

NATURAL AND ANTHROPOGENIC DRIVERS OF OYSTER REEF COMMUNITY
DYNAMICS

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A thesis submitted to the faculty at the University of North Carolina at Chapel Hill in partial fulfillment of the requirements for the degree of Master of Science in the Department of Marine Sciences.

Chapel Hill
2018

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ABSTRACT

Maxwell L. Tice-Lewis: Natural and anthropogenic drivers of oyster reef community dynamics
in a temperate estuarine context
(Under the direction of F. Joel Fodrie)

Understanding how natural and anthropogenic alterations to local abiotic conditions influence oyster reef distribution and associated community assembly is critical to achieving conservation and restoration goals. Between 2013-2015, we surveyed 6 intertidal and 5 subtidal oyster reefs encompassing salinity gradients in two estuaries, measured salinity and temperature, and culled historical salinity records to determine the relative importance of tidal emersion, salinity, and temperature on oyster communities and multidecadal changes to oyster community variability. We found tidal emersion was the most important driver of differences in oyster density, species richness, and community structure followed by salinity. We found that channel dredging and sea level rise have likely caused a multidecadal trend in rising salinity in the Newport River Estuary, and decreases in spatial community turnover on oyster reefs between Wells (1961) and our surveys. This has resulted in a “coastal squeeze” in oyster community spatial variability and oyster cultivation area.

To my parents and grandparents, who always nurtured my fascination with underwater life and believed in my academic success.

ACKNOWLEDGEMENTS

I first thank Dr. Joel Fodrie, who gave me the opportunity to be a part of the Fodrie lab in 2013, and created a climate of hard work, motivation, and creative thought that allowed me to grow as a scientist and person throughout this process. I thank Dr. Niels Lindquist for his copious knowledge and willingness to provide advice on the relationship between salinity and oyster populations, as well as the history of oyster research and restoration in the Beaufort area. I thank Antonio Rodriguez for his insights into the effects of climate change on the estuarine environment, and his continually positive outlook on my research. I also want to thank Quin Walker, Gray Redding, and Stacy Zhang for their help in the field, and spending hundreds of hours identifying oyster reef fauna.

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CHAPTER 1: ABIOTIC DRIVERS OF OYSTER-REEF COMMUNITY DYNAMICS IN TWO TEMPERATE ESTUARIES

Introduction

Biogenic habitats are among the most ecologically important features in global ecosystems. Species that form biogenic habitats, or foundation species, such as corals and plants engineer complex structures underpinning diverse faunal assemblages. The distribution of foundation species and associated faunal assemblages are regulated by the interactions between spatiotemporally varying abiotic physiological stressors, competition (Connell 1961), predation (Paine 1966), disturbance (Dayton 1971), and facilitation (Bruno et al. 2003). Anthropogenic disturbance threatens ecosystems through direct harvest and use, landscape modifications, climate change, pollution, and resource extraction, which may physically deteriorate biogenic habitats or cause shifts in abiotic conditions thereby changing environmental suitability for the foundation species and associated biota (Lenihan and Peterson 1998). Biodiversity enhances ecosystem functioning, services (Worm et al. 2006) and resilience (Elmqvist et al. 2003). In the face of global losses of biogenic habitats (Beck et al. 2011), it is critical to understand how biogenic habitats and associated communities vary in response to natural abiotic gradients to enhance the efficacy of restoration and conservation efforts and resilience to future environmental perturbations.

In the marine realm, biogenic habitats such as seagrasses, mangroves, coral reefs, and oyster reefs occur in dynamic coastal areas, where abiotic gradients, such as tidal exposure, may vary greatly at multiple temporal and geographic scales. Tidal zonation is a critical component of

shoreline environments that structures communities across both abiotic and biogenic habitat types (Connell 1961, Pennings and Bertness 2001, Fodrie et al. 2014). Natural gradients in salinity are an inherent feature of the estuarine environments (Pritchard 1952) that heavily influence community structure, in habitats such as marshes, oyster reefs, and mudflats (Odum 1988, Wells 1961, Ysebaert and Herman 2002). In North Carolina, Wells (1961) sampled oyster-reef communities across a polyhaline-euhaline salinity gradient, revealing distinct oyster-associated species distribution patterns correlating directly with location along the salinity gradient. Oyster patches in higher salinity areas exhibited higher species richness than areas with lower means and higher variability (Wells 1961, Ortega and Sutherland 1992). Freshwater disturbances, referred to as freshets, occur after intense precipitation events, rapidly altering the salinity throughout an estuary, and can lead to massive die-offs of oyster associated fauna, and even oysters themselves (Wells 1961). These reoccurring events (hurricanes and seasonally varying rainfall) can serve as a reset to the oyster community, allowing oyster-reefs lying close to predator and pest salinity tolerance thresholds a release from those negative biotic interactions (Fodrie et al. 2014, Walles et al. 2016). Wet and dry seasons may have a substantial impact on oyster community structure as a whole; communities may shift differentially in response to interannual precipitation variability depending on their location in the estuary, and community variability among locations may increase or decrease depending on precipitation (Tolley et al. 2006). Eastern oysters (*Crassostrea virginica*) are an ecologically and socially important biogenic reef forming species, occurring from mesohaline to euhaline salinity regimes (Galtsoff 1964), and inhabiting subtidal and intertidal inundation regimes (Baggett et al. 2015). Oyster-reefs are considered among the most diverse biogenic habitats in temperate estuarine waters, providing substrate, refuge, and resources to infaunal and epifaunal invertebrates (including

decapods, non-decapod crustaceans, bivalves, gastropods, porifera, bryozoans, cnidarians, etc.), juvenile and resident fishes, all of which attract larger transient predatory fishes during foraging (Wells 1961, Meyer and Townsend 2000, Lenihan et al. 2001, Lehnert and Allen 2002, Grabowski et al. 2005, Tolley and Volety 2005).

Over latitudinal gradients, eastern oysters exhibit differences in distribution relative to the tidal gradient. In the South Atlantic Bight, oysters are typically intertidal (Byers et al. 2015), while north of North Carolina, oysters are predominantly subtidal in distribution. In North Carolina, both subtidal and intertidal oysters occur in single estuarine systems. Researchers have noted for centuries that in euhaline, lower portions of estuaries, oysters are predominantly intertidal, whereas subtidal oysters are only found in mesohaline areas with more freshwater inflow (Winslow 1886, Grave 1901). The restriction of oyster populations to the intertidal in euhaline regions occurs as a result of largely marine, desiccation intolerant predators and competitors that limit oyster survival at the settlement and early post-settlement life history stages in the subtidal (Mackin 1946, Chestnut and Fahy 1953, Fodrie et al. 2014, Johnson and Smee 2014). Competitors limiting the settlement and recruitment of oysters in subtidal euhaline zones include sessile bivalves, sponges, ascidians, and bryozoans (MacDougall 1943). In fact, the benefit of reduced competition for resources in the intertidal outweighs the costs associated with tidal emersion (Bishop and Peterson 2005). Oyster predators and bioeroders such as *Muricid* oyster drills (Mackin 1946, Chestnut and Fahy 1953), decapods (Grabowski 2004, Grabowski et al. 2008, O'Connor et al. 2008) and Clionid sponges (Wells 1959) are typically marine adapted, possessing higher salinity tolerance thresholds. Despite this knowledge, many oyster restoration efforts (mainly subtidal) have not taken into account the abiotic and biotic limitations to successful oyster-reef sustainability, leading to oyster community failure and in the

worst cases, specifically when carbonate substrates are utilized, creation of nursery habitat for oyster enemies (N. Lindquist, *personal communication*).

Oyster restoration success relies on a combination of long-term reef persistence and the reef's effectiveness at providing desired ecosystem services, such as biogenic habitat formation or shoreline buffering (Baggett et al. 2015). Baggett et al. (2015) determined the three variables most important when evaluating oyster restoration success: Water temperature, salinity, and dissolved oxygen (DO). In a reply, Walles et al. (2016) argued that tidal emersion is at least as important as temperature, salinity and DO. In temperate, shallow estuaries, where temperatures rarely reach fatal extremes and wind-driven circulation dominate, temperature and DO are likely of lessened importance than salinity and tidal emersion in driving oyster reef persistence and functioning (Walles et al. 2016). Despite researchers addressing the role of tidal emersion and salinity in determining oyster-reef distribution since before the 19th century (eg. Winslow 1889), there exists clear contention over these important abiotic drivers in the present day. We asked the question, in two shallow, temperate estuaries, how do oyster abundances, faunal diversity, and community structure vary according to the abiotic variables of temperature, salinity, and tidal emersion? In the shallow temperate estuarine systems studied (less than 2m average depths), it is highly unlikely that DO reaches sufficiently low levels to cause faunal mortality such that we did not include this variable in the study (Kirby-Smith and Costlow 1989). We combined repetitive oyster-reef community sampling and continuous environmental monitoring between 2013-2015 across 11 intertidal and subtidal reefs in two temperate estuaries spanning polyhaline-euhaline salinity gradients to determine the relative influence of temperature, salinity, and tidal emersion.

Materials and Methods

Study sites

Oyster reef sites selected for surveys occur within the Newport River Estuary (NPRE) and North River Estuary (NRE) in Carteret County, NC (Figure 1.1). The NPRE, located north of Morehead City and Beaufort, was selected to revisit habitats originally sampled by HW Wells from 1955 to 1956. The NPRE is a shallow, well-mixed system with an average residence time of 6 days or 12 tidal cycles (Jennings et al. 1970, Kirby-Smith and Costlow 1989). In addition, there is a long history of shellfishing in the upper NPRE, and has been an area of focus for oyster research since the 19th century. The North River Estuary was selected due its reputed oyster productivity (Grave 1904), protection from mechanized harvesting, and five recent cultch plantings (3-5 years old) created by the N.C. Division of Marine Fisheries. No information is available for mixing and exchange rates in the North River. Notably, both study sites are important areas for commercial and recreational oyster harvesting.

In the NPRE, five sites were selected to from most saline and least salinity-variable to least saline and most salinity-variable. The NPRE sites were (site names in parentheses indicate river basin: NP or NR, low numbers are closer to Beaufort Inlet, and tidal regime, “I” for intertidal and “S” for subtidal): Pivers Island (NP1I), Newport River Yacht Club (NP2I), White Rock (NP3I), Cross Rock Intertidal (NP4I) and Cross Rock Subtidal (NP4S). Six sites were selected for sampling in the NRE, encompassing a similar linear distance to Beaufort Inlet as that of the NPRE, such that broad comparisons between the two basins are possible. Sites listed from highest to lowest salinity are Carrot Island Subtidal (NR1S), Carrot Island Intertidal (NR1I), North River Marsh (NR2I), South Artificial (NR3S), North Artificial (NR4S), and North Natural (NR5S). NR1I is a marsh-fringing reef located in the Rachel Carson National Estuarine

Research Reserve, and is naturally occurring. Immediately adjacent to NR1I, and part of the same reef complex, exists a shallow subtidal reef (NR1S). Many adult oysters at NR1S appear to be of intertidal origin, dislodged at some point in time and forming this subtidal continuation of the larger intertidal patch. Upstream, NR2I is a naturally occurring intertidal patch reef within the North River Marsh complex. The first restored subtidal reef in our sampling regime, termed NR3S (NCDMF 2011 cultch planting # 11-011) exists south of the Highway 70 Bridge running over the North River. North of the bridge is the restored subtidal site NR4S, the NCDMF 2010 cultch planting # 10-012. The freshest site, NR5S, is a natural subtidal reef immediately south from the mouth NRE narrows.

Continuous temperature and salinity monitoring

Water temperature and conductivity were monitored continuously (10-minute intervals) in the Newport and North River Estuaries from September 2013 to December 2015 at each of the 9 sampling locations (a single logger was used at locations with both intertidal and subtidal sites). Odyssey brand conductivity and temperature loggers (<http://odysseydatarecording.com>) were used, with a detection range of 3 to 60 mS/cm, accuracy of +/- 3% (0-40.2 ppt range). Loggers were suspended 0.25 m off the bottom immediately adjacent to reef sites within perforated 2" diameter PVC pipes anchored in the sediment.. Data was downloaded in the field monthly from November 2013 – June 2014 and bimonthly from August 2014 – December 2015. After each download, loggers were calibrated using three KCl standards (0.1M, 0.3M, and 0.5M), representative of the natural salinity range found in estuarine systems (Dataflow Systems Ltd, personal communication). A calibration run was completed with fouling organisms intact to assess the degree of calibration drift that had occurred over the logging period. After this run, all

fouling organisms were scraped from the logger housing and an additional calibration run was completed to serve as a beginning reference for the subsequent salinity sampling period.

Raw conductivity data (mS/cm) were converted from conductivity to salinity (ppt) using the appropriate algorithms developed by Fofonoff and Millard (1983) and integrated into Microsoft Excel by Douglass (2010). Calibration drift was assumed to have begun immediately following deployment and ending at logger retrieval. Raw salinity was corrected for linear drift using calibration curves from before and after deployment.

Oyster-reef sampling

To assess oyster abundances and the structure of associated faunal assemblages, each site was sampled bimonthly beginning in August of 2013 and ending December of 2015, using the methodology of Grabowski et al. (2005). To sample a reef, three 0.1m² quadrats (0.325 m x 0.325 m) were thrown haphazardly. An aluminum core was placed in the middle of each quadrat to 10cm past the surface of the mud layer, and the contents were placed in plastic woven sandbags. Shell material within the quadrat surrounding the core was excavated to the anoxic mud interface and sieved in the field. Oysters, mussels, and barnacles were counted in the field. Large, mobile fauna within the field sieve were placed in plastic bags, brought to the lab and frozen for later sorting and enumeration. These frozen samples excluded amphipods, isopods, and sessile fouling organisms, which were included in the core samples. Core samples were brought to the UNC-CH Institute of Marine Sciences for sorting and enumeration. Core samples were sieved in water tables, placed in salt water filled jars, and refrigerated at ~40°C until sorting could be completed, no longer than one month post-collection. This method of sample storage was chosen for two reasons: A number of fauna attach to the shells of oysters, and freezing samples would render these organisms too fragile for enumeration. Second, fauna living within

porous bioeroded shells will exit the shell or become exposed when dissolved oxygen becomes depleted in the jar (S. Fegley, personal communication). In quadrat and core samples, all fauna visible without microscopy were identified to the lowest possible taxonomic level using appropriate field guides, dichotomous keys, and online resources. Fauna not immediately identifiable, often due to high within-sample diversity and density, such as amphipods, were fixed in 10% formalin solution and transferred to 70% ETOH for long-term preservation and later enumeration. Additionally, microscopic photography using a Leica EC4 camera was utilized to aid in identification. All fauna were counted and identified to the lowest possible taxonomic level, abundances were recorded and standardized to 0.1m². Oysters in core samples were measured (no more than 20 oysters) from the hinge to the outer edge of the right valve and meats were dried and weighed. Response variables inferred from these methods include species richness and evenness, abundance, oyster length, and oyster biomass.

Statistical analyses

To test if temporal trends in salinity existed over the 2013-2015 sampling periods, four linear regression models were run using temperature (°C) as the independent variable and salinity (ppt) and salinity standard deviation (ppt) as dependent variables, for each estuary. Salinity and temperature values used were calculated as the mean of all salinity and temperature measurements in the 1-month leading up to each oyster-reef sample.

To test the question, do oyster density and species richness vary between intertidal and subtidal reefs and over estuarine salinity gradients, we employed univariate Mann-Whitney U tests for each salinity regime (low, 0-23 ppt; mid, 24-29 ppt; high, 30-35 ppt) and tidal emersion regime (intertidal or subtidal) and *post-hoc* Mann-Whitney U tests with Bonferroni correction to test for pairwise differences between factorial combinations. Mann-Whitney U tests were

employed because pooled oyster density and richness data did not meet ANOVA assumptions for equality of variances (Welch's test, $p < 0.05$) and normality.

To evaluate any potential relationships between species richness and living oyster abundance, separate linear regressions were conducted for intertidal and subtidal samples.

All univariate statistical analyses were completed using the base R statistical software and JMP Pro 13.0 (SAS).

Non-metric dimensional scaling (NMDS) ordination was conducted to explore patterns in the data related to temperature (season), salinity and tidal emersion factors. NMDS is an ordination technique based on Bray-Curtis dissimilarity matrices and is a valuable tool for exploring relationships between biological communities that may be structured by environmental gradients (Clarke and Warwick 2001). Abundance data were 4th-root transformed to down-weight the occurrence of common species (Clarke and Warwick 2001). An NMDS plot was determined acceptable for use if stress values were > 0.2 .

Envfit analysis was used to determine correlations between community data and abiotic variables. Variables found to be significantly correlated with the community data were included in subsequent tests for differences between factor groups. Differences in communities based on salinity and tidal emersion were tested with one-way Analysis of Similarities (ANOSIM). After the initial test, *post-hoc* ANOSIM tests were used to test differences between individual salinity-tidal emersion factor combinations. SIMPER analysis was utilized for salinity-tidal emersion factors, which calculates dissimilarity percentages between treatments and percent contribution of individual species to those dissimilarities. Multivariate statistical tests and ordination, including ANOSIM, SIMPER tests and nonmetric multidimensional scaling were completed in PRIMER (Plymouth Routines in Multivariate Ecological Research) Version 6.

Results

Salinity and temperature

Continuous salinity measurements from September 2013 – December 2015 revealed the expected salinity gradients in the Newport and North Rivers; sites closer to Beaufort Inlet have higher salinity means and lower standard deviations (Table 1.1). Salinity variance displayed a significant negative relationship with mean salinity at a given site ($F=129.8$, $p<0.001$) (Figure 1.2). In the Newport River, higher temperatures correlated with higher salinities ($F=6.069$, $p=0.0166$) (Figure 1.3a) and lower variability (standard deviation) ($F=6.091$, $p=0.0165$) (Figure 1.3b), indicating higher salinities in summer months and lower salinities in winter months. The North River showed no significant temporal trend in salinity mean ($F=0.6694$, $p=0.4159$) (Figure 1.3c) or standard deviation ($F=0.6694$, $p=0.4159$) (Figure 1.3d).

