OCS Study
BOEM 2018-051

Documenting fish response to seismic surveying and establishing a baseline soundscape for reefs in Onslow Bay, North Carolina

Final Report

US Department of the Interior
Bureau of Ocean Energy Management
Office of Renewable Energy Programs

U.S. Department of Commerce
National Oceanic and Atmospheric Administration
National Centers for Coastal Ocean Science

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Prepared under Cooperative Agreement
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Cooperative Agreement M13AC00006
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Published by
US Department of the Interior
Bureau of Ocean Energy Management
Office of Renewable Energy Programs

US Department of Commerce
National Oceanic and Atmospheric Administration
National Centers for Coastal Ocean Science

October 15, 2018

DISCLAIMER

Research collaboration and funding were provided by the US Department of the Interior, Bureau of Ocean Energy Management, Environmental Studies Program, Washington, DC, under Agreement Number M13AC00006. This report has been technically reviewed by BOEM and it has been approved for publication. The views and conclusions contained in this document are those of the authors and should not be interpreted as representing the opinions or policies of the US Government, nor does mention of trade names or commercial products constitute endorsement or recommendation for use.

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US Department of the Interior
Bureau of Ocean Energy Management
Office of Renewable Energy Programs
381 Elden Street, MS HM-1328
Herndon, VA 20170

U.S. Department of Commerce
National Technical Information Service
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Springfield, Virginia 22161
Email: bookstore@ntis.gov

CITATION

Paxton A.B., J.C. Taylor, D.P. Nowacek, J. Dale, E. Cole, C.M. Voss, B. Degan, E. Ebert, and C.H. Peterson. 2018. Documenting fish response to seismic surveying and establishing a baseline soundscape for reefs in Onslow Bay, North Carolina. U.S. Department of the Interior, Bureau of Ocean Energy Management, Office of Renewable Energy Programs, Herndon, VA and Department of Commerce, National Oceanic and Atmospheric Administration, National Centers for Coastal Ocean Science, Springfield, VA. OCS Study BOEM 2018-051. 62 p.

ACKNOWLEDGMENTS

We acknowledge the assistance of several colleagues and research partners who aided in the conceptualization of this project, the survey design and interpretation. We thank R. Gaesser, H. Lemoine, and R. Rosemond for field assistance and data processing for the soundscape monitoring component. We thank E. Ebert, D. Freshwater, A. Pickett, L. Bullock, K. Egan, J. Vander Pluym, J. Fleming, E. Pickering, R. Purifoy and crew from Olympus Dive Center, and T. Leonard and crew from Discovery Diving for field assistance for the soundscape monitoring component. We thank Y. Azevedo, P. Oliviera, A. Requarth, L. Revels, S. Richardson, D. Rouse, T. Uruganti, and K. Wiedbusch for soundscape monitoring video processing. For the seismic survey component, we thank thank J. Vander Pluym, A. Adler, R. Mays, R. Purifoy, and crew from Olympus Dive Center for field assistance. Cover photos (top panel and bottom right) by John McCord and David Sybert (UNC – Coastal Studies Institute

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Equation 1: db_{meas} = the measured dB value; p_ {meas} = the measured pressure for a given file; p_{ref} = the reference pressure for that unit, i.e., from the calibration tone. So, based on the 1 kHz calibration tone from the SoundTrap, we are able to report a calibrated dB level for each recording. 19

ABBREVIATIONS AND ACRONYMS

Abbreviation / Acronym	Definition
ANOSIM	Analysis of Similarities
AOI	Wind Energy Area of Interest
AR	Artificial Reef
BOEM	Bureau of Ocean Energy Management
CPA	Closest Point of Approach
dB	Decibel
EFH	Essential Fish Habitat
G&G	Geophysical and Geotechnical
GB	Gigabyte
hr	Hour
kg	Kilogram
kHz	Kilohertz
LDEO	Lamont-Doherty Earth Observatory
m	Meter
min	Minute
ms	Millisecond
NC	North Carolina
nMDS	Nonmetric Multidimensional Scaling
OCS	Outer Continental Shelf
PCA	Principal Components Analysis
SD	Secure Digital
sec	Second
US	United States

1. Introduction

The Bureau of Ocean Energy Management (BOEM) is responsible for oversight and management of the development of offshore energy resources on the outer continental shelf (OCS). A large proportion of the Atlantic OCS blocks deemed likely suitable for energy development is located offshore of North Carolina (NC). Prior to making OCS blocks available for lease, BOEM must satisfy criteria of the Outer Continental Shelf Lands Act, of which Section 1346 mandates the conduct of environmental and socioeconomic studies to assess and manage any environmental impacts on the human, marine, and coastal environments anticipated by construction, development, or operational activities. Offshore energy development, such as installing wind turbine infrastructure and using seismic surveys to explore for oil and gas deposits, introduces noise to the marine environment. Knowledge of how these noises influence fish in their natural environments is limited but understanding possible impacts has important management implications (Popper and Hastings 2009, Popper et al. 2014, Nowacek et al. 2015).

Hardbottom reefs that occur on the NC continental shelf support a diverse community of fishes and present an opportunity to test how underwater sound affects reef fish. These hardbottom reefs are defined as Essential Fish Habitat (EFH) by the Magnusson-Stevens Fisheries Conservation and Management Act (1996) because they function as nursery, foraging, and spawning grounds, as well as refuge and nearshore connectivity corridors for fishery species (Deaton et al. 2010). Hardbottom reefs include natural hard substrate of exposed rock and consolidated sediments, as well as architecturally unique man-made structures, such as shipwrecks and artificial reefs. Natural reefs include flat pavements, rubble fields, and substantial ledge systems with up to several meters of vertical relief that are subject to dramatic state changes due to sediment dynamics and other physical processes (Riggs et al. 1996, Renaud et al. 1996, 1999). Artificial reefs and shipwrecks vary in architecture, as these man-made structures range from piled concrete pipes to large sunken ships. There is a particularly high concentration of shipwrecks in NC coastal waters, which are commonly referred to as the 'Graveyard of the Atlantic,' because they form the resting grounds for thousands of shipwrecks from the past 500 years that were casualties of changing barrier island geomorphology, as well as war (Stick 1989). In addition to shipwrecks, the state of NC intentionally sinks man-made structures, such as ships, bridge rubble, airplanes, boxcars, concrete pipes, and numerous other items to enhance habitat for fish and invertebrates as part of the NC Artificial Reef Program (North Carolina Department of Natural Resources and Community Development - Division of Marine Fisheries 1988).

Natural hardbottom and artificial reefs, henceforth jointly referred to as hardbottom or temperate reefs, serve as living resources because they, with the exception of shipwrecks, form federally-designated EFH for reef-associated fishes, as well as habitat for invertebrates and macroalgae. These reefs draw recreational and commercial fishers, as well as divers to these areas (Parker Jr. 1990, Parker Jr. and Dixon 1998, Whitfield et al. 2014). Fish in the snapper-grouper complex that use hardbottom are of particular concern because of their recreational and commercial value and their depressed numbers for several exploited populations in the region (Deaton et al., 2010).

Benthic invertebrates and macroalgae, where they occur, may provide important biogenic habitat structure and prey for fishes on hardbottom (Peckol and Searles 1983, 1984, Renaud et al. 1997, 1999). Temperate reefs of NC are not only ecologically valuable but are also important to coastal economies and cultures.

Here, we present findings from two related research components where we documented the marine soundscape and associated fish communities on hardbottom reefs of the NC continental shelf within Onslow Bay:

> Component 1 – Documenting Fish Response to Seismic Surveying: During September 2014, we conducted opportunistic monitoring on four offshore temperate reefs within Onslow Bay, NC to determine the response of reef fishes to a planned scientific seismic survey. We deployed video cameras and hydrophones to record fish communities and associated acoustic signatures before and during seismic surveying. Results from this component were published in the peer-reviewed journal *Marine Policy* in April 2017 (Paxton et al. 2017).
>
> Component 2 – Establishing a Baseline Soundscape: Over a ten-month period beginning in September 2015, we documented the marine soundscape and associated fish communities on artificial and natural reefs of the NC continental shelf by installing fish and soundscape monitoring stations off the coast of NC. The objective was to establish opti-sonic monitoring stations in the vicinity of hardbottom to sample the soundscape and fish community assemblages and provide a baseline record of bioacoustics and anthropogenic noise in Onslow Bay, NC. Results from this component are preliminary.

2. Documenting Fish Response to Seismic Surveying (Component 1)

2.1. Background

Marine seismic surveys emit high intensity, low frequency sounds (> 230 dB re 1μ Pa) from airgun arrays downward into the water column (Hildebrand 2009). The resultant sound waves penetrate the seafloor to provide imagery of the underlying geology. These surveys can detect reservoirs of oil and natural gas, determine site-specific suitability for installation of offshore renewable energy infrastructure, evaluate sources of minerals for commercial extraction or sand for use in beach nourishment, and/or provide information on the substructure for geological research. Noise from seismic surveying can alter marine mammal vocalizations and foraging rates, and can lead to marine mammal displacement (Miller et al. 2009, Pirotta et al. 2014, Blackwell et al. 2015); however, there remain unanswered questions regarding how wild fish respond to seismic survey noise. Understanding whether fish are affected through alterations in behaviors associated with feeding, growth and survival has important conservation and management implications.

Acute impacts to individual fish from seismic noise, including damage to sensory ear hair cells, can occur with close-range exposure to low-frequency, high-intensity sounds in laboratory settings (McCauley et al. 2003, Popper et al. 2005). Impulsive sounds similar to those from

seismic surveys, such as noise made by pile driving, can cause mild to lethal injuries ranging from swim bladder rupture to hematoma and hemorrhaging (Popper and Hastings 2009, Halvorsen et al. 2012, Popper et al. 2014). Behavioral responses of fish to noise are more difficult to quantify but may include changes in abundance in particular habitats (Slotte et al. 2004), changes in swimming patterns or feeding (Purser and Radford 2011, Hawkins et al. 2014), as well as physiological stress leading to mortality (Popper and Hastings 2009). In contrast, in two studies that were specific to noise associated with seismic surveying, there were no marked changes in fish physiology or behavior (Popper et al. 2005, Song et al. 2008). Reductions in fish catches can persist for up to five days after seismic activity (Skalski et al. 1992, Slotte et al. 2004, Løkkeborg et al. 2012). Aside from those mentioned previously, most studies on fish response to seismic noise occur in laboratory settings; underwater observations of fish in their natural environment during seismic surveys are rare (Popper and Hastings 2009). Wardle et al. (Wardle et al. 2001) experimentally exposed fish *in situ* to noise from airguns and observed startle responses but did not detect other changes in behavior or abundance.

To determine whether reef-associated fishes in their natural environment respond to marine seismic surveying, we conducted opportunistic monitoring of a seismic survey offshore of NC. Seismic surveying was conducted by Columbia University – Lamont-Doherty Earth Observatory (LDEO) aboard the R/V *Marcus G. Langseth* (*Cruise Report: Eastern North American Margin Community Seismic Experiment, Cruise MGL1408, R/V Marcus G Langseth* 2014). During the first leg of this marine seismic survey in September 2014, the R/V *Langseth* completed several lines offshore of NC from the lower continental slope (>5000 m deep) to shelf waters (< 35 m deep, Figure 2-1; Cruise MGL1408 2014). The second line of the survey was conducted in northeastern Onslow Bay in close proximity to temperate reefs (Figure 2-1; Cruise MGL1408 2014). In coordination with investigators from LDEO, we deployed passive monitoring units to record underwater sounds and video of fishes occupying temperate reefs prior to, during, and immediately after the R/V *Langseth* surveys on the continental shelf. Here, we report our findings of this short-term investigation. Results from this component were published in the peer-reviewed journal *Marine Policy* in April 2017 (Paxton et al. 2017).

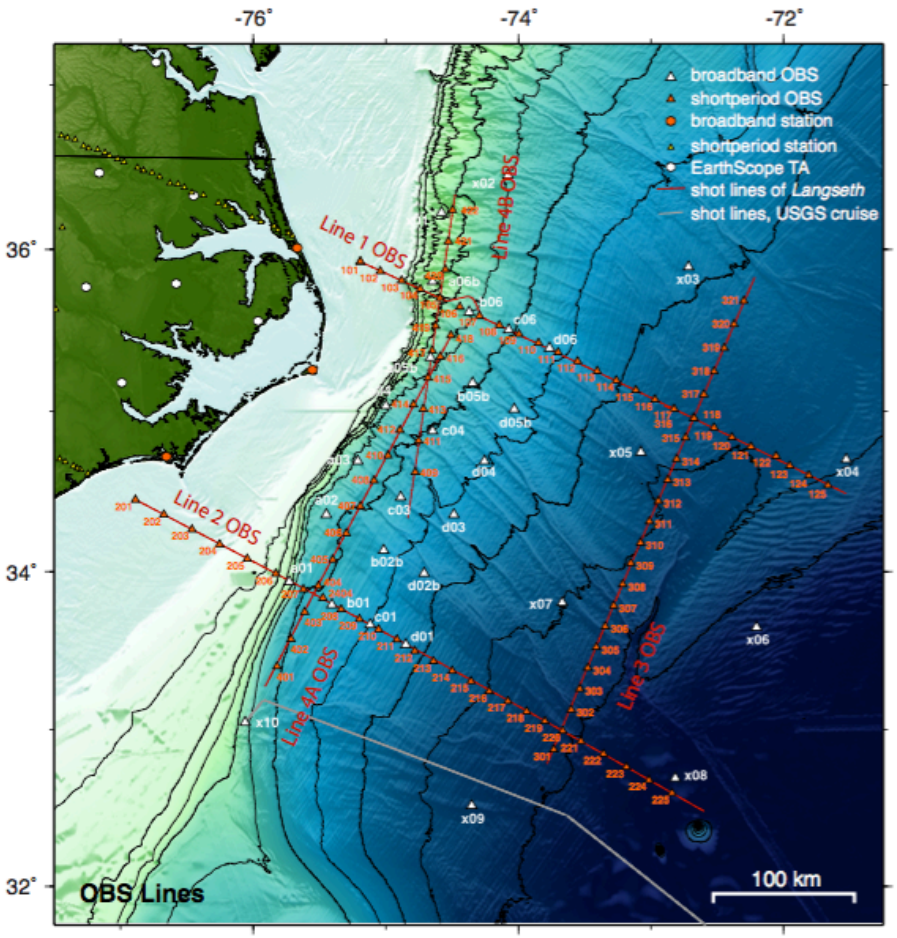

Figure 2-1: Map of survey lines for the R/V *Langseth* cruise in 2014 (map from Cruise Report, Eastern North Atlantic Margin Community Seismic Experiment, Cruise MGL1408, R/V Marcus G. Langseth, September 16 – October 18, 2014).

2.2. Methods

2.2.1. Site Selection

We selected four reefs to monitor within continental shelf waters that were close to the anticipated cruise track of the R/V *Langseth* (Figure 2-2). These four temperate reefs lie within northeastern Onslow Bay at depths ranging from 22 to 33 m (Figure 2-2). Three of the reefs are natural reefs with known ledges and high structural complexity (210 Rock, West Rock, 10 Fathom Rock), and one is an artificial reef (US Navy Cable Layer Aeolus). These reefs have been the focus of various marine fisheries and ecological studies for several decades, with notable abundances of snapper and grouper species and other commercially important species (Parker and Dixon 1998, Whitfield et al. 2014).

At each of the four hardbottom reef sites, scientific divers established temporary passive monitoring stations. Each passive monitoring station included a GoPro audio/video cameras (GoPro, US) with attached intervalometers (cam-do, US), which recorded 10-sec long time lapse videos every 20 min (Table 2-1). Autonomous acoustic recorders (hydrophones, SoundTrap 202 recorders, Ocean Instruments, New Zealand) recorded ambient sounds, including those of biological and anthropogenic origins. The hydrophones where deployed at two of the four monitoring sites, one at West Rock and the other at US Navy Cable Layer *Aeolus* (Figure 2-2; Table 2-2). These hydrophones were set to collect data continuously at a 96-kHz sampling frequency. This frequency and the lack of any duty cycling had potential to record for a duration of approximately 14 days, a duration well within the time window of the scheduled seismic survey. These recorders have several features that are ideal for this work: (1) at the beginning of each deployment they create a calibration tone, which allows for simple and robust calibrated measurements of the sounds recorded; and (2) they are compact, rechargeable and can be initiated easily at sea. At each site, the array of instruments was mounted on a conical metal frame measuring approximately 0.5 m high and 0.3 m in diameter at the base. Each frame was anchored with 60-80 kg of lead. All monitoring equipment was deployed at the monitoring sites on the morning of September 17 and retrieved on September 25, 2014.

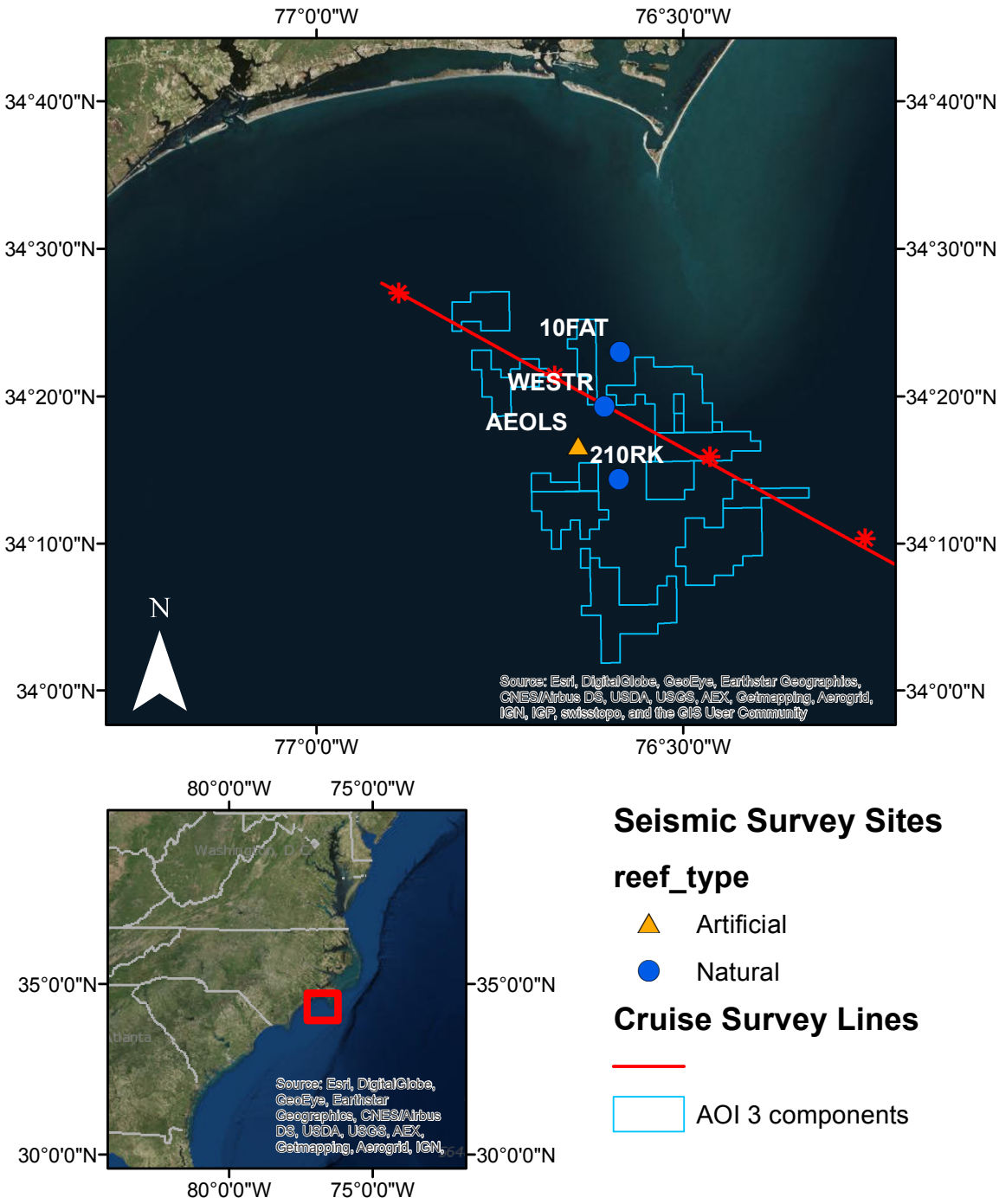

Figure 2-2: Map of seismic survey monitoring sites. Red line and asterisks represent the planned R/V *Langseth* seismic survey cruise track. Blue outlines represent components retained within Wind Energy Area of Interest (AOI) 3 based on previous studies of EFH in Onslow Bay. The R/V *Langseth* surveyed the line twice, first shoreward then offshore.

