ABSTRACT

RICCI, SHANNON WHITNEY. Spatiotemporal Soundscape Patterns and Processes in an Estuarine Reserve. (Under the direction of Dr. David Eggleston).

A soundscape is the collection of physical, biological, and anthropogenic sources of sound of an environment, and provides valuable sensory information for organisms living in those environments. Estuarine habitats such as seagrass beds, salt marshes, and oyster reefs, likely have unique soundscape characteristics due to the variation in the diversity and abundance of resident soniferous species, as well as physical sources of sound from the structural aspects of these habitats. Though these habitats are well studied, their soundscapes are not. The emerging field of marine soundscape ecology aims to investigate how the soundscapes of different habitats change over time and space, and how this spatial and temporal variability influences organisms living in these environments. This thesis characterized spatiotemporal patterns and processes in the soundscape of a mosaic of structurally complex benthic habitats in an estuarine reserve, and assessed the role of specific soundscape characteristics of habitat-associated sound in promoting settlement in oyster (Crassostrea virginica) larvae.

The first parts of this study characterized spatiotemporal patterns in the summer soundscape in a coastal estuarine reserve, Middle Marsh, within Back Sound, North Carolina, and identified the likely processes underlying those patterns. Soundscapes can change greatly over time, with the greatest changes occurring on daily and seasonal timescales. Though previous studies have examined soundscapes over short periods of time with the greatest changes (e.g., day vs night), there is increasing interest in recording soundscapes over longer timescales with high temporal replication. This study characterized temporal patterns and processes in the soundscapes of a coastal estuarine reserve to determine how the soundscape
changes on daily, lunar, or monthly cycles, and how abiotic and biotic factors influence these temporal changes. Temporal variability in the higher frequency band was correlated with the nightly peak in snapping shrimp activity, whereas the lower frequency band was influenced by both biological activity (e.g., fish chorusing) as well as tidal water levels.

Within estuaries, there are habitat mosaics of seagrass, oyster reef, salt marsh, and soft-bottom habitats that provide essential fish habitat, but are difficult to study with traditional sampling gear. Underwater soundscapes can be a valuable indicator of habitat type and quality, and can be used to determine presence of soniferous fish species. This part of the study characterized spatial patterns and process in the soundscape, and focused on relating characteristics of the soundscape (e.g., SPLs in high and low frequency bands, amount of fish chorusing) to patterns in the seascape (percent cover of estuarine habitats). While habitat composition was not related to spatial patterns in fish-driven, low frequency sounds, it did explain some variation in spatial patterns of snapping-shrimp driven high frequency sounds.

Previous studies have shown that habitat-associated sounds promote settlement for oyster larvae, but have not examined which components of the habitat-associated sound elicits that response. In the final part of this study, the oyster reef soundscape was divided into a lower frequency component and a higher frequency component to test whether fish-associated low frequency sounds, or snapping-shrimp associated higher frequency sounds results in a higher proportion of larval oyster settlement.

Together, these studies investigating temporal and spatial patterns in the soundscape, and the influence of habitat-associated sound on the settling stage of reef-building oyster larvae, illustrate the importance of comprehensive soundscape studies. Soundscapes are complex, and exploring their patterns and underlying processes is essential to understanding
its role in larval settlement, as well as its potential to rapidly assess habitat quality and biodiversity in marine systems.
Spatiotemporal Soundscape Patterns and Processes in an Estuarine Reserve

by
Shannon Whitney Ricci

A thesis submitted to the Graduate Faculty of
North Carolina State University
in partial fulfillment of the
requirements for the degree of
Master of Science

Marine, Earth, and Atmospheric Sciences

Raleigh, North Carolina

2015

APPROVED BY:

_______________________________  _________________________________
Dr. DelWayne Bohnenstiehl        Dr. John Fear

_______________________________
Dr. David Eggleston
Chair of Advisory Committee
DEDICATION

To my mom Cheryl, my dad Merritt, my sister Savannah, and my husband Tom. Thank you for your constant love, support, and encouragement to achieve my dreams.
Shannon Whitney Ricci (born Brown) grew up in Lake Peekskill, NY, a small town an hour north of NYC. She first fell in love with the ocean during family summer trips to Block Island, a small island off the coast of Rhode Island. One summer, her parents took her “Marsh Mucking” with the Block Island Nature Conservancy. It was there, knee-deep in water, with a bucket full of critters, that she found out that marine biologists did this for a living. Throughout her time at school in Putnam Valley she continued to dream of becoming a marine biologist. What started as a dream for many of the students in her class (“When I grow up I want to be…”), became what she actually pursued in college.

Her undergraduate years were at the University of Maine at Orono, where she majored in Marine Biology. As a sophomore she joined Dr. Sara Lindsay’s lab and began, much to her surprise, working with worms. After working on various projects involving marine worms, Shannon discovered her deep interest and love for all of the amazing marine invertebrates, each uniquely extraordinary. The summer following her sophomore year, Shannon received a NSF Research Experience for Undergraduates, where she studied horseshoe crabs in the Delaware Inland Bays with Dr. Doug Miller from the University of Delaware. After a long summer of late nights counting masses of spawning horseshoe crabs and long hot days digging up horseshoe crab eggs, she traveled to Walpole, ME where she spent the first semester of her junior year at the Darling Marine Science Center. It was here that she explored the gorgeous rocky intertidal and mudflats of Downeast Maine. Back in Orono, she completed an undergraduate honors thesis, co-advised by Dr. Lindsay and Dr. Paul Rawson, where she investigated treatments to rid oysters of mud worms.
After graduating in 2012, Shannon returned home to New York and began volunteering at an environmental education center on the Hudson River. She loved teaching students about the fish and invertebrates they collected while seining or exploring the marsh. In the spring of 2013 she moved to Edgewater, Maryland for an internship in the education center at the Smithsonian Environmental Research Center (SERC). During her time at SERC, Shannon was able to share her passion for marine science with students and the public through all manner of ways ranging from leading canoe trips through a marsh to helping students with their first plankton tow and microscope observations.

Upon completion of her internship, Shannon was accepted into the master’s program at North Carolina State University, where she began researching an entirely new topic: underwater soundscape ecology. She was awarded the 2014 NC Sea Grant/NC Coastal Reserve Coastal Research Fellowship to characterize soundscapes in the Rachel Carson Estuarine Research Reserve. In addition to these field experiments, she also spent many long nights in Jordan Hall counting larvae for settlement experiments investigating the response of oyster larvae to oyster reef soundscapes.

Recently married to her high school sweetheart, Shannon is drawing nearer to her next milestone: receiving her master’s degree. She looks forward to pursuing a career where she can bridge her interests in environmental education and research to inspire children and adults to find their own love of the wonderfully weird animals of the marine world.
ACKNOWLEDGMENTS

This thesis is the result of many years of encouragement and support from the best family and friends someone could ask for. To my mom and dad, Cheryl and Merritt Brown, thank you for supporting me and giving me the chance to choose my own path in life. To Savannah, my sister- thanks for putting up with my scientist quirks, and I promise you I do more than just “sift-sand” for a living. My husband Tom has been essential in keeping me sane throughout my time here, and I am so glad to have him as my partner in life.

To my current and past advisors- thank you for believing in my abilities as a scientist and encouraging me to always push further. I am grateful for the expertise, support and guidance of my master’s advisor, Dr. David Eggleston. I admire his ability to see where everything fits in to a big picture, which was always comforting in our meetings. To Dr. Del Bohnenstiehl- I am so appreciative for his patience as I tried, failed, then somewhat succeeded in, learning how to code in MATLAB. It is through the collaboration of Del, Dave, and myself that I saw the benefit of this department of Marine, Earth, and Atmospheric Sciences. I would also like to thank Dr. John Fear for providing his insight and expertise as manager of coastal reserve sites. In addition, I’d like to thank the administrative staff, for their role in helping me receive the materials and information I needed to carry out my projects.

This project could not have been completed without help from my fabulous lab members. First, a huge thanks goes to Jordan Byrum for being a top-notch boat captain and navigating our way through the shifting channels of Middle Marsh. I am especially thankful for Ashlee Lillis for the years she spent developing the soundscape ecology program here,
and for her support and guidance as I learned how to develop my own questions to add to the
great work she had done. Thank you to Doreen McVeigh for bringing me to one of the best
places in Raleigh, the NC Museum of Natural Sciences, and for working with me to become
a better communicator of science. To Seth Theuerkauf, thank you for making the time spent
learning MATLAB, struggling through statistics, and surviving some wicked field work
conditions, more enjoyable than going at it alone. To the rest of my lab members, Katelyn
Theuerkauf, Olivia Caretti, Pat Lyon, and Kayelyn Simmons: thank you for help, and
distractions, during the final push of working on this thesis. I’d also like to acknowledge
zipties, an honorary member of this group. A wise Eggleston lab member once said, “I think
75% of all marine science is just zipties”. Without them, this project, and many other marine
science endeavors, would not be possible.

Finally, this work would not be possible without funding contributions from NC Sea
Grant, NC Coastal Reserve, and the National Science Foundation.
# TABLE OF CONTENTS

LIST OF TABLES ........................................................................................................... ix

LIST OF FIGURES ......................................................................................................... x

CHAPTER 1
TEMPORAL SOUNDSCAPE PATTERNS AND PROCESSES IN AN
ESTUARINE RESERVE ................................................................................................. 1

ABSTRACT ..................................................................................................................... 1

INTRODUCTION ............................................................................................................ 2

MATERIALS AND METHODS ....................................................................................... 4

  Site selection and characterization ................................................................. 4
  Acoustic sampling .............................................................................................. 5
  Acoustic analysis ............................................................................................... 6
  Environmental data collection and analysis .................................................... 8

RESULTS ...................................................................................................................... 9

  General temporal patterns in SPLs ................................................................. 9
  Processes underlying temporal patterns in SPLs .............................................. 10
    Abiotic variables ............................................................................................. 10
    Biotic variables: Fish chorusing ................................................................. 11
    Biotic variables: Snapping shrimp .............................................................. 12

DISCUSSION ................................................................................................................. 13

  Processes underlying temporal patterns in SPL .............................................. 13
  Study implications ............................................................................................ 15

CONCLUSIONS ............................................................................................................ 18

LITERATURE CITED ................................................................................................... 20

CHAPTER 2
SOUNDSCAPES TO SEASCAPES: DO SPATIAL PATTERNS IN
ESTUARINE HABITATS RELATE TO SPATIAL PATTERNS IN
SOUNDSCAPES? ........................................................................................................ 41

ABSTRACT .................................................................................................................... 41

INTRODUCTION ........................................................................................................... 42

MATERIALS AND METHODS ................................................................................... 45

  Site Selection and Characterization ............................................................... 45
  Acoustic Analysis .............................................................................................. 47
  Statistical Analysis ............................................................................................ 49

RESULTS ...................................................................................................................... 49

  Spatial patterns in SPLs and fish chorusing ............................................... 49
  Model selection ............................................................................................... 50

DISCUSSION ................................................................................................................. 51

  Spatial patterns in SPLs and fish chorusing ............................................... 52
LIST OF TABLES

Chapter 1

Table 1. Deployment start and end times for each site and deployment ...........23

Table 2. Correlation SPLs in the lower band (150-1500 Hz) at each site ..........23

Table 3. Correlation between SPLs in the higher band (7-43 kHz)
at each site...........................................................................................................24

Table 4. Results of correlation between SPL in the lower band (150-1500 Hz)
at all sites and water level (meters above mean lower low water) at Beaufort
for three deployments. ..............................................................................................24

Table 5. Results of correlation between SPL in the higher band (7-43 kHz)
at all sites and water level (meters above mean lower low water) at Beaufort
for three deployments. ..............................................................................................25

Table 6. Correlation between the higher frequency band and snap counts
for all sites during each deployment ..........................................................................25

Chapter 2

Table 1. Hydrophone deployment start date and time, and end date and time
for each site. ..............................................................................................................61

Table 2. Results of multiple regression analyses using stepwise selection to test
the relationship between soundscape characteristics (dB SPLs in a lower
frequency or higher frequency band) and site habitat characteristics
(percent cover of oyster reef, seagrass, and marsh) in the RCERR. ..................61

Table 3. Results of regression analyses to test the relationship between
soundscape characteristics (dB SPLs in a lower frequency or
higher frequency band) and the amount of marsh edge at each site in the RCERR
in 10 m, 25 m, and 100 m radius circles around each site. .................................62
LIST OF FIGURES

Chapter 1

Figure 1. Map of US Southeast Coast with inset map of Middle Marsh study sites .................................................................26

Figure 2. GIS map of Middle Marsh study sites with habitat layers ..........27

Figure 3. Diagram of tidal phase angles ............................................................28

Figure 4. Sound pressure level in the lower frequency band (150-1500Hz) over time with associated wavelet scaleogram for each deployment ..............29

Figure 5. Sound pressure level in the higher frequency band (150-1500Hz) over time with associated wavelet scaleogram for each deployment ..........31

Figure 6. SPLs in each frequency band, lower and the higher (7-43Hz), versus tidal phase angle ......................................................33

Figure 7: Scatterplots of acoustic variables versus water temperature ........34

Figure 8: Mean nightly SPLs for each lunar quarter for the lower (150-1500Hz) and higher (7-43kHz) frequency bands ........................................36

Figure 9: Percent of total files with fish chorusing at each phase angle of the tidal cycle ........................................................................37

Figure 10. Total files per day of fish chorusing for each day of deployments 1-3 .................................................................38

