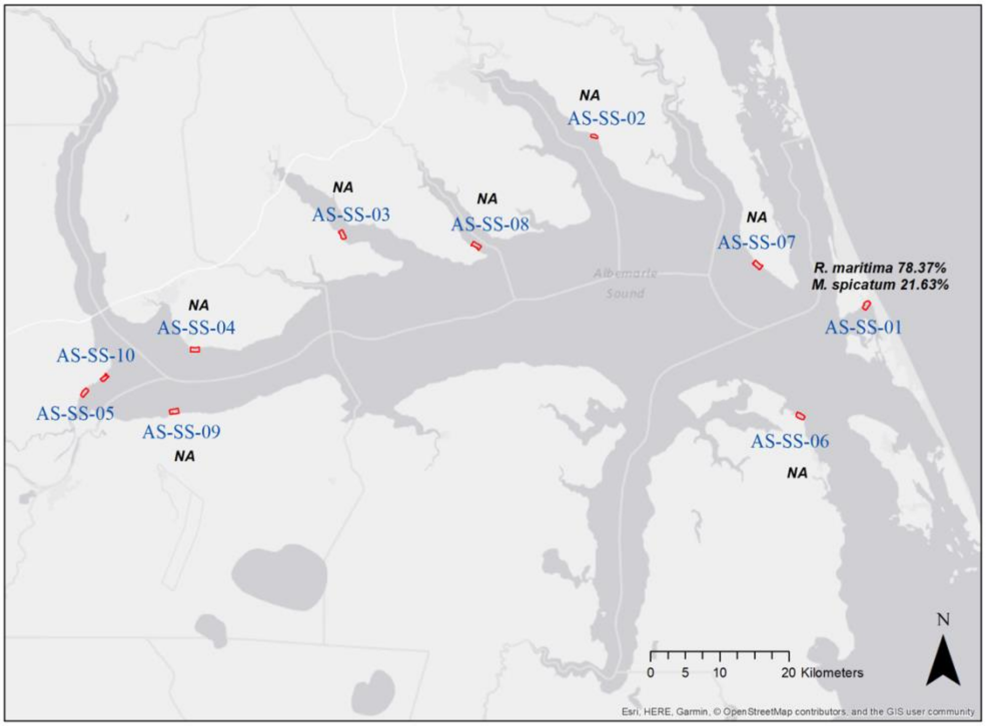
APPENDIX B-1. 2019 Albemarle Sound sentinel site composition map based on the percentage of each species found in cores. NA's represent sentinel sites that were not surveyed during the year. Sentinel sites with no NA or species are sites that were surveyed but did not contain SAV.

Albemarle Sound 2018



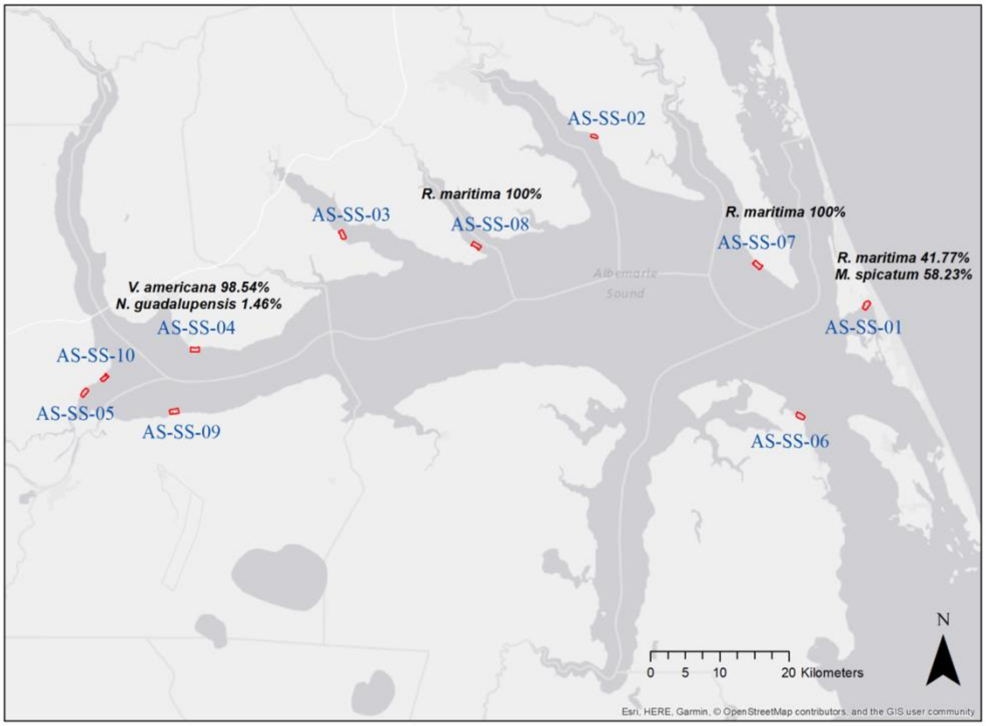
APPENDIX B-2. 2018 Albemarle Sound sentinel site composition map based on the percentage of each species found in cores. NA's represent sentinel sites that were not surveyed during the year. Sentinel sites with no NA or species are sites that were surveyed but did not contain SAV.

Albemarle Sound 2017

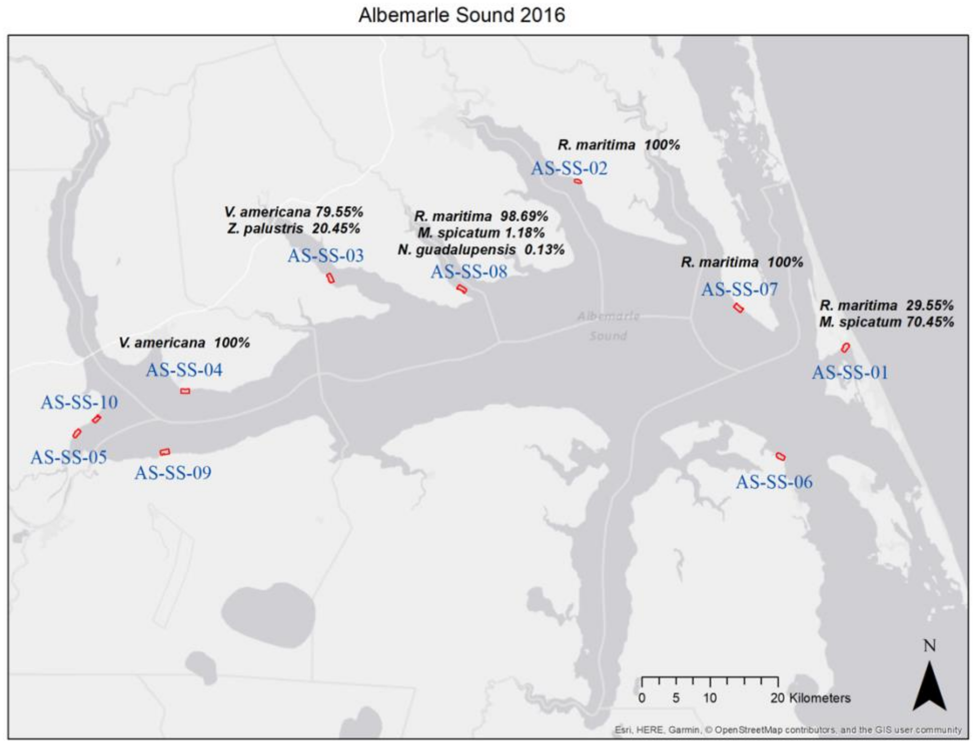


APPENDIX B-3. 2017 Albemarle Sound sentinel site composition map based on the percentage of each species found in cores. NA's represent sentinel sites that were not surveyed during the year. Sentinel sites with no NA or species are sites that were surveyed but did not contain SAV.

Albemarle Sound 2016 Fall

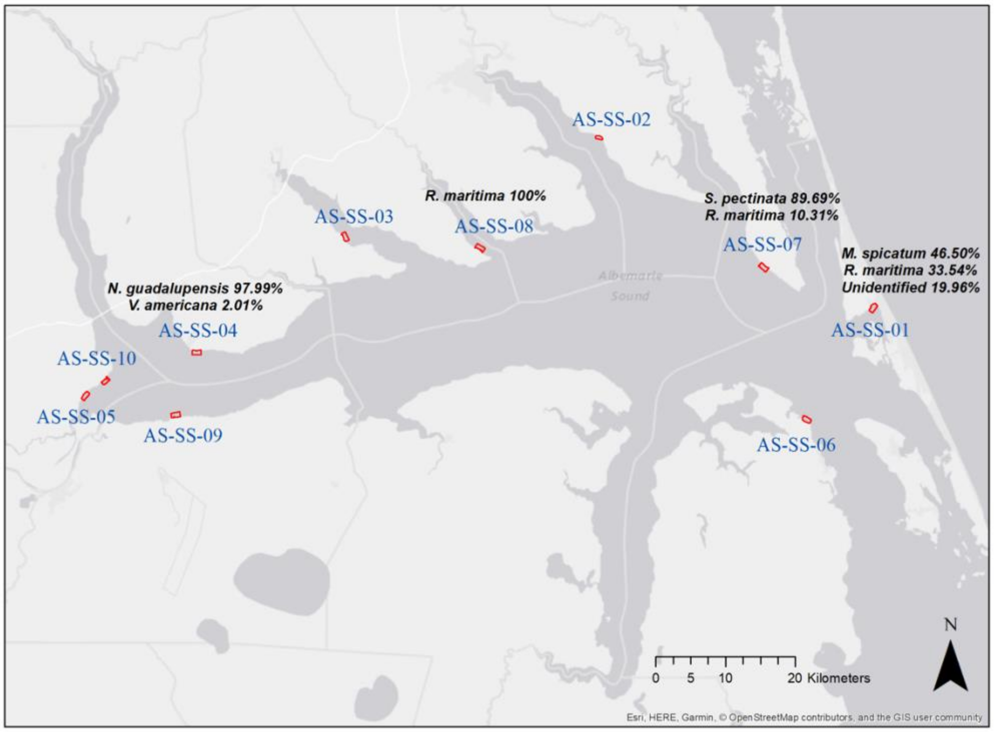


APPENDIX B-4. Fall 2016 Albemarle Sound sentinel site composition map based on the percentage of each species found in cores. NA's represent sentinel sites that were not surveyed during the year. Sentinel sites with no NA or species are sites that were surveyed but did not contain SAV.



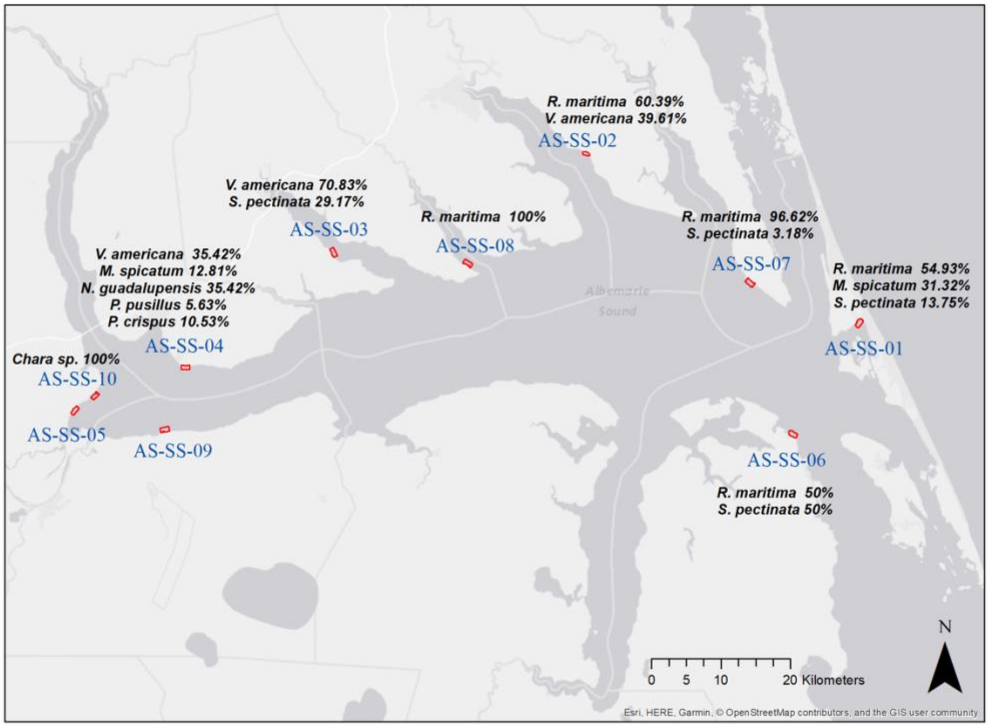
APPENDIX B-5. Spring 2016 Albemarle Sound sentinel site composition map based on the percentage of each species found in cores. NA's represent sentinel sites that were not surveyed during the year. Sentinel sites with no NA or species are sites that were surveyed but did not contain SAV.

