Exploring differences in ecological functions between Nominated SHA (Strategic Habitat Areas) and non-SHA marsh habitat in western Pamlico Sound where marsh inundation is meteorologically driven

Final report to the North Carolina Division of Marine Fisheries for research funded by the North Carolina Marine Resources Fund (CRFL)

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# Table of Contents

List of Tables ......................................................................................................................... iii
List of Photographs .................................................................................................................. iii
Table of Figures ....................................................................................................................... iv
Summary .................................................................................................................................. 1
Introduction ............................................................................................................................ 2
Methods .................................................................................................................................... 5
  Selection of study sites ......................................................................................................... 5
  Study site layout .................................................................................................................... 7
  Marsh elevation mapping ....................................................................................................... 7
  Water-level monitoring .......................................................................................................... 8
  Marsh inundation computations ............................................................................................. 9
  Vegetation sampling ............................................................................................................. 10
  Faunal sampling ................................................................................................................... 10
  Data and statistical analyses ................................................................................................. 11
Results ..................................................................................................................................... 12
  Marsh elevations .................................................................................................................. 12
  Water-level records ............................................................................................................. 9
  Role of marsh surface topography and consequent inundation depth and duration .......... 10
  Vegetation .......................................................................................................................... 11
  Fish and other nekton use of marsh sites ......................................................................... 15
    Minnow pot sampling ........................................................................................................ 15
      Univariate analyses .......................................................................................................... 15
      Permanent versus temporary residents ................................................................. 18
      Multivariate analyses ..................................................................................................... 24
    Pit trap sampling .............................................................................................................. 30
      Univariate analyses ......................................................................................................... 30
      Multivariate analyses ..................................................................................................... 32
    Bottomless liftnet sampling .............................................................................................. 38
Discussion ............................................................................................................................... 39
References ............................................................................................................................... 39
Appendix I ............................................................................................................................... 47
Appendix II .............................................................................................................................. 48
List of Tables

Table 1. The 10-month mean percent of time that sampling stations were flooded in Yr 1 strata: Otter Creek (stratum 02), Berry’s Bay (03), Far Creek (04), and Middleton Creek (05) between September 2013 and July 2014, and in Yr 2 strata: North Wysocking Bay (12), Mt. Pleasant (14), Lone Tree Creek (18), and East Bluff (19) between September 2014 and July 2015. All strata were located in western Pamlico Sound, North Carolina, with one altered (A) and one unaltered control (C) site in each stratum. Each site had 3 transects (A, B, and C) with sampling stations at 4 distances from estuarine edge (1, 10, 25, and 50 m). ............................................................................................................................................... 11

Table 2. List of fish species considered to be permanent vs temporary residents of the marshes in western Pamlico Sound, North Carolina ........................................................................................................................................ 19

List of Photographs

Photo 1. Representative photograph showing the estuarine edge of marsh sites in western Pamlico Sound that are impacted by frequent waves and therefore have edges dominated by Spartina alterniflora .......................................................................................................................................... 12

Photo 2. Representative photograph showing the estuarine edge of marsh sites in western Pamlico Sound that are dominated by Juncus roemerianus .......................................................................................................................................... 12
Table of Figures

Figure 1. Map of the Pamlico Sound, North Carolina (Region 2) showing nominated SHAs outlined in red. The dashed blue rectangle shows the actual study area that includes an abundance of both nominated SHA and non-SHA marsh habitat.......................................................... 3

Figure 2. Map of western Pamlico Sound, North Carolina, showing the 8 strata in Region 2 within which the nekton use of marsh habitat was assessed. Each stratum had one highly altered marsh site paired with one minimally altered marsh site, and a water-level station operating throughout the year when that stratum was repeatedly sampled.......................................................... 6

Figure 3. Photo of a water-level station in western Pamlico Sound (left) and an illustration of the way in which the pressure transducers are mounted within each station (right). .................................................. 9

Figure 4. Mean elevation of the marsh surface, along 50-m long transects from the estuarine edge to marsh interior, for 8 altered and 8 unaltered study sites in western Pamlico Sound, North Carolina between July 2013 and June 2015.......................................................... 13

Figure 5. Box plot showing the mean and variability of the elevations of all altered and unaltered sites along 50-m-long transects from estuarine edge to marsh interior at study sites in western Pamlico Sound, North Carolina in 2013-2015. Asterisks denote mild outliers and circles denote extreme outliers; these outliers are due to a relatively steep slope at the upland transition at the altered site in Far Creek. .................................................................................................................. 14

Figure 6. Digital elevation models (DEMs) of the altered (left) unaltered control (center) sites in the 8 stratum with a graph (right) showing the mean elevation (± standard error) of triplicate transects extending from the estuarine edge to 50 m into the marsh interior. Yellow diamonds indicate the location of sampling stations along triplicate transects at each site. Individual DEMs can be found in Appendix II. ........................................................................................................ 8

Figure 7. Water levels for Otter Creek, Berry’s Bay, Far Creek, and Middleton Creeks in western Pamlico Sound, North Carolina from September 2013 through August 2014.................................................. 9

Figure 8. Water levels for North Wysocking Bay, Mount Pleasant Bay, Lone Tree Creek, and East Bluff in western Pamlico Sound, North Carolina from September 2014 through July 2015. .............................. 9

Figure 9. Total biomass of all species of vegetation from 8 altered and 8 unaltered marsh sites (left) and at 1 m, 10 m, 25 m, and 50 m from estuarine edge (right) in western Pamlico Sound, North Carolina in 2014 and 2015. Levels not sharing the same letter are significantly different. ........................................................................ 13

Figure 10. Total biomass of Juncus roemerianus from 8 altered and 8 unaltered marsh sites (left) and at 1 m, 10 m, 25 m, and 50 m from estuarine edge (right) in western Pamlico Sound, North Carolina in 2014 and 2015. Levels not sharing the same letter are significantly different. Letters are color-coded according to the categories shown in the figure legend; letters are not shown for category levels that did not differ significantly. ........................................................................ 14

Figure 11. Shoot density of all species of vegetation from 8 altered and 8 unaltered marsh sites at 1 m, 10 m, 25 m, and 50 m from estuarine edge in western Pamlico Sound, North Carolina in 2014 and 2015. Levels not sharing the same letter are significantly different. ........................................................................ 14
**Figure 12.** The mean abundance of organisms sampled with minnow pots at altered and unaltered sites (left) and at 1 m, 10 m, 25 m, and 50 m from estuarine edge (right) at marsh sites from September 2013 to June 2015 in western Pamlico Sound, North Carolina. Levels not sharing the same letter are significantly different. Letters are color-coded according to the categories shown in the figure legend; letters are not shown for category levels that did not differ significantly.

**Figure 13.** The means of the total length (TL) of organisms sampled with minnow pots at altered and unaltered sites (left) and at 1 m, 10 m, 25 m, and 50 m from estuarine edge (right) at marsh sites from September 2013 to June 2015 in western Pamlico Sound, North Carolina. Levels not sharing the same letter are significantly different. Letters are color-coded according to the categories shown in the figure legend; letters are not shown for category levels that did not differ significantly.

**Figure 14.** The mean biomass of organisms sampled with minnow pots at altered and unaltered sites (left) and at 1 m, 10 m, 25 m, and 50 m from estuarine edge (right) at marsh sites from September 2013 to June 2015 in western Pamlico Sound, North Carolina. Levels not sharing the same letter are significantly different. Letters are color-coded according to the categories shown in the figure legend; letters are not shown for category levels that did not differ significantly.

**Figure 15.** Summed fish abundance of permanent and temporary fishes as defined in Table 2. Total numbers of fishes are computed for all sampling periods by adding catches from minnow pots across the 4 distances into the marsh for each of the 3 transects.

**Figure 16.** Summed fish biomass of permanent and temporary fishes as defined in Table 2. Total mass of fishes are computed for all sampling periods by adding catches from minnow pots across the 4 distances into the marsh for each of the 3 transects.

**Figure 17.** GLM of the relationship between fish abundance in minnow pots and marsh inundation depth in western Pamlico Sound, North Carolina, from fall 2013 through spring 2015.

**Figure 18.** GLM of the relationship between the total length of fishes in minnow pots and marsh inundation depth in western Pamlico Sound, North Carolina, from fall 2013 through spring 2015.

**Figure 19.** GLM of the relationship between fish biomass in minnow pots and marsh inundation depth in western Pamlico Sound, North Carolina, from fall 2013 through spring 2015.

**Figure 20.** GLM of the relationship between fish abundance in minnow pots and duration of marsh inundation in western Pamlico Sound, North Carolina, from fall 2013 through spring 2015.

**Figure 21.** GLM of the relationship between the total length of fishes in minnow pots and duration of marsh inundation in western Pamlico Sound, North Carolina, from fall 2013 through spring 2015.

**Figure 22.** GLM of the relationship between fish biomass in minnow pots and duration of marsh inundation in western Pamlico Sound, North Carolina, from fall 2013 through spring 2015.

**Figure 23.** Non-metric Multi-Dimensional Scaling (nMDS) ordination of the faunal communities sampled at 8 altered and 8 unaltered marsh habitats in western Pamlico Sound, North Carolina, based upon the abundance of each species sampled with minnow pots in 2013 – 2015.

**Figure 24.** Non-metric Multi-Dimensional Scaling (nMDS) ordination of the faunal communities sampled at 1, 10, 25, and 50 m from the estuarine edge in marsh habitats in western Pamlico Sound, North Carolina, based upon the abundance of each species sampled with minnow pots in 2013 – 2015.
Figure 25. Non-metric Multi-Dimensional Scaling (nMDS) ordination of faunal communities sampled in fall and spring in western Pamlico Sound, North Carolina, based upon the abundance of each species sampled with minnow pots in 2013 – 2015.

Figure 26. Non-metric Multi-Dimensional Scaling (nMDS) ordination of faunal communities showing similarities among the communities in 13 marshes sites in areas nominated as SHAs and 3 non-SHA in western Pamlico Sound, North Carolina, based upon the abundance of each species sampled with minnow pots in 2013 – 2015.

Figure 27. Non-metric Multi-Dimensional Scaling (nMDS) ordination of fish communities showing similarity profiles linked by green spheres for the communities in 8 altered and 8 unaltered marsh habitats in western Pamlico Sound, based upon the fish abundance in minnow pots in 2013 – 2015. Labels note whether samples represent nominated SHA or non-SHA marshes.

Figure 29. Non-metric Multi-Dimensional Scaling (nMDS) ordination of the faunal communities sampled at 8 altered and 8 unaltered marsh habitats in western Pamlico Sound, North Carolina, based upon the biomass of each species sampled with minnow pots in 2013 – 2015.

Figure 30. nMDS ordination of the faunal communities sampled at 1, 10, 25, and 50 m from the estuarine edge in marsh habitats in western Pamlico Sound, North Carolina, based upon the biomass of each species sampled with minnow pots in 2013 - 2015.

Figure 31. nMDS ordination of fish communities showing similarity profiles linked by green spheres for fish biomass in pit traps in 8 altered and 8 unaltered marsh habitats in western Pamlico Sound, in 2013 – 2015. Levels not sharing the same letter are significantly different. Letters are color-coded according to the categories shown in the figure legend; letters are not shown for category levels that did not differ significantly.

Figure 32. The mean abundance of organisms sampled with pit traps at altered and unaltered sites (left) and at 1 m, 10 m, 25 m, and 50 m from estuarine edge (right) at marsh sites from September 2013 to June 2015 in western Pamlico Sound, North Carolina. Levels not sharing the same letter are significantly different. Letters are color-coded according to the categories shown in the figure legend; letters are not shown for category levels that did not differ significantly.

Figure 33. The mean total length of organisms sampled with pit traps at altered and unaltered sites (left) and at 1 m, 10 m, 25 m, and 50 m from estuarine edge (right) at marsh sites from September 2013 to June 2015 in western Pamlico Sound, North Carolina. Levels not sharing the same letter are significantly different. Letters are color-coded according to the categories shown in the figure legend; letters are not shown for category levels that did not differ significantly.

Figure 34. The mean biomass of organisms sampled with pit traps at altered and unaltered sites (left) and at 1 m, 10 m, 25 m, and 50 m from estuarine edge (right) at marsh sites from September 2013 to June 2015 in western Pamlico Sound, North Carolina. Levels not sharing the same letter are significantly different. Letters are color-coded according to the categories shown in the figure legend; letters are not shown for category levels that did not differ significantly.

