Using Acoustic Indices to Determine Changes in Biodiversity off the Coast of Cape Hatteras, NC

by

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Executive Summary

The ability to monitor environmental health is a growing imperative resulting from global climate change and loss of essential habitats, including along the coast. Coastal environments are extremely susceptible to degradation from anthropogenic activity but provide a plethora of ecosystem services including storm buffering, increased water quality, and nursery areas for economically valuable species. These environments are also relatively inaccessible and extremely difficult to examine over long periods of time. To combat these issues, ecological indicators are a common method used to quantify the overall health of an ecosystem by examining specific environmental characteristics such as biodiversity. Healthy environments are thought of as sustainable and resilient to stress, and these traits have been directly correlated with the biological composition or diversity of an ecosystem.

Analysis of biodiversity is traditionally done with visual surveys that can be time consuming, expensive, require trained professionals and are limited in the scope of the analysis that can be completed. The use of passive acoustic monitoring can eliminate these restrictions as acoustic recorders are easy to deploy, analysis does not require trained specialists and the recordings can be done over much larger spatial and temporal scales. Because many marine organisms use sound for either social communication, foraging or reproductive activities, acoustic monitoring of coastal environments can provide valuable information about the species present and therefore the overall ecosystem health. From these acoustic recordings, bioacoustics indices such as the Acoustic Complexity Index (ACI) can be calculated to help infer biodiversity based from the complexity or variability of the acoustic scene. This provides an efficient way to rapidly assess acoustic biodiversity present in an ecosystem and monitor changes throughout the environment over time.

Looking specifically at five locations off the coast of Cape Hatteras, this project calculated the ACI on low-frequency acoustic data collected over the course of a year to determine if these values corresponded with biological activity present, and how this changed over time based on the expected biological composition of the study site known from fish and marine mammal surveys. Results showed that within each location there was a least one month that was significantly different that the rest of the year, and closer examination of each month between locations also determined that at least one location varied significantly from the other locations.

The values were plotted separately for each month in the year and each day within the month to distinguish times where the ACI values were substantially higher or lower, and determine the cause of

the variability. At most locations, times where the acoustic complexity increased dramatically were the result of short duration disturbance, either from vessel activity or a source that could not be aurally determined, that caused a brief increase in fish vocalizations. The most common species identified in these recording included Atlantic spadefish (*Chaetodipterus faber*), Grey triggerfish (*Balistes capriscus*), Channel bass (*Sciaenops ocellatus*) and Black drum (*Pogonias cromis*). These species were found in most locations at various times throughout the year. There was no substantial difference of observed fish species between locations, however one species, Sheepshead (*Arhosargus probatocephalus*) was only observed at locations in relatively shallow water (21 -26 meters depth). This is consistent with what is known about their lifestyle, as they are primarily found in coastal waters so would not likely travel that far away from the shore.

Baleen whale species such as the North Atlantic right whale (*Eubalaena glacialis*), humpback whale (*Megaptera novaeangliae*), fin whale (*Balaenoptera physalus*) and minke whale (*Balaenoptera acutorostrata*), were the focus of this analysis as the data collected were at lower frequencies (below 1000 Hz), and while marine mammal vocalizations did not contribute to the sharp peaks in complexity, increases in the average values seen in the fall at the hydrophones located in deeper waters could have occurred as these whales migrated through this region travelling between their feeding and breeding grounds.

Through this project, the potential of the ACI as a way to distinguish acoustically relevant or important times and areas in the marine environment was verified and this information could have important implications for management of noise pollution. Masking is an impact of increased ambient noise due to anthropogenic activity that has been well documented and was also observed during this analysis at hydrophones located in deeper waters where larger vessels could be a potential concern. More information needs to be collected to characterize ambient noise levels in this study area using appropriate metrics to fully understand how noise is changing over time. More information regarding fish species and how they may respond to human generated noise would also be beneficial, especially if the vocalizations are indicative of spawning activity, masking could have potentially harmful population effects.

1. Introduction

1.1 Habitat Monitoring

Broad-scale climate change and the resultant decline in the health of ecosystems around the world pose a threat to the future health and stability of our environment as well as the welfare of humans (Harris, 2014, Harris 2016, Barber et. al. 2009). Coastal habitats are especially susceptible, as they contain irreplaceable natural resources and provide habitat for many species (Harris 2014). These coastal environments are essential for human health as well, for many coastal habitats can provide increased water quality, buffering from storm activity and nursery functions for economically vital species (Rapport 1998). The declining health of environments globally has increased the need for more accurate and efficient ways to monitor changes in ecosystem health over time (Rombouts et. al. 2013).

Ecosystem monitoring projects have typically focused on the use of indicators such as biodiversity to measure the health of an ecosystem (Rapport 1998, Harris 2014). Healthy environments are typically thought of as sustainable and resilient to stress, and the biological composition or diversity of an ecosystem is thought to be indicative of these features (Rapport 1998). Monitoring biological activity is an important aspect of conservation that can help indicate changes in the overall health of an environment which can help inform management efforts and allow easier ecosystem monitoring over time (Parks et. al. 2014, Rapport 1998).

Although biodiversity has been identified as a useful indicator of environmental health, it is a difficult quantity to efficiently determine, especially in the marine environment (Thomas 2012). Traditional biodiversity surveys are done with visual transects that can be time consuming, labor intensive, expensive, invasive, susceptible to human bias and typically require trained specialists to identify species (Harris 2014, Mann 2012, Lammers et. al. 2008, Parks et. al. 2014, Mellinger et. al. 2007). These surveys are especially challenging in the marine environment as the species of interest spend the majority of their time underwater, and visual surveys are often limited by light and weather conditions (Heenehan 2016, Harris 2014, Mann 2012, Harris 2016). Also, many marine species utilize camouflage techniques to avoid predators, can be missed visually due to their small size, or they are only active at night when light is a limiting factor (Harris 2014, Harris 2016, Thomas 2012).

These surveys can also be limited in the spatial and temporal coverage they can accomplish. Many marine habitats are isolated and widely distributed and therefore inaccessible for complete biodiversity surveys (Lammers et. al. 2008). This is an issue faced by many governmental and nongovernmental conservation agencies who require long term monitoring of various global environments to effectively make decisions (Gedamke et. al. 2016). Especially in habitats critical for endangered or threatened species, long-term monitoring in remote areas is essential for preservation of habitat (Gedamke et. al. 2016, NRC 1994, NRC 2000).

To combat these issues, passive acoustic monitoring has increased in popularity as an efficient way to monitor changes in biodiversity and environmental composition over time. Sound is an integral part of the marine environment that is used by most organisms, including both fish and marine mammals, to perceive their environment (Staaterman et. al. 2014, Harris 2014, NRC 1994, Nowacek et. al. 2007). This is especially true in areas of low visibility where the animals may rely heavily on sound as a means of communication (Thomas 2012, Heenehan 2016, Harris 2016, Harris 2014). Passive acoustic monitoring can help provide data in real-time over much larger temporal and spatial scales than traditional marine survey methods (Harris 2014, Mellinger et. al. 2007, Parks et. al. 2014, Harris 2016). Sound travels much further in the water column than light, and advances in hydrophone technology have made it possible to take long-term recordings (Mann 2012, Gage & Axel 2014, Thomas 2012, NRC 2000). Acoustic monitoring of biodiversity is also less expensive, non-invasive, involves a single interpreter that minimizes error and can be done remotely (Mann 2012, Harris 2014, Mellinger et. al. 2007, Parks et. al. 2014, Harris 2016).

1.2 Noise Pollution

In recent years, management of noise from non-biological sources has become a growing topic of concern, especially in protected areas designated for the preservation of endangered and threatened species (Barber et. al. 2009, Hatch et. al. 2008, NRC 1994, NRC 2000, NRC 2005, Slabbekoorn et. al 2010). The ocean can be a noisy environment, with most marine animals producing sound for various purposes, and non-biological natural contributions including wind, waves, precipitation and geologic events (Staaterman et. al. 2014, NRC 1994, Parks et. al. 2014, Miksis-Olds et. al. 2012). Over the past few decades, however, ambient noise levels have increased due to human activity (Gedamke et. al. 2016, Rice et. al. 2014, Hatch et. al. 2008). The major contributors to this increase in sound levels in the ocean have been identified as sounds originating from vessel activity, marine construction, geophysical surveys, military sonar, scientific surveys and even aircrafts (Nowacek et. al. 2007, Gedamke et. al. 2016, Miksis-Olds 2012). Particularly in the Northern Hemisphere, commercial shipping has greatly increased and can dominate the low-frequency bandwidth in underwater environments (Hatch et. al. 2008).

Impacts of noise on marine organisms have become the focus of many research projects as a result of increased noise pollution concerns. Studies have shown the potential for acute and chronic impacts of sound on marine life, and could potentially result in either temporary or permanent hearing loss (for a review, see Nowacek et. al. 2007). Noise can also alter animal behavior resulting in displacement from their environment, movement away from sound source or increased intensity and frequency of vocalizations in response to increased noise exposure (Nowacek et. al. 2007, Slabbekoorn et. al. 2010, Barber et. al. 2009, Weilgart 2007). A phenomenon called masking is a great concern with noise pollution, as increased background noise levels have been shown to reduce organisms' ability to perceive sounds and communicate with conspecifics (Barber et. al. 2009, Hatch et. al. 2008, Rice et. al. 2014, Gage & Axel 2014, Slabbekoorn et. al. 2010, NRC 2000). This reduced transmission or perception of acoustic signals could reduce reproductive success of populations, and could impact individual survival by reducing response to signals indicating predators or other threats (Barber et. al. 2009, Hatch et. al. 2008, Rice et. al. 2014, Gage & Axel 2014, Slabbekoorn et. al. 2010). Mass stranding and mortality events of marine mammals have also been linked to behavioral changes resulting from exposure to increased noise pollution, prompting the need for mitigation efforts of anthropogenic sounds (Weilgart 2007, NRC 2005).

Ocean noise in the United States is typically regulated primarily by the National Oceanic and Atmospheric Association (NOAA) whose authority comes from: the Marine Mammal Protection Act (MMPA), the Endangered Species Act (ESA), the Magnuson-Stevens Fishery Conservation and Management Act (MSA), and the National Marine Sanctuaries Act (NMSA). Under the MMPA and the ESA, NOAA has the ability to monitor and mitigate any potential threat to the health of all marine mammals and endangered or threatened species in the marine realm that could cause a decline in the health and function of the population. Overall, these laws allow NOAA to designate areas or habitats that have been identified as biologically or ecologically significant for at-risk species. To reduce impacts of noise pollution on these organisms, NOAA utilizes acoustic thresholds and mitigation procedures such as power down or shutdown zones, seasonal or area limitations, and noise abatement measures. They have also identified the importance of monitoring both species and habitats to fully understand impacts of anthropogenic noise pollution. (Gedamke et. al. 2016)

1.3 Acoustic Indices in the Marine Environment

An emerging method of quantifying biodiversity rapidly and efficiently is the use of bioacoustics or ecoacoustic indices calculated from passive acoustic data (Harris 2016, Staaterman et. al. 2014, Pierreti et. al. 2011, Farina et. al. 2011, Parks et. al. 2014). As previously mentioned, recording temporal changes in biodiversity can be extremely time consuming, expensive and invasive to the environment the divers are attempting to assess (Harris 2014, Mann 2012, Lammers et. al. 2008, Parsons et. al. 2016, Mellinger et. al. 2007). Information about biodiversity in a region can be correlated with the overall health of the ecosystem, so finding a way to measure biodiversity in the field can be very beneficial (Rapport 1998). Statistical indices have been developed as a way to quickly quantify biological and acoustic diversity in acoustic recordings.