Figure 11. Files with fish chorusing for each time of day for all deployments ....39

Figure 12. Mean snap rate for each time of day ..............................................40

Figure 13. Percent excess snap rate for each day from deployment 1-3 ..........40

Chapter 2

Figure 1. Map of US Southeast Coast with inset map of Middle Marsh study sites .................................................................63
Figure 2. GIS map of Middle Marsh study sites with habitat layers ..................64

Figure 3. Map showing spatial patterns in mean nighttime SPLs in the low frequency (150-1500Hz) band at each site for each deployment ...............65

Figure 4. Map showing spatial patterns in fish chorusing at each site for silver perch, oyster toadfish, and other fish .............................................66

Figure 5. Map showing spatial patterns in mean nighttime SPLs in the high frequency (7-43kHz) band at each site for each deployment ..................68

Figure 6. Regression plot for percent cover of seagrass and SPLs in the high frequency band (7-43kHz) ........................................................................69

Figure 7. Regression plots for amount marsh and SPLs in the high frequency band (7-43kHz) ........................................................................70

Chapter 3

Figure 1. Map of oyster reef sites in Pamlico Sound, NC .........................82

Figure 2. Diagram of laboratory experiment set-up ...................................82

Figure 3. Spectral composition of sound treatments .................................83

Figure 4. Mean oyster settlement for 48-hour trials .................................84

Figure 5. Mean oyster settlement for 24-hour trials .................................84

Figure 6. Mean oyster settlement for each treatment across all trials ............85

Figure 7. Mean oyster settlement for each batch of larvae received ..........85
CHAPTER 1

TEMPORAL SOUNDSCAPE PATTERNS AND PROCESSES IN AN ESTUARINE RESERVE

ABSTRACT

Habitat-related soundscapes can play an important role in the ecology of estuarine systems. Comprehensive measurements of acoustic variability within estuaries advances our understanding of how sound influences ecological processes at a range of scales, and informs the potential use of these data as an indicator of habitat quality. This study characterized temporal patterns in the summer soundscape of a coastal estuarine reserve, Middle Marsh, within Back Sound, North Carolina, and identified the likely processes underlying these soundscape patterns. Passive acoustic recorders were deployed at eight sites in the reserve, and sampled two minutes every 20 minutes over three months in the summer of 2014. Time-series analysis of sound pressure levels (SPLs) indicates SPLs of higher frequency sounds (7-43kHz) had a periodicity of once per day, with SPLs ~10 dB higher at night than during the day. This diurnal pattern in the high-frequency soundscape is correlated with biological snapping shrimp noise, with an average excess of ~12% more snaps detected at night. The same analysis for SPLs of lower frequency sounds (150-1500Hz) revealed a periodicity of twice per day, and SPLs varied by as much as 20 dB between daily maximum and daily minimum SPLs. Temporal variability in the lower frequency band is correlated with both biological sound sources (fish chorusing), as well as tidal water level, which may influence the presence or absence of fish, and impact sound propagation. The peaks in both the higher frequency band (once per day at night) and the lower frequency band (twice per day, with
high tide) coincide with periods of higher biological activity during nighttime high tides. Sampling mobile fish and snapping shrimp within this relatively complex mosaic of seagrass, saltmarsh, oyster reef and soft-bottom habitats within Middle Marsh, and sampling at night, is extremely difficult with traditional sampling gear such as trawls and throw traps. Soundscape monitoring provides an effective alternative method to assess the reserve’s roll as essential fish habitat, and highlights the ecological functions and services provided by this estuarine habitat.

INTRODUCTION

A soundscape is a composite of all biological (biophony), physical (geophony), and anthropogenic (anthrophony) sounds within a given habitat or area (Pijanowski et al. 2011). Soundscape ecology is an emerging field in which researchers characterize the sounds of an environment, investigate how those sounds affect organisms living in that environment, and use characteristics of the soundscape to infer habitat quality (Farina 2014, Lillis et al. 2014). Soundscapes can also be used to infer what organisms are present, their relative abundance, and what they may be doing.

Much of the research in marine soundscape characterization has focused on temporal patterns, which are key to understanding how organisms use a particular habitat(s) and why (Radford et al. 2008b, Staaterman et al. 2014). While many temporal soundscape investigations have used recordings from discrete blocks of time (e.g., dusk) (Radford et al. 2008b, Lillis et al. 2014), there is increasing evidence from subtropical coral reefs in Australia (Radford et al. 2014), New Zealand (Radford et al. 2010), Panama (Kennedy et al.
2010), and the Florida Keys, USA (Staaterman et al. 2014), as well as oyster reefs in North Carolina, USA (Lillis et al. 2014), that soundscapes change over different time scales (days, weeks, months, lunar cycles) in response to environmental conditions. Understanding these temporal patterns requires long-term, simultaneous sound recordings made with high temporal fidelity (Staaterman et al. 2014).

Few studies, to our knowledge, have investigated temporal patterns in the soundscape of a mosaic of intertidal, estuarine habitats. Such habitats are important as they are used as essential fish habitat for ecologically important species, like the silver perch (Bairdiella chrysoura), and economically important species, like the spotted seatrout (Cynoscion nebulosus) (Mok & Gilmore 1983, Locascio & Mann 2008, Luczkovich et al. 2008). Passive acoustics provides a cost-effective way to get rapid information on biodiversity and habitat that are potentially critical for ecological management and fisheries research. This study investigates the soundscape of the Middle Marsh ecosystem within the Rachel Carson Estuarine Research Reserve in Back Sound, North Carolina, which has been well studied from a faunal and water quality perspective (Fear 2008). The goals of this investigation were to 1) characterize temporal patterns in the summer soundscape in this coastal estuarine reserve at multiple sites, and 2) identify what processes and environmental variables influence temporal soundscape patterns across the entire reserve.
METHODS AND MATERIALS

Site Selection and Characterization

Soundscape characterization was conducted within Middle Marsh, a portion of the Rachel Carson Estuarine Research Reserve (RCERR), a National Estuarine Research Reserve located in Back Sound, near Beaufort Inlet, NC (Figure 1). Middle Marsh is subject to semi-diurnal tides, and is located ~ 5 km from the nearby Beaufort Inlet that connects Back Sound to the Atlantic Ocean. Mean salinity at the site is high year round, and ranges from > 34 in summer to 32 in winter, with relatively little influence of the nearby North River estuary (Elis 1998). Water depth in Middle Marsh varies from 0.1 to 0.4 m at low tide, to 1.2-1.5 m at high tide, though there are deeper channels adjacent to shallower habitats (Eggleston et al. 1999). The reserve contains a variety of estuarine habitats including seagrass beds, salt marshes, oyster reefs, and unstructured soft bottom (NC DENR, 2001).

In 2006, the North Carolina Estuarine Research Reserve (NCNERR) conducted habitat-mapping analysis in the Rachel Carson Reserve (http://portal.ncdenr.org/web/crp/habitat-mapping). Using the updated, ground-truthed habitat data from 2013 (NCNERR), and a submerged aquatic vegetation (SAV) map from 2008 (NCDENR, APNEP), we generated a spatially-referenced map of the different habitat types in the RCERR (Figure 2). This map was then refined through subsequent field reconnaissance, resulting in our selection of 16 possible sites in MM that were suitable for hydrophone deployment. Our main criteria for suitability were that (i) hydrophones remained submerged at maximum low tide, and (ii) that the sites were acoustically independent, with the direct propagation of sound between sites inhibited by the bathymetry of the marsh. Eight
of 16 possible sites were then randomly chosen as deployment sites for the duration of the study. An analysis of spatial patterns of soundscapes in Middle Marsh, and descriptions of habitat types in each habitat can be found in (Ricci et al.-Chapter 2, Spatial patterns).

**Acoustic sampling**

Underwater sound was recorded at the eight sites simultaneously. An underwater recording system (SoundTrap, Ocean Instruments New Zealand) was strapped vertically to a metal post and positioned ~0.15m above the seafloor, and in water of sufficient depth so that it remained submerged during spring low tides. At low tide, the SoundTraps were submerged at least 0.15 m, and at high tide, the SoundTraps were submerged in approximately 1 m of water, depending on the magnitude of the tide. Sampling occurred during the months of June, July, and August 2014 in three deployments, with gaps in the data representing times where the SoundTraps were not recording due to malfunction or maintenance (Table 1). A two-minute recording was taken once every 20 minutes (sample rate: 96 kHz) for the duration of the deployments to characterize differences in the soundscape to variations in diurnal patterns. The SoundTrap analog signal is digitized at a fixed rate of 288 kHz. A digital anti-alias filter, with a cutoff frequency of 0.45 times the desired sample rate, is then applied before decimation. Consequently, the useable (-3 dB) bandwidth of these recordings is 0.020-43.0 kHz.
Acoustic Analysis

Each two-minute recording was processed in MATLAB using purpose-written code. These recordings were then partitioned into a lower (150-1500Hz) and a higher (7-43kHz) frequency bands. The lower frequency band included the range that most fish vocalizations occur, including the frequency range of the silver perch (*Bairdiella chrysoura*), a dominant sound producer in this area (Mok & Gilmore 1983, Ramcharitar et al. 2006). The higher frequency band was selected to exclude energy associated with perch chorusing frequencies, and to include energy in frequencies associated with snapping shrimp (Au & Banks, 1998). The gap between these two selected frequency bands included frequencies where fish calls and snaps overlapped, as well as frequencies that were not recorded due to SoundTrap malfunction. Since the acoustic time series are likely to be influenced by time-varying biotic and abiotic factors, wavelet scaleograms were produced to determine strength and persistence of the periodicity in sound pressure levels (SPLs) in both frequency bands (Grinstead et al. 2004).

Spectrograms of individual recordings were used to help identify soniferous species, and patterns in fish chorusing. Files with fish chorusing were identified and compared to archived sound files (e.g., [http://www.dosits.org/science/soundsinthesea/commonsounds/](http://www.dosits.org/science/soundsinthesea/commonsounds/)). Periods of fish chorusing were defined by times when the chorus lasted the duration of the recording, and persisted for multiple recordings.

To further examine temporal and spatial patterns in snapping shrimp acoustic activity, individual snaps were identified using the envelope correlation method described by Bohnenstiehl et al. (in review). The snap kernel was derived from a suit of snaps recorded
locally within Middle Marsh, with the procedure operating on a 7-43 kHz band passed waveform. An amplitude threshold of 107 dB re 1 µPa (peak-to-peak) was applied to the detection catalog; this corresponds to the 90% quantile of the background sound levels observed throughout the monitoring period for all sites.

To quantify diurnal patterns in the acoustic activity of snapping shrimp, the number of snaps detected during the day is compared to those detected at night. For each day, the percent excess snaps occurring at night is calculated as: \(100 \times \frac{(N_o - N_e)}{N_t}\), where \(N_o\) is the number of snaps observed at night and \(N_e\) is the expected number given the fraction of nighttime recordings and the total number of snaps detected daily \(N_t\) (Bohnenstiehl et al., in review). Positive values of percent excess snaps indicate greater snap activity at night; whereas, negative values indicate greater activity during daylight hours. Daytime and nighttime periods are defined based on the local times of sunrise and sunset.

To study temporal patterns in the soundscape of Middle Marsh, the eight sites were used as sub-samples of the overall soundscape. We used the median value of each of three acoustic variables: (i) SPL in the higher frequency band, (ii) SPL in the lower frequency band, and (iii) snap rate among the eight sites at each time point, to produce a single time series representing all eight sites. We examined spatial coherence in SPLs among sites to justify pooling among sites for temporal analyses of SPLs (Tables 2 & 3). The range of correlation coefficients between SPLs in the low band at each site was from 0.41 to 0.75. Correlation coefficients between SPLs in the higher frequency band at each site ranged from 0.43 to 0.79, with the exception of Site 2 and Site 7, which had less snapping shrimp activity than other sites.
Environmental Data Collection and Analysis

The soundscape of a habitat or local area can be driven, in part, by abiotic variables such as temperature, wind speed and direction, tidal currents and water levels (i.e., depth) (Urick 1983). Water level data were retrieved from a nearby NOAA water-level recording station located ~ 5 km away from Middle Marsh in Beaufort, NC (Station ID: 8656483). These data were interpolated to match the sampling frequency of our acoustic data, from one sample every six minutes to one sample every 20 minutes. The correlation between the water level time series and the time series of acoustic variables was determined to examine the effect of water level (tides) on underwater sound. The tidal phase of each two-minute acoustic recording also was estimated from these water level data, with measurements made at high tide having a phase angle of 0°, and those made at low tides having phase angle of 180°/180° (Figure 3).

The water quality monitoring site maintained within Middle Marsh was not used due to missing data, so water temperature data were retrieved from the NERRS Shackleford Banks water quality monitoring site approximately 2.5 km from Middle Marsh, maintained by the National Park Service and the NCNERR (34.68692 /-76.64364). The Shackleford Banks data were interpolated to match the sampling frequency of our acoustic data, from one sample every 15 minutes to one sample every 20 minutes. The correlation between the water temperature time series (in degrees Celsius), and the time series of acoustic variables was determined to examine the effect of water temperature on underwater sound.

Wind speed and direction data were collected from the same NOAA station as the water level data. The wind speed and direction were converted to an alongshore wind
velocity (U), and a cross-shelf wind velocity (V). Corresponding wind velocity and SPLs (in either the lower or higher frequency band) were taken at each hour on the hour. The correlation between these time series was determined to examine the effect of wind velocity on underwater sound. Data on current velocities at our sites within Middle Marsh were not recorded.