Albemarle Sound 2015 Fall



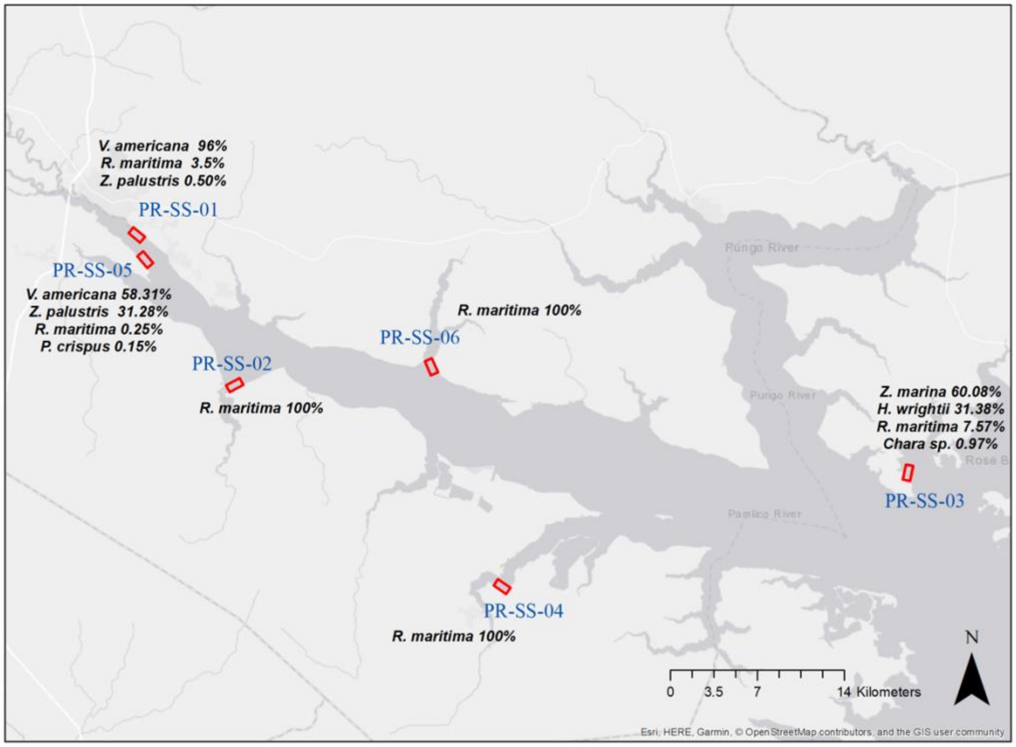
APPENDIX B-6. Fall 2015 Albemarle Sound sentinel site composition map based on the percentage of each species found in cores. NA's represent sentinel sites that were not surveyed during the year. Sentinel sites with no NA or species are sites that were surveyed but did not contain SAV.

Albemarle Sound 2015



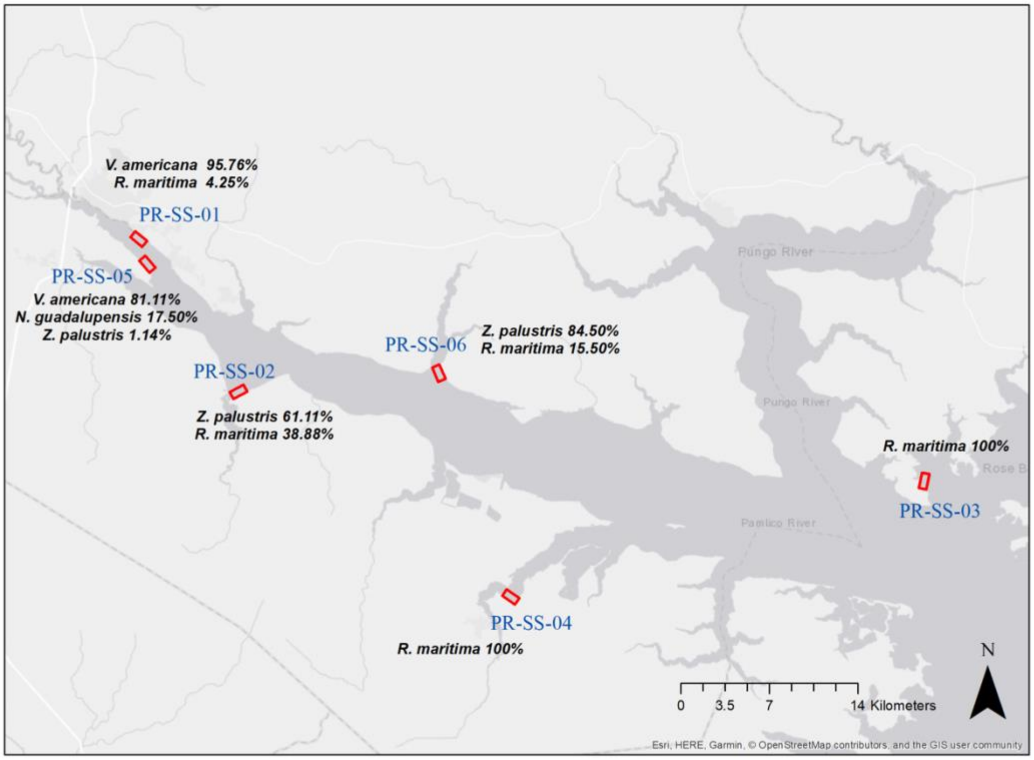
APPENDIX B-7. Spring 2015 Albemarle Sound sentinel site composition map based on the percentage of each species found in cores. NA's represent sentinel sites that were not surveyed during the year. Sentinel sites with no NA or species are sites that were surveyed but did not contain SAV.

Pamlico River Estuary 2019



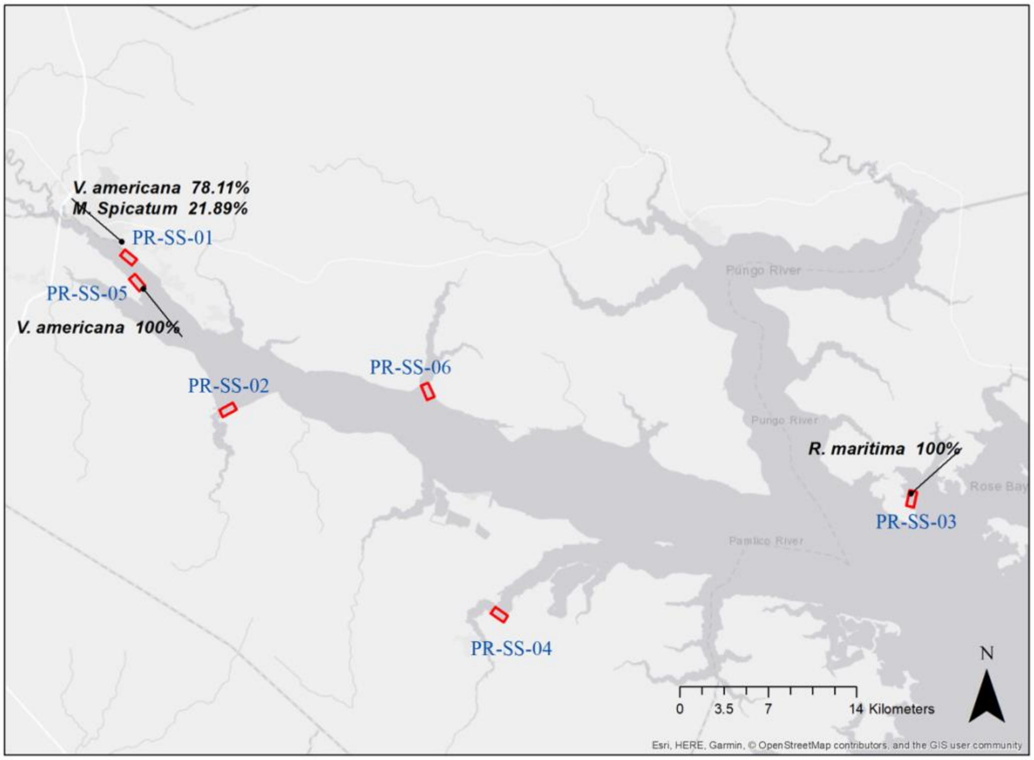
APPENDIX B-8. 2019 Pamlico River Estuary sentinel site composition map based on the percentage of each species found in cores. NA's represent sentinel sites that were not surveyed during the year. Sentinel sites with no NA or species are sites that were surveyed but did not contain SAV.

Pamlico River Estuary 2017



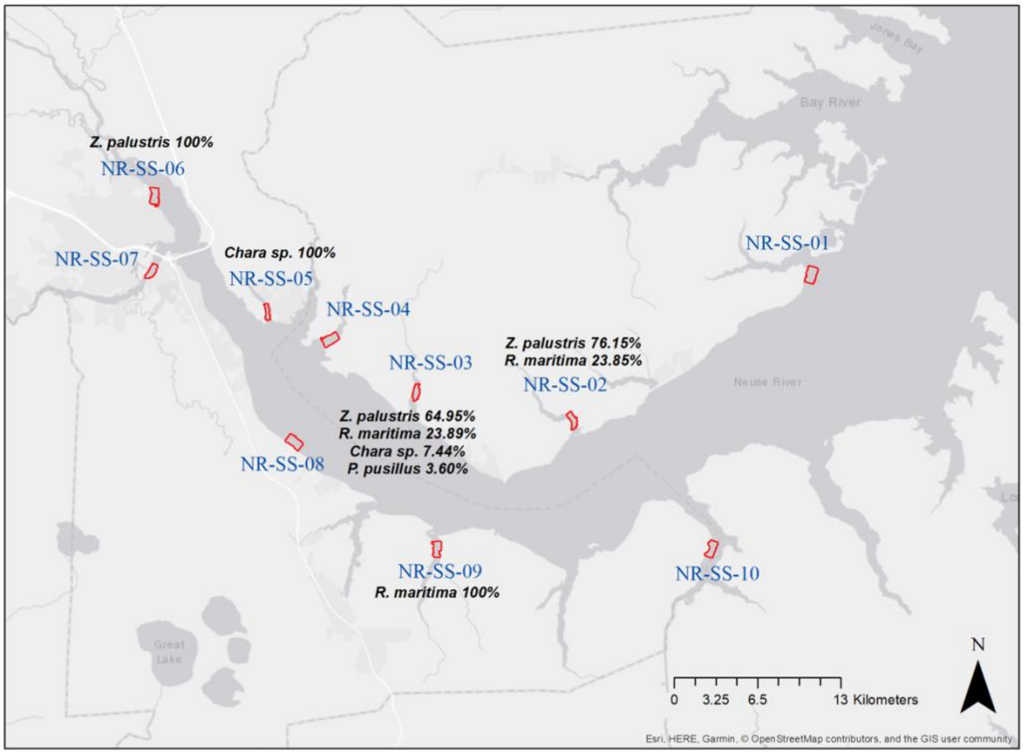
APPENDIX B-9. 2017 Pamlico River Estuary sentinel site composition map based on the percentage of each species found in cores. NA's represent sentinel sites that were not surveyed during the year. Sentinel sites with no NA or species are sites that were surveyed but did not contain SAV.

Pamlico River Estuary 2016



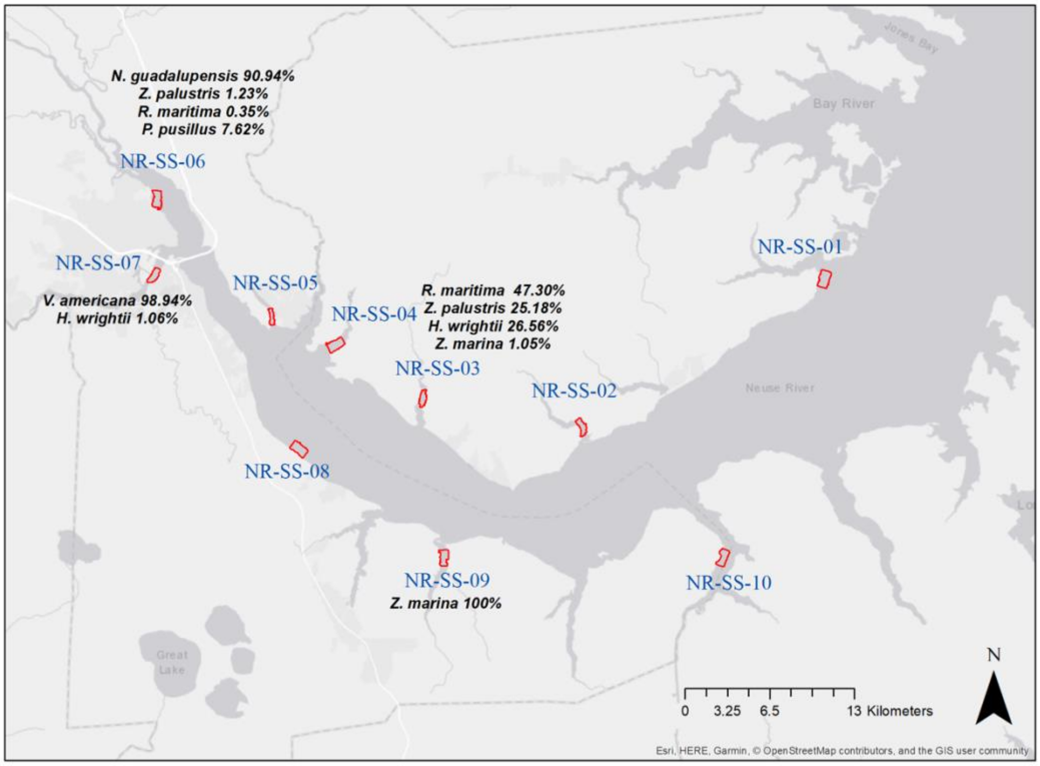
APPENDIX B-10. 2016 Pamlico River Estuary sentinel site composition map based on the percentage of each species found in cores. NA's represent sentinel sites that were not surveyed during the year. Sentinel sites with no NA or species are sites that were surveyed but did not contain SAV.