Figure 35. Non-metric Multi-Dimensional Scaling (nMDS) ordination of the faunal communities sampled at 8 altered and 8 unaltered marsh habitats in western Pamlico Sound, North Carolina, based upon the abundance of each species sampled with pit traps in 2013 – 2015.
Figure 36. nMDS ordination of the faunal communities sampled at 1, 10, 25, and 50 m from the estuarine edge in marsh habitats in western Pamlico Sound, North Carolina, based upon the abundance of each species sampled with pit traps in 2013 – 2015. ................................................................. 33

Figure 37. Non-metric Multi-Dimensional Scaling (nMDS) ordination of faunal communities sampled in fall and spring in western Pamlico Sound, North Carolina, based upon the abundance of each species sampled with pit traps in 2013 – 2015. .................................................................................................................. 33

Figure 38. Non-metric Multi-Dimensional Scaling (nMDS) ordination of faunal communities showing similarities among the communities in 13 marshes sites in areas nominated as SHAs and 3 non-SHA in western Pamlico Sound, North Carolina, based upon the abundance of each species sampled with pit traps in 2013 – 2015. .................................................................................................................. 34

Figure 39. Non-metric Multi-Dimensional Scaling (nMDS) ordination of fish communities showing similarity profiles linked by green spheres for the communities in 8 altered and 8 unaltered marsh habitats in western Pamlico Sound, North Carolina, based upon the fish abundance in pit traps in 2013 – 2015. Labels note whether samples represent nominated SHA or non-SHA marshes. ........................................... 35

Figure 40. Non-metric Multi-Dimensional Scaling (nMDS) ordination of the faunal communities sampled at 8 altered and 8 unaltered marsh habitats in western Pamlico Sound, North Carolina, based upon the biomass of each species sampled with pit traps in 2013 – 2015. .................................................................................................................. 36

Figure 41. nMDS ordination of the faunal communities sampled at 1, 10, 25, and 50 m from the estuarine edge in marsh habitats in western Pamlico Sound, North Carolina, based upon the biomass of each species sampled with pit traps in 2013 – 2015. .................................................................................................................. 36

Figure 42. Non-metric Multi-Dimensional Scaling (nMDS) ordination of faunal communities sampled in fall and spring in western Pamlico Sound, North Carolina, based upon the biomass of each species sampled with pit traps in 2013 – 2015. .................................................................................................................. 37

Figure 43. nMDS ordination of faunal communities showing similarities among the communities in 13 marshes sites in areas nominated as SHAs and 3 non-SHA in western Pamlico Sound, North Carolina, based upon the biomass of each species sampled with pit traps in 2013 - 2015. ......................................................... 37

Figure 44. nMDS ordination of fish communities showing similarity profiles linked by green spheres for the communities in 8 altered and 8 unaltered marsh habitats in western Pamlico Sound, North Carolina, based upon the fish biomass in pit traps in 2013 – 2015. Labels note whether samples represent nominated SHA or non-SHA marshes. .................................................................................................................. 38
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Summary

Sampling of Juncus roemerianus marshes on the shores of western Pamlico Sound revealed from measurements of marsh surface elevations with Real Time Kinematic GPS technology that these expansive marshes occupy broad platforms extending with relatively little elevation variation far from the estuarine shoreline. On the basis of continuous water level records from stations ground-truthed to NAVD88, the hydrography allows estimation of maximum inundation and duration of inundation at stations along parallel triplicate transects extending from 1 m, 10 m, 25 m, and to 50 m into the marsh at replicated shoreline strata, made up in each of 2 seasons (fall and spring) in each of 2 years (Fall 2013 – Spring 2015) of 8 pairs of sites, one minimally altered and the other highly altered based on analyses used to nominate SHAs (Strategic Habitat Areas). These pairs of sites are located in proximity to each other and in positions experiencing similar exposure to and fetch from prevailing winds, which dictate inundation regime along this estuarine shoreline. Aboveground production of plant biomass by Juncus roemerianus is high along the entire 50-m transect with a mean standing stock biomass of 1522 g m$^{-2}$ (1109 g m$^{-2}$ at 1 m, 1664 g m$^{-2}$ at 10 m, 1598 g m$^{-2}$ at 25 m, and 1712 g m$^{-2}$ at 50 m) in these marshes that extend even further back from the estuary edge. Deploying minnow pots and pit traps to sample fish and crustacean use quantitatively along this distance gradient from marsh edge revealed substantial use of stations well beyond the marsh edge. Fish that are targets of recreational fisheries and transients in the marsh, such as red drum (Sciaenops ocellatus), speckled trout (Cynoscion nebulosus), gray snapper (Lutjanus griseus), American eel (Anguilla rostrata), and Atlantic croaker (Micropogonias undulatus), occurred throughout the marsh, as did higher numbers of resident, prey fishes – largely multiple species of Fundulus. Abundance, total length, and biomass of fishes revealed higher use of interior distances into the marsh than at marsh edge, in stark contrast to studies of Spartina alterniflora marshes on the Gulf of Mexico coast where inundation was greater near marsh edges. ANOVA and PERMANOVA analyses revealed no significant difference between total fish abundance, lengths, and biomass between highly altered and minimally altered sites, whereas the effects of distance away from marsh edge was repeatedly significant, indicating greater use of interior habitat than at 1 m and 10 m from the estuarine edge. Fish community analyses of minnow pot and pit trap contents using non-metric Multidimensional Scaling (nMDS) resulted in showing no significant fish community change with habitat condition distinctions (SHA alteration scores in Region 2), whereas distance from marsh edge was statistically significant, showing non-random repeated community associations among different distances, as well as fish composition changes between fall and spring seasons. Our study results imply need for reconsideration of the SHA designation in the marshes of western Pamlico Sound and additionally reveal that both resident forage fishes and transient fish species, targeted in the recreational fishery, fail to show peak uses at the marsh margin, but instead penetrate deep within the marsh to 25 m and even 50 m towards the marsh interior.
Introduction

In recognition of the reliance of fish on the habitats that produce them, the North Carolina General Assembly passed the Fisheries Reform Act of 1997 with a provision requiring the Department of Environment and Natural Resources (DENR) to prepare and then implement a Coastal Habitat Protection Plan (CHPP). The legislative intent for the CHPP is long-term enhancement of coastal fish populations dependent upon explicitly identified critical fish habitats: perhaps most important among these are coastal marshes (MEA 2005). Identified as critical habitat for recreationally and commercially important species of fish and crustaceans, coastal marshes generate exceptionally high primary and secondary production as well as provide structural refuge habitat, supporting the food web that includes many recreationally important fishes, such as red drum (Daniel 1988, SAFMC 1990), speckled trout (Minello et al. 1989), two species of flounder (Burke 1995), and perhaps striped bass (Markle and Grant 1970).

Acknowledging the importance of coastal marshes for supporting production of fish and shellfish, the North Carolina Division of Marine Fisheries (NC DMF) identified and nominated Strategic Habitat Areas (SHAs) to include high-value coastal marshes, as mandated by the Coastal Habitat Protection Plan (Fig. 1). SHAs represent priority areas for protection on the basis of their exceptional condition and/or their critical ecological functions in supporting coastal fish and shellfish species. The Habitat Section of the North Carolina Division of Marine Fisheries has nominated SHAs for Regions 1, 2, and 3, and assessments are under way for Region 4 (Deaton et al. 2010). Approximately 11% of the Pamlico Sound region (Region 2) has been nominated as SHAs (Fig. 1) (NCDMF 2011). Because determination of the proposed SHAs was made using MARXAN modeling (with subsequent input from a scientific advisory committee), there is need to test the accuracy of the inferences on special habitat value with actual data. Important unanswered questions are: (1) how have human activities impacted the ecological functions of coastal marsh habitats; and (2) how well are such impacts incorporated in outcomes of the current SHA-determination process? Such information is needed to know which habitat areas to protect with most vigor to sustain and enhance fish populations and to support the broader coastal ecosystem. Our study tested whether differences exist in the productivity and habitat functions of highly and minimally altered marsh habitats; hence, we test key assumptions of the SHA-designation process as applied to Region 2 coastal marshes. Our project results are intended to aid DMF in adapting this process for the identification of future SHAs and provide the Marine Fisheries Commission (MFC) with information needed to adopt appropriate conservation and restoration measures.
Pamlico Sound, the Nation’s largest lagoonal (enclosed) estuary, harbors salt and brackish marshes that are irregularly inundated. On the shores of Pamlico Sound, seasonal wind forcing floods the marsh habitat and provides access to fishes and nektonic crustaceans for relatively long periods of time (days to weeks). *Juncus roemerianus* is the dominant marsh macrophyte in this region (Wilson 1962). The research done to date in the southeast U.S. assessing the value of coastal marsh habitat to the production of fishes has been conducted almost exclusively in regularly flooded *Spartina alterniflora*-dominated marshes, even though 60% of the 105,866 hectares of coastal marsh within North Carolina is dominated by *J. roemerianus* (NOAA 1990) and the vast majority of marsh acreage in Region 2 is *J. roemerianus*. There exists a basic understanding that marshes provide a key role in the early life stages of estuarine nekton (Kneib 1997, Able and Hagan 2003, Minello et al. 2003) and that the extent of marsh flooding is positively related to fishery production (Zimmerman et al. 2000); however, the processes by and degree to which nekton, including recreationally valued fishes, benefit from irregularly flooded, largely *J. roemerianus*, marsh habitat are not well understood.

Research to better understand the extent of marsh use by early life stages of recreationally and commercially valued fishes and crustaceans is necessary in North Carolina because of ongoing threats to marsh habitat and general ignorance of the importance of irregularly flooded marsh. Rozas and Zimmerman (2000) and Minello et al. (2003) reported that fish and nektonic crustacean abundances in *S. alterniflora* marshes in the Gulf of Mexico are uniformly higher at the low edge nearest the estuarine tributary that supplies the incoming water and serves as a source for immigrating nekton. In a recent examination of flooding patterns of southern U.S. marshes, Minello et al. (2012) found a positive relationship between flooding duration and habitat use of marshes by blue crabs and penaeid shrimps. Baker et al. (2013) showed that the marsh contribution to trophic support of nekton was positively correlated to access of the vegetated marsh surface, which is regulated by hydrodynamics. If marsh use (habitat value) increases with inundation time, juvenile fishes and nekton would be expected to
penetrate deeply into the western Pamlico Sound marsh platforms where long flood durations suggest that aquatic organisms may benefit from a broader extent of the coastal marsh, not just the marsh edge. Minello et al. (2012) actually propose that the fisheries value of any given area of marsh habitat might be calculated by the duration of marsh inundation. In addition, there exists insufficient data on species that use the Pamlico Sound as nursery habitat, which may serve an uncommonly broad range of species since this large lagoonal estuary lies near the ocean convergence of the Gulf Stream and Labrador Currents.

As sea-level rise continues, apparently at an intensifying pace (Romero-Lankao et al. 2014, Wong et al. 2014), demand is growing to construct bulkheads, revetments, or dikes to prevent estuarine shoreline erosion, flooding, and property damage. The viability of coastal wetlands is particularly threatened by sea-level rise with dire consequences to coastal communities (Wong et al. 2014). The presence of such erosion control structures results in escalating marsh loss as this habitat becomes increasingly squeezed between rising waters of the estuary and a fixed vertical wall, potentially leading to a walled estuary ultimately lacking any intertidal habitats: even worse, the vertical wall prevents marsh transgression upslope (Titus 1998, Peterson et al. 2008). In addition, any irregularly flooded marsh, typically high marsh habitat that is landward of a vertical erosion control structure is doomed when cut off from the nourishing and necessary exchanges of tidal waters with the estuary. Although coastal marsh habitat is highly valued for its many ecosystem services (MEA 2005, Nellemann et al. 2009) and enjoys legislative protections in the U.S. at both federal and state levels under the mantra of “no net loss of wetlands”, its acreage continues to decline and thus so do its ecosystem services.

Because the role of marsh habitat in the early life stages of key fish species is critical to sustaining high production of these valued vertebrates, a quantitative understanding of how fishes use marshes that are inundated irregularly and how use of such marshes differs between areas that are minimally versus moderately altered by human activities is necessary to prioritize protection afforded by SHA designation. Of the top 5 recreationally-important fish species in North Carolina, coastal marsh appears to be critical for direct provision of forage and refuge habitat for at least 4 recreationally fished species (Sciaenops ocellatus (red drum), Cynoscion nebulosus (speckled trout), Paralichthys lethostigma (southern flounder) and Paralichthys dentatus (summer flounder) and, based upon dietary information, this list may also include Morone saxatilis (striped bass). Coastal marsh provides indirect support of these and other recreationally caught fishes by supporting the production of forage fish and crustaceans important in the diets of these and other recreationally targeted fish. Information on direct habitat provision and indirect support by enhancing prey production is needed to provide better scientific support for identifying SHAs and to facilitate management to sustain and enhance stocks of recreationally important fishes and crustaceans.