A Kruskal-Wallis test was used to examine the effects of lunar quarter (LQ1= lunar days 27-4; LQ2=lunar days 5-11; LQ3= lunar days 12-19; LQ4= lunar days 20-26) on mean nightly SPLs (dB) in both the lower frequency band and the higher frequency band. For each deployment day, we determined the mean of nighttime (sunset to sunrise) SPLs (in linear units), and for each lunar quarter we took the mean of the mean nighttime SPLs (in linear units) across deployment days in the respective lunar quarter.

RESULTS

General Temporal Patterns in SPLs

Sound pressure levels in Middle Marsh showed cyclical peaks in SPLs in both the lower and higher frequency bands, though the periodicity of these peaks differed between the two bands. For example, daily maximum SPLs in the lower frequency band were 20-30 dB greater than daily minimum SPLs (Figure 4). The difference between daily maximum and daily minimum SPLs in the higher band was 5-15 dB (Figure 5). The lower frequency SPL band had a periodicity of two cycles per day (Figure 4). The higher frequency SPL band had a periodicity of one cycle per day (Figure 5). Wavelet analysis showed that the strength and persistence of these periodicities varied throughout the three deployments. The periodicity
was lessened during the neap tide after the new moon, when there was a low range in water levels, as well as reduced amounts of fish chorusing during this lunar period (Figure 4 & 5, Figure 10). The periodicity was also interrupted by Hurricane Arthur, a category 2 hurricane that made landfall in Beaufort, NC the evening of 7/3/14 through the early morning hours of 7/4/14 (Figure 4 & 5).

**Processes Underlying Temporal Patterns in SPLs**

*Abiotic variables.* -- The lower frequency band had a strong positive correlation with water level, a relationship that was strongest for Deployment I (Table 4). The higher frequency band had a very weak, yet positive correlation with water level (Table 5). Assigning a phase angle to each part of the tidal cycle revealed SPLs in the lower frequency band were approximately 10-15dB greater around high tide (0° tidal phase) than at low tide (-180°/180° tidal phase) (Figure 6A). In addition, SPLs during a falling tide (0-180° tidal phase) were approximately 5 dB higher than during a rising tide (-180-0° tidal phase). SPLs in the higher frequency band did not vary by more than a few decibels throughout a tidal cycle (Figure 6B).

Water temperatures exhibited a quartile range of 24-29° (21-36°C full range) throughout the three deployments in the summer of 2014, with an average temperature of 27°C. The lower frequency band had a weak, yet positive, relationship with water temperature (Figure 7A). Both the higher frequency band and snap rate had relatively weak, negative relationships with water temperature (Figure 7B,C).
There was a slightly negative correlation between SPLs and cross-shelf (V) wind velocity for both the higher frequency band \((r = -0.26, p<0.01)\), and the lower frequency band \((r = -0.09, p<0.01)\). The relationship between alongshore wind velocity (U) and SPLs was not significant for the higher frequency band, and was negatively correlated with the lower frequency band \((r=-0.15, p<0.01)\).

There was no significant difference in mean nightly SPLs in the lower frequency band among lunar quarters, though nighttime SPLs were slightly elevated during new (LQ1) and full moons (LQ3) (Figure 8A, \(p=0.73\)). Mean nightly SPLs in the higher frequency band were not significantly different between lunar quarters (Figure 8B, \(p=0.43\)).

**Biotic variables: Fish chorusing.** - The largest peaks in SPLs in the lower band coincided with choruses characteristic of large aggregations of soniferous fish species. Silver perch \((Bairdiella chrysoura)\) choruses occurred during falling high tides \((0-100^\circ\) tidal phase\), with the most choruses present at \(20^\circ\) to \(40^\circ\) tidal phase (Figure 9). Oyster toadfish \((Opsanus tau)\) choruses occurred on both sides of the high tide, but were present in similar amounts from \(-60^\circ\) to \(60^\circ\) tidal phase (Figure 9). Other, unidentified fish choruses also occurred on both sides of the high tide (Figure 9). There were changes in the amount of fish chorusing by each group (perch, toadfish, other/unidentified) throughout the study period. Silver perch were most prominent in Deployment I, with choruses dying off by mid-August (Figure 10). The perch choruses may also have been influenced by lunar phase, as choruses were less intense between the full and new moon phases, but would increase in duration and intensity around the new and full moon (Figure 10). Perch choruses occurred at night, after sunset,
through the early morning hours, and were not present during daytime recordings (Figure 11). Persistent toadfish choruses were not observed in Deployment I, and were most numerous during Deployment II, peaking in mid-July (Figure 10). Toadfish chorusing was present at all times of day, though there were fewer occurrences during the mid-day hours (10:00-14:00) than in evening and early morning hours (Figure 11). Other, unidentified fish choruses were present through all three deployments, with fewer fish choruses between the full and new moon phases (Figure 10). These fish choruses were identified at all times of day, but were observed most from 6:00 to 21:00, with fewer observations during the early morning hours (0:00 to 5:00), and with the onset of perch chorusing (Figure 11).

*Biotic variables: Snapping shrimp.* - The higher frequency band was selected to represent snapping shrimp activity (Lillis et al. 2014, Bohnenstiehl et al. in review). There was a strong, positive correlation between snap count and the higher frequency band indicating that changes in SPLs in the higher frequency band can be explained by snapping shrimp activity (Table 6). Mean snap rate was greater in the evening and early morning hours than in daylight hours, a pattern that intensified from Deployment I to Deployment III (Figure 12). Median snap amplitude remained relatively constant throughout a 24-hour period for all three deployments. Percent excess shrimp snaps was positive for all three deployments, indicating more snaps occurred at night than during the day (Figure 13).
DISCUSSION

Sound pressure levels in the high frequency band peaked once per day, driven by diurnal activity of snapping shrimp, whereas sound pressure levels in the low frequency band peaked twice per day, driven by water levels and fish chorusing. The temporal patterns and processes in the soundscape of Middle Marsh illustrate the complexity of soundscapes in intertidal estuarine habitats.

Processes underlying temporal patterns in SPL

Sound pressure levels in the low frequency band peaked twice per day, coinciding with higher tidal amplitudes and periods of fish chorusing. The acoustic data do not allow us to determine if this is due to fish calling more at higher tide, or if at high tide sound propagates farther and the pattern we see is a result of integrating more distance sound sources at high water levels. Other studies of fish activity in intertidal environments reveal greater fish activity occurring at higher water levels. For example, in other marsh systems mummichog (*Fundulus heteroclitus*) and white perch (*Morone americana*) move into the flooded marsh with the tide for spawning to take advantage of food resources (McGrath & Austin 2009, Meyer & Posey 2009). In Middle Marsh, SPLs in the lower frequency band peaked twice per day surrounding the high tide, and fish chorusing for several fish species was centered around the high tide phase. Chorusing is associated primarily with spawning (Mok & Gimore 1983) and given that other fish activities, like feeding, also appear to center around high tide (Kleypas & Dean 1983, McGrath & Austin 2009, Meyer & Posey 2009), perhaps these soniferous fish species also use flooded habitats for spawning.
Unlike toadfish and other fish chorusing, perch chorusing occurred only at nighttime during falling tides. Activity during nighttime high tides is consistent with other silver perch activities including feeding and spawning. Synchronizing spawning during high water of spring tides is common for many marine fish species, and is thought to reduce predation pressure on eggs, as they will disperse out of the habitats with the receding tide (Gibson 1992). Silver perch are nocturnal feeders and use flooded habitats as foraging areas, as inferred by fuller stomachs for fish captured at nighttime ebb tides than during the day (Kleypas & Dean 1983). Perch are also nocturnally active on seagrass banks, particularly at dusk and into the first hours of darkness, and around high tide, primarily on the second rise and first fall (Sogard et al. 1989). Though sound production by silver perch is associated with spawning and not feeding, those previous studies illustrate that perch use and move into flooded habitats. A previous study found that both maximum daily sound production, and appearance of eggs and larvae for the silver perch occurred between 17:00 and 22:00 hours, further supporting that silver perch are active at night in these estuarine habitats (Mok & Gilmore 1983).

In addition to the overall temporal pattern of a peak in SPLs in the lower band at night high tides, there was evidence of temporal partitioning of the soundscape, with spotted seatrout (Cynoscion nebulosus) calling before perch on most nights. The spotted seatrout chorus was either masked or ended with the onset of the perch chorus. Previous acoustic studies investigating spotted seatrout and silver perch observed that when large groups of seatrout were recorded early, perch activity was delayed and occurred later in the evening (Mok & Gilmore, 1983). Spotted seatrout and silver perch have similar spawning group
distributions, and it appears here, as has previously been observed, that these species may
adjust their calling schedules to avoid overlap (Mok & Gilmore 1983, Locascio & Mann
2008). More recently, Ruppe et al. (2015) suggested that nocturnal fish calls have a clear
distinction in frequencies because fish at night are deprived of visual cues.

Diurnal patterns in snapping shrimp activity heavily influences peaks in SPLs in the
high frequency band. More snaps were observed at night than during the day, which is
consistent with other summertime recordings of snapping shrimp activity (Radford et al.
2008, Bohnenstiehl et al. in review). Radford et al. (2008) suggest snapping shrimp are more
active at night to avoid predation in daylight hours. Shrimp also snap more frequently in the
summer as a result of warmer water temperatures that increase their activity (Radford et al.,
2008; Bohnenstiehl et al. in review). Recent work within nearby Pamlico Sound, however,
shows that in winter months, the diurnal pattern of snapping shrimp acoustic activity
reverses, with more snaps occurring during the day than at night (Bohnenstiehl et al. in
review).

**Study implications**

In both the high frequency and the low frequency sound bands, a peak in SPLs
occurred at night, indicating greater acoustic activity for the fish and invertebrates producing
sounds in Middle Marsh. Under the cloak of darkness, snapping shrimp and fish species are
able to produce sounds with a likely reduced risk of being detected by predators. Soniferous
fish, like the silver perch and spotted seatrout, produce sounds associated with spawning to
attract mates (Mok & Gilmore 1983). This loud, nightly chorus of species-specific sound
production is a benefit for these fish, most of which are broadcast spawners, because there is an advantage for them to produce these large aggregations, driven by cues of darkness, as well as sound production, to come together and spawn (Locascio & Mann 2008).

Like many soundscape studies of coastal marine environments, nighttime summer soundscapes were observed to be 20 dB louder than daytime soundscapes, and the greatest differences in recordings between habitats are often, although not always, at night (Radford et al. 2008, Radford et al. 2010). This feature of coastal soundscapes has many implications with respect to enhancement of a larval settlement cue for drifting and swimming larvae. Many marine organisms, including crabs, fish, and oysters, have a dispersive larval stage prior to settling in their adult habitats, and dusk is the time when the nocturnal settling stages become active in the water column (Jeffs et al. 2003, Radford et al. 2008, Simpson et al. 2008, Lillis et al. 2013). In both laboratory and field experiments, settling stages of coral, crabs, coral reef fish, and oysters respond to their habitat-associated soundscapes, with a higher proportion of larvae settling in those treatments (Jeffs et al. 2003, Simpson et al. 2008, Stanley et al. 2011, Vermeji et al. 2010, Lillis et al. 2013).

This study also highlights the use of acoustics to determine when, where, and which species are using estuarine habitats. Knowledge of spawning habitat and stock estimates is essential for conservation of fish stocks (Luczkovich et al. 2008, Rowell et al. 2015). Traditional sampling methods for the determination of spawning sites and times (e.g., collecting larvae) require tremendous labor, and are often not reliable as there is uncertainty of species identification at early life stages and gear efficiency among varying habitats (Kellison et al. 2003, Luczkovich et al. 2008). Other methods, which include collecting fish
and examining gonadal condition, may not be accurate as some fish collected at a sampling site may not be at their final spawning site (Luczkovich et al. 2008, Rowell et al. 2015).

Though there is potential gear bias associated with acoustic monitoring, including only being able to record soniferous species, passive acoustic recordings provide a cost-effective, informative, non-invasive way to identify critical spawning habitats for sound-producing species (Locascio & Mann 2008, Luczkovich et al. 2008, Rowell et al. 2015). Sounds produced by soniferous fish are species- and activity-specific, which allows for fish identification, as well as characterization of their activity through the use of passive acoustic recorders, providing a unique way to gain knowledge of fish spawning habitat (Mok & Gilmore 1983, Luczkovich et al. 2008, Rowell et al. 2015). Though there have been studies aimed at identifying fish spawning sites through passive acoustics, methods were not consistent, and there does not appear to be a consensus on how these surveys should be conducted. A benefit of longer-term soundscape studies with high temporal, and spatial resolution, like the present study, is the ability to get a more complete picture of when and how various soniferous species are using the study area. Studies with high temporal resolution allows for information on chorus duration, start and end times, and whether other variables, like tides and lunar cycles, influence variability in sound production (Locascio & Mann 2008). Short, snapshot recordings at multiple sites may miss important aspects of acoustic activity; for example, the partitioning of the soundscape between spotted seatrout and silver perch would be missed with a single nightly recording window. This study period was longer than others, however, it is important to note the limitations of a single season study. For example this summer soundscape characterization did not illustrate the seasonal
pattern observed in snapping shrimp activity, and missed peak spawning periods of
soniferous fish species that spawn in late spring (May), like the weakfish, or late summer
(September), like the red drum (Bohenstiehl et al. in review, Luczkovich et al. 2008). It is
necessary to have multiple, simultaneous, recordings to understand how and when these fish
species are using these habitats, to better inform management of stocks, and conservation of
these essential habitats.