Neuse River Estuary 2019



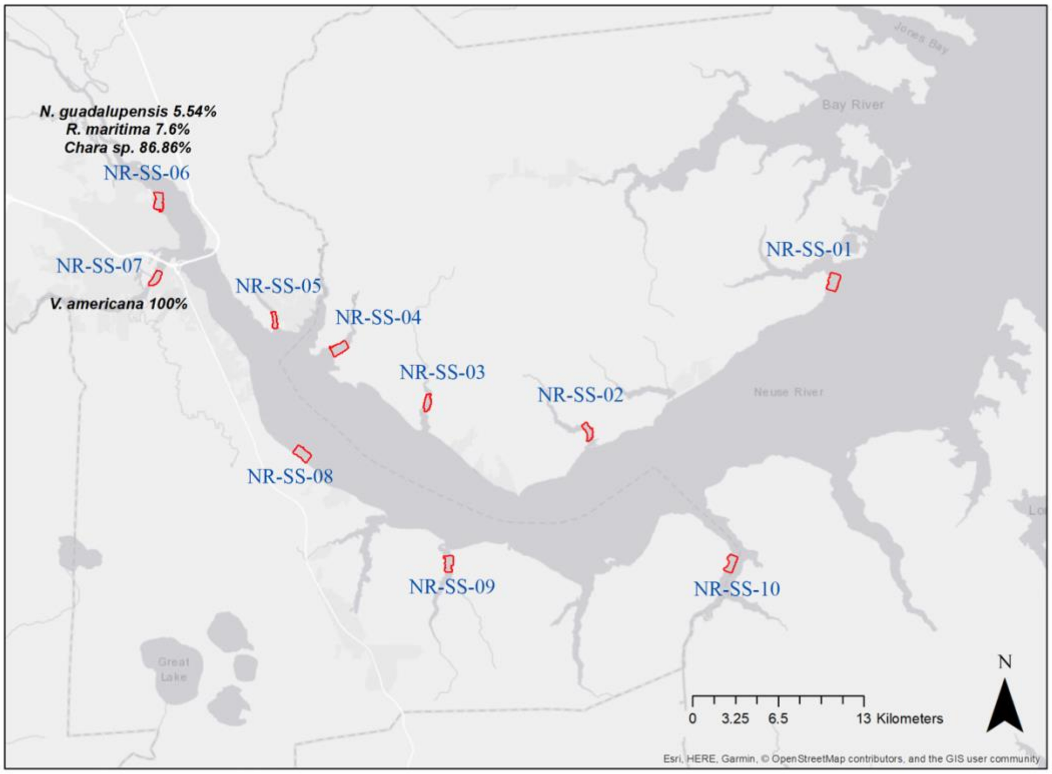
APPENDIX B-11. 2019 Neuse River Estuary sentinel site composition map based on the percentage of each species found in cores. NA's represent sentinel sites that were not surveyed during the year. Sentinel sites with no NA or species are sites that were surveyed but did not contain SAV.

Neuse River Estuary 2018



APPENDIX B-12. 2018 Neuse River Estuary sentinel site composition map based on the percentage of each species found in cores. NA's represent sentinel sites that were not surveyed during the year. Sentinel sites with no NA or species are sites that were surveyed but did not contain SAV.

Neuse River Estuary 2017



APPENDIX B-13. 2017 Neuse River Estuary sentinel site composition map based on the percentage of each species found in cores. NA's represent sentinel sites that were not surveyed during the year. Sentinel sites with no NA or species are sites that were surveyed but did not contain SAV.

APPENDIX C: SENTINEL SITE QUADRAT SUMMARY TABLES

Albemarle Sound, Pamlico River Estuary and Neuse River Estuary summary sentinel site characteristics based on quadrat and cores. Sites with NA species indicate that no SAV species were found during core sampling. AS sampling time period was from 2015-2019, PRE from 2016-2017 and 2019, NRE from 2017-2019.

APPENDIX C-1. Albemarle Sound sentinel site characteristic summary table from cores and quadrats 2015-2019. Cumulative species found at sentinel sites from 2015-2019.

Sentinel Site	**Peak Abundance Year**	**Mean % Cover Range**	**Mean % Freq. Range**	**Dry Biomass Range (g)**	**Species**
AS_SS_01	2015	32 – 92	40 – 94	10 – 87	*R. maritima, M. spicatum, V. americana, H. wrightii, S. pectinata*
AS_SS_02	2015	4.8 – 11	12 – 32	0.04 – 1.5	*R. maritima, V. americana*
AS_SS_03	2016	0 – 55	0 – 71	0 – 0.4	*V. americana, Z. palustris, S. pectinata*
AS_SS_04	2015	0 – 48	0 – 84	0 – 11.2	*V. americana, M. spicatum, N. guadalupensis, P. perfoliatus, P. crispus*
AS_SS_05	NA	0	0	0	NA
AS_SS_06	2015	0 – 0.05	0 – 1.6	0 – 2.3	*R. maritima, S. pectinata*
AS_SS_07	2019	0 – 69	0 – 35	0 – 21.6	*R. maritima, Chara sp., S. pectinata*
AS_SS_08	2016	0 – 64	0 – 53	0 – 27.1	*R. maritima, N. guadalupensis, M. spicatum*
AS_SS_09	NA	0	0	0	NA
AS_SS_10	2015	0 – 0.27	0 – 10	0	*Chara sp.*

APPENDIX C-2. Pamlico River Estuary sentinel site characteristic summary table from cores and quadrats 2016, 2017 and 2019. Cumulative species found at sentinel sites from 2016, 2017 and 2019.

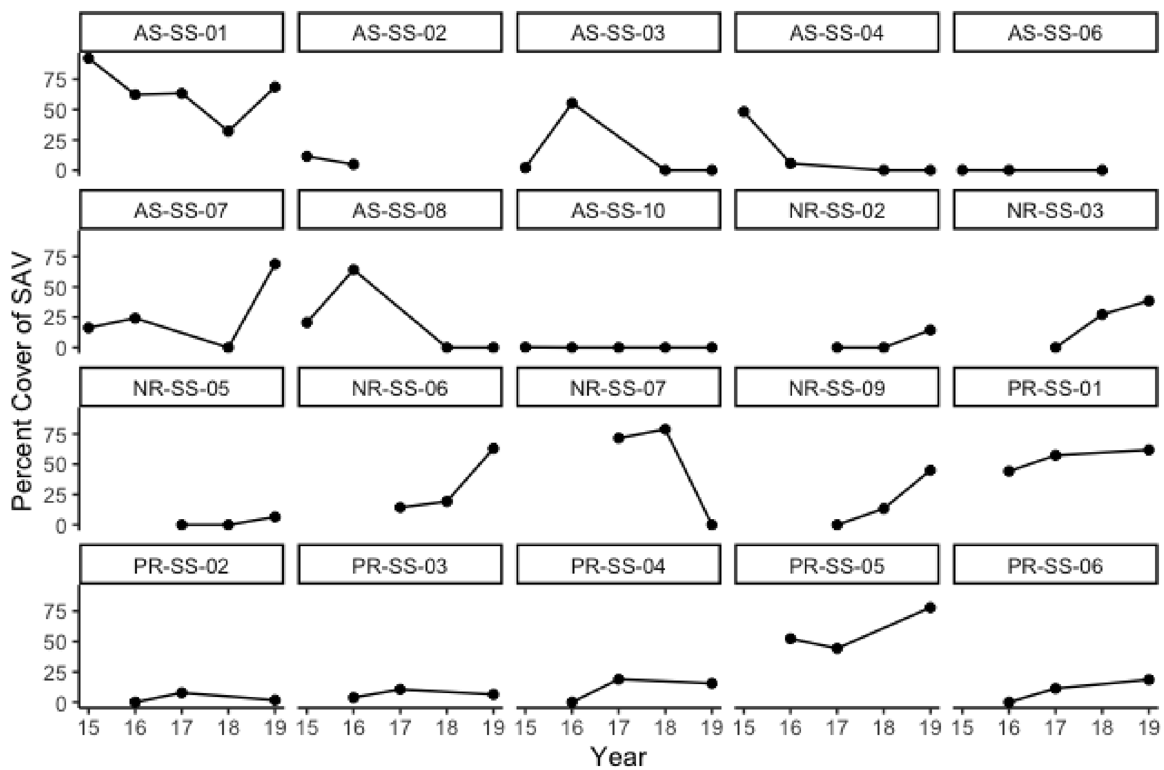
Sentinel Site	Peak Abundance Year	Mean % Cover Range	Mean % Freq. Range	Dry Biomass range (g)	Species
PR_SS_01	2019	44 – 62	39 – 68	18 – 49	*R. maritima, V. americana, Z. palustris, M. spicatum*
PR_SS_02	2017	0 – 7.7	0 – 27	0 – 3.5	*R. maritima, Z. palustris*
PR_SS_03	2017	3.8 – 11	30 – 33	0.6 – 6.4	*R. maritima, Z. palustris, H. wrightii, Chara sp.*
PR_SS_04	2017	0 – 19	0 – 64	0 – 17.6	*R. maritima*
PR-SS_05	2019	44 – 78	46 – 89	9.6 – 68	*R. maritima, V. americana, Z. palustris, N. guadalupensis, P. crispus*
PR_SS_06	2019	0 – 19	0 – 42	0 – 9.8	*R. maritima, Z. palustris*

APPENDIX C-3. Neuse River Estuary sentinel site characteristic summary table from cores and quadrats 2017-2019. Cumulative species found at sentinel sites from 2017- 2019.

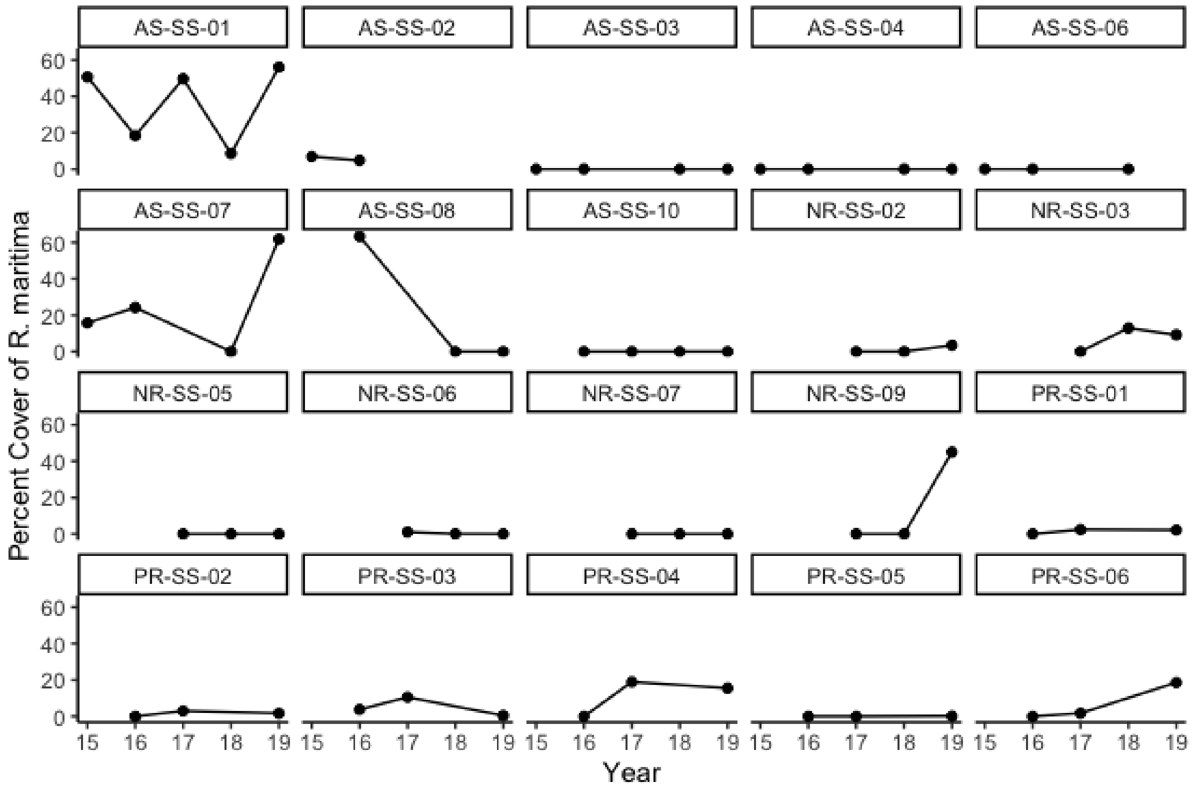
Sentinel Site	Peak Abundance Year	Mean % Cover Range	Mean % Freq. Range	Dry Biomass range (g)	Species
NR_SS_01	NA	0	0	0	NA
NR_SS_02	2019	0 – 14	0 – 56	0 – 8.8	*R. maritima, Z. palustris*
NR_SS_03	2019	0 – 38	0 – 63	0 – 17.5	*R. maritima, Z. palustris, P. pusillus, Chara sp.*
NR_SS_04	NA	0	0	0	NA
NR_SS_05	2019	0 – 6.5	0 – 20	0 – 2.7	*Chara sp.*
NR_SS_06	2019	14 - 63	13 - 55	4.1 – 24.6	*Z. palustris, R. maritima, N. guadalupensis, P. pusillus*
NR_SS_07	2018	0 - 79	0 - 91	0 – 115.2	*V. americana, H. wrightii*
NR_SS_08	NA	0	0	0	NA
NR_SS_09	2019	0 - 45	0 - 92	0 – 51.7	*R. maritima, Z. marina*
NR_SS_10	NA	0	0	0	NA