The objectives of this project were: (1) to gather quantitative information on the spatial and temporal patterns of use of this irregularly inundated marsh habitat by fishes and crustaceans, comparing sets of a highly altered and minimally altered marsh in each of 8 geospatially-defined strata (4 comparisons in each of the 2 yrs), in which the non-SHA sites are inferred to be substantially altered largely by changes in hydrology from nearby ditching, draining, and other agricultural activities; (2) to test the hypothesis that the landward extent of penetration by nekton into marsh habitat increases with inundation duration, thereby evaluating the proposition in Minello et al. (2012); (3) to develop a more complete understanding of how water depth and distance from marsh edge covary with duration of inundation to determine fish and crustacean use; (4) to collect data on the use by fishes and crustaceans of J. roemerianus-dominated marsh habitat so as to make comparisons with S. alterniflora, which comprises almost all literature on coastal marsh; and (5) to measure end-of-season peak standing stock biomass and shoot density of each marsh macrophyte as proxies for the ecosystem services of primary
production, water quality treatment, and refuge function so as to allow novel contrasts with analogous published data by distance from marsh edge in regularly flooded marshes of varying macrophyte composition.

Methods
Selection of study sites

We first studied maps of the entire western Pamlico Sound study area (Fig. 1) so as to subdivide it into a collection of about 1.5-km stretches (strata) of shoreline segments of similar aspect and fetch exposure. We searched for strata that included a pair of separate but nearby sites, one identified by the DMF Habitat Team as unaltered and the other as human modified so as to allow pairwise testing of human impacts. Finding pairs of sites within each stratum is critical to the study design because water levels throughout the study region are largely determined by wind direction, speed, and duration, and it was important to delineate strata with stretches of shoreline that experienced similar inundation regimes despite differences in human intervention. In total, 19 strata were considered and randomly assigned a number. We visited each stratum, accepting or eliminating it from consideration based on the following criteria: (1) inclusion of boat-accessible marsh sites with both regionally high (“altered”) and low (“control”) alteration scores; (2) similar energy regime and fetch between altered and control sites; (3) similar topography and upland connectivity between altered and control sites; and (4) permission to use both altered and control sites. To maximize replication, we chose to sample 8 sites comprising 4 strata in each of 2 years, for a total of 16 study sites. Four strata, each with one altered and one control marsh site, were established in Yr 1 (Jul 2013 – Jun 2014) and another four in Yr 2 (Jul 2014 – Jun 2015). In Yr 1, Otter Creek, Berry’s Bay, Far Creek, and Middleton Creek strata were sampled. In Yr 2, North Wysocking Bay, Lone Tree Creek, Mt. Pleasant Bay, and East Bluff were sampled (Fig 2).

A key component of the SHA-selection process for all regions of North Carolina is the determination of alteration scores, which were calculated by the MARXAN algorithms (as per Appendix B in NCDMF 2011). Alteration factors are human activities that impact the estuarine and marine environment. The alteration factors that were most prominent and impacted marsh habitat in western Pamlico Sound were: (1) hydrology obstructed by culverts, water control structures, impoundments, channelized streams, ditching and draining, and canal infrastructure; (2) water-quality alterations derived from discharge of major and minor National Pollution Discharge Elimination Systems, animal operations, developed land-use, and agricultural land-use; (3) physical conversion of habitat such as wetlands lost to development or agriculture and riparian upland lost to development or agriculture. By comparing marsh sites with low vs. high alteration scores, as derived from MARXAN, we were able to test how well alterations scores relate to the habitat provisioning function of the irregularly inundated marshes in Pamlico Sound. Additional ecosystem functions may contribute to whether given habitats qualify as SHAs. Importantly, in Region 2, all of the Primary Nursery Areas (PNAs) were included as nominated SHAs because they had been selected on the basis of intense sampling and by scientifically based evidence of being highly productive fish and shellfish nursery areas (Deaton et al. 2011).
Figure 2. Map of western Pamlico Sound, North Carolina, showing the 8 strata in Region 2 within which the nekton use of marsh habitat was assessed. Each stratum had one highly altered marsh site paired with one minimally altered marsh site, and a water-level station operating throughout the year when that stratum was repeatedly sampled.
Study site layout

Triplicate 10-m wide x 50-m long parallel transects were established at each site, starting from and running perpendicular to the estuarine edge. In general, transects were spaced 5 to 15 m apart within each site. PVC poles were placed at 1, 10, 25, and 50 m distances from the estuarine edge and PVC-stemmed flags were placed 5 m either side of the PVC pole to clearly delineate sampling stations for the year when each site was studied.

Marsh elevation mapping

Vertical benchmarks were established at each site and on each water-level station at the beginning of the study year when sampling occurred in that stratum (either fall 2013 or fall 2014). At each marsh site, heavy-duty, 0.76-m long, mobile-home auger anchors were inserted into the substrate approximately 20 m from the estuarine edge, leaving the head exposed above the marsh surface. For each water-level station, a #14, 5-cm-long, round-head stainless steel screw was inserted, leaving about 2 cm protruding, at the approximate mid-point of the aerial portion of the station post. The elevation of each benchmark was determined using a Trimble RTK-GPS (Trimble 5800). Elevation of all benchmarks was recorded multiple times in each season for the sites and water-level stations in those strata being studied during that year to obtain RTK-GPS data from satellites with changing orientations to improve accuracy of determining these vertical elevations. The mean of the RTK-GPS data in each season was used to convert the relative elevations to mNAVD88, as determined by laser leveling.

A Topcon (RL-H3C) laser level was used to determine the elevation along each 10-m wide by 50-m long transect at each site, resulting in a 5-m grid of elevations that was referenced to the site benchmark and, therefore, to NAVD88. A separate elevation survey of each site was conducted in early spring of the study year, using the backpack module of the RTK-GPS to record elevation every 0.5-m, creating a high-resolution grid. The data obtained using the laser level were used to compute the elevations of sampling stations, from which inundation depths and durations were computed. The mean of the 3 elevation points for each sampling station, at the center and 5-m each side of center, served as station elevation. The RTK-GPS database was used to create a digital elevation model for all sites.

Digital elevation models (DEMs) were created for each site with the data obtained from RTK-GPS measuring elevations continuously every 0.5 m, resulting in approximately 1200 elevation points for each site. The RTK-GPS derived elevations were expressed as XY Data (point data) in ESRI ArcGIS (v. 10.3.1). Spatial Analyst tools were employed to create hydrologically accurate DEMs. An inverse distance weighting file (IDW) was created from the elevation point data (z-values), using a radius of 12 possible points and a search radius of one half the maximum distance between points. Surface contours were interpolated from the IDW, using a contour interval of 0.02m. The chosen contour interval was the minimum value to be used without possible interference from vertical errors associated with the initial collection of elevation data. A polygon was drawn around the strata to create a boundary for the analysis, reducing error in interpolations the greater the distance away from the elevation point data. The point data, contours, and polygon were all used to create each DEM in the form of a raster file using the Topo to Raster tool. The default settings were used for the Topo to Raster tool with an exception of the usage of the Enforce_With_Sink option. Sampling site locations were overlaid upon the DEM in the form of a feature class shapefile.
Water-level monitoring

One water level station was established in each stratum, recording barometric pressure and water levels in the year that stratum was sampled (Fig. 2). A pressure-treated 10-cm x 10-cm x 4.9-m wooden post was jet-washed into the submerged sediments, with approximately 2.5 m remaining above the estuarine floor, to support the water-level station. For the Far Creek stratum, a piling of an existing dock was used instead of a newly installed post. Onto each post or piling, a vertical benchmark was established, as described above, and used to reference the water-level data set to NAVD88. Two, eyed boat cleats were bolted onto each post or piling through which stainless steel hose clamps were used to secure a 5-cm diameter slotted PVC-pipe housing of sufficient length to allow the lower transducer, recording water level, to remain submerged and the upper transducer, recording barometric pressure, to remain aerial (Fig. 3). Within the PVC housing, two Onset HOBO pressure transducers (U20-001-01-Ti) were suspended using stainless steel cable from an eye bolt in the cap as shown in Fig. 3. All water-level stations were positioned within 5 m of the shoreline, were labeled with our contact information, and had reflectors, so as to not be a navigation hazard. Station housings were periodically removed for cleaning: the cleat and hose clamp system worked well to maintain the vertical stability of each housing. A reference water-level reading, measuring the distance from the station benchmark to the water level, was recorded with the date and time on every occasion when pressure transducers were deployed and retrieved. The transducers recorded barometric or water pressure every 6 min and data were downloaded at least every 2 months, except during the 4-mo winter-time period between the fall and spring faunal sampling seasons when 12-min readings were recorded. In addition, the submerged transducer recorded water temperature and the aerial transducer recorded air temperature at these same intervals.
Marsh inundation computations

To compute the mean inundation depth of all sampling stations for each sampling period, we created a formula within a copy of the water level record in which the mean elevation of each sampling station (Appendix I) was subtracted from the water level elevation of the respective stratum, for a given sampling period. Subsequently, the mean of all inundation depths for a given sampling station was calculated for each sampling period and added as a factor within the faunal database. Similarly, a separate copy of the water level record was used to determine duration of inundation for each station prior to sample collection. Here, we created an “If, Then” statement such that if the water level elevation was greater than 2 cm above the elevation of a given station, a “1” was entered into the cell and a “0” was entered otherwise. From this database, we determined the number of hours that a given sampling station was inundated prior to sample collection. For the first sampling period of each study year, we determined the minimum number of hours that a given station was inundated instead of the exact number of hours, because we lacked knowledge of water level before our stations were installed. We were conservative in our estimates of inundation duration prior to sample collection by including only inundation depths that were greater than 2 cm, the possible level of error in RTK-GPS measurements. While our record of instrument error was generally less than 1 cm, we elected to allow for 1 cm of error in our determination of elevation at both the site and water-level station benchmarks. In addition, our casual observations documented that small fishes, typically juvenile *Fundulus* sp., occurred regularly with 2 cm of water upon the marsh surface.
Vegetation sampling

Triplicate 25-cm x 25-cm (0.0625 m$^2$) quadrats were used to haphazardly harvest samples of vegetation with each sampling station of marsh sites in August of the year studied. Emergent vegetation was clipped at the marsh surface, placed into labeled plastic bags, and kept cool prior to further processing in the UNC-IMS lab. Aboveground material was sorted by species, then shoots were counted, lengths measured, and biomass assessed by weighing after drying at 85$^\circ$C until weights stabilized. Shoot density and vegetation community demographics were analyzed for patterns that differed with alteration category (highly vs minimally altered) and distance from marsh edge. Separately, the percent cover of each vegetation species, bare sediment, or wrack was visually estimated for each 10-m x 10-m section of each transect.

Faunal sampling

Quantitative sampling of nekton in tall vegetation (i.e., *J. roemerianus*) and in meteorologically-dominated inundation regimes is challenging; therefore, we employed 3 different sampling devices, each of which had different advantages. Unbaited minnow pots (Layman and Smith 2001) shrouded with 1-mm mesh screening captured mobile fishes and invertebrates over undetermined, but presumably large, areas of attraction. Pit traps (23 cm x 18 cm x 5 cm trays) (e.g., Able and Hagan 2003) with tops of edges flush with the sediment surface were deployed to sample early life-stages of fish and invertebrates from unknown, but presumably large, distances. Bottomless liftnets with dimensions of 1.5 m x 2.5 m were constructed with 3-mm delta mesh walls (Rozas 1992, Raposa and Roman 2001) to provide quantitative data on densities per unit area for fish and other nekton; however, we found this gear to be of limited utility because we required that the vegetation within the sampling station remain intact. Therefore, in each year during each sampling period, we deployed duplicate pit traps and duplicate minnow pots at each of 4 distances (1, 10, 25 and 50 m from the marsh edge) along the 3 transects, at each of 8 marsh sites. In Yr 1, we deployed bottomless liftnets at the 1 and 10 m stations of 4 sites. We attempted to replicate liftnet sampling in Yr 2; however, strong northeast winds during our first sampling period that year destroyed 4 and damaged 3 of the 8 nets, making use of this gear not feasible. Because pit traps required excavating substrate to inset the trap, their randomly assigned location remained the same within each sampling station for the year it was sampled. The minnow pots were placed randomly for each sampling period. We conducted 3 sampling events in fall and 2 in the subsequent spring of each study year, with sampling consisting of 2 24-hr soaks and 1 long soak between 96-168 hours, dependent upon weather conditions. The sampling effort for the spring and fall in Yr 1 and Yr 2 sampling events was remarkably similar, allowing us to evaluate annual and seasonal differences.