A few indices including acoustic entropy (H), acoustic richness (AR) and acoustic complexity (ACI) have been used in various studies to analyze acoustic soundscapes and changes in diversity over time (Parks et. al. 2014, Farina et. al. 2011, Staaterman et. al. 2014, Harris 2014, Pierreti et. al. 2011, Bertucci et. al. 2016, Depraetere et. al. 2012, Harris 2016). This form of analysis was developed primarily for bird calls in terrestrial environments, but has recently begun to be tested in the marine realm, and has shown promise for distinguishing between acoustic environments at different sites and changes over time (Harris 2014, Harris 2016, Depraetere et. al. 2012, Pierreti et. al. 2011, Staaterman et. al. 2014). Acoustic entropy (H) has been shown to be a useful measure of the diversity of species present in an area, but can be impacted by heavy background noise due to anthropogenic activity (Parks et. al. 2014). To elaborate on this index and accommodate for increasing noise levels, the acoustic richness index was developed and has shown great results in habitats with higher anthropogenic sounds and lower biological sounds (Depraetere et. al. 2012, Harris 2016, Bertucci et. al. 2016, Harris 2014). Finally, the acoustic complexity index (ACI) was developed to show variations in biological activity within sites, rather than just species diversity, and can be a useful site comparison metric that is also robust to anthropogenic sounds (Pierreti et. al. 2011, Harris 2014, Harris 2016, Farina et. al. 2011).

Using acoustic data collected off the coast of Cape Hatteras in North Carolina, this study attempted to characterize the biological composition of this area using the ACI to monitor spatial and temporal changes in acoustic complexity. Acoustic studies in coastal North Carolina have focused primarily on estuarine systems and locations within Pamlico Sound, and on how species use certain acoustic signatures for larval settlement cues (Lillis et. al. 2014a, Lillis et. al. 2014b). Studies have also characterized the ambient noise levels in area and how they compare to other locations along the East Coast of the U.S, but they have focused on low frequency sounds (Rice et. al. 2014, Wiggins 2015. To date, there are no biodiversity analyses for this part of North Carolina utilizing acoustic indices, even though it is home to numerous fish and marine mammal species (Garrison et. al. 2003, Daniel 2014).

The goal of this study is to determine if there are any spatial or temporal patterns in acoustic diversity and what the potential causes of this variability may be, and to identify times during the year and locations throughout the study area where animal activity is greatest.

2. Methods

2.1 Study Area and Acoustic Data Collection

Acoustic recordings were collected in North Carolina off the coast of Cape Hatteras (see Figure1).

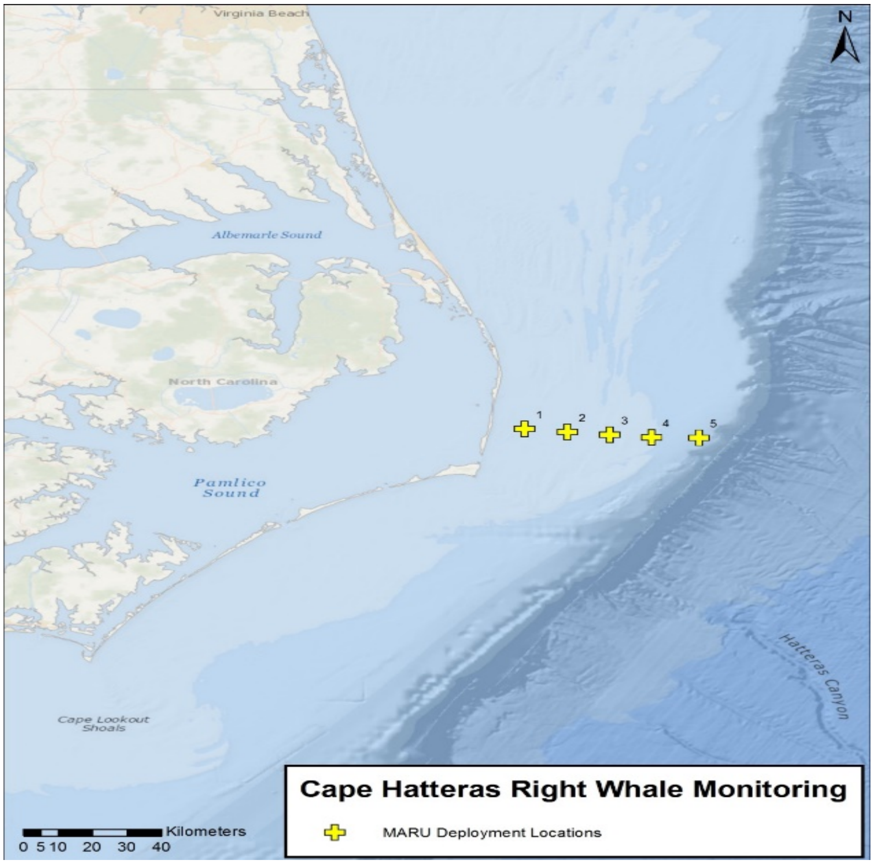

Figure 1. Map indicating the locations of the hydrophones off the coast of Cape Hatteras in North Carolina.

Cape Hatteras is located on Hatteras Island on the outer banks of North Carolina. It is a National Seashore managed by the National Parks Service established to preserve the barrier island for protection against storm surges (National Parks Service 2016). The National Seashore and its adjacent waters are home to a number of animals including sea birds, reptiles, sea turtles and a various marine mammals species like the bottlenose dolphin (*Tursiops truncates*) and common dolphin (*Delphinus delphis*), and migratory marine mammal species such as the Northern right whale (*Eubalaena glacialis*), humpback whale (*Megaptera novaeangliae*), minke whale (*Balaenoptera acutorostrata*), pilot whale (*Globicephala sp.*) and dwarf sperm whales (*Kogia sp.*) (Garrison et. al. 2003, Marine Mammal Stranding Network of the North Carolina Central Coast 2016).

Hatteras Island also hosts a number of fishing ports where people can fish either inshore from the pier or surf, or they can charter a boat from various companies that will take them to the inlets, the sound, or offshore to the Gulf Stream (National Parks Service 2016, Big Tahuna Sport Fishing 2017, Oregon Inlet Fishing Center 2017). These services are available year round and can be chartered for a full day or just a few hour of offshore fishing (Oregon Inlet Fishing Center 2017). There are over 35 species of fish that are either seasonally or permanently present in this area, including Channel bass, Atlantic croaker, Black drum, and Grey triggerfish (Daniel 2014).

Acoustic data were collected by an array of five Marine Autonomous Recording Units (MARUs), bottom mounted “pop-up” recorders developed by the Cornell Bioacoustics Research Program (The Cornell Lab of Ornithology 2017a, Parks et. al. 2008). The recorders were equipped with HTI-94-SSQ hydrophones with a sensitivity of -151.2 dB re 1 uPa, a flat frequency response between 15-585 Hz and a sampling frequency of 2000 Hz. They were deployed 10 km apart starting at the coast and going out to the continental shelf where the depth of the water ranged from 21 meters to 90 meters where low-frequency sounds were collected continuously from October 2013 to August 2015 (Figure 1) (Hatch et. al. 2012, The Cornell Lab of Ornithology 2017).

Originally, these recordings were used in a North Atlantic Right Whale monitoring project to examine their migration patterns along the North Carolina Coast so a high-pass filter and a low-pass filter were applied at 10 and 1000 Hz, respectively, to match the known vocalization range of the whales. There is a gap in the data from June 2014 to October 2013 when the MARUs were not deployed because right whales are not present in this region during that time. Data were saved to external hard drives as 15 minute multichannel AIF files with each channel in the files corresponding to a different hydrophone (e.g. channel one is data from hydrophone one, channel two is data from hydrophone two, etc).

2.2 Acoustic Complexity Index

The Acoustic Complexity Index (ACI) was created initially for terrestrial applications in order to rapidly assess and quantify bird vocalizations by computing the variation in sound intensities (Pieretti et. al. 2011).

$$ACI_{tot} = \sum_{l=1}^{q} ACI_{(\Delta f l)} \tag{1}$$

Equation (1) is the final formula in a series of steps described in Pieretti et. al. 2011 where the absolute difference between two adjacent intensity values in a single frequency bin are found and added together for the first temporal step of the recording. The result of this calculation is then divided by the total sum of intensity values to find relative intensity in a single temporal step and frequency bin. The formula shown in Equation (1) corresponds to the total value of the ACI for all of the frequency bins in the entire recording. This index works on the hypothesis that many biotic sounds have greater variability of intensities compared to anthropogenic noises, whose intensities are comparatively constant, and therefore biotic sounds can be extracted from acoustic recordings (Pieretti et. al. 2011).

I used this metric as an attempt to quantify biodiversity for each of the five locations in my study site. In order to calculate the complexity index, I first had to convert the original AIF files to WAV format using a custom written MATLAB code (version R2016b). I then calculated the ACI for each 15 minute WAV file using the Seewave package in R 1.0.136 (Sueur et. al. 2008) and the values were plotted by month for each location as a boxplot. This allowed me to identify months of interest and plot them separately to examine further the months and days of interest which I compared to spectrograms created using the default setting in Raven Lite 1.0 (see Charif et. al. 2006). To verify whether this was an accurate representation of relative biodiversity I compared the temporal and spatial variation calculated with the ACI to expected biodiversity variations based on fish and marine mammal surveys performed in the area. Due to the data being filtered between 10 and 800 Hz, only baleen whale species were considered when estimating biodiversity, as odontocete species tend to have peak frequencies above 1000 Hz and would not be detected in these acoustic recordings (Discovery of Sounds in the Sea 2016). Fish present in acoustic recording were identified by comparing my recordings to audio files available on Macaulay Library, developed by the Cornell Lab of Ornithology, and the website Discovery of Sounds in the Sea (The Cornell Lab of Ornithology 2017b, Discovery of Sounds in the Sea 2016).

2.3 Statistical Analysis

To further compare differences in ACI values between hydrophone locations, or depths, and time, I used the kruskal-wallis test, a non-parametric alternative to ANOVA (Parks et. al. 2014, Lix et. al. 1996). This test does not depend on the data being normally distributed and was used to test the null hypothesis that acoustic diversity is equal between all sites and months (Parks et. al. 2014, Lix et. al. 1996). I tested for differences in ACI values between months within each channel and between channels for each month.

3. Results

3.1 Biodiversity Analysis

The ACI values showed no obvious temporal trend for each hydrophone (Figure 2). At hydrophones four and five the index values began to increase starting in May and June, while at hydrophone two the complexity values were at their highest in March and April. Statistical analysis showed that there was a significant difference between months at each location (p-value < 0.05). The highest complexity values were observed at hydrophone four and occurred in November, while the lowest complexity values were observed again in November, but at hydrophone two (Table 1). Comparison of individual months across hydrophone locations also showed significant differences with depth (p-value < 0.05).

	Minimum	**Maximum**	**Median**
Hydrophone 1	140.6	249.0	155.5
Hydrophone 2	76.08	275.2	153.7
Hydrophone 3	123.4	273.8	153.5
Hydrophone 4	104.6	310.3	154.7
Hydrophone 5	101.9	250.2	151

Table 1. Minimum, maximum and median ACI values for each of the five hydrophones. Values are shown for all months over the study period.

The baleen whales known to migrate through this area are North Atlantic right whales (*E. glacialis*), humpback whales (*M. novaeangliae*), minke whales (*B. acutorostrata*), and fin whales (*Balaenoptera physalus*) (Marine Mammal Stranding Network of the North Carolina Coast 2016, Roberts et. al. 2016). These species have been documented travelling from their winter breeding grounds in the Southern Atlantic to their summer feeding grounds in the Northern Atlantic and would be expected to cross through the mid-Atlantic sometime between December and February (Rice et. al. 2014, Stevick et. al. 2003, NOAA Fisheries 2015). Knowing this, it was expected that the biodiversity observed would begin to increase in the winter for at least some of the locations.

Of the over 35 fish species found in coastal Carolina waters, 19 of them are known to be soniferous (Daniel 2014, The Cornell Lab of Ornithology 2017b). These species are: Weakfish (*Cynoscion regalis*), Atlantic croaker (*Micropogonias undulates*), Pigfish (*Orthopristis chrysoptera*), Kingfish (*Menticirrhus sp.*), Northern puffer (*Sphoeroides maculatus*), Pinfish (*Lagodon rhomboides*), Atlantic spadefish (*Chaetodipterus faber*), Black drum (*Pogonias cromis*), Channel bass (*Sciaenops ocellatus*), Sheepshead (*Arhosargus probatocephalus*), Bluefish (*Pomatomus saltatrix*), Silver perch (*Bairdiella chrysoura*), Amber jack (*Seriola sp.*), Gag grouper (*Mycteroperca microlepis*), White grunt (*Haemulon plumierii*), Vermilion snapper (*Rhomboplites aurorubens*), Black sea bass (*Centropristis striata*), and Grey triggerfish (*Balistes capriscus*).