APPENDIX D: CHANGE IN QUADRAT PERCENT COVER WITH TIME

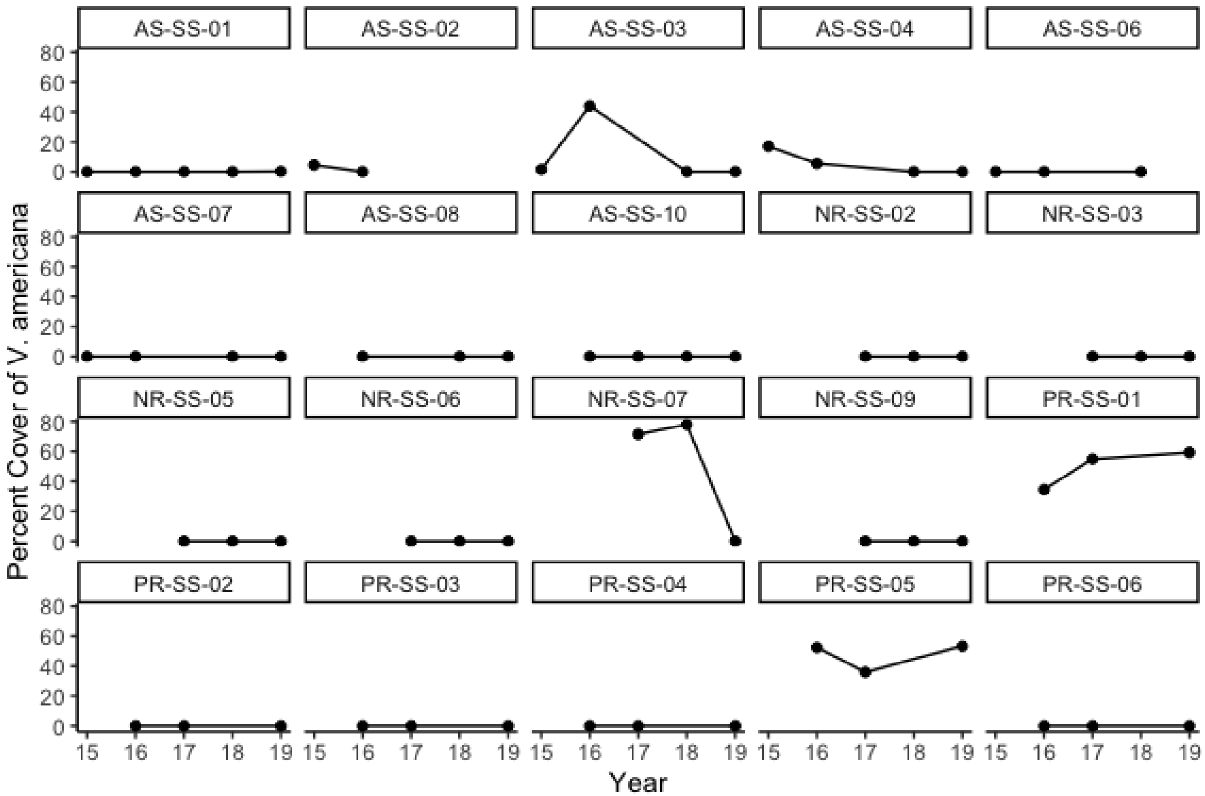
The change in SAV quadrat percent cover from sentinel site surveys from 2015-2019 (AS) 2016, 2017 and 2019 (PRE) and 2017-2019 (NRE). Six sentinel sites omitted that never contained SAV for any of the surveyed years (AS-05, 09) and (NR-01,04,08,10). Only spring and summer quadrat percent cover are shown (AS 2015 and 2016 Fall sites omitted as they were not surveyed during the peak SAV biomass season).



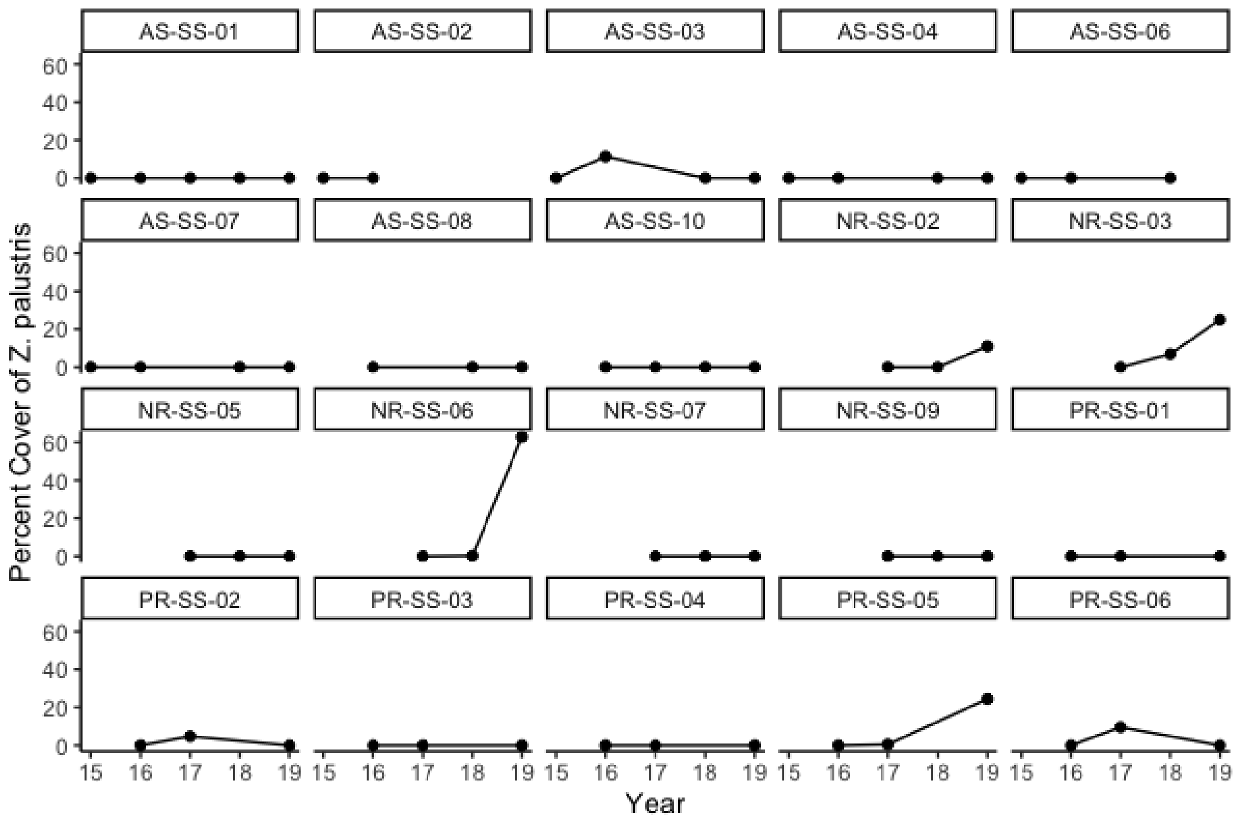
APPENDIX D-1. The change in quadrat percent cover for all SAV species combined through the monitoring years at each sentinel site (AS) 2015-2019; PRE 2016, 2017 and 2019; and NRE 2017-2019). Six sentinel sites omitted that never contained SAV for any of the surveyed years (AS-05, 09) and (NR-01,04,08,10). Only spring and summer quadrat percent cover points are shown (AS 2015 and 2016). The fall sites for those two years were omitted as they were not surveyed during the peak SAV biomass season.



APPENDIX D-2. The change in quadrat percent cover for *Ruppia maritima* through the monitoring years at each sentinel site (AS) 2015-2019; PRE 2016, 2017 and 2019; and NRE 2017-2019). Six sentinel sites omitted that never contained SAV for any of the surveyed years (AS-05, 09) and (NR-01,04,08,10). Only spring and summer quadrat percent cover points are shown (AS 2015 and 2016). The fall sites for those two years were omitted as they were not surveyed during the peak SAV biomass season.



APPENDIX D-3. The change in quadrat percent cover for *Vallisneria americana* through the monitoring years at each sentinel site (AS) 2015-2019; PRE 2016, 2017 and 2019; and NRE 2017-2019). Six sentinel sites omitted that never contained SAV for any of the surveyed years (AS-05, 09) and (NR-01,04,08,10). Only spring and summer quadrat percent cover points are shown (AS 2015 and 2016). The fall sites for those two years were omitted as they were not surveyed during the peak SAV biomass season.



APPENDIX D-4. The change in quadrat percent cover for *Zannichellia palustris* through the monitoring years at each sentinel site (AS) 2015-2019; PRE 2016, 2017 and 2019; and NRE 2017-2019). Six sentinel sites omitted that never contained SAV for any of the surveyed years (AS-05, 09) and (NR-01,04,08,10). Only spring and summer quadrat percent cover points are shown (AS 2015 and 2016). The fall sites for those two years were omitted as they were not surveyed during the peak SAV biomass season.

APPENDIX E: ANOVA STATISTICAL OUTPUT TABLES FOR COVER, FREQUENCY AND BIOMASS VS. WATER DEPTH

Analysis of variance for water depth tested as a factor against SAV measurements of cover, frequency and biomass. The output tables are separated by the species analyzed. An arcsine square-root transformation was performed on cover and frequency measurements (proportions). While a log10 transformation was used for biomass data to improve assumptions of ANOVA tests. Table abbreviations: DF= degrees of freedom, F=f-statistic, P=P-value and ω^2 = omega squared. 52 SAV positive sentinel sites were used for this analysis that have four measured water depths each (0.25, 0.5, 0.75 and 1m) (N=52 surveys * 4 water depths= 208 observations). Some shallow water depths were not allowed to be surveyed due to bulk heads and docks resulting in NA's.

APPENDIX E-1. Output of analysis of variance for water depth tested as a factor against SAV measurements of cover, frequency and biomass with all SAV species data found from this study. DF= degrees of freedom, F= the f-statistic, P=P-value and effect size measured by omega squared (ω^2).

Combined SAV Species	Variable	DF	F	P	ω^2
Arcsine Sqrt Cover	Water Depth	3	8.166	**<0.001**	0.10
	Residual	185			
Arcsine Sqrt Frequency	Water Depth	3	10.294	**<0.001**	0.13
	Residual	181			
Log10 Dry Biomass	Water Depth	3	3.675	**0.013**	0.04
	Residual	176			

APPENDIX E-2. Output of analysis of variance for water depth tested as a factor against SAV measurements of cover, frequency and biomass for *R. maritima*. DF= degrees of freedom, F= the f-statistic, P=P-value and effect size measured by omega squared (ω^2).

Ruppia maritima	Variable	DF	F	P	ω^2
Arcsine Sqrt Cover	Water Depth	3	1.195	0.313	0.003
	Residual	185			
Arcsine Sqrt Frequency	Water Depth	3	1.935	0.1255	0.01
	Residual	181			
Log10 Dry Biomass	Water Depth	3	0.566	0.6375	-0.0072
	Residual	176			

APPENDIX E-3. Output of analysis of variance for water depth tested as a factor against SAV measurements of cover, frequency and biomass for *V. americana*. DF= degrees of freedom, F= the f-statistic, P=P-value and effect size measured by omega squared (ω^2).

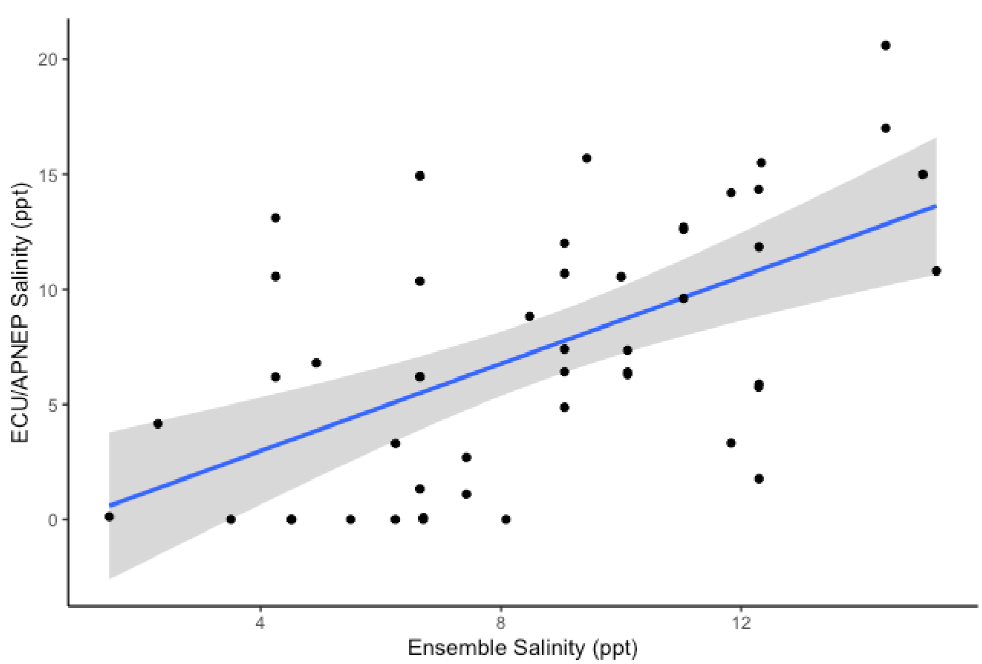
Vallisneria americana	Variable	DF	F	P	ω^2
Arcsine Sqrt Cover	Water Depth	3	2.71	**0.0465**	0.03
	Residual	185			
Arcsine Sqrt Frequency	Water Depth	3	2.724	**0.0457**	0.03
	Residual	181			
Log10 Dry Biomass	Water Depth	3	2.322	0.07679	0.02
	Residual	176			

APPENDIX E-4. Output of analysis of variance for water depth tested as a factor against SAV measurements of cover, frequency and biomass for *Z. palustris*. DF= degrees of freedom, F= the f-statistic, P=P-value and effect size measured by omega squared (ω^2).