Oyster densities

Across both the Newport and North Rivers, oyster densities were higher on natural intertidal reefs than on subtidal reefs in all salinity regimes (Mann-Whitney, $Z=-9.8$, $p<0.0001$). Intertidal low salinity (<20 ppt average salinity) oyster-reefs supported 41.2% lower oyster densities ($128.36/0.1\text{m}^2$) than reefs at intermediate salinity regimes ($194.96/0.1\text{m}^2$) (Mann-Whitney, $Z=2.19$, $p=0.029$). Oyster densities on high salinity intertidal reefs ($146.67/0.1\text{m}^2$) were 28.27% lower and 13.31% higher than densities at intermediate and low salinity locations respectively (Figure 1.4). Subtidal oyster densities at low salinities and at intermediate salinities were similar. Subtidal high salinity oyster densities were significantly high than intermediate salinity subtidal densities ($Z=-4.55$, $p<0.001$) as well as low salinity subtidal densities ($Z=-3.41$, $p=0.0006$) (Figure 1.4). Oyster abundance on intertidal reefs showed no significant relationship

with mean salinity (Figure 1.8a). Subtidal oyster-reefs also showed no significant trend in oyster abundance with salinity (Figure 1.8b). *Species richness*

In the Newport and North Rivers, intertidal oyster-reef species richness varied very little between low, intermediate, and high salinity regimes (Figure 1.5). On subtidal oyster reefs at high salinities, species richness was highest of any salinity and tidal emersion regime, averaging 20.08 species/0.1m². Intermediate salinity, subtidal oyster-reefs were significantly less diverse than high salinity subtidal reefs (Wilcoxon, $Z=-3.796$, $p=0.0001$) and significantly more diverse than low salinity, subtidal reefs (Wilcoxon, $Z=-4.039$, $p<0.0001$). High salinity subtidal reefs were also significantly more diverse than low salinity subtidal reefs over the course of the study (Wilcoxon, $Z=-5.034$, $p<0.0001$). No significant correlations were found between 1-month pre-sample averaged salinity and species richness on intertidal reefs (Figure 1.8c). Subtidal reef species richness displayed a significant positive linear relationship with salinity ($F=21.92$, $p<0.0001$) (Figure 1.8d).

Oyster density was a poor predictor of species richness in intertidal oyster-reef settings across salinities (Figure 1.6a). However, in subtidal settings, there was a strong linear correlation between oyster density and species richness ($F=17.85$, $p<0.0001$) (Figure 1.6b).

Faunal composition

In total, 273 species were collected and identified from August 2013 – December 2015, comprising 12 phyla. Major groups found on oyster-reefs in this study include errant polychaetes (Subclass: Errantia; 29 species), sedentary polychaetes (Subclass: Sedentaria; 25 species), bivalves (Class: Bivalvia; 54 species), anthozoans (Class: Anthozoa; 6 species) fishes (Class: Actinopterygii; 12 species), decapods (Order: Decapoda; 35 species), amphipod crustaceans (Order: Amphipoda; Infraorders: Gammaridea, Corophiida, Caprellida, Talitrida; 22 species),

isopod crustaceans (Order: Isopoda; 5 species), gastropods (Class: Gastropoda: 26 species), barnacles (Class: Maxillopoda: 6 species, Hexanauplia; 1 species), oligochaetes (Order: Haplotaxida; 1 species), Collembola (1 species), porifera (Class: Demospongiae; 3 species), and bryozoans (Gymnolaemata; 4 species). Other groups were collected in this study, but not typically common included sea spiders (Class: Pycnogonida), peanut worms (phylum: Sipuncula), flatworms (Class: Rhabditophora), and ascidians (Class: Ascidiacea).

Community structure response abiotic gradients

NMDS analysis revealed natural groupings based on abiotic environmental variables. Communities showed no obvious grouping based on season (Fall, Winter, Spring, Summer) and envfit showed no significant temporal correlation ($R^2=0.008$, $p=0.899$). There was a strong grouping of communities based on tidal emersion regime (intertidal or subtidal) and envfit revealed a strong correlation between the tidal emersion vector and the community data ($R^2=0.37$, $p<0.001$). Dispersion between samples was more pronounced in subtidal oyster-associated faunal communities than with intertidal communities, suggesting intertidal communities were more similar to one another. NMDS ordination by salinity bin (low, mid, high) revealed clustering of communities based on salinity differences (Figure 1.7) and envfit salinity correlation was significant ($R^2=0.34$, $p<0.001$). Ordination based on a combined salinity and exposure factor guided subsequent multivariate analyses to understand differences in community structure across estuarine salinity gradients between tidal emersion regimes. Across group combinations of salinity and tidal emersion, community structure varied significantly (ANOSIM, global $R=0.66$ $p>0.01$). Subsequent pairwise comparisons (*post-hoc*) reveal significant differences in community structure between all combinations except for high salinity and mid salinity intertidal reefs (ANOSIM, global $R=0.042$, $p=0.285$). Three species

characterized the high salinity intertidal, polychaetes, *Maryphysa sanguinea*, *Cirratulus grandis*, and the bivalve, *Sphenia antillensis* that were not abundant in the mid salinity intertidal, while mid salinity intertidal reefs were characterized by just one species not abundant in the high intertidal, the polychaete *Nereiphylla fragilis* - these faunal differences were insufficient for statistically significant differences in community structure. All pairwise comparisons were significant at the $p < 0.001$ level, except for the comparison between low salinity intertidal and mid salinity intertidal reef community structure, for which $p = 0.008$.

SIMPER analysis for oyster-reef communities pooled by exposure regime (intertidal, subtidal) reveals intertidal sites were on average, 74.43% dissimilar to subtidal sites (Table 1.2). Excluding Porifera and Bryozoa (presence/absence), on intertidal reefs across salinity regimes, 14 species accounted for 90% of the cumulative similarity, listed in order of percent contribution: *Alitta succinea*, *Eurypanopeus depressus*, *Panopeus herbstii*, *Geukensia demissa*, *Boonea impressa*, *Balanomorpha* spp., *Brachidontes exustus*, *Anurida maritima*, *Loxothylacus panopeae*, *Polydora websteri*, *Melita nitida*, *Marphysa sanguinea*, *Amphipod* spp., and *Sphaeroma quadridentata*. Across salinities on subtidal oyster locations, 24 species account for 90% of the cumulative similarity, listed in order of percent contribution: *Alitta succinea*, *Marphysa sanguinea*, *Hydroides dianthus*, *Corophiid* spp., *Urosalpinx cinerea*, *Amphipod* spp., *Panopeus herbstii*, *Eurypanopeus depressus*, *Balanomorpha* spp., *Alpheus heterochaelis*, *Dyspanopeus sayi*, *Gobiosoma bosc*, *Mercenaria mercenaria*, *Mytilid* sp., *Boonea impressa*, *Polydora websteri*, *Sedentaria* sp., *Terebellid* sp., *Amphibalanus eburneus*, *Crepidula fornicata*, *Anomia simplex*, *Diodora cayenensis*, *Doriopsilla pharpha*, and *Rhepoxynius epistomium*.

One-way ANOSIM showed temperature to be an insignificant factor in structuring oyster-reef communities in this study.

Discussion

Our results indicate that across wide-ranging salinity gradients and between intertidal and subtidal regimes, substantial differences exist in oyster densities, faunal richness, and community composition. Oysters had much higher densities on intertidal than subtidal regimes. In the intertidal, intermediate salinities appear to offer the ideal conditions for high oyster densities, likely due to fewer predators and competitors capable of tolerating the environmental extremes of the intertidal zone. However, relatively high densities were found across salinities at intertidal reefs. Conversely, subtidal reef densities were highest when augmented by dislodged shell from adjacent intertidal areas, the example being at NR1S (eroded from NR1I). Over the polyhaline-euryhaline salinity gradient sampled in this study, only NP4S showed evidence of long-term subtidal oyster persistence. Oyster-reef community structure was most strongly affected by tidal elevation, secondarily by salinity regime, and temporal trends were insignificant, such that in temperate, shallow systems, managers should primarily consider tidal emersion, salinity and their interaction when determining sites for oyster restoration.

In the context of oyster-reef restoration, our results offer insights into the relative importance of environmental factors deemed critical in Baggett et al. (2015) and Walles et al. (2015) for consideration when planning and monitoring oyster restoration. Baggett et al. (2015) argues that water temperature, salinity, and dissolved oxygen are universal environmental variables to be used to evaluate the potential efficacy of a restoration project, and Walles et al. (2015) argued for the inclusion of tidal emersion into this framework. Data in this study are largely in agreement with Walles et al. (2015) that tidal emersion and salinity are critical factors in determining oyster-reef dynamics.

In the NRE and NPRE basins, tidal emersion and then salinity were the most important variables in determining oyster density, richness, and community structure. Higher oyster densities on intertidal reefs (mean: 148.55 oysters/ 0.1m²) versus on subtidal reefs (mean: 27.99 oysters/ 0.1m²) is not unusual in the study region. Powers et al. (2009) observed a similar differences in oyster density between intertidal restored reefs in Back Sound, NC and subtidal restored reefs in the Neuse-Pamlico varied substantially between intertidal and subtidal reefs, regardless of salinity; intertidal reefs supported higher densities than those in the subtidal. The intertidal oyster densities in our study are similar to those reported in other NC intertidal oyster surveys in the literature (Grabowksi et al. 2005, Powers et al. 2009, Zeigler et al. 2017, Hanke et al. 2017).

An inherent goal in restoring oyster populations is to achieve high densities, or abundances that successfully recruit future generations. Our results suggest that salinity and tidal emersion are both essential factors in determining oyster density patterns and that there is a strong interactive effect between tidal emersion and salinity. Between tidal emersion regimes, oyster densities showed differing patterns regarding positioning along the salinity gradient. Although intertidal mid salinity oyster densities were higher than those at low salinities, the low salinity intertidal reef (NP4I) in our survey displayed high densities relative to those in the subtidal NP4S nearby. High oyster densities on the high salinity subtidal reef at NR1S were likely skewed by subsidies from the adjacent intertidal reef. We could not determine with certainty which oysters originated in the intertidal or subtidal at this site, however, boring sponge and oyster drills likely exert sufficient top down pressure to limit recruitment (Fodrie et al. 2014), and for this reason, it would be unwise to place subtidal oyster restoration efforts in euhaline environments, even if natural scattered subtidal reefs exist nearby. Subtidal reefs must

be in a sufficiently low and variable salinity regime, such as that at NP4S, where oysters were entirely recruited from subtidal origin, to escape negative influences of marine adapted pests (Wells 1961, Fodrie et al. 2014). While densities were lower (25.07 oysters/m² on average) at NP4S than on NP4I, these reefs show evidence of long-term persistence and provide a habitat function for species with tolerance for salinity variability, such as Xanthid crabs, Melitid and Corophiid amphipods, the oyster-commensal polychaete, *Polydora websteri*, and the Nereid worm, *Alitta succinea*.

Tidal emersion had an interactive effect with salinity regime on species richness, in that the strength of the salinity effect was dependent upon whether a reef was intertidal or subtidal. In the intertidal, tidal emersion dampens the expected influence of salinity on species richness, which is that high salinity environments are less stressful to largely marine adapted oyster reef associated fauna, resulting in higher species richness and trophic complexity (Wells 1961, Odum 1988). Species adapted to the high stress of the intertidal may display resistance to multiple stressors, such that species assemblages in highly stressful environments resemble one another (Vinebrooke et al. 2004). This is potentially occurring on intertidal oyster reefs, where adaptations of intertidal organisms enhance their ability to cope with intertidal stressors, such as desiccation and heat variability.

While richness varied little between intertidal reefs in different salinities, community structure varied significantly between high and low salinity and between mid and low salinity intertidal communities. While these differences were low in magnitude relative to variability across subtidal salinity regimes, the presence of differences indicates salinity plays a role, albeit smaller than tidal emersion, in structuring intertidal communities. Studies in the literature considering oyster reef community variability over salinity gradients have been conducted on

subtidal reefs, so there is little known about the individual and interactive effects of salinity and tidal emersion on oyster community. In effect, the stress of intertidal environments is stronger than that of salinity in ordering oyster reef species assemblages, oyster density and species richness.

Surprisingly, temporal variation did not correlate with patterns in oyster community structure in the NRE and NPRE. We rank temperature as the third most important abiotic variable in regulating oyster reef community dynamics, due to the lack of temporal and community correlation as well as qualitative observations regarding a lack of temperature extremes in the study region. While temperature surely plays a role in the timing of individual species spawning events, including oysters, community level variation was not detectable in our analysis. Wells (1961) described temporally characteristic communities on NPRE oyster reefs for winter and summer months. Of the 11 winter and 19 summer “sub-community” species documented in Wells study, only four species were detected in our modern study, each considered very rare occurrences. Temporal variability may be more important on subtidal oyster reef communities, as the larger species pools are capable of expressing more variability. This is reflected in Wells account of seasonal community variability; the most common winter inhabitant, *Tubularia crocea*, a subtidally occurring hydroid, provided secondary habitat and food for multiple amphipod species, a nudibranch species, and *Mytilus edulis*, the blue mussel. Such temporally defined communities likely exist on oyster reefs today and are certainly relevant to understanding oyster community variability, however, our analysis reveals it is likely more important to study within specific tidal emersion-salinity regime contexts.

Temperature variability in our study region rarely reaches the extremes necessary to cause oyster mortality. Where in the Northeast, ice scour prevents intertidal oysters from thriving

through winter months, constricting oyster distributions to subtidal environments, temperatures in the NC study region are not low enough to cause this phenomenon. High summer temperatures can enhance transmission of oyster pathogens *Haplosporidium nelson* (MSX) (Andrews 1966) and *Dermocystidium marinum* (Dermo) (Andrews and Hewatt 1957), but there was no evidence of oyster mortality from these diseases in our collections. In line with Walles et al. (2016), we maintain the notion that temperature extremes are unlikely to play a major role in broad scale oyster success in temperate regions, and that tidal emersion and salinity are the most critical abiotic variables for managers considering oyster restoration and conservation.

DO is likely a less significant variable affecting oyster reef ecology in our shallow well-mixed system, but this factor is potentially of great importance in deeper estuaries. For instance, in the Neuse River, where depths may reach 6m or more, subtidal reefs with crests deeper than 3m are likely to experience periods of hypoxia or anoxia during summer months when surface algal production and bottom water bacterial respiration are high (Lenihan and Peterson 1998). Even in these deeper systems where low oxygen bottom layers commonly occur, tidal emersion is a primary factor, as hypoxia is only an issue in the subtidal (Walles et al. 2016). Salinity may be considered less important than DO as a subtidal reef may be restored in the proper mesohaline salinity regime, but placed at depths where low DO causes significant mortality.

Variations in salinity have been shown to influence community structure (Tolley et al. 2006) and species-specific responses (Wells 1961, Kimbro et al. 2017). The NPRES exhibited a stronger association between salinity and temperature than the NRE, indicating that stronger temporal salinity trends are present in the NPRES, which may influence temporal variability in community structure (Wells 1961, Tolley et al. 2006). Outflow from the NRE is lower than that of the NPRES (Grave 1904), which helps to explain the differing temporal salinity trends. This

was supported by our salinity data; there was a ~4.5ppt difference in mean salinity and ~2ppt difference in standard deviation, NP4S fresher and more dynamic than NR5S despite being 2.7km closer Beaufort Inlet. These salinity differences were apparent in our collections of oysters and associated fauna. At NR5S and NP4S, the freshest subtidal sites (Table 1.1), live oysters and shell material contained the complex galleries characteristic of previous *Cliona* infestation and at NR5S, dead shells from oyster drills, *Urosalpinx cinerea* and *Eupleura caudata* were regularly found.

Because we were interested in observations across contiguous salinity gradients, we were not able to control for reef size, height, shape, or complexity, as these factors are influenced by estuarine gradients in salinity and tidal exposure that informed site selection. However, we do recognize the importance of spatial factors and these reef characteristics. The size, height, and shape of oyster-reef structures can alter the physical flow regime of the local environment, which facilitates growth and settlement of future generations (Lenihan 1999). In addition, the structural complexity of a reef is of fundamental importance, affecting interspecific interaction strength (Grabowski 2004); higher complexity resulting in greater size distributions of individual species (Margiotta et al. 2016) and potentially higher species richness. Furthermore, in intertidal oyster habitats, reef elevation determines reef expansion rates; there exists an intertidal elevation where reef growth is optimal, likely by the general exclusion of dessication intolerance of oyster predators and competitors while having sufficient submersion for resource acquisition (Ridge et al. 2015). Thus, elevational differences among the intertidal reefs may have contributed to the observed differences in oyster density. In fact, the patch reefs at NP3I and NR2I showed highly compacted shell and accumulated sediment at the reef center, indicating these reefs may have reached elevations greater than that of the optimal growth zone (Bahr and Lanier 1981, Ridge et

al. 2015). Densities at these two reefs were lower than those at NP1I and NP2I, but were higher than NP4I, indicating densities were not significantly reduced by high elevation at the whole-reef scale. Elevation may have played a role at NP4I, in contributing to the high densities of *Geukensia demissa*, which were often more abundant than the oysters themselves. This was particularly so at the crest of this reef (M. Tice-Lewis *personal observation*). In North Carolina, Hanke et al. (2017) determined major oyster-reef taxa displayed community responses to differing reef edge distances and species-specific responses to reef size. *Brachidontes exustus*, *Geukensia demissa*, *Eurypanopeus depressus*, and *Panopeus herbstii* were all more abundant at the farthest edge distance, and *G. demissa* were significantly more dense on reefs with widths of 5 meters.

The strong association between salinity and species richness on subtidal oyster-reefs is an important consideration for fisheries management and is consistent with the literature (Wells 1961, Tolley et al. 2005). As oysters provide refuge for a high diversity of soft bodied invertebrates (Wells 1961, this study), they are highly suitable foraging grounds for juvenile and adult fish species, including those of recreational and commercial value (Lenihan et al. 2001). While the single euryhaline subtidal reef (NR1S) was the most diverse site by a wide margin, the relative area of high salinity subtidal reefs compared to intertidal reefs in the NRE and NPRE is small, but may play important ecological roles than suggested by spatial scale. Shallow subtidal reefs in high salinity areas may eventually succumb to predators and bioeroders, but in certain systems could be continuously or periodically replenished by intertidal subsidies. In a restoration context however, euhaline, subtidal reefs are ill-advised ventures.

Oyster restoration projects should be tailored such that placement is optimized to maximize performance toward the specific restoration objectives (Baggett et al. 2015).

Theoretically, subtidal oyster reefs may have a higher potential to augment biological communities than those in the intertidal, because relatively few marine species are adapted for tolerating the extreme environmental insult of an intertidal existence (as observed at NR1S). For instance, fish use of subtidal reefs is increased over that of intertidal reefs, because resident fish may remain on subtidal structures throughout the tidal cycle, whereas intertidal fish assemblages are largely transient in nature (Lehnert and Allen 2002). However, across salinity regimes, our results suggest that intertidal reef restoration yields more diverse community assemblages than those in low salinity subtidal environments, where subtidal reef restoration efforts are most likely to succeed. Although the most species rich assemblages in our study occurred in subtidal high and intermediate salinity areas, those reefs are largely transient efforts, as NC-DMF has largely ignored the qualitative and quantitative knowledge that salinity thresholds limit subtidal oyster distribution.

A deep understanding of factors affecting oyster reef persistence and oyster-reef faunal assemblages in restoration target areas is critical for the success of restoration efforts (Baggett et al. 2015, Walles et al. 2016). The relevant abiotic factors for specific oyster restoration and conservation focal areas are likely to be different along latitudinal gradients, and these factors should be studied and understood by managers and practitioners to maximize successful attempts at restoration.