It has been shown that fish produce sounds for multiple purposes, such as spawning, so for the purposes of this project it was assumed that acoustic activity would most likely increase during this period. Spawning for most fish species usually occurs in late spring and summer, but some fish are known to spawn later in the year with peaks in activity around September or October (Daniel 2014). Because data were not available for the summer months, this activity was expected to increase in late spring (May and June) and early autumn (October and November), depending on the species present.

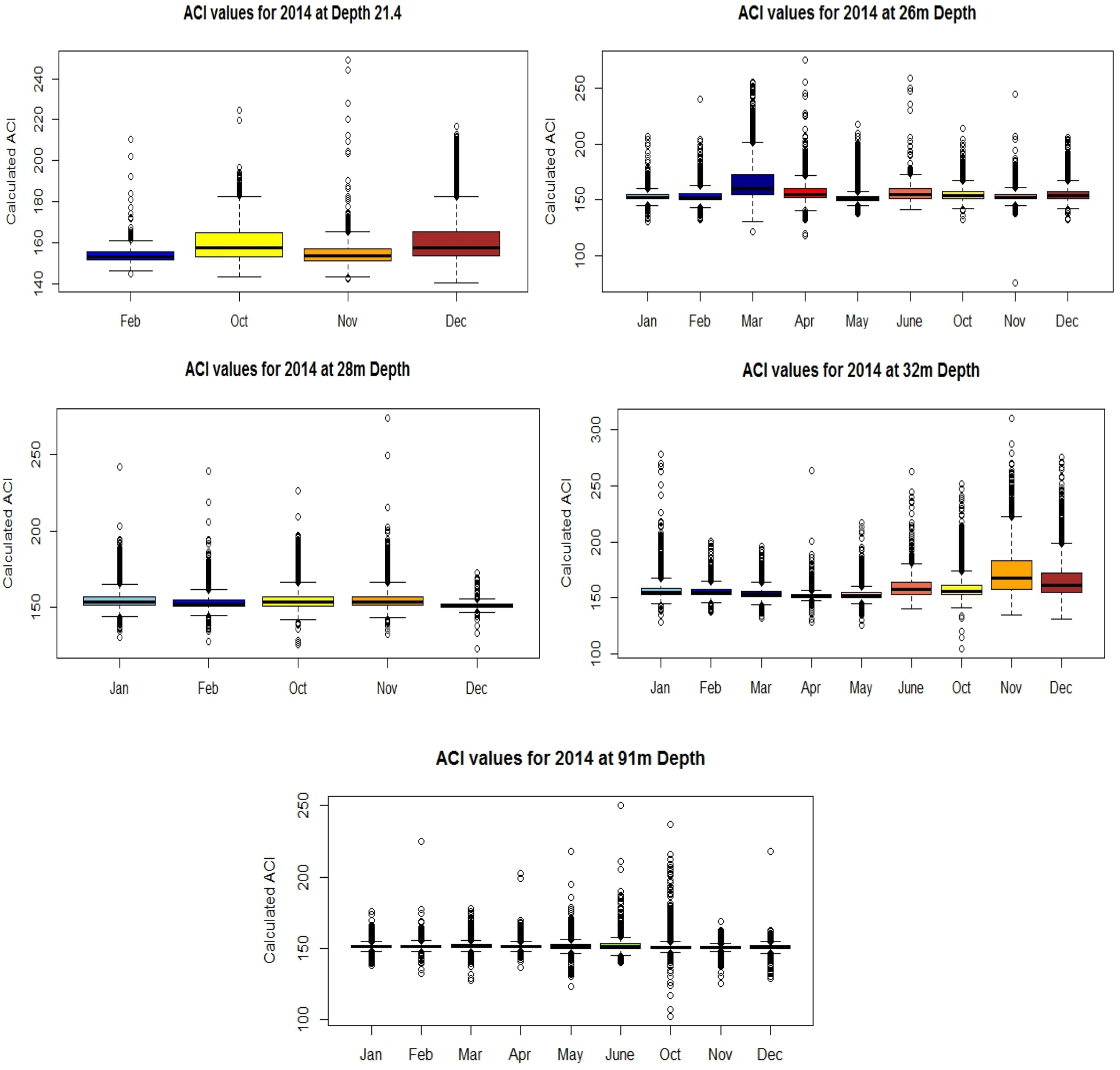

Figure 2. ACI values for each month separated by hydrophone location starting with hydrophone one (*top-left panel*) and going to hydrophone five (*bottom panel*). Data missing from hydrophones one and three were due to inability to retrieve the data or technical malfunction. Note the difference in the range ACI values on the y-axis for the plots.

3.2 Analysis of ACI variation

The use of acoustical indices is a growing field in soundscape studies as a quick way to quantify biodiversity in an area (Parks et. al. 2014, Pieretti et. al. 2011, Harris 2014, Harris 2016). The ACI in particular measures the variation in intensities over changing frequencies, and has shown potential to be useful in areas affected by constant anthropogenic noise and be used as a method of distinguishing biological activity from increased human activity (Farina et. al. 2011, Pierreti et. al. 2011, Harris 2016, Harris 2014). To test whether or not increases in ACI values were indicative of biological sounds rather than non-biological activity, peaks in ACI values for each hydrophone were identified and compared to spectrograms of the original audio files. Multiple months for each location were analyzed to further explain temporal variation observed in these values.

At hydrophone one, the highest ACI value was observed in November, and the distribution had a median of 153.5 and a maximum of 249.0 (Figure 2). In October, the range of ACI values observed was lower, and the maximum for this month was 224.6 while the median was 157.6 (Figure 2). To determine the cause of this difference in complexity values, the monthly plots were examined in Figure 3. In November, the ACI values remained consistent between about 145 and 180 for a majority of the month, but there was a large jump in values on the 22 (Figure 3). The values in October show a similar range for the majority of the month as November except for the 6 and 8 where the values increased drastically, especially on the 8 (Figure 3). The isolated daily plots for November 22 and October 8 both showed that the increase in complexity was a result of a short-term disturbance occurring in the afternoon (Figure 4). Aural and visual analysis of the spectrograms for these time indicated that a boat drove through the area on both days followed by an increase in fish vocalizations (Figures 5 and 6). Potential fish species identified in the audio files for November 22 were Grey triggerfish, Channel bass, Atlantic spadefish, Bluefish and Atlantic croaker (Figure 6). On October 8, the Atlantic croaker, Channel bass and Grey triggerfish were observed along with Black drum, but not Bluefish or Atlantic spadefish (Figure 5).

ACI values for hydrophone two showed higher values in April with a median of 155.2 and a maximum of 275.2 (Figure 2). Comparatively, values in November were lower with a median of 152.60 and a maximum of 244.50 (Figure 2). The monthly plots for April and November showed that April was more variable as it fluctuated between 275 and 120 throughout the month while November remained consistently between 140 and 200 except for a few outliers (Figure 7). The first day of both months was determined to be the first major increase in ACI values, and examination of the daily plot showed that the complexity for April 1 increased gradually from 05:00 to 13:00 before it decreased again while November 1 showed a singular, short duration increase in complexity (Figure 8). Aural and visual analysis of the audio files for April 1 showed no apparent cause in the increase, and potential fish species identified in the recordings were Black drum, Atlantic spadefish, Grey triggerfish, Bluefish and Atlantic croaker (Figure 9). On November 1, there appeared to be a lot of wind activity throughout the audio file and the increase in ACI values were the result of increased fish activity (Figure 10). Atlantic spadefish, Grey triggerfish and Black drum were also identified in the audio files for November 1, along with Gag grouper, and Sheepshead.

Looking at the greatest increase in ACI values on April 12 at hydrophone two showed a short-term peak around 22:00 and analysis of the audio file showed no apparent acoustic disturbance (Figures 11 and 12). There was heavy wind noise present in the audio files, but no intense, impulsive sounds or vessel activity (Figure 12). Potential fish species that were identified included Atlantic croaker, Gag grouper, Grey triggerfish, Channel bass and Sheepshead (Figure 12). The second large peak in November occurred on the 21 and was also a short-duration increase, but this was mostly likely the result of a boat passing through at that time (Figures 11 and 13). The potential fish species present based on aural and visual inspection of the audio files for November 21 also included Channel bass, Grey triggerfish and Sheepshead with the addition of Black drum and Gag grouper (Figure 13).

At hydrophone three, the highest value of 273.8 was seen in November with a median value of 153.5, while the maximum value for January was 241.7 and the median was 153.6 (Figure 2). Both monthly plots showed similar variability and in January one large increase in ACI values was observed on the 9, and the values for November increased substantially on the 5 (Figure 14). Analysis of the daily plots showed both these patterns were due to short-term increases in fish activity with no apparent acoustic cause based on analysis of the audio files (Figures 15, 16 and 17). The audio files for January did, however, show higher wind activity than November (Figures 16 and 17). The potential fish species identified in the audio files for January 9 were Black drum, Atlantic croaker, Atlantic spadefish, Grey triggerfish and Channel bass (Figure 16). On November 5, Black drum, Channel bass, Atlantic croaker and Atlantic spadefish were also identified as well as Sheepshead and Gag grouper (Figure 17).

Hydrophone four showed the highest ACI values of all the locations, with a maximum complexity value of 310.3 in November and a median value of 167.9 (Figure 2). The values for March were much less variable and had a median of 152.6 and a maximum of 195.6 (Figure 2). The monthly plot for March showed a few occurrences throughout the month where the ACI values increased, but they did not fluctuate as much or reach a value as high as November (Figure 18). The first major increase was observed on March 10 and November 5, both showing relatively gradual increases towards the end of the day around 22:00 (Figure 19). Inspection of the audio files for March 10 showed some vessel activity but it was relatively quiet compared to other boats detected throughout the recordings (Figure 20). The fish species identified in this file included Grey triggerfish, Black drum, Atlantic spadefish and Atlantic croaker (Figure 20). No vessel activity was found in the audio files for November 5, and the fish species identified included Grey triggerfish, Black drum, Atlantic spadefish and Atlantic croaker, as well as Black drum and Channel bass (Figure 21). The second increase in ACI values occurred on November 22, while there was a large decrease in ACI values on March 25 (Figure 22). There was no masking of biological activity on March 25 so the apparent cause for this decrease in complexity is unknown (Figure 23). Wind noise was present in the files and a few fish species like Atlantic spadefish, Black drum and Gag grouper were identified (Figure 23). On November 22 a loud vessel noise was observed in the audio files which was followed by increased vocalizations of Atlantic spadefish, Black drum, Gag grouper, Grey triggerfish, Atlantic croaker and Channel bass (Figure 24).

At hydrophone five, the greatest range of ACI values was observed in October where there was a maximum value of 236.7, a minimum of 101.9 and a median of 150.3 (Figure 2). November showed a much lower range of values with a minimum of 125.3, a maximum of 168.8 and a median of 150.3 (Figure 2). The monthly plots showed various increases in ACI values in October and one large decrease in complexity on October 17 (Figures 25 and 26). The values for November were generally lower than those in October, but there was also a large decrease which occurred on November 8 (Figures 25 and 26). Both these decreases were the result of vessel noise passing through the area that effectively masked biological activity (Figures 27 and 28). No fish species could be successfully identified from aural or visual inspection of the audio files for these two days.

Figure 29 shows spectrograms from four of the five locations at the same point in time. This image is used to illustrate a sound that was produced and picked up by multiple hydrophones at the same time. This has implications for analyzing the ambient and complexity levels of this environment, as the analysis treated each location separately when they may not be entirely distinct soundscapes.