CONCLUSIONS

Underwater soundscapes are inherently complex, and relationships between acoustic
and environmental variables are difficult to disentangle for complex, shallow estuarine
habitats. Despite these difficulties, soundscape analysis illustrates the importance of these
habitats as essential fish spawning habitats. Patterns in the low frequency band and fish
chorusing revealed the interacting role of nighttime and tidal phase on fish behavior and
habitat use in Middle Marsh, while the diurnal pattern in snapping shrimp activity adds to our
understanding of this ubiquitous sound-producer. Future characterizations of intertidal,
estuarine soundscapes must consider the influence of water level and other environmental
variables. In addition, the development of fish call detectors will greatly advance the use of
passive acoustics in management and conservation efforts.

ACKNOWLEDGEMENTS

We thank J Byrum, D McVeigh, S Theuerkauf, B Puckett, and O Phillips for assistance in the
field. Funding for this project was provided by NSF (OCE-1234688) to DBE and DB, NC
Sea Grant (RMRD56/12-HCE-2) to DBE, and a NC Coastal Reserve/NC Sea Grant College Program *Coastal Research Fellowship* to SR.


McGrath P, Austin HA (2009) Site fidelity, home range, and tidal movements of white perch during the summer in two small tributaries of the York River, Virginia. Trans Am Fish Soc 138:966-974


Table 1: Deployment start date and time, and end date and time for each site

<table>
<thead>
<tr>
<th>Site</th>
<th>Deployment I (6/12/14 17:00)</th>
<th>Deployment II (7/9/14 17:00)</th>
<th>Deployment III (8/8/14 17:00)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>End Date</td>
<td>End Date</td>
<td>End Date</td>
</tr>
<tr>
<td>1</td>
<td>7/8/14 7:00</td>
<td>7/28/14 17:00</td>
<td>8/31/14 5:00</td>
</tr>
<tr>
<td>2</td>
<td>7/7/14 20:00</td>
<td>8/7/14 9:00</td>
<td>8/31/14 5:00</td>
</tr>
<tr>
<td>3</td>
<td>7/7/14 20:00</td>
<td>8/7/14 9:00</td>
<td>8/31/14 5:00</td>
</tr>
<tr>
<td>4</td>
<td>6/23/14 14:00</td>
<td>8/7/14 9:00</td>
<td>8/31/14 5:00</td>
</tr>
<tr>
<td>5</td>
<td>7/3/14 0:00</td>
<td>8/4/14 20:00</td>
<td>8/31/14 5:00</td>
</tr>
<tr>
<td>6</td>
<td>7/8/14 7:00</td>
<td>--</td>
<td>8/31/14 5:00</td>
</tr>
<tr>
<td>7</td>
<td>7/8/14 7:00</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>8</td>
<td>7/8/14 7:00</td>
<td>8/4/14 20:00</td>
<td>--</td>
</tr>
</tbody>
</table>

Table 2: Correlation between SPLs in the lower band (150-1500Hz) at each site.

<table>
<thead>
<tr>
<th></th>
<th>Site 1</th>
<th>Site 2</th>
<th>Site 3</th>
<th>Site 4</th>
<th>Site 5</th>
<th>Site 6</th>
<th>Site 7</th>
<th>Site 8</th>
<th>all sites</th>
</tr>
</thead>
<tbody>
<tr>
<td>Site 1</td>
<td>1.00</td>
<td>0.46</td>
<td>0.56</td>
<td>0.56</td>
<td>0.52</td>
<td>0.52</td>
<td>0.41</td>
<td>0.64</td>
<td>0.69</td>
</tr>
<tr>
<td>Site 2</td>
<td>1.00</td>
<td>0.69</td>
<td>0.54</td>
<td>0.61</td>
<td>0.67</td>
<td>0.53</td>
<td>0.60</td>
<td>0.79</td>
<td></td>
</tr>
<tr>
<td>Site 3</td>
<td>1.00</td>
<td>0.58</td>
<td>0.57</td>
<td>0.67</td>
<td>0.51</td>
<td>0.66</td>
<td>0.80</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Site 4</td>
<td>1.00</td>
<td>0.65</td>
<td>0.65</td>
<td>0.51</td>
<td>0.63</td>
<td>0.77</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Site 5</td>
<td>1.00</td>
<td>0.75</td>
<td>0.62</td>
<td>0.64</td>
<td>0.83</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Site 6</td>
<td>1.00</td>
<td>0.66</td>
<td>0.61</td>
<td>0.87</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Site 7</td>
<td>1.00</td>
<td>0.46</td>
<td>0.73</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Site 8</td>
<td>1.00</td>
<td>0.79</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>all sites</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1.00</td>
<td></td>
</tr>
</tbody>
</table>
Table 3: Correlation between SPLs in the higher band (7-43kHz) at each site. Correlation coefficients marked with an asterisk (*) are not significant at p<0.05.

<table>
<thead>
<tr>
<th></th>
<th>Site 1</th>
<th>Site 2</th>
<th>Site 3</th>
<th>Site 4</th>
<th>Site 5</th>
<th>Site 6</th>
<th>Site 7</th>
<th>Site 8</th>
<th>all sites</th>
</tr>
</thead>
<tbody>
<tr>
<td>Site 1</td>
<td>1.00</td>
<td>0.37</td>
<td>0.44</td>
<td>0.59</td>
<td>0.59</td>
<td>0.63</td>
<td>0.04*</td>
<td>0.63</td>
<td>0.68</td>
</tr>
<tr>
<td>Site 2</td>
<td>1.00</td>
<td>0.38</td>
<td>0.36</td>
<td>0.40</td>
<td>0.45</td>
<td>0.04*</td>
<td>0.37</td>
<td>0.45</td>
<td></td>
</tr>
<tr>
<td>Site 3</td>
<td>1.00</td>
<td>0.53</td>
<td>0.46</td>
<td>0.52</td>
<td>0.09</td>
<td>0.50</td>
<td>0.52</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Site 4</td>
<td>1.00</td>
<td>0.70</td>
<td>0.75</td>
<td>0.09</td>
<td>0.76</td>
<td>0.79</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Site 5</td>
<td>1.00</td>
<td>0.74</td>
<td>0.10</td>
<td>0.75</td>
<td>0.90</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Site 6</td>
<td></td>
<td>1.00</td>
<td>0.08</td>
<td>0.79</td>
<td>0.84</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Site 7</td>
<td></td>
<td></td>
<td>1.00</td>
<td>0.08</td>
<td>0.23</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Site 8</td>
<td></td>
<td></td>
<td></td>
<td>1.00</td>
<td>0.90</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>all sites</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1.00</td>
</tr>
</tbody>
</table>

Table 4: Results of correlation between SPL in the lower band (150-1500 Hz) at all sites and water level (meters above mean lower low water) at Beaufort for three deployments. Correlation coefficient reported is the bootstrap mean plus or minus the standard deviation of the bootstrap means.

<table>
<thead>
<tr>
<th></th>
<th>Deployment I June</th>
<th>Deployment II July</th>
<th>Deployment III August</th>
</tr>
</thead>
<tbody>
<tr>
<td>Site 1</td>
<td>0.42 ± 0.02</td>
<td>0.33 ± 0.03</td>
<td>0.46 ± 0.03</td>
</tr>
<tr>
<td>Site 2</td>
<td>0.62 ± 0.02</td>
<td>0.44 ± 0.02</td>
<td>0.53 ± 0.02</td>
</tr>
<tr>
<td>Site 3</td>
<td>0.73 ± 0.01</td>
<td>0.68 ± 0.01</td>
<td>0.51 ± 0.02</td>
</tr>
<tr>
<td>Site 4</td>
<td>0.59 ± 0.02</td>
<td>0.64 ± 0.01</td>
<td>0.65 ± 0.02</td>
</tr>
<tr>
<td>Site 5</td>
<td>0.67 ± 0.02</td>
<td>0.73 ± 0.02</td>
<td>0.72 ± 0.01</td>
</tr>
<tr>
<td>Site 6</td>
<td>0.74 ± 0.01</td>
<td>0.69 ± 0.01</td>
<td>--</td>
</tr>
<tr>
<td>Site 7</td>
<td>0.56 ± 0.02</td>
<td>--</td>
<td>0.55 ± 0.02</td>
</tr>
<tr>
<td>Site 8</td>
<td>0.57 ± 0.01</td>
<td>0.64 ± 0.02</td>
<td>--</td>
</tr>
<tr>
<td>All Sites (median)</td>
<td>0.78 ± 0.01</td>
<td>0.77 ± 0.01</td>
<td>0.76 ± 0.01</td>
</tr>
</tbody>
</table>
Table 5: Results of correlation between SPL in the higher band (7-43 kHz) at all sites and water level (meters above mean lower low water) at Beaufort for three deployments. Correlation coefficient reported is the bootstrap mean plus or minus the standard deviation of the bootstrap means.

<table>
<thead>
<tr>
<th>Site</th>
<th>Deployment I June</th>
<th>Deployment II July</th>
<th>Deployment III August</th>
</tr>
</thead>
<tbody>
<tr>
<td>Site 1</td>
<td>0.21 ± 0.02</td>
<td>0.23 ± 0.03</td>
<td>0.22 ± 0.02</td>
</tr>
<tr>
<td>Site 2</td>
<td>0.23 ± 0.02</td>
<td>0.14 ± 0.02</td>
<td>0.12 ± 0.03</td>
</tr>
<tr>
<td>Site 3</td>
<td>0.20 ± 0.01</td>
<td>0.18 ± 0.02</td>
<td>0.11 ± 0.02</td>
</tr>
<tr>
<td>Site 4</td>
<td>0.24 ± 0.03</td>
<td>0.08 ± 0.03</td>
<td>0.05 ± 0.02</td>
</tr>
<tr>
<td>Site 5</td>
<td>0.18 ± 0.03</td>
<td>0.11 ± 0.03</td>
<td>0.07 ± 0.02</td>
</tr>
<tr>
<td>Site 6</td>
<td>0.13 ± 0.03</td>
<td>0.14 ± 0.02</td>
<td>--</td>
</tr>
<tr>
<td>Site 7</td>
<td>-0.20 ± 0.02</td>
<td>--</td>
<td>-0.10 ± 0.02</td>
</tr>
<tr>
<td>Site 8</td>
<td>0.05 ± 0.02</td>
<td>0.03 ± 0.02</td>
<td>--</td>
</tr>
<tr>
<td>All Sites (median)</td>
<td>0.07 ± 0.03</td>
<td>0.07 ± 0.02</td>
<td>0.09 ± 0.02</td>
</tr>
</tbody>
</table>

Table 6: Correlation between the higher frequency band and snap counts for all sites during each deployment. Correlation coefficient reported is the bootstrap mean plus or minus the standard deviation of the bootstrap means.

<table>
<thead>
<tr>
<th>Site</th>
<th>Deployment I</th>
<th>Deployment II</th>
<th>Deployment III</th>
</tr>
</thead>
<tbody>
<tr>
<td>Site 1</td>
<td>0.71 ± 0.02</td>
<td>0.77 ± 0.01</td>
<td>0.76 ± 0.01</td>
</tr>
<tr>
<td>Site 2</td>
<td>0.71 ± 0.02</td>
<td>0.79 ± 0.01</td>
<td>0.80 ± 0.01</td>
</tr>
<tr>
<td>Site 3</td>
<td>0.72 ± 0.01</td>
<td>0.71 ± 0.01</td>
<td>0.72 ± 0.01</td>
</tr>
<tr>
<td>Site 4</td>
<td>0.76 ± 0.01</td>
<td>0.73 ± 0.01</td>
<td>0.77 ± 0.01</td>
</tr>
<tr>
<td>Site 5</td>
<td>0.77 ± 0.01</td>
<td>0.80 ± 0.01</td>
<td>0.86 ± 0.01</td>
</tr>
<tr>
<td>Site 6</td>
<td>0.74 ± 0.02</td>
<td>0.72 ± 0.02</td>
<td>--</td>
</tr>
<tr>
<td>Site 7</td>
<td>0.12 ± 0.02</td>
<td>--</td>
<td>0.40 ± 0.03</td>
</tr>
<tr>
<td>Site 8</td>
<td>0.85 ± 0.01</td>
<td>0.87 ± 0.01</td>
<td>--</td>
</tr>
<tr>
<td>All sites</td>
<td>0.67 ± 0.01</td>
<td>0.77 ± 0.01</td>
<td>0.76 ± 0.01</td>
</tr>
</tbody>
</table>
Figure 1: Map of Southeast US Coast and study sites (inset) in Middle Marsh (RCERR). The town of Beaufort, NC is located at -76.658W, 34.718N.
Figure 2: Map of Middle Marsh in the RCERR showing habitat layers and deployment sites.

Legend
- Sites
- SAV
- Marsh
- Reef
- Unstructured
Figure 3: Definition of tidal phase angle. High tide is assigned the phase angle of 0°. Falling to low tide, the phase angle increases to 180°. Rising to high tide the phase angle is negative and increases from -180° to 0°.
Figure 4: SPL in the lower frequency band (150-1500 Hz) over time with associated scaleogram for Deployments I, II, and III. In the SPL over time panel, vertical dashed lines denote midnight of each day and open/filled circles indicate full/new moons respectively. Scaleograms illustrate periodicity in the time series with areas in red showing a stronger periodicities, and cooler colors representing weaker periodicities. Areas outlined in black are significant at the 95% confidence level. Shaded areas represent data that cannot be resolved due to the length of the time series. These scaleograms show the periodicity of peaks in SPLs in days (period of 0.5 = peak in SPL twice per day)
Figure 5: SPL in the higher frequency band (7-43kHz) over time with associated scaleogram for Deployments I, II, and III. In the SPL over time panel, vertical dashed lines denote midnight of each day and open/filled circles indicate full/new moons respectively. Scaleograms illustrate periodicity in the time series with areas in red showing a stronger periodicities, and cooler colors representing weaker periodicities. Areas outlined in black are significant at the 95% confidence level. Shaded areas represent data that cannot be resolved due to the length of the time series. These scaleograms show the periodicity of peaks in SPLs in days (period of 0.5 = peak in SPL twice per day).
Figure 6: SPLs in the lower frequency band (150-1500Hz) versus phase angle (A.) and SPL in the higher frequency band (7-43 kHz) versus phase angle (B.) over three deployments for all sites. Quadratic trend line was fit to the median SPL at each phase angle.
Figure 7: Scatter plot of acoustic variables, including SPLs in the lower band (A), SPLs in the higher band (B), and snap rate (C) versus water temperatures (°C) at Shackleford Banks. The mean bootstrapped estimate of the correlation coefficient (r) ± the standard deviation of the estimates is reported for each relationship.
A. \( r = 0.0473 \pm 0.0159 \)

B. \( r = -0.3140 \pm 0.0113 \)

C. \( r = -0.1919 \pm 0.0115 \)
Figure 8: Mean nightly dB SPLs in the lower frequency band (A) and higher frequency band (B) for all sites for each lunar quarter in Deployments I-III. Error bars are the 68% confidence interval.
Figure 9: Percent of total files with fish chorusing at each phase angle of the tidal cycle (0=high tide, -180/-180=low tide) for all sites over Deployments I-III. Each color represents a different site. Top panel is for perch (*Bairdiella chrysoura*) choruses, middle panel is for toadfish (*Opsanus tau*) choruses, and the bottom panel is for other or unidentified fish choruses, including fish choruses from spotted seatrout (*Cynoscion nebulosus*) and Atlantic croaker (*Micropogonias undulates*).
Figure 10: Files per day (72 total files each recording day) of fish chorusing for each day of Deployments I, II, and III for all sites. Top panel is for perch (*Bairdiella chrysoura*) choruses, middle panel is for toadfish (*Opsanus tau*) choruses, and the bottom panel is for other or unidentified fish choruses, including fish choruses from spotted seatrout (*Cynoscion nebulosus*) and Atlantic croaker (*Micropogonias undulates*). Open/filled circles indicate full/new moons respectively.
Figure 11: Files with fish chorusing for each time of day (00:00 to 23:40, every 00:20) for all sites through deployments I, II, and III. Shaded gray boxes illustrate the range in sunrise or sunset times throughout the three deployments. Top panel is for perch (*Bairdiella chrysoura*) choruses, middle panel is for toadfish (*Opsanus tau*) choruses, and the bottom panel is for other or unidentified fish choruses, including fish choruses from spotted seatrout (*Cynoscion nebulosus*) and Atlantic croaker (*Micropogonias undulates*).
Figure 12: Mean snap rate (snaps per minute) for each time of day (00:00 to 23:40, every 00:20) for all sites. Shaded gray boxes illustrate the range in sunrise or sunset times throughout the three deployments.

Figure 13: Percent excess snap rate throughout deployments I, II, and III for all sites. Bars show the daily percent excess values that are significant at the 95% confidence level. Gaps in the graph are times when the SoundTraps were taken out to offload data.
CHAPTER 2

SOUNDSCAPES TO SEASCAPES: DO SPATIAL PATTERNS IN ESTUARINE HABITATS RELATE TO SPATIAL PATTERNS IN SOUNDSCAPES?

ABSTRACT

Structurally complex estuarine habitats, such as seagrass beds, salt marshes, and oyster reefs are used by fish as habitat for foraging, avoiding predators, and spawning. These habitats often appear as a complex mosaic of all three habitat types, and sampling fish within these habitat mosaics to assess their role as essential fish habitat (EFH) can be difficult with traditional sampling gear. Moreover, soundscape characteristics can provide information on the types of habitats in a location, and there is increasing interest in whether these soundscape characteristics reflect the complexity and quality of habitats. This study characterized spatial patterns of an underwater soundscape within a mosaic of estuarine habitats at a coastal reserve in North Carolina, USA. Passive acoustic recorders were deployed at eight sites in the reserve, and sampled over three months during summer 2014. Nighttime, high-tide sound pressure levels (SPLs in dB) in a higher frequency (7-43kHz) and a lower frequency band (150-1500Hz) varied spatially throughout the reserve. Multiple regression models revealed that site habitat characteristics (percent cover of seagrass, oyster reef, and salt marsh) did not explain spatial patterns in the primarily fish-driven low frequency band, though there was spatial variation in fish chorusing. The snapping shrimp driven high frequency band had a negative relationship with percent cover of seagrass at smaller spatial scales (10 to 25 m), and a positive relationship with percent cover of marsh
and marsh edge at larger spatial scales (100m). Though habitat composition could not explain spatial patterns in soundscape characteristics alone, this study provides a baseline characterization of soundscapes in an estuarine reserve, and revealed that there are differences in the soundscapes in this reserve, even over relatively short (~400m) spatial scales. Underwater soundscapes may provide a cost-effective and non-invasive way to characterize the role of estuarine habitats as EFH. Development of additional soundscape characteristics (e.g., acoustic diversity metrics, fish call detectors), and accurate habitat composition data could make passive acoustics a valuable technique to rapidly assess habitat quality and biodiversity in marine systems.

INTRODUCTION

Soundscape ecology is an emerging field in which researchers characterize the sounds of an environment, investigate how those sounds affect organisms living in that environment, and use characteristics of the soundscape to infer habitat quality (Farina 2014, Lillis et al. 2014). A soundscape is the combination of the sounds of the environment, including biological, physical, and human produced sources of sound (Pijanowskii et al. 2011). The collection of these three sources produces a characteristic ‘sonic profile’ for a given location.

There is increasing interest in studying soundscapes of marine and terrestrial habitats and applying soundscape metrics as a tool to manage habitats and assess ecosystem health (Sueur et al. 2008, Tucker et al. 2014). Soundscape ecologists can use acoustic recordings to infer what organisms are present, their relative abundance, and what they may be doing. The objectives of this study were to quantify the relationship between estuarine habitat
characteristics and soundscape metrics at multiple spatial scales, and to identify essential fish habitat use within a complex mosaic of shallow, structured habitats.

A central task in conservation biology is determining biodiversity estimates, and most traditional methods are time consuming, and may be difficult to achieve in certain landscapes (Sueur et al. 2008). Spatial patterns in the landscape influence the composition and distribution of species, and recent work in soundscape ecology aims to consider how the landscape impacts the sound production and vice-versa (Sueur et al. 2014). Terrestrial studies investigating the relationship between landscape and soundscape variables have been successful in developing and testing acoustic diversity metrics, as high quality habitat and landscape attribute data is available for many systems, and biological surveying methods are generally well developed (Sueur et al. 2008, Joo et al. 2011, Pekin et al. 2012, Sueur et al. 2014, Tucker et al. 2014). Initial patterns in these systems reveals that degraded habitat with less structure are often less diverse acoustically than intact, highly structured areas (Sueur et al. 2008, Joo et al. 2011, Pekin et al. 2012). Some studies in marine systems compared soundscape metrics to broad seascape metrics (e.g. one habitat type versus another), and found that soundscapes are habitat-associated and have distinct sound signatures (Radford et al. 2010, Radford et al. 2014, Lillis et al. 2014). Others considered more specific seascape variables (percent cover of habitat type, depth), however, there was a lack of concurrent recordings and minimal temporal replication (Kennedy et al. 2010, McWilliam & Hawkins 2013).

Few studies to our knowledge have investigated spatial patterns in the soundscapes of a mosaic of intertidal, estuarine habitats, and most soundscape studies investigating spatial
patterns are not characterized with concurrent recordings of high temporal resolution (Kennedy et al. 2010, Radford et al. 2010, McWilliam & Hawkins 2013, Radford et al. 2014). Seascape ecology is a growing scientific field where concepts from landscape ecology are used to evaluate ecological consequences of spatial patterns and structural changes in marine habitats (Bostrom et al. 2011, Pittman et al. 2011, Wedding et al. 2011). A seascape can be defined as the mosaic of habitat patches that make up a spatially heterogeneous area (Bostrom et al. 2011, Davis et al. 2014). In coastal areas, these include semi-terrestrial marsh habitats, as well as subtidal and intertidal coral and oyster reef habitats (Bostrom et al. 2011). Most studies, including soundscape studies, look at defined habitat types (e.g. coral reefs, oyster reefs, unstructured bottom, or seagrass), without considering the possible effect of seascape composition and diversity (Drew & Eggleston 2008). Previous studies provide evidence that spatial configuration and composition of habitat mosaics have a significant influence on marine organisms (Drew & Eggleston 2008). Further investigation into sources of sounds, and the relationship between soundscapes and habitats and biological communities is needed to assess the potential of passive recordings as a monitoring tool for habitat types and quality, and essential fish habitat (EFH) (Kennedy et al. 2010, McWilliam & Hawkins 2013, Lillis et al. 2014). Given that estuarine habitats are used as EFH for ecologically important species such as silver perch (*Bairdiella chrysoura*), and economically important species such as spotted seatrout (*Cyanoscion nebulosus*) (Mok & Gilmore 1983, Locascio & Mann 2008, Luczkovich et al. 2008), and these habitats often occur as a mosaic of all three habitat types, there is a need to explore the relationship between the seascape and the soundscape in this system to gain an understanding of the value of soundscape
characterization as a tool to fully characterize ecological functions and services provided by these habitats.

The Middle Marsh system within the Rachel Carson Estuarine Research Reserve in Back Sound, North Carolina, USA provides an excellent study system because it contains habitat mosaics of seagrass, oyster reef, salt marsh, and soft-bottom habitats that vary naturally in terms of their relative percent cover. In addition, there is inherent interest in monitoring this reserve site, and providing baseline information on soundscape patterns and processes that could inform future monitoring or conservation efforts in the reserve. The aim of this study was to quantify soundscape patterns and processes within the Middle Marsh system.

MATERIALS AND METHODS

Site Selection and Characterization

Soundscape characterization was conducted within Middle Marsh, a portion of the Rachel Carson Estuarine Research Reserve (RCERR), a National Estuarine Research Reserve located in Back Sound, near Beaufort Inlet, NC (Figure 1). Middle Marsh is subject to semi-diurnal tides, and is located ~ 5 km from the nearby Beaufort Inlet that connects Back Sound to the Atlantic Ocean. Mean salinity at the site is high year round, and ranges from > 34 in summer to 32 in winter, with relatively little influence of the nearby North River estuary (Elis 1998). Water depth in Middle Marsh varies from 0.1 to 0.4 m at low tide, to 1.2-1.5 m at high tide, though there are deeper channels adjacent to shallower habitats.
(Eggleston et al. 1999). The reserve contains a variety of estuarine habitats including seagrass beds, salt marshes, oyster reefs, and unstructured soft bottom (NC DENR, 2001).

In 2006, the North Carolina Estuarine Research Reserve (NCNERR) program conducted habitat-mapping analysis in the Rachel Carson Reserve (http://portal.ncdenr.org/web/crp/habitat-mapping). Using the updated, ground-truthed habitat data from 2013 (NCNERR), and a submerged aquatic vegetation (SAV) map from 2008 (NCDENR, APNEP), we generated a spatially-referenced map of the different habitat types in the RCERR (Figure 2). This map was then refined through subsequent field reconnaissance, resulting in our selection of 16 possible sites in Middle Marsh that were suitable for hydrophone deployment. Our main criteria for suitability were that (i) hydrophones remained submerged at maximum low tide, and (ii) that there was acoustic independence between hydrophone sites (i.e., no direct acoustic path between them). Eight of 16 possible sites were then randomly chosen as deployment sites for the duration of the study. To ground-truth the GIS habitat layers, the bottom type was recorded every meter for 25 meters in six of eight randomly selected compass directions (N, S, E, W, NE, SE, NW, SW) from the hydrophone.

This field habitat characterization occurred twice, once before the start of Deployment I on 6/12/14 and again before the start of Deployment III on 8/7/14. Three of the six transects in each field characterization were used for habitat characterization of the 10 meter radius around each site, and the remaining three were used for habitat characterization of the 25 m radius around each site. The percent cover of each habitat type reported for the
10 m and 25 m radius is the average percent cover of each habitat type (marsh, seagrass, oyster reef) for each field characterization event (n=6 transects per radius).

In addition to the percent cover of habitat data calculated in the field for the 10 m and 25 m radius buffers, the GIS-based NCNERR habitat data, and the NCDENR/APNEP SAV data were used to calculate percent cover of marsh, seagrass, and oyster reef in 10 m, 25 m, and 100 m buffer zones around each hydrophone site. The GIS data were also used to determine the amount of marsh edge (in meters) in each of those buffer zones by adding together the edge length of each marsh patch present in the zones. Marsh edge is an important foraging and refuge habitat for a variety of estuarine species (Kneib 1984).

**Acoustic Analysis**

Underwater sound recordings were taken at all eight sites over three deployments (Table 1). Acoustic sampling was conducted using underwater recording systems (SoundTrap, Ocean Instruments New Zealand) set to record at all sites simultaneously for two minutes every 20 minutes (sample rate: 96kHz). Detailed methods for acoustic sampling may be found in Ricci et al. (Chpt 1).

Underwater sound recordings from each site were then partitioned into a low frequency band (150-1500 Hz) and a high frequency band (7-43 kHz). The low frequency band included the range of frequencies that most fish vocalizations occur (Mok & Gilmore 1983, Ramcharitar et al 2006, Luczkovich et al. 2008), whereas the high frequency band was selected to include energy in frequencies associated with snapping shrimp (Au & Banks 1998, Bohnenstiehl et al. in review). Sound pressure levels (SPLs) in the low frequency band
in Middle Marsh are influenced by both time of day and water level, with highest SPLs occurring at nighttime high tides (Ricci et al. Chpt 1). SPLs in the high frequency band were not influenced by tidal amplitude; however, they were loudest at nighttime due to an increase in snapping shrimp activity (Ricci et al. Chpt 1). We attempted to reduce variation in our soundscape response variables due to factors other than habitat characteristics or location within Middle Marsh by selecting time periods with the highest average SPLs. In this case, we chose mean SPLs recorded during nighttime (recordings made between sunset and sunrise) and high tides (recordings made between tidal phase -40° to 40°). This selection also ensured that habitats of interest were underwater and being recorded.

Spectrograms of individual recordings were used to help identify soniferous species, and patterns in fish chorusing. Files with fish chorusing were identified and compared to archived sound files (e.g., http://www.dosits.org/science/soundsinthesea/commonsounds/). Periods of fish chorusing were defined by times when the chorus lasted the duration of the recording, and persisted for multiple recordings. To examine spatial patterns in fish chorusing, we determined the total number of files with choruses for silver perch (*Bairdiella chrysoura*), oyster toadfish (*Opsanus tau*), or other fish (includes spotted seatrout, *Cynoscion nebulosus*, and Atlantic croaker, *Micropogonias undulatus*) at each of eight sites, and then determined the percentage of total files recorded at each site with fish (perch, toadfish, other fish) chorusing.
**Statistical Analysis**

We employed a series of stepwise selection, multiple regression models to determine the relationship between acoustic properties of the soundscape (mean nighttime high tide decibel (dB) SPLs in a higher and lower frequency band for each deployment), and site characteristics (percent cover of seagrass, marsh, oyster reef, amount of marsh edge) within a given hydrophone deployment site. Separate statistical analyses conducted according to (i) spatial scale (10 m, 25 m, 100 m radius around the site) and (ii) habitat data source (GIS versus empirical field data). Habitat types that were not represented in at least four of the eight sites were not included in the full model.

**RESULTS**

**Spatial Patterns in SPLs and Fish Chorusing**

Mean nighttime high tide sound pressure levels in the lower frequency band (150-1500 Hz) varied between deployments and sites. (Figure 3). Differences between sites were most evident between “inner” versus “outer” sites. Inner sites were defined as sites that were farther away from the perimeter of Middle Marsh (Sites 4, 5, 7), or sites that were blocked from deeper channels by sand flats (Site 6). Outer sites were sites located on the perimeter of Middle Marsh (Sites 1, 2, 3, 8). In general, sites became quieter from early to late summer (deployments 1 to 3). Sites 3 and 8 were the loudest sites, and Sites 1 and 2 were the quietest sites (Figure 3). These patterns in mean nighttime SPLs coincided with patterns in fish chorusing. Presence of fish chorusing varied by site, with sites around the edges of Middle Marsh (Sites 1, 2, 3, 8) having higher percentages of total files with fish chorusing than sites
toward the inside of Middle Marsh (Sites 4, 5, 6, 7) (Figure 4). Site 3 had the second highest percentage of perch (*Bairdiella chrysoura*) chorus files (6.04% of total files), and the highest percentage of other fish chorus files (39.65%), yet had very few files with toadfish (*Opsanus tau*) chorusing (0.18% of all files at that site) (Table A.B.1). Sites 5, 6, and 7 had the least amount of fish chorusing. Choruses of spotted seatrout (*Cynoscion nebulosus*, represented in “other fish”) were often associated with silver perch choruses, occurring a few hours before the onset of the perch chorus (Figure 4, Ricci et al. Ch.1).

Mean nighttime high tide SPLs in the higher frequency band (7-43 kHz) also varied between sites and deployments (Figure 5). In general, SPLs in the higher frequency band became louder from Deployment I to Deployment III. Sites towards the center of Middle Marsh (Sites 4, 5, 6, 7) had the highest SPLs in this frequency band, whereas outer sites (Sites 1, 2, and 3) had lower SPLs in this higher frequency band (Figure 5).

**Model Selection**

Though there were spatial patterns in mean nighttime SPLs and presence of fish chorusing, site habitat characteristics were not useful in predicting SPLs in the lower frequency band (Table 2). At the high frequency band, spatial patterns in SPLs could be explained by site habitat characteristics. For example, on smaller spatial scales (10 m, 25 m), percent cover of seagrass was significant in predicting SPLs in the higher frequency band (Table 2). This relationship between percent cover of seagrass and SPLs in the higher frequency band is negative, and sites with higher percent cover of seagrass were quieter than sites without seagrass (Figure 6). At larger spatial scales (and when using GIS habitat data),
percent cover of marsh was the only significant predictor of SPLs in the higher frequency band.

To further investigate this positive relationship between percent cover of marsh and SPLs, we conducted regression analysis between the amount of marsh edge in 10 m, 25 m, and 100 m radii around each site and SPLs in each frequency band. Marsh edge was a marginally significant explanatory variable at 10 m for the lower frequency band, but was not significant at other spatial scales (Table 3). For the higher frequency band, marsh edge was not significant at the 10 m spatial scale, but was significant at 25 m and 100 m spatial scales. As the amount of marsh edge increased, SPLs in the higher frequency band increased (Figure 7).

DISCUSSION

This is the first study we are aware of that has quantified spatial variation in the soundscape of an estuarine system containing a mosaic of complex habitat types, as well as the processes driving this variation and scale-dependence of these processes. Certain locations within the Middle Marsh estuarine reserve were louder, and contained greater fish chorusing, than other areas. These locations corresponded with location within the seascape more than specific habitat variables, such as percent cover of structurally complex habitats. In cases where there was a positive or negative relationship between sound pressure levels and percent cover of a given habitat, the relationship was dependent upon the spatial scale of habitat measurements, and whether the frequency band was relatively high or low. For example, at high frequencies (e.g., snapping shrimp), at scales of 10m and 25m, there was a
negative relationship between increasing seagrass cover and sound pressure levels. At high frequencies and scales of 25m and 100m, there was a positive relationship between increasing marsh edge and sound pressure levels. The likely mechanisms underlying these patterns are described below.

Spatial patterns in SPLs and fish chorusing

There was spatial variability in fish chorusing hotspots; however, habitat composition did not explain spatial variation of SPLs in the lower frequency band. The focal fish species in this study (silver perch, oyster toadfish, and spotted seatrout) have certain habitat preferences. For example, perch are commonly found within estuaries in seagrass beds and open sandy bottoms (Mok & Gilmore, 1983), while oyster toadfish prefer shallow water, and create nests under rocks, shells, or other sheltered habitat (Gudger 1908). Spotted seatrout also inhabit seagrass beds or relatively deep channels in estuaries, with a preference for deeper water adjacent to vegetated shallow areas when spawning (Mok & Gilmore 1983). Despite these habitat preferences, particularly for seagrass beds or shallow vegetated areas adjacent to deep channels, there was not a relationship between SPLs in the frequencies these fish call in, and the percent cover of habitat types throughout Middle Marsh. These results suggest that seascape patterns, such as proximity of channels connecting deep water and shallow, structured habitats might be a better predictor of fish distribution and abundance patterns than habitat variables per se (e.g., Drew & Eggleston 2008).

Habitat composition did explain some variation in SPLs in the high frequency band. For example, percent cover of seagrass in 10m and 25m radii around each site was significant
in predicting SPLs in the higher frequency band—an increase in seagrass cover resulted in a decrease in SPLs. Though snapping shrimp have been collected in seagrass beds, they are more prevalent in oyster reef habitats, which could explain reduced SPLs in the high frequency band in areas with seagrass (Glancy et al. 2003). One other explanation could be that there is a transmission loss of high frequency tones through seagrass, with more loss occurring in summer when seagrass biomass is greater (Quintana-Rizzo et al. 2006, Wilson et al. 2013). Percent cover of marsh, and length of marsh edge were also useful in predicting SPLs in the high frequency band. Since the high frequency band was meant to capture snapping shrimp activity, increases in SPLs in the high frequency band with increasing marsh habitat suggest that snapping shrimp are using marsh as habitat. Snapping shrimp have been collected from marsh edge and lower intertidal marsh, though the occurrence of snapping shrimp in marsh edge or non-vegetated habitats was related to presence of oyster clumps within these “non-oyster” habitats (Yozzo & Smith 1998, Glancy et al. 2003, Zeug et al. 2007, Shervette & Gelwick 2008). Percent cover of oyster reef was relatively low throughout Middle Marsh, but this relationship between marsh and marsh edge and SPLs in the high frequency band may reveal that there is more oyster reef habitat presence in Middle Marsh than is mapped out. Alternatively, snapping shrimp could reside in marsh or marsh edge habitats, but could be missed by traditional sampling methods (Yozzo & Smith 1998, Glancy et al. 2003).

Though spatial soundscape patterns cannot be explained by habitat type alone, other seascape characteristics may play a role in determining local soundscape characteristics. Study sites were adjacent to deeper channels, some of which were connected to other routes
in and out of Middle Marsh. These deep channels could be corridors for fish movement to feeding and spawning areas, with animals counting on the tide to get in and out of shallower areas. A previous study in Middle Marsh discussed that larger fish reside in deeper channels and move into shallower habitats at night to feed on fish and benthic organisms (Baille et al. 2015). Foraging, and spawning, environments expand and shrink with the flow and ebb of tides, with incoming tides and channels providing access to target habitats (Kneib & Wagner 1994). Intertidal creeks serve as the major routes for tidal migratory nekton to move from subtidal channels to marshes (Bretsch & Allen 2006), indicating that the spatial patterns in fish activity observed in this study could be more related to accessibility to these highways than to habitat type itself.

This study was one of the first to consider the influence of habitat mosaics on patterns of sound production, on a longer time scale in a marine reserve. Previous studies would use non-simultaneous, short duration, recordings to map fish habitat use over large areas (Luczkovich et al. 2008, Gannon & Gannon 2009, Walters et al. 2009). Others characterized fish use of an area using a single hydrophone over the course of one month (Locascio & Mann 2008). Though these studies provided valuable information, borrowing methods from soundscape characterization like those used in this study (e.g, increasing the number of sites sampled, using simultaneous recordings over longer time periods) revealed interesting temporal and spatial patterns of fish activity, which would have been missed without spatial and temporal replication of recordings.

There is a need to understand what species are present and when, as well as their behaviors to inform and map essential fish habitat; however, most studies that have applied
soundscape measures to inform EFH have not conducted simultaneous recordings, and lack long-term recordings (Rountree et al. 2006). For example, spotted seatrout calls at night, which means a soundscape survey conducted during the day would miss this activity (Mok & Gilmore 1983). Multi-day, and longer, sound recording periods allow for a better characterization of what species are calling and when. A benefit of using sounds to delineate EFH and monitor fish species, is that sounds produced by fish are species specific, can be correlated with the size of the individual, or size of the aggregation, and are often associated with a specific activity (spawning) (Mok & Gilmore 1983, Locascio & Mann 2008, Luczkovich et al. 2008). Long-term, replicated soundscape characterization can document activity associated with reproduction for soniferous fishes, thus informing managers of essential spawning locations over time (Locascio & Mann 2008).

Though there are benefits of using passive acoustic recorders to fully characterize marine habitats, there are issues that will limit the use of passive acoustics and soundscape characterization in management and conservation applications in the near future. One major limitation is that development of soundscape diversity metrics and identifying species-specific vocalizations, requires some level of specialized expertise. Unlike terrestrial soundscape characterization, the study of marine soundscapes and the potential acoustic metrics that can be used to rapidly characterize the biodiversity or health of an area, are still being developed. The soundscapes of many habitats have not been characterized, and there is a lack of data linking the characteristics of habitats that have been characterized to seascape and biological data. Studies like this current one illustrate that sound is important, yet complex, and with further advancements in methods for both, the collection and processing
of acoustic data should become a viable technique to rapidly assess biodiversity and habitat quality of marine systems.
LITERATURE CITED


Table 1: Hydrophone deployment start date and time, and end date and time for each site. “——” denotes site that was not recorded due to hydrophone malfunction.

<table>
<thead>
<tr>
<th>Site</th>
<th>Deployment I (6/12/14 17:00)</th>
<th>Deployment II (7/9/14 17:00)</th>
<th>Deployment III (8/8/14 17:00)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>7/8/14 7:00</td>
<td>7/28/14 17:00</td>
<td>8/31/14 5:00</td>
</tr>
<tr>
<td>2</td>
<td>7/7/14 20:00</td>
<td>8/7/14 9:00</td>
<td>8/31/14 5:00</td>
</tr>
<tr>
<td>3</td>
<td>7/7/14 20:00</td>
<td>8/7/14 9:00</td>
<td>8/31/14 5:00</td>
</tr>
<tr>
<td>4</td>
<td>6/23/14 14:00</td>
<td>8/7/14 9:00</td>
<td>8/31/14 5:00</td>
</tr>
<tr>
<td>5</td>
<td>7/3/14 0:00</td>
<td>8/4/14 20:00</td>
<td>8/31/14 5:00</td>
</tr>
<tr>
<td>6</td>
<td>7/8/14 7:00</td>
<td>8/4/14 20:00</td>
<td>--</td>
</tr>
<tr>
<td>7</td>
<td>7/8/14 7:00</td>
<td>--</td>
<td>8/31/14 5:00</td>
</tr>
<tr>
<td>8</td>
<td>7/8/14 7:00</td>
<td>8/4/14 20:00</td>
<td>--</td>
</tr>
</tbody>
</table>

Table 2: Results of multiple regression analyses using stepwise selection to test the relationship between soundscape characteristics (dB SPLs in a lower frequency or higher frequency band) and site habitat characteristics (percent cover of oyster reef, seagrass, and marsh) in the RCERR. Habitat variables were excluded from the full model tested if <4 sites did not have a certain habitat type. Percent cover of estuarine habitat types were calculated using both data collected in the field in 10 and 25 m radius circles around each site, as well as GIS data (from NCDENR) in 25 and 100 m radius circles around each site. SPLs (dB) in each frequency band were calculated from the nightly mean at high tide for each deployment. No terms to add or remove indicates there were no significant variables in the model, or the model was not significant at p<0.05.

<table>
<thead>
<tr>
<th>Radius</th>
<th>Data source</th>
<th>Model tested</th>
<th>Lower Frequency Band (150-1500Hz)</th>
<th>Higher Frequency Band (7-43kHz)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10 m</td>
<td>Field</td>
<td>SPL=reef * SAV</td>
<td>No terms to add/remove</td>
<td>Seagrass (-0.31± 0.08)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>R²=0.44 ± 0.13</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>p=0.0011</td>
</tr>
<tr>
<td>25 m</td>
<td>Field</td>
<td>SPL=reef* SAV * marsh</td>
<td>No terms to add/remove</td>
<td>Seagrass (-0.39 ± 0.13)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>R²=0.33 ± 0.14</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>p=0.0061</td>
</tr>
<tr>
<td>25 m</td>
<td>GIS</td>
<td>SPL= SAV * marsh</td>
<td>No terms to add/remove</td>
<td>Marsh (0.23 ± 0.08)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>R²=0.272 ± 0.11</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>p=0.0153</td>
</tr>
<tr>
<td>100 m</td>
<td>GIS</td>
<td>SPL= SAV * marsh</td>
<td>No terms to add/remove</td>
<td>Marsh (0.27 ± 0.09)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>R²=0.347 ± 0.11</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>p=0.0050</td>
</tr>
</tbody>
</table>
Table 3: Results of regression analyses to test the relationship between soundscape characteristics (dB SPLs in a lower frequency or higher frequency band) and the amount of marsh edge at each site in the RCERR in 10 m, 25 m, and 100 m radius circles around each site. Marsh edge lengths were calculated using GIS habitat data from NCDENR for the RCERR. SPLs (dB) in each frequency band were calculated from the nightly mean at high tide for each deployment. Results show the parameter estimate, $R^2$, and $p$-value for the model. No terms to add or remove indicates there were no significant variables in the model, or the model was not significant at $p<0.05$.

<table>
<thead>
<tr>
<th>Radius</th>
<th>Lower Frequency Band (150-1500Hz)</th>
<th>Higher Frequency Band (7-43kHz)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10 m</td>
<td>$0.190 \pm 0.088$ $R^2=0.197 \pm 0.149$ $p=0.0438$</td>
<td>No terms to add/remove</td>
</tr>
<tr>
<td>25 m</td>
<td>No terms to add/remove</td>
<td>$0.053 \pm 0.014$ $R^2=0.433 \pm 0.010$ $p=0.00118$</td>
</tr>
<tr>
<td>100 m</td>
<td>No terms to add/remove</td>
<td>$0.007 \pm 0.001$ $R^2=0.607 \pm 0.090$ $p=3.19e-5$</td>
</tr>
</tbody>
</table>
Figure 1: Map of Southeast US Coast and study sites (inset) in Middle Marsh located within the Rachel Carson Estuarine Research Reserve (RCERR). The town of Beaufort, NC is located at -76.658W, 34.718N.
Figure 2: Map of Middle Marsh showing habitat layers and deployment sites.
Figure 3: Mean nighttime, high tide SPLs in the lower frequency (150-1500 Hz) band at each site for Deployments I-III.
Figure 4: Percentage of files with fish chorusing at each site for Deployments I through III. Circle size is representative of the percentage of total files with fish chorusing (see Appendix for a table with all percentages). Files with perch chorusing (top) ranged from 0.029% to 7.64% of all files. Files with toadfish chorusing (middle) ranged from 0% to 9.1%. Files with other fish (bottom), including spotted seatrout (*Cyanoscion nebulosus*) and Atlantic croaker (*Micropogonias undulates*), ranged from 0.35% to 39.65% of total files.
Figure 5: Mean nighttime, high tide SPLs in the higher frequency (7-43 kHz) band at each site for Deployments I-III.
Figure 6: SPL in the higher frequency band (7-43 kHz) at each site over three deployments, versus percent cover of seagrass at each site in a 10 m radius (A), and a 25 m radius (B).
Figure 7: SPL (dB) in the higher frequency band (7-43 kHz) at each site over three deployments, versus amount of marsh edge (in meters) at each site in a 25 m radius (A), and a 100 m radius (B).
CHAPTER 3

SETTLEMENT RESPONSE OF OYSTER LARVAE TO DIFFERENT FREQUENCY BANDS OF HABITAT-ASSOCIATED OYSTER REEF SOUND

ABSTRACT

One of the central questions of larval biology is, what cues do larvae use to locate and settle on preferred substrates? Marine organisms with a biphasic life history, like the eastern oyster (*Crassostrea virginica*), are particularly interesting to study with respect to this question because their desired habitat is often patchy, and they are not strong swimmers. Lillis et al. (2013) found that oyster larvae settle in response to oyster reef sound versus the sounds of adjacent unstructured, soft-bottom habitats or no sound controls in complimentary laboratory and field experiments. The main objective of this study was to determine which frequencies of oyster-reef sounds elicit a settlement response in larval oysters. We hypothesized that a greater proportion of oyster larvae would settle when exposed to the higher frequency (≥2kHz) component of oyster reef sound than a lower frequency (150-1000 Hz) component of oyster reef noise, and no sound treatment. There was no significant difference in proportion of larvae settled between the sound treatments for all trial lengths. One explanation for no treatment effect in this experiment is that sound levels were increased within the tank so that sound levels in the larval containers matched the original recording. We do not know how particle acceleration associated with this increase in sound level may have impacted the larvae. Additional experiments testing the settlement and behavioral response of oyster larvae to sound characteristics like frequencies and sound levels, are critical to understanding the role and scale over which sound acts as a cue for this species.
INTRODUCTION

The larvae of many marine organisms, including crabs, oysters and reef fish, have a planktonic dispersal phase in which the larvae must use cues to identify suitable, often patchy, habitat (Vermeij et al. 2010, Lillis et al. 2014b). Though these larvae probably use a suite of cues to find suitable habitat, recent work in soundscape ecology has focused on how sound facilitates habitat selection and settlement behavior in the larvae of marine fish and invertebrates (Lammers et al. 2008, Radford et al. 2008, Lillis et al. 2013). For example, coral reef fish swim preferentially towards traps broadcasting sounds from their desired habitat. This response to habitat associated sound is also present in oyster reef systems; recent larval settlement experiments show a higher proportion of oyster larvae settle in response to oyster reef sound versus off-reef sound (Lillis et al. 2013, 2015).

Soundscape characterization of coral reef, oyster reef, unstructured, and other marine habitats reveals that there are differences in acoustic properties between habitats, such as the intensity of sounds in specific frequency bands, supporting the notion of habitat-specific soundscapes (Radford et al. 2010, Stanley et al. 2012, Lillis et al. 2013). Soniferous species that inhabit coral and oyster reefs, like snapping shrimp, have characteristic high frequency, broadband snaps (1.5-20kHz) (Jeffs et al. 2003, Simpson et al. 2008, Kennedy et al. 2010, Stanley et al. 2012, Lillis et al. 2013, McWilliam & Hawkins 2013, Bohnenstiehl et al. in review). These structured habitats (e.g., reefs) often have higher sound pressure levels (SPLs) and higher frequencies than unstructured habitats that do not harbor soniferous species (Simpson et al. 2008, Radford et al. 2010). Alternatively, offshore and off-reef recordings can have lower power and lower frequency (100-800Hz) acoustic profiles than reef habitats.
likely due to the absence of resident sound producing species (Kennedy et al. 2010, Lillis et al. 2013). In addition, the characteristic acoustic properties of a habitat are tightly linked to proximity to the habitat type being characterized (Lillis et al. 2014b), therefore the spatial scale at which these habitat-associated sounds can be used as a cue is essential to further understanding the role of sound in larval settlement.

The eastern oyster (*Crassostrea virginica*) is a benthic marine invertebrate that has a biphasic life history with a planktonic dispersal phase. Recent larval settlement experiments show that a higher proportion of oyster larvae settled in response to oyster reef sound than to off-reef sound (Lillis et al. 2013, 2015). Because habitat associated sounds can contain a variety of sound sources that depend on habitat type, and because the distinct soundscape is closely linked to proximity to the habitat, identification of the particular components of the soundscape that stimulate a response remains an emerging subject of research (Simpson et al. 2008, Stanley et al. 2012, Lillis et al. 2013, Lillis et al. 2014b). Simpson et al. (2008) found that when exposed to either the higher frequency component (570-2000Hz) or the lower frequency component (<570Hz) of reef noise, the settlement stage of coral reef fish prefer the high frequency component. One proposed benefit of responding to the high frequency (invertebrate-generated) component of reef noise is that low frequency sounds are often those of fish, which may be transient and originating from suboptimal habitat (Simpson et al. 2008). Additionally, invertebrate fauna are dependent on a complex reef structure, and a higher level of invertebrate generated sound may indicate reef quality (Simpson et al. 2008). Therefore, the main objective of this study was to determine the frequencies that stimulate a settlement response in oyster (*Crassostrea virginica*) larvae. We hypothesize that a greater
proportion of oyster larvae will settle when exposed to the higher frequency component of oyster reef noise when compared to lower frequency and silent (no sound playback) treatments.

MATERIALS AND METHODS

To test the response of oyster larvae to distinct frequency bands of oyster reef noise, laboratory playback experiments were conducted using the full bandwidth of original reef recordings with three treatments: silent (no sound played back), higher frequency (original recording high-pass filtered ≥2kHz), and lower frequency (original recording bandpass filtered 150-1000 Hz).

Sound recordings for playback experiments

Subtidal oyster reef soundscapes were recorded as a part of a 2010 study in Pamlico Sound, NC, USA (Lillis et al. 2013) (Figure 1). These subtidal oyster reef reserves were established by the North Carolina Division of Marine Fisheries (NCDMF), in an effort to restore the oyster population in Pamlico Sound. A detailed description of the acoustic sampling procedure for these habitats is given by Lillis et al. (2013, 2014 a,b).

A fifteen-minute sound clip of reef noise at dusk in July that was recorded at the West Bay (WB) reserve was used in these playback experiments. The selected clip was filtered into a higher frequency component (≥2kHz), and a lower frequency component (150-1000 Hz). Sound clips were played continuously for the duration of each trial using Clark Synthesis Aquasonic AQ339 Underwater Loudspeakers (20Hz-17kHz range) placed at the
bottom of the tank and located 0.2m below larval containers (Figure 2). Speakers were connected to a laptop with an audio player. Soundproofing foam was placed underneath each study tank to reduce transfer of sound between treatments.

**Source and Maintenance of Larvae**

Eyed pediveliger-stage oyster larvae from U.S. East Coast hatcheries were obtained for the laboratory settlement experiments. Larvae were wrapped in a damp cloth and shipped in a cooler from the hatchery. Upon arrival at the Marine Ecology and Conservation laboratory at NC State University in Raleigh, North Carolina, larvae were placed in filtered seawater and kept at room temperature for the duration of the experiment.

**Playback settlement experiments**

Prior to each trial, sound treatments were randomly allocated to each tank recordings of the sound treatment within the larval container were taken using a Brüel and Kjaer (B&K) miniature hydrophone (sensitivity of ± 1 dB re 1V/µPa over the 0.1 Hz to 20 kHz frequency range). Two hydrophones were used to make 1-minute tank recordings- one positioned in the center of the container, and another outside the container to measure the recording in the tank. These recordings were used to determine the sound pressure levels inside and outside of the container and to match, as much as possible, the acoustic spectra of the original recordings. All recordings were adjusted to be played back at the same sound pressure level as the original, full bandwidth recording (119dB) inside the containers to eliminate the effect of sound pressure levels on larval settlement. Experiments occurred in the dark, and each
container contained oyster shell substrate, given the preference of oysters to settle on shells of conspecifics.

Actively swimming larvae were randomly selected and placed into cylindrical, acoustically transparent, 80mL containers filled with filtered seawater, with 100 larvae per container. Each container of larvae was assigned in a random fashion to each treatment tank (20L cylindrical water bath), with four groups of larvae per tank (Figure 2). The treatments included: full bandwidth, higher frequency ($\geq 2\text{kHz}$), lower frequency (150-1000 Hz), and silent (no playback) with N=2 tanks per treatment, for a total of eight tanks per trial. Two sets of experiments were conducted with varying trial length. In the first set, sound trials lasted 48 hours, and in the second set, trials lasted 24 hours. At the end of each trial, containers and shell substrates were examined under a dissection microscope and attached (settled) larvae were counted. Larvae were considered settled if they remained attached after agitation with a pipette. A total of seven 48-hour trials, and seven 24-hour trials were conducted.

**Data Analysis**

An ANOVA was used to test for differences in mean settlement for all treatments, blocked by trial and larval batch. Significant differences in mean settlement between treatments (all pairwise comparisons) were tested using the Ryan-Einot-Gabriel-Welsch multiple range test (REGWQ procedure in SAS).
RESULTS

**Sound treatments**

The underwater speakers produced sound spectra that were similar to the original recordings however, some speakers were more consistent than others in reproducing the sound treatments (Figure 3). There was a notch (i.e., no sound) in acoustic power in the 1000-3000 Hz frequency range in sound coming from the speakers (Figure 3B). Though there were some differences in the sound spectra between original and playback recordings, the treatments captured the desired differences in sound pressure levels in the higher and lower frequency bands (i.e., the higher frequency treatment had lower sound pressure levels at lower frequencies and the lower frequency treatment had lower sound pressure levels at higher frequencies) (Figure 3). The mean sound levels produced in the containers for all sound treatments (full bandwidth, higher, and lower) was 119.8 dB re 1 µPa, which was consistent with the sound level of the original recording (119.9 dB re 1 µPa). The silent, or no sound treatment, had a mean sound level of 99.6 dB re 1 µPa.

**Larval settlement playback experiment**

There were no significant differences in mean larval oyster settlement between treatments for 48 hour, 24 hour and all trials (Figures 4 – 6, F=0.09 p=0.9647). There was no significant interaction between treatment and trial (F=1.14 p=0.2713). Mean oyster larval settlement differed significantly between trials (F=400.92 p<0.0001). Mean oyster larval settlement also different significantly between batches (Figure 7).
DISCUSSION

The results of this study are inconclusive. Lillis et al. (2013, 2015) showed in both laboratory and field experiments, that oyster larvae settle in response to habitat-associated sounds from oyster reefs. We expected to produce similar results in this experiment and see a greater amount of settlement in treatments with the full bandwidth oyster reef recordings than those with no sound. However, we did not see any significant difference in oyster settlement between any of the sound treatments in both 48-hour and 24-hour trials.

One of the initial concerns with this experimental design was the 48-hour trial length. Though this was the trial length used by Lillis et al. (2013), there was relatively high (>70%) settlement in all treatments which could mean our trial length was too long and larvae settled regardless of treatment due to age. This resulted in decreasing the trial length to 24 hours for the last seven trials. A subsequent reduction in trial length from 48h to 24h reduced mean settlement rate to ~30%, but did not reveal any treatment effect on oyster larval settlement.

Methods used in this playback experiment were similar to those used by Lillis et al. (2013). One exception was that we were able to measure the Sound Pressure Level (SPL) in the larval containers. This allowed us to set the SPL of each treatment to be the desired level in the larval container, not just in the tank. Acrylic containers, like the ones used in this study, are said to be acoustically transparent (Lillis et al., 2013; Stanley et al., 2011), however after measuring SPLs in the tank and in the larval container, the sound treatments needed to be increased in volume so the SPL in the container matched the SPL of the original recording. In some cases, the differences in SPL between tank and container was 10 dB re 1 µPa, which meant that perhaps the acrylic containers were not as transparent as initially
thought. Upon further preliminary laboratory tests with containers made of different plastics, we found softer plastic containers to be more acoustically transparent than the harder plastic (acrylic) containers (Lillis and Ricci, unpublished results, 2014). Turning up the volume for each treatment allowed us to better match the original SPL of the reef sound, however, we do not know how, or if, the resulting changes in particle acceleration could have impacted larval oyster settlement.

Determining threshold sound levels and component frequencies that elicit a settlement response is important in understanding the role of sound as a cue in larval settlement (Simpson et al. 2008, Stanley et al. 2011, Lillis et al. 2013). Though this first attempt at looking at which frequency components of habitat-associated sound elicit the settlement response in oyster larvae was inconclusive, future work should improve methods and test both sound pressure levels and frequency bands that may be relevant to larval oyster settlement. Laboratory playback experiments would benefit from a fully soundproofed room, absent of noise from heaters, fans, and other room noise. In addition experimental containers should be tested for acoustic transparency, to avoid the issue of having to increase the volume of sound treatments. Lillis et al. (2014) found that with distance from an oyster reef, SPLs in distinct frequency bands, particularly high frequency noise, decrease in intensity. These field soundscape measurements provide valuable insight into the design of future laboratory experiments.
LITERATURE CITED


Figure 1: Map of oyster reef sites in Pamlico Sound from Lillis et al. 2014b.

Figure 2: Larval settlement experiment laboratory setup. Larval containers contained seawater made from Instant Ocean, to avoid any chemical cues, and a small piece of oyster shell of standard size and shape to provide a settlement substrate for larval within the container. The containers were placed in a seawater bath that contained an underwater speaker at the bottom of the tank.
Figure 3: Spectral composition of sound in experimental tanks for the silent (A), full (B), higher frequency (C), and lower frequency (D) treatments. The red line represents the spectrum of the playback recording within the larval containers. The black line represents the spectrum of the original or filtered recordings. Power spectral density estimated via Welch’s method (Hamming window, 50% overlap).
Figure 4: Mean oyster settlement in each treatment (silent, full, higher, lower) for the 48-hour trials. Settlement was measured as the percentage (number out of 100) of larvae settled at end of trial length. Each bar is an average of the replicates (N=2) of each treatment type. Error bars represent the standard deviation.

Figure 5: Mean oyster settlement in each treatment (silent, full, higher, lower) for the 24-hour trials. Settlement was measured as the percentage (number out of 100) of larvae settled at end of trial length. Each bar is an average of the replicates (N=2) of each treatment type. Error bars represent the standard deviation.
Figure 6: Mean oyster settlement in silent, full recording, higher frequency, and lower frequency reef sound treatments for all trials (N=14 trials, N=112 per treatment). Error bars represent the standard deviation.

Figure 7: Mean oyster settlement in silent and reef sound treatments by batch of larvae. Proportion settled was averaged for each treatment across a batch (N=2 trials, 16 containers per treatment for Batches 1 and 3, N=3 trials, 24 containers/treatment for Batch 2).
APPENDICES
Appendix A

Table A.A.1 Expanded deployment details including SoundTrap used at each site for each deployment, the SoundTrap serial number and gain calibration, as well as deployment start and end times. Using the calibration factors below, the data can be read into MATLAB and response corrected as follows: \[ y, fs = \text{audioread('FILENAME.wav')}; y = (y - \text{mean}(y)) * \text{calibration}. \]

Deployment I

<table>
<thead>
<tr>
<th>Site</th>
<th>SoundTrap #</th>
<th>Serial #</th>
<th>Low gain Calibration (µPa/count)</th>
<th>Start</th>
<th>End</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>8</td>
<td>67399703</td>
<td>184.9</td>
<td>6/12/14 17:00</td>
<td>7/8/14 7:00</td>
</tr>
<tr>
<td>2</td>
<td>3</td>
<td>67379211</td>
<td>187.9</td>
<td>6/12/14 17:00</td>
<td>7/7/14 20:00</td>
</tr>
<tr>
<td>3</td>
<td>5</td>
<td>67903499</td>
<td>186.1</td>
<td>6/12/14 17:00</td>
<td>7/7/14 20:00</td>
</tr>
<tr>
<td>4</td>
<td>4</td>
<td>67690520</td>
<td>186.1</td>
<td>6/12/14 17:00</td>
<td>6/23/14 14:00</td>
</tr>
<tr>
<td>5</td>
<td>2</td>
<td>67383320</td>
<td>187.3</td>
<td>6/12/14 17:00</td>
<td>7/3/14 0:00</td>
</tr>
<tr>
<td>6</td>
<td>6</td>
<td>67657752</td>
<td>185.4</td>
<td>6/12/14 17:00</td>
<td>7/8/14 7:00</td>
</tr>
<tr>
<td>7</td>
<td>7</td>
<td>67641354</td>
<td>184.4</td>
<td>6/12/14 17:00</td>
<td>7/8/14 7:00</td>
</tr>
<tr>
<td>8</td>
<td>1</td>
<td>67670026</td>
<td>185.4</td>
<td>6/12/14 17:00</td>
<td>7/8/14 7:00</td>
</tr>
</tbody>
</table>

Deployment II

<table>
<thead>
<tr>
<th>Site</th>
<th>SoundTrap #</th>
<th>Serial #</th>
<th>Low gain Calibration (µPa/count)</th>
<th>Start</th>
<th>End</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>67670026</td>
<td>185.4</td>
<td>7/9/14 17:00</td>
<td>7/28/14 17:00</td>
</tr>
<tr>
<td>2</td>
<td>6</td>
<td>67657752</td>
<td>185.4</td>
<td>7/9/14 17:00</td>
<td>8/7/14 9:00</td>
</tr>
<tr>
<td>3</td>
<td>7</td>
<td>67641354</td>
<td>184.4</td>
<td>7/9/14 17:00</td>
<td>8/7/14 9:00</td>
</tr>
<tr>
<td>4</td>
<td>8</td>
<td>67399703</td>
<td>184.9</td>
<td>7/9/14 17:00</td>
<td>8/7/14 9:00</td>
</tr>
<tr>
<td>5</td>
<td>3</td>
<td>67379211</td>
<td>187.9</td>
<td>7/9/14 17:00</td>
<td>8/4/14 20:00</td>
</tr>
<tr>
<td>6</td>
<td>2</td>
<td>67383320</td>
<td>187.3</td>
<td>7/9/14 17:00</td>
<td>8/4/14 20:00</td>
</tr>
<tr>
<td>7</td>
<td>4</td>
<td>67690520</td>
<td>186.1</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>8</td>
<td>5</td>
<td>67903499</td>
<td>186.1</td>
<td>7/9/14 17:00</td>
<td>8/4/14 20:00</td>
</tr>
</tbody>
</table>
## Deployment III

<table>
<thead>
<tr>
<th>Site</th>
<th>SoundTrap #</th>
<th>Serial #</th>
<th>Low gain Calibration (µPa/count)</th>
<th>Start</th>
<th>End</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>8</td>
<td>67399703</td>
<td>184.9</td>
<td>8/8/14 17:00</td>
<td>8/31/14 5:00</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>67383320</td>
<td>187.3</td>
<td>8/8/14 17:00</td>
<td>8/31/14 5:00</td>
</tr>
<tr>
<td>3</td>
<td>4</td>
<td>67690520</td>
<td>186.1</td>
<td>8/8/14 17:00</td>
<td>8/31/14 5:00</td>
</tr>
<tr>
<td>4</td>
<td>5</td>
<td>67903499</td>
<td>186.1</td>
<td>8/8/14 17:00</td>
<td>8/31/14 5:00</td>
</tr>
<tr>
<td>5</td>
<td>6</td>
<td>67657752</td>
<td>185.4</td>
<td>8/8/14 17:00</td>
<td>8/31/14 5:00</td>
</tr>
<tr>
<td>6</td>
<td></td>
<td></td>
<td></td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>7</td>
<td>7</td>
<td>67641354</td>
<td>184.4</td>
<td>8/8/14 17:00</td>
<td>8/31/14 5:00</td>
</tr>
<tr>
<td>8</td>
<td>3</td>
<td>67379211</td>
<td>187.9</td>
<td>--</td>
<td>--</td>
</tr>
</tbody>
</table>
Appendix B

Table A.B.1: Percentage of total files (recorded at each site) with fish chorusing

<table>
<thead>
<tr>
<th>Site</th>
<th>Total files recorded</th>
<th>Perch (<em>Bairdiella chrysoura</em>)</th>
<th>Oyster toadfish (<em>Opsanus tau</em>)</th>
<th>Other fish (includes spotted seatrout <em>C.nebulosus</em>, Atlantic croaker <em>M. undulates</em>)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5529</td>
<td>2.93</td>
<td>1.3</td>
<td>2.88</td>
</tr>
<tr>
<td>2</td>
<td>5496</td>
<td>6.04</td>
<td>0</td>
<td>14.26</td>
</tr>
<tr>
<td>3</td>
<td>5496</td>
<td>7.64</td>
<td>0.18</td>
<td>39.65</td>
</tr>
<tr>
<td>4</td>
<td>4470</td>
<td>1.36</td>
<td>6.11</td>
<td>4.94</td>
</tr>
<tr>
<td>5</td>
<td>4965</td>
<td>0.22</td>
<td>0.54</td>
<td>0.36</td>
</tr>
<tr>
<td>6</td>
<td>3725</td>
<td>0.0805</td>
<td>0.62</td>
<td>2.17</td>
</tr>
<tr>
<td>7</td>
<td>3464</td>
<td>0.029</td>
<td>0</td>
<td>0.35</td>
</tr>
<tr>
<td>8</td>
<td>3725</td>
<td>7.33</td>
<td>9.1</td>
<td>3.38</td>
</tr>
</tbody>
</table>
Figure A.B.1: Representative spectrograms of silver perch (A), oyster toadfish (B), and spotted seatrout (C), fish choruses.
Figure A.B.2. Example of output for each recording. Top panel is the waveform, middle panels are spectrograms (full bandwidth, then focus on lower frequencies), and the bottom panel is the spectra. Figures like this were used to identify presence/absence of periods of fish chorusing.