Table 1.1. Sampling locations in the Newport and North River estuaries. Mean and standard deviation of salinity, measured in parts per thousand, were calculated with continuously logged salinity (10-minute intervals) from September 2013-December 2015.

River	Site	Latitude Longitude	Mean Salinity (ppt)	Standard deviation of Salinity (ppt)
Newport	Cross Rock Intertidal (NP4I)	34°45.107'N 76°40.567'W	18.46465709	8.265350276
Newport	Cross Rock Subtidal (NP4S)	34°46.107'N 76°40.567'W	18.46465709	8.265350276
Newport	White Rock Replacement (NP3I)	34°46.143'N 76°40.580'W	22.67329856	6.609997913
Newport	Yacht Club (NP2I)	34°46.143'N 76°40.580'W	25.40386569	5.553255296
Newport	Pivers Island (NP1I)	34°43.263'N 76°40.551'W	31.76738418	3.513213165
North	North Natural (NR5S)	34°48.353'N 76°37.162'W	23.16618036	6.554795572
North	North Artificial (NR4S)	34°47.727'N 76°36.573'W	26.27751036	5.642571388
North	South Artificial (NR3S)	34°46.624'N 76°36.583'W	28.51026454	5.342901763
North	North River Marsh (NR2I)	34°43.124'N 76°36.497'W	29.61960021	3.557393213
North	Carrot Island Intertidal (NR1I)	34°42.175'N 76°38.235'W	32.26905878	3.468236913
North	Carrot Island Subtidal (NR1S)	34°42.180'N 76°38.254'W	32.26905878	3.468236913

Table 1.2. SIMPER analysis results showing the major species and species groups responsible for community similarities within groups. Numbers denote the average density of each species.

Average abundances of species accounting for 90% similarity within Salinity-Exposure groups.						
Species	Low Salinity Intertidal	Mid Salinity Intertidal	High Salinity Intertidal	Low Salinity Subtidal	Mid Salinity Subtidal	High Salinity Subtidal
Cnidarians						
<i>Actiniaria sp.</i>						0.31
Errant polychaetes						
<i>Alitta succinea</i>	2.22	2	1.6	1.52	1.45	1.43
<i>Nereis falsa</i>						0.74
<i>Nereiphylla fragilis</i>		0.67				
<i>Oxydromus obscura</i>					0.68	
<i>Harmothoe aculeata</i>						0.47
<i>Marphysa sanguinea</i>			0.82	0.59	1.41	1.24
<i>Lumbrineris sp.</i>						0.78
Sedentary polychaetes						
<i>Amphitrite ornata</i>						0.33
<i>Terebellid sp.</i>					0.74	0.72
<i>Cirratulus grandis</i>			0.78			
<i>Hydroides dianthus</i>					3.16	1.29
<i>Sabellaria sp.</i>						1.1
<i>Polydora websteri</i>	1.78	1.48	0.53	1.05		
<i>Piromis eruca</i>						0.53
<i>Sedentaria sp.</i>					0.79	0.6
Decapods						
<i>Alpheus heterochaelis</i>					0.78	0.96
<i>Eurypanopeus depressus</i>	1.5	2.04	1.58	0.45	1.17	0.59
<i>Panopeus herbstii</i>	1.03	1.49	1.43		0.9	1.32
<i>Dyspanopeus sayi</i>					0.72	0.64
<i>Menippe mercenaria</i>						0.41
<i>Pinnixa chaetoptera</i>						0.41
<i>Megalobrachium soriatum</i>						0.34
Amphipods						
<i>Amphipod spp.</i>	1.34		0.66	0.81	1.08	1.66
<i>Melita nitida</i>	1.86	1.25				
<i>Rhepoxynius epistomus</i>						1.06
<i>Corophiid spp.</i>				1.55	1.48	0.76
Isopods						

Continued on next page.

Table 1.2 continued.

Average abundances of species accounting for 90% similarity within Salinity-Exposure groups.						
Species	Low Salinity Intertidal	Mid Salinity Intertidal	High Salinity Intertidal	Low Salinity Subtidal	Mid Salinity Subtidal	High Salinity Subtidal
<i>Sphaeroma quadridentatum</i>	2.32	1.02				
Tanaids						
<i>Tanaid sp.</i>						0.65
Barnacles						
<i>Amphibalanus eburneus</i>				0.67		
<i>Balanomorpha spp.</i>	1.09	0.88	0.89	1.52		0.97
<i>Loxothylacus panopeae</i>	0.71	1.23	0.59			
Bivalves						
<i>Anadara transversa</i>						0.33
<i>Anomia simplex</i>					0.58	0.36
<i>Brachidontes exustus</i>		0.93	1.12			0.52
<i>Geukensia demissa</i>	1.58	1.51	0.87			
<i>Mytilid sp.</i>	2.64	2.63	2.16	0.71		0.9
<i>Sphenia fragilis</i>			0.44			0.81
<i>Mercenaria campechiensis</i>				0.34		
<i>Mercenaria mercenaria</i>				0.58		
<i>Leiosolenus bisulcata</i>						0.71
<i>Anurida maritima</i>		1.88	1.1			
Gastropods						
<i>Pyrgocythara plicosa</i>					0.45	
<i>Seila adamsi</i>						0.62
<i>Urosalpinx cinerea</i>				0.37	1.2	0.84
<i>Boonea impressa</i>	1.8	1.84	1.19			0.96
<i>Chaetopleura apiculata</i>						0.82
<i>Crepidula fornicata</i>						0.61
<i>Diodora cayenensis</i>					0.36	0.8
<i>Doriopsilla pharpa</i>					0.57	0.52
Fish						
<i>Gobiosoma bosc</i>				0.36	0.44	
Ascidians						
<i>Molgula manhattensis</i>					0.45	
Brittle stars						
<i>Ophiothrix angulata</i>						0.66

Table 1.3. SIMPER analysis results showing % dissimilarity between different salinity-tidal emersion treatment groups. Higher % dissimilarity indicates community structure is more different between a set of two oyster-reef environmental contexts (salinity-tidal emersion groups).

Comparison	% dissimilarity
Low-Inter & Mid-Inter	47.43
High-Inter & Mid-Inter	49.26
Low-Inter & High-Inter	57.36
High-Sub & Mid-Sub	67.07
Low-Inter & Low-Sub	71.23
Low-Sub & Mid-Inter	73.25
High-Inter & High-Sub	73.52
Low-Sub & High-Inter	73.97
High-Inter & Mid-Sub	74.44
Low-Sub & Mid-Sub	74.72
Mid-Inter & Mid-Sub	74.97
Mid-Inter & High-Sub	75.39
Low-Inter & Mid-Sub	77.38
Low-Inter & High-Sub	77.51
Low-Sub & High-Sub	79.42

Figure 1.1. Oyster-reef sampling locations in the Newport and North River Estuaries.

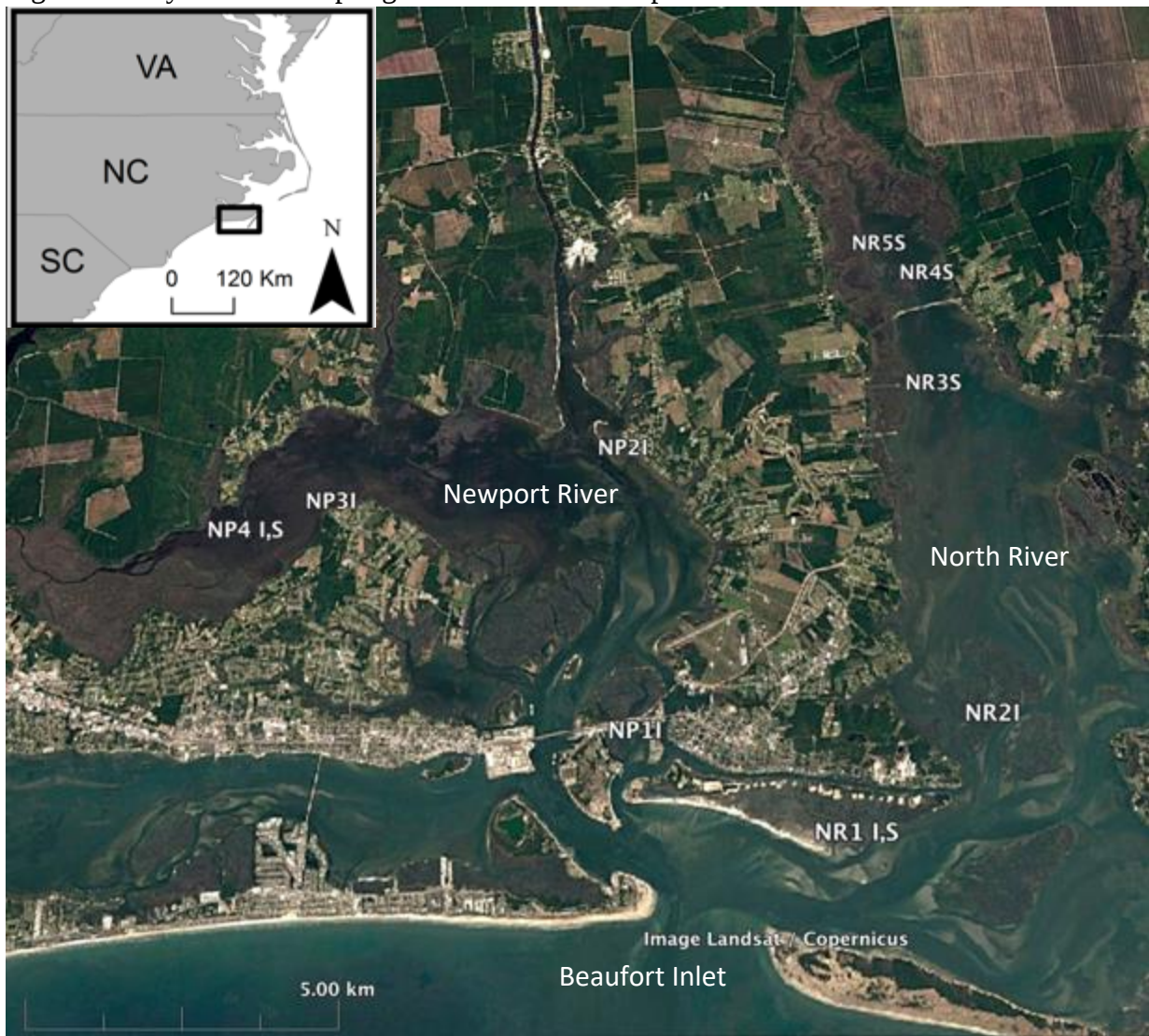


Figure 1.2. Relationship between mean salinity and salinity variation (standard deviation). Means and standard deviations were calculated from the 1-month (28 days) prior to sampling. The linear regression model ($p < 0.001$) indicates a significant negative relationship between mean salinity and salinity variation.

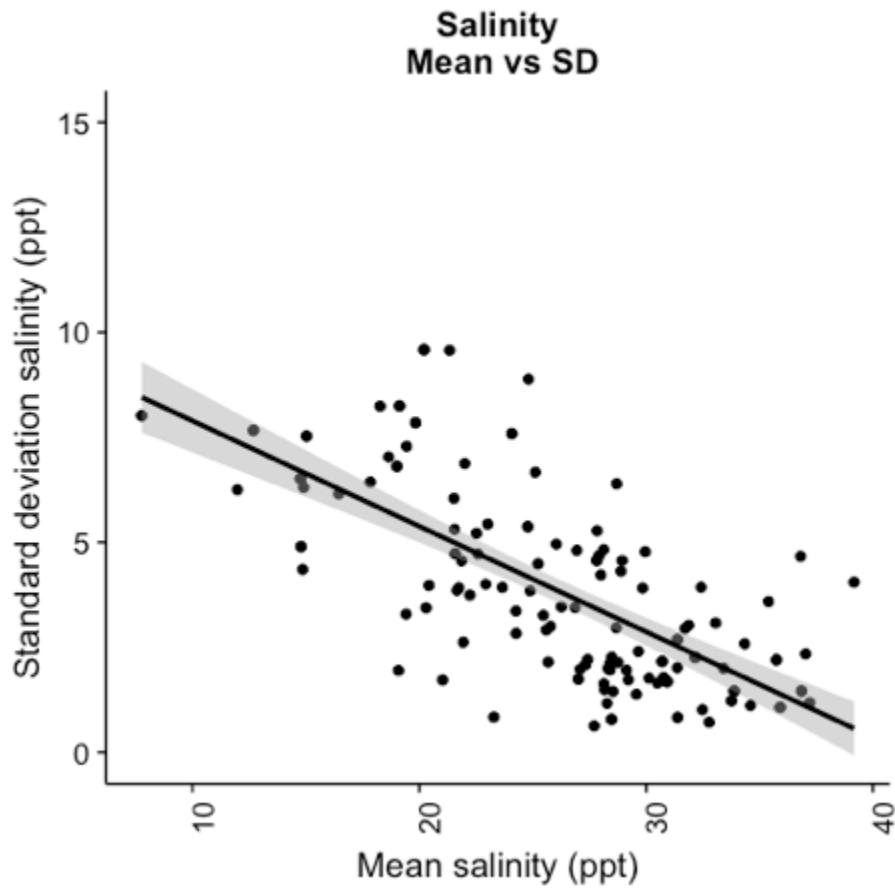


Figure 1.3. (a) Relationship between month pre-sample average temperature and average salinity in the Newport River (b) Relationship between month pre-sample average temperature and standard deviation of salinity in the Newport River (c) Relationship between month pre-sample average temperature and average salinity in the North River (d) Relationship between month pre-sample average temperature and standard deviation of salinity in the North River.

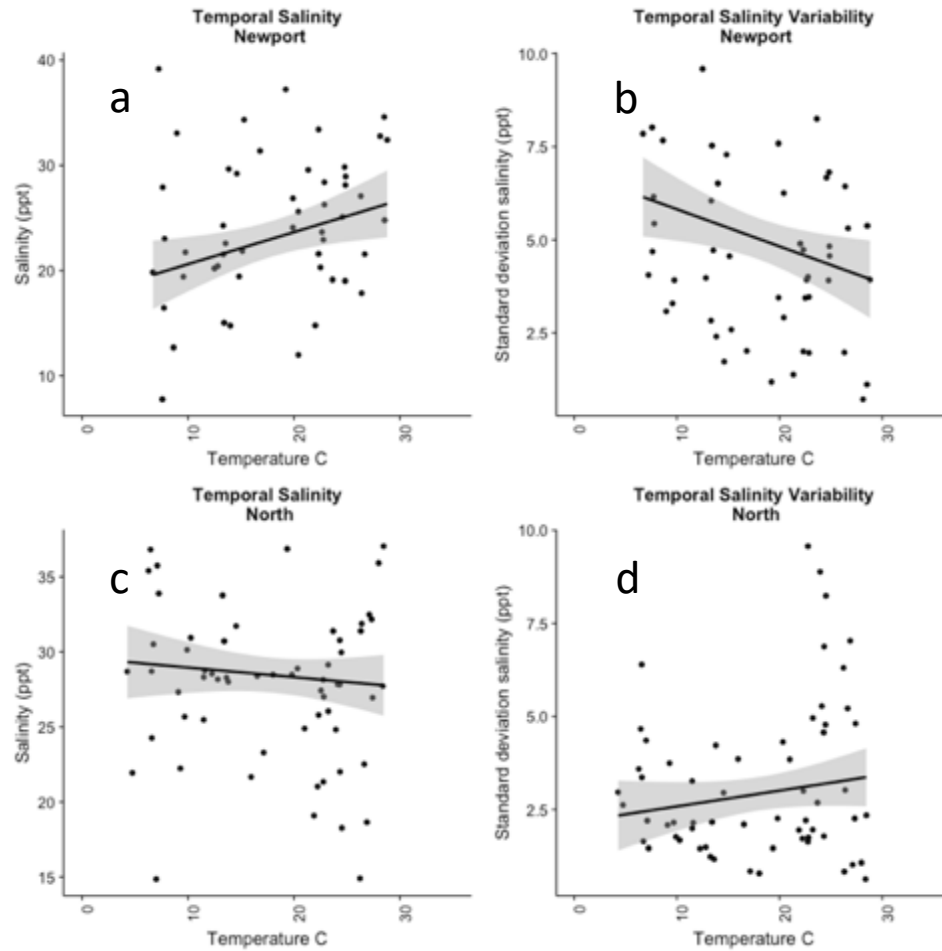


Figure 1.4. Mean oyster densities at intertidal (dark blue) and subtidal (light blue) oyster-reefs in high, mid, and low salinity regimes. Lowercase letters denote statistically different oyster densities (pairwise Kruskal-Wallis, $p > 0.05$).

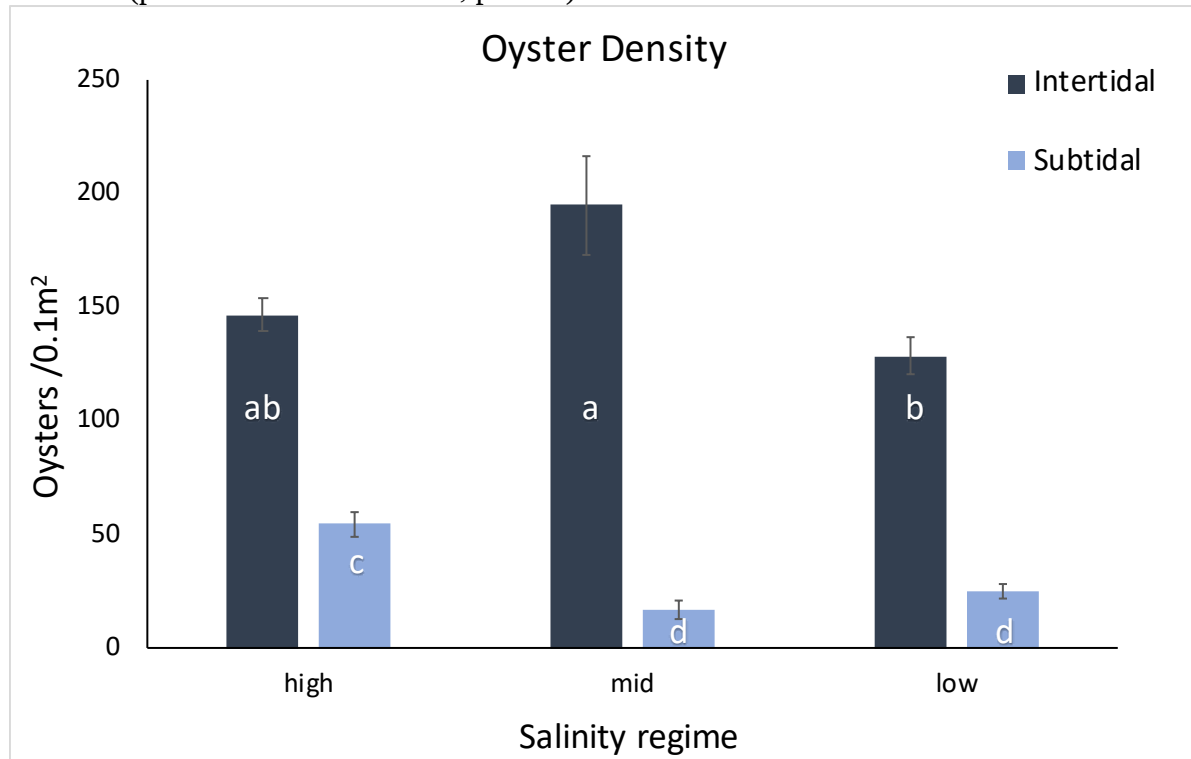


Figure 1.5. Mean species richness (bars: +/- standard error) at intertidal (dark blue) and subtidal (light blue) oyster-reefs in high, mid, and low salinity regimes. Lowercase letters denote statistically different oyster densities (pairwise Kruskal-Wallis Test, $p > 0.05$).