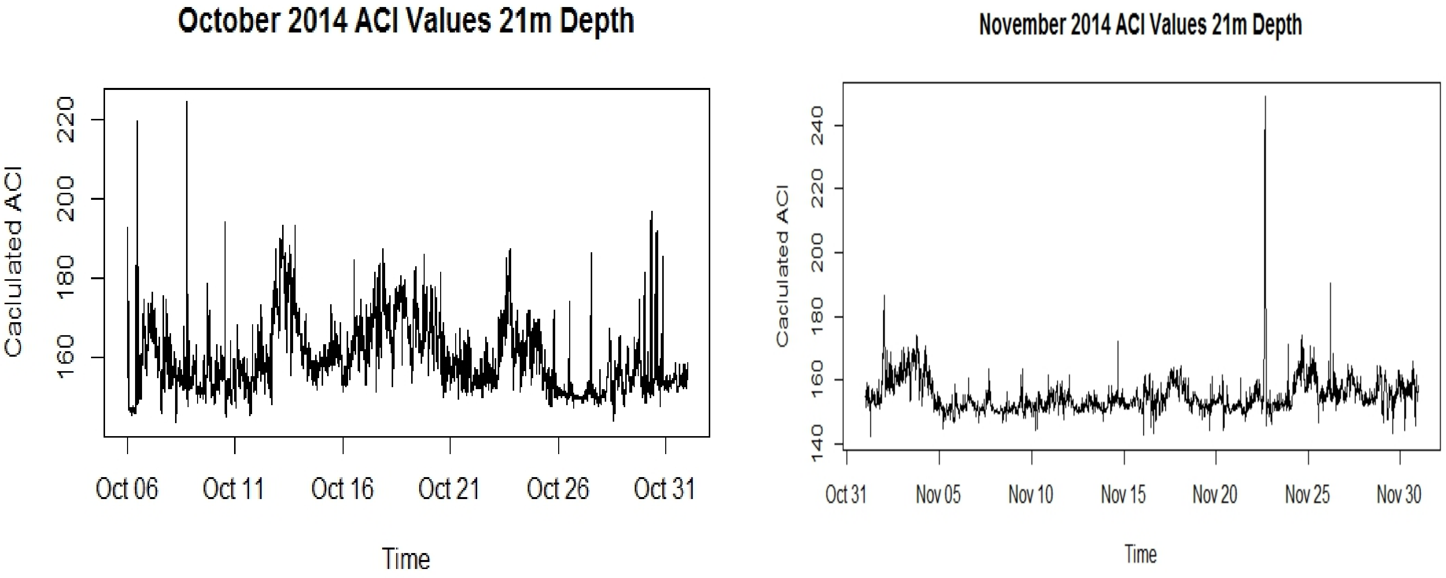

Figure 3. ACI Values for the months of October (*left*) and November (*right*) at hydrophone one. Note the difference in the y-axes. Values in October showed much more variability with large peaks occurring at the beginning of the month while November has relatively consistent with only one large peak towards the end of the month.

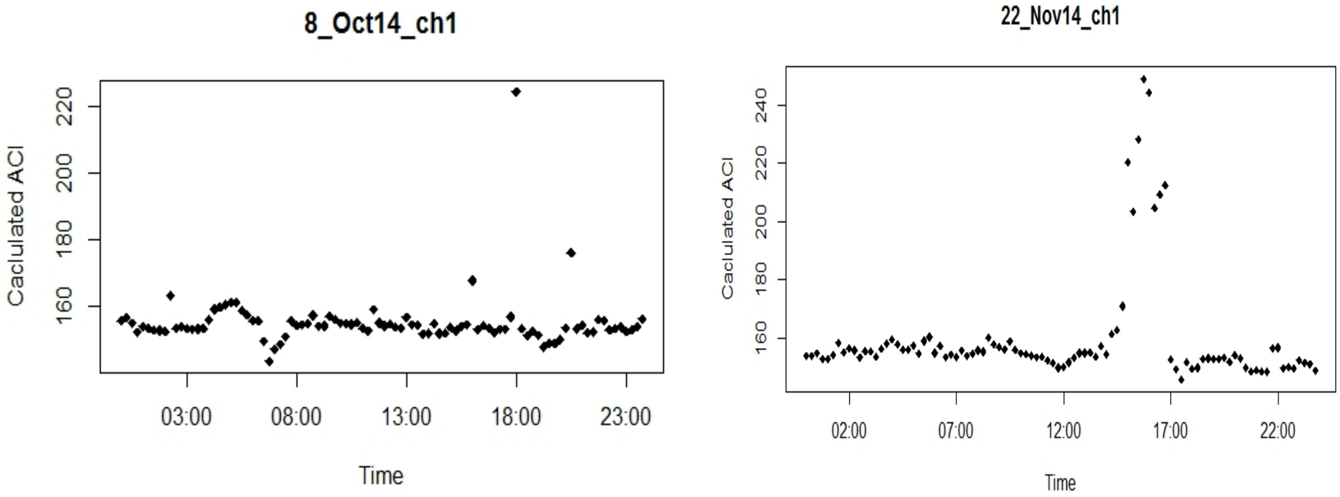

Figure 4. ACI values for the 8 of October (*left*) and the 22 of November (*right*) at hydrophone one isolated for further comparison of individual days. Note the difference in the y-axes. October 8 shows one large peak around 18:00 that rapidly decreased and November 22 shows a larger increase between 14:00 and 16:00.

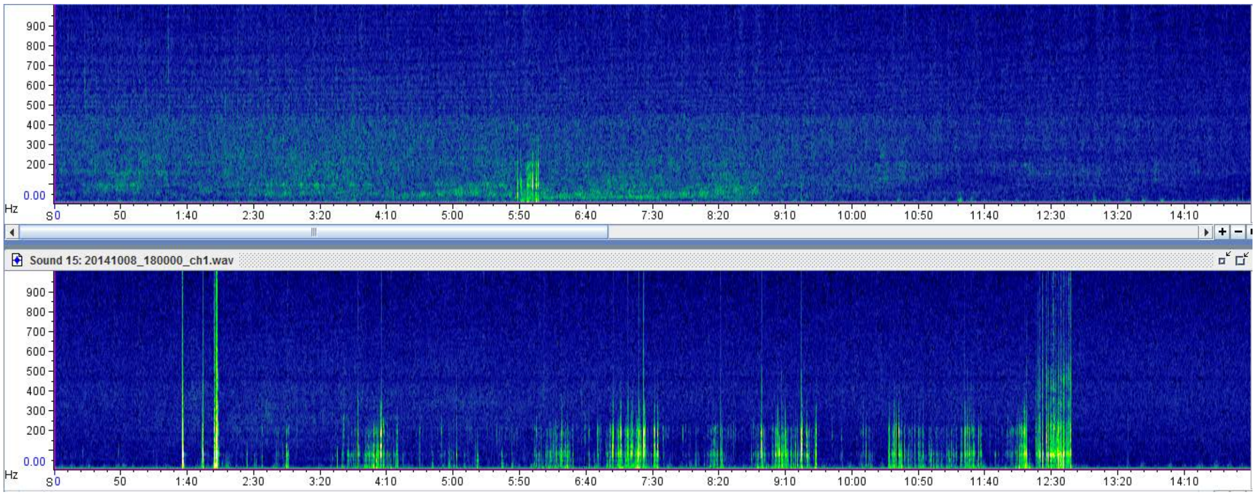

Figure 5. Spectrograms from the 8 of October at hydrophone one. The top image starts at time 17:45 and the bottom image starts at time 18:00. Vessel activity can be seen in the top image as the mostly horizontal band below 200 Hz followed by increased fish vocalizations in both spectrograms. Fish species present include Atlantic croaker, Black drum, Channel bass and Grey triggerfish.

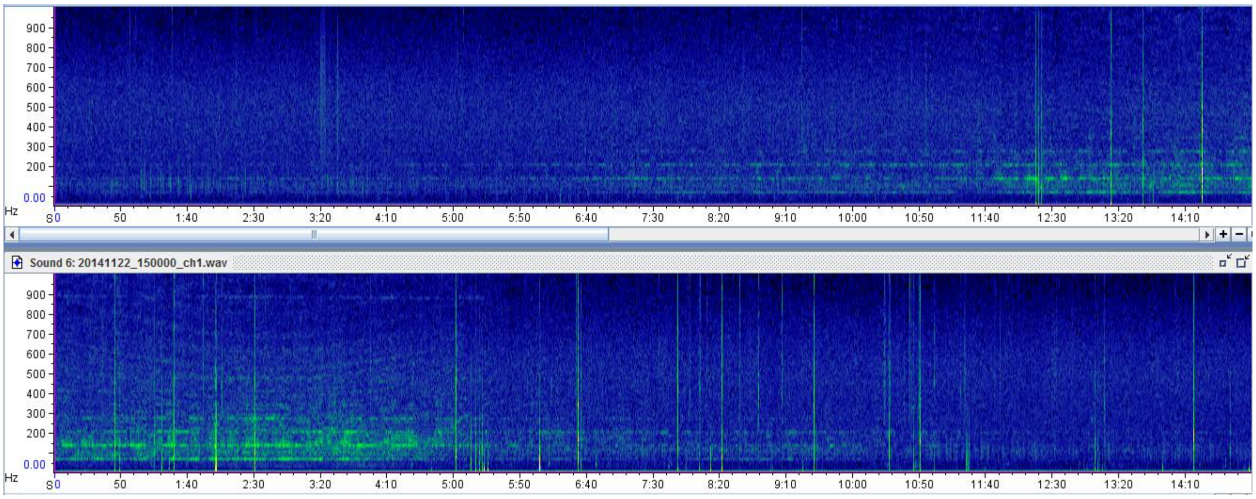

Figure 6. Spectrograms for the 22 November at hydrophone one. The top image starts at time 14:45 and the bottom image starts at time 15:00. Boat noise can be seen starting at the end of the top spectrogram through the bottom spectrogram along with increase fish vocalizations starting after the boat noise begins. Fish identified were Grey triggerfish, Channel bass, Atlantic spadefish, Bluefish and Atlantic croaker.

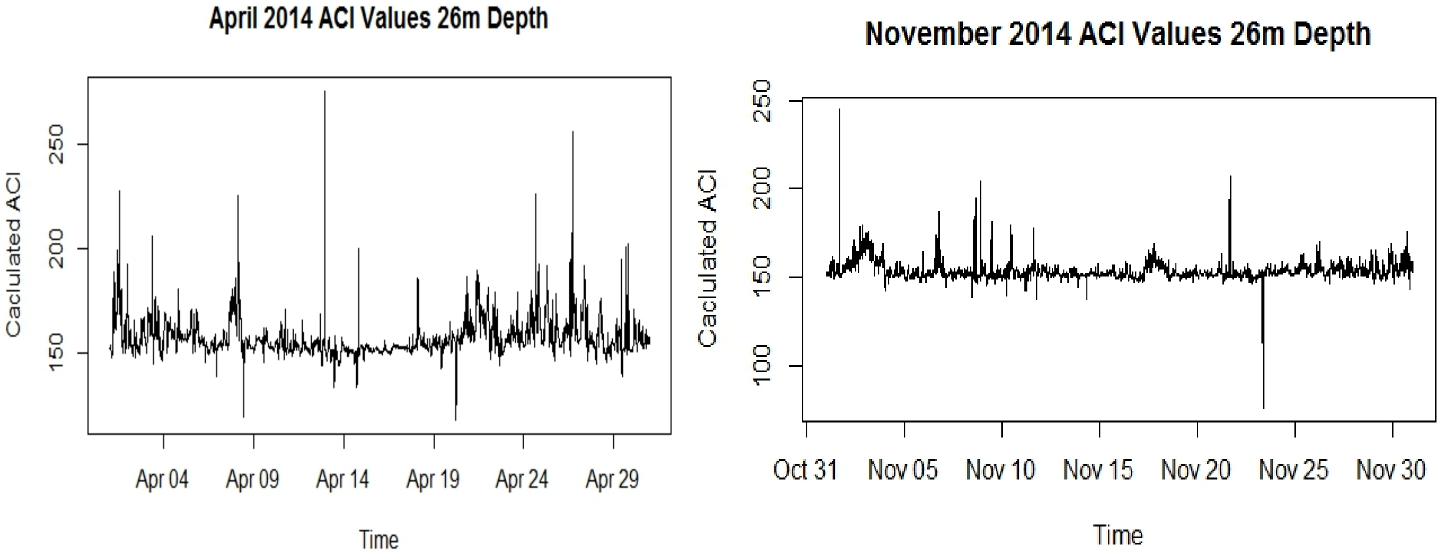

Figure 7. ACI Values for the months of April (*left*) and November (*right*) at hydrophone two. Note the difference in the range of ACI values on the y-axis. In April, multiple increases in ACI values can be observed throughout the month, most notably around the 1, 12 and 26. Values for November also show a few increases one the 1 and 21 as well as a large decrease around the 23.