All specimens were identified to the lowest taxonomic level possible, counted and measured (total length for fish and shrimp or carapace length for crabs), and mass recorded on site, then released. Unidentified specimens were bagged with labels, held in a cooler filled with ice, and returned to the lab for identification. Salinity of the estuarine water was recorded for each site at least once during each sampling period, except when our refractometer was in disrepair.
Data and statistical analyses

Electronic GPS data were downloaded and reviewed for completeness. Data from HOBO pressure loggers (transducers) were downloaded regularly to ensure available memory capacity; Onset HOBOware Pro software (v. 3.7.2) was used to compute water levels in each stratum using reference measurements of water level relative to station benchmarks and the Onset barometric pressure assistant functions. Hard copies of data from field sampling and lab identification and measurements of fauna were reviewed for completeness and entered electronically into Excel workbooks. All data were reviewed for completeness and entered into Excel spreadsheets, where pivot tables were used to again verify completeness and a 20% sub-sample of each database was spot-checked for accuracy. Data analyses were conducted using Systat (v 13.00.05, Systat Inc. 2009), JMP (v 9, SAS 2010), PRIMER (v 7, Primer-E Ltd 2015), and PERMANOVA (Anderson 2001) software packages. Data from all sampled organisms were analyzed separately by trap type. The faunal metrics analyzed were the mean abundance, total lengths, and biomass for each station. All datasets from minnow pots and pit traps, except the fish biomass from minnow pots, met the assumptions of normality and passed the O’Brien test of unequal variances. The fish biomass from minnow pots was analyzed using the non-parametric Kruskal-Wallis test by ranks; yet, these results exhibited the same patterns found using parametric methods. Therefore, for simplicity, we report here the parametric (2-way ANOVA) and non-parametric (2-way PERMANOVA) results in which the effects of condition (highly altered “altered” vs. minimally altered “control”) and distance from estuarine edge (1, 10, 25, and 50 m) were compared. In addition, we conducted 3-way PERMANOVA tests adding season (fall vs. spring) as a third factor to the 2-way PERMANOVA test described above. One-way PERMANOVA tests were used to compare differences in the community assemblages in minnow pots for the 13 nominated SHA sites vs. the 3 non-SHA sites, and separately for 6 independent comparisons of 3 randomly chosen nominated SHA sites vs. the 3 non-SHA sites. Kruskal-Wallis tests of ranks was used to analyze the data sets from the bottomless liftnets, for which the effects of condition (altered vs. control) and distance from estuarine edge (1 m vs. 10 m) were tested separately. Additional analyses of the fish communities sampled in minnow pots was conducted using the summed abundance, summed total lengths, and summed biomass as metrics of fish use of marsh habitats in western Pamlico Sound.

Multivariate analyzes were conducted using non-metric multidimensional scaling (nMDS) of the datasets from minnow pots and pit traps. PRIMER offers a graphical and statistical description of the relationships among biotic communities (Clarke 1993). Non-metric multidimensional scaling (nMDS) is an ordination of the similarities found among biotic communities that may differ in composition. Each of the datasets used for PRIMER and PERMANOVA analyses were fourth-root (or Log(X+1)) transformed to down-weight the importance of highly abundant species prior to analysis. Pairwise tests in PERMANOVA were used to explore further differences between communities comparing treatments and seasons. PERMANOVA extends the resemblance-based methods of PRIMER to allow for more complex statistical modeling of multivariate data; it is a nonparametric, multivariate analysis of variance based on permutations of Bray-Curtis or other similarity matrices (Anderson 2001). We used 2 groups of PERMANOVA tests to examine whether nominated SHA marshes differed from non-SHA marshes in community composition. Because it was essential to compare highly altered and minimally altered sites with similar inundation regimes, many of our study sites were located in PNAs, of which all were nominated as SHAs in Region 2. This resulted in a total of 13 nominated SHA sites and 3
non-SHA sites, an unbalanced design. Therefore, we also compared SHA vs non-SHA communities by randomly selecting 3 SHA sites for PERMANOVA tests with the 3 non-SHA sites, repeating these permutation tests 6 times and reporting the mean P-values.

To tease out similarities among the fish communities we sampled, we use the similarity profile analysis (SIMPROF) described in Clarke et al. (2008). Here, SIMPROF is a method of exploratory data analysis that employs null hypothesis testing to detect structure in ecological communities. A similarity profile is constructed from a set of species abundance and composition data by first creating a similarity matrix using an ecologically appropriate resemblance measure (e.g., Bray-Curtis similarity). The lower tridiagonal of this matrix is then unwrapped as a vector and sorted by magnitude. This sorted list of similarities provides a profile of the structure underlying the observed data and is visualized using a simple line plot. An iterative permutation procedure, based on randomly shuffling the original raw data, is then used to generate a mean permuted similarity profile, which represents the profile expected under the null model. The congruence between the observed similarity profile and the null model is measured using the pi statistic, as the sum of the absolute differences between corresponding elements of the two profiles. The pi statistic is then assessed via a permutation test, if it is statistically significant the null hypothesis that no multivariate structure exists within the data is rejected at the appropriate alpha level. Stated another way, similarity profile analysis examines whether the similarities observed in the data are smaller or larger than those expected by chance.

General linear models (GLMs) were used to explore relationships between the abundance, total length, and biomass of fishes sampled using minnow pots and: (1) the mean inundation depth of each sampling station during each sampling period, as well as (2) the duration (hrs) that a station was inundated with ≥ 2 cm depth prior to sample collection.

**Results**

**Marsh elevations**

The Region 2 marshes along western Pamlico Sound are best described as broad platforms with limited topography. The mean elevation of all marsh sites was 0.061 mNAVD88, ranging from -0.225 mNAVD88 at the lowest edge to 0.472 mNAVD88 the highest point. Across all strata, marsh elevation did not differ (P=0.58) between altered and unaltered sites, using distance from edge as a covariate (Figs. 4 and 5). Within strata, altered sites were significantly higher in elevation than at paired unaltered sites at Otter Creek (P<0.0001), Far Creek (P<0.0001), North Wysocking Bay (P=0.003), and East Bluff (P=0.04), but not for the other site pairs (Fig. 6A – 6H). The most strikingly different marsh site elevation was detected at the altered site in Far Creek, near the town of Engelhard, where the best option for positioning coincided with a location where the marsh interior exhibited a sharply increasing slope near an upland area: these few anomalously high points are clearly distinguishable in Figs. 5 and 6.
Figure 4. Mean elevation of the marsh surface, along 50-m long transects from the estuarine edge to marsh interior, for 8 altered and 8 unaltered study sites in western Pamlico Sound, North Carolina between July 2013 and June 2015.
Figure 5. Box plot showing the mean and variability of the elevations of all altered and unaltered sites along 50-m-long transects from estuarine edge to marsh interior at study sites in western Pamlico Sound, North Carolina in 2013-2015. Asterisks denote mild outliers and circles denote extreme outliers; these outliers are due to a relatively steep slope at the upland transition at the altered site in Far Creek.
Figure 6A. Digital elevation models (DEMs) of the altered (left) unaltered control (center) sites in the Otter Creek stratum with a graph (right) showing the mean elevation (± standard error) of triplicate transects extending from the estuarine edge to 50 m into the marsh interior. Yellow diamonds indicate the location of sampling stations along triplicate transects at each site. Individual DEMs can be found in Appendix II.
Figure 6B. Digital elevation models (DEMs) of the altered (left) unaltered control (center) sites in the Berry’s Bay stratum with a graph (right) showing the mean elevation (± standard error) of triplicate transects extending from the estuarine edge to 50 m into the marsh interior. Yellow diamonds indicate the location of sampling stations along triplicate transects at each site. Individual DEMs can be found in Appendix II.
Figure 6C. Digital elevation models (DEMs) of the altered (left) unaltered control (center) sites in the Far Creek stratum with a graph (right) showing the mean elevation (± standard error) of triplicate transects extending from the estuarine edge to 50 m into the marsh interior. Yellow diamonds indicate the location of sampling stations along triplicate transects at each site. Individual DEMs can be found in Appendix II.
Figure 6D. Digital elevation models (DEMs) of the altered (left) unaltered control (center) sites in the Middleton stratum with a graph (right) showing the mean elevation (± standard error) of triplicate transects extending from the estuarine edge to 50 m into the marsh interior. Yellow diamonds indicate the location of sampling stations along triplicate transects at each site. Individual DEMs can be found in Appendix II.
Figure 6E. Digital elevation models (DEMs) of the altered (left) unaltered control (center) sites in the North Wysocking Bay stratum with a graph (right) showing the mean elevation (± standard error) of triplicate transects extending from the estuarine edge to 50 m into the marsh interior. Yellow diamonds indicate the location of sampling stations along triplicate transects at each site. Individual DEMs can be found in Appendix II.
Figure 6F. Digital elevation models (DEMs) of the altered (left) unaltered control (center) sites in the Mt. Pleasant Bay stratum with a graph (right) showing the mean elevation (± standard error) of triplicate transects extending from the estuarine edge to 50 m into the marsh interior. Yellow diamonds indicate the location of sampling stations along triplicate transects at each site. Individual DEMs can be found in Appendix II.
Figure 6G. Digital elevation models (DEMs) of the altered (left) unaltered control (center) sites in the Lone Tree Creek stratum with a graph (right) showing the mean elevation (± standard error) of triplicate transects extending from the estuarine edge to 50 m into the marsh interior. Yellow diamonds indicate the location of sampling stations along triplicate transects at each site. Individual DEMs can be found in Appendix II.
Figure 6H. Digital elevation models (DEMs) of the altered (left) unaltered control (center) sites in the East Bluff stratum with a graph (right) showing the mean elevation (± standard error) of triplicate transects extending from the estuarine edge to 50 m into the marsh interior. Yellow diamonds indicate the location of sampling stations along triplicate transects at each site. Individual DEMs can be found in Appendix II.
Water-level records

Graphs of water levels for Yr 1 (Fig. 7) and Yr 2 (Fig. 8) show that seasonal patterns, driven by winds, dominate over the daily tidal signals in determining water levels in western Pamlico Sound.

The mean elevations of the water-level station benchmarks, based upon RTK-GPS measurements, for each season were used to compute water levels in each season a given stratum was studied. Any differences in benchmark elevation between the fall and subsequent spring, the 4-mon winter period, were averaged and used to compute water levels for that time period. For most stations, the fall to spring difference in benchmark elevation was < 8 cm; however, this difference was 13.5 cm and 15.1 cm at Berry’s Bay and Otter Creek, respectively, probably due to heaving of the station pole caused by ice in Yr 1.

**Figure 7.** Water levels for Otter Creek, Berry’s Bay, Far Creek, and Middleton Creeks in western Pamlico Sound, North Carolina from September 2013 through August 2014.

**Figure 8.** Water levels for North Wysocking Bay, Mount Pleasant Bay, Lone Tree Creek, and East Bluff in western Pamlico Sound, North Carolina from September 2014 through July 2015.
Role of marsh surface topography and consequent inundation depth and duration

Our carefully executed topographic surveys by RTK-GPS of all marsh sites, when combined with our continuous water-level recording for about 1 year in each stratum allowed us to create valuable hydrographic databases in which we assigned site-specific records to each sampling station in every marsh site. We first computed mean elevation relative to NAVD88 for each sampling site (Appendix I). This allowed us to utilize our continuous water-level record to compute two variables of likely great significance in affecting the spatially explicit use of the marsh habitat by fishes, crustaceans, and other mobile faunal groups. The two variables of such likely significance are: (1) the mean inundation depth during each faunal sampling period; and (2) the duration of ≥ 2-cm of water coverage prior to sample collection for each faunal sampling period, at each sampling station within each site.