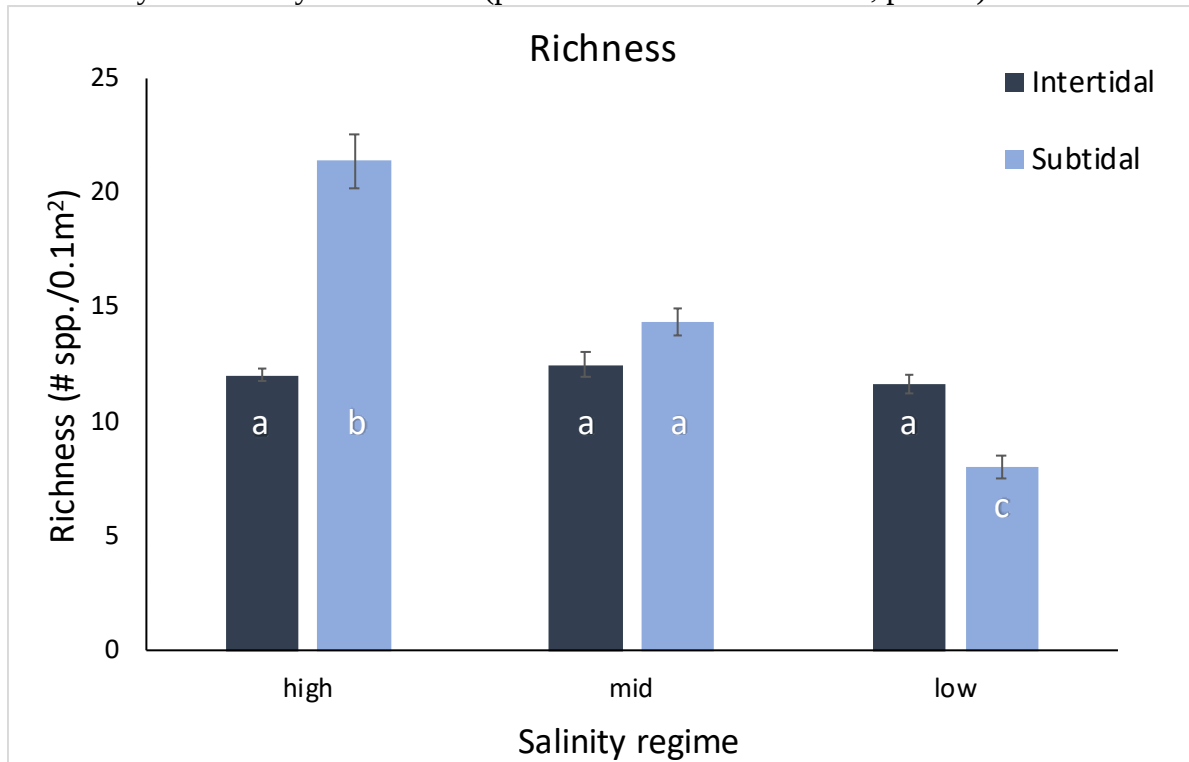


Figure 1.6. (a) Relationship between oyster abundance and species richness on intertidal oyster-reefs. (b) Relationship between oyster abundance and species richness on subtidal oyster-reefs.

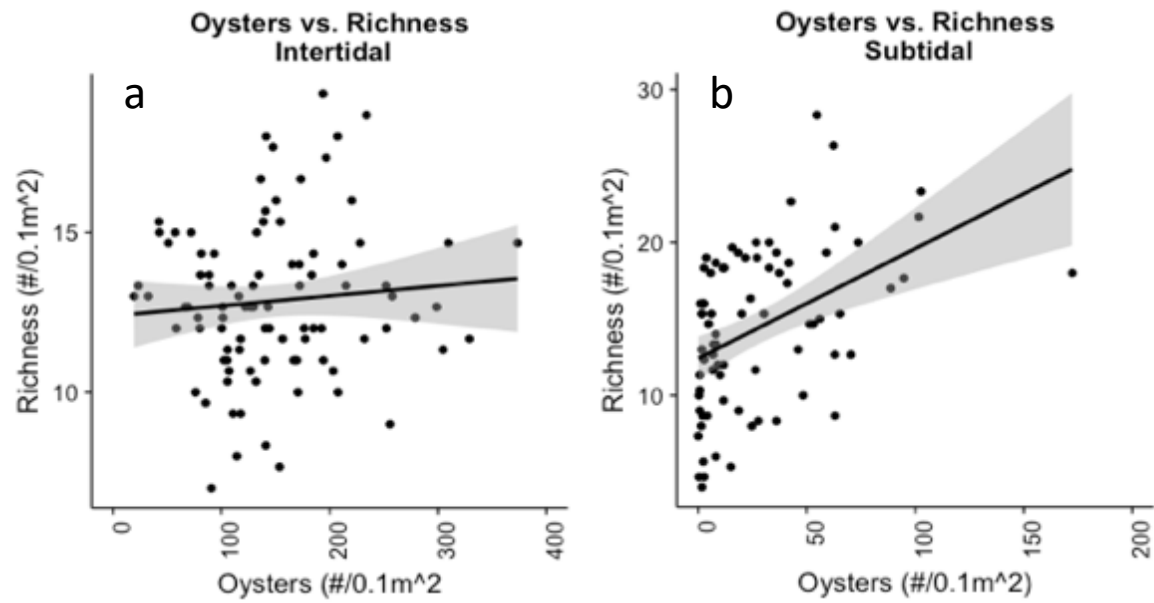


Figure 1.7. Nonmetric multidimensional scaling plot (NMDS) of all samples. Blue-toned triangles represent subtidal communities and red-toned triangles represent subtidal communities. Darker colors indicate high salinity sites, middle colors indicate mid salinity sites, and lighter colors indicate low salinity sites. Ellipses represent 95% confidence intervals for each factor group.

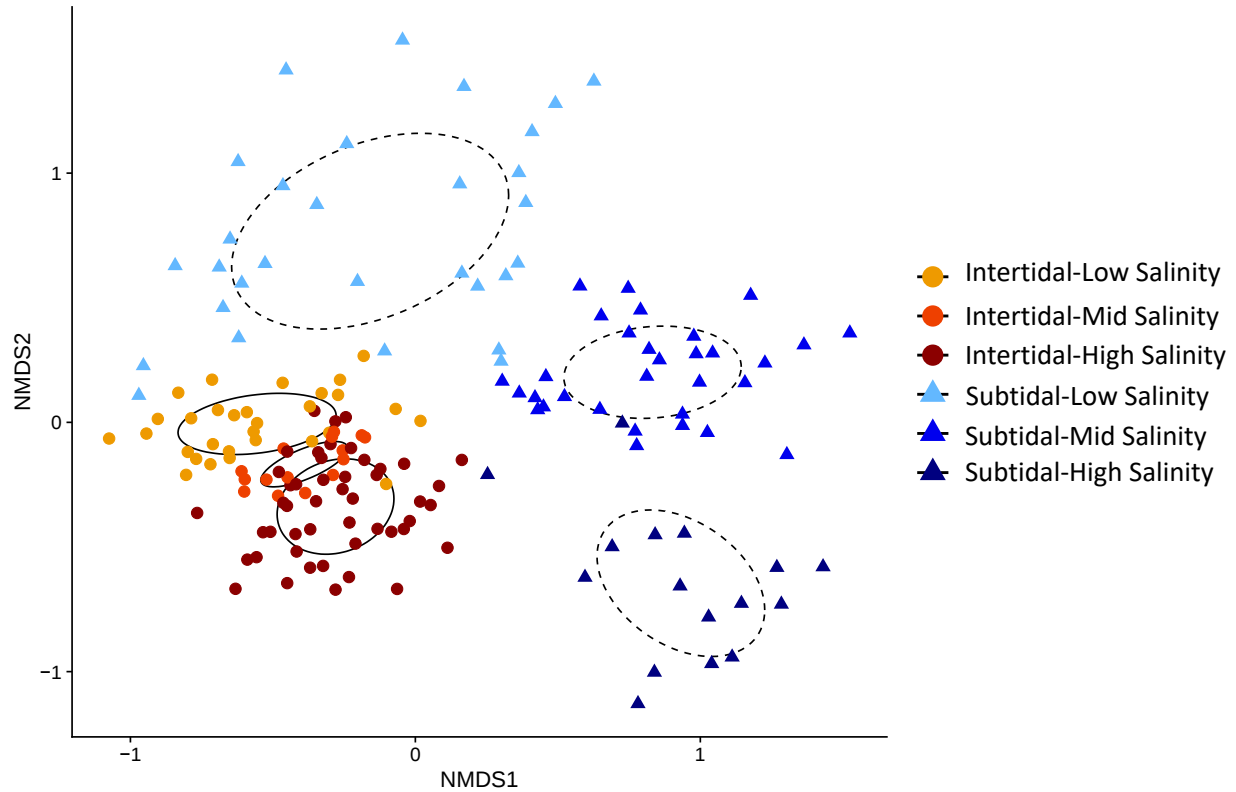
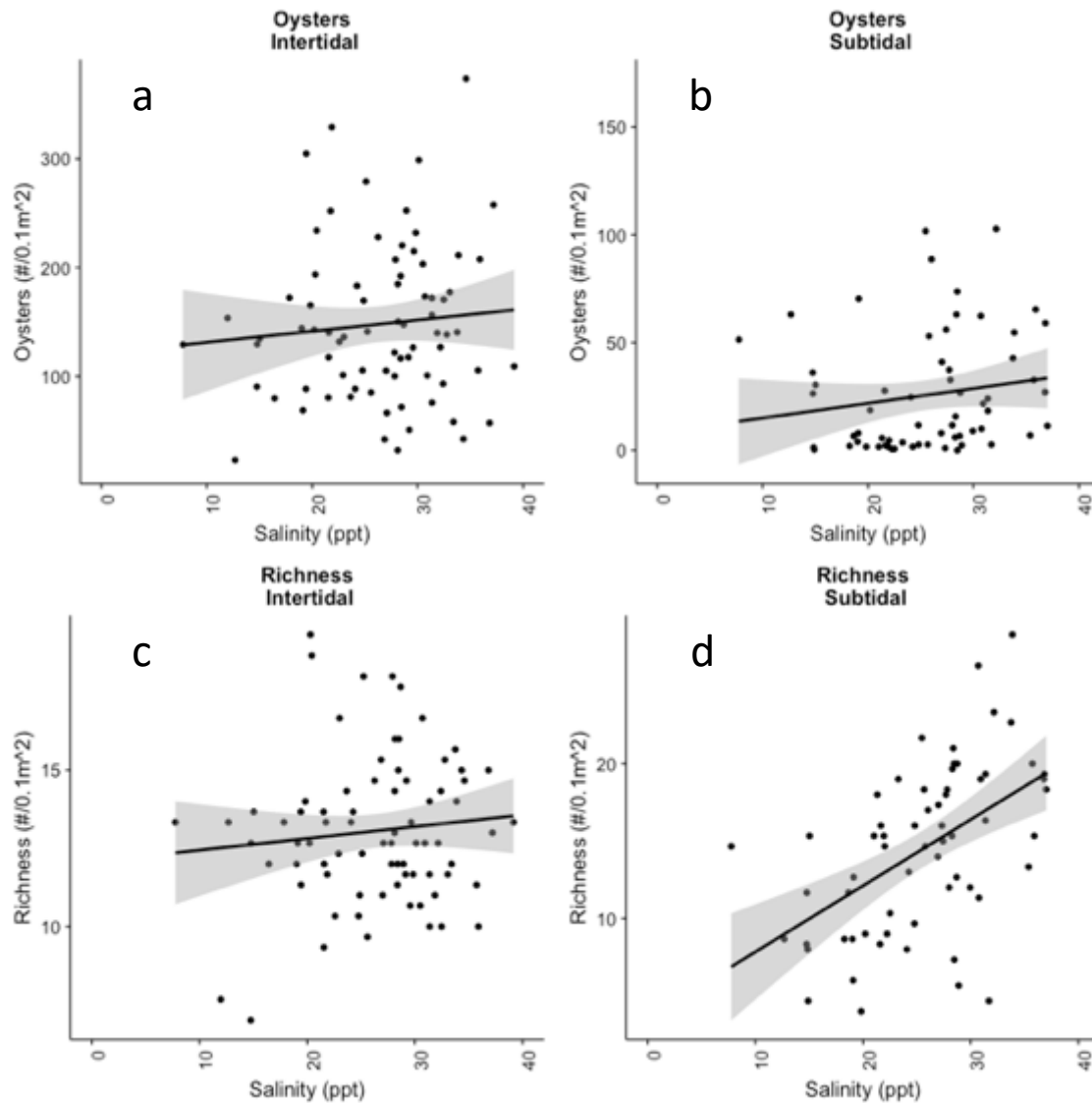


Figure 1.8. (a) Relationship between pre-sample month averaged salinity and oyster density at intertidal sites. (b) Relationship between pre-sample month averaged salinity and oyster density at subtidal sites. (c) Relationship between pre-sample month averaged salinity and species richness at intertidal sites. (d) Relationship between salinity and species richness at subtidal sites.



REFERENCES

- Andrews, J. D. (1966). Oyster mortality studies in Virginia V. Epizootiology of MSX, a protistan pathogen of oysters. *Ecology*, 47(1), 19-31.
- Andrews, J. D., & Hewatt, W. G. (1957). Oyster mortality studies in Virginia. II. The fungus disease caused by *Dermocystidium marinum* in oysters of Chesapeake Bay. *Ecological Monographs*, 27(1), 1-25.
- Baggett, L. P., Powers, S. P., Brumbaugh, R. D., Coen, L. D., DeAngelis, B. M., Greene, J. K., ... & Bushek, D. (2015). Guidelines for evaluating performance of oyster habitat restoration. *Restoration Ecology*, 23(6), 737-745.
- Bahr, L. M., & Lanier, W. P. (1981). *The ecology of intertidal oyster-reefs of the South Atlantic coast: a community profile*(No. 81/15). US Fish and Wildlife Service.
- Beck, M. W., Brumbaugh, R. D., Airoidi, L., Carranza, A., Coen, L. D., Crawford, C., ... & Lenihan, H. S. (2011). Oyster-reefs at risk and recommendations for conservation, restoration, and management. *Bioscience*, 61(2), 107-116.
- Bruno, J. F., Stachowicz, J. J., & Bertness, M. D. (2003). Inclusion of facilitation into ecological theory. *Trends in Ecology & Evolution*, 18(3), 119-125.
- Byers, J. E., Grabowski, J. H., Piehler, M. F., Hughes, A. R., Weiskel, H. W., Malek, J. C., & Kimbro, D. L. (2015). Geographic variation in intertidal oyster-reef properties and the influence of tidal prism. *Limnology and Oceanography*, 60(3), 1051-1063.
- Chestnut, A. F., & Fahy, W. E. (1953). Studies on the vertical distribution of setting of oysters in North Carolina.
- Clarke, K. R., & Warwick, R. M. (2001). Change in marine communities. *An approach to statistical analysis and interpretation*.
- Coen, L. D., Brumbaugh, R. D., Bushek, D., Grizzle, R., Luckenbach, M. W., Posey, M. H., ... & Tolley, S. G. (2007). Ecosystem services related to oyster restoration. *Marine Ecology Progress Series*, 341, 303-307.
- Connell, J. H. (1961). The influence of interspecific competition and other factors on the distribution of the barnacle *Chthamalus stellatus*. *Ecology*, 42(4), 710-723.
- Dame, R. F. (1976). Energy flow in an intertidal oyster population. *Estuarine and Coastal Marine Science*, 4(3), 243-253.
- Davis, H. C. (1958). Survival and growth of clam and oyster larvae at different salinities. *The Biological Bulletin*, 114(3), 296-307.

- Dayton, P. K. (1971). Competition, disturbance, and community organization: the provision and subsequent utilization of space in a rocky intertidal community. *Ecological Monographs*, 41(4), 351-389.
- Elmqvist, T., Folke, C., Nyström, M., Peterson, G., Bengtsson, J., Walker, B., & Norberg, J. (2003). Response diversity, ecosystem change, and resilience. *Frontiers in Ecology and the Environment*, 1(9), 488-494.
- Fodrie, F. J., Rodriguez, A. B., Baillie, C. J., Brodeur, M. C., Coleman, S. E., Gittman, R. K., ... & Theuerkauf, E. J. (2014). Classic paradigms in a novel environment: inserting food web and productivity lessons from rocky shores and saltmarshes into biogenic reef restoration. *Journal of applied ecology*, 51(5), 1314-1325.
- Fofonoff, N. P., & Millard Jr, R. C. (1983). Algorithms for the computation of fundamental properties of seawater.
- Galtsoff, P. S. (1964). The American oyster *Crassostrea virginica* (Gmelin). *US Fish Wildlf. Serv. Fish. Bull.*, 64, 1-480.
- Grabowski, J. H., Hughes, A. R., Kimbro, D. L., & Dolan, M. A. (2005). How habitat setting influences restored oyster-reef communities. *Ecology*, 86(7), 1926-1935.
- Grabowski, J. H., & Powers, S. P. (2004). Habitat complexity mitigates trophic transfer on oyster-reefs. *Marine Ecology Progress Series*, 277, 291-295.
- Grabowski, J. H., & Peterson, C. H. (2007). Restoring oyster-reefs to recover ecosystem services. *Ecosystem engineers: plants to protists*, 4, 281-298.
- Grabowski, J. H., Hughes, A. R., & Kimbro, D. L. (2008). Habitat complexity influences cascading effects of multiple predators. *Ecology*, 89(12), 3413-3422.
- Grabowski, J. H., Brumbaugh, R. D., Conrad, R. F., Keeler, A. G., Opaluch, J. J., Peterson, C. H., Piehler, M. F., Powers, S. P. & Smyth, A. R. (2012). Economic valuation of ecosystem services provided by oyster-reefs. *BioScience*, 62(10), 900-909.
- Grave, C. (1901). *The oyster-reefs of North Carolina: a geological and economic study*. Place of publication not identified.
- Grave, C., Moore, H. F., & Glaser, O. C. (1904). *Investigations for the promotion of the oyster industry of North Carolina* (Vol. 556). Govt. print. off..
- Hanke, M. H., Posey, M. H., & Alphin, T. D. (2017). The effects of intertidal oyster-reef habitat characteristics on faunal utilization. *Marine Ecology Progress Series*, 581, 57-70.
- Hopkins, S. H. (1962). Distribution of species of *Cliona* (boring sponge) on the Eastern Shore of Virginia in relation to salinity. *Chesapeake Science*, 3(2), 121-124.