Table 2-1: Components of monitoring stations, collecting passive acoustic and video data, deployed on temperate reefs in Onslow Bay, North Carolina in September 2014.

Hardware Equipment	Manufacturer	Model	Description	Recording Parameters
Hydrophone	Ocean Instruments	SoundTrap 202	Record ambient sound; deployed at West Rock and *Aeolous*	Continuous recording at 96 kHz
Video camera	GoPro	Hero 2, 3, 3+	Record video of fish	10 sec video every 20 min
Time-lapse controller	cam-do	Intervalometer	Control the time schedule of fish videos	10 sec video every 20 min

Table 2-2: Reefs where passive acoustic and video monitoring stations were established on temperate reefs in Onslow Bay, North Carolina in September 2014. Monitoring gear denotes the array of instruments (hydrophone = SoundTrap; video = GoPro (number deployed)) that were deployed on each reef.

Site Name	Site Code	Latitude (ddm)	Longitude (ddm)	Latitude (dd)	Longitude (dd)	Reef Type	Depth (m)	Monitoring Gear
US Navy Cable Layer Aeolus (AR-305)	AEOLS	34 16.700	-76 38.592	34.2783	-76.6432	Artificial	31	SoundTrap; GoPros (1)
10 Fathom Rock	10FAT	34 23.035	-76 35.173	34.3839	-76.5862	Natural	22	GoPros (2)
210 Rock	210RK	34 14.380	-76 35.250	34.2397	-76.5875	Natural	33	GoPros (2)
West Rock	WESTR	34 19.320	-76 36.430	34.3220	-76.6072	Natural	25	SoundTrap; GoPros (2)

2.2.2. Continuous Acoustic Data and Seismic Recordings

Acoustic data from the two hydrophones were processed and then five shots were aggregated for each of nine selected time points. Shots were processed in groups of five to obtain a 'local average' to smooth fine scale variation that occurs in the propagation conditions. The time points were chosen relative to the closest point of approach (CPA) on both the landward and seaward components of the survey path. The five shots closest to the CPA that were not clipped were processed, and other locations were chosen to compare the received signals from the reefs, e.g., the more distant sampling locations gave similar propagation paths to the reefs, while the closer locations were subject to very different parts of the non-uniform source beam pattern (Tolstoy et al. 2009). On acoustic recordings from the reef located 0.7 km from the path of the seismic surveying vessel, the noise of the seismic shots overloaded the recorders when the ship was at its CPA. Using the known source sound level of the survey vessel's airgun array (Tolstoy et al. 2009), the anticipated broadband level of received sound at the reef was calculated based on two models, spherical spreading and cylindrical spreading (Urick 1983a). All acoustic values reported are in dB re 1μ Pa peak-peak.

Each autonomous acoustic receiver recorded tracks in segments approximately 2 hrs long and 1.9 GB in size throughout the deployment, and with the two recorders we logged a total of 212 such files. These files were downloaded, verified and archived upon retrieval of the units and return to the laboratory. The West Rock recorder had a software malfunction and reset the clock to the year 1970 after the initial calibration tone, so synchronizing the recorders to real time, and thus to each other, was a significant task. Synchronization was key as one of the tasks was to compare at the two sites the received levels of individual pulses. To synchronize the recorders we used Adobe Audition© we followed a series of steps:

1. Identified peak events in the sound tracks when the ship came closest to each recorder, i.e., we assumed that the loudest pulses would occur when the ship was closest to a given recorder. With a combination of certain propagation conditions and depth of source and receiver, the closest pulses may not be the loudest (Madsen et al. 2006). However, in these shallow depths and relatively short ranges, the closest pulses should be the loudest (Urick 1983b).
2. Marked the shots around the peak events and during the transition of shot interval from ~90 sec intervals (every 225 m at 3.5 kts) to 20-30 sec (50 m linear distance between shots), which occurred after the ship turned at the end of the track line and began the offshore-bound leg (Figure 2-2). This change in transmission timing from 90 to 30 sec shot intervals was a straightforward and well-documented point in the survey in recordings on both sensors, and thus made an ideal point in time for synchronization.
3. Assembled a continuous sound track for each recorder, resulting in a multi-track session. The multi-track session facilitated the automated processing of the pulses.
4. Aligned shots and recorder times for the two continuous sound tracks.

During a period of time, as the ship came closest to the recorders ('peak events'), the airgun shots exceeded the dynamic range of the recorder and the waveforms clipped due to the high source level. As a result, approximately 17 min of data were noted but excluded from analyses as these clipped waveforms cannot be accurately analyzed for frequency or amplitude information. When the waveform is clipped in the time domain, it means that the recorder is no longer able to measure the amplitude of the signal because the actual amplitude is at or above the recorder's capability. This occurrence is unfortunate but not surprising given the intensity of the seismic signals.

To compare levels at the two locations resulting from the same airgun shot, 5 shots were aggregated at 9 time points for both recorders. Five shots were sampled at each of the following time points (Note: Change in shot interval occurred after the turnaround point):

Point 2: 2:22:07 h before closest point of approach (CPA) (90-sec shot interval)

Point 3: 5 samples prior to clipping, i.e., when the ship was as close to the recorders as possible with us still being able to measure (90-sec sec shot interval)

Point 4: 5 samples after clipped signals (90-sec sec shot interval)

Point 5: 2:22:07 after CPA (90-sec shot interval)

Point 6: 4:44:15 after CPA (90-sec shot interval) Calculated as the mid point between the CPA events and the assumed furthest distance and vessel turnaround point

Point 7: 2:22:07 prior to return trip CPA (30-sec shot interval)

Point 8: 5 samples prior to clipping during seaward leg (30-sec sec shot interval)

Point 9: 5 samples after clipping (30-sec sec shot interval

Point 10: 2:22:07 after CPA (30sec shot interval)

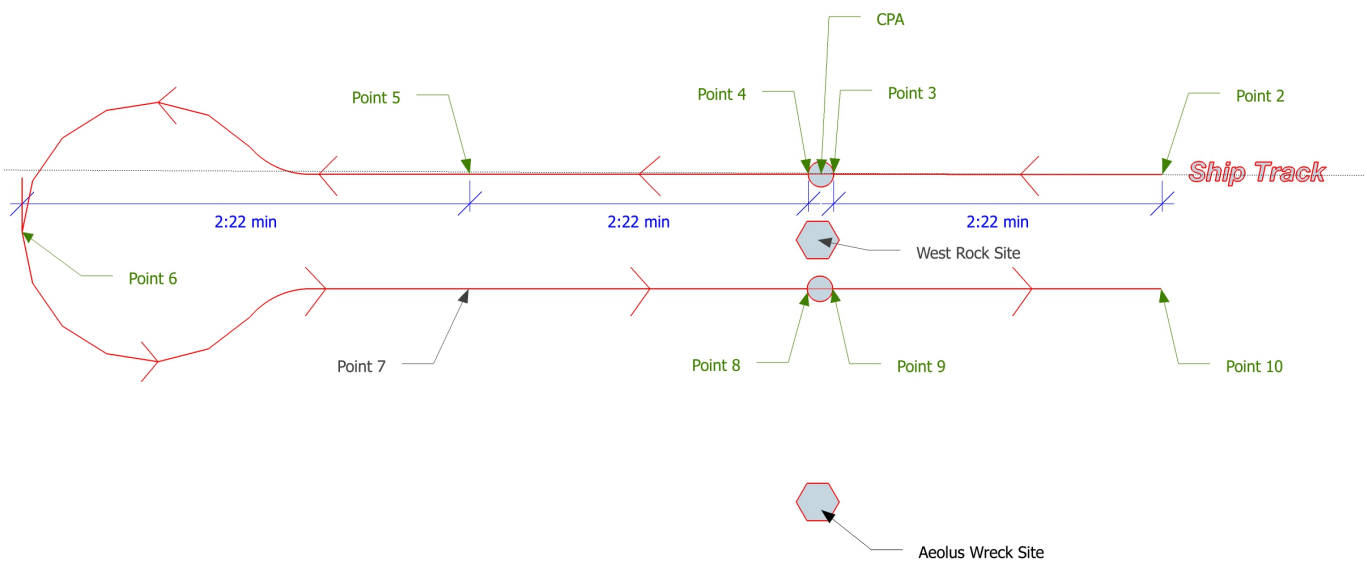

Figure 2-3: Schematic of the R/V *Langseth* track relative to the position of acoustic recording stations. Drawing is not to scale nor geographically representative of the location of shots relative to positions of recording stations.

Shots were not audible at the 4:44:15 time mark before arriving at the recorder and after passing. It was assumed the ship was in deeper water and the signal energy propagating up into the shallow water was sufficiently attenuated to the point we were unable to detect it. We used this time period to identify pre-survey periods to sample ambient noise levels with the SoundTraps at both locations. For the analyses, we used 5-30-sec samples prior to and after the survey vessel past the stations:

1. West Rock recorder – pre-survey: clips taken from 1st track after deployment track (i.e., after the deployment team departed the area). We used 5 x 30-sec samples from the start of the track starting on the minute.

2. West Rock recorder – post-survey: clips taken from the track recorded prior to retrieval track, and again, 5 x 30 sec samples from the start of the track starting on the minute.
3. *Aeolus* – pre survey: clips taken from 1st track after deployment track (i.e., after the deployment team departed the area). We used 5 x 30 sec samples from the start of the track starting on the minute.
4. *Aelous* – post survey: clips were taken from the final track as the recorder filled SD card. There were no sounds recorded from the retrieval. We used 5 x 30 sec samples from the start of the track, starting on the minute.

To obtain calibrated values for the measured signals we utilized the calibration tones provided by the SoundTrap, and each unit includes calibration information. We take a measurement of the time domain waveform from that calibration tone, and then compare that level with the measured level from a given sound. To obtain calibrated measurements of the seismic signals, we used a 100-ms time window, the nominal duration of the pulses. It is important to note that the signals can be longer than that depending on the amount of reverberation, but within that 100-ms window, it is assumed that at least 95% of the energy in the pulse is captured. The denominator values in Equation (1) are the raw amplitudes of the 1-kHz peak in the spectrogram of the calibration tones generated by the SoundTrap. The last factor is the "measured pressure", which is the variable for which we want the calibrated measurement, i.e., the amplitude of the signal. It is obtained by solving the definition:

$$Equation\ (1): dB_\{meas\} = 20 * log10(p_\{meas\}/p_\{ref\})\ for\ p_\{meas\}$$

Equation 1: db_{meas} = the measured dB value; p_ {meas} = the measured pressure for a given file; p_{ref} = the reference pressure for that unit, i.e., from the calibration tone. So, based on the 1 kHz calibration tone from the SoundTrap, we are able to report a calibrated dB level for each recording.

2.2.3. Time-Lapse Videography of Reef Fishes

At each of the four reefs, video monitoring was conducted to document any response of reef-associated fish to the seismic survey conducted by the R/V *Langseth* (Figure 2-2). The video cameras were set to record 10-sec videos every 20 min throughout the deployment. Several of the cameras malfunctioned while deployed and did not record as programmed. On the artificial reef *Aeolus*, the video camera did not record. On one of the natural reefs, West Rock, the video camera only recorded for two days prior to the seismic survey. On another natural reef, 10 Fathom Rock, sampling began late on the first day, because of the deployment time, and there was insufficient replication to resolve fish response to seismic surveying on this reef. On the fourth reef, 210 Rock, which was located 7.9 km from the CPA of the seismic surveying vessel, videos recorded for three days before and one day during seismic surveying. Logistical constraints prevented collection of data following seismic surveying.

Each 10-sec video clip was processed by recording the maximum number of fish in the frame (maxN), identified to the lowest taxonomic level possible. Behavior was recorded and classified as feeding, resting, schooling, or swimming. Airgun shots from the seismic survey and/or sounds

from boats were also recorded. If fish changed behavior when a noise source was heard, a qualitative description of the type of behavioral shift was also recorded. To prevent observer bias, fish were first counted with video sound turned off; then sound was turned back on to detect whether shots were present. Underwater visibility was estimated, and the occurrence and time of sunset and sunrise were also documented. These data were recorded in Microsoft Excel spreadsheets.

Fish data obtained from video recordings were analyzed in R (R Development Core Team 2015). We plotted hourly untransformed fish abundance for the three reefs where we successfully collected video recordings for all fish, for the subset of fish federally-designated as part of the snapper-grouper complex, and for candidate fish species.

For 210 Rock, which was the reef where we had the most extensive time series that also included airgun shots detectable in the video recordings, we conducted more rigorous analyses. The time series of hourly untransformed fish abundance for 210 Rock was plotted for each of three days before and the following day during seismic surveying to visualize daily abundance patterns. The smoothed conditional mean of the hourly fish abundance for the combined three days before seismic activity and the accompanying standard error, as well as the smoothed conditional mean of hourly fish abundance on the day with seismic activity, was also calculated. The resulting two curves and the standard error were compared to determine whether the temporal pattern of fish abundance differed from before to during seismic surveying.

Two different statistical tests determined if the pattern in daily fish abundance on 210 Rock differed before versus during seismic surveying. First, an analysis of means for variance (ANOMV) with a Levene transformation (Wludyka and Nelson 1997, Pallmann 2015) tested the equality of variance in fish counts on three days pre-seismic surveying and one day during seismic surveying. ANOMV determined whether daily means for variance in fish counts were significantly different than the grand mean for variance. Second, ANOVA followed by post-hoc pairwise t-test on box-cox transformed fish counts (Venables and Ripley 2002) tested for daily differences in fish abundance during the four-hour evening period (1600-2000) of typically greatest fish occupation. The percent change in fish occupation of the reef based on the average evening fish abundance on three days without seismic surveying and the evening fish abundance on the following day with seismic noise was also computed.

2.3. Results and Discussion

The R/V *Langseth* survey was conducted along the Outer Continental Shelf. We focused our observations and analysis during time periods when the vessel was traversing survey Line 2. Shot logs for these survey lines reference OBS001 and MCS002, reciprocal lines that traversed onto the shallow shelf in Onslow Bay, offshore of NC. The OBS (Onshore) line used shot intervals of 225 m or about 60-90 seconds. The return line (MCS) used shot spacing of 50 m or about 18-20 seconds. Readers are directed to the R/V *Langseth* cruise report MGL1408 for further details of system specifications during data acquisition (MGL1408 Cruise Report 2014).

The R/V *Langseth* approached the shallow shelf waters on 20 September 2015 at 0530 UTC (

Figure 2-4; Table 2-3). The R/V *Langseth* passed as close as 695 m away from the West Rock station at 1147 UTC during the shoreward pass (OBS001) and as close as 716 m away from the West Rock station during the offshore pass (MCS002) at 2112 UTC.

Table 2-3. Measures of closest proximity of seismic airgun array to observation stations.

Station	Closest Pass (shoreward, m)	Date Time	Closest Pass (offshore, m)	Date Time
West Rock	697	11:47 UTC	716	21:12 UTC
10 Fathom Rock	6272	11:58 UTC	6257	21:03 UTC
Aeolus	6530	11:51 UTC	6547	21:08 UTC
210 Rock	7940	11:06 UTC	7953	21:55 UTC

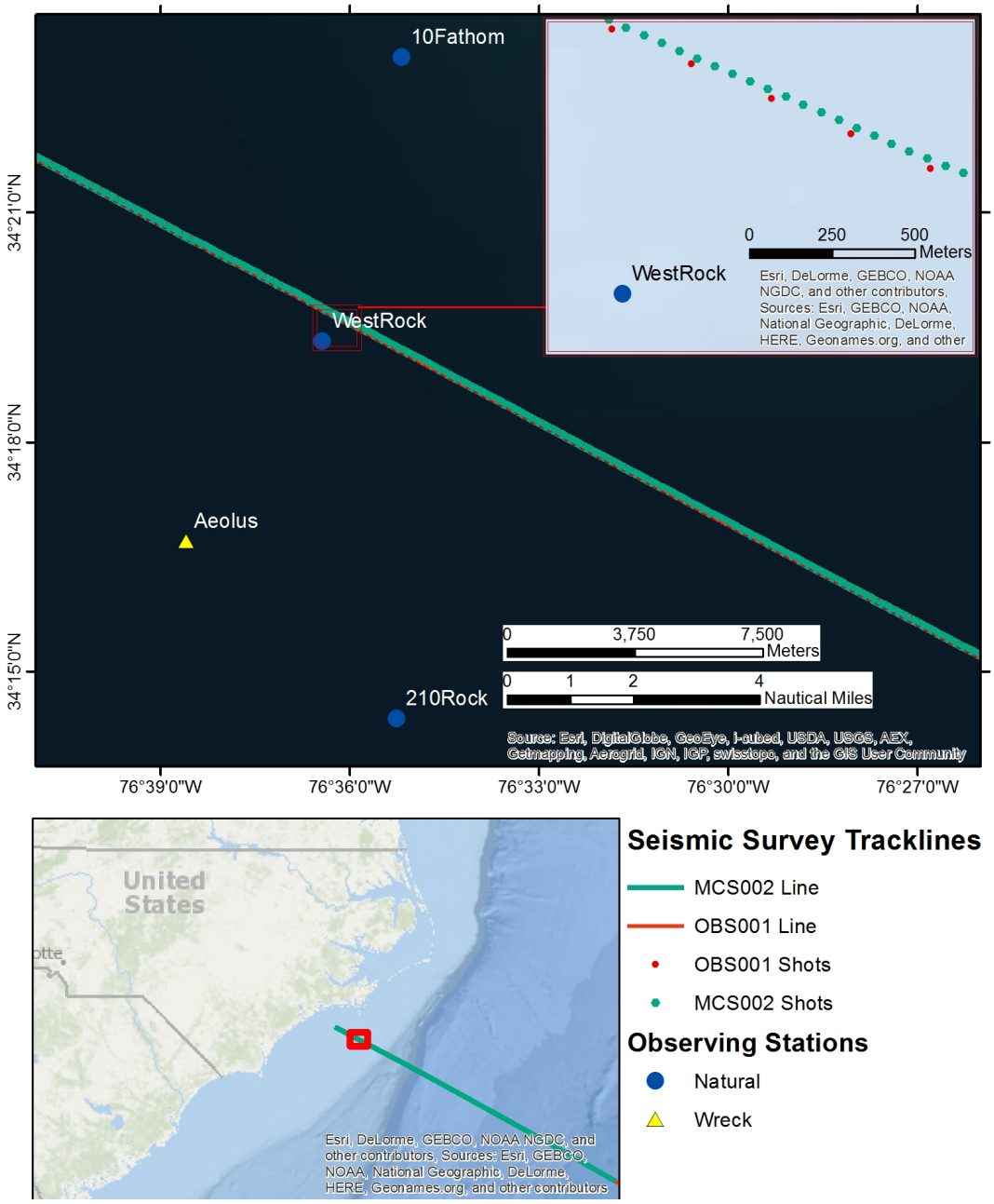

Figure 2-4: Seismic survey track lines for the R/V *Langseth* OBS001 and MCS002 (see cruise report). Nearly identical reciprocal passes result in lines that appear superimposed in the map showing four observation stations. Inset shows locations of airgun shots in proximity to the West Rock site.