The mean, minimum, and maximum inundation depths at each sampling station for each sampling period are embedded within the SHA CRFL faunal database of fishes. Mean inundation depths ranged from 0.00 cm to 38.9 cm with a mean of 16.9 cm. Minimum depths ranged from 0.00 cm to 31.1 cm with a mean of 11.2 cm. And maximum depths ranged from 0.00 cm to 54.4 cm with a mean of 24.2 cm. The minimum, maximum, and mean depths of marsh inundation were highly significantly (each P<0.0001) higher in the fall than in the spring of each study year, and also highly significantly (each P<0.001) higher in Yr 2 than in Yr 1, with a significant (each P<0.001) interaction between factors arising from much higher water levels in fall of Yr 2. The duration of marsh inundation (above 2-cm depth) prior to collecting fishes was significantly (P<0.001) longer during the spring seasons with greater durations of inundation occurring in Yr 2 (P<0.001), and an interaction between these factors driven by the much longer inundation durations in spring of Yr 2 compared to all other comparisons.

The 10-month mean percent of time that each sampling station was flooded from September to July of respective study years is shown in Table 1. For Yr 1, the grant period enabled us to document a 12-month water-level record, which yielded about an additional 6% in percent of time flooded over all Yr-1 sampling stations. We suspect that a similar pattern would be evident in Yr-2 water-level records had we been able to continue monitoring water levels in these strata.
Table 1. The 10-month mean percent of time that sampling stations were flooded in Yr 1 strata: Otter Creek (stratum 02), Berry’s Bay (03), Far Creek (04), and Middleton Creek (05) between September 2013 and July 2014, and in Yr 2 strata: North Wysocking Bay (12), Mt. Pleasant (14), Lone Tree Creek (18), and East Bluff (19) between September 2014 and July 2015. All strata were located in western Pamlico Sound, North Carolina, with one altered (A) and one unaltered control (C) site in each stratum. Each site had 3 transects (A, B, and C) with sampling stations at 4 distances from estuarine edge (1, 10, 25, and 50 m).

<table>
<thead>
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<th>Site</th>
<th>Station: % time flooded</th>
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Vegetation

Sampling of marsh macrophytic vegetation confirmed the visual impressions of these western Pamlico Sound marshes. Plant biomass and shoot density are consistently high, and are dominated by *Juncus roemerianus* everywhere (Photo 1) except at the estuarine edge on unaltered sites (Photo 2), where a virtual monoculture of *Spartina alterniflora* occupies the lower edge where exposure to wave energy is relatively high.
Photo 1. Representative photograph showing the estuarine edge of marsh sites in western Pamlico Sound that are impacted by frequent waves and therefore have edges dominated by *Spartina alterniflora*

Photo 2. Representative photograph showing the estuarine edge of marsh sites in western Pamlico Sound that are dominated by *Juncus roemerianus*
Spartina patens and Distichlis spicata contributed as well to the marsh vegetation biomass and shoot density, but represented small fractions of each data set. Total aboveground biomass of marsh vegetation was about 10% higher on the more altered sites than where site condition was less altered (P=0.003; Fig. 9). Our ability to detect such a proportionately small difference is facilitated by the generally high uniformity of marsh aboveground biomass and low variance from site to site. A 2-way ANOVA on total marsh aboveground biomass as a function of site condition and distance from edge revealed a significant effect of site condition (P<0.001) but no influence of distance from edge and no factor interaction (Fig. 9). Aboveground biomass of Juncus alone failed to exhibit a significant response to site condition in a t-test (P=0.42). In a 2-way ANOVA, testing for effects of distance from edge and site condition (Fig. 10), these two factors interacted (P<0.0007). Tukey’s HSD post hoc contrasts revealed that his interaction was driven by significantly lower Juncus biomass in the less altered than in the altered treatment at the 1-m distance. The explanation for this interaction is the replacement of Juncus by Spartina alterniflora in the 1-m station at less altered sites where wave action is most intense (Photos 1 and 2). The 2-way ANOVA of the shoot density of all species of vegetation differed by site condition (P<0.0001), and with distance from estuarine edge (P<0.0001), with an interaction between these factors (P=0.006) (Fig. 11). A 2-way ANOVA on the shoot density of Juncus did not differ by site condition (P=0.70), but did with distance from estuarine edge (P=0.0002), and also revealed a significant interaction between site condition and distance from edge (P=0.003) and Tukey’s HSD post hoc contrasts revealed that the cause of the interaction was the failure of the 50-m station to exhibit a pattern of higher shoot density in the most altered site conditions, a pattern held in common at the 1-, 10-, and 25-m distances. Hence, shoot density of Juncus generally reflects enrichment associated with the alteration conditions like overall marsh plant density.

Figure 9. Total biomass of all species of vegetation from 8 altered and 8 unaltered marsh sites (left) and at 1 m, 10 m, 25 m, and 50 m from estuarine edge (right) in western Pamlico Sound, North Carolina in 2014 and 2015. Levels not sharing the same letter are significantly different.
Figure 10. Total biomass of *Juncus roemerianus* from 8 altered and 8 unaltered marsh sites (left) and at 1 m, 10 m, 25 m, and 50 m from estuarine edge (right) in western Pamlico Sound, North Carolina in 2014 and 2015. Levels not sharing the same letter are significantly different. Letters are color-coded according to the categories shown in the figure legend; letters are not shown for category levels that did not differ significantly.

Figure 11. Shoot density of all species of vegetation from 8 altered and 8 unaltered marsh sites at 1 m, 10 m, 25 m, and 50 m from estuarine edge in western Pamlico Sound, North Carolina in 2014 and 2015. Levels not sharing the same letter are significantly different.
Fish and other nekton use of marsh sites

Minnow pot sampling

Univariate analyses

Our indices of fish abundance suggested possible patterns of importance, but these patterns were not statistically significant. Graphs of how mean fish abundance changed with site condition designation and distance from marsh edge implied some intriguing patterns, yet main effects of site condition (P=0.33), distance (P=0.19), and their interaction (P=0.97) failed to exhibit statistical significance. The non-significant pattern for site condition implied an undetected approximately 10% greater fish abundance on altered (non-SHA) than on minimally altered (SHA) sites (Fig. 12). The non-significant pattern for mean fish abundance per sample with distance from edge implied about a progressive 20% increase in moving from 1 to 25 and 50 m from the marsh edge (Fig. 12). Mean total lengths of fishes per sample likewise failed to show significant impacts of site condition (P=0.23), distance from edge (P=0.30) or their interaction (P=0.97), yet total fish length per sample again implied an undetected 10% greater level in non-SHA, altered sites than in SHAs and increased progressively from 1 to 25 and 50 m distances by about 10% (Fig. 13). The third index of fish abundance, average biomass per sample failed to display any suggestion of a pattern of differences between altered and minimally altered (SHA) sites and across distances from the marsh edge (Fig. 14), conforming with the ANOVA results (P=0.81 for site condition; P=0.95 for distance effects, and P=0.50 for their interaction).

Mean abundance of marine invertebrates captured in minnow pots did not differ with site condition (P=0.66), but did (P=0.003) with distance because of significantly higher abundances nearest to the estuarine edge (Fig. 12), with no interaction between these factors (P=0.42). Although the total lengths of marine invertebrates per sample followed a pattern similar to mean abundance, neither site condition (P=0.87), nor distance from edge (p=0.11) differed significantly, with no interaction between factors (P=0.80) (Fig 13). The mean biomass of marine invertebrates was strikingly similar between altered and unaltered sites (P=0.81), among the distances from estuarine edge (P=0.95), with no interactions between these factors (P=0.50) (Fig 14).

Mean abundance of terrestrial invertebrates differed with site condition (P=0.02) because about 15% more individuals occurred in samples at the less altered (SHA) sites, but not with distance from estuarine edge (P=0.96), or their interaction (P=0.66) (Fig. 12). Mean total lengths of this faunal group did not differ by site condition (P=0.55), distance from edge (p=0.67), or factor interaction (P=0.59) (Fig. 13). Finally, mean biomass of terrestrial invertebrates did not differ with site condition (P=0.93), distance from edge (P=0.55), or the interaction between factors (p=0.38). Although non-significant, the mean biomass of terrestrial invertebrates exhibited a pattern suggesting about a 50% decline from marsh edge to the site 50 m deep into the marsh (Fig. 14).

Mean abundance of terrestrial vertebrates (mainly snakes and rodents) did not differ significantly with site condition (P=0.76), distance from estuarine edge (p=0.88), or their interaction (P=0.43). Nevertheless, highest sample densities tended to appear 25 m into the marsh interior where the marshes were most frequently inundated, with the second greatest abundance nearest the edge (Fig. 12). Mean total lengths and mean biomass of terrestrial invertebrates suggested a possible pattern of greater values in the altered than in minimally altered sites, although these differences were not statistically significant. The longest total lengths for this faunal group were found 25 m into the marsh
with the second longest mean at the marsh edge; however, mean total lengths of terrestrial vertebrates did not differ significantly with site condition (P=0.63), distance from edge (P=0.79), or interaction between factors (P=0.41) (Fig. 13). Mean biomass of terrestrial vertebrates did not differ with site condition (P=0.58), distance from edge (P=0.90), or interaction between factors (P=0.30) (Fig. 14). Despite lack of statistical significance for any of the habitat use metrics for marsh vertebrates, this repetition of a common pattern with highest usage at 25 m from the marsh edge for all metrics offers some confidence that this pattern with distance from edge may be real.

**Figure 12.** The mean abundance of organisms sampled with minnow pots at altered and unaltered sites (left) and at 1 m, 10 m, 25 m, and 50 m from estuarine edge (right) at marsh sites from September 2013 to June 2015 in western Pamlico Sound, North Carolina. Levels not sharing the same letter are significantly different. Letters are color-coded according to the categories shown in the figure legend; letters are not shown for category levels that did not differ significantly.
Figure 13. The means of the total length (TL) of organisms sampled with minnow pots at altered and unaltered sites (left) and at 1 m, 10 m, 25 m, and 50 m from estuarine edge (right) at marsh sites from September 2013 to June 2015 in western Pamlico Sound, North Carolina. Levels not sharing the same letter are significantly different. Letters are color-coded according to the categories shown in the figure legend; letters are not shown for category levels that did not differ significantly.

Figure 14. The mean biomass of organisms sampled with minnow pots at altered and unaltered sites (left) and at 1 m, 10 m, 25 m, and 50 m from estuarine edge (right) at marsh sites from September 2013 to June 2015 in western Pamlico Sound, North Carolina. Levels not sharing the same letter are significantly different. Letters are color-coded according to the categories shown in the figure legend; letters are not shown for category levels that did not differ significantly.
**Permanent versus temporary residents**

Fishes sampled by minnow pots can be divided into permanent marsh residents and temporary residents (Table 2). Permanent residents include several species of killifish, mostly in the *Fundulus* genus, whereas the temporary residents include many fishes that attain larger sizes and are targeted by recreational (and in some cases commercial) fishermen. This list of harvested species for western Pamlico Sound *Juncus roemerianus* marshes included American eel, silver perch, speckled trout, spot, red drum, striped mullet, and several species characteristic as adults of the rocky reefs on the continental shelf, such as gray snapper.

Total abundance of fishes caught by minnow pots exhibited highly significant patterns in ANOVAs, comparing fish group (resident vs. transient) and site condition and then fish group to distance from marsh edge (Figure 15). Permanent residents were about 6 times more abundant ($P<0.0001$) than transient fishes, and fish abundances at the altered sites were about 30% greater than at the minimally altered sites (Fig 15). In contrast to earlier studies of marsh use by temporary or “transient” fish species, we found that the use of marsh habitat did not differ between permanent and temporary fish species. A 2-way ANOVA showed that the abundance of permanent fish residents did not differ by site condition ($P=0.20$), nor with distance from the estuarine edge ($P=0.15$), with no interaction between these factors. Similarly, the abundance of temporary fish residents did not differ with site condition ($P=0.20$), nor by distance from the estuarine edge ($P=0.21$), with no interaction between these factors. Despite the lack of statistical significance, both temporary and permanent resident fishes displayed mean abundances about 20-30% higher at the 25-m and 50-m interior sampling stations (Fig. 15).