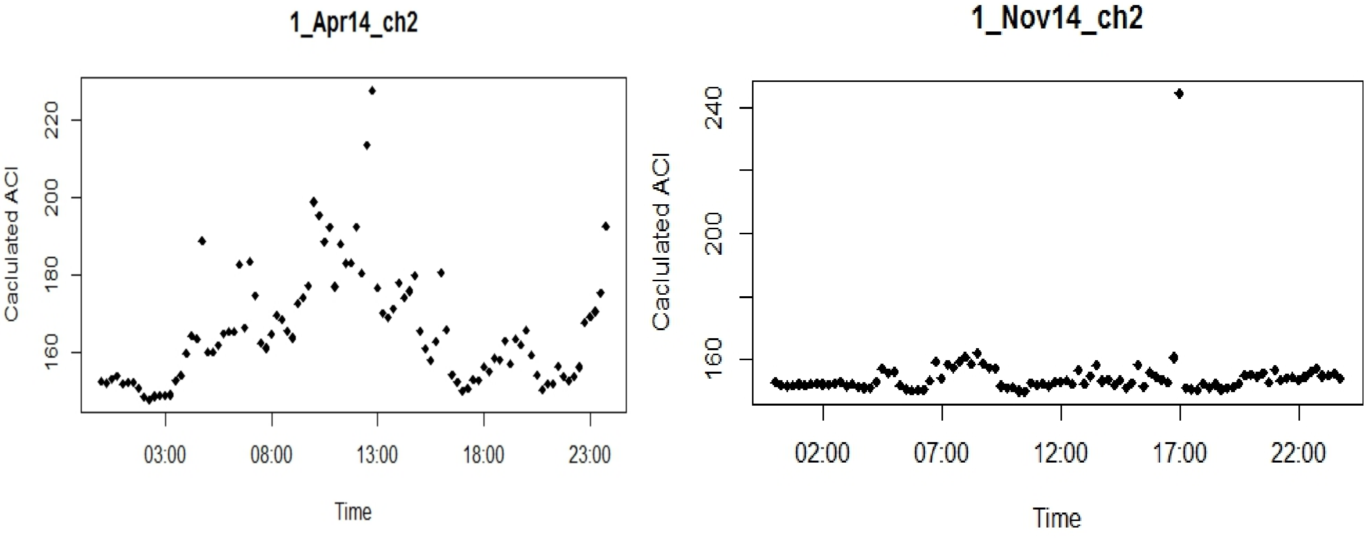

Figure 8. ACI Values for the 1 of April (*left*) and the 1 of November (*right*) at hydrophone two, isolated for further comparison of individual days. Note the difference in the y-axes. The increase in complexity on April 1 was relatively gradual, beginning to increase around 04:00 and decreasing again after 13:00. November 1 only showed one sharp increase in complexity between 16:00 and 17:00.

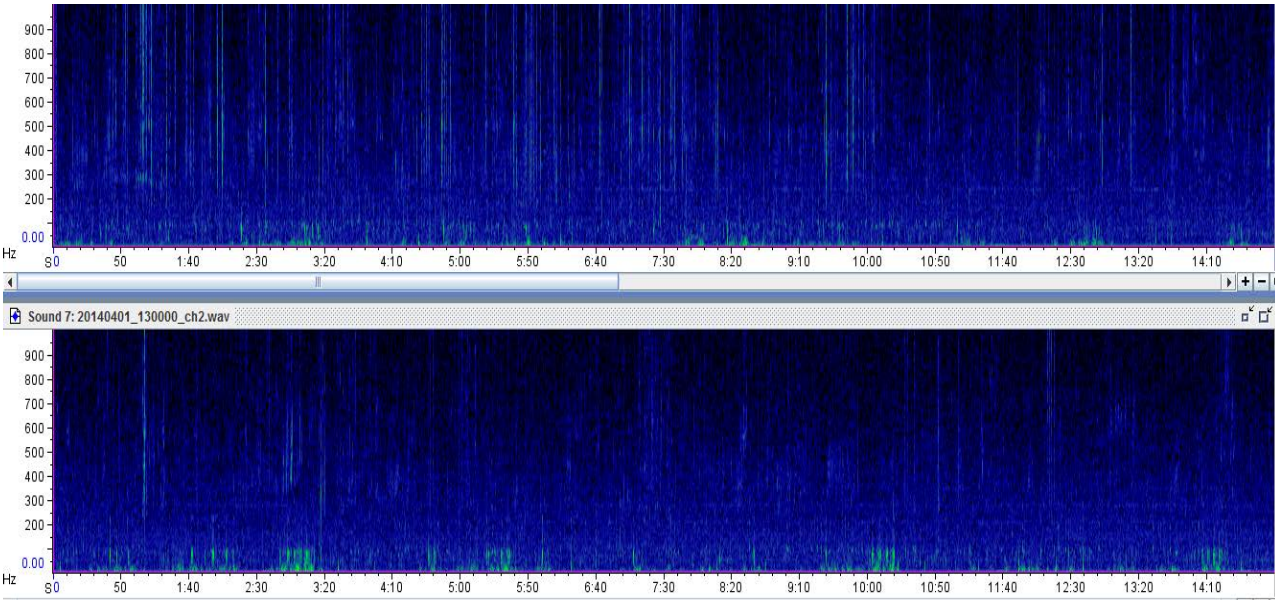

Figure 9. Spectrograms from the 1 April at hydrophone two. The top image starts at time 13:00 and the bottom image starts at time 13:15. Wind noise and fish vocalizations can be heard throughout the recordings but do not appear to be as intense as seen in other spectrograms. Fish present include Black drum, Atlantic spadefish, Grey triggerfish, Bluefish and Atlantic croaker.

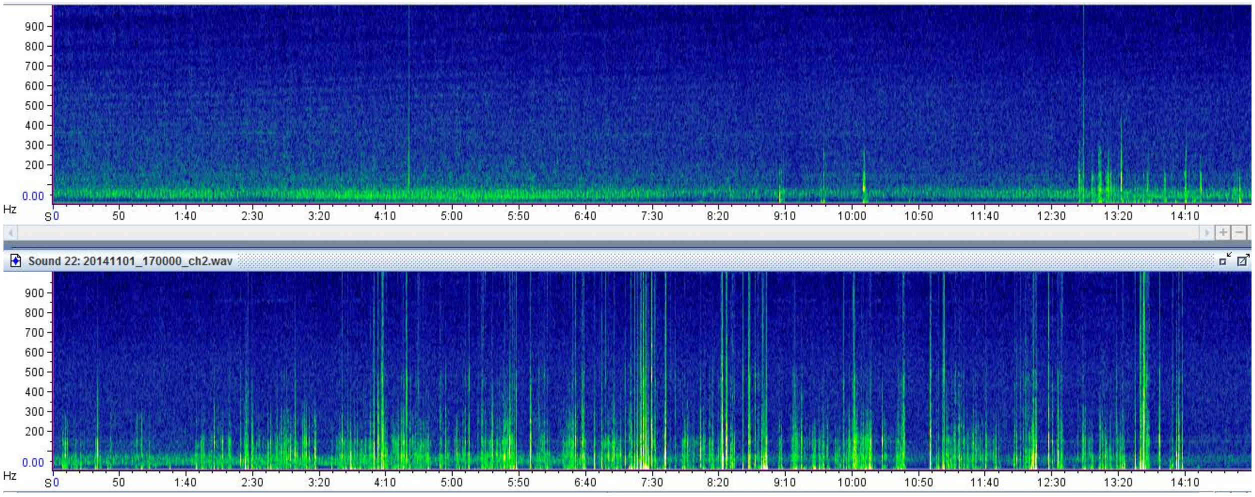

Figure 10. Spectrograms for the 1 of November at hydrophone two. The top image starts at time 16:45 and the bottom image starts at time 17:00. Loud wind noise can be seen throughout both spectrograms and fish noise increases starting at the end of the top spectrogram and into the second. Fish identified were Channel bass, Gag grouper, Atlantic spadefish, Grey triggerfish, Black drum and Sheepshead.

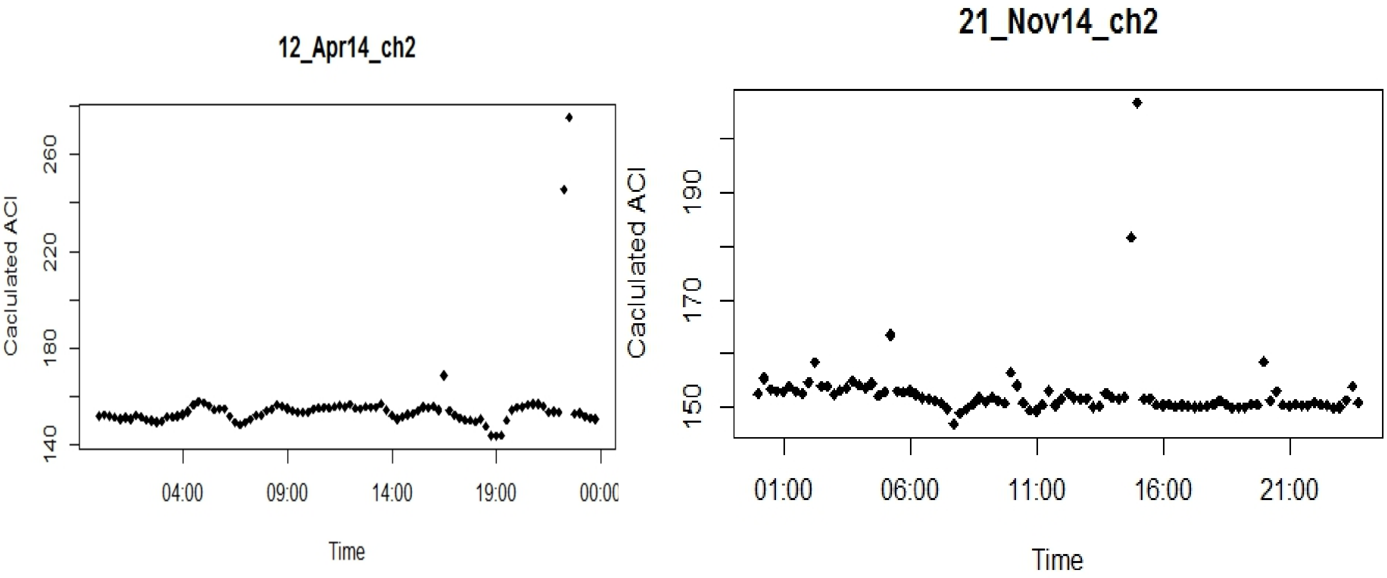

Figure 11. ACI values for the 12 of April (*left*) and the 21 of November (*right*) at hydrophone two, isolated for further comparison. Note the difference in the y-axes. A rapid increase in complexity values can be observed at the end of the day on April 12, around 22:00, while the increase in values in November was seen in the afternoon at approximately 14:00.

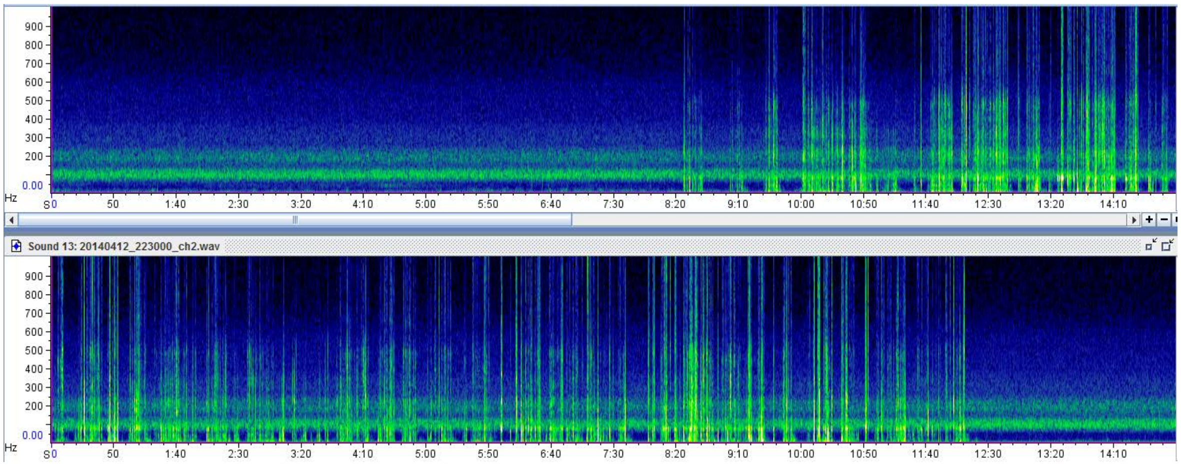

Figure 12. Spectrograms from 12 April at hydrophone two. The top image starts at time 22:15 and the bottom image starts at time 22:30. Heavy wind noise can be observed throughout both spectrograms while fish noise can be seen increasing towards the end of the first image and into the second. Fish species identified were Atlantic croaker, Gag grouper, Grey triggerfish, Channel bass and Sheepshead.