2.3.1. Acoustic Signatures of Seismic Surveys

Noise levels on the two reefs designated as Essential Fish Habitat and located closest to the seismic survey track, 0.7 and 6.5 km away, exceeded 170 dB re 1μ Pa (Fig. 2). The peak levels that actually occurred at the sites are unknown because the noise overloaded the recorders. Using a sound source level of 258.6 dB re 1μ Pa (Tolstoy et al. 2009), the received sound was estimated using two different models, spherical spreading and cylindrical spreading (Urick 1983a). Based on a spherical spreading model, the corresponding received sound level on the closest reef would have been 202 dB re 1μ Pa, whereas based on the cylindrical spreading model, the received level would have been 230 dB re 1μ Pa. Realized peak sound levels likely fall between those predicted by spherical and cylindrical spreading models (Nowacek et al. 2013). The high intensity of this low-frequency sound is consistent with previous measurements (Guerra et al. 2011a, Racca et al. 2015). The intensity of the noise is of significant concern because laboratory experiments indicate that fish experience recoverable injuries and/or potentially mortal injuries at noise levels > 207 dB re 1μ Pa peak (Popper et al. 2014).

Ten-second videos were recorded every 20 min for three days before and through the day with seismic surveying on a 33-m-deep reef located 7.9 km from the closest approach of the seismic survey vessel. Although a hydrophone did not record sound on this reef, based on spherical spreading and a source sound of 258.6 dB re 1μ Pa the estimated noise experienced on this reef was 181 dB re 1μ Pa when the survey vessel was closest. Using a second model based on cylindrical spreading, the received sound level was 220 dB re 1μ Pa on the reef. Realized peak sound levels probably lie between the predictions of these two spreading models (Nowacek et al. 2013).

The sound fields at time point 1 (i.e., ambient levels, see definition above) for the two sites, West Rock (the site closest to the survey line) and *Aeolus* were quite similar across the spectrum analyzed (Figure 2-5). For time point 2, the samples taken more than 2 hours before and 20 km away from the closest point of approach (CPA), we see similar acoustic signatures for the two sites (Figure 2-6). At time point 3, just before the signals started overloading the recorders (i.e., as close to CPA as we were able to record), we see significant differences in the West Rock vs. *Aeolus* pulse signatures (Figure 2-7). The levels at West Rock are much higher, 20-40 dB at the main seismic frequencies, than at *Aeolus*. Also, interestingly, the levels at the relatively high frequencies (>1 kHz) are as high or higher at *Aeolus* than at West Rock despite being further from the seismic array. This is most likely due to the lateral footprint of sound from the seismic array. We also see non-uniformities at *Aeolus* with the levels dropping out occasionally through the spectrum, and these features are likely due to interference patterns created in the propagation by features in the environment, e.g., sand ridges and structure on the seafloor. Once the survey vessel passed the CPA, we analyzed another set of pulses for comparison with those from just before. Figure 2-8 shows the levels for pulses just after CPA (time point 4) (after the clipped signals), and we see a difference when compared to before the CPA. For example, the low frequency levels at *Aeolus* are approaching those at West Rock, and we have lost the interference structure and some of the high frequency energy.

At time point 5, >2 hours after CPA, the levels and spectra at the two sites are back to being quite similar (Figure 2-9). For time point 6, which is the at the point where the R/V *Langseth* was turning to make the return pass over the area, we still see noise levels at the seismic frequencies being 30-40 dB above ambient and 10-20 dB above ambient at the higher frequencies (Figure 2-10). The ship was 30 km from the West Rock location at the time of these recordings. Time point 7 was taken on the return trip (seaward bound) at the approximately the same distance from West Rock and *Aeolus* as time point 5. We see the levels at the seismic frequencies increased relative to time point 6 and similar to what they were for time point 5 (Figure 2-11). As the R/V *Langseth* again approached the recording sites, we see the levels increase substantially to about 160 dB at the low frequencies and up to 140 dB for the higher frequencies (Figure 2-12). At time point 9, just after CPA and clipped recordings, we see again ca. 160 dB in the low frequencies at West Rock, while at the same frequencies at *Aeolus* the levels are somewhat less, ca. 140 dB (Figure 2-13). This result is consistent with a downward oriented beam pattern, thus sending more energy straight down toward the West Rock station and less toward *Aeolus*. The higher frequencies (>1 kHz) at *Aeolus* are comparable to those at West Rock for this time point, which is also consistent with expectations as the *Aeolus* site receives the lateral beam, which often contains higher frequencies. For the final time point, 10, we see the levels decrease again as the *Langseth* moves away and the recorders are receiving the end-fire of the array (

Figure 2-14). Lastly, we see ambient levels at the two sites return to previously recorded ambient levels (Figure 2-15). The slight elevation in low frequencies for the ambient recording is likely due to wind/wave generated noise, fish chorusing, or both.

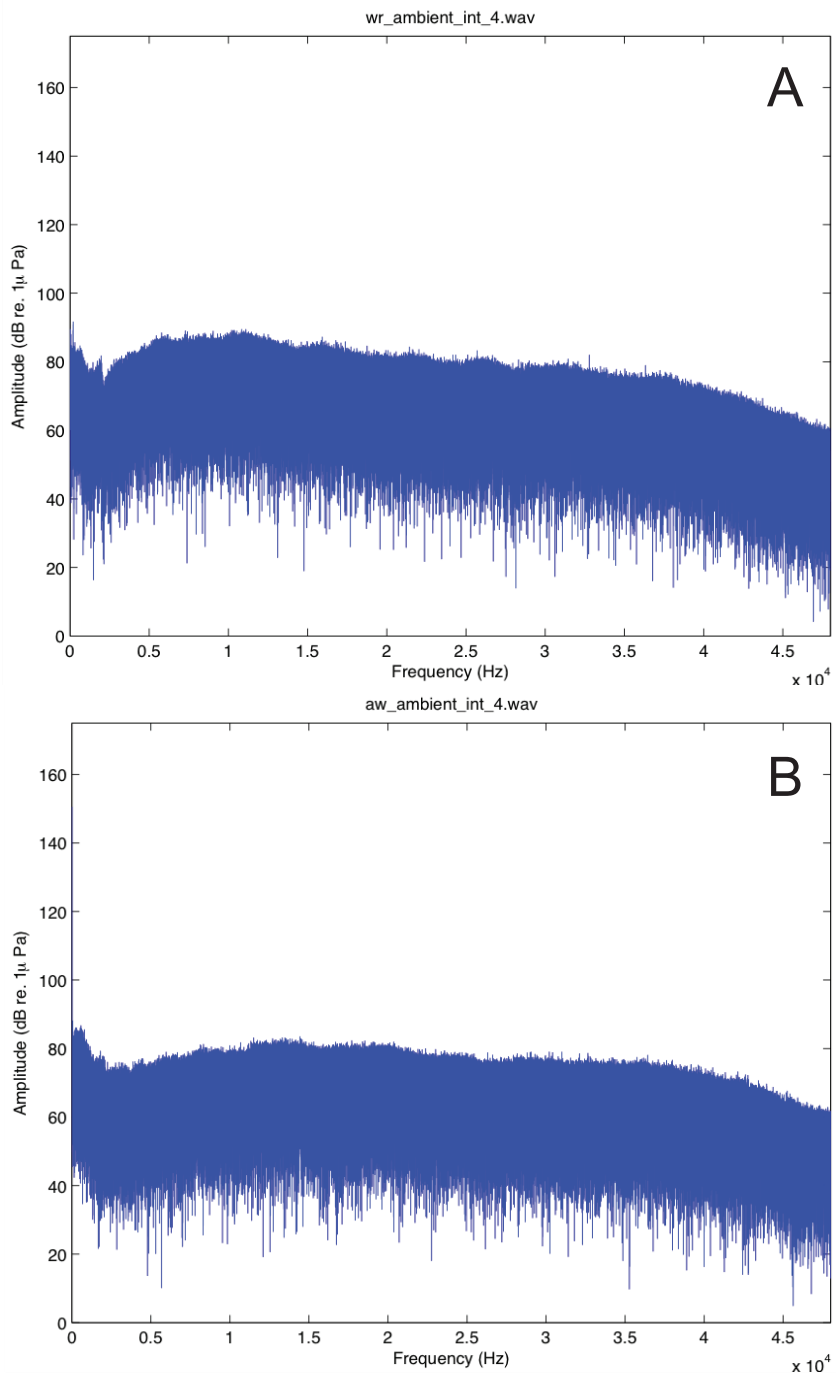

Figure 2-5: A) Ambient noise levels pre-survey at West Rock for time point 1 . B) Ambient noise levels pre-survey at *Aeolus* for time point 1. For this and successive figures, the x-axis shows frequency (Hz) and the y-axis shows amplitude (dB re: 1 uPa) for spectrum levels (dB re: 1 μPa/sqrt(Hz)). The spectrum levels were calculated using a 100 ms window, the nominal duration of the seismic pulses. Ambient noise levels shown here are fairly typical. Due to some Gibbs Phenomenon from the discontinuity at the low frequency edge of the data (i.e., <10 Hz), there are some anomalous peaks in that area.

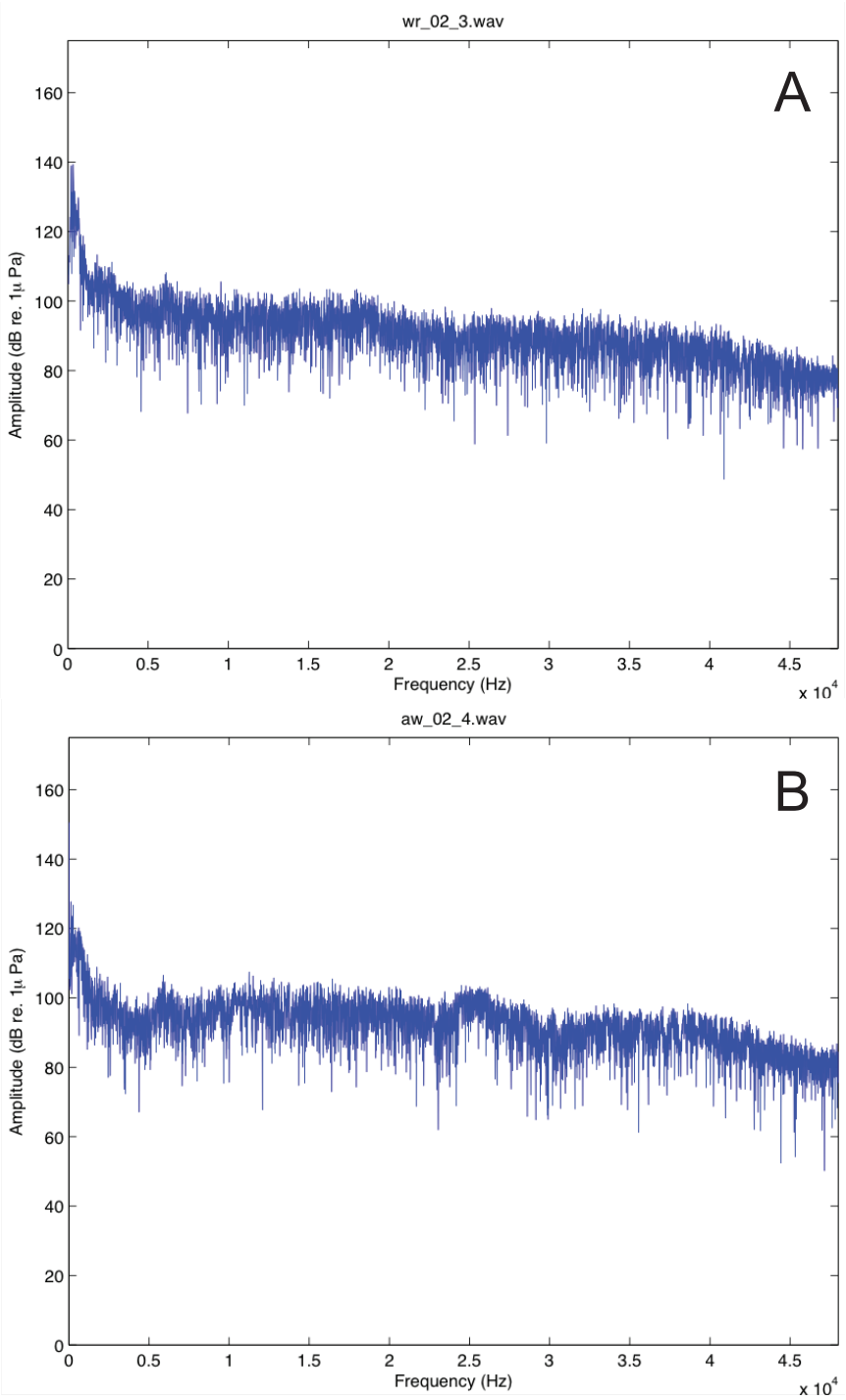

Figure 2-6: A) Levels (dB re: 1 μPa/sqrt(Hz)) for time point 2 at West Rock. This recording at time point 2 is more than 2 hours before the CPA, and given the energy increase relative to ambient, particularly at the low frequencies, the seismic energy is already reaching West Rock. Time point B) Levels recorded at *Aeolus* at time point 2. See Figure 2-5 for full description of figure parameters. We see an acoustic signature that is similar to the West Rock location for this time point.

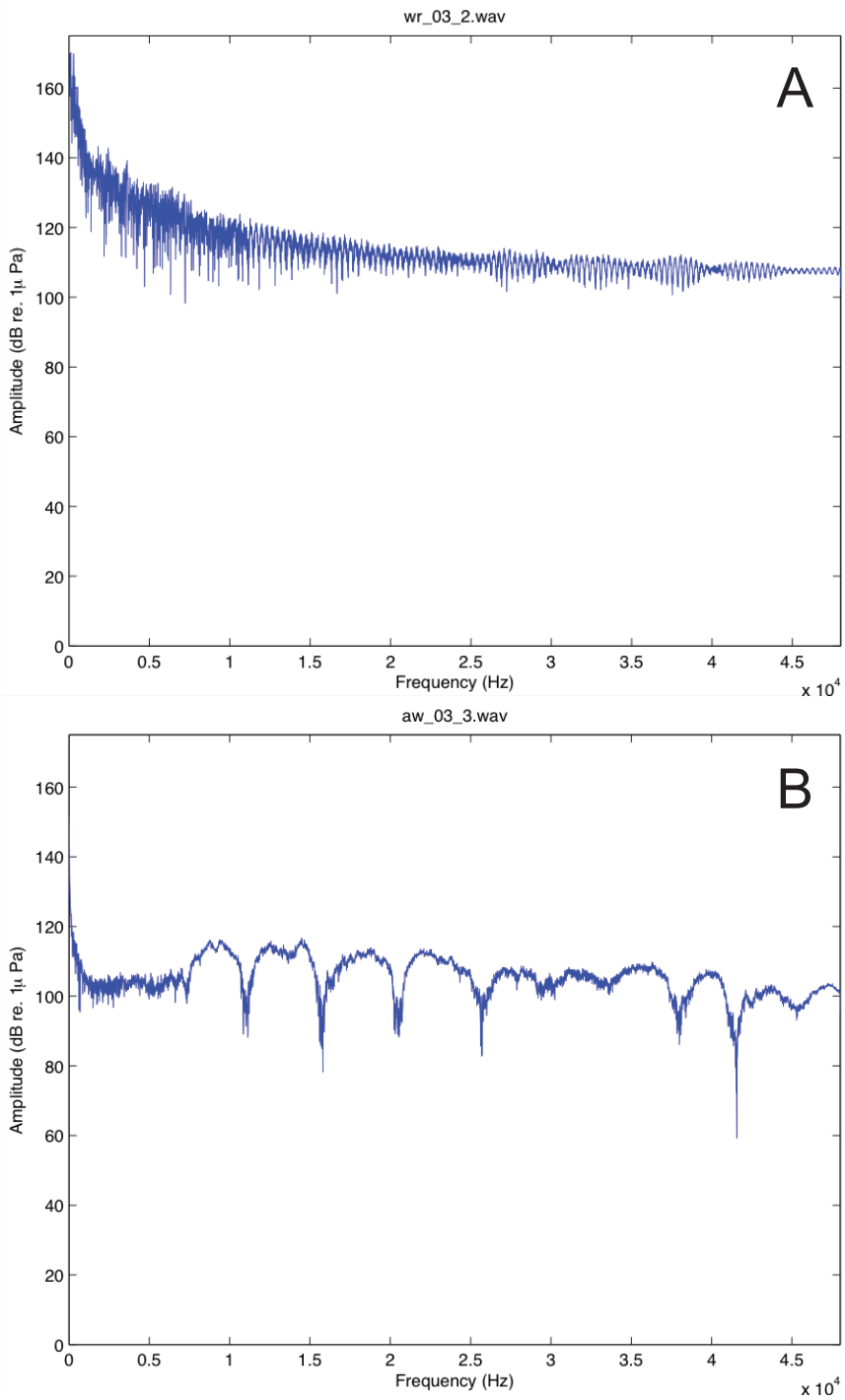

Figure 2-7: A) Seismic pulse signature at West Rock for time point 3. Note the significant increase in level compared to the time point 2, including energy at the higher frequencies. These levels were the highest we were able to measure because the pulses that occurred around the CPA overloaded the recorder. B) Seismic pulse levels at time point 3 at *Aeolus*. We now see significant differences between the pulse signatures at the two sites. The scalloped structure we see in the mid to high frequencies likely results from a combination of multi-path travel and features (e.g., ridges) occurring between the two sites.

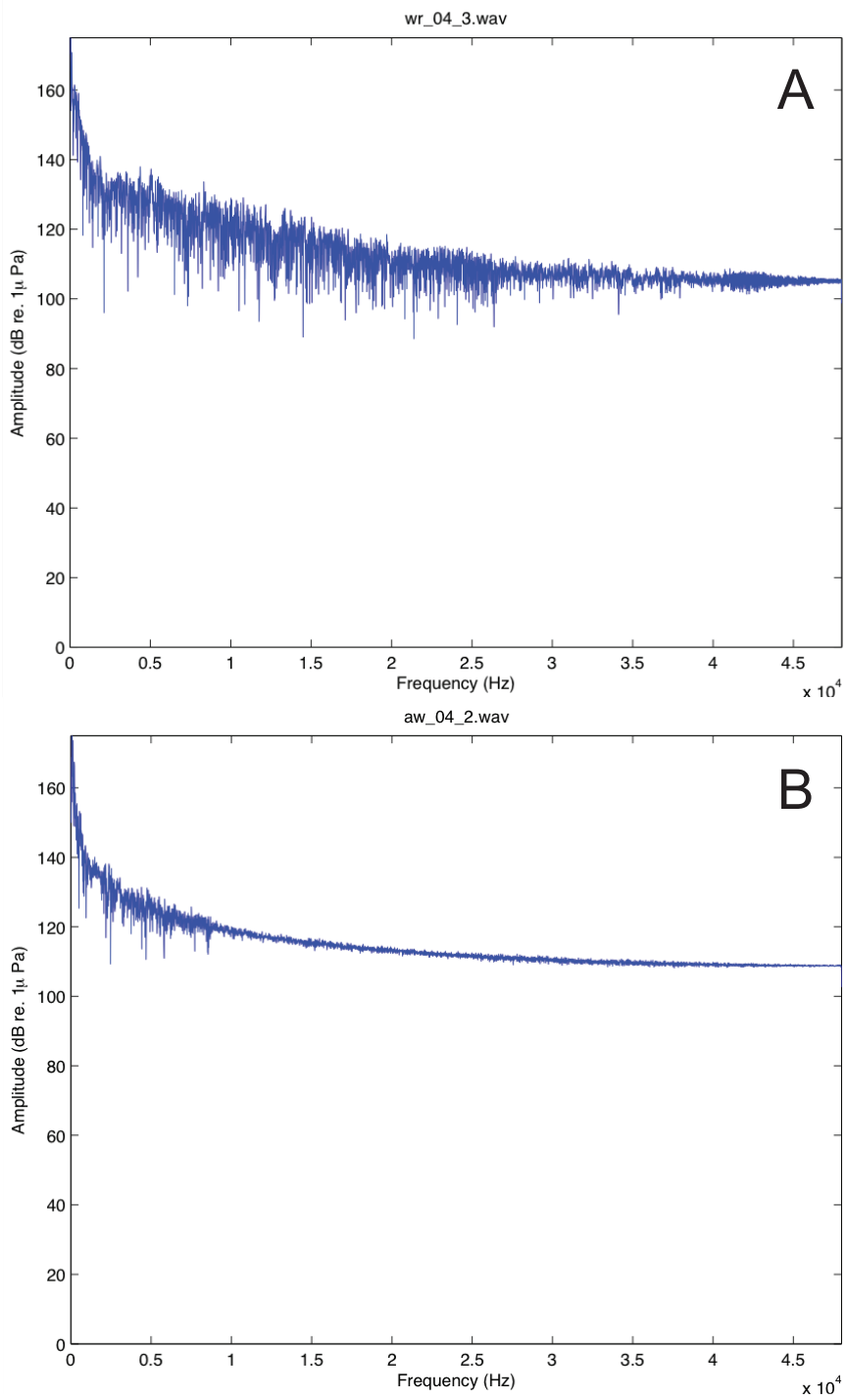

Figure 2-8: A) Levels and spectrum for seismic pulses at time point 4 at West Rock. We see significantly elevated levels at the low, seismic frequencies (20-200 Hz). B) Levels and spectrum at *Aeolus* for time point 4.