Identically designed ANOVAs evaluating how summed fish biomass differed between permanent and temporary fish species and, first, site condition and, then second, distance from marsh edge (Fig. 16) revealed similar outcomes to the analogous statistical analyses of total fish abundance data. Fish group average biomass values differed, with permanent residents about 10 (minimally altered) to 200% (altered) more biomass than transient fishes ($P<0.0001$) (Fig. 16). Also similar to the fish abundance findings, the biomass of permanent fish species did not differ by site condition ($P=0.07$), nor by distance ($P=0.33$), with no interaction. And the biomass of temporary fish species did not differ by site condition ($P=0.76$), nor by distance ($P=0.39$), with no interaction. Again, permanent residents displayed greater summed biomass than transient fishes and summed biomass was about 10 to 30 times higher at 25-m and 50-m stations than at the two stations nearer the marsh edge (Fig. 16).
Table 2. List of fish species considered to be permanent vs temporary residents of the marshes in western Pamlico Sound, North Carolina

<table>
<thead>
<tr>
<th>Permanent resident fish species</th>
<th>Temporary resident fish species</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Cyprinodon variegatus</em></td>
<td><em>Anguilla rostrata</em></td>
</tr>
<tr>
<td><em>Fundulus confluentus</em></td>
<td><em>Bairdiella chrysoura</em></td>
</tr>
<tr>
<td><em>Fundulus heteroclitus</em></td>
<td><em>Citharichthys spiopterus</em></td>
</tr>
<tr>
<td><em>Fundulus luciae</em></td>
<td><em>Cynoscion nebulosus</em></td>
</tr>
<tr>
<td><em>Fundulus majalis</em></td>
<td><em>Dormitator maculatus</em></td>
</tr>
<tr>
<td><em>Fundulus sp.</em> (juvenile)</td>
<td><em>Gobionellus oceanicus</em></td>
</tr>
<tr>
<td><em>Gambusia affinis</em></td>
<td><em>Gobiosoma bosc</em></td>
</tr>
<tr>
<td></td>
<td><em>Lutjanus griseus</em></td>
</tr>
<tr>
<td></td>
<td><em>Menidia beryllina</em></td>
</tr>
<tr>
<td></td>
<td><em>Micropogonias undulatus</em></td>
</tr>
<tr>
<td></td>
<td><em>Mugil curema</em></td>
</tr>
<tr>
<td></td>
<td><em>Sciaenops ocellatus</em></td>
</tr>
</tbody>
</table>

Figure 15. Summed fish abundance of permanent and temporary fishes as defined in Table 2. Total numbers of fishes are computed for all sampling periods by adding catches from minnow pots across the 4 distances into the marsh for each of the 3 transects.
We used General Linear models (GLMs) across all sampling periods to determine the possible relationship between metrics of fish use from minnow pot data, and both inundation depth and inundation duration. Total fish abundance proved positively related (P<0.001) to mean inundation depth during active sampling of the deployed minnow pot (soak time) at each site, although the relationship explained only 26 percent of the variance (Fig. 17). A similar positive relationship with mean inundation depth was detected for the summed total length of fishes ($r^2 = 0.27$, P<0.001) (Fig. 18) and summed sample biomass of fish ($r^2 = 0.27$, P<0.001) (Fig. 19). The patterns of significance in all three relationships between inundation depth and a metric of fish abundance from minnow pot sampling were driven by results of a single sampling period, fall 2014. This sampling period included the meteorological conditions that facilitated extensive flooding of the marshes of western Pamlico Sound, especially at East Bluff. Analogous GLM analyses revealed similarly weak, yet statistically significant, positive relationships between the number of hours that a given sampling station was inundated with ≥ 2 cm water depth prior to sample collection, and summed fish abundance ($r^2 = 0.03$; P=0.02) (Fig. 20), the summed total length of fishes ($r^2 = 0.03$; P=0.02 (Fig. 21), and summed fish biomass ($r^2 = 0.02$; P=0.03) (Fig 22). Hence, for each of these metrics of fish biomass, mean inundation depth explained a higher percentage of the variance than inundation duration.
Figure 17. GLM of the relationship between fish abundance in minnow pots and marsh inundation depth in western Pamlico Sound, North Carolina, from fall 2013 through spring 2015.

Figure 18. GLM of the relationship between the total length of fishes in minnow pots and marsh inundation depth in western Pamlico Sound, North Carolina, from fall 2013 through spring 2015.
Figure 19. GLM of the relationship between fish biomass in minnow pots and marsh inundation depth in western Pamlico Sound, North Carolina, from fall 2013 through spring 2015.

Figure 20. GLM of the relationship between fish abundance in minnow pots and duration of marsh inundation in western Pamlico Sound, North Carolina, from fall 2013 through spring 2015.
Figure 21. GLM of the relationship between the total length of fishes in minnow pots and duration of marsh inundation in western Pamlico Sound, North Carolina, from fall 2013 through spring 2015.

Figure 22. GLM of the relationship between fish biomass in minnow pots and duration of marsh inundation in western Pamlico Sound, North Carolina, from fall 2013 through spring 2015.
Multivariate analyses

Multivariate (community-level) analyses from the PRIMER statistical package conducted to test for potential statistical significance in species composition differences as a function of distance from marsh edge as compared to site condition (altered vs. unaltered) and to test whether the non-SHA and SHA site designations produced communities of detectably different species compositions yielded compelling visual and statistical results. First, an nMDS on mean abundances of all fishes sampled revealed that the centroid (mid-point point of similarity indices) of community composition by site was virtually overlapping for minimally altered (SHA eligible) and modified (highly altered habitat) communities (Fig. 23). In addition, the graphical distribution of points reflecting community composition reveals that the set of altered sites extends to a detectably wider distribution well beyond the set of sites chosen to represent minimal alteration. In other words, minimally altered sites exhibit less extreme variation in fish composition by abundance in minnow pots. Second, the analysis of similarity among this same set of sites color-coded for distance to marsh edge (Fig. 24) revealed that fish community composition in minnow pots may indeed shift its mean leftwards from marsh edge points (1 m) to subsequently greater distances (10, 25, and 50 m). Even more compelling than inferences on changing centroids of composition space, the variances among fish community composition differ by shrinking from 1 m sites, to sites at 10, 25, and 50 m (Fig. 24).

Figure 23. Non-metric Multi-Dimensional Scaling (nMDS) ordination of the faunal communities sampled at 8 altered and 8 unaltered marsh habitats in western Pamlico Sound, North Carolina, based upon the abundance of each species sampled with minnow pots in 2013 – 2015.
Figure 24. Non-metric Multi-Dimensional Scaling (nMDS) ordination of the faunal communities sampled at 1, 10, 25, and 50 m from the estuarine edge in marsh habitats in western Pamlico Sound, North Carolina, based upon the abundance of each species sampled with minnow pots in 2013 – 2015.
**Figure 25.** Non-metric Multi-Dimensional Scaling (nMDS) ordination of faunal communities sampled in fall and spring in western Pamlico Sound, North Carolina, based upon the abundance of each species sampled with minnow pots in 2013 – 2015.

Three-way PERMANOVA tests of how faunal abundances in minnow pots showed no difference in community assemblages by site condition (P=0.26) (Fig. 23), but these communities differed with distance from marsh edge (P=0.002) (Fig. 24) and with season (fall vs. spring) (P=0.001) (Fig. 25). A 1-way PERMANOVA test showed no distinction between designated SHA and non-SHA sites (P=0.40) (Fig. 26). Analogous 2-way PERMANOVA tests on only the fish abundances in minnow pots revealed a marginally non-significant effect of distance from marsh edge (P=0.068) and no effect of condition (P=0.318). One-way PERMANOVA tests of whether community composition differed in comparing the summed fish abundances in minnow pots from the 13 nominated SHA sites to all 3 nominated non-SHA sites was done six times so that the variance associated with the specific starting points could be displayed by the range in P-values of the outcomes. Of the six randomly altered permutations of the data, only one P-value revealed significance (P=0.038), whereas the other five ranged in P-value from 0.056 to 0.064). Subsequent tests of how these minnow pot fish abundances differed in community composition between the 3 non-SHAs and 3 randomly chosen nominated SHAs was done six times with different randomly chosen sets of 3 SHAs, revealing a single significant outcome (P=0.012) versus 5 non-significant outcomes ranging from P=0.059 to P=0.809.
Figure 26. Non-metric Multi-Dimensional Scaling (nMDS) ordination of faunal communities showing similarities among the communities in 13 marshes sites in areas nominated as SHAs and 3 non-SHA in western Pamlico Sound, North Carolina, based upon the abundance of each species sampled with minnow pots in 2013 – 2015.

Figure 27. Non-metric Multi-Dimensional Scaling (nMDS) ordination of fish communities showing similarity profiles linked by green spheres for the communities in 8 altered and 8 unaltered marsh habitats in western Pamlico Sound, based upon the fish abundance in minnow pots in 2013 – 2015. Labels note whether samples represent nominated SHA or non-SHA marshes.
When this same set of tests on community composition were conducted on total biomass of fauna from minnow pots, the 3-way PERMANOVA test revealed no difference by site condition \( (P=0.24) \) (Fig. 28), yet faunal biomass differed with distance from edge \( (P=0.003) \) with the 10-m stations having significantly lower biomass and less biodiversity than at all other distances, and by season \( (P=0.001) \) with community assemblages in fall being more diverse than those found in spring. The 2-way PERMANOVA tests showed distance from marsh edge to be marginally non-significant \( (P=0.084) \) (Fig. 29), while site condition again was non-significant at \( P=0.114 \). Analogous testing done on only the biomass of fishes from minnow pots produced a marginally non-significant effect of distance from marsh edge at \( P=0.09 \) and a marginally non-significant condition effect with \( P=0.078 \). Subsequent testing of whether the fish communities in 13 nominated SHA marshes differed from the 3 non-SHA marshes demonstrated significance in each of six randomly initiated runs (average \( P=0.025 \)), with all 6 random groupings with P-values ranging from \( P=0.02 \) to \( P=0.031 \) (Fig. 30). Finally, testing to evaluate whether the 3 non-SHAs differed in fish community composition by biomass from the 3 non-SHA marshes done by randomly selecting 6 different combinations of 3 nominated SHA sites produced one significant difference \( (P=0.009) \) and 5 non-significant outcomes \( (P\text{-values ranging from } P=0.070 \text{ to } P=0.313) \), with all six averaging \( P=0.155 \).

**Figure 28.** Non-metric Multi-Dimensional Scaling (nMDS) ordination of the faunal communities sampled at 8 altered and 8 unaltered marsh habitats in western Pamlico Sound, North Carolina, based upon the biomass of each species sampled with minnow pots in 2013 – 2015.
Figure 29. nMDS ordination of the faunal communities sampled at 1, 10, 25, and 50 m from the estuarine edge in marsh habitats in western Pamlico Sound, North Carolina, based upon the biomass of each species sampled with minnow pots in 2013-2015.

Figure 30. nMDS ordination of fish communities showing similarity profiles linked by green spheres for fish biomass in pit traps in 8 altered and 8 unaltered marsh habitats in western Pamlico Sound, in 2013–2015. Labels note whether samples represent nominated SHA or non-SHA marshes.
Marine invertebrates, mostly crab species, dominated faunal sampling using pit traps. The mean abundance of marine invertebrates was significantly greater in unaltered than in highly altered sites ($p=0.03$), yet did not differ with distance from edge ($p=0.10$), with no interaction between these two factors ($p=0.15$) (Fig. 31). The mean total length of marine invertebrates did not differ with site condition ($p=0.31$), nor distance from edge ($p=0.49$), and no interaction between factors emerged during analysis ($p=0.76$) (Fig. 32). As with total lengths, the biomass of marine invertebrates did not differ with site condition ($p=0.27$) or distance from edge ($p=0.16$), and no interaction existed between factors ($p=0.71$) (Fig. 33).

The mean total abundance of terrestrial invertebrates did not differ with site condition ($p=0.91$), nor distance from edge ($p=0.16$), with no interaction between factors ($p=0.58$) (Fig. 31). The mean total length of this group did not differ with site condition ($p=0.70$), nor distance from edge ($p=0.06$), although greatest extreme lengths appeared in interior marsh: no interaction between factors emerged ($p=0.80$) (Fig. 32). Mean biomass of terrestrial invertebrates did not differ with site condition ($p=0.41$), nor distance from marsh edge ($p=0.43$), with no interaction between factors ($p=0.42$) (Fig. 33).

We found no terrestrial vertebrates in pit traps during this study.