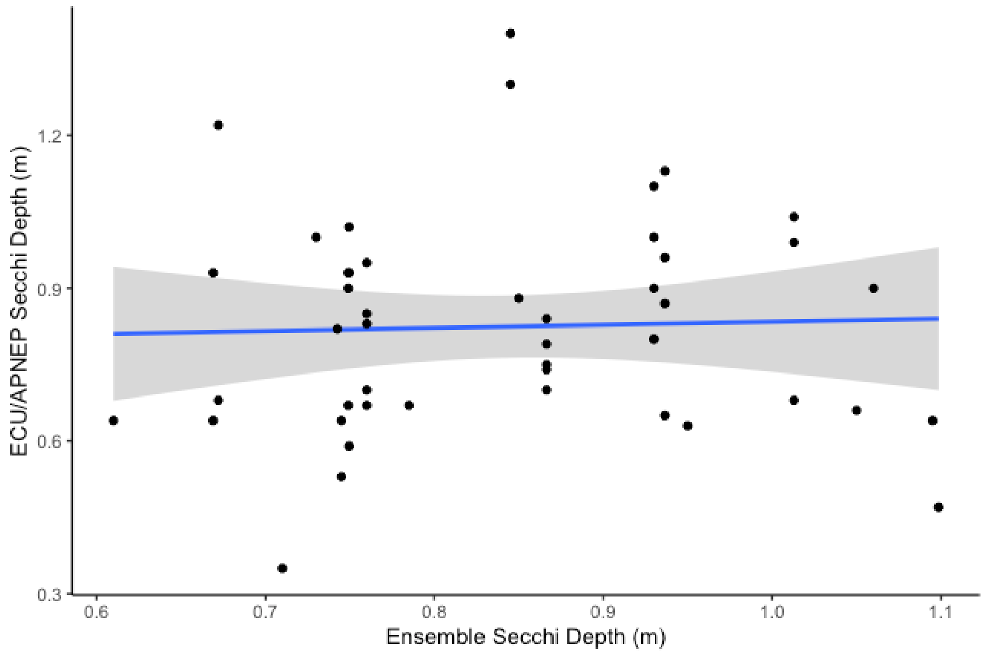
Zannichellia palustris	Variable	DF	F	P	ω^2
Arcsine Sqrt Cover	Water Depth	3	0.762	0.5163	-0.0037
	Residual	185			
Arcsine Sqrt Frequency	Water Depth	3	1.965	0.1208	0.02
	Residual	181			
Log10 Dry Biomass	Water Depth	3	1.347	0.2605	0.0057
	Residual	176			

APPENDIX F: CORRELATION OF ENSEMBLE WATER QUALITY AND ECU/APNEP MEASUREMENTS

A Pearson correlation coefficient (r) was determined for the ensemble water quality data set and ECU/APNEP point measurements. The yearly average ensemble data for salinity was positively correlated with a correlation coefficient value of 0.577 with the ECU/APNEP salinity measurements. The correlation coefficient value for Secchi depth between the two water quality data sets showed no correlation with a R value of 0.035. This is likely due to the fact that the ensemble Secchi depth measurements come from water quality stations that are in the middle of the river while sentinel sites in this study are located near the shore. Only sites comparable were in the PRE and NRE (N=48) since ensemble water quality information was not available in the AS.



APPENDIX F-1. The correlation of the ensemble salinity measurements and ECU/APNEP salinity point measurements are positively associated with one another (r= 0.577).



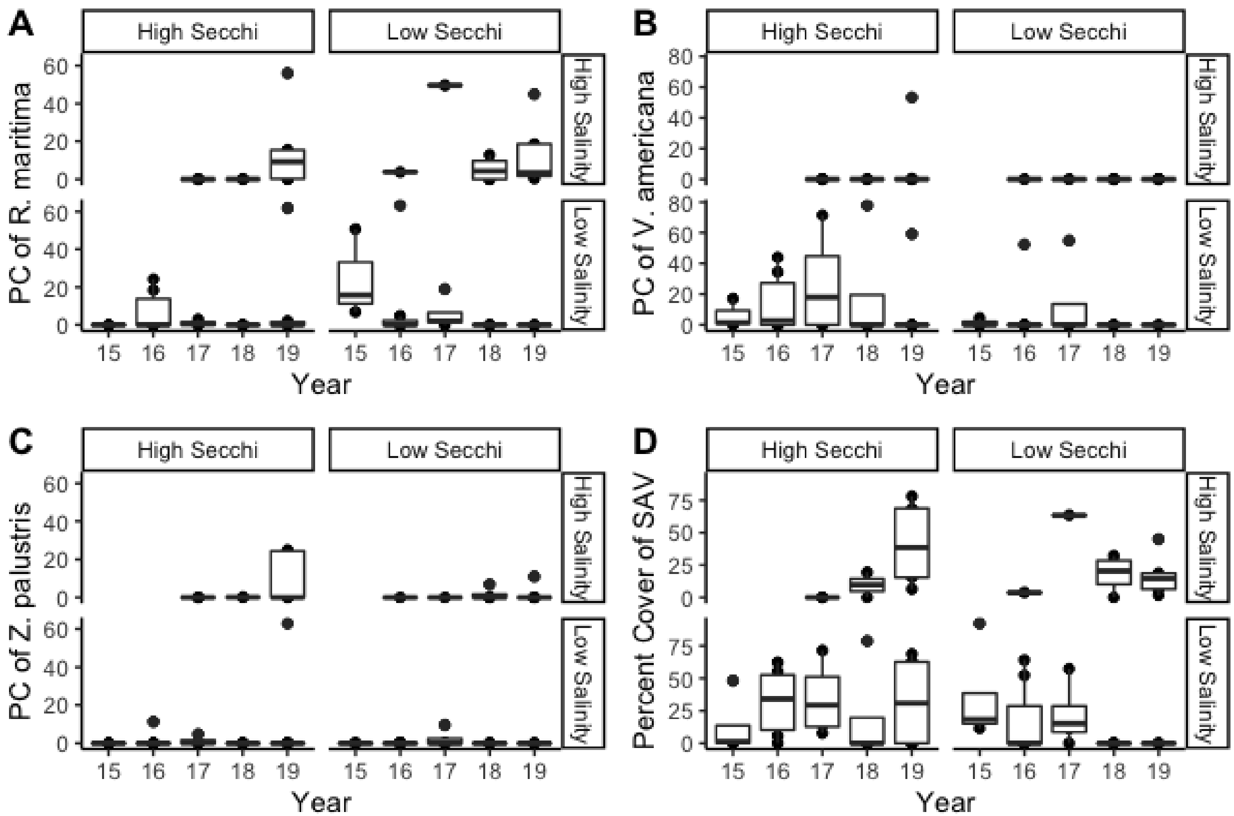
APPENDIX F-2. The correlation between the ensemble Secchi depth and ECU/APNEP Secchi depth point measurements r=0.0035.

APPENDIX F-3. Secchi depth and salinity data from PRE and NRE 2016-2019 sentinel site surveys used to create correlation plots between ECU/APNEP data and the ensemble data set.

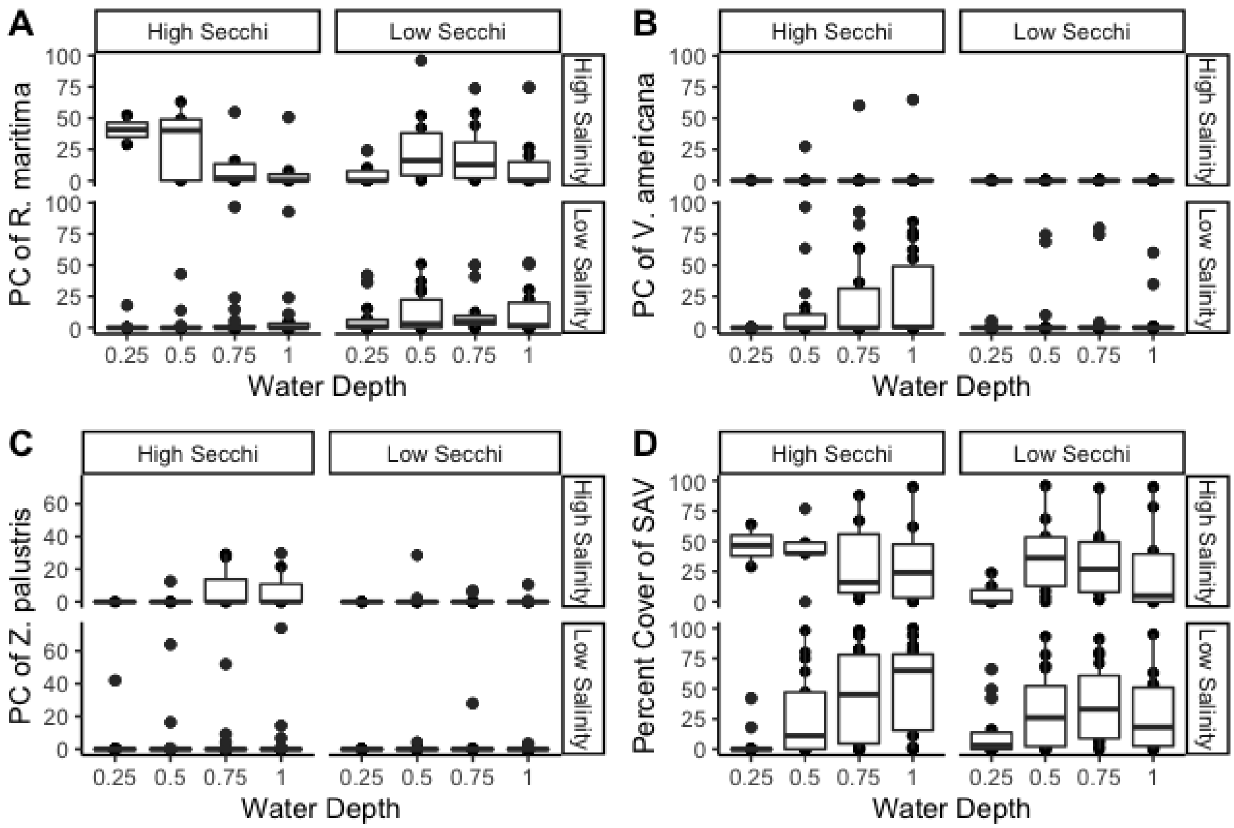
Sentinel Site (SS)	Year	ECU/APNEP Salinity (ppt)	Ensemble Salinity (ppt)	ECU/APNEP Secchi Depth (m)	Ensemble Secchi Depth (m)
NR_SS_01	2019	14.19	11.83	0.74	0.8665
NR_SS_02	2019	10.36	6.65	0.59	0.7495
NR_SS_03	2019	7.4	9.06	0.79	0.8665
NR_SS_04	2019	3.32	11.83	0.7	0.8665
NR_SS_05	2019	6.2	6.65	0.84	0.8665
NR_SS_06	2019	0.12	1.48	1.02	0.7495
NR_SS_07	2019	1.33	6.65	0.75	0.8665
NR_SS_08	2019	6.19	4.245	0.93	0.7495
NR_SS_09	2019	10.69	9.06	0.68	0.672
NR_SS_10	2019	14.99	15.025	1.22	0.672
PR_SS_01	2019	4.87	9.06	0.93	0.749
PR_SS_02	2019	10.56	4.245	0.67	0.749
PR_SS_03	2019	12	9.06	0.64	0.61
PR_SS_04	2019	14.93	6.65	0.93	0.669
PR_SS_05	2019	6.41	9.06	0.9	0.749