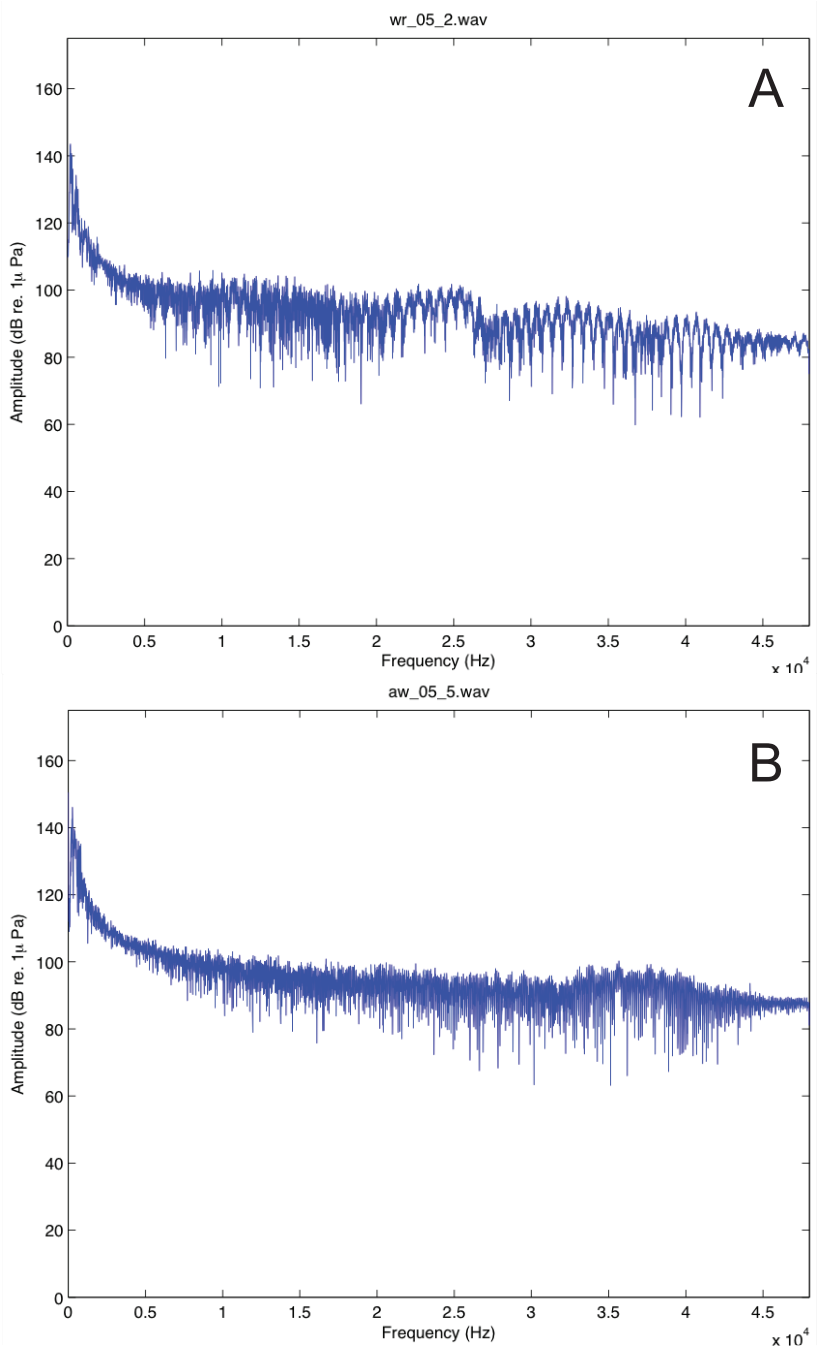

Figure 2-9: A) Levels and spectrum at West Rock for time point 5. We see, more than 2 hours after CPA, that the low frequency levels have diminished but are still well above ambient (see Figure 2-5). B) Levels and spectrum for *Aeolus* at time point 5. Levels are similar to those at West Rock as the source is now distant.

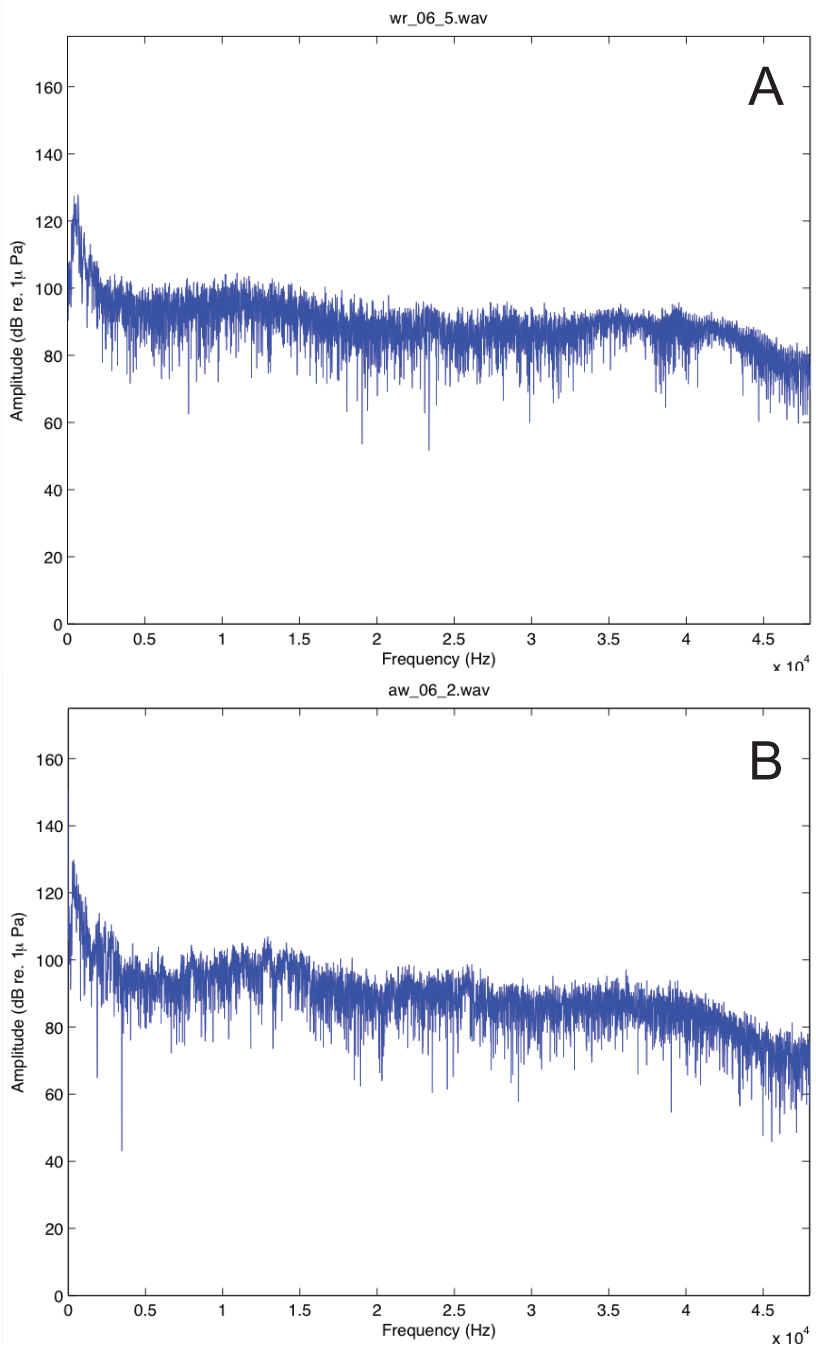

Figure 2-10: A) Levels and spectrum at West Rock for time point 6. B) Levels and spectrum at *Aeolus* for time point 6.

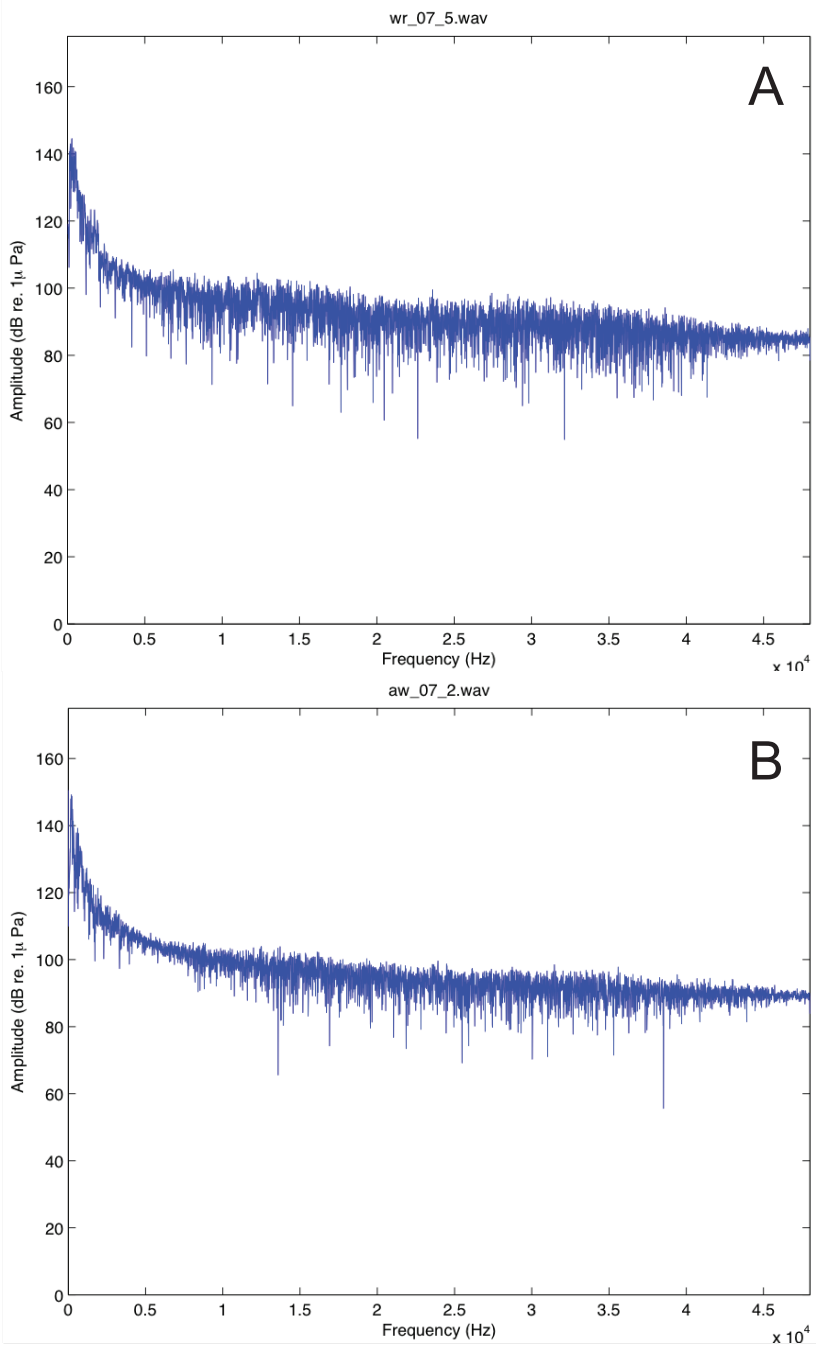

Figure 2-11: A) Levels and spectrum at West Rock for time point 7. We see a signature that is similar to time point 5. B) Levels and spectrum at *Aeolus* for time point 7.

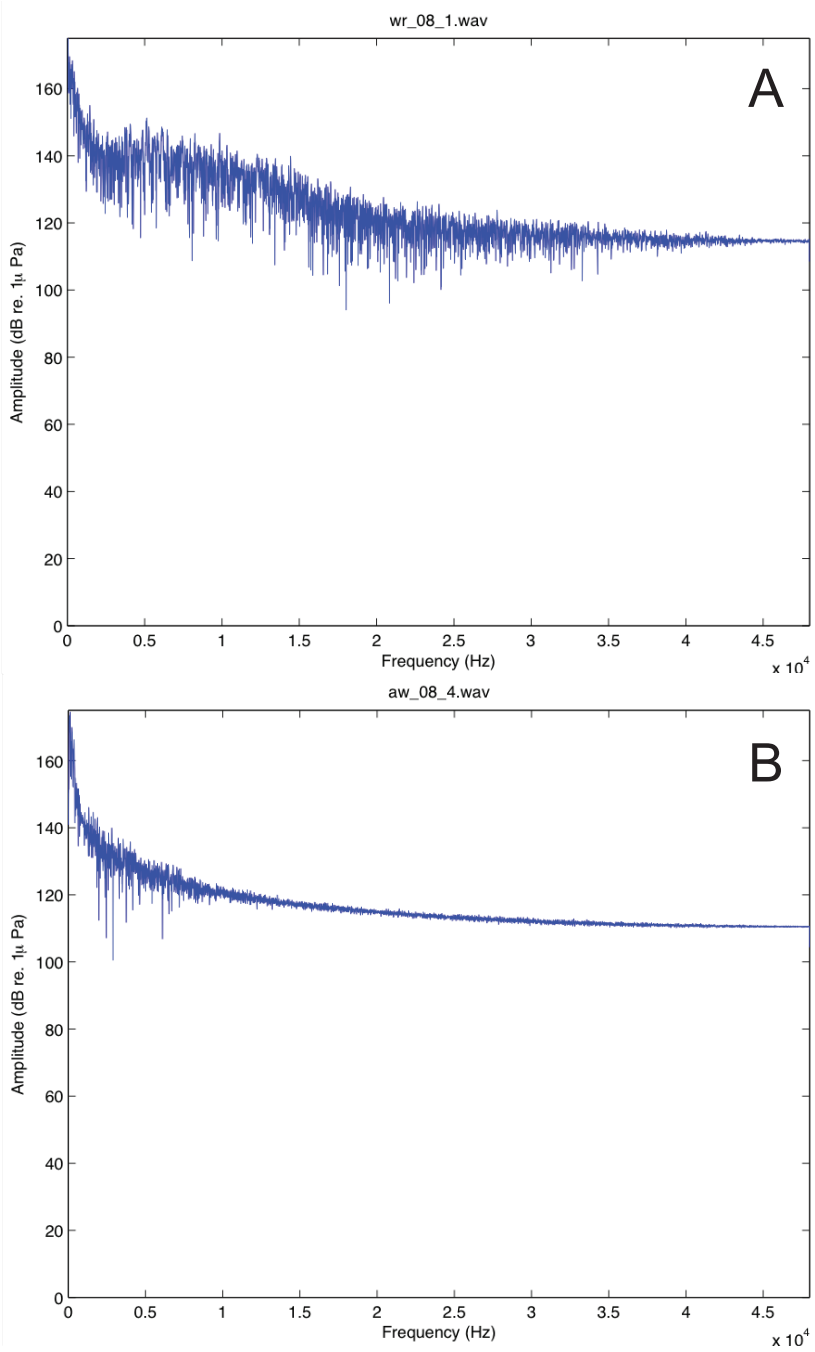

Figure 2-12: A) Levels for West Rock at time point 8, the pulses sampled just before the recorders started overloading during the seaward transect. We see the levels at the seismic frequencies elevated as they were for time point 4, the corresponding sample for the shoreward line. B) Levels at *Aeolus* for time point 8.

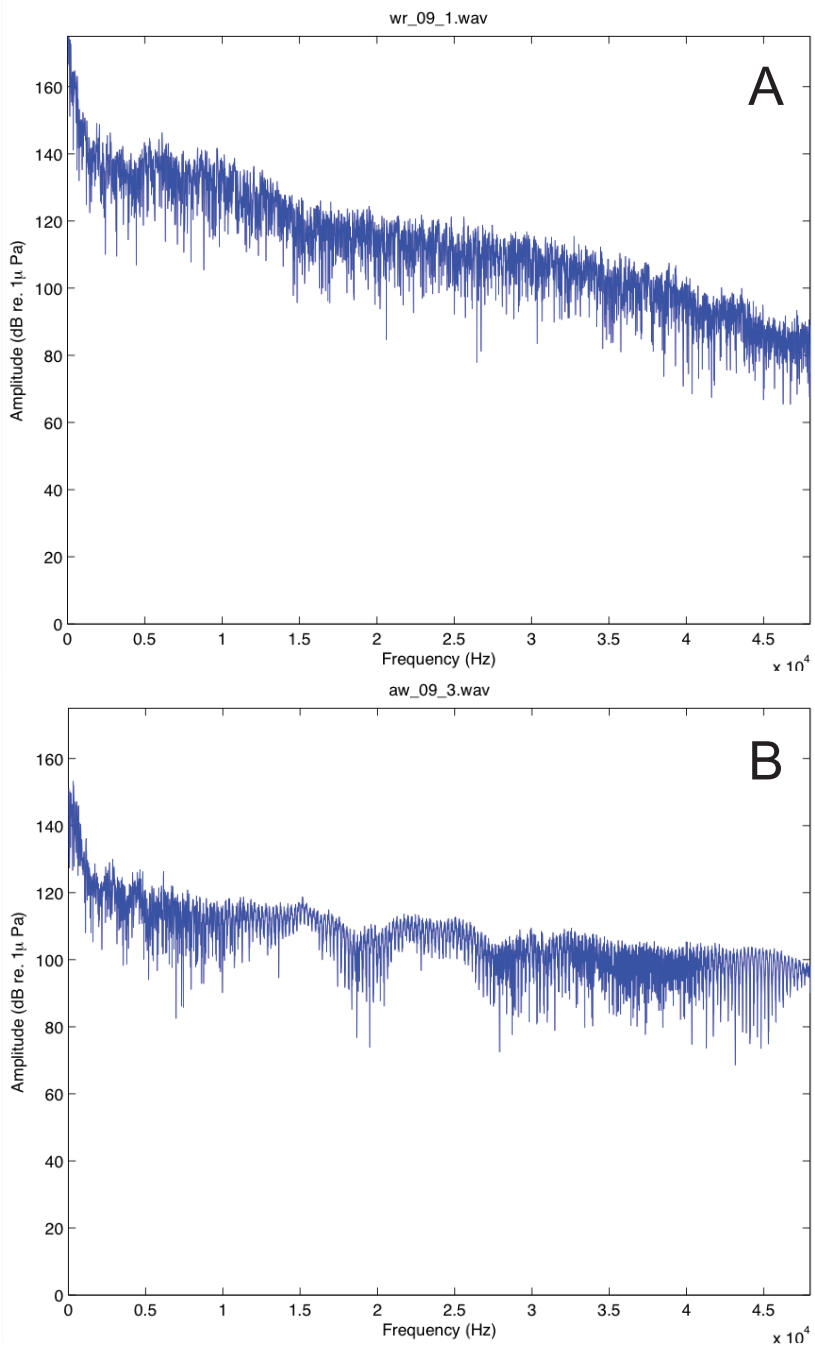

Figure 2-13: A) Levels recorded at West Rock for the time point just after CPA and the clipped recordings. B) Levels recorded at *Aeolus* for time point 9. The levels at the low frequencies are lower than at West Rock for this time point, which is consistent with a downward oriented beam of the seismic array.