**Figure 31.** The mean abundance of organisms sampled with pit traps at altered and unaltered sites (left) and at 1 m, 10 m, 25 m, and 50 m from estuarine edge (right) at marsh sites from September 2013 to June 2015 in western Pamlico Sound, North Carolina. Levels not sharing the same letter are significantly different. Letters are color-coded according to the categories shown in the figure legend; letters are not shown for category levels that did not differ significantly.
**Figure 32.** The mean total length of organisms sampled with pit traps at altered and unaltered sites (left) and at 1 m, 10 m, 25 m, and 50 m from estuarine edge (right) at marsh sites from September 2013 to June 2015 in western Pamlico Sound, North Carolina. Levels not sharing the same letter are significantly different. Letters are color-coded according to the categories shown in the figure legend; letters are not shown for category levels that did not differ significantly.

**Figure 33.** The mean biomass of organisms sampled with pit traps at altered and unaltered sites (left) and at 1 m, 10 m, 25 m, and 50 m from estuarine edge (right) at marsh sites from September 2013 to June 2015 in western Pamlico Sound, North Carolina. Levels not sharing the same letter are significantly different. Letters are color-coded according to the categories shown in the figure legend; letters are not shown for category levels that did not differ significantly.
Multivariate analyses

PERMANOVA tests of how distance from marsh edge and site condition conducted on pit trap faunal abundances revealed similar results to those obtained from analysis of the minnow pot catches. Three-way PERMANOVA tests of how faunal abundances in pit traps showed no difference in community assemblages by site condition (P=0.19) (Fig. 34), but these communities differed with distance from marsh edge (P=0.001) (Fig. 35) and with season (fall vs. spring) (P=0.002) (Fig. 36). Testing of the significance of community composition differences in total faunal abundance from pit traps showed in comparing the 13 designated SHAs to the 3 non-SHAs revealed significance at P=0.007. The similar test of the 3 non-SHA sites against each of 6 random selections of 3 SHA sites also revealed significant differences in community composition in every combination, with a p-value averaging P=0.008 (Fig. 37).

Non-metric MDS

Figure 34. Non-metric Multi-Dimensional Scaling (nMDS) ordination of the faunal communities sampled at 8 altered and 8 unaltered marsh habitats in western Pamlico Sound, North Carolina, based upon the abundance of each species sampled with pit traps in 2013 – 2015.
Figure 35. nMDS ordination of the faunal communities sampled at 1, 10, 25, and 50 m from the estuarine edge in marsh habitats in western Pamlico Sound, North Carolina, based upon the abundance of each species sampled with pit traps in 2013 – 2015.

Figure 36. Non-metric Multi-Dimensional Scaling (nMDS) ordination of faunal communities sampled in fall and spring in western Pamlico Sound, North Carolina, based upon the abundance of each species sampled with pit traps in 2013 – 2015.
Non-metric MDS
Transform: Fourth root
Resemblance: S17 Bray-Curtis similarity
SHA/nonSHA
SHA
nonSHA
2D Stress: 0.15

Figure 37. Non-metric Multi-Dimensional Scaling (nMDS) ordination of faunal communities showing similarities among the communities in 13 marshes sites in areas nominated as SHAs and 3 non-SHA in western Pamlico Sound, North Carolina, based upon the abundance of each species sampled with pit traps in 2013 – 2015.

Analogous 2-way PERMANOVA tests of only the fish communities, based on abundances of each fish species sampled using pit traps, showed no difference by site condition (P=0.88), but differed significantly with distance from estuarine edge (P=0.019), and showed no interaction. One-way PERMANOVA tests of whether community composition differed in comparing the summed fish abundances in pit traps from the 13 nominated SHA sites to all 3 nominated non-SHA sites was done six times so that the variance associated with the specific starting points could be displayed by the range in P-values of the outcomes. All six of the randomly altered permutations of the data revealed no significance with a mean P=0.47; P-values ranged from 0.46 to 0.48 (Fig. 38). Subsequent tests of how these pit trap fish abundances differed in community composition between the 3 non-SHAs and 3 randomly chosen nominated SHAs was done six times with different randomly chosen sets of 3 SHAs, again revealing no significant outcomes with a mean P=0.52 and range of outcomes from P=0.16 to P=0.94.
Figure 38. Non-metric Multi-Dimensional Scaling (nMDS) ordination of fish communities showing similarity profiles linked by green spheres for the communities in 8 altered and 8 unaltered marsh habitats in western Pamlico Sound, North Carolina, based upon the fish abundance in pit traps in 2013 – 2015. Labels note whether samples represent nominated SHA or non-SHA marshes.

When this same set of tests of faunal community composition differences was applied to pit trap biomass data, a 3-way PERMANOVA test of faunal biomass in pit traps showed no difference in community assemblages by site condition (P=0.36) (Fig. 39), but these communities differed with distance from marsh edge (P=0.001) (Fig. 40) and with season (fall vs. spring) (P=0.001) (Fig. 41). Contrasts of faunal community biomass from pit trap data revealed in results of PERMANOVAs that the 3 designated non-SHAs differed from the 13 designated SHAs (at P=0.002) (Fig. 42). This outcome was confirmed through subsequent PERMANOVA on faunal biomass contrasting the 3 non-SHAs to 6 random selections of designated SHAs, each of which displayed significance at an average p-value of P=0.007. Analogous 2-way PERMANOVA tests of only the fish communities, based on biomass of each fish species found in pit traps, showed no difference by site condition (P=0.67), nor differed with distance from estuarine edge (P=0.15), and showed no interaction. One-way PERMANOVA tests of whether community composition differed in comparing the summed fish biomass in pit traps from the 13 nominated SHA sites to all 3 nominated non-SHA sites repeated six times so that the variance associated with the specific starting points could be displayed by the range in P-values of the outcomes. All six of the randomly altered permutations of the data revealed no significance with a mean P=0.88, ranging from 0.86 to 0.89 (Fig. 43). Subsequent tests of how these pit trap fish biomass differed in community composition between the 3 non-SHAs and 3 randomly chosen nominated SHAs was done six times with different randomly chosen sets of 3 SHAs, again revealing no significant outcomes with a mean P=0.46, and a range of outcomes from P=0.17 to P=0.82.
Figure 39. Non-metric Multi-Dimensional Scaling (nMDS) ordination of the faunal communities sampled at 8 altered and 8 unaltered marsh habitats in western Pamlico Sound, North Carolina, based upon the biomass of each species sampled with pit traps in 2013 – 2015.

Figure 40. nMDS ordination of the faunal communities sampled at 1, 10, 25, and 50 m from the estuarine edge in marsh habitats in western Pamlico Sound, North Carolina, based upon the biomass of each species sampled with pit traps in 2013 – 2015.
Figure 41. Non-metric Multi-Dimensional Scaling (nMDS) ordination of faunal communities sampled in fall and spring in western Pamlico Sound, North Carolina, based upon the biomass of each species sampled with pit traps in 2013 – 2015.

Figure 42. nMDS ordination of faunal communities showing similarities among the communities in 13 marshes sites in areas nominated as SHAs and 3 non-SHA in western Pamlico Sound, North Carolina, based upon the biomass of each species sampled with pit traps in 2013 - 2015.
**Figure 43.** nMDS ordination of fish communities showing similarity profiles linked by green spheres for the communities in 8 altered and 8 unaltered marsh habitats in western Pamlico Sound, North Carolina, based upon the fish biomass in pit traps in 2013 – 2015. Labels note whether samples represent nominated SHA or non-SHA marshes.

**Bottomless liftnet sampling**

We found the bottomless liftnets to be extremely cumbersome for working in densely vegetated marshes dominated by *J. roemerianus*, which reached over 1 m in height across several of our study sites. Among our key objectives was to document which aquatic species utilize marsh habitat in western Pamlico Sound; therefore, it was critical that we did not destroy the macrophytic vegetation at our sampling stations. Hence, our ability to sample well the small fishes at inundated sampling stations with dense stands of vegetation was less successful than described in Raposa and Roman (2001). We were successful in maintaining sampling with bottomless liftnets in Yr 1; however, a strong nor’easter storm during the first sampling event in Yr 2 destroyed half of these nets, yielding only a limited dataset for analysis. No fishes were found in the liftnet samples; however, marine invertebrates, mainly *Uca minax* (fiddler crabs) and *Callinectes sapidus* (blue crabs), were present in sufficient numbers to warrant analysis. The Kruskal-Wallis ranks tests of marine invertebrate abundance (*P*=0.22) and total lengths (*P*=0.14) did not differ with site condition; however, marine invertebrate biomass was significantly greater in altered vs. unaltered sites (*P*=0.003). Kruskal-Wallis tests of marine invertebrate abundance (*P*=0.027) was significantly greater at 1 m than for 10 m from the estuarine edge of the marsh sites sampled in Yr 1; however, total lengths for this faunal group did not differ significantly in total lengths (*P*=0.05) or in biomass (*P*=0.22).
Discussion

The US Clean Water Act of 1974 provides a blueprint for the protection of coastal wetlands, driven by the recognition that coastal marshes provide several ecosystem services that sustain water quality by acting as natural buffers that modify and treat waters flowing from land into the rivers, then sounds, and seas. Marshes extract plant nutrients, converting them into marsh macrophyte biomass. They help extract microbial pathogens from the run-off into rivers, estuaries and the sea, reducing exposures to humans. And by physically buffering storm-water flows, coastal marshes suppress erosion while also inducing deposition of suspended sediments and thereby suppressing estuarine sedimentation.

In addition to their role in treating storm water, coastal marshes provide many other ecosystem services as a habitat for many groups of organisms. These include several vertebrates, among which are endangered birds like several rails, endangered reptiles, and several commercially and recreationally valuable fishes and their prey (Greenberg et al. 2006). Coastal marshes also serve as an important carbon sink as they induce organic deposition and sustain carbon burial, thereby leading to sequestration of carbon, preventing it from being re-released into the atmosphere where it would induce further global warming. Accordingly, several reviews of ecosystem services have suggested that coastal marsh has higher value than any other estuarine habitat (MEA 2003). Several of these valuable ecosystem services relate to enhancing fish and fisheries, resulting in the North Carolina CHPP Team’s emphasis on appreciation, protection, and restoration of the coastal marsh habitat. Here we explore some of the values of a type of coastal marsh, Juncus roemerianus-dominated, that is so prominent in northeastern North Carolina along the shores of the broader Pamlico Sound estuary, and is seriously threatened by sea-level rise and increasing frequency of intense storms (Titus and Richman 2001). Despite a rich literature on the functioning of coastal marsh, the vast majority of previous research has targeted Spartina alterniflora marshes, while much still remains unknown about the functioning and ecosystem services provision of Juncus roemerianus marshes.

In one process designed to protect coastal marsh from losses during land development, the USACE holds the responsibility for approving delineation of coastal marsh that is required to be protected by the 401 Section of the Clean Water Act. Surveys are made to identify and map the boundaries of coastal marsh habitat before development begins on a site so as to ensure its protection. Because of rising sea levels and heightened risk of damage and greater flooding from more frequent and more flashy, more intense coastal storms, the shorelines of our nation’s estuaries and sounds are rapidly being hardened by construction of bulkheads, rock revetments, and other engineered structures designed to protect private property (Gittman et al. 2014). Once a hardened shoreline structure has been positioned on shore, it cuts off connectivity between any coastal marsh landward of the structure and the waters of the estuary below (Titus 1998, Peterson et al. 2008). If any functional coastal marsh exists above the bulkhead, its services to the aquatic ecosystem are eliminated, leading to a need for great care in delineating the boundaries of coastal marsh before construction. The guidance from the USACE draws the horizontal line that dictates the closest approach allowed to the estuary for siting a bulkhead at the lower edge of the Juncus roemerianus distribution along classically zoned higher-salinity marsh shorelines. This decision may not reflect the valuable services that Juncus roemerianus provides. Our new data on functioning of broad Juncus roemerianus marshes in lower-salinity regions helps identify perhaps the most important services that would be lost by cutting off such a brackish Juncus marsh from contact with estuarine waters by construction of bulkheads at various distances from the marsh edge and thereby sacrificing Juncus ecosystem services from all positions deeper into the marsh.
The brackish *Juncus roemerianus* marshes occupying broad geomorphic platforms along the western Pamlico Sound shores occupy a different environmental setting from the high-salinity *Juncus roemerianus* marshes that can cover higher ground on otherwise *Spartina alterniflora* marsh shorelines. In other words, the habitat characteristics and functions that we document from this study of *Juncus roemerianus* marshes along western Pamlico Sound may or may not apply well to *Juncus* in other settings. Nevertheless, our demonstration of the depth of penetration by game fish and their bait fish prey into the broad *Juncus* marsh platforms is revealing in itself because the ecosystem services of this type of coastal marsh have not been widely studied or previously quantified and we show high value to fisheries deserving of habitat protection for this marsh system. The topography of these *Juncus* platform marshes is vital to their role in promoting fish production. Specifically, we show that the elevation of the marsh floor is sufficiently constant and low that even positions far from the estuarine shoreline and thus from the sources of fish are regularly used by fish – both game fish themselves as juveniles and forage fishes, especially in the genera *Cyprinodon* and *Fundulus*. Marsh floor elevation dictates depth of water cover and duration of water coverage and thereby directly determines abundances of and uses by fishes. Even at 50 m into the marsh, and by casual inspection at 100 m and even further wherever topographic elevation is low enough, fish can be found making use of the broad *Juncus* marsh (pers. obs., C. Voss). Features that we document here, specifically the high density of *Juncus roemerianus* shoots, the great height of the shoots, often exceeding 1 m, and the unpleasant and dangerous sharp point on the shoot tip, along with an abundance of snakes and occasional alligators and black bears, have inhibited study and suppressed even observation of fish uses in these broad brackish platform marshes. We show that where estuarine waters penetrate, fish are using these marshes, which may lead to additional study of such marshes elsewhere (e.g., Gulf of Mexico).