- Johnson, K. D., & Smee, D. L. (2014). Predators influence the tidal distribution of oysters (*Crassostrea virginica*). *Marine biology*, 161(7), 1557-1564.
- Kimbrow, D. L., White, J. W., Tillotson, H., Cox, N., Christopher, M., Stokes-Cawley, O., ... & Stallings, C. D. (2017). Local and regional stressors interact to drive a salinization-induced outbreak of predators on oyster-reefs. *Ecosphere*, 8(11).
- Kirby-Smith, W. W., & Costlow, J. D. (1989). *The Newport River estuarine system*. UNC Sea Grant College Program.
- La Peyre, M. K., Gossman, B., & La Peyre, J. F. (2009). Defining optimal freshwater flow for oyster production: effects of freshet rate and magnitude of change and duration on eastern oysters and *Perkinsus marinus* infection. *Estuaries and Coasts*, 32(3), 522-534.
- Lehnert, R. L., & Allen, D. M. (2002). Nekton use of subtidal oyster shell habitat in a southeastern US estuary. *Estuaries*, 25(5), 1015-1024.
- Lenihan, H. S., & Peterson, C. H. (1998). How habitat degradation through fishery disturbance enhances impacts of hypoxia on oyster reefs. *Ecological applications*, 8(1), 128-140.
- Lenihan, H. S. (1999). Physical-biological coupling on oyster-reefs: how habitat structure influences individual performance. *Ecological Monographs*, 69(3), 251-275.
- Lenihan, H. S., Peterson, C. H., Byers, J. E., Grabowski, J. H., Thayer, G. W., & Colby, D. R. (2001). Cascading of habitat degradation: oyster-reefs invaded by refugee fishes escaping stress. *Ecological Applications*, 11(3), 764-782.
- Mackin, J. G. (1946). *A study of oyster strike on the seaside of Virginia*. Commonwealth of Virginia, Division of Purchase and Printing.
- Margiotta, A. M., Shervette, V. R., Hadley, N. H., Plante, C. J., & Wilber, D. H. (2016). Species-specific responses of resident crabs to vertical habitat complexity on intertidal oyster-reefs. *Journal of Experimental Marine Biology and Ecology*, 477, 7-13.
- McDougall, K. D. (1943). Sessile Marine Invertebrates of Beaufort, North Carolina: A Study of Settlement, Growth, and Seasonal Fluctuations among Pile-Dwelling Organisms. *Ecological Monographs*, 13(3), 321-374.
- Menge, B. A., & Sutherland, J. P. (1976). Species diversity gradients: synthesis of the roles of predation, competition, and temporal heterogeneity. *The American Naturalist*, 110(973), 351-369.
- Meyer, D. L., & Townsend, E. C. (2000). Faunal utilization of created intertidal eastern oyster (*Crassostrea virginica*) reefs in the southeastern United States. *Estuaries*, 23(1), 34-45.

- Newell, R. I. (1988). Ecological changes in Chesapeake Bay: are they the result of overharvesting the American oyster, *Crassostrea virginica*. *Understanding the estuary: advances in Chesapeake Bay research*, 129, 536-546.
- Newell, R. I., Fisher, T. R., Holyoke, R. R., & Cornwell, J. C. (2005). Influence of eastern oysters on nitrogen and phosphorus regeneration in Chesapeake Bay, USA. In *The comparative roles of suspension-feeders in ecosystems* (pp. 93-120). Springer, Dordrecht.
- O'Connor, N. E., Grabowski, J. H., Ladwig, L. M., & Bruno, J. F. (2008). Simulated predator extinctions: predator identity affects survival and recruitment of oysters. *Ecology*, 89(2), 428-438.
- Odum, W. E. (1988). Comparative ecology of tidal freshwater and salt marshes. *Annual Review of Ecology and Systematics*, 19(1), 147-176.
- Paine, R. T. (1966). Food web complexity and species diversity. *The American Naturalist*, 100(910), 65-75.
- Pennings, S. C., & Bertness, M. D. (2001). Salt marsh communities. *Marine community ecology*, 289-316.
- Powers, S. P., Peterson, C. H., Grabowski, J. H., & Lenihan, H. S. (2009). Success of constructed oyster-reefs in no-harvest sanctuaries: implications for restoration. *Marine Ecology Progress Series*, 389, 159-170.
- Pritchard, D. W. (1952). Estuarine hydrography. In *Advances in geophysics* (Vol. 1, pp. 243-280). Elsevier.
- Ridge, J. T., Rodriguez, A. B., Fodrie, F. J., Lindquist, N. L., Brodeur, M. C., Coleman, S. E., ... & Theuerkauf, E. J. (2015). Maximizing oyster-reef growth supports green infrastructure with accelerating sea-level rise. *Scientific reports*, 5, 14785.
- Rodney, W. S., & Paynter, K. T. (2006). Comparisons of macrofaunal assemblages on restored and non-restored oyster-reefs in mesohaline regions of Chesapeake Bay in Maryland. *Journal of Experimental Marine Biology and Ecology*, 335(1), 39-51.
- Stachowicz, J. J., Bruno, J. F., & Duffy, J. E. (2007). Understanding the effects of marine biodiversity on communities and ecosystems. *Annu. Rev. Ecol. Evol. Syst.*, 38, 739-766.
- Tolley, S. G., Volety, A. K., & Savarese, M. (2005). Influence of salinity on the habitat use of oyster-reefs in three southwest Florida estuaries. *Journal of Shellfish Research*, 24(1), 127-137.
- Tolley, S. G., & Volety, A. K. (2005). The role of oysters in habitat use of oyster-reefs by resident fishes and decapod crustaceans. *Journal of Shellfish Research*, 24(4), 1007-1012.

- Tolley, S. G., Volety, A. K., Savarese, M., Walls, L. D., Linardich, C., & Everham Iii, E. M. (2006). Impacts of salinity and freshwater inflow on oyster-reef communities in Southwest Florida. *Aquatic Living Resources*, 19(4), 371-387.
- Vinebrooke, R., L Cottingham, K., Norberg, M. S., I Dodson, S., C Maberly, S., & Sommer, U. (2004). Impacts of multiple stressors on biodiversity and ecosystem functioning: The role of species co-tolerance. *Oikos*, 104(3), 451-457.
- Walles, B., Fodrie, F. J., Nieuwhof, S., Jewell, O. J., Herman, P. M., & Ysebaert, T. (2016). Guidelines for evaluating performance of oyster habitat restoration should include tidal emersion: reply to Baggett et al. *Restoration ecology*, 24(1), 4-7.
- Wells, H. W. (1959). Boring sponges (Clionidae) of Newport River, North Carolina. *Journal of the Elisha Mitchell Scientific Society*, 75(2), 168-173.
- Wells, H. W. (1961). The fauna of oyster beds, with special reference to the salinity factor. *Ecological Monographs*, 31(3), 239-266.
- Winslow, F. (1886). Report on the waters of North Carolina, with reference to their possibilities for oyster culture; together with the results of the surveys directed by the resolution of the general assembly, ratified March 11, 1885. *Raleigh: North Carolina State Printer and Binder*.
- Worm, B., Barbier, E. B., Beaumont, N., Duffy, J. E., Folke, C., Halpern, B. S., ... & Sala, E. (2006). Impacts of biodiversity loss on ocean ecosystem services. *science*, 314(5800), 787-790.
- Ysebaert, T., & Herman, P. M. (2002). Spatial and temporal variation in benthic macrofauna and relationships with environmental variables in an estuarine, intertidal soft-sediment environment. *Marine Ecology Progress Series*, 244, 105-124.
- Ziegler, S. L., Grabowski, J. H., Baillie, C. J., & Fodrie, F. J. (2017). Effects of landscape setting on oyster-reef structure and function largely persist more than a decade post-restoration. *Restoration Ecology*.

CHAPTER 2: MULTIDECADAL CHANGES TO ESTUARINE GRADIENTS AND OYSTER-REEF COMMUNITIES: A COASTAL SQUEEZE FOR OYSTERS?

Introduction

The ecological responses to natural or anthropogenic environmental shifts are heavily dependent on the spatiotemporal scale of variability. Understanding how faunal communities respond to multiple scales of environmental variability is crucial to identify natural and anthropogenic-driven shifts in those communities, such that habitat conservation efforts are focused at appropriate spatial scales and siting tools that allow for long-term habitat persistence and function. Due to increased anthropogenic CO₂ inputs in the 20th and 21st centuries, researchers and environmental stewards are desperately trying to understand the potential for, and consequences of, ecological change under future climatic scenarios. Space-for-time approaches have commonly used relatively short-scale climatic perturbations, such as El Niño Southern Oscillation (ENSO) indices, or leveraging artificial temperature gradients to examine ecological community response to future thermal scenarios under climate change (Sagarin et al. 1999). For instance, ENSO cause ecological communities to shift drastically due to associated physical transport and thermal stress (Dayton and Tegner 1984, Chavez et al. 2003). However, these "pulsed" changes may not reflect the gradual shift in abiotic factors under "press" disturbances that impact recruitment, survival, and phenological aspects of community structure (Barry et al. 1995). Ecological studies spanning multi-decadal timescales are a rare, yet valuable tool for understanding how gradual environmental changes influence biological communities. In particular, long-term studies that compare historical datasets to current observations, allow for

assessments of change in the studied parameter(s) over time and its potential ecological impacts. This situation decreases the potential for speculative conclusions that ignore the potential for environmental complexity to impact communities over long time scales (Sagarin et al. 1999). Relevant temporal scales of environmental change attributable to climate change are often multi-decadal, such as with relative sea level rise (RSLR), the combined effect of global SLR and regional subsidence or uplift (Church and White 2005). When possible, taking advantage of historical datasets offers an effective way of assessing the potential for future ecological change by leveraging knowledge of what has already changed over multi-decadal timescales.

The impacts of climate change may be magnified in dynamic coastal and estuarine environments where biological communities exist across rapidly changing abiotic gradients (concentrated at meter-to-kilometer scales). For instance, abiotic stress gradients are known to influence intertidal communities by selecting for species with physiological tolerance to desiccation and temperature variability, and physical tolerance to wave energy that vary substantially within relatively narrow (meter scale) elevational gradients (Helmuth et al. 2002, Harley and Helmuth 2003). Multi-decadal studies comparing rocky intertidal communities in the 1930's to those in the 1990's have uncovered both small-scale (meters in elevation) and large-scale (biogeographic, and related to poleward range extensions) community shifts in response to temperature and potentially RSLR (Barry et al. 1995), reflected by increased abundances of southern invertebrate species and decreased abundances of northern invertebrate species. Additionally, anthropogenic activity is prevalent in coastal areas, which may include land use change, channel dredging, pollution, and mechanical harvesting of organisms for fisheries (Beck et al. 2011). These anthropogenic stressors in combination with climate change, may be

redundant, additive or synergistic in their influence(s) on coastal communities already subject to the inherently stressful existence in a dynamic estuarine system.

Estuarine biogenic habitats such as saltmarshes, seagrasses, and oyster reefs harbor diverse faunal assemblages and are at particular risk for climate change and other anthropogenic impacts, because they often occur along relatively narrow environmental interfaces, such as in the intertidal zone, and across chemical gradients such as salinity. For salt marshes, the combination of RSLR and upland development can result in what has been termed “coastal squeeze”, or the constriction of available intertidal space for salt-tolerant plants. Erosive wave energy, increased tidal magnitude, and altered sediment dynamics force transgressive movement of foundation species to maintain proper positioning within the intertidal. Eventually, transgression is hindered by artificial coastal defenses or high relief, squeezing the width of suitable intertidal habitat (Doody 2013, Pontee 2013). *Crassostrea virginica* oyster reefs commonly fringe seaward salt marsh margins (Bahr and Lanier 1981) in estuarine systems, and are thereby subjected to similar physical forces imposed by RSLR but are considerably less studied in relation to coastal squeeze than saltmarshes. Oyster reefs are more resistant to erosive forces imposed by RSLR than marshes (Ridge et al. 2017a), and while marshes can adjust their position vertically via passive sediment trapping, augmenting below ground biomass, and increasing above ground production (Morris et al. 2002), oysters accrete via individual vertically oriented growth (Chestnut and Fahy 1953, Bishop and Peterson 2006). Intertidal oysters respond rapidly to fluctuations in sea level (month to year timescales) via accretion or erosion across reef crests and (Ridge et al. 2017b), and over longer timescales can vertically accrete at rates outpacing projected RSLR (Rodriguez et al. 2014).

In temperate and subtropical estuaries, such as in North Carolina, oysters may occupy both intertidal and subtidal zones within estuaries, their distributions strongly regulated by salinity regime and aerial exposure (Baggett et al. 2015, Walles et al. 2016). Intertidal oyster reefs may be distributed throughout suitable salinity ranges (10-35ppt), subtidal oysters are constrained to areas with mesohaline salinity regimes because freshwater inputs to these regions act to exclude abundant marine-adapted predators and competitors (i.e. pests) of oysters (Fodrie et al. 2014, Powers et al. 2009 Walles et al. 2016). Further, the habitat function of oyster reefs is influenced by salinity, as community composition on oyster reefs varies with salinity regime and seasonal variability in freshwater outflow (Wells 1961, Tolley et al. 2006). Because RSLR and channel dredging impact tidal magnitudes, and thus the volume of saltwater entering estuaries on flood tides, there will be salinity increases occurring along coastlines that will impact oyster-reef distributions, as well as the associated communities.

The Newport River Estuary (NPRES hereafter) in North Carolina offers an opportunity to investigate oyster reef associated faunal communities over multidecadal timescales, because it has been an active site of environmental monitoring and oyster research for over a century. In the latter 19th and early 20th centuries, oyster distribution mapping described extensive intertidal and subtidal oyster reefs throughout the NPRES, and indicated subtidal oyster reefs could occur upstream of a line crossing the Newport approximately 12 km from Beaufort Inlet, the nearest connection with the ocean. Caswell Grave indicated region upstream in the NPRES, above which subtidal oyster cultivation was feasible, due to periodic freshets that limit the extent of common oyster enemies, termed here "Grave line" (Grave 1904). Grave (1901) qualitatively stated that subtidal oyster reefs provide habitat for taxonomically diverse invertebrate assemblages, but the exceptional diversity of oyster-associated faunal assemblages of the NPRES was first quantified

by Wells (1961), who found over 300 species across the polyhaline-euhaline salinity gradient, and a positive association between salinity and faunal diversity. It is unknown how saltwater intrusion will impact the oyster reef community diversity gradients found by Wells, but long term changes in sea level may impact estuarine salinity dynamics at a given location, thus having the potential to impact the distribution biogenic structures and their associated communities.

I revisited historical oyster reef sampling stations in the NPRES to quantify spatiotemporal patterns of oyster community variability over multidecadal timescales. Specifically, I integrated 1955-1956 oyster reef survey data (Wells 1961) and modern oyster reef surveys (2013-2015), with long-term salinity data in the NPRES to answer the following questions: (1) Have NPRES oyster reef communities changed in composition over six decades since Wells? (2) Has community turnover across the estuarine salinity gradient become altered potentially to SLR-linked salinity changes? (3) For any changes I observed across the estuary or over time, which faunal groups account for differences between/among reefs?

Materials and Methods

Study Area

The NPRE, is a shallow (1m mean low water depth), tidally influenced (2m range) temperate estuary. From its headwaters (freshwater source), it flows in an eastward direction for approximately 8.5 km, or half of its length, before curving southward toward Beaufort Inlet which serves as its ocean connection (Kirby-Smith and Costlow 1989). The river-dominated portion of the NPRE, termed the “narrows”, is composed of a 20m wide channel of 2-4m depth with dense marsh growth on either bank. East of the “narrows”, the estuary widens where the tide dominates riverine outflow (Kirby-Smith and Costlow 1989). This upper portion of the estuary has historically supported natural and planted subtidal and natural intertidal oyster populations (Winslow 1889, Grave 1901, Grave 1904, Wells 1961).

To assess changes in the Newport River estuary since Wells (1961), sampling locations were selected that optimally replicated Wells’ original sites. Wells sampled oyster associated communities at five sites in the NPRE spanning the polyhaline-euhaline salinity gradient, to better understand oyster community assemblage distributions respective to salinity. In the lower, euhaline portion of the estuary, Wells (1961) sampled two sites: Shark Shoal and Pivers Island ; and in the upper, polyhaline portion of the estuary, he sampled three sites: Gallants Point, White Rock, and Cross Rock (Figure 2.1).

Wells (1961) provided brief site descriptions of the landscape context of these reefs and their general characteristics in 1955, which are summarized here. Nearest to Beaufort Inlet was Shark Shoal, consisting of predominantly intertidal oysters growing on a large man-made rock jetty. There is no present-day analogue to Shark Shoal. Pivers Island (PI hereafter) consisted of a rock jetty and shell substrate on either side of a narrow channel with shell material extending a

half meter below low tide. PI reef is located in an embayment that receives seawater directly through a dredged channel leading to Beaufort Inlet. It receives riverine input indirectly as water must move through Gallants Channel, mixing with oceanic water in the embayment. The close proximity to Beaufort Inlet combined with indirect connectivity to the main NPRES channel leads to relatively stable salinities at this site, and requires extreme meteorological events to achieve low salinity tolerance thresholds for characteristic fauna (Wells 1961). Today, PI is entirely intertidal, largely fringing *Spartina alterniflora* marsh. Live oysters were not typical of the shallow subtidal in our surveys, so the eroded, scattered shell of the sublittoral was not part of our repeat sampling efforts. Wells' Gallants Point reef was located approximately 2.1 km from Pivers Island, bar-shaped and composed of intertidal oysters, and has disappeared over the last half century. No present-day analogue exists for Gallants Point. The first scientific reports of Newport River oyster distributions noted the intertidal oysters at White Rock (Winslow 1889, Grave 1901). This patch reef located directly north of the middle channel, 12.5 km from Beaufort Inlet, no longer exists. For the purposes of multi-decadal temporal comparison, I instead sampled a nearby patch reef, termed White Rock Replacement (WRR), which is located 13.1 km from Beaufort Inlet on the southern side of the middle channel. Cross Rocks (CR) was the freshest location sampled in Wells (1961), 14.7 km upstream of Beaufort Inlet. The site is composed of a large bar shaped oyster reef oriented with its long axis perpendicular to the mean flow as well as subtidal oyster carpeting the adjacent, seaward mudflat. Because Wells (1961) did not discern between intertidal and subtidal faunal collections, intertidal and subtidal reefs were treated in combination for modern sampling. Therefore, out of the Wells' 5 sites, I was able to sample three extant locations to make multi-decadal scale comparisons (Table 2.1).