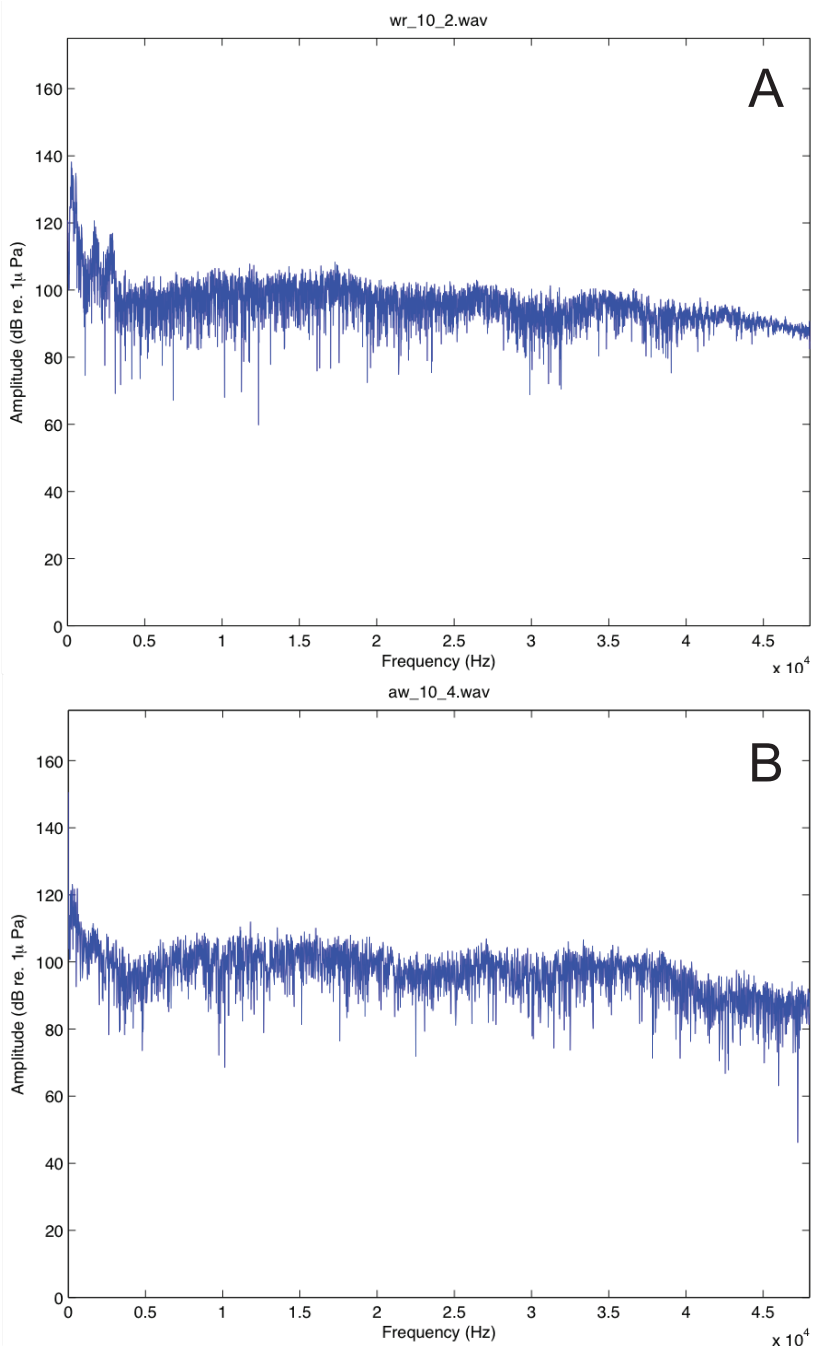

Figure 2-14: A) Levels at West Rock for time point 10. The 10-20 dB elevation above ambient is still present though the vessel passed the CPA more than two hours previously. B) Levels at *Aeolus* for time point 10. Levels are similar to those at West Rock for this time point, which is consistent with the vessel being almost equidistant from the two stations.

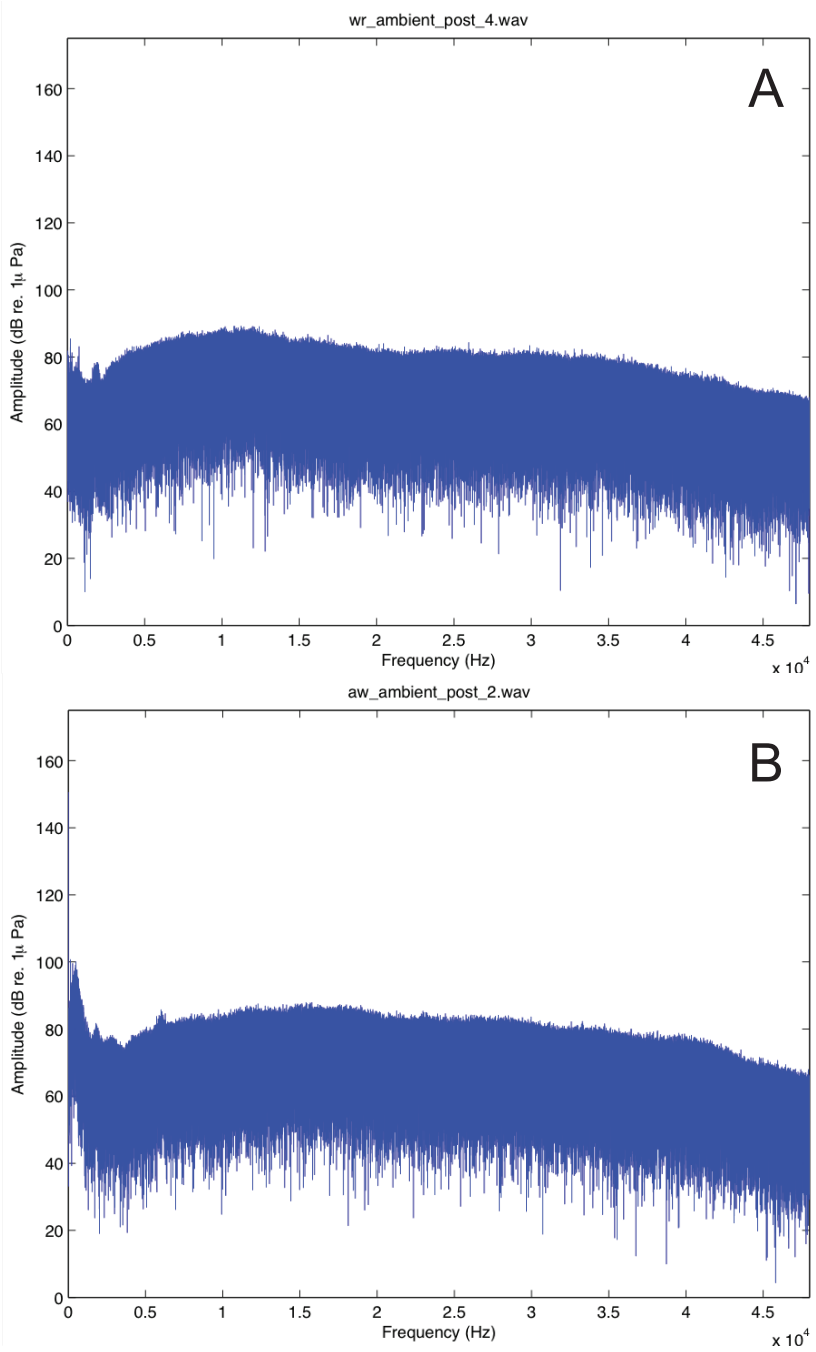

Figure 2-15: A) Post-survey ambient levels at West Rock. Note the ~80 dB mean across most of the spectrum, which is ca. 20 dB lower than during the survey. B) Post-survey ambient levels at *Aeolus*. The elevated levels at low frequencies are likely due to sound energy from wind/waves and/or fish chorusing.

We also analyzed the SoundTrap data from West Rock and *Aeolus* for biological sounds, primarily focused on fishes and any marine mammals that could be heard in the recordings. We scored the hourly presence of dolphins, cusk eels and all other "grunting" fishes for periods before, during and after the survey. Discriminating between fish species is difficult and time consuming and beyond the scope of this project. We scored 20 hours pre-seismic, 5 hours during the seismic survey, and 5 hours from 2 days after the survey concluded; the 5-hour samples were matched in time so as to sample the same time of day to control for diel differences. Generally, cusk eels were present in every hour of recordings analyzed, while the dolphins and other fish were present in high numbers pre-seismic, but less so during and after; the small sample sizes precluded any statistical testing of these data.

2.3.2. Video Recordings of Fish Responses to Seismic Surveys

We recorded 303 video segments across three natural reefs located in northwestern Onslow Bay from September 17 through September 20, 2015 (

Table 2-4). The video camera located on the artificial reef malfunctioned; therefore, we were not able to obtain an adequate sample size. However, at the three natural hardbottom reefs (210 Rock, 10 Fathom Rock, and West Rock), we counted a total of 13,632 fishes across all videos (Figure 2-16). These fishes encompassed 49 species and 23 families (Table 2-5; Table 7-1) and represented six general trophic groups: carnivores, herbivores, invertivores, omnivore, piscivores, and planktivores. Notably, 17 species that are part of the federally managed snapper-grouper complex were recorded during our monitoring efforts (Table 2-5; 7 – Appendix 1, Table 7-1).

The three most abundant fishes were *Haemulon aurolineatum* (tomtate)*, Diplodus holbrookii* (spottail pinfish)*,* and *Decapterus spp.* (scad species) (Table 2-5). *Haemulon aurolineatum* were present in 60.5% of the videos, whereas *Diplodus holbrookii* were present in 29.0% of the videos (Table 2-5). Of the fishes in the snapper-grouper complex, excluding *Haemulon aurolineatum*, *Mycteroperca microlepis* (gag grouper) were the most abundant and commonly occurring fish (5.6%), followed by *Centropristis striata* (black sea bass; 4.2%), *Centropristis ocyurus* (bank sea bass; 3.8%), and *Mycteroperca phenax* (scamp grouper; 1.0%) (Table 2-5).

Table 2-4: Number of videos recorded per reef on each of four survey dates.

	Reef		
Survey Date	**210 Rock**	**10 Fathom Rock**	**West Rock**
September 17, 2014	34	14	13
September 18, 2014	39	31	42
September 19, 2014	46	33	0
September 20, 2014	37	14	0
Total videos per site	**156**	**92**	**55**
Total videos	**303**		

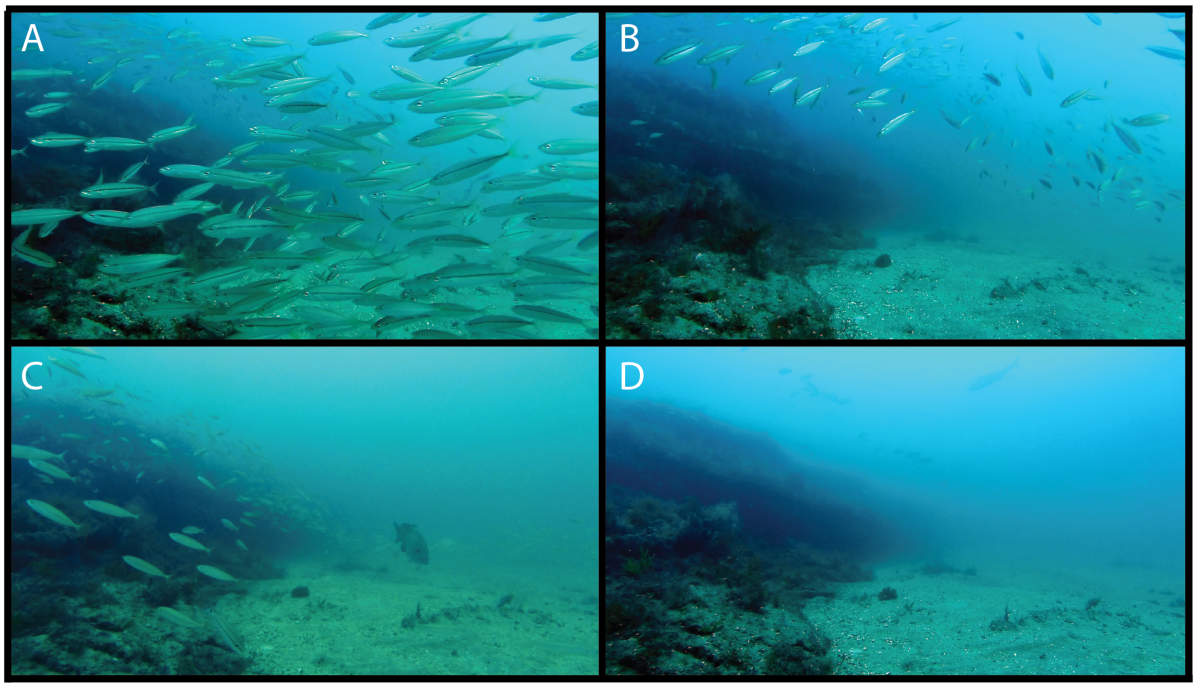

Figure 2-16: Fish documented at 210 Rock in videos represented 23 families. Shown here are A) *Decapterus spp.* (scad); B) *Decapterus spp.* (scad) and *Haemulon aurolineatum* (tomtate); C) Serranidae (grouper) and *Decapterus spp.* (scad); D) *Seriola dumerili* (greater amberjack) and *Rhomboplites aurorubens* (vermillion snapper).

Table 2-5: Fish abundance by species within alphabetically ordered families, as well as percent occurrence across all three natural temperate reefs. Abundance is provided as a total (sum) and mean of individuals.

Family	Genus	Species	Common _Name	Snapper_ Grouper	total_ abund	mean abund	percent_ occurrence
Acanthuridae	*Acanthurus*	*chirurgus*	Doctorfish		1	0.003	0.350
Balistidae	*Balistes*	*capriscus*	Gray Triggerfish	YES	1	0.003	0.350
Carangidae	*Decapterus*	spp.	Round or mackeral scad		7492	26.196	17.832
	Caranx	*ruber*	Bar Jack	YES	12	0.042	1.049
	Seriola	*dumerili*	Greater Amberjack	YES	5	0.017	1.049
	Carangidae	spp.	Unknown Jack	YES	1	0.003	0.350
	Carangoides	*bartholomaei*	Yellow Jack		1	0.003	0.350
Carcharhinidae	Carcharhinus	spp.	Unknown Shark		2	0.007	0.699
Chaetodontidae	*Chaetodon*	*ocellatus*	Spotfin Butterflyfish		1	0.003	0.350
Ephippidae	*Chaetodipterus*	*faber*	Atlantic Spadefish	YES	4	0.014	0.350
Haemulidae	*Haemulon*	*aurolineatum*	Tomtate	YES	3270	11.434	60.490
	Haemulon	*plumieri*	White Grunt	YES	2	0.007	0.699
Labridae	*Halichoeres*	*bivittatus*	Slippery Dick		55	0.192	11.189
	Halichoeres	spp	Unknown Wrasse		40	0.140	10.140
	Thalassoma	*bifasciatum*	Bluehead Wrasse		2	0.007	0.350
	Halichoeres	*garnoti*	Yellowhead Wrasse		1	0.003	0.350
Lutjanidae	*Rhomboplites*	*aurorubens*	Vermillion Snapper	YES	13	0.045	0.699
	Lutjanus	*griseus*	Gray snapper	YES	1	0.003	0.350
Monacanthidae	*Stephanolepis*	*hispidus*	Planehead Filefish		7	0.024	2.098
Osteichthyes sp.	*Osteichthyes*	sp.	Unknown Fish Species		54	0.189	5.594
Pomacanthidae	*Holacanthus*	*bermudensis*	Blue Angelfish		11	0.038	3.846
Pomacentridae	*Chromis*	*scotti*	Purple Reef Fish		51	0.178	17.483
	Stegastes	*variabilis*	Cocoa Damselfish		7	0.024	2.098
	Stegastes	*leucostictus*	Beaugregory Damselfish		1	0.003	0.350
Scaridae	*Sparisoma*	*atomarium*	Green Blotch Parrotfish		1	0.003	0.350
	Sparisoma	*aurofrenatum*	Redband Parrotfish		1	0.003	0.350
Sciaenidae	*Pareques*	*umbrosus*	Cubbyu		2	0.007	0.699
	Sciaenidae	spp.	Unknown Drum		2	0.007	0.699
Scombridae	Scombrid	spp.	Mackerel / Tunny		5	0.017	1.748
Scorpaenidae	*Pterois*	*volitans*	Lionfish		34	0.119	9.790
Serranidae	*Mycteroperca*	*microlepis*	Gag	YES	21	0.073	5.594
	Centropristis	*striata*	Black Sea Bass	YES	14	0.049	4.196

Family	Genus	Species	Common _Name	Snapper_ Grouper	total_ abund	mean abund	percent_ occurrence
	Centropristis	*ocyurus*	Bank Sea Bass	YES	14	0.049	3.846
	Rypticus	*maculatus*	White Spotted Soapfish		8	0.028	2.448
	Mycteroperca	*phenax*	Scamp	YES	4	0.014	1.049
	Diplectrum	*formosum*	Sand Perch		2	0.007	0.699
	Serranus	*subligarius*	Belted Sandfish		1	0.003	0.350
Sparidae	*Diplodus*	*holbrookii*	Spottail Pinfish		1049	3.668	29.021
	Calamus	spp.	Unknown Porgy	YES	7	0.024	2.448
	Archosargus	*probatocephalus*	Sheepshead		5	0.017	1.748
	Pagrus	*pagrus*	Red Porgy	YES	2	0.007	0.699
	Stenotomus	*caprinus*	Longspine Porgy	YES	1	0.003	0.350
	Stenotomus	*chrysops*	Scup	YES	1	0.003	0.350
Sphyraenidae	*Sphyraena*	*barracuda*	Barracuda		3	0.010	1.049
Synodontidae	*Synodus*	spp.	Lizardfish		3	0.010	1.049
Tetraodontidae	*Sphoeroides*	*spengleri*	Bandtail Puffer		1	0.003	0.350
Triglidae	*Prionotus*	spp.	Unknown Searobin		1	0.003	0.350

2.3.3. Diel Patterns of Fish Abundance

The abundance of fishes on the natural reefs varied considerably over the course of the day from sunrise to sunset (Figure 2-17). Generally, fish abundance was highest in the mid-morning and during the evening hours between 1600 and 2000, perhaps corresponding to crepuscular behavior of reef-associated fishes (Figure 2-17A). On the deepest natural reef, 210 Rock, we recorded the greatest number of videos over four consecutive days compared to the other two sites, and, curiously, the fish abundance did not peak twice a day (Figure 2-17B). Rather, at 210 Rock, the mean fish abundance was less than 50 fish per video until 1600 (Figure 2-17B). At 1600, the mean fish abundance increased, reaching a maximum average of nearly 150 fishes per video segment (Figure 2-17B). On 10 Fathom Rock, the fish abundance peaked several times during the day (Figure 2-17C), and at West Rock, fish abundance reached maxima in the evening and at mid-day (Figure 2-17D). These patterns suggest that fish abundance on these temperate hardbottom reefs is highest during the evening hours as the light availability decreases on the reefs, yet exhibits variability across different hardbottom reefs.