While the importance of marsh bathymetry and water coverage at some level and for some temporal duration is presumed to be instrumental in allowing fish to access to and use of *Juncus* marsh habitat in these broad platforms (Baker et al. 2013) extending such large distances away from the estuarine edge, we gathered quantitative data and conducted explicit statistical tests of several relationships between alternative water-level metrics and faunal, especially fish, use of this broad marsh habitat. For all fishes sampled by minnow pots, we found by application of General Linear Models (GLMs) that variation in total abundance, mean total length, and mean biomass was better explained by mean water depth during sampling than by mean duration of water depth in excess of 2 cm prior to sampling. These results may imply how water coverage acts mechanistically, for example by suggesting that fish may be advected into the marsh in part passively by physical flow of rising waters as opposed to being attracted behaviorally by stands of more deeply flooded marsh. Nevertheless, conditions of greater marsh flooding in this platform *Juncus roemerianus* marsh system lead to largely correlated hydrologic parameters, such as flooding duration, maximum water height, and mean water height. Each of these hydrologic parameters could be involved in affecting the capacity of fishes to use the habitat and the quantitative numbers of fish present making use of the marsh (e.g., Minello et al. 2013, Baker et al. 2013).

Among the sampled fish, we distinguished permanent residents from transients (temporary residents) on the basis of common knowledge that the killifishes remain in the *Juncus* marsh for long periods of time, perhaps for their life time, whereas the juveniles of game fish and others that attain substantially larger adult size utilize marsh habitat only when young and small, then move ontogenetically out into open waters of the estuary for further growth and development, and ultimately too for reproduction.
The long-term presence of the killifishes in the marsh probably reflects a dual motivation for finding structural refuge from their predators, while also taking advantage of abundant invertebrate prey resources within the *Juncus* marsh. Many studies of fish demography have revealed higher rates of predation on smaller than on larger fishes, which implies strong selection for predator avoidance such as occupying some sort of predation refuge. The abundant use of these *Juncus* platform marshes by juvenile game fishes is also likely to be a reflection of the value of the emergent vegetation as a structural refuge against predators, such as herons, egrets, and rails, all of which we encountered regularly in sampling these *Juncus roemerianus* marshes. Among the transient fishes, several are game fish, such as croaker, silver perch, American eel, speckled trout, red drum, and grey snapper. All game fishes that appeared in our minnow pots were small juveniles, whose use of the *Juncus* marsh habitat is probably dually motivated by predator avoidance within the structural refuges provided by the emergent marsh and by abundant benthic invertebrate prey. Thus, while the game fish ultimately become larger and prey upon *Fundulus, Cyprinodon*, and other bait fishes, they use the *Juncus* marshes largely as protective nursery habitat at sizes when they may be trophic equivalents of the permanent bait fishes. Several temporary residents even represent additional bait fishes, such as silversides, white mullet, and two gobies that may not spend their entire life span within the *Juncus* marsh habitat.

Our new data on aboveground standing stock biomass and shoot density of *Juncus roemerianus* during 2 seasons, fall and spring, in each of 2 years in this brackish, wide marsh platform flooded by wind and other meteorological forces can be contrasted to previous studies of the emergent structure of *Juncus roemerianus* marshes in different types of shoreline habitat and under different hydrological conditions. Aboveground production of plant biomass by *Juncus roemerianus* is high along the entire 50-m transect with a mean standing stock biomass of 1522 g m\(^{-2}\) (1109 g m\(^{-2}\) at 1 m, 1664 g m\(^{-2}\) at 10 m, 1598 g m\(^{-2}\) at 25 m, and 1712 g m\(^{-2}\) at 50 m) in these marshes that extend even further back from the estuarine edge. We were surprised that the aboveground standing stock biomass of all vegetation was significantly greater at the highly altered sites compared to the minimally altered sites (Fig. 9); however, this was likely due to high inorganic nitrogen levels coming from the canals that drain agricultural fields in this region. Apparently, there is a net benefit of agricultural runoff in this region that acts to fertilized marsh macrophytes that sufficiently offsets any negative effects to vegetation resulting from other agricultural chemicals, such as the defoliants used by local farmers in growing cotton crops. As expected from visual observations of marshes dominated by *Juncus roemerianus* (Christian et al. 1990), the standing stock biomass of *Juncus* is differed little with distance from the estuarine edge.

Perhaps the most surprising species of fish included among our samples of fishes using the extensive *Juncus roemerianus* marsh platform habitat of the western shores of Pamlico Sound is the gray snapper, *Lutjanus griseus*. This discovery thereby establishes a functional linkage between rocky reef habitats of the continental shelf and the soft-sediment, vegetated marsh habitats of the far (western) shore of Pamlico Sound. Many coastal marsh habitats include natural hard substrata in the form of oyster reefs, typically at or close to the marsh edge, a coastal habitat of high use and value to demersal fishes (Peterson et al. 2003, Wong et al. 2007), but no such substantial oyster-reef margin appeared in the western Pamlico Sound platform marshes that we characterized and sampled. After using the *Juncus roemerianus* marsh for some of its early juvenile development, gray snappers enter the wide Pamlico Sound and presumably migrate eastward to an inlet, such as Ocracoke Inlet, gaining access to the ocean over the continental shelf of Raleigh Bay. However, Raleigh Bay contains very limited rocky reef habitat as compared its high cover of Onslow Bay and Long Bay to the south. Hence, it is possible that the
juvenile gray snappers must not only navigate across Pamlico Sound to the sea to find adult habitat but also then swim south a substantial distance when reaching the sea. Gray snappers have a second common name, mangrove snappers, implying that the juveniles leaving the platform marshes of *Juncus roemerianus* in western Pamlico Sound may find some suitable vegetation habitat within the sound instead of or in addition to navigating to rocky reefs of the continental shelf. With advancing global warming, further biogeographic changes in mangrove distributions will occur, as has already happened in the northern Gulf of Mexico with widespread invasion first by black mangroves. Gray snappers may be pre-adapted to a warming Pamlico Sound.

Perhaps the most compelling motivation for this study of western Pamlico Sound platform marsh habitat structure, production, and use by fishes especially, but also by other groups of mobile vertebrates and invertebrates, was to help provide information to state government managers charged under the North Carolina Coastal Habitat Protection Plan with protection and enhancement of coastal habitats that directly and indirectly support fish production and propagation. The process is one based upon assembling reliable information on habitat condition and functions by location so that SHAs, Strategic Habitat Areas, could be identified for critical habitats for fishes, including explicitly the wetland edge habitat type that we focused on in this study. Specifically, the North Carolina Division of Marine Fisheries Regional Advisory Committee for Region 2 established Natural Resource Targets (NRTs), so as to include in SHA selection criteria, habitat areas that are known to have a high ecological function based on habitat condition. Among NRT types was wetland edge based upon previous evidence that wetland shorelines serve to provide important foraging and refuge habitat for juveniles of some priority fish species (Deaton et al. 2011). Our fish sampling design and analyses of results revealed that fishes penetrate into the western Pamlico Sound *Juncus roemerianus* marshes at least 50 m from the estuarine edge and that inundation depth, not distance from edge, is the key factor in explaining fish/nekton use of marsh habitat (Figs. 17 – 19). We found that important fishery species, such as red drum (*Sciaenops ocellatus*), speckled trout (*Cynoscion nebulosus*), gray snapper (*Lutjanus griseus*), American eel (*Anguilla rostrata*), and Atlantic croaker (*Micropogonias undulatus*), not just marsh residents, penetrate deep into the inundated marsh when inundated (Figs. 12 - 16) and that inundation depths remained higher away from the estuarine edge as a consequence of lower elevations (Fig. 6 and Appendix I).

During the SHA selection process, the North Carolina Division of Marine Fisheries Regional Advisory Committee for Region 2 established Natural Resource Targets (NRTs), so as to include in SHA selection criteria habitat areas that are known to have a high ecological function. Among NRT types was wetland edge based upon previous evidence that wetland shorelines serve to provide important foraging and refuge habitat for juveniles of some priority fish species (Deaton et al. 2011). Our findings show that fishes penetrate into the marsh at least 50 m from the estuarine edge and inundation depth and duration are important determinants of fish abundance in the platform *Juncus roemerianus* marshes. In these western Pamlico Sound platform marshes, the deepest water depths and longest durations of water coverage are not found at the marsh edge. Instead, the sampling stations 25 and 50 m into the marsh tended to have the deepest and longest inundation as well as highest uses by marsh fishes.

Our findings help resolve questions that arose during the Region 2 SHA selection process about whether PNAs, Primary Nursery Areas, for which fish sampling evidence exists documenting their high value as nursery habitat, should be included as SHAs (Deaton et al. 2011). During the SHA nomination process for Region 2, committee members expressed concern that if some PNAs were nominated as SHAs, the PNAs excluded from SHA designation could be misinterpreted as having less value in their fish support
functions; however, the goals for the selection of PNAs and SHAs differ sufficiently that valuable nursery habitat could exist and be important to manage without being named a SHA. One important reason that some primary nursery areas may persist as non-SHAs is the administrative mandate to limit total habitat area receiving SHA designation.

We performed two sets of analyses designed to test with our sampling data how well the process determining minimally vs. highly altered sites succeeded in allowing tests of fish response to habitat condition in the wide *Juncus roemerianus* marshes of western Pamlico Sound. First, an nMDS on mean abundances of all fishes sampled in minnow pots showed that while the centroid of community composition by site was virtually overlapping for minimally altered (SHA eligible) and modified (highly altered habitat) communities, the highly altered sites displayed much wider variance than those sites experiencing minimal alteration (Fig. 23). Hence, our condition assignment using alteration scores and incorporated in MARXAM modeling of condition appears to be meaningful. Second, our 1-factor PERMANOVA on total abundances of all fishes in minnow pot samples comparing the community composition of the 13 designated SHAs to the 3 non-SHAs revealed an average p-value over the 6 different permutations (replications) of 0.056, implying marginally significant differences between SHAs and non-SHAs. Repetition of this testing process using total fish biomass from these same minnow pot deployments discovered an average significance of the string of 6 different permutations of p = 0.025. Thus, these formal tests of community composition difference at least partially validate the current SHA designation process.
References


South Atlantic Fishery Management Council (SAFMC). 1990. The Atlantic coast red drum fishery management plan, including an environmental impact statement and regulatory impact review. SAFMC, Charleston, SC in cooperation with MAFMC, Dover, Delaware, 106p.


Appendix I.

Mean elevation (mNAVD88) of sampling stations for Yr 1 strata: Otter Creek (stratum# 02), Berry’s Bay (03), Far Creek (04), and Middleton Creek (05) recorded in October 2013, and for Yr 2 strata: North Wysocking Bay (12), Mt. Pleasant (14), Lone Tree Creek (18), and East Bluff (19) recorded in October 2014. All sites were located in western Pamlico Sound, North Carolina, with 1 altered (A) and 1 unaltered control (C) site in each stratum. Each site had 3 transects (A, B, and C) with sampling stations at 4 distances from estuarine edge (1, 10, 25, and 50 m).

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Appendix II.

High-resolution images of digital elevation models for each of the altered unaltered marsh sites within the 8 stratum examined in western Pamlico Sound, North Carolina in 2013 – 2015.