Salinity data collection

More than 100 years of marine research conducted in the Beaufort area by the United States Fisheries Commission, Duke University Marine Lab, the University of North Carolina at Chapel Hill's Institute of Marine Sciences (UNC IMS), and North Carolina Division of Marine Fisheries (NC-DMF) has resulted in a long-term library of environmental data for the NPRE. To investigate multi-decadal salinity trends, I gathered historical salinity data from these sources to establish a salinity time series that covered 102 years between 1913 and 2015. At PI, Hoyt (1920) measured PI salinity daily from 1913-1914, and reported monthly maximum, minimum, and mean values. Gutsell (1930) measured PI salinity daily from 1924-1928, and also provided monthly maximum and minimum values. Wells (1961) sampled salinity monthly from January 1955 to October 1956 at nine sites over the NPRE polyhaline-euhaline salinity gradient. Wells point sampled salinity from PI, WR, Turtle Rock (located directly north of this study's 2013-2015 WRR site), and CR, which were utilized in this analysis. PI, WR, WRR and CR site-proximal salinity data from 1965-2015 were acquired from the NC-DMF Shellfish Sanitation program (NC-DMFSS). NC-DMFSS routinely measures point salinities at 0.6 m depth at set sites along the Newport River to regulate shellfish harvesting in the context of water quality related to human health. The earliest salinity records from DMFSS were from 1965, and their sampling operations have continued to the present-day with some modifications to routine sampling sites. Because no single DMFSS site for a given study area was located directly adjacent to the oyster reef sites, and some sampling sites slightly changed location over time, we chose to pool salinity sites based on proximity to the location of a particular oyster reef. By combining salinity data collected within a particular section of the estuary, , higher sample sizes were achieved and multi-decadal trends were better represented. For the PI area, salinity

measurements were aggregated from DMFSS sites within 500 m of 34°43'14.66"N. For the WR-WRR area (WR area hereafter), DMFSS salinity measurements within 500 meters of the respective site were considered (for WR, 76°43'28.97"W and WRR, 76°44'0.68"W). At CR, salinity measurements taken at DMFSS site 500 m from 76°45'0.62"W.

Oyster community collection

To sample oyster associated communities, Wells utilized a simple sampling methodology. At each site except White Rock, 1-gallon buckets were filled with intertidal and shallow subtidal oyster clusters and associates at low tide. He identified all fauna to the lowest taxonomic level possible. At White Rock, oyster communities were sampled using a scallop dredge at high tide. Wells did not explain why this was done in lieu of the 1-gallon bucket sampling procedure. Wells sampled PI, WR, and CR 14, 6 and 15 times, respectively, between 1955 and 1956. All seasons are represented in Wells' PI and CR samplings. He only sampled White Rock from July-September each year of the 2-year sampling period (Table 2.2).

My sampling of oyster-reef associated fauna began in July 2013 and was repeated monthly at each site until April 2014, at which time, sampling was done bimonthly and until December 2015. WRR was not sampled in October, 2015 due to exceedingly high water caused by Hurricane Matthew (Table 2.2). Two additional sampling trips (on top of the regular schedule) were made in October 2015 to Pivers Island and Cross Rocks to document the impact of freshets (freshwater input events) on the oyster communities. These two additional samples are included in the multi-decadal comparisons to Wells (1961), as the earlier study included communities impacted by hurricanes Connie and Diane in August, 1955 (Wells 1961). A detailed description of faunal collection is provided in Chapter 1: METHODOLOGY.

Statistical analyses:

Multi-decadal (century to half-century) time series of salinity metrics in the NPPE were analyzed to evaluate long-term NPPE salinity trends. Yearly minimum, maximum, and mean (when possible) salinities were calculated from the raw salinity data for each site area (CR, WR-WRR, and PI). For PI, least-squares regressions were conducted for yearly minimum and maximum salinities for the time periods 1913-2015 and 1955-2015, while mean salinity regression was only possible for 1955-2015. WR and WRR salinities were pooled to provide spatial consistency between environmental and community analyses. For both WR-WRR and CR, least squares regression analyses were conducted for yearly minimum, maximum, and mean salinities from 1955-2015. For each site, we aimed to utilize as much salinity data as were available for establishing time series trends.

A series of univariate and multivariate statistical analyses were employed to examine multi-decadal salinity changes and alterations to oyster community structure at different spatial scales. We analyzed oyster community datasets from 1955-1956 and 2013-2015 using multivariate statistical techniques with the purpose of determining the influence of time and salinity on oyster community turnover across the NPPE salinity gradient. Wells (1961) reported oyster community samples presence/absence of species that occurred in 20% of samples. To accurately compare communities in 2013-2015 to those of Wells, we converted all abundance data to presence/absence, and filtered out species that did not occur in 20% or more of samples (across sites) during the two-year sampling period. A conservative approach was employed such that species present in Wells (1961) samples that did not occur in 20% of 2013-2015 samples were kept in the 2013-2015 dataset for comparative analyses. In 2013-2015 samples, for some species, taxonomic resolution was lower than that of Wells (1961), so to avoid inflation of

community differences between the two studies, species were grouped into their respective families for analyses in both Wells and my datasets.

Nonmetric multidimensional scaling (NMDS) was used to visualize and identify natural groupings in the oyster community data from Wells (1961) and 2013-2015, as well as assess the role of salinity in driving community composition. Samples were coded by study (Wells or 2013-2015) and site within each study (PI, WR, CR) to explore oyster community patterns across an estuarine salinity gradient and over 60 years between sampling efforts. Environmental and temporal variables of salinity, Julian day, and year were fitted to the data using *envfit* in the R ‘vegan’ package, to determine how these variables possibly correlated with observed community groupings within the NMDS space. The salinity value corresponding to a particular sample were calculated as the site-specific mean salinity during the season and year of sampling. *Envfit* analysis indicates the statistical significance of environmental vector correlations with community data. Only significant environmental variables, expressed as vectors, were included in the final ordination.

To determine if NPRES communities varied between studies and sites, a crossed permutational MANOVA (PERMANOVA) was conducted, with study, site, and study*site as fixed factors. A pairwise PERMANOVA post-hoc procedure was used to determine significant differences in group means between pairs of study-site groupings. P-values were Bonferroni corrected for multiple comparisons. To determine which taxonomic groups (families) were driving differences between study groups and within-study site differences, similarity percentages (SIMPER) analyses were performed using the ‘vegan’ R package (Dixon 2003). To determine if there were differences in community turnover (beta-diversity) across the NPRES salinity gradient between studies, permutational analysis of homogeneity of dispersions

(PERMDISP) was conducted on all samples, using study as the independent factor (Anderson 2006). PERMDISP pairwise tests with Bonferroni correction were conducted for all study-site groups to determine within site community variability over space and time. PERMDISP tests served an additional purpose to indicate whether PERMANOVA tests met the assumptions of homogeneity of multivariate dispersions.

To quantify variability (site-site beta-diversity) changes in oyster communities between paired sampling locations at different locations along the salinity gradient, as well as over the 60 year gap between studies, I calculated Jaccard dissimilarity scores (*1-Jaccard index*). Jaccard scores were generated for each site comparison for Wells (1961) and the 2013-2015 family-level presence/absence data using the ‘vegan’ package in R (Dixon 2003). To determine the significance of observed differences in site-to-site community variability, nonparametric paired Kruskal-Wallis tests were performed for each site pair (CR-WR, WR-PI, CR-PI) between studies. This nonparametric approach was chosen as variances were heteroscedastic between site-pair groups.

To gain further insight into the potential for salinity to drive changes in community turnover, we conducted a single mantel test, which determines whether a significant correlation exists between two difference matrices, in this case the community dissimilarity (*1-Jaccard index*) matrix and the salinity difference matrix ($\alpha = 0.05$). This was conducted using all sample pairs.

Results

Multi-decadal salinity trends

Since the earliest available salinity measures were taken in 1913, NPRE salinity dynamics have shifted in terms of extremes and averages. At CR, the farthest upstream site in the NPRE, yearly mean, maximum, and minimum salinity displayed an increasing trend between 1955 and 2015 (Least-squares regressions: Mean: $R^2=0.37$, $P<0.001$; Max: $R^2=0.19$, $P<0.01$; Min: $R^2=0.19$, $P<0.01$) (Figure 2.2A). Yearly mean, maximum and minimum salinities similarly increased at WR-WRR between 1955 and 2015 (Mean: $R^2=0.3304$, $P<0.001$; Max: $R^2=0.2496$, $P<0.001$; Min: $R^2=0.1945$, $P<0.001$) (Figure 2.2B). At PI, closest to Beaufort Inlet, yearly maximum and mean did not shift over either 1913-2015 or 1955-2015 spans. Minimum yearly salinity showed a significant linear increase over the 1913-2015 time span (Least squares regression: $R^2=0.323$, $P<0.001$) but not at the shorter time span from 1955-2015 (Figure 2.2C).

Community comparison: Wells vs. 2013-2015

In general, fewer numbers of taxa were collected in 2013-2015 NPRE surveys (36 families total) than in 1955-1956 surveys (54 families total) by Wells (1961). At PI, WR, and CR, Wells collected 52, 44, and 33 families, respectively. Between 2013-2015, 27, 26, and 26 families were collected at PI, WRR, and CR respectively. During 1955-1956, across all samples at all sites, dominant oyster associated taxa (in 30% or more samples within specified group) included species from 8 phyla and 33 families, while in 2013-2015, only 15 families from 4 phyla were dominant across all sites. Dominant taxa across all three sites that both studies included were barnacles; Balanidae (*Amphibalanus eburneus*), amphipods; Melitidae (*Melita nitida*, *Dulichella appendiculata*), xanthid crabs; Panopeidae (*Panopeus herbstii* and *Eurypanopeus depressus*), Gastropods; Pholadidae (*Crepidula* spp.) and Pyramidellidae (*Boonea*

impressa), polychaetes; Nereididae (*Alitta succinea*, *Nereis falsa*), and bivalves (Mytilids, *Geukensia demissa* and *Brachidontes exustus*) (Table 2.3). Spionid polychaetes (*Polydora websteri*) were dominant at all sites across both studies except PI in 1955-1956(25% of samples). Anemones (Actiniaria spp.) and Venerids (ie. *Mercenaria* spp.), were dominant at all sites in both studies except CR in 1955-1956(13.3% and 0% respectively).

I observed differences between NPRE oyster faunal communities between 1955-1956 and 2013-2015 with respect to group means as well as variability within and among groups (Figure 2.3). Sample year and salinity were significantly correlated with community variability (Envfit: Year: $R^2=0.6698$, $P<0.001$; Salinity: $R^2=0.3655$, $P<0.001$). Grouping communities by study (1955-1956 or 2013-2015) revealed within study group clustering, and less dispersion (distance to study group centroid) occurring among 2013-2015 communities than in those from Wells (1961). PERMDISP analysis ($F=0.952$, $P<0.001$) validated this observation as NPRE community variability at the landscape scale was higher in 1955-1956 (average distance to group centroid: 0.4464) than in 2013-2015 (average distance to group centroid: 0.3821). Families contributing (Top 50% cumulative contribution) to the community variability between Wells and the current study include hydrozoa (Campanulariidae), bryozoans (Electridae, Victorellidae, and Bugulidae), sedentary polychaetes (Sabellariidae and Spionidae), burrowing amphipods (Corophiidae), sessile protists (Folliculinidae), anemones (Actiniaria spp.), errant polychaetes (Phyllodocidae and Eunicidae), bivalves (Veneridae), Nemertea, gastropods (Pyramidellidae), isopods (Sphaeromatidae), and parasitic barnacles (Sacculinidae).

Study groups varied substantially and correlated with the “year” vector axis. Wells and 2013-2015 study group means were 56.5% different, indicating landscape-scale shifts in oyster reef associated community composition over multi-decadal timescales are plausible

(PERMANOVA: $df=1$, $F=18.67$, $p<0.001$). Dispersions were heterogeneous between 1955-1956 and 2013-2015 groups. Because study group dispersions differed, multivariate statistical tests to compare group means could not accurately assess whether groups differed due to differences between group multivariate means or group variability. However, qualitative separation between the group centroids indicated differences between study-grouped communities likely exist due to both dispersion and mean differences (Figure 2.3).

Coding communities by study and site, clustering of samples within site groups was evident, oriented in multivariate space along the axis of the salinity environmental fit vector. Overall, community turnover along the NPRE salinity gradient was higher during Wells' study than in our more recent study, meaning NPRE oyster associated beta-diversity has declined over time. At a higher site specific resolution, beta-diversity between site pairs declined in the 2013-2015 survey are consistent with observations at the landscape-scale. In 1955-1956, Jaccard dissimilarity between CR and WR sites (49.05% different) was significantly higher than Jaccard dissimilarity between 2013-2015 CR and WRR sites (Dunn test: $Z=-5.89$, $P<0.001$). The five families contributing the most to variability between CR and WR in 1955-1956 include Sphaeromatidae, Alcyonidiidae, Bugulidae, Campanulariidae, and Folliculinidae whereas the top five contributing taxa to 2013-2015 CR-WRR difference (36.95% difference) were Corophiidae, Eunicidae, Phyllodocidae, Pyramidellidae, and Actiniaria spp. (SIMPER analysis, Table 4). Community dissimilarity between WR and PI in 1955-1956 (51.90% different) was also larger than the dissimilarity between WRR and PI in 2013-2015 (37.57% different) (Dunn test: $Z=-6.62$, $P<0.001$). Oyster-reef community variability (ie. Sample 1 vs. sample 2 Jaccard dissimilarities) was significantly correlated with salinity variation between sites (ie. Sample 1 – sample 2 salinity) (Mantel $R=0.16$, $p=0.001$), indicating salinity differences between sites play a

role in driving community turnover. The five families contributing the most to variability between WR and PI in 1955-1956 included Halichondriidae, Muricidae, Eunicidae, Schizoporellidae, and Membraniporidae whereas the top five contributing taxa to 2013-2015 CR-WRR difference were Sphaeromatidae, Melitidae, Spionidae, Sacculinidae, and Phyllodocidae (SIMPER analysis, Table 4). At the largest distance between sites, between CR and PI, community differences in 1955-1956 (60.99% different) were significantly larger than in 2013-2015 (47.85% different) (Dunn test: $Z=-7.23$, $P<0.001$) (Figure 2.3). The five families contributing the most to variability between CR and PI in 1955-1956 included Clionaidae, Eunicidae, Halichondriidae, Phyllodocidae, and Actiniaria spp. whereas the top five contributing taxa to 2013-2015 CR-PI difference were Corophiidae, Eunicidae, Sphaeromatidae, Melitidae, and Spionidae.

Within-site variability differed among sites over multi-decadal time scales and the spatial scale related to the NPRE salinity gradient. (PERMDISP: $F=3.809$, $P=0.006$). *Post-hoc* pairwise tests for differences in dispersion between study-site group pairs indicate no difference between 2013-2015 site dispersions, but in Wells' communities, WR (mean dispersion, 0.44) was much more disperse than either PI (mean dispersion, 0.27) or CR (mean dispersion, 0.33) (Figure 2.4). Significantly different oyster-associated communities occurred at different sites across studies (PERMANOVA: $F=13.32$, $R^2=0.47$, $P<0.001$).

Discussion

Substantial changes have occurred in oyster associated communities and salinity gradient in the NPRES since Wells' 1961 study. At the whole estuary scale, community assemblages shifted in composition, and most notably, those communities reported in Wells (1961) were more heterogeneous over space than reefs sampled between 2013-2015. This result indicates turnover (beta-diversity) of NPRES communities has decreased between sites over time. This decrease in community variability is correlated with multi-decadal decreases in salinity heterogeneity among NPRES sites (due to estuarine-wide salinity increases), following the widely accepted idea that environmental heterogeneity promotes community turnover or beta-diversity (Harrison et al. 2010). These shifts in community turnover were potentially due to both decreases in subtidal reef habitat, indicated by fewer subtidal fauna in 2013-2015 collections, as well as individual species shifts to upper NPRES sites not documented in Wells (1961).

Mechanisms of community change

Qualitatively, I documented loss of oyster reef habitat based on descriptions in Grave (1901) and Wells (1961), including intertidal reefs at Gallants Point and White Rock, and subtidal reef at PI (based on Wells' site descriptions and our field observations). Families accounting for the most variability between the same sites over the 60-year time span were predominantly subtidal taxa. Out of 25 families that explained more than 2% of the variability over time at PI, 17 families were characteristically subtidal in distribution, and all 25 families were found at higher frequency during Wells' time (1961). At WR and WRR, 10 out of 23 families that explained more than 2% of the variability over time were subtidal in distribution. While WR and WRR are technically different sites, there were no signs of subtidal oyster reef at WRR. At WR, Wells described scattered subtidal oyster clusters, and at ~0.5 m of maximum

exposure at low tide, the top of WR was covered with sand and dead shell, indicative of a reef that has reached its vertical growth potential (Bahr and Lanier 1981). At CR, where subtidal oyster reef existed during our 2013-2015 sampling, 5 of the 12 families contributing 2% or more of the variability between Wells' and my samples over time were subtidal, and four of those, all sessile invertebrates, were never collected in my samples and one, Gobiidae, was more abundant in 2013-2015. While our analysis operated on the family level, it was notable that at CR, Wells collected Balanid barnacles of the species, *Amphibalanus eburneus* and *Amphibalanus improvisus*, while 2013-2015 collections only found the former. Gordon (1969) noted distributional differences based on salinity; *Amphibalanus improvisus* populations were limited to salinities below 15 ppt. These patterns, along with the absence of subtidal reef in 2013-2015 at Pivers Island and Gallants Point, (that had existed during Wells sampling) may be indicative of salinity shifting toward higher values.

Seasonal freshets are control mechanisms on the long-term persistence of oyster pests (I.e. Predators, bioeroders, and sessile fouling organisms) on subtidal oyster reefs in polyhaline portions of estuaries (Wells 1961). The duration and magnitude of freshets determine the degree of disturbance imposed on the biological community present on an oyster reef. Changes in precipitation, upstream modifications that influence freshwater outflow, human modifications to inlets, and RSLR can all influence the duration and magnitude of seasonal freshets, and as a result, the ability for subtidal oyster reefs to persist in a particular estuarine location. Wells described substantial mortality of oysters and associated taxa at PI, the second most saline sampling site in his study following three successive hurricanes, during which measured salinities were 16.5, 15, and 10 ppt. During 2013-2015 sampling, two freshets were recorded, and salinity during the second, more severe freshet in November 2015 reached 15 ppt. Oyster

mortality was not observed following this freshet. It is probable that oyster communities in Wells' study were more severely affected due to the prolonged low-salinity period associated with three successive hurricanes, and that the relatively short-lived freshets in the modern study were not of high enough magnitude or longevity to cause observable levels of faunal mortality.