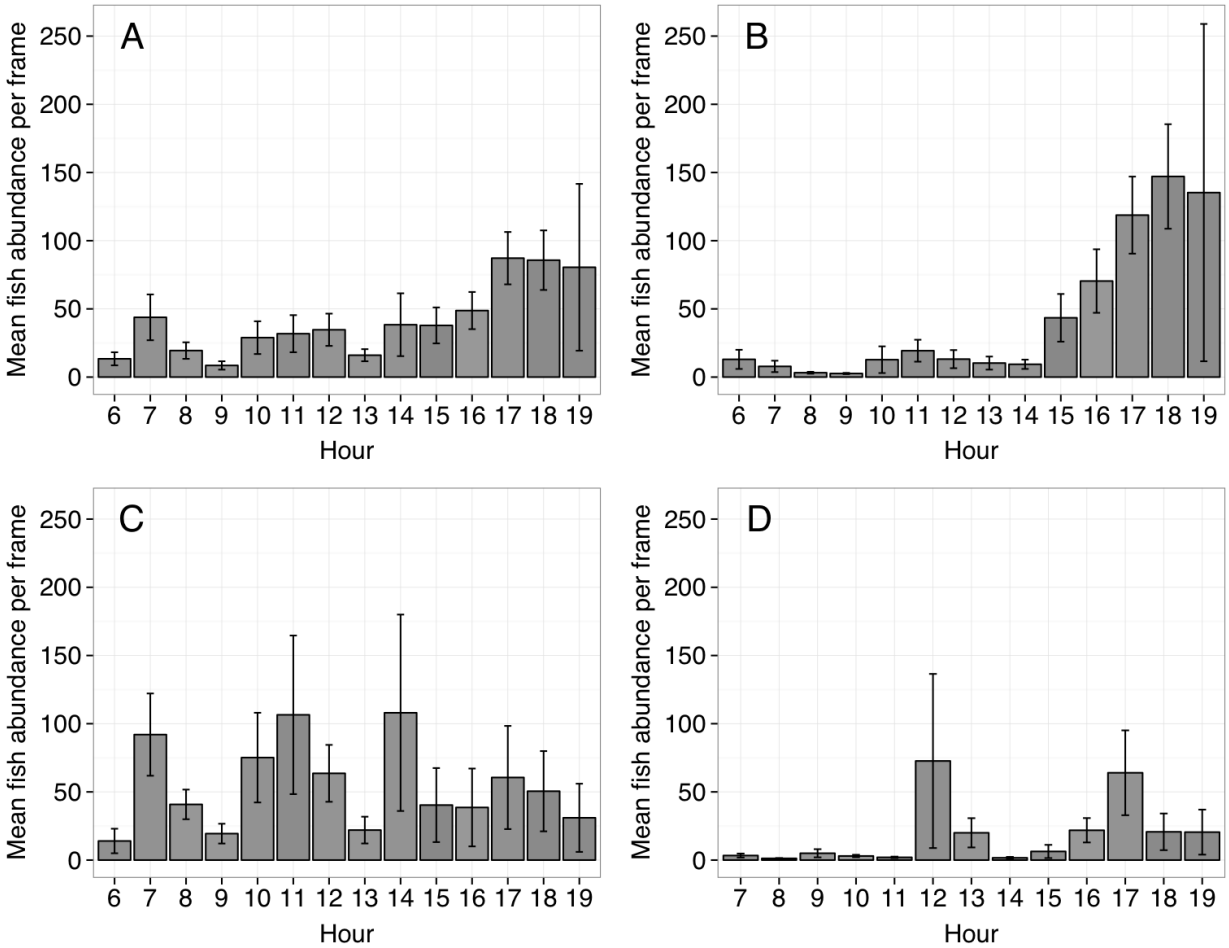

Figure 2-17: Fish abundance per video by hour of the day on A) three natural reefs, B) 210 Rock, C) 10 Fathom Rock, and D) West Rock. Hours are in 24 hour time, with 6 representing 0600 and 19 representing 1900. The bar colors correspond to different hours of the day. The error bars represent standard error.

Reef-associated fishes in the federally managed snapper-grouper complex displayed a similar diel pattern in abundance (Figure 2-18). Across all three reefs, snapper-grouper abundance was highest during the morning, noon, and evening hours (Figure 2-18A), while at individual reefs, there was higher variability (Figure 2-18 B-D). Individual species of fish exhibited different trends in abundance throughout the day. The planktivorous scad *(Decapterus spp.)* gradually increased in abundance until it peaked in the evening hours (Figure 2-19A). In contrast, *Haemulon aurolineatum* (tomtate), which is federally managed as a species in the snapper-grouper complex, reached maxima at three separate times throughout the day: morning, noon, and evening (Figure 2-19B). Mean abundance of *Diplodus holbrookii*, a reef generalist and omnivore, remained stable throughout the day (Figure 2-19C). *Mycteroperca microlepis* (gag grouper), which is managed as part of the snapper-grouper complex and is often targeted by commercial and recreational fishermen, peaked in the morning and evening hours (Figure 2-19D).

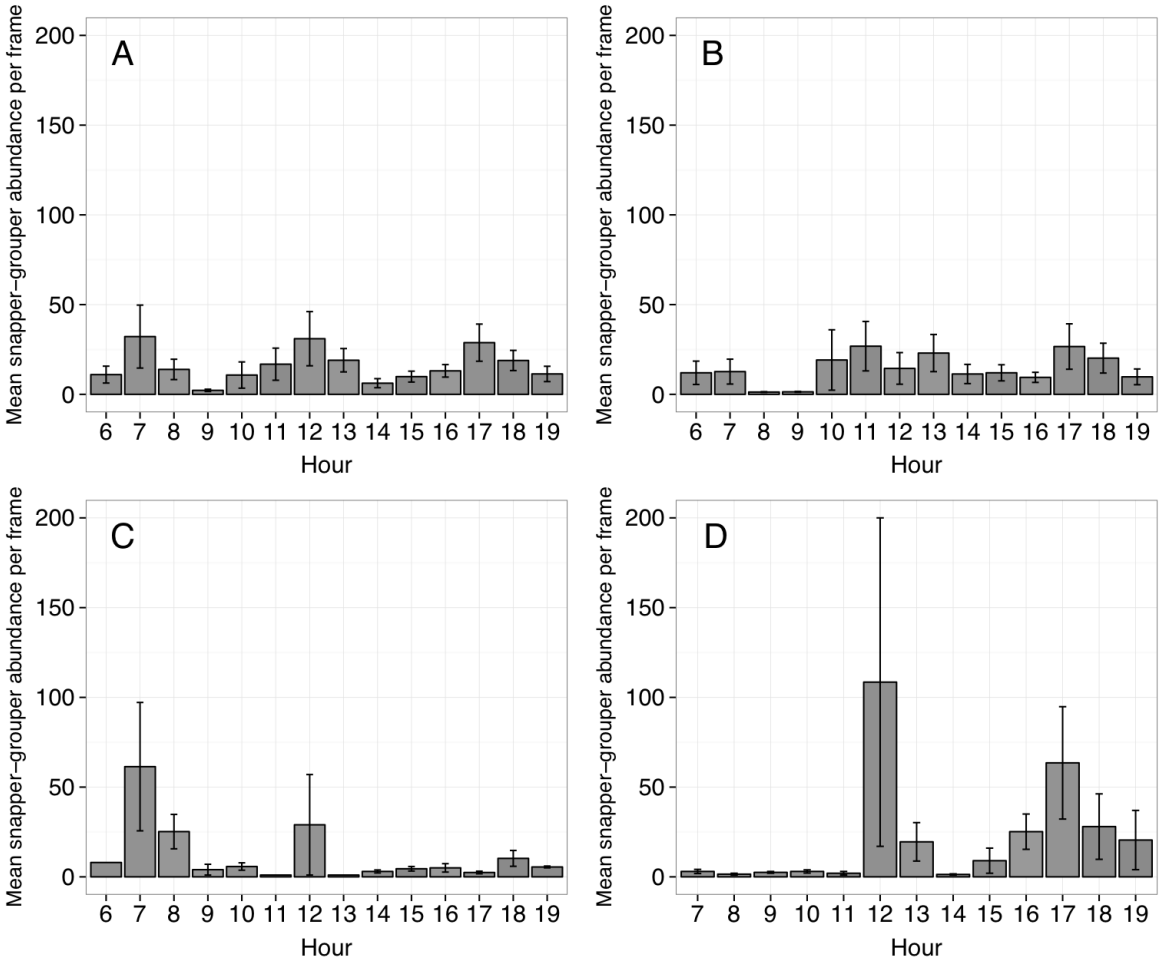

Figure 2-18: Snapper-grouper abundance per video by hour of the day on A) three natural reefs, B) 210 Rock, C) 10 Fathom Rock, and D) West Rock. Hours are in 24 hour time, with 6 representing 0600 and 19 representing 1900. The bar colors correspond to different hours of the day. The error bars represent standard error.

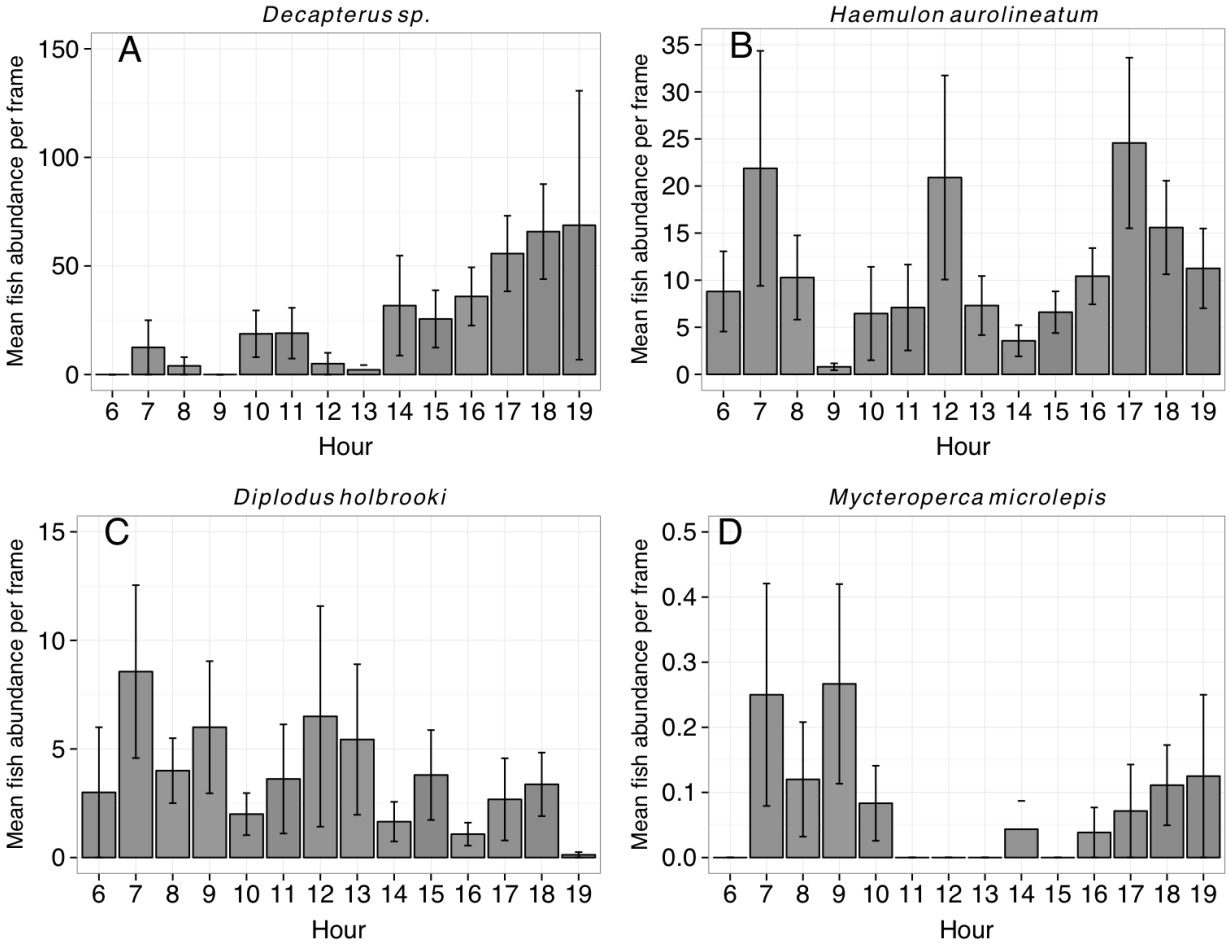

Figure 2-19: Mean abundance per video by hour of the day for four of the most abundant species on the three natural reefs. A) *Decapterus sp.,* B) *Haemulon aurolineatum,* C) *Diplodus holbrookii,* and D) *Mycteroperca microlepis.* Hours are in 24 hour time, with 6 representing 0600 and 19 representing 1900. The y-axis scales are different for each species of fish. The bar colors correspond to different hours of the day. The error bars represent standard error.

2.3.4. Response of Fish Abundance to Seismic Surveys

Thirteen seismic airgun shots coincided with video recordings from two of the natural reefs (eight on 210 Rock and five on 10 Fathom Rock). Due to camera malfunction, the West Rock camera did not record videos during passage of the seismic survey. At the shallower natural reef, 10 Fathom Rock, airgun shots were detected on three separate days. Prior to the airgun shots, we did not record videos of fish for a full day because sampling began at 1400, so there was insufficient replication to resolve the fish response to seismic surveys at this reef. Here, we present results from 210 Rock where we recorded videos from three days before and one day during active seismic surveying.

On 210 Rock, the reef monitored by video camera before and during the seismic survey, fish occupation during three days prior to the seismic survey exhibited a daily pattern of increasing

abundance during the evening, as compared to morning and afternoon (Figure 2-20). On the following day with airgun noise, this pattern in fish use did not emerge from observations across periods of the day. Fish abundance remained low for the entire day, with the exception of one outlying observation during evening (Figure 2-21) The outliers were predominately comprised of *Haemulon aurolineatum* (tomtate), a grunt that consumes benthic invertebrates and zooplankton, and *Decapterus spp.* (scad), a forage fish that eats zooplankton. Reductions in fish abundances during seismic surveying proved statistically significant using two different statistical tests. First, the mean variance in fish counts on each of the three days without seismic noise was greater than the corresponding mean variance on the day with seismic surveying (via analysis of means for variance (ANOMV) with Levene transformation, $p = 0.047$; Figure 2-22). The statistically significant differences in fish abundance between the single day with and the three days without seismic noise were driven by data from a four-hour evening period (1600-2000 local time). Whether fish occupation of the reef differed during the evening across all days was further tested. The total number of fish occupying the reef during evening declined by 78% when exposed to seismic noise (ANOVA followed by post-hoc pairwise t-test with Box-Cox transformation, $F_{3,36} = 4.74$, $p = 0.007$).

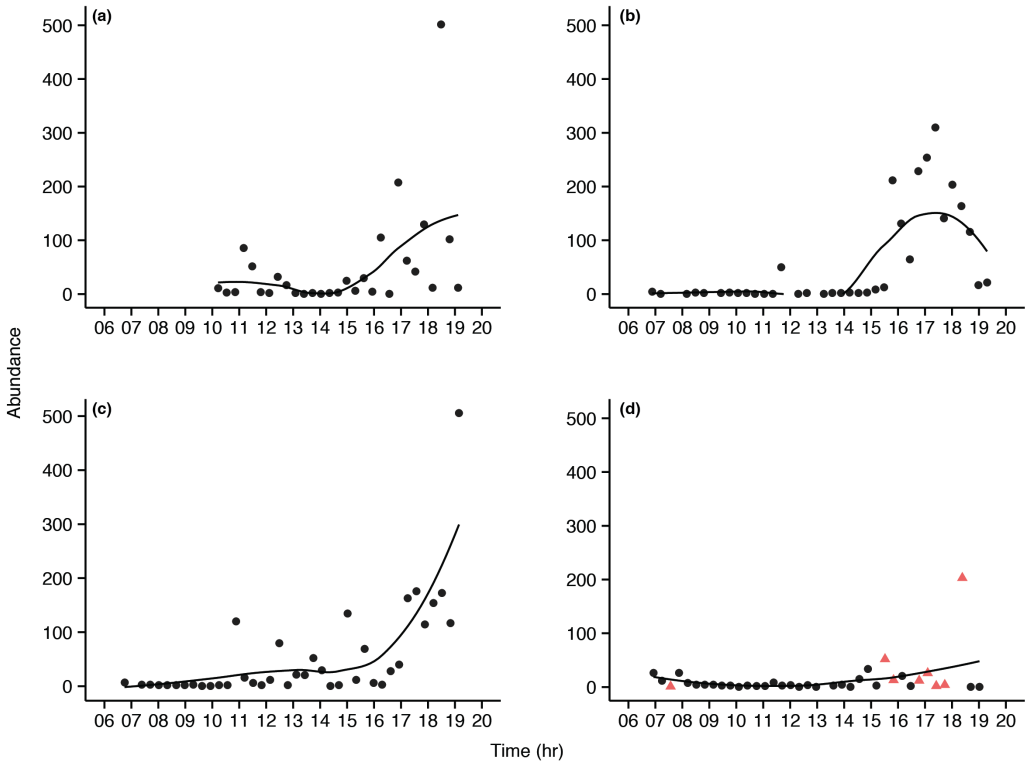

Figure 2-20: Hourly time series of fish abundance on natural rocky reef (210 Rock) on four separate days: A) September 17, 2014; B) September 18, 2014; C) September 19, 2014; D) September 20, 2014. Each point represents fish abundance in a single video clip. Although seismic surveying was active on September 20, seismic activity was not audible on all collected videos. The color and shape of each point corresponds to whether seismic activity was audible on the video (red triangles) or not audible (black circles). Black lines are smoothed conditional means. Figure from Paxton et al. (2017) – *Marine Policy.*

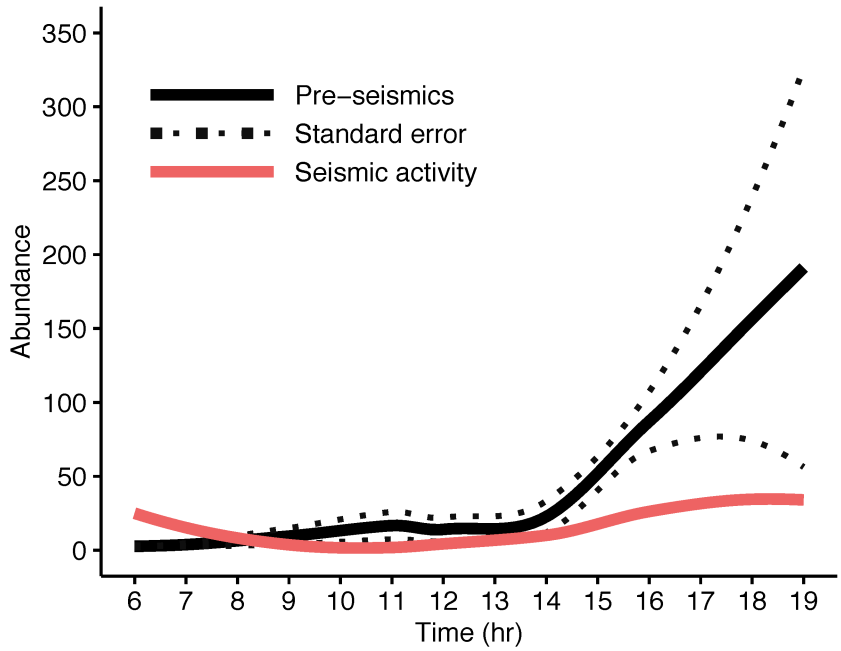

Figure 2-21: Hourly fish abundance on the reef 7.9 km from the closest approach of the seismic survey ship during three days before (solid black line) and on one day during the height of seismic activity near the reef (red line). The solid black line is the smoothed conditional mean and the black dotted lines are standard error of the hourly fish abundance for three days before seismic surveying. The red line is the smoothed conditional mean of hourly fish abundance on the day with seismic activity. Figure from Paxton et al. (2017) – *Marine Policy.*