PR_SS_06	2019	13.1	4.245	0.64	0.669
NR_SS_01	2018	14.34	12.29	0.64	1.095
NR_SS_02	2018	5.75	12.29	0.65	0.9365
NR_SS_03	2018	7.35	10.105	0.47	1.0985
NR_SS_04	2018	10.8	15.25	1.13	0.9365
NR_SS_05	2018	6.3	10.105	0.87	0.9365
NR_SS_06	2018	6.4	10.105	0.99	1.013
NR_SS_07	2018	1.77	12.295	0.96	0.9365
NR_SS_08	2018	8.82	8.475	1.04	1.013
NR_SS_09	2018	5.89	12.295	0.68	1.013
NR_SS_10	2018	11.84	12.295	0.82	0.7425
NR_SS_01	2017	17.01	14.405	0.8	0.93
NR_SS_02	2017	12.7	11.04	0.8	0.93
NR_SS_03	2017	9.6	11.04	1.1	0.93
NR_SS_04	2017	15.5	12.335	0.9	0.93
NR_SS_05	2017	6.8	4.925	1	0.93
NR_SS_06	2017	1.1	7.425	1.4	0.845
NR_SS_07	2017	2.7	7.425	1.3	0.845
NR_SS_08	2017	12.6	11.04	0.9	1.06
NR_SS_09	2017	15.7	9.43	1	0.73
NR_SS_10	2017	20.6	14.405	0.8	0.93
PR_SS_01	2017	0	4.51	0.7	0.76
PR_SS_02	2017	3.3	6.2435	0.95	0.76
PR_SS_03	2017	12	14.3	0.85	0.76
PR_SS_04	2017	0	8.0834	0.67	0.76
PR_SS_05	2017	0	4.51	0.83	0.76
PR_SS_06	2017	0	6.24	0.66	1.05
PR_SS_01	2016	0	6.705	0.88	0.85
PR_SS_02	2016	4.16	2.29	0.35	0.71
PR_SS_03	2016	10.55	10	0.53	0.745
PR_SS_04	2016	0	5.495	0.67	0.785
PR_SS_05	2016	0.08	6.705	0.63	0.95
PR_SS_06	2016	0	3.505	0.64	0.745

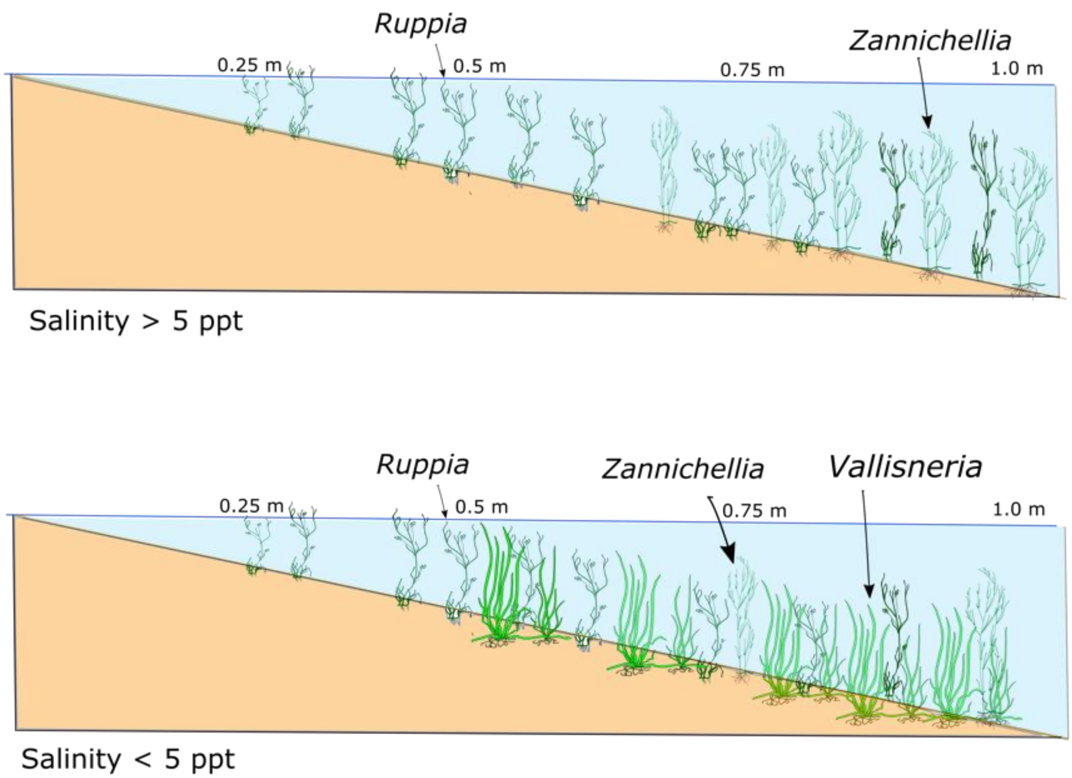
APPENDIX G: DOMINANT SAV SPECIES PERCENT COVER INTERACTION WITH SALINITY AND SECCHI DEPTH



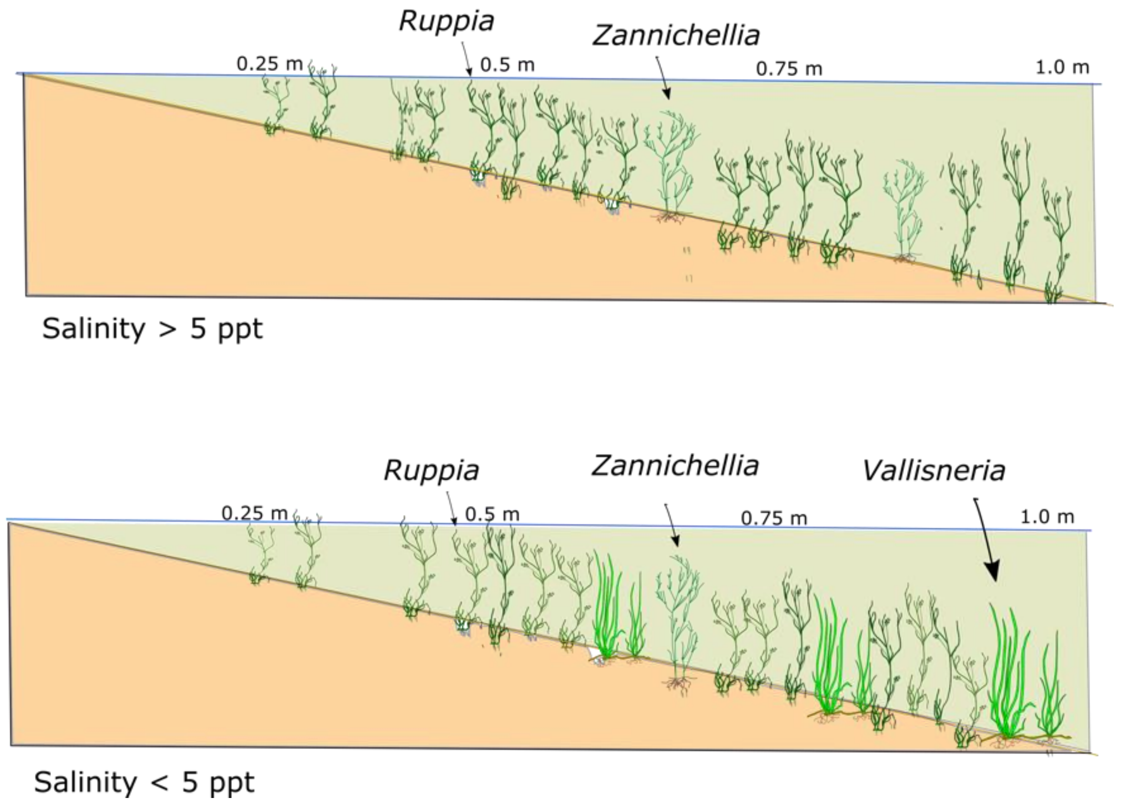
APPENDIX G-1. The percent cover (PC) for dominant SAV species (A) *Ruppia maritima*, (B) *Vallisneria americana*, (C) *Zannichellia palustris* and (D) all SAV species combined, separated by high salinity (>5 ppt), low salinity (<5 ppt) salinity, high Secchi depth (>0.77 m) and low Secchi depth (<0.77 m). Sentinel site survey year is on the x-axis. The threshold values were selected as they are the median values for all observed sentinel survey salinities and Secchi depths.



APPENDIX G-2. The percent cover (PC) for dominant SAV species (A) *Ruppia maritima,* (B) *Vallisneria americana,* (C) *Zannichellia palustris* and (D) all SAV species combined, separated by high salinity (>5 ppt), low salinity (<5 ppt) salinity, high Secchi depth (>0.77 m) and low Secchi depth (<0.77 m). The measured sentinel site water depths (0.25, 0.50, 0.75 and 1.0) are on the x-axis. The threshold values were selected as they are the median values for all observed sentinel survey salinities and Secchi depths.



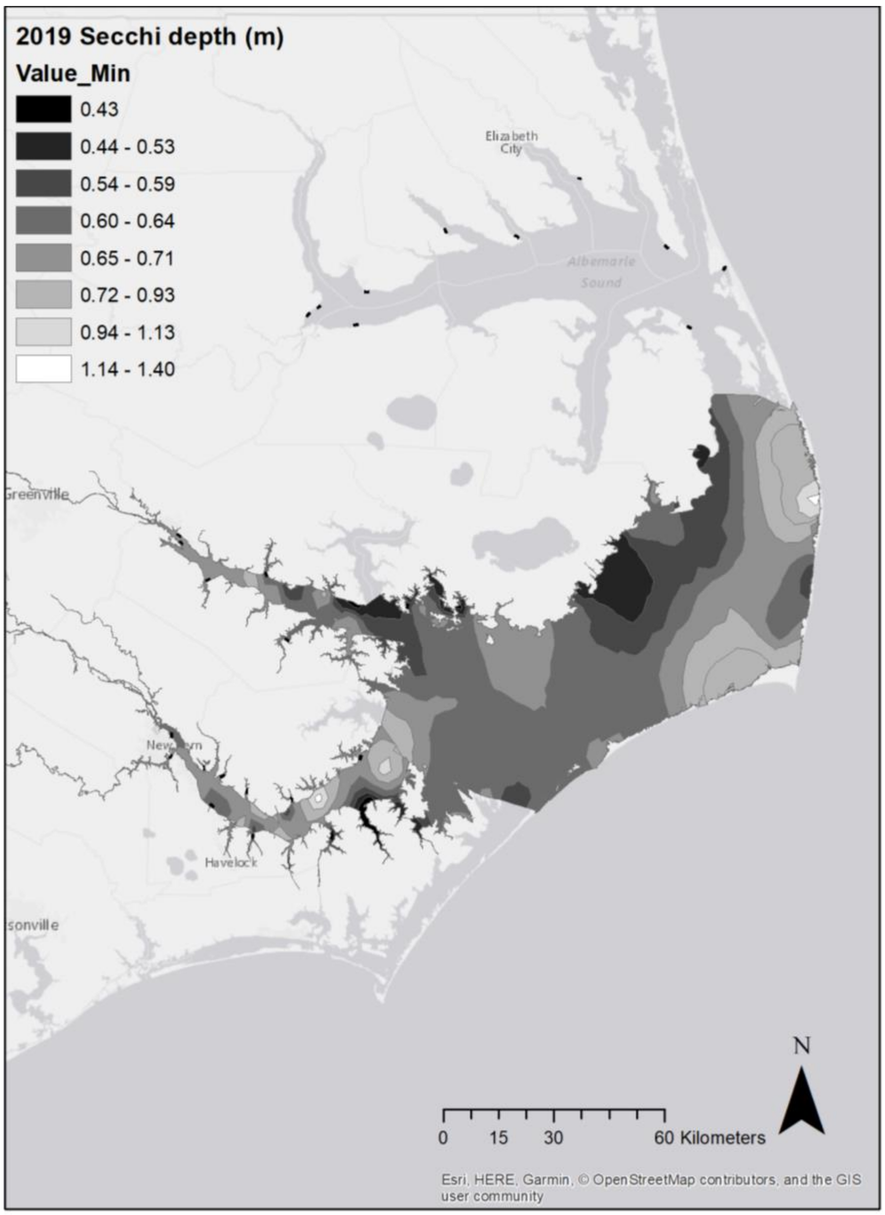
APPENDIX G-3. High Secchi depth (>0.77 m) visual representation of sentinel sites under high and low salinity conditions focusing on three dominant SAV species *Ruppia maritima, Vallisneria americana and Zannichellia palustris* distribution with respect to water depth.