Wells' site descriptions and faunal collections with numerous subtidal taxa suggest that PI, which had an average salinity of 30.7 ppt between 1955-1956, harbored subtidal oysters. It is possible that subtidal oyster clusters in Wells' collection were dislodged individuals from the intertidal, as we have observed this phenomenon at the edges of other high-salinity oyster reef sites along Carrot Island near Beaufort Inlet. The rising trend of minimum yearly salinity at PI may explain the disappearance of subtidal oysters, as freshwater perturbations (freshets hereafter) were documented in Wells (1961) as contributing heavy mortality oysters and associated fauna. If these oysters did originate in the subtidal, one can speculate that the increased minimum salinities associated with RSLR and channel dredging contributed to the degradation of subtidal oysters by facilitating commensalism, predation, and competition.

The observed decreasing shift in community variability between sites may have attributed to two possible scenarios: (1) taxa move upriver to follow shifting salinity regimes (2) salinity increases facilitate predation and bioerosion of subtidal reefs, depleting available habitat and thus the number of species that require subtidal environments. There is evidence that some combination of the above two scenarios has occurred over the 6 decade time span between studies. In 1955-1956, 10 families had upper estuarine limits at WR, and 5 of those families upper limits shifted to CR. Two of those five families, Caprellids and Leptochellids, are chiefly subtidal species and often associated with algae and mossy bryozoans (Ruppert and Fox 1988). These families were only found at CR in 2013-2015, suggesting both suitability of salinity and

presence of subtidal habitat are interactive for this species moving up-estuary. The other 3 families, Venerids (*Mercenaria spp.*), Eunicids (*Marphysa sanguinea*), and Terebellids are largely infaunal, and occurred at all sites in 2013-2015 (all are predominantly intertidal), indicating subtidal habitat loss is not a culprit. The polychaete, *Marphysa sanguinea* (Eunicidae) was shown to grow less readily in salinities of 15ppt as well as more variable salinities in laboratory experiments (Garcês and Pereira 2011) and salinity-temperature experiments conducted by Davis and Calabrese (1964) suggest the larvae of the hard clam, *Mercenaria mercenaria* are limited at salinities less than 15ppt during in summer temperature conditions. Salinities at CR averaged 16.19 ± 2.24 ppt in 1955-1956 and 21.88 ± 0.95 ppt in 2013-2015, suggesting that a once suboptimal salinity regime at CR has shifted to be suitable for these species, thereby allowing distributions to expand spatially up-estuary. Due to difficulty in identifying Terebellids, and the potential for multiple species within the family to possess differing salinity tolerances, no comments can be made on up-estuary expansion of this family. Atlantic oyster drill, *Urosalpinx cinerea* (Muricidae) distribution shifted up-estuary since Wells (1961); in 1955-1956 this species had an upper estuarine limit at Gallants Point and in 2013-2015 surveys, occurred as far up-estuary as WRR (~ 7 km upstream from Gallants Point). The distributional expansion of *U. cinerea* to WRR is notable because it is known to be limited by low salinities (Federighi 1931, Wells 1961, Manzi 1971), particularly during warm summer months when storm-related freshets pulse the estuary with freshwater. Oyster drills are also typically limited to subtidal or low intertidal zones, so their occurrence at WRR in 2013-2015, where the reef is chiefly intertidal and not at Wells' WR, is somewhat surprising, but further suggests upstream movement of species under increasing salinities. WR mean $\pm$ SD salinity specific to 1955-1956 (23.46 ± 1.38 ppt) was only slightly lower and more variable than the

mean $\pm$ SD salinity specific to 2013-2015 (23.61 ± 1.25), but multidecadal salinity trends suggest salinity at this site is rising over time, of particular importance, minimums, which which limit the upstream distribution of oyster drills.

Factors influencing salinity changes

Relative sea level may have contributed to the general salinity increases in the NPRE (Figure 2.2). Data from NOAA Tide Gauge at Pivers Island (Station ID 8656483) indicate an RSLR rate of 3.04 ± 0.35 mm/year between 1953-2015 (182.4 mm over the 60 years between studies) due to global SLR and local subsidence. This rise in sea level may lead to saltwater intrusion, meaning saltwater reaches higher into an estuary, which has been documented in Louisiana saltmarsh systems (Boesch et al. 1994), and even converted some marsh systems into open water (Titus 1988). However, RSLR in Louisiana is nearly triple (NOAA station ID 8761724) those found in the present NC study area, such that the role of RSLR is likely of greater in causing saltwater intrusion in Louisiana saltmarsh systems than in the NPRE. In addition, it is difficult to determine the degree to which RSLR is contributing to the observed salinity increases in the study system because it requires measurements of bottom elevation changes over time to determine changes in estuarine volume.

Inlet and shipping channel dredging of shipping channels over the past century could have also contributed to the pattern of increasing salinity in the NPRE. Mechanical dredging of navigational channels alters coastline bathymetry and as a result, localized hydrodynamics, including the range and timing of tides (Zervas 2003), flushing, and residence time, potentially leading to saltwater intrusion (Johnston 1981). Beaufort Inlet has been dredged to new depths at least 5 times since 1911 (Years and depths: 1911, 6.0m; 1936, 9.1m; 1961, 10.7m; 1978, 12.2m; 1994, 13.7m), a cumulative inlet depth increase of 50.5% from 1936-1994 (currently maintained

at 12.2m) (Zervas 2003). Zervas (2003) examined tide range and timing trends at four NOAA stations corresponding to major shipping ports on the Atlantic coast, subject to substantial dredging efforts over the 20th century, including at Pivers Island in Beaufort, NC. At Pivers Island, mean high water (MHW) increased at a rate of 5.33 mm/yr and mean low water (MLW) increased at a rate of 1.92 mm/yr between the mid-1970's and 2003 with a ~0.1m/yr increase in mean tidal range. Zhu et al. (2015) modelled the influence of channel widening and deepening on Tampa Bay (12m channel depth, 1.35m maximum tidal range) tidal constituents, and found that the mean tidal range increases and that under maximum salt flux, nontidal salinity increases are as high as 4 psu. Mean tidal range is increasing at both Tampa Bay and the NPRES systems in response to dredging, and the Zhu et al. (2015) model found substantially increasing salinities as a response, so it is highly likely that the salinity increases in the NPRES are at least in part driven by dredging. Given the scope and resolution of our salinity data for the NPRES over 6 decades, we cannot be certain to what degree dredging practices and RSLR have caused the observed increases in salinity, but evidence from the literature suggest that these may be additive causes for the salinity changes observed in this study.

Drought can be a cause of heightened salinity in estuarine systems due to decreased freshwater inputs from riverine sources and runoff, and may exacerbate the influence of RSLR on saltwater intrusion (Hull and Titus 1986). During some drought periods (as defined by the Palmer Drought Severity Index), salinity variation between maximum and minimum yearly values decreased, with lower or normal maximums and higher minimums. Notable drought periods where this occurred were 1977-1978, 1987-1988, 2002-2003, and 2012-2013. During each of these drought periods, the mean sea level (MSL) was below the model predicted value from the NOAA-sourced RSLR trendline. Drought conditions were present during Wells (1961)

surveys, and his data deviated from the aforementioned pattern in 1955-1956; salinity was variable and relatively high. Notable years with high salinity were 1984-1986 (drought in 1986), 1993-1994, 2008 (winter drought), 2011, and 2015. Each of these time periods saw higher MSL compared to the RSLR linear trendline, indicating that indeed, MSL is likely the predominating factor determining interannual and long term changes in salinity. Drought conditions under lower MSL may function to decrease the occurrence of freshwater perturbations that lead to low salinity aberrations.

A “coastal squeeze” for subtidal oysters

I propose that a form of coastal narrowing is taking place in the NPRES system, where oyster reefs are growing increasingly intertidal in distribution, and their associated faunal communities are growing less variable over the spatial scale of the estuary, some species moving to previously unattainable upper estuary locations. Given the current rate of RSLR for the study area, it is probable that oyster community turnover may continue to decrease over time, and that eventually, the farthest upriver site, CR, may succumb to the indirect effects of salinity increases, predation, bioerosion, and competition.

Continued declines in oyster populations and compressed intertidal distribution could have negative effects on higher trophic level production in estuaries. Subtidal oyster reefs play an important role as nursery habitat for juvenile fish, foraging grounds for commercially important larger fish species and decapods (Lenihan et al. 2001). Coastal narrowing from continued losses to available subtidal habitat from saltwater intrusion may also impact social welfare of fishing communities, as upper estuarine areas, such as that of the NPRES, have been important sites of subtidal oyster cultivation for over a century (Winslow 1889).

The area of the upper NPRE open to shellfishing and the area with suitable salinity regime for subtidal oyster persistence have declined with rising salinity and human population growth during the 20th century. Prior to 1969, the entire NPRE was open to shellfishing, but following the failure of the Newport Sewage Treatment plant in 1969, the entire river was closed. Following intensive sampling by the FDA and EPA in the following years, in 1979, the NC Division of Health Services, Shellfish Sanitation permanently prohibited all shellfishing immediately upstream of Cross Rock reef. In 1998, an additional 400 acre area was designated as permanently closed to shellfishing, in 2005 an additional 103 acres, and finally in 2016 an additional 38 acres were closed permanently, totaling 541 acres since 1972 (North Carolina Division of Marine Fisheries Department of Environmental Quality 2016).

Simultaneously, salinity increases in the upper NPRE have allowed in the upstream movement of oyster pests, in particular oyster drills, which limit subtidal oyster distribution. As a result, the Grave (1904) line (threshold for subtidal oyster cultivation upstream), effectively moved from a point immediately east of WR reef, to the latitudinal point at WRR reef. The linear distance of this movement, following the main estuarine channel is 1.2 km, and the loss of suitable subtidal oyster habitat due to salinity increases is approximately 550 acres. Using Google Earth polygons, we approximated the subtidal oyster cultivation area lost to salinity increases and human waste pollution. Starting at the point at which the NPRE widens from the narrows section, west of Cross Rock, the total area available to subtidal oyster cultivation in 1904 was approximately 1,400 acres. The sum of the area lost due to salinity increases and shellfish sanitation closures is 1091 acres, leaving 210 acres suitable for subtidal oyster cultivation, an 85% decrease in the historical area available to the oyster fishermen in the NPRE. This estimate is certainly conservative. The area directly west of Cross Rock has infilled with

sediment such that the available bottom habitat has decreased over the 20th century, due to developments upstream in the town of Newport. Our calculations are based on the current area, so the amount of subtidal oyster habitat lost since Caswell Grave's 1904 survey and Harry Wells 1950's survey is likely larger than the estimates detailed above.

This squeeze of available oyster habitat and cultivation area is certainly not unique to our study system, and is probably occurring to various degrees in all areas that have experienced a combination of dredging and RSLR, and population growth. Not only are estuarine biogenic communities influenced by salinity regime shifts and habitat losses, but the coastal fishing communities that rely on estuarine shellfish resources. Thus, our results of long term change in oyster communities over shifting estuarine salinity gradient can inform coastal restoration on a broad scale. Efforts to restore oysters back to historical levels will be complicated by the impacts of climate change. Thus, efforts should be tailored to adapt to the inevitable salinity changes that will continue to change the chemical landscape of estuaries as a result of RSLR and waterway modifications. On the east coast of the United States, oyster restoration efforts have largely been subtidally focused, and in North Carolina, exemplified by often failed efforts in the Pamlico Sound by NC-DMF (Lindquist *personal communication*). Future restoration efforts should examine the potential for RSLR induced salinity changes to threaten restoration success, and maximize success by focusing efforts in the intertidal or estuaries with high outflow to limit high salinity induced degradation processes.

Table 2.1. Summary of Newport River oyster reef site characteristics. Insights from Grave (1901) are provided to give historical context to oyster reef sites relevant to the multi-decadal analysis.

Site	Grave 1901	Wells 1955- 1956	Current study 2013-2015	Inlet Distance (km)	Reef Type	Aerial exposure regime
Shark Shoal	Not noted	Extant	Nonextant	1.8	Jetty	Intertidal, scattered subtidal
Pivers Island	Not noted	Extant	Extant	3.47	Fringing	Intertidal, scattered subtidal
Gallants Point	Not noted	Extant	Nonextant	6.61	Bar	Intertidal, scattered subtidal
White Rock	Extant	Extant	Nonextant	12.5	Patch	Intertidal, scattered subtidal
White Rock Replacement	Extant	Not noted	Extant	13.1	Patch	Intertidal

Table 2.2. Sampling months during each year at sites used for comparison between Wells (1961) 1955-1956 oyster communities and 2013-2015 communities in the NPRES.

Sites		Pivers Island (PI)	White Rock (WR) White Rock Rep. (WRR)	Cross Rock (CR)
Wells (1961)	1955	7,8,9,10,11	7,8,9,10,11	7,8,9,10,11
	1956	1,2,3,4,5,6,7,9	1,2,3,4,5,6,7,9	1,2,3,4,5,6,7,9
Current 2013-2015	2013	7,8,10,12	7,8,10,12	7,8,10,12
	2014	2,4,6,8,10	2,4,6,8,10	2,4,6,8,10
	2015	1,3,5,7,10,10,12	1,3,5,7,12	1,3,5,7,10,10,12

Table 2.3. List of taxonomic families observed at oyster reefs by Wells (1961) and during 2013-2015 surveys. Values on the right side of the table represent the frequency of occurrence over all samples and at each site within each study period.

Taxonomic group	1955-1956				2013-2015			
	All	PI	WR	CR	All	PI	WRR	CR
ARTHROPODA								
Amphipoda								
Caprellidae	0.4	0.857	0.333	0	0.043	0	0	0.125
Corophiidae	0.714	0.571	0.667	0.867	0.37	0	0.143	0.938
Gammaridae	0.4	0.643	0.333	0.2	0.065	0.063	0.071	0.063
Melitidae	0.971	1	1	0.933	0.674	0.375	0.786	0.875
Tanaidacea								
Leptocheliidae	0.2	0.357	0.333	0	0.043	0	0	0.125
Isopoda								
Sphaeromatidae	0.543	0.214	0.333	0.933	0.674	0.125	1	0.938
Decapoda								
Diogenidae	0.171	0.357	0.167	0	0.022	0.063	0	0
Panopeidae	0.857	1	0.667	0.8	1	1	1	1
Diptera								
Tabanidae	0.114	0	0.167	0.2	0.022	0.063	0	0
Maxillopoda								
Balanidae	0.914	0.929	1	0.867	0.826	0.813	0.786	0.875
Chthamalidae	0.143	0.357	0	0	0	0	0	0
Sacculinidae	0	0	0	0	0.587	0.438	0.786	0.563
Pycnogonida								
Nymphonidae	0.114	0.286	0	0	0	0	0	0
BRYOZOA								
Cheilostomatida								
Bugulidae	0.457	0.643	0.667	0.2	0.043	0.063	0.071	0
Electridae	0.686	0.286	1	0.933	0	0	0	0
Membraniporidae	0.371	0.857	0.167	0	0	0	0	0
Schizoporellidae	0.371	0.857	0.167	0	0	0	0	0
Ctenostomatida								
Alcyonidiidae	0.229	0.214	0.667	0.067	0	0	0	0
Nolellidae	0.057	0	0.333	0	0	0	0	0
Victorellidae	0.371	0.286	0.333	0.467	0	0	0	0
CILIOPHORA								
Heterotrichea								
Folliculinidae	0.714	0.929	0.667	0.533	0	0	0	0
CLITELLATA								
Oligochaeta								
Tubificidae	0	0	0	0	0.239	0.063	0.214	0.438
CNIDARIA								
Anthozoa								
Actiniaria spp.	0.514	1	0.333	0.133	0.413	0.375	0.5	0.375
Gorgoniidae	0.257	0.571	0.167	0	0	0	0	0
Hydrozoa								
Campanulariidae	0.514	0.5	0.667	0.467	0	0	0	0

Continued on next page

Table 2.3. Continued.

Taxonomic group	1955-1956				2013-2015			
	All	PI	WR	CR	All	PI	WRR	CR
Frequency of occurrence								
MOLLUSCA								
Bivalvia								
Anomiidae	0.229	0.357	0.333	0.067	0.022	0.063	0	0
Arcidae	0.086	0.214	0	0	0	0	0	0
Mytilidae	0.914	1	0.5	1	1	1	1	1
Pholadidae	0.171	0.429	0	0	0	0	0	0
Tellinidae	0	0	0	0	0.087	0	0.071	0.188
Veneridae	0.286	0.571	0.333	0	0.565	0.375	0.571	0.75
Gastropoda								
Buccinidae	0.029	0.071	0	0	0	0	0	0
Calyptraeidae	0.286	0.357	0.5	0.133	0.217	0	0.429	0.25
Cerithiidae	0.229	0.571	0	0	0	0	0	0
Columbellidae	0.486	0.929	0.5	0.067	0.022	0	0.071	0
Fasciariidae	0.2	0.5	0	0	0	0	0	0
Fissurellidae	0.286	0.714	0	0	0.022	0.063	0	0
Muricidae	0.343	0.857	0	0	0.087	0.063	0.214	0
Pyramidellidae	0.6	1	0.333	0.333	0.63	0.75	0.786	0.375
NEMERTEA								
Nemertea spp.	0.486	0.857	0.5	0.133	0.152	0	0.214	0.25
PLATYHELMINTHES								
Polycladida								
Stylochidae	0.286	0.429	0.167	0.2	0	0	0	0
POLYCHAETA								
Errantia								
Eunicidae	0.4	0.929	0.167	0	0.587	0.938	0.786	0.063
Nereididae	1	1	1	1	1	1	1	1
Phyllodocidae	0.514	1	0.333	0.133	0.457	0.438	0.786	0.188
Syllidae	0.343	0.643	0.333	0.067	0.022	0.063	0	0
Sedentaria								
Capitellidae	0.429	0.714	0.333	0.2	0.087	0.063	0	0.188
Sabellariidae	0.514	0.929	0.5	0.133	0	0	0	0
Sabellidae	0.486	0.857	0.5	0.133	0.065	0.125	0	0.063
Serpulidae	0.6	1	0.5	0.267	0.239	0.25	0.357	0.125
Spionidae	0.743	0.786	0.5	0.8	0.522	0.25	0.643	0.688
Terebellidae	0.343	0.786	0.167	0	0.13	0.125	0.214	0.063
PORIFERA								
Demospongia								
Chalinidae	0.171	0.286	0.333	0	0	0	0	0
Clionidae	0.486	1	0.333	0.067	0.022	0.063	0	0
Halichondriidae	0.371	0.929	0	0	0	0	0	0
TUNICATA								
Ascidacea								
Molgulidae	0.4	0.571	0.5	0.2	0.022	0	0.071	0
Styelidae	0.229	0.286	0.5	0.067	0	0	0	0
VERTEBRATA								
Actinopterygii								
Gobiidae	0.143	0.071	0.167	0.2	0.196	0	0.143	0.438

Figure 2.2. Historical trends in yearly mean, maximum, and minimum salinity across NPRES sampling sites. WR measurements were pooled between WR and WRR sites. CR and WR time series are from 1955-2015. PI time series considers salinity data from 1913 through 2015. Statistics associated with yearly maximum and minimum least-squares regression and associated lines of best fit are color coded on each panel. Points represent the mean salinity for each year when data was available, and lines extending from each mean point represent the salinity range.