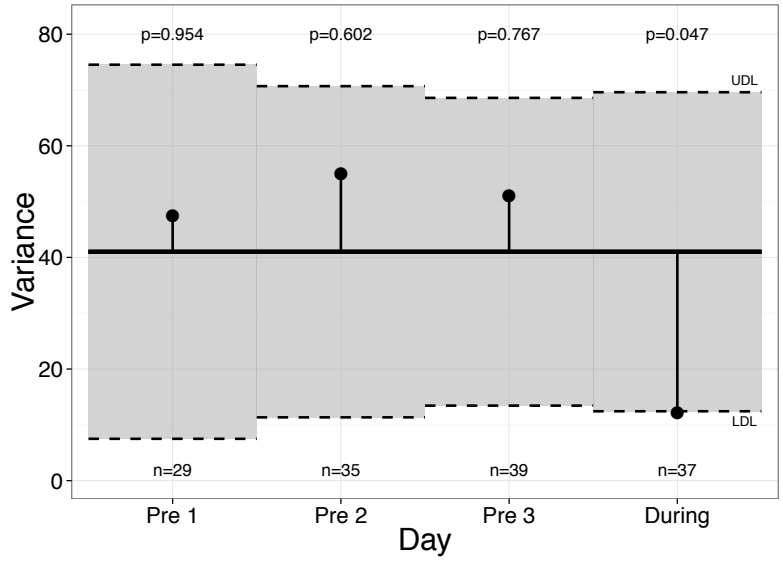

Figure 2-22: Test of equality of variance in fish counts on three days pre-seismic surveying and one day during seismic surveying, based on analysis of means for variance (ANOMV) with Levene transformation. Daily means for variance (black points) are contrasted with grand mean for variance (solid black horizontal line). P-values indicate whether daily means of variance are significantly different from grand mean, as do horizontal dashed lines that represent 95% confidence limits. On the day with seismic surveying, variance in fish counts was significantly lower than on each of three days before, driven by reduced abundance. N is number of videos. Figure from Paxton et al. (2017) – *Marine Policy.*

2.3.5. Response of Fish Behavior to Seismic Surveys

In addition to counting fish, video recordings were examined to assess whether fish exhibited behaviors that could help understand the change in reef use. Noises from seismic surveying were audible as discrete airgun shots in video recordings, allowing association of any observed behavioral responses with timing of individual shots. Eight shots were audible on video from 210 Rock. The other shots occurred at 30 to 90-s intervals and did not coincide with the recording schedule. Only one observed fish, a *H. aurolineatum*, exhibited an apparent behavioral response to an airgun shot by swimming away from a ledge. From the lack of abundant fish observed during evening when repeatedly exposed to seismic noise, it is presumed that at least some reef-associated fishes left the reef.

2.4. Conclusions

The amplitudes and spectra recorded for the seismic air gun pulses at our two sites are entirely consistent with expectations, based on the literature (Guerra et al. 2011b), specifically the increase in ambient noise levels present virtually throughout the survey even with the vessel ca. 25 nm away from our recording stations. This result is notable due to the shallow water environment in which the survey occurred. Noise levels at relatively high frequencies (1-4 kHz) were elevated by 20-40 dB re: 1 μPa / sqrt (Hz) at the time of the pulses. In the seismic frequencies (20-1000 Hz), we recorded levels >160 dB re: 1 μPa / sqrt (Hz) at the time closest to CPA that we were able to analyze. The pulses at CPA were too loud for our equipment to sample properly. Finally, the activity of cusk eels seems to have remained consistent while that of dolphins and some species of fishes appears to have diminished during and after the survey, though these results are inconclusive due to small sample sizes.

We monitored the response of reef-associated fish to high-intensity, low-frequency sound created by repeated airgun deployments from a seismic survey on the continental shelf of NC. Although working with limited data, we provide evidence that during exposure to seismic noise, the prevailing pattern of heavy fish use of reefs during the evening was suppressed. Our finding is notable because it goes well beyond detection of a startle response from individual fish (Wardle et al. 2001), instead suggesting a multi-species response to airgun noise. The Magnuson-Stevens Fishery Conservation and Management Act (2007) mandates protection of reefs as Essential Fish Habitat. Reducing opportunities for fish to aggregate causes concern as this could reduce options for foraging, mating, or other important life history functions. Though we do not have observations to indicate the duration of the effect we observed, our research results augment and confirm issues raised by marine mammal experts (Nowacek et al. 2015) and suggest that concerns associated with marine seismic surveys appear to be realistic and well-founded.

3. Establishing a Baseline Soundscape (Component 2)

3.1. Methods

3.1.1. Site Selection

To establish a baseline soundscape for hardbottom habitats in offshore NC waters that are habitat for a diverse group of tropical, temperate, coastal-pelagic, and migratory species, we monitored five temperate reefs. Monitoring stations included two natural reefs (210 Rock and West Rock) and three artificial reefs (USCGC *Spar,* US Navy Cable Layer *Aeolus,* and *Ashkhabad*) (Figure 3-1; Table 3-1). The *Spar* and *Aeolus* were purposely sunk as artificial reefs. The *Ashkhabad* is a Russian freighter sunk during 1942 when it was torpedoed by a German U-boat.

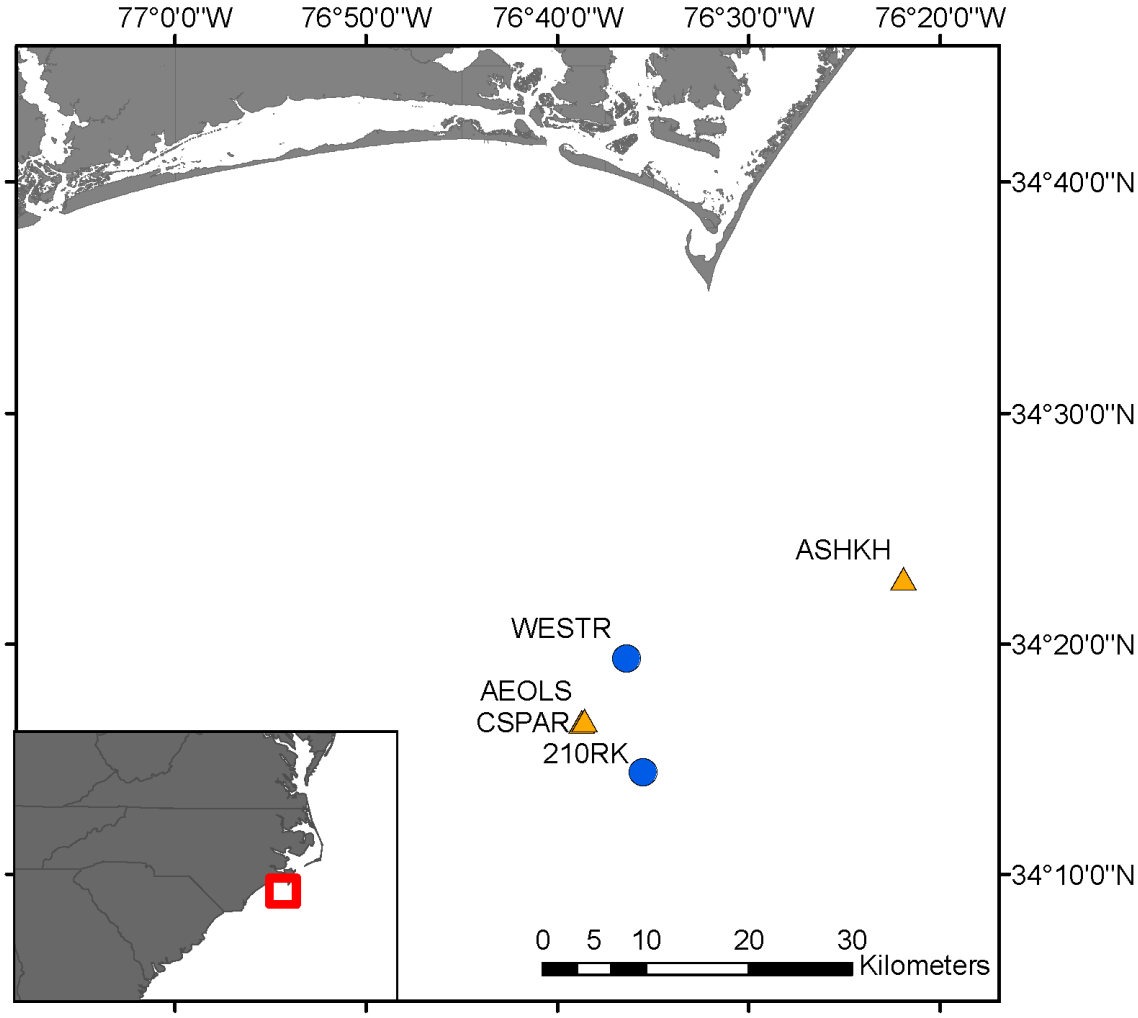

Figure 3-1: Location of soundscape monitoring stations in Onslow Bay, NC where video and acoustic data were collected. Stations include natural (blue circles) and artificial (orange triangles) reefs. *Spar and* Aeolus are several hundred meters apart. Information on each reef, referenced by site code, is contained in Table 3.

Table 3-1: Information on soundscape monitoring stations in Onslow Bay, NC.

Site_name	Site_code	Latitude_dd	Longitude_dd	Reef_type	Depth_m
210 Rock	210RK	34.2408	-76.5923	natural	32
West Rock	WESTR	34.3231	-76.6066	natural	26
USCGC *Spar*	CSPAR	34.2771	-76.6455	artificial	34
USNCL *Aeolus*	AEOLS	34.2783	-76.6432	artificial	35
Ashkhabad	ASHKH	34.3807	-76.3655	artificial	19

3.1.2. Data Collection

We deployed high-frequency ambient opti-sonic recording devices (HARD-ROCS) on the five monitoring stations on the continental shelf. These opti-sonic arrays (OSAs) included a hydrophone, video camera, and temperature logger (Figure 3-2). The hydrophones (SoundTrap 202 recorders, Ocean Instruments, New Zealand) recorded biological and anthropogenic sounds between 20Hz and 60kHz (sample rate = 96 kHz) continuously, while the video cameras recorded time-lapse videography during the day and, with an LED light, the night (8 - Appendix 1). The video camera units are composed of GoPro Hero3+ Black cameras encased in aluminum housings and attached to programmable intervalometers, supplemental batteries, and LED lights (The Sexton Corporation, Oregon, United States). These video units, recorded 20-s videos every 20-30 min during the daytime (example: 8 – Appendix 1, Video 1), with the LED activated during the latter 10 s of each video (example: Video 2). Temperature loggers (Onset, Water Temperature Pro v2 Data Logger – U22-001) measured the water temperature on the reefs. We mounted the OSAs on conical metal frames (0.5 m high, 0.3 m base diameter) anchored with 60-80 kg of lead and deployed them on the reefs for two-week periods in November 2015, January 2016, April 2016, June 2016, and August 2016. These two-week deployments in each of five months spanned a 10-month period.

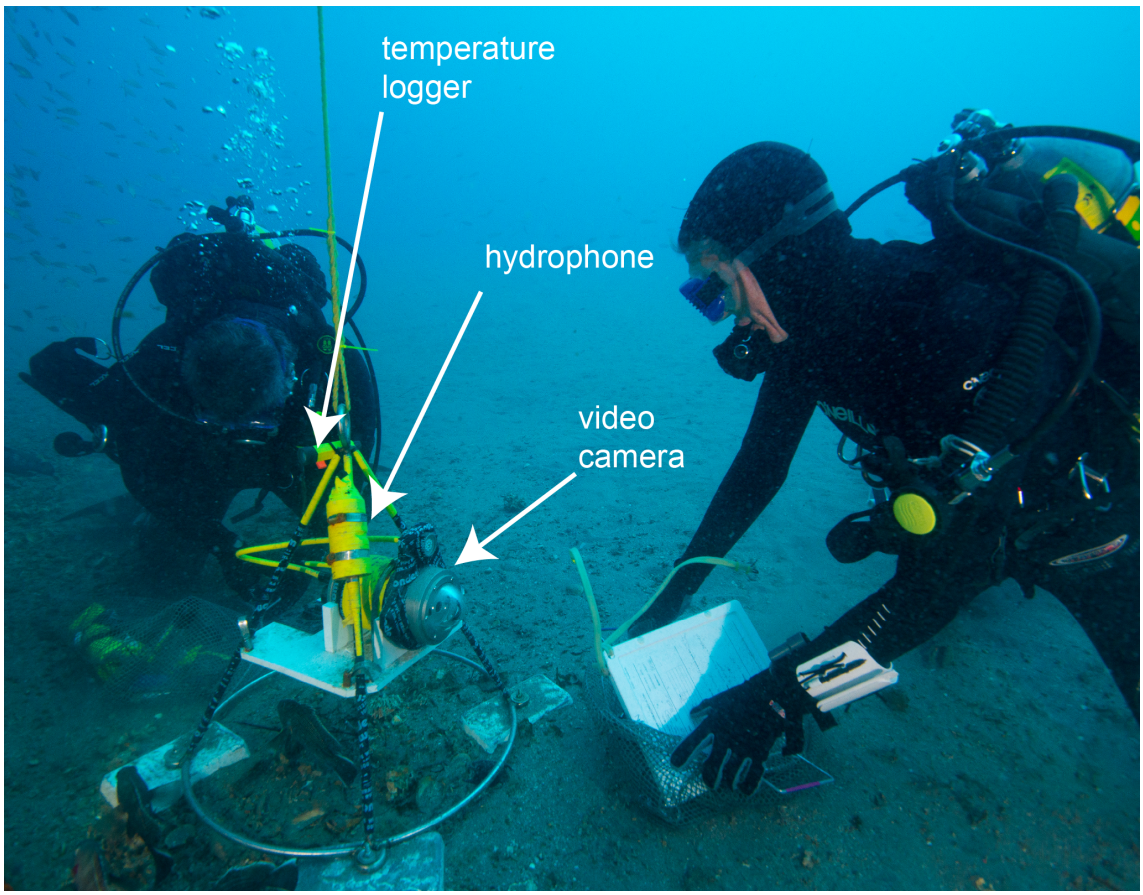

Figure 3-2: Soundscape monitoring instruments, including a video camera, hydrophone, and temperature logger, were mounted on a weighted, conical frame and deployed at each temperate reef. The instruments are covered in colored electrical tape to prevent fouling.

3.1.3. Video Processing and Analysis

Experts reviewed the last 10 seconds of each video recording when the LED light was active, ensuring identical length for each video regardless of whether the video was recorded during the day or night. We used each 10-sec video recording to identify fish to the lowest taxonomic level possible, count the maximum number of fish in the frame by species, and record additional behavioral attributes, such as group size (individual or school), movement (swimming or stationary), behavior (foraging, refuging, other), and position in the water column (0-1 m above reef, 1-2 m above reef, >2 m above reef). If fish were unidentifiable in the videos, they were classified as either unknown fish or unknown schooling fish. We also recorded environmental data, including visibility and whether vessel noise was present or absent. Time and date were extracted from timestamps on each video. All data were entered in a Microsoft Access Database.

For the first sampling period, we processed nearly all videos collected at each reef. We used these processed data to create species accumulation curves using PRIMER (Clarke and Gorley 2006). The number of videos where the species accumulation curve plateaued indicated the minimum number of videos we should process to fully encompass the community of fish present on the reefs. We used the number of videos where the plateau began for reefs in the first sampling period to determine how many videos to process in future sampling periods.

Video processing has been conducted by project investigators, one intern, and eight undergraduate students. Interns and students were trained by the project investigators. Some undergraduate students have processed video as volunteers, whereas others have processed videos for independent research credit through the University of North Carolina at Chapel Hill. Preliminary analysis of processed videos has been conducted by creating time-series visualizations in R (R Development Core Team 2016). Full data analysis was not in the original scope of the project.

3.1.4. Acoustics Processing and Analysis

Ambient bioacoustics and anthropogenic noise collected by hydrophones have not been processed or analyzed because it was not in the original scope of work.

3.2. Preliminary Results

We collected 11,690 videos of fish on the soundscape monitoring stations over five sampling periods that spanned 10 months (Table 3-2; 8 - Appendix 1). Of the collected videos, we processed 2,327 videos (Table 2). In the processed videos, we documented 232,949 fish (Appendix 2). These fish belong to 77 species representing 33 families (8 - Appendix 1, Table 8-1).

The species accumulation curve for videos in the first sampling period indicated that when processing videos from the second through fifth sampling rounds, we should process at least 400 videos for each reef to adequately sample the fish community (Figure 3-3). Based on the species accumulation curve, we randomly subsampled the total number of videos collected to select 400 from each reef to process.

Preliminary visualizations of hourly mean fish abundance by reef type (Figure 3-4) indicate several emerging patterns for processed videos. Artificial reefs support higher numbers of fish than natural reefs (Figure 3-4).

Table 3-2: Number of videos collected for each reef during each sampling period. Number of videos collected (#_col) and processed (#_pro) are provided for each reef and for the study duration. Entries with * represent sampling effort on shipwreck named the *Ashkhabad,* where current was too strong to deploy video cameras.