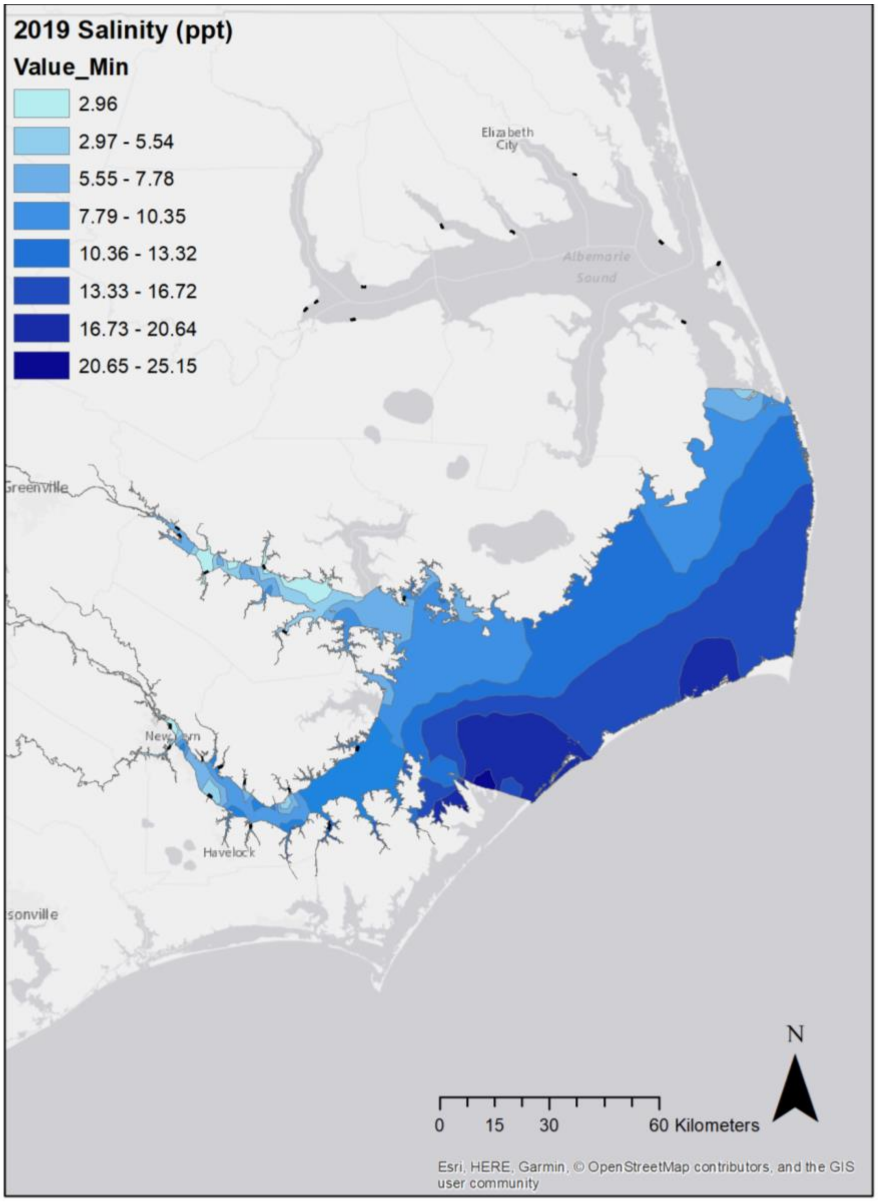
APPENDIX G-4. Low Secchi depth (<0.77 m) visual representation of sentinel sites under high and low salinity conditions focusing on three dominant SAV species *Ruppia maritima, Vallisneria americana and Zannichellia palustris* distribution with respect to water depth.

APPENDIX H: INVERSE DISTANCE WEIGHTED INTERPOLATION MAPS FOR SALINITY AND SECCHI DEPTH

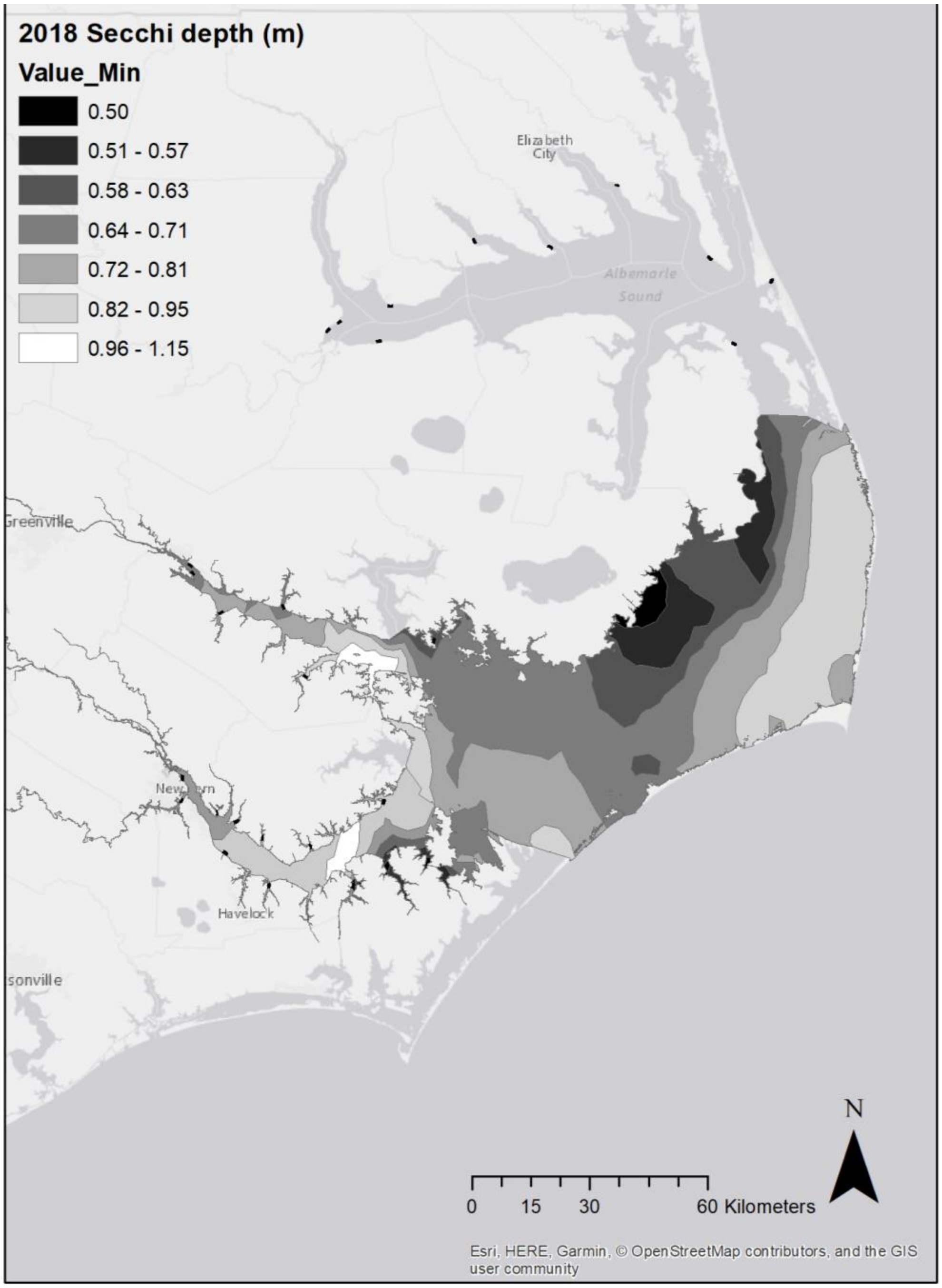
Inverse distance weighted interpolation map created in ArcMap 10.5.1 using water quality measurements collected from the ensemble data set for PRE and NRE sentinel site survey years (2016-2019).



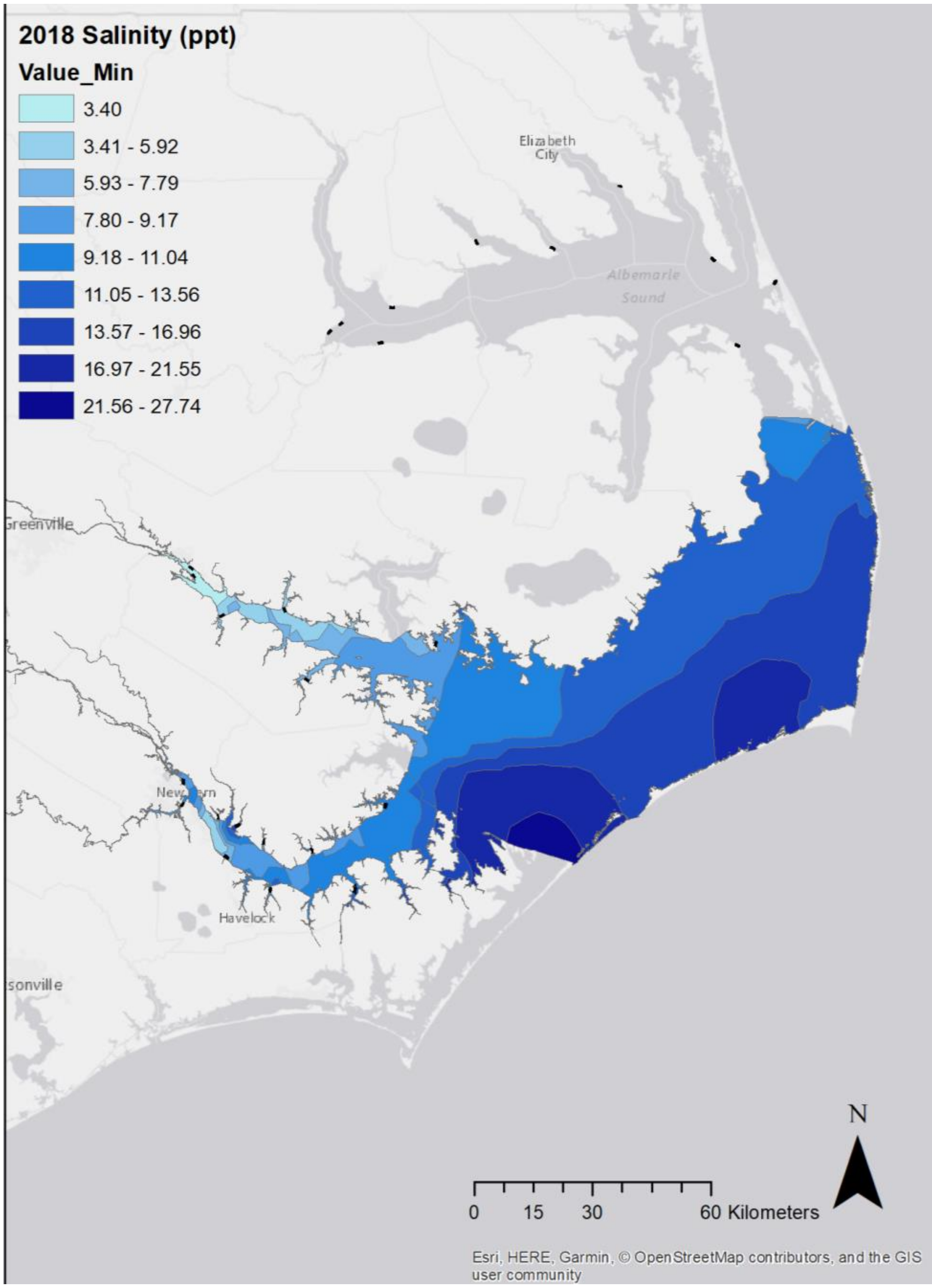
APPENDIX H-1. 2019 Inverse distance weighted interpolation prediction surface for Secchi depth (m) using the ensemble water quality data set.



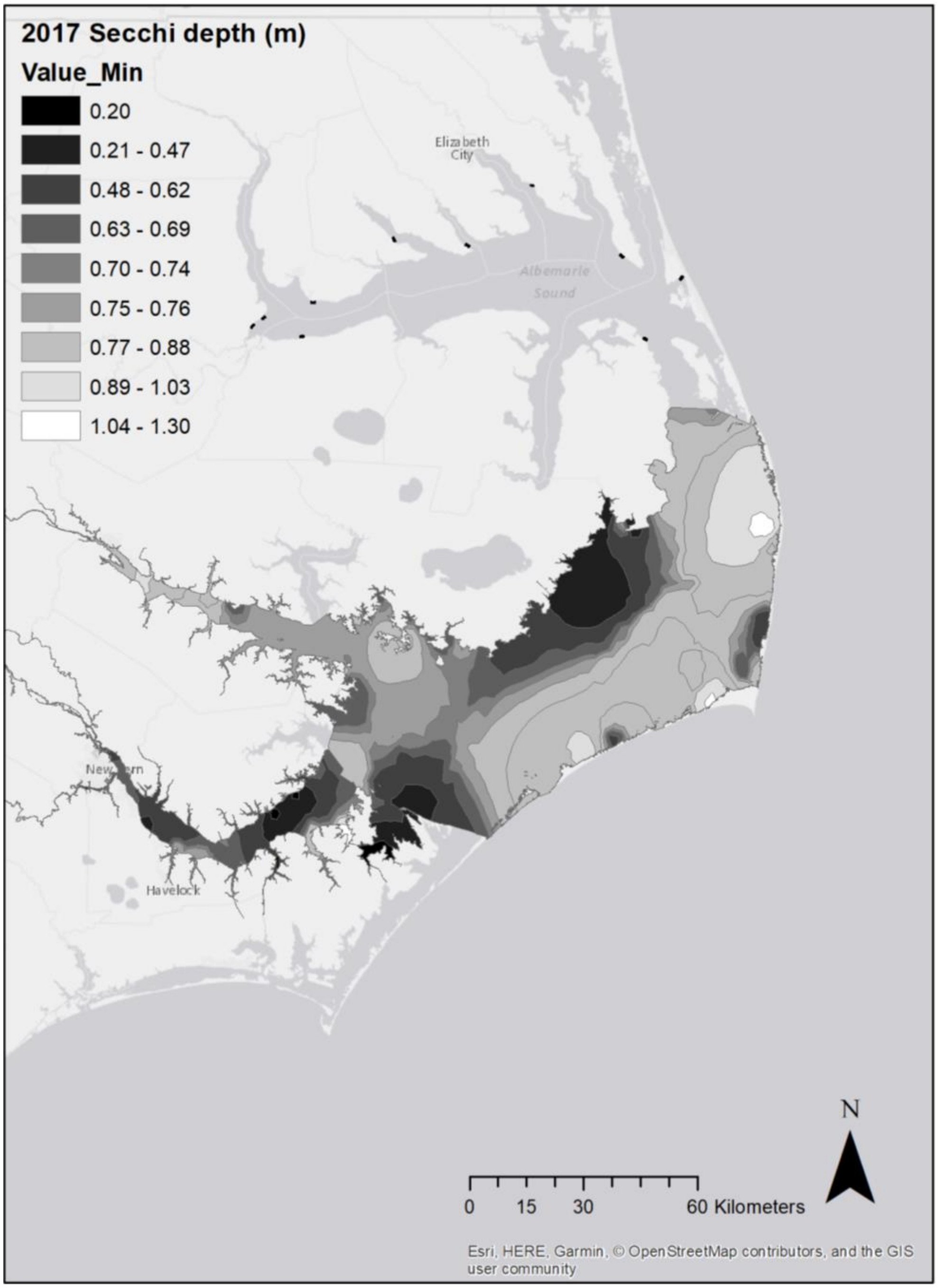
APPENDIX H-2. 2019 Inverse distance weighted interpolation prediction surface for salinity (ppt) using the ensemble water quality data set.



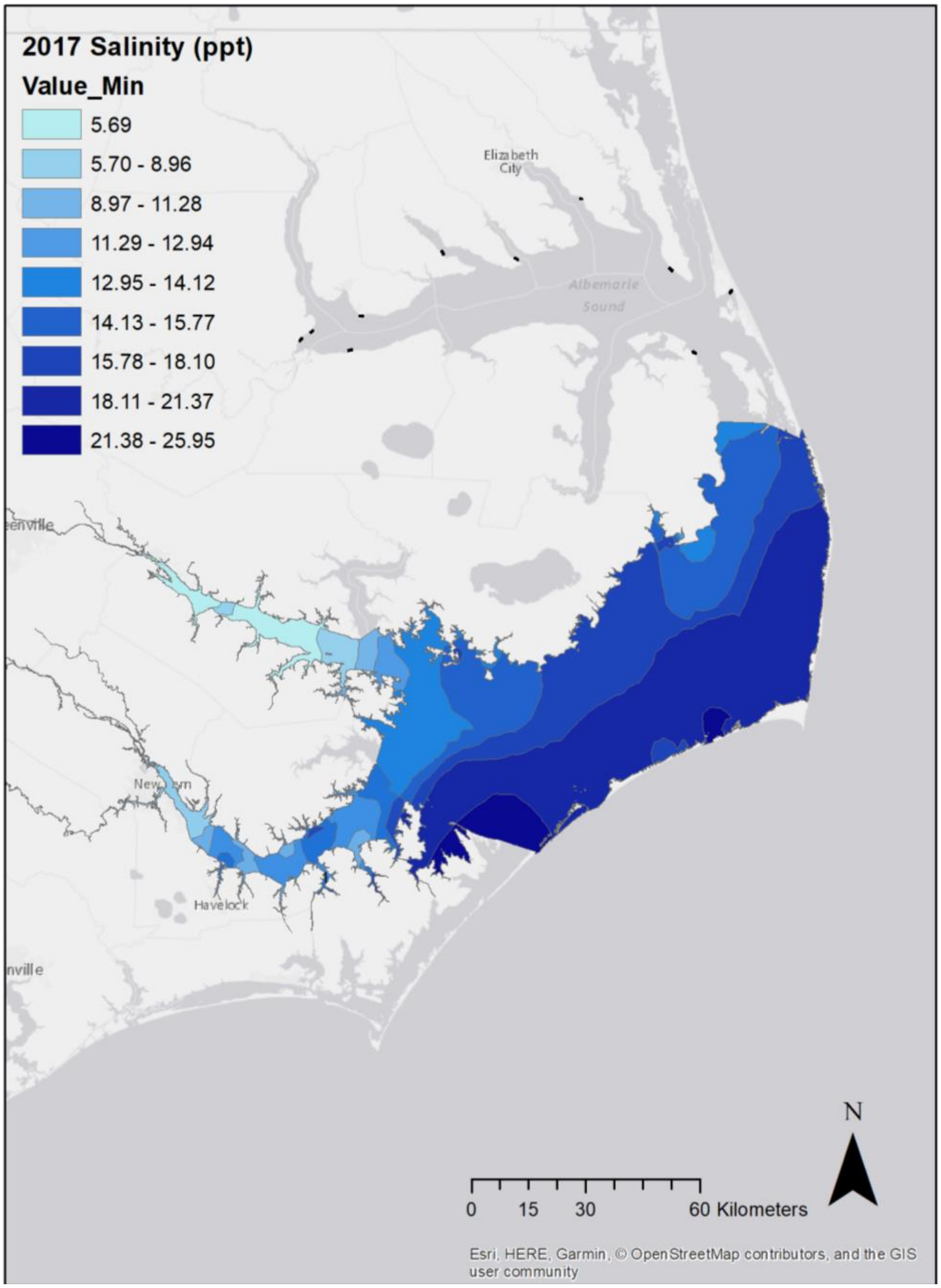
APPENDIX H-3. 2018 Inverse distance weighted interpolation prediction surface for Secchi depth (m) using the ensemble water quality data set.



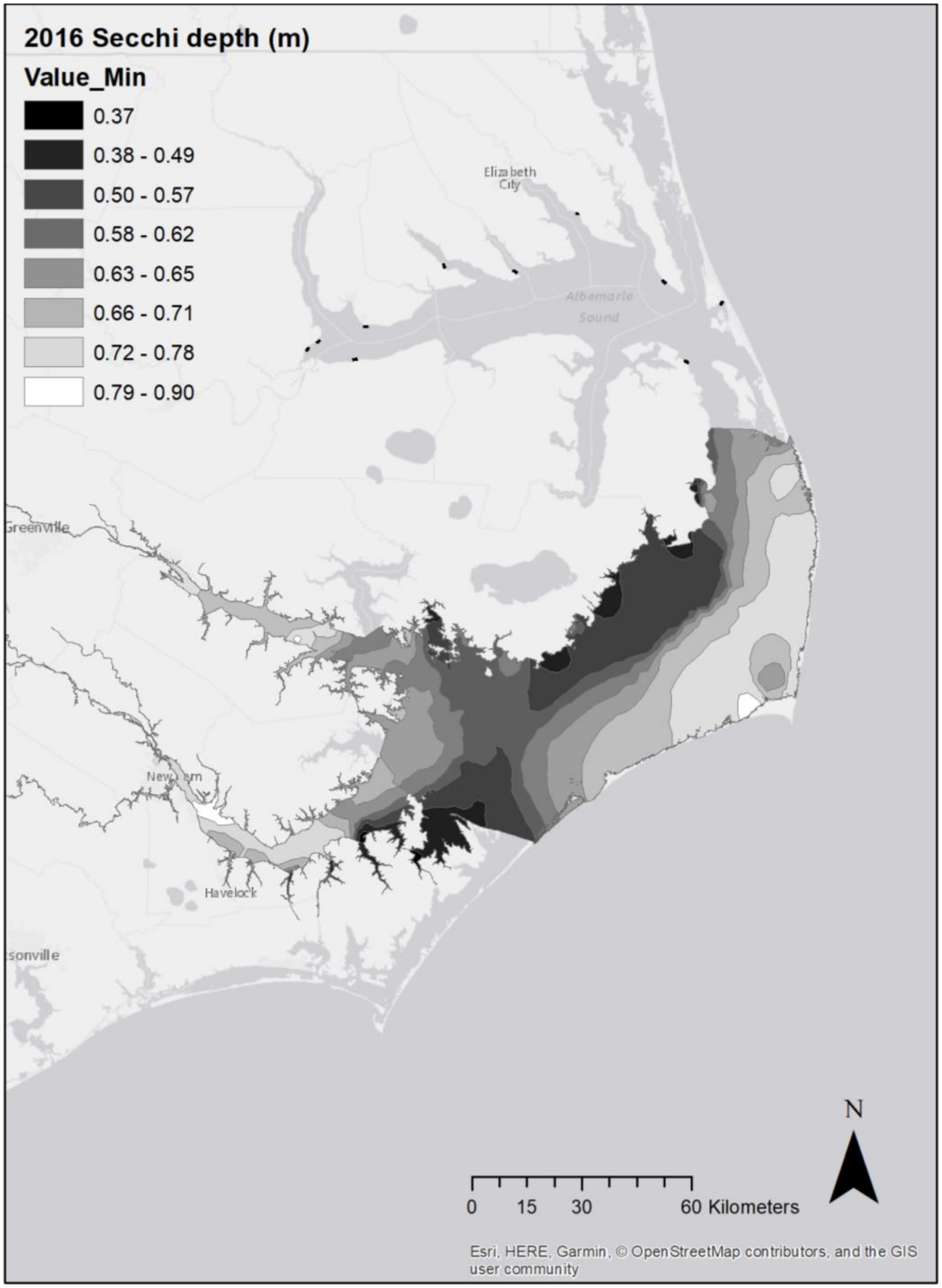
APPENDIX H-4. 2018 Inverse distance weighted interpolation prediction surface for salinity (ppt) using the ensemble water quality data set.



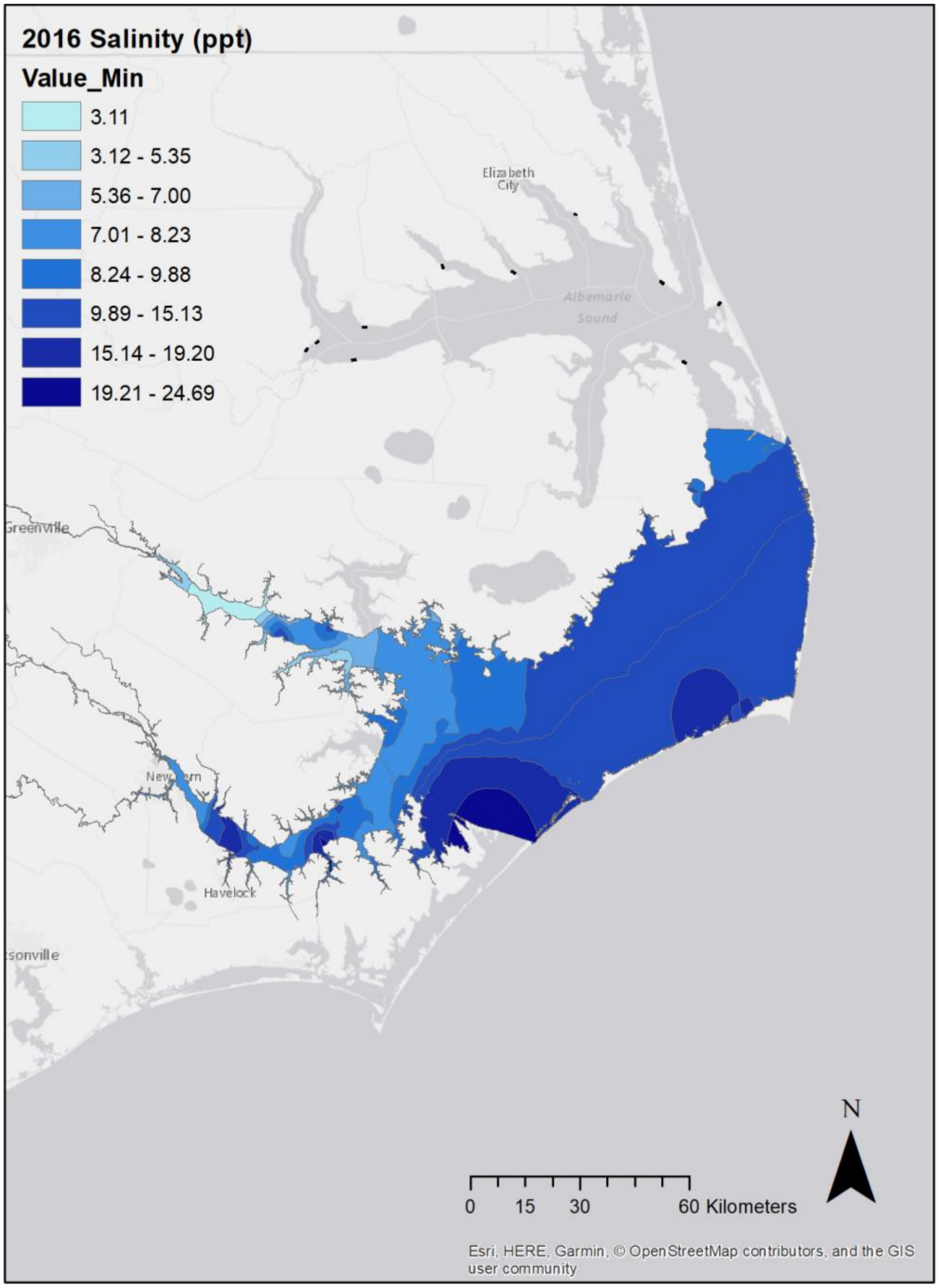
APPENDIX H-5. 2017 Inverse distance weighted interpolation prediction surface for Secchi depth (m) using the ensemble water quality data set.



APPENDIX H-6. 2017 Inverse distance weighted interpolation prediction surface for salinity (ppt) using the ensemble water quality data set.



APPENDIX H-7. 2016 Inverse distance weighted interpolation prediction surface for Secchi depth (m) using the ensemble water quality data set.



APPENDIX H-8. 2016 Inverse distance weighted interpolation prediction surface for salinity (ppt) using the ensemble water quality data set.