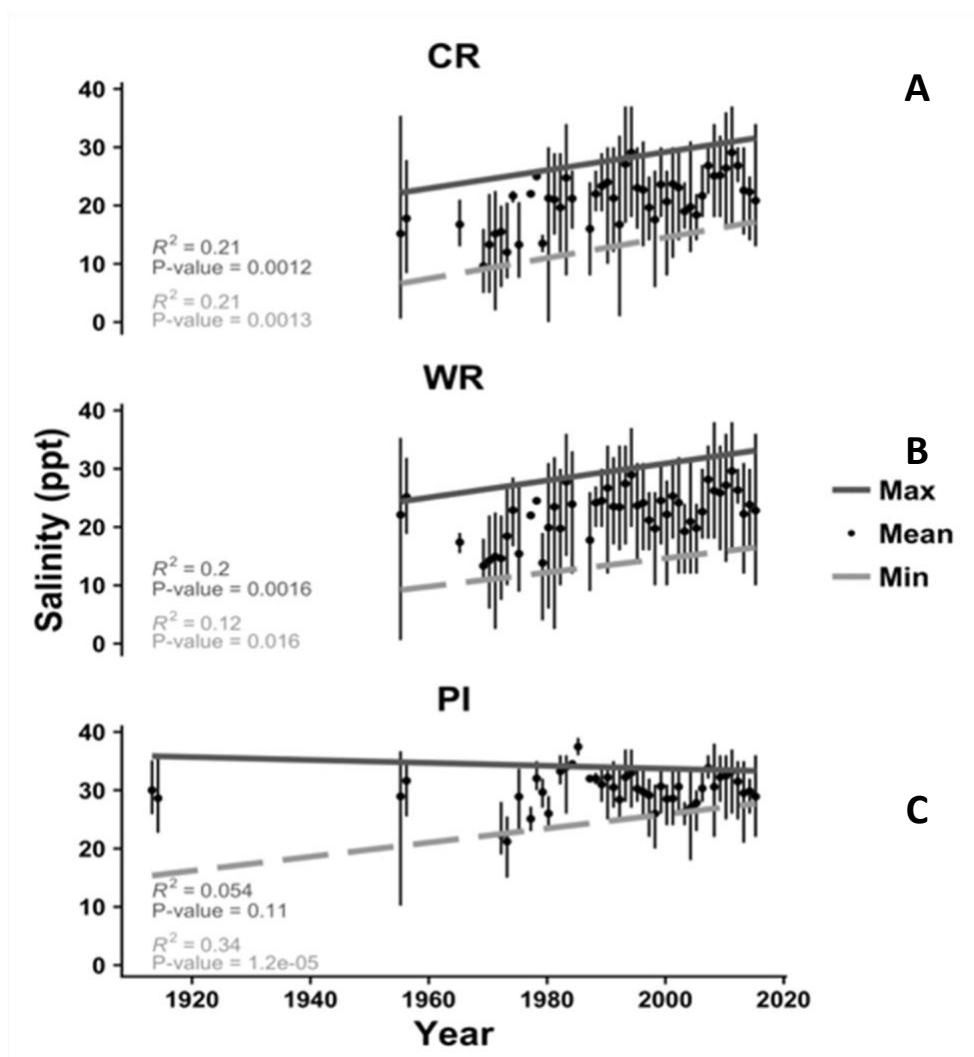


Figure 2.3. NMDS (Nonmetric multidimensional scaling) plot of 1955-1956 (Wells 1961) and 2013-2015 (current study) oyster associated communities in the NPRES. Warm colors correspond to Wells samples and cool colors correspond to current samples. Darker colors correspond to sites closer to the Beaufort Inlet. Vectors labeled “Year” and “Salinity” explained significant portions of community variability (Envfit). Ellipses represent 95% confidence intervals. Stress < 0.2.

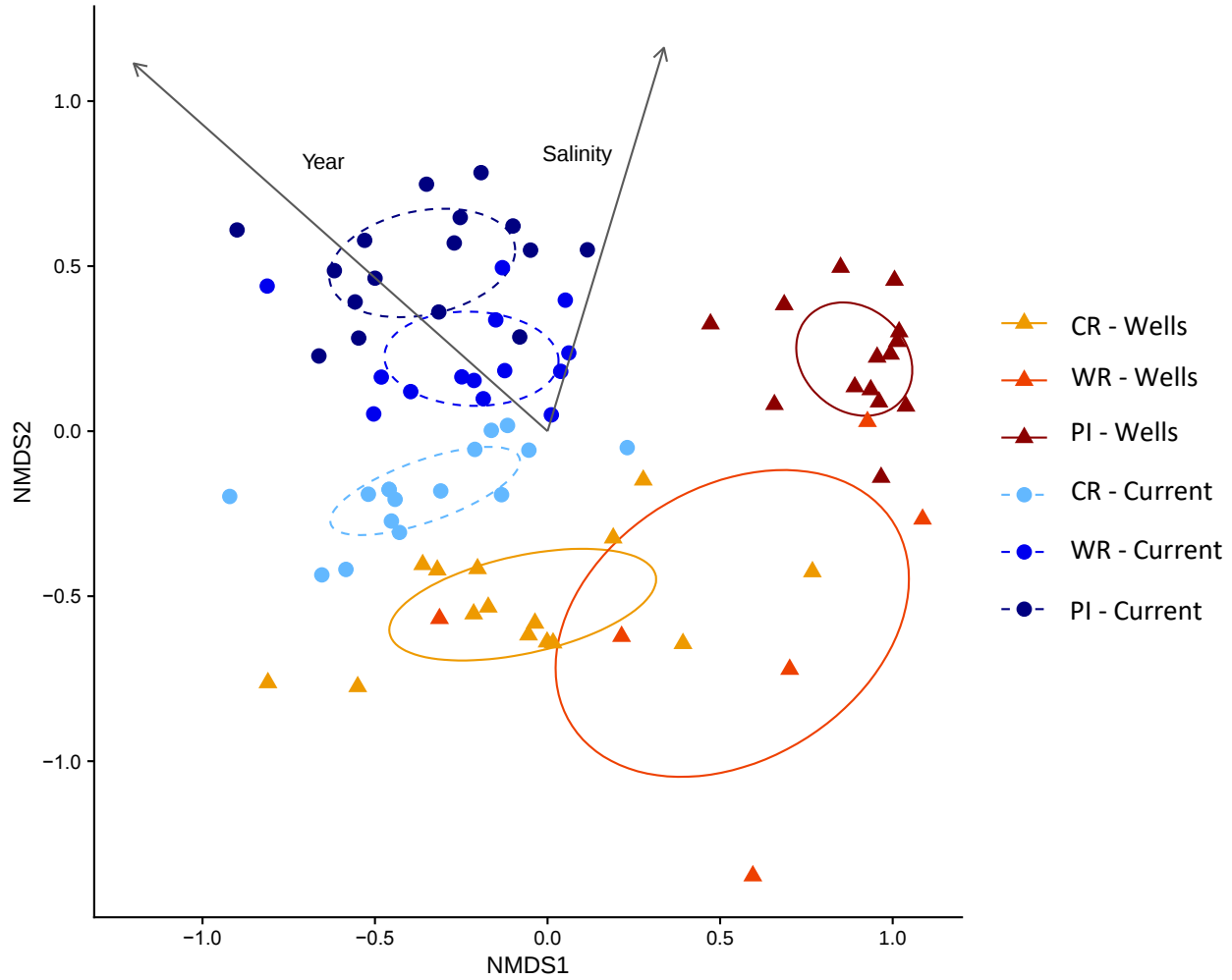
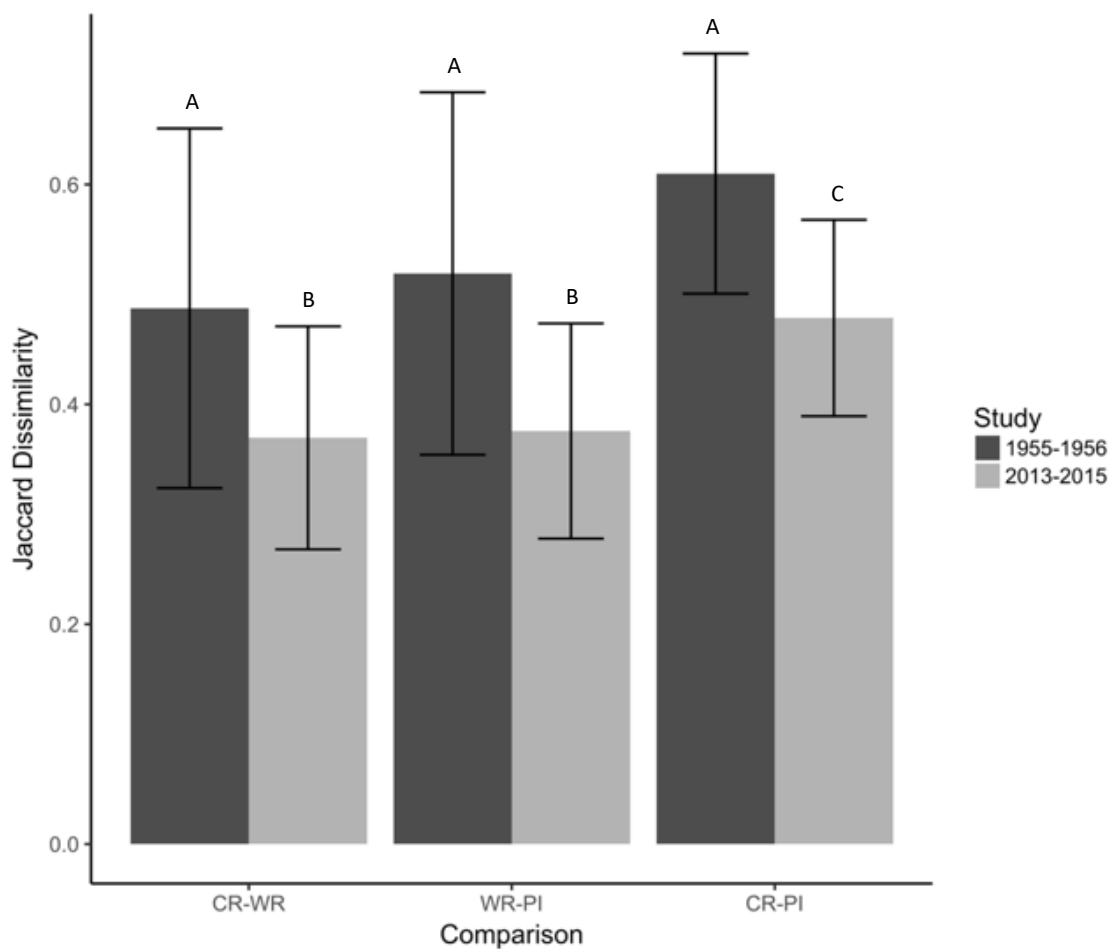


Figure 2.4. Jaccard dissimilarity between site pairs for 1955-1956 Wells sampled communities (dark grey bars) and 2013-2015 communities (light grey bars), mean $\pm$ 1SD. “WR” is used to represent both White Rock and White Rock Replacement. Site comparisons are arranged in order of increasing site-site distance.



REFERENCES

- Bahr, L. M., & Lanier, W. P. (1981). *The ecology of intertidal oyster reefs of the South Atlantic coast: a community profile*(No. 81/15). US Fish and Wildlife Service.
- Barry, J. P., Baxter, C. H., Sagarin, R. D., & Gilman, S. E. (1995). Climate-related, long-term faunal changes in a California rocky intertidal community. *Science*, 267(5198), 672-675.
- Boesch, D. F., Josselyn, M. N., Mehta, A. J., Morris, J. T., Nuttle, W. K., Simenstad, C. A., & Swift, D. J. (1994). Scientific assessment of coastal wetland loss, restoration and management in Louisiana. *Journal of Coastal Research*, i-103.
- Beck, M. W., Brumbaugh, R. D., Airoidi, L., Carranza, A., Coen, L. D., Crawford, C., ... & Lenihan, H. S. (2011). Oyster reefs at risk and recommendations for conservation, restoration, and management. *Bioscience*, 61(2), 107-116.
- Chavez, F. P., Ryan, J., Lluch-Cota, S. E., & Niquen, M. (2003). From anchovies to sardines and back: multidecadal change in the Pacific Ocean. *science*, 299(5604), 217-221.
- Church, J. A., & White, N. J. (2006). A 20th century acceleration in global sea-level rise. *Geophysical research letters*, 33(1).
- Davis, H. C., & Calabrese, A. (1964). Combined effects of temperature and salinity on development of eggs and growth of larvae of *M. mercenaria* and *C. virginica*. *Fishery Bulletin*, 63(3), 643-655.
- Dayton, P. K., & Tegner, M. J. (1984). Catastrophic storms, El Niño, and patch stability in a southern California kelp community. *Science*, 224(4646), 283-285.
- Doody, J. P. (2013). Coastal squeeze and managed realignment in southeast England, does it tell us anything about the future?. *Ocean & coastal management*, 79, 34-41.
- Federighi, H. (1931). Salinity Death-Points of the Oyster Drill Snail, *Urosalpinx Cinerea* Say. *Ecology*, 12(2), 346-353.
- Fodrie, F. J., Rodriguez, A. B., Baillie, C. J., Brodeur, M. C., Coleman, S. E., Gittman, R. K., ... & Theuerkauf, E. J. (2014). Classic paradigms in a novel environment: inserting food web and productivity lessons from rocky shores and saltmarshes into biogenic reef restoration. *Journal of applied ecology*, 51(5), 1314-1325.
- Garcês, J. P., & Pereira, J. (2011). Effect of salinity on survival and growth of *Marphysa sanguinea* Montagu (1813) juveniles. *Aquaculture international*, 19(3), 523-530.
- Goodwin, C. R. (1991). *Tidal-flow, circulation, and flushing changes caused by dredge and fill in Hillsborough Bay, Florida*(No. 2376). USGPO,.

- Gordon, C. M. (1969). The apparent influence of salinity on the distribution of barnacle species in Chesapeake Bay (Cirripedia). *Crustaceana*, 139-142.
- Grabowski, J. H., Hughes, A. R., Kimbro, D. L., & Dolan, M. A. (2005). How habitat setting influences restored oyster reef communities. *Ecology*, 86(7), 1926-1935.
- Grave, C. (1901). *The oyster reefs of North Carolina: a geological and economic study*. Place of publication not identified.
- Grave, C., Moore, H. F., & Glaser, O. C. (1904). *Investigations for the promotion of the oyster industry of North Carolina* (Vol. 556). Govt. print. off..
- Gutsell, J. S. (1931). *Natural history of the bay scallop* (No. 1100). US Government Printing Office.
- Harley, C. D., & Helmuth, B. S. (2003). Local-and regional-scale effects of wave exposure, thermal stress, and absolute versus effective shore level on patterns of intertidal zonation. *Limnology and Oceanography*, 48(4), 1498-1508.
- Harrison, S., Vellend, M., & Damschen, E. I. (2011). 'Structured'beta diversity increases with climatic productivity in a classic dataset. *Ecosphere*, 2(1), 1-13.
- Hull, C. H. J., & Titus, J. G. (Eds.). (1986). *Greenhouse effect, sea level rise, and salinity in the Delaware Estuary*. United States Environmental Protection Agency.
- Helmuth, B., Harley, C. D., Halpin, P. M., O'donnell, M., Hofmann, G. E., & Blanchette, C. A. (2002). Climate change and latitudinal patterns of intertidal thermal stress. *Science*, 298(5595), 1015-1017.
- Hoyt, W. D. (1920). *Marine algae of Beaufort, NC, and adjacent regions* (Vol. 876). Govt. Print. Off..
- Johnston, S. A. (1981). Estuarine dredge and fill activities: a review of impacts. *Environmental management*, 5(5), 427-440.
- Kirby-Smith, W. W., & Costlow, J. D. (1989). *The Newport River estuarine system*. UNC Sea Grant College Program.
- Lenihan, H. S., Peterson, C. H., Byers, J. E., Grabowski, J. H., Thayer, G. W., & Colby, D. R. (2001). Cascading of habitat degradation: oyster reefs invaded by refugee fishes escaping stress. *Ecological Applications*, 11(3), 764-782.
- Manzi, J. J. (1970). Combined effects of salinity and temperature on the feeding, reproductive, and survival rates of *Eupleura caudata* (Say) and *Urosalpinx cinerea* (Say)(Prosobranchia: Muricidae). *The Biological Bulletin*, 138(1), 35-46.

- Pontee, N. (2013). Defining coastal squeeze: A discussion. *Ocean & coastal management*, 84, 204-207.
- Ridge, J. T., Rodriguez, A. B., & Fodrie, F. J. (2017). Salt marsh and fringing oyster reef transgression in a shallow temperate estuary: Implications for restoration, conservation and blue carbon. *Estuaries and Coasts*, 40(4), 1013-1027.
- Rodriguez, A. B., Fodrie, F. J., Ridge, J. T., Lindquist, N. L., Theuerkauf, E. J., Coleman, S. E., ... & Kenworthy, M. D. (2014). Oyster reefs can outpace sea-level rise. *Nature climate change*, 4(6), 493.
- Ruppert, E. E., & Fox, R. S. (1988). Seashore animals of the Southeast: a guide to common shallow-water invertebrates of the southeastern Atlantic Coast.
- Sagarin, R. D., Barry, J. P., Gilman, S. E., & Baxter, C. H. (1999). Climate-related change in an intertidal community over short and long time scales. *Ecological monographs*, 69(4), 465-490.
- Titus, J. G. (1988). Sea level rise and wetland loss: an overview. *Titus, JG Greenhouse Effect, Sea Level Rise, and Coastal Wetlands. US Environmental Protection Agency. Washington, DC*, 186.
- Wells, H. W. (1961). The fauna of oyster beds, with special reference to the salinity factor. *Ecological Monographs*, 31(3), 239-266.
- Winslow, F. (1889). *Report on the Sounds and Estuaries of North Carolina: With Reference to Oyster Culture*. United States Coast and Geodetic Survey.
- Zervas, C. E. (2003). Long term changes in tidal response associated with the deepening of navigational channels. In *Proc., Coastal Zone* (Vol. 3).

APPENDIX 1.1: TIME SERIES OF OYSTER ABUNDANCE, LENGTH, AND DIVERSITY