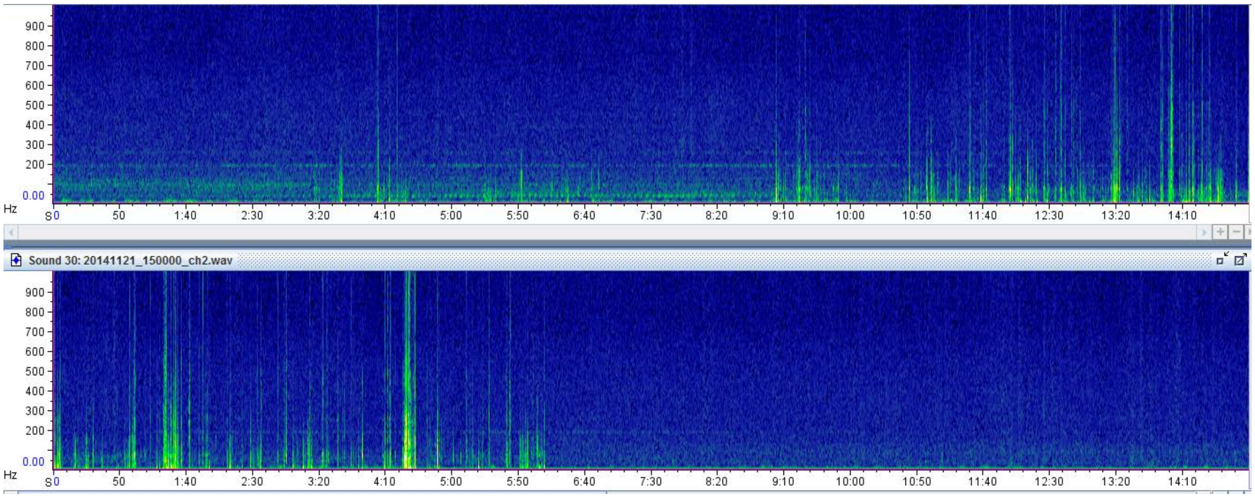

Figure 13. Spectrograms for the 21 of November at hydrophone two. Top image starts at time 14:45 and bottom image starts at time 15:00. Boat noise can be observed in the top spectrogram followed by increased fish vocalizations. Fish present were Black drum, Sheepshead, Grey triggerfish, Channel bass and Gag grouper.

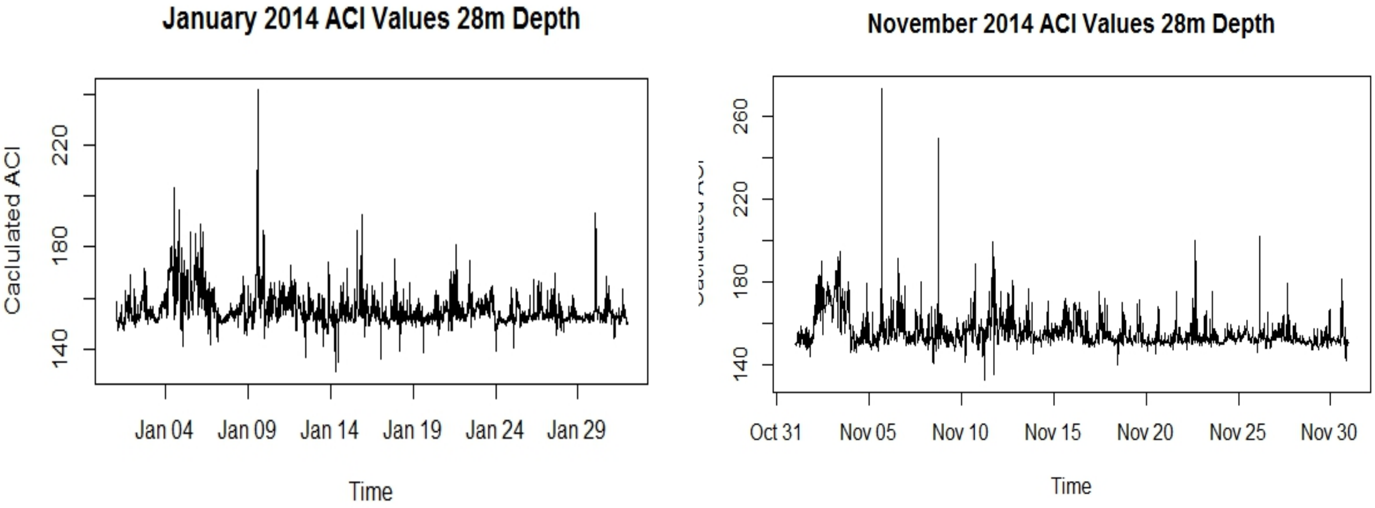

Figure 14. ACI Values for the months of January (*left*) and November (*right*) at hydrophone three. Note the difference in the y-axes. In January a sharp increase in values can be observed on January 9, while November shows two large peaks on the 5 and 8.

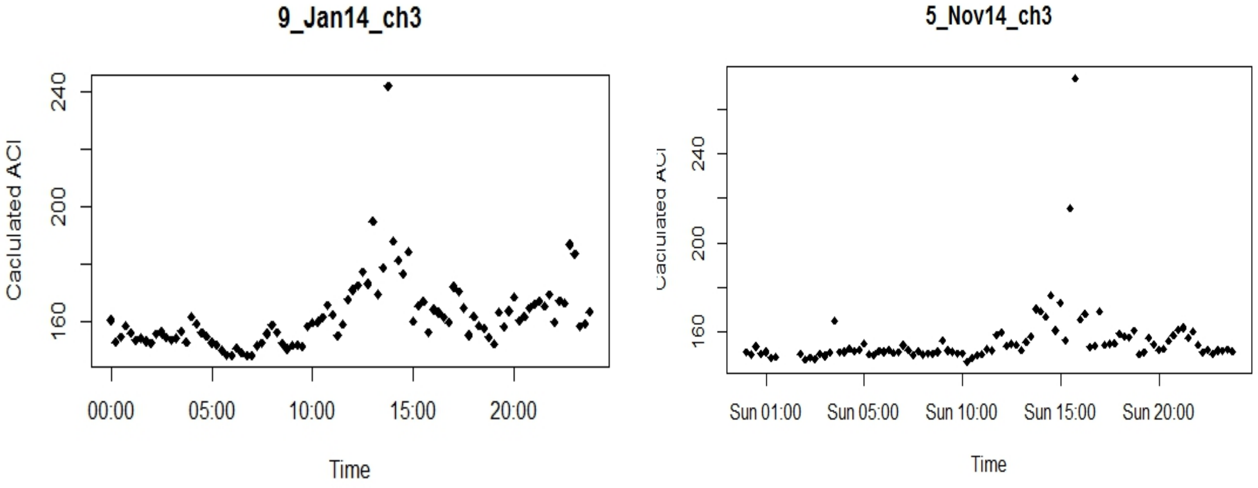

Figure 15. ACI values for the 9 of January (*left*) and the 5 of November (*right*) at hydrophone three, isolated for further comparison. Note the difference in the y-axes. ACI values increase on January 9 between 10:00 and 16:00 with a peak around 14:00 while the increase in ACI values in November occurred more rapidly between 13:00 and 16:00 with a peak around 15:00.

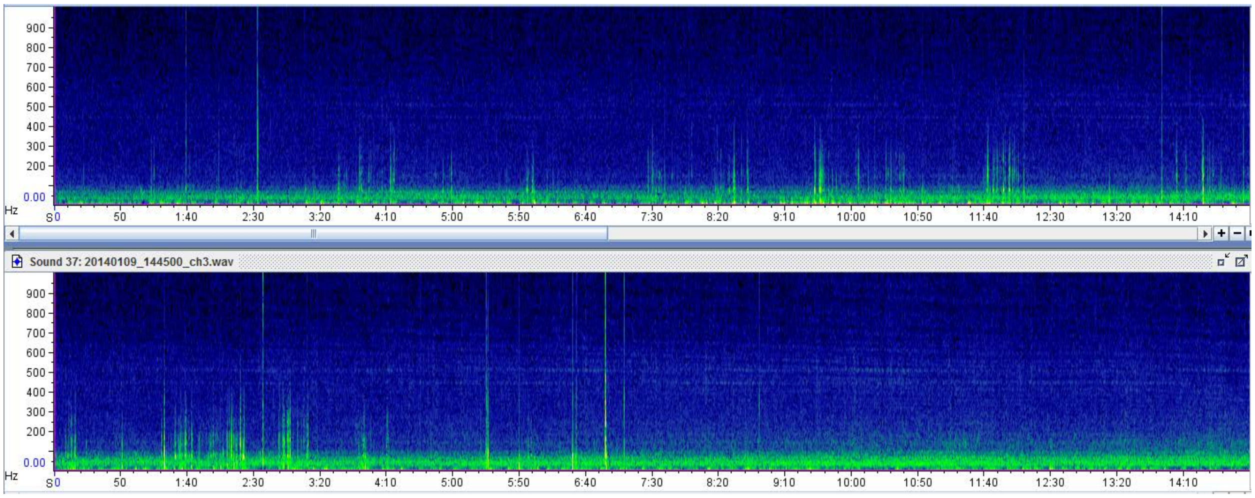

Figure 16. Spectrograms for the 9 of January at hydrophone three. Top image starts at time 14:30 and bottom image starts at time 14:45. Heavy wind noise and fish vocalizations can be seen in both spectrograms. Fish present included Black drum, Atlantic croaker, Atlantic spadefish, Grey triggerfish and Channel bass.

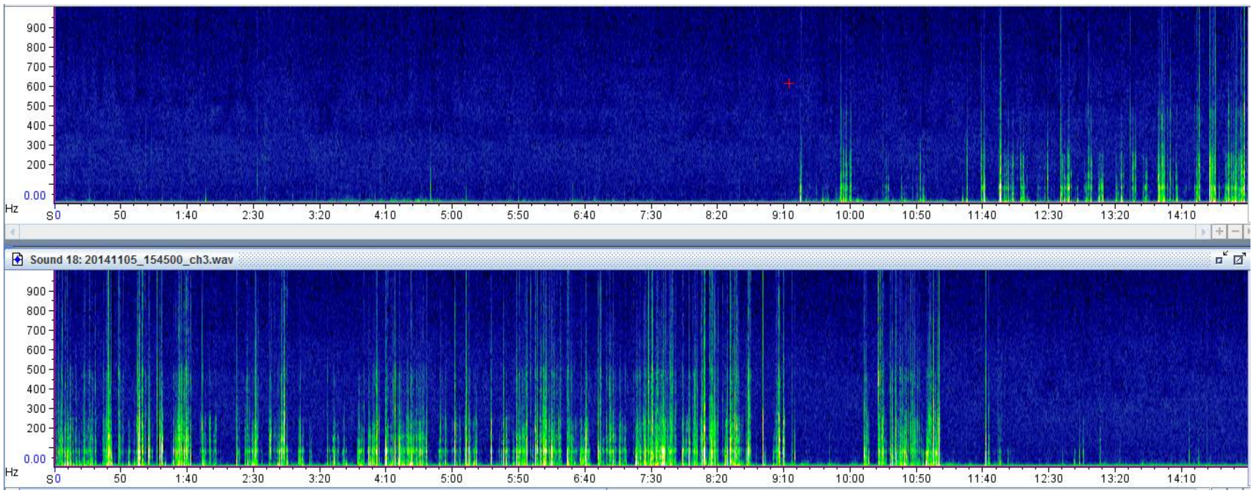

Figure 17. Spectrograms for the 5 November at hydrophone three. The top image starts at time 15:30 and the bottom image starts at time 15:45. Increasing fish noise can be observed starting at the end of the first spectrogram and continuing into the second. Fish identified were Gag grouper, Black drum, Atlantic croaker, Sheepshead, Channel bass and Atlantic spadefish.

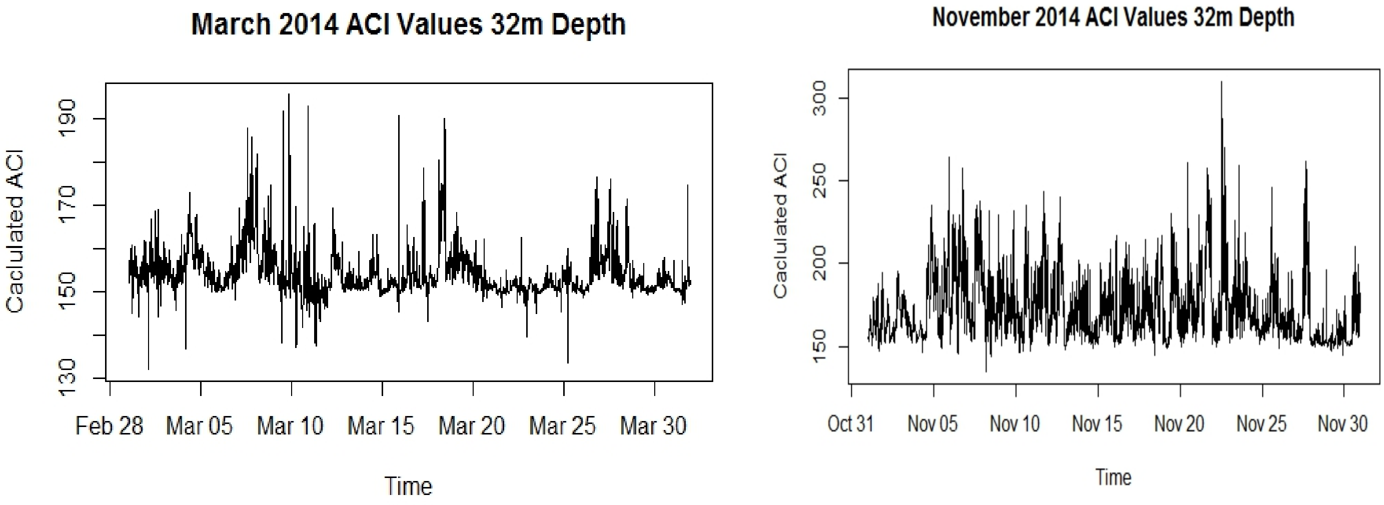

Figure 18. ACI values for the months of March (*left*) and November (*right*) at hydrophone four. Note the difference in the y-axes. Values for both months varied, but a few peaks in March can be seen around the 10 and 19 with sharp decreases at the beginning of the month and on the 25, while the largest peaks in values can be seen in November on the 5 and the 22.

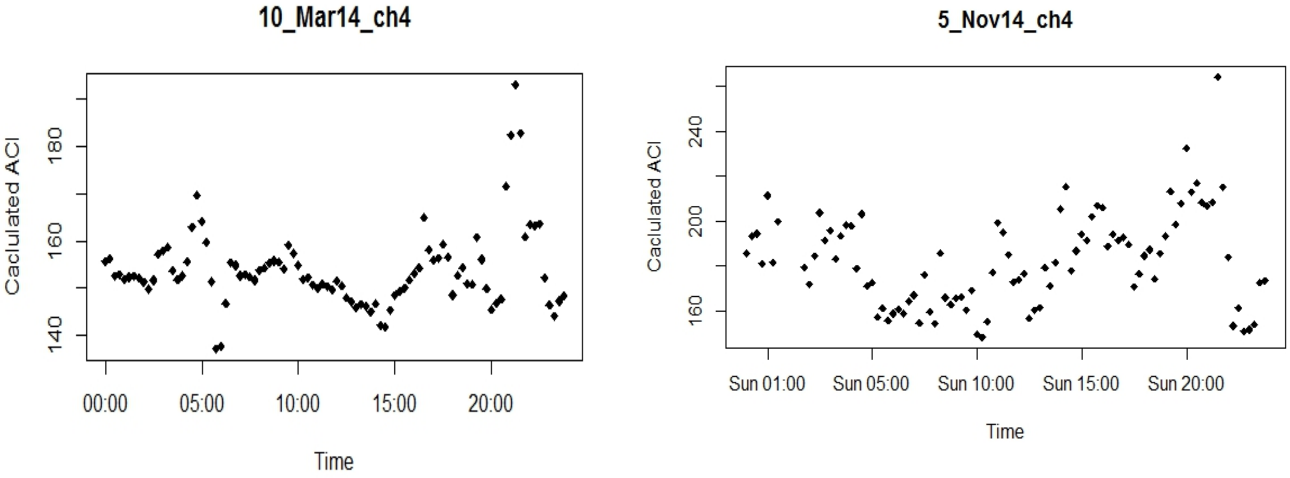

Figure 19. ACI values for the 10 of March (*left*) and the 5 of November (*right*) at hydrophone four, isolated for comparison. Note the difference in the y-axes. Values on March 10 increased rapidly at the end of the day around 21:00 while values for November fluctuated throughout the day and increased substantially around 21:00.

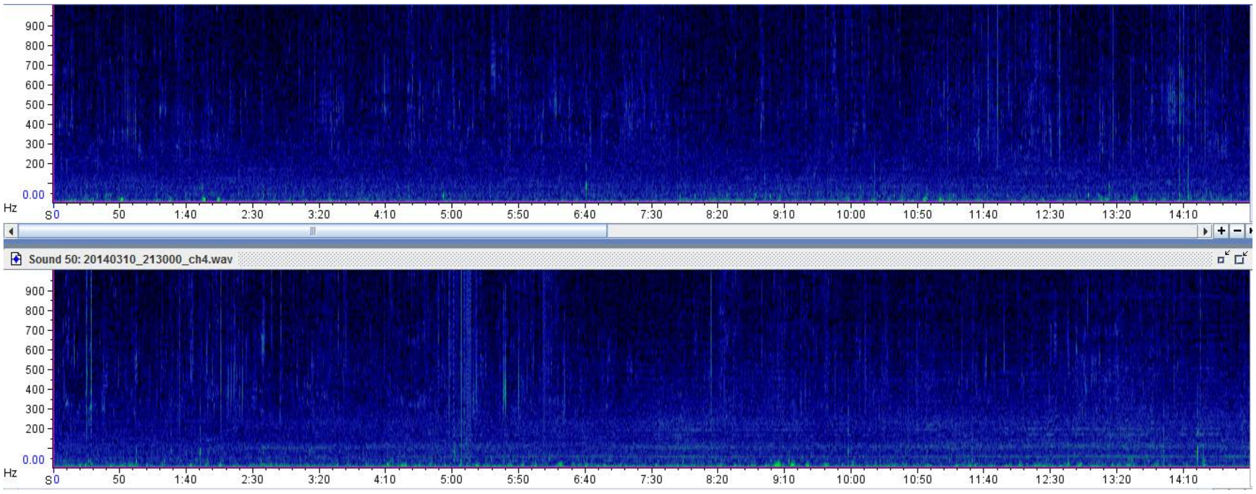

Figure 20. Spectrograms for the 10 of March at hydrophone four. Top spectrogram starts at time 21:15 and bottom spectrogram starts at 21:30. A few fish vocalizations can be seen throughout the spectrograms and some wind noise, although it less intense relative to other audio files examined. Fish identified were Grey triggerfish, Black drum, Altantic spadefish and Atlantic croaker.

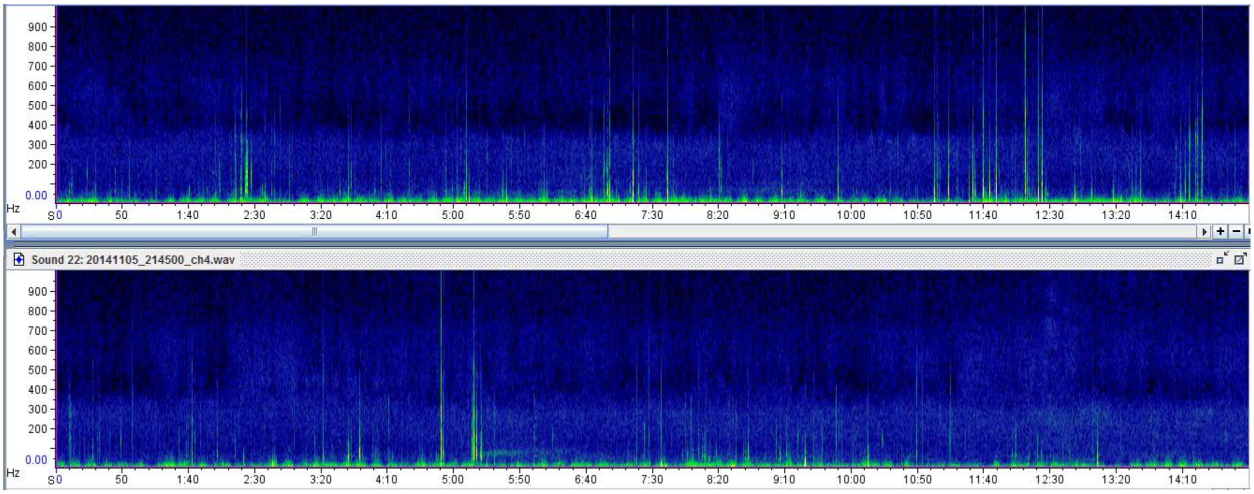

Figure 21. Spectrograms for the 5 November at hydrophone four. The top image starts at time 21:30 and the bottom image starts at time 21:45. Fish vocalizations can be seen throughout both spectrograms. Fish present included Atlantic spadefish, Grey triggerfish, Gag grouper, Black drum, Atlantic croaker and Channel bass.

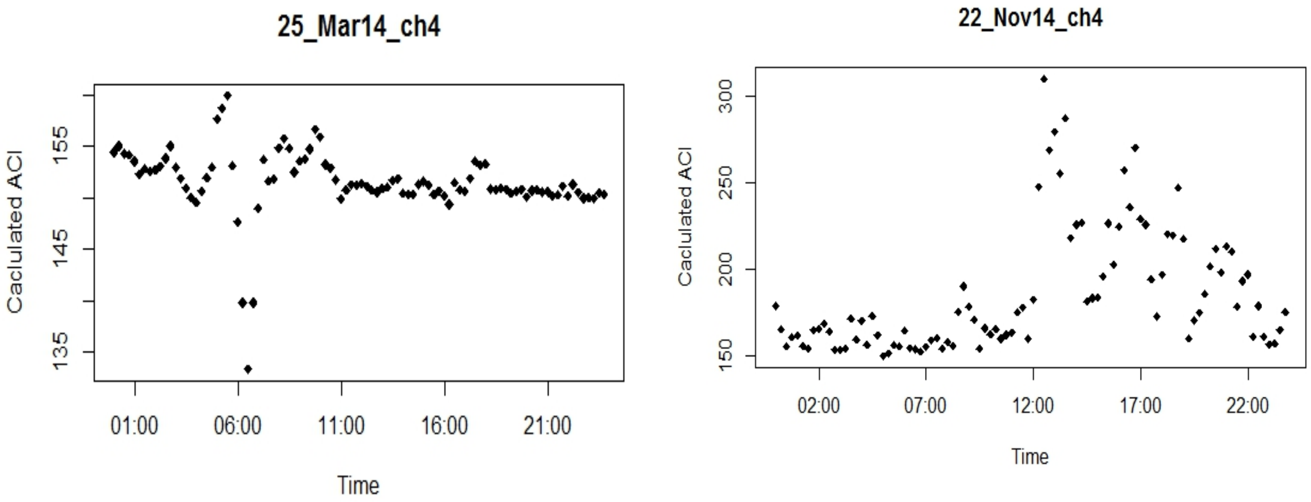

Figure 22. ACI values for the 25 of March (*left*) and the 22 of November (*right*) at hydrophone four, isolated for further comparison. Note the difference in the y-axes. A sharp decrease in values can be seen on March 25 between 06:00 and 07:00 while the values on November 22 increase drastically around 12:00 and gradually decline throughout the rest of the day.

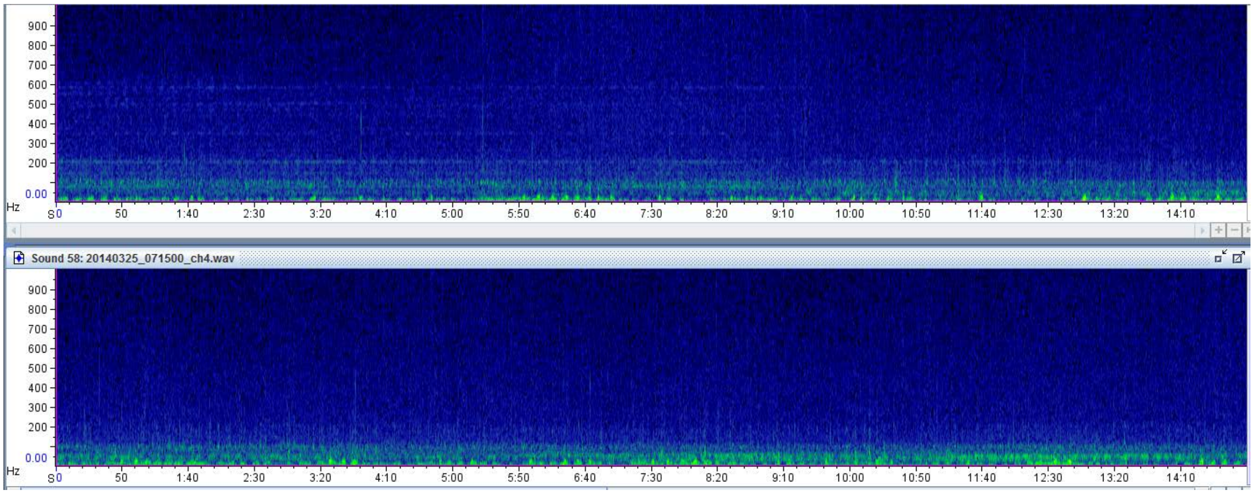

Figure 23. Spectrograms from the 25 of March at hydrophone four. Top image starts at time 07:00 and bottom image starts at time 07:15. Some fish vocalizations can be seen in both spectrograms. Fish present included Atlantic spadefish, Black drum and Gag grouper.

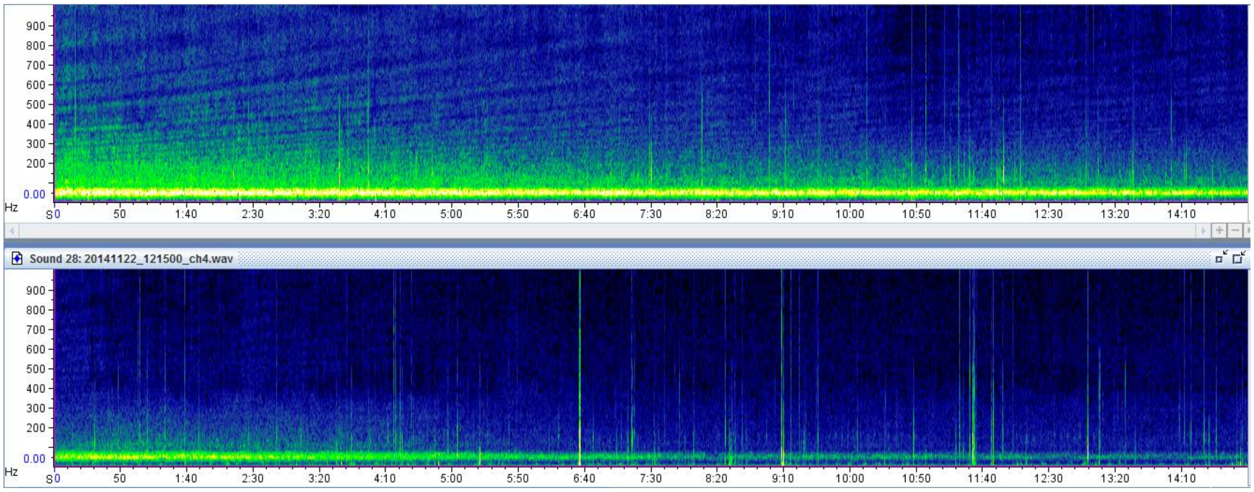

Figure 24. Spectrograms from the 22 November at hydrophone four. The top image starts at time 12:00 and the bottom image starts at time 12:15. Heavy boat noise can be seen starting in the top spectrogram and continuing through the second as the intensity decreases. Fish vocalizations can be seen after the intensity of the boat noise decreases. Fish identified were Black drum, Gag grouper, Atlantic spadefish, Channel bass and Grey triggerfish.

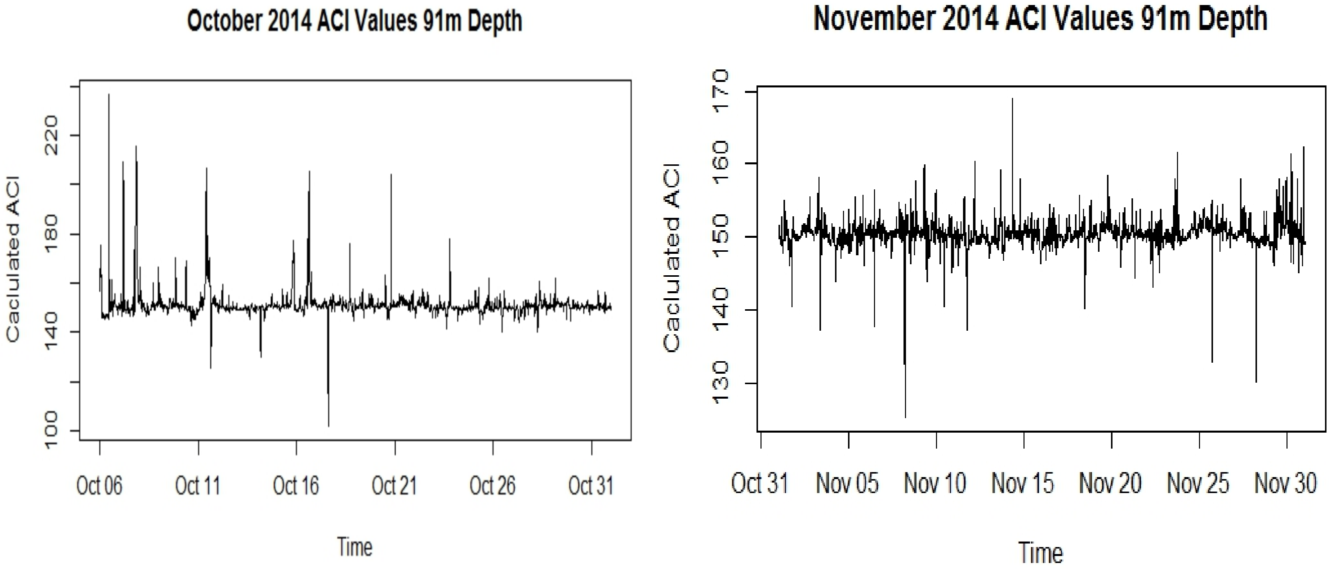

Figure 25. ACI values for the months of October (*left*) and November (*right*) at hydrophone five. Note the difference in the y-axes. Values for October showed increases at the beginning of the month, around the 12 and the 16 with a large decrease around the 17. Values for November showed one peak in values around the 14 with substantial decreases that can be seen on the 8, 25 and 28.

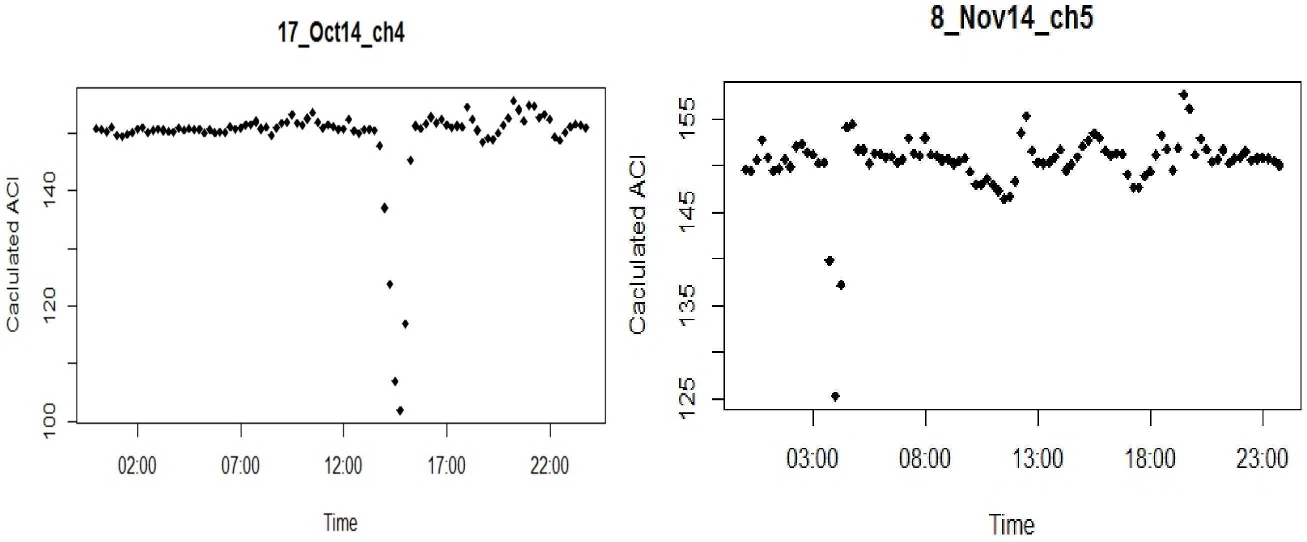

Figure 26. ACI values for the 17 of October (*left*) and the 8 of November (*right*) at hydrophone five, isolated for further comparison. Note the difference in the y-axes. The decrease in October occurred between 13:00 and 15:00 while the decrease in November occurred between 03:00 and 05:00.

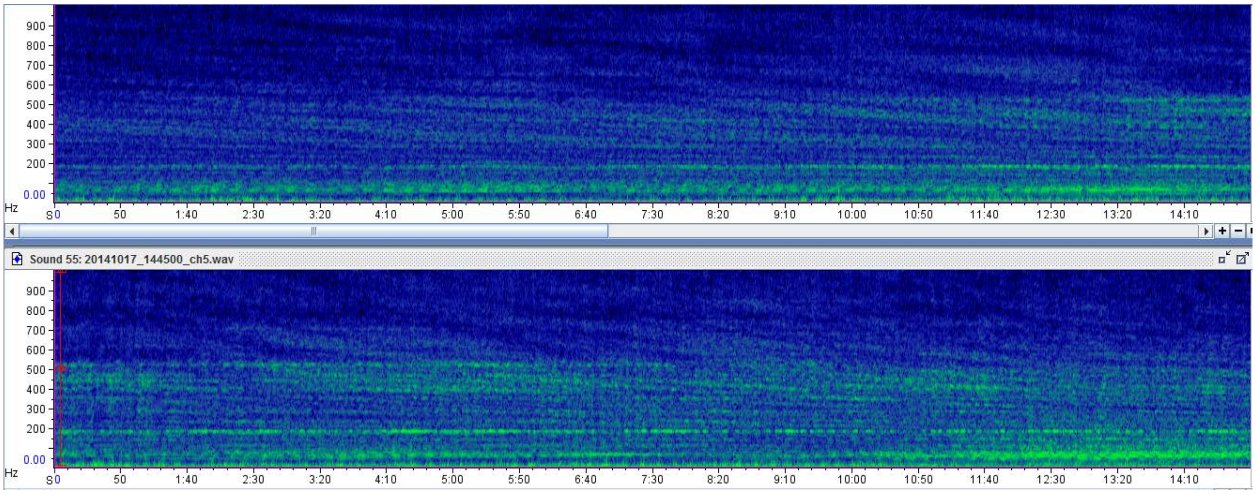

Figure 27