Sampling rounds			210 Rock		West Rock		*Spar*		*Aeolus*		totals	
Sampling_ period	Date_ deployed	date_ retrieved	#_ col	#_ pro	#_ col	#_ pro	#_ col	#_ pro	#_ col	#_ pro	#_ col	#_ pro
1	11/2/15	11/16/15	665	591	150	76	706	351	0*	0*	1521	1018
2	1/14/16	1/25/16	644	181	793	0	780	91	0*	0*	2217	272
3	4/11/16	4/25/16	237	237	925	380	164	136	345	284	1671	1037
4	6/9/16	6/27/16	1154	0	1026	0	1293	0	877	0	4350	0
5	8/15/16	8/29/16	640	0	5	0	640	0	646	0	1931	0
Totals			3340	1009	2899	456	3583	578	1868	284	11690	2327

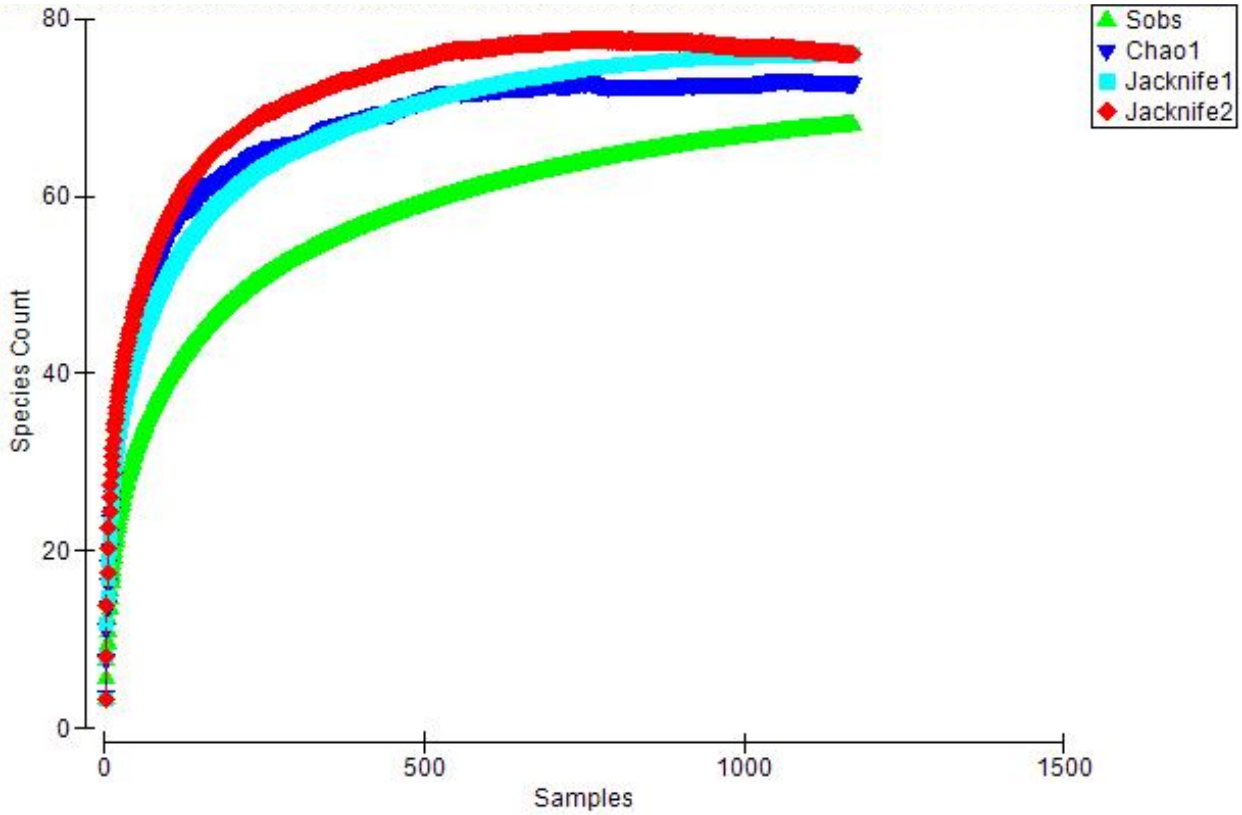

Figure 3-3: Species accumulation curve for videos processed from the first sampling period.

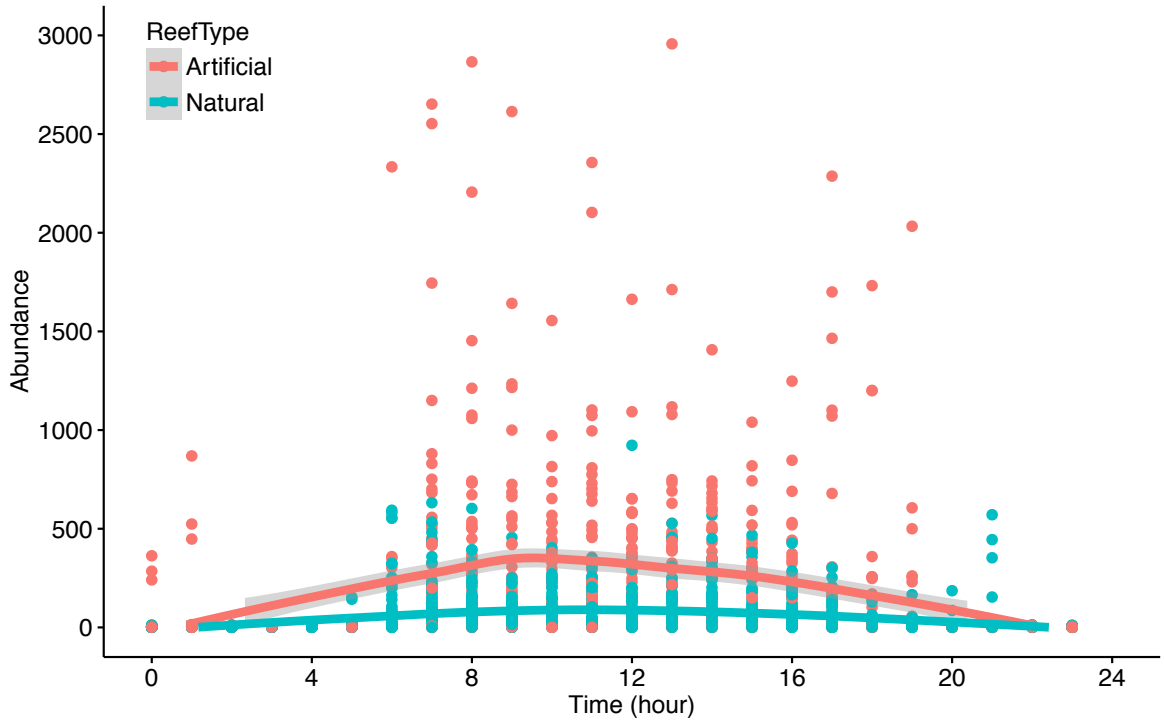

Figure 3-4: Time series of hourly mean fish abundance on temperate reefs. Red line and points correspond to artificial reefs. Blue line and points correspond to natural reefs.

3.2.1. Acoustic Results

We collected 2,934 GB of continuous acoustic data on the soundscape monitoring stations over the course of the study (Table 3-3). These data have not been processed.

Table 3-3: Acoustic data collected for each reef during each sampling round. Number of .wav audio files collected (#_files) and the corresponding total size of these files in gigabytes (Size_GB) are provided for each reef and for the study duration. Entries with * were recorded on a shipwreck named the *Ashkhabad.*

Sampling rounds			210 Rock		West Rock		*Spar*		*Aeolus*		totals	
Sampling_ period	Date_ deployed	Date_ retrieved	#_ files	Size_ GB	#_ files	Size_ GB	#_ files	Size_ GB	#_ files	Size_ GB	#_ files	Size_ GB
1	11/2/15	11/16/15	68	130	81	155	84	160	0	0	233	445
2	1/14/16	1/25/16	80	153	90	173	100	192	81*	153*	351	671
3	4/11/16	4/25/16	73	140	89	171	88	168	88	168	338	647
4	6/9/16	6/27/16	66	125	79	151	86	165	80	153	311	594
5	8/15/16	8/29/16	65	119	78	150	83	159	78	149	304	577
Totals			352	667	417	800	441	844	327	623	1537	2934

3.3. Future Analysis

We have collected and stored data on a hardbottom soundscape and the associated fish community for 2 weeks in 5 non-consecutive months spanning a 10-month period. If funding becomes available, we would like to complete the analysis and synthesis of the video and acoustic data collected for this baseline soundscape study of hardbottom habitat.

4. Expenditures

Post-processing of data from the R/V *Langseth* seismic survey accounted for 72% of the budget and included salaries, benefits, Duke University Marine Laboratory subcontractor, and acoustic software licenses. Soundscape data acquisition accounted for 28% of the budget. Soundscape expenses included supplies, truck use, and boat charters.

5. Conclusions

This report details our findings from opportunistically monitoring how fish respond to a marine seismic survey and our establishment of monitoring stations to collect baseline soundscape data and accompanying data on fish communities. While the findings from the first component, monitoring the marine seismic survey, are published in the peer-reviewed journal *Marine Policy,* data processing and analysis are ongoing for the baseline soundscape data. Our unique dataset on the soundscape and fish communities of temperate reefs of Onslow Bay, NC can be used to quantitatively assess deviations from the baseline soundscape condition that may be expected with offshore energy development and related activities.

6. References

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7. Appendices for Documenting Fish Response to Seismic Surveying

7.1. Appendix 1: Fish Species List

Table 7-1: Fish species list from 303 videos recorded on three natural reefs in Onslow Bay from September 17 – 20, 2014.

Family	Genus	Species	Common_Name	Snapper-Grouper Complex
ACANTHURIDAE	*Acanthurus*	*chirurgus*	Doctorfish	
BALISTIDAE	*Balistes*	*capriscus*	Gray Triggerfish	YES
CARANGIDAE	*Carangidae*	*spp*	Unknown Jack	YES
	Carangoides	*bartholomaei*	Yellow Jack	
	Caranx	*ruber*	Bar Jack	YES
	Decapterus	*spp.*	Decapterus Species	
	Seriola	*dumerili*	Greater Amberjack	YES
CARCHARHINIDAE	*Carcharhinus*	*spp*	Unknown Shark	
CHAETODONTIDAE	*Chaetodon*	*ocellatus*	Spotfin Butterflyfish	
EPHIPPIDAE	*Chaetodipterus*	*faber*	Atlantic Spadefish	YES
HAEMULIDAE	*Haemulon*	*aurolineatum*	Tomtate	YES
	Haemulon	*plumieri*	White Grunt	YES
LABRIDAE	*Halichoeres*	*garnoti*	Yellowhead Wrasse	
	Halichoeres	*spp*	Unknown Wrasse	
	Halichoeres	*bivittatus*	Slippery Dick	
	Thalassoma	*bifasciatum*	Bluehead Wrasse	
LUTJANIDAE	*Lutjanus*	*griseus*	Gray snapper	YES
	Rhomboplites	*aurorubens*	Vermillion Snapper	YES
MONACANTHIDAE	*Aluterus*	*schoepfi*	Orange Filefish	
	Stephanolepis	*hispidus*	Planehead Filefish	
UNKNOWN	*Osteichthyes*	*spp.*	Unknown Fish Species	
POMACANTHIDAE	*Holacanthus*	*bermudensis*	Blue Angelfish	
POMACENTRIDAE	*Chromis*	*scotti*	Purple Reef Fish	
	Stegastes	*leucostictus*	Beaugregory Damselfish	
	Stegastes	*variabilis*	Cocoa Damselfish	
SCARIDAE	*Sparisoma*	*aurofrenatum*	Redband Parrotfish	
	Sparisoma	*atomarium*	Green Blotch Parrotfish	
SCIAENIDAE	*Pareques*	*umbrosus*	Cubbyu	
	Sciaenidae	*spp.*	Unknown Drum	
SCOMBRIDAE	*Scomberomorus*	*cavalla*	King Mackerel	
	Scombrid	*spp*	Mackerel / Tunny	
SCORPAENIDAE	*Pterois*	*volitans*	Lionfish	
SERRANIDAE	*Centropristis*	*ocyurus*	Bank Sea Bass	YES
	Centropristis	*striata*	Black Sea Bass	YES
	Diplectrum	*formosum*	Sand Perch	

Family	Genus	Species	Common_Name	Snapper-Grouper Complex
	Mycteroperca	*microlepis*	Gag	YES
	Mycteroperca	*phenax*	Scamp	YES
	Rypticus	*maculatus*	White Spotted Soapfish	
	Serranus	*subligarius*	Belted Sandfish	
SPARIDAE	*Archosargus*	*probatocephalus*	Sheepshead	
	Calamus	*spp*	Unknown Porgy	YES
	Diplodus	*holbrookii*	Spottail Pinfish	
	Pagrus	*pagrus*	Red Porgy	YES
	Stenotomus	*caprinus*	Longspine Porgy	YES
	Stenotomus	*chrysops*	Scup	YES
SPHYRAENIDAE	*Sphyraena*	*barracuda*	Barracuda	
SYNODONTIDAE	*Synodus*	*spp*	Lizardfish	
TETRAODONTIDAE	*Sphoeroides*	*spengleri*	Bandtail Puffer	
TRIGLIDAE	*Prionotus*	*spp*	Unknown Searobin	

8. Appendices for Establishing a Baseline Soundscape

8.1. Appendix 1: Representative fish videos

Video 1: Daytime video recording from natural reef, 210 Rock (see attached file 210RK_GOPR1896.MP4).

Video 2: Nighttime video recording from natural reef, 210 Rock (see attached file Video2_210RK_GOPR2796.MP4). The video is dark for 10 s before the LED light turns on, as programmed.

Video 3: Daytime video recording from natural reef, West Rock (see attached file Video3_WESTR_GOPR9538.MP4).

Video 4: Daytime video recording from artificial reef, US Navy Cable Layer *Aeolus* (see attached file Video4_AEOLS_GOPR3743.MP4).

Video 5: Daytime video recording from artificial reef, US Navy Cable Layer *Aeolus* (see attached file Video5_AEOLS_GOPR3891.MP4).

Video 6: Daytime video recording from artificial reef, US Navy Cable Layer *Aeolus* (see attached file Video6_AEOLS_GOPR3933.MP4).

Video 7: Daytime video recording from artificial reef, USCGC *Spar* (see attached file Video7_CSPAR_GOPR5265.MP4).

Video 8: Daytime video recording from artificial reef, USCGC *Spar* (see attached file Video8_CSPAR_GOPR5681.MP4).

Video 9: Nighttime video recording from artificial reef, USCGC *Spar* (see attached file Video9_CSPAR_GOPR9481.MP4).

Video 10: Nighttime video recording from artificial reef, USCGC *Spar* (see attached file Video10_CSPAR_GOPR9649.MP4).

8.2. Appendix 2: Species List

Table 8-1:Species list from 2,327 processed videos on temperate reefs of the NC continental shelf. Bold text indicates fish in the federally managed snapper-grouper complex. Abundance values indicate the total number of individuals of each species observed across the 2,327 processed videos.

Family	Genus	species	Common_Name	Abundance
Acanthuridae	*Acanthurus*	*chirurgus*	Doctorfish	1
Anguillidae	*Anguilla*	*rostrata*	American Eel	2
Apogonidae	*Apoginidae*	sp.	Unknown Cardinalfish	211
Apogonidae	*Apogon*	*pseudomaculatus*	Two Spot Cardinal Fish	28
Balistidae	***Balistes***	***capriscus***	**Grey Triggerfish**	**6**
Blenniidae	*Blenniidae*	sp.	Unknown Blenny	1
Carangidae	*Decapterus*	sp.	Scad Species	9127
Carangidae	*Carangidae*	sp.	Unknown Jack	1686
Carangidae	*Decapterus*	*punctatus*	Round Scad	575
Carangidae	***Seriola***	***dumerili***	**Greater Amberjack**	**53**
Carangidae	*Carangoides*	*bartholomaei*	Yellow Jack	33
Carangidae	***Seriola***	***fasciata***	**Lesser Amberjack**	**10**
Carangidae	***Seriola***	***rivoliana***	**Almaco Jack**	**7**
Carcharhinidae	*Carcharhinus*	*plumbeus*	Sandbar Shark	6
Chaetodontidae	*Chaetodon*	*ocellatus*	Spotfin Butterflyfish	1
Cheloniidae	*Caretta*	*caretta*	Loggerhead Turtle	21
Dasyatidae	*Dasyatis*	*americana*	Southern Sting Ray	7
Echeneidae	*Remora*	*remora*	Remora	4
Ephippidae	***Chaetodipterus***	***faber***	**Atlantic Spadefish**	**520**
Haemulidae	***Haemulon***	***aurolineatum***	**Tomtate**	**67854**
Haemulidae	***Haemulonidae***	**sp.**	**Unknown Grunt**	**3003**
Haemulidae	***Haemulon***	***album***	**White Margate**	**152**
Haemulidae	*Anisotremus*	*surinamensis*	Black Margate	18
Haemulidae	***Haemulon***	***plumieri***	**White Grunt**	**18**
Labridae	*Halichoeres*	*bivittatus*	Slippery Dick	233
Labridae	*Labridae*	sp.	Unknown Wrasse	141
Labridae	*Halichoeres*	*caudalis*	Painted Wrasse	8
Labridae	*Bodianus*	*rufus*	Spanish Hogfish	6
Labridae	*Halichoeres*	*radiatus*	Pudding Wife	1
Labridae	***Lachnolaimus***	***maximus***	**Hogfish**	**1**
Labridae	*Tautoga*	*onitis*	Tautog	1

Lutjanidae	***Rhomboplites***	***aurorubens***	**Vermillion Snapper**	**5565**
Lutjanidae	***Lutjanus***	***griseus***	**Gray Snapper**	**47**
Lutjanidae	***Lutjanidae***	**sp.**	**Unknown Snapper**	**7**
Lutjanidae	***Lutjanus***	***campechanus***	**Red Snapper**	**1**
Lutjanidae	***Lutjanus***	***cyanopterus***	**Cubera snapper**	**1**
Monacanthidae	*Aluterus*	*monoceros*	Unicorn Filefish	441
Monacanthidae	*Stephanolepis*	*hispidus*	Planehead Filefish	26
Monacanthidae	*Monacanthidae*	sp.	Unknown Filefish	18
Monacanthidae	*Cantherhines*	*macrocerus*	Whitespotted Filefish	3
Mullidae	*Pseudupeneus*	*maculatus*	Spotted Goatfish	1
Muraenidae	*Anguilliformes*	sp.	Unknown Eel	4
Octopodidae	*Octopodidae*	sp.	Unknown Octopus	2
Odontaspididae	*Carcharias*	*taurus*	Sandtiger Shark	996
Osteichthyes	*Osteichthyes*	sp.	Unknown Schooling Fish	134132
Osteichthyes	*Osteichthyes*	sp.	Unknown Fish Species	3887
Phycidae	*Urophycis*	*earllii*	Carolina Hake	5
Pomacanthidae	*Holacanthus*	*bermudensis*	Blue Angelfish	39
Pomacanthidae	*Holacanthus*	*ciliaris*	Queen Angelfish	4
Pomacentridae	*Pomacentridae*	sp.	Unknown Damselfish	573
Pomacentridae	*Stegastes*	*partitus*	Bicolor Damselfish	102
Pomacentridae	*Stegastes*	*leucostictus*	Beaugregory	3
Rachycentridae	*Rachycentron*	*canadum*	Cobia	3
Rhincodontidae	*Ginglymostoma*	*cirratum*	Nurse Shark	3
Sciaenidae	*Pareques*	*iwamotoi*	Blackbar Drum	150
Sciaenidae	*Pareques*	*umbrosus*	Cubbyu	45
Scorpaenidae	*Pterois*	*volitans*	Lionfish	218
Serranidae	***Centropristis***	***striata***	**Black Sea Bass**	**1866**
Serranidae	***Mycteroperca***	***microlepis***	**Gag**	**117**
Serranidae	***Centropristis***	***ocyurus***	**Bank Sea Bass**	**99**
Serranidae	*Rypticus*	*maculatus*	White Spotted Soapfish	73
Serranidae	*Serranus*	*subligarius*	Belted Sandfish	40
Serranidae	***Serranidae***	**sp.**	**Unknown Seabass**	**17**
Serranidae	*Diplectrum*	*formosum*	Sand Perch	13
Sparidae	*Diplodus*	*holbrookii*	Spottail Pinfish	422
Sparidae	***Sparidae***	**sp.**	**Unknown Porgy**	**100**
Sparidae	***Stenotomus***	**sp.**	**Longspine Porgy/Scup**	**47**
Sparidae	***Stenotomus***	***chrysops***	**Scup**	**41**
Sparidae	***Calamus***	***calamus***	**Saucereye Porgy**	**35**
Sparidae	*Archosargus*	*probatocephalus*	Sheepshead	14
Sparidae	*Archosargus*	*rhomboidalis*	Sea Bream	11

Sparidae	***Calamus***	***nodosus***	**Knobbed Porgy**	**9**
Sparidae	***Calamus***	***bajonado***	**Jolthead Porgy**	**2**
Sparidae	***Stenotomus***	***caprinus***	**Longspine Porgy**	**1**
Sphyraenidae	*Sphyraena*	*barracuda*	Barracuda	2
Synodontidae	*Synodus*	*foetens*	Inshore Lizardfish	2
Tetraodontidae	*Sphoeroides*	*spengleri*	Bandtail Puffer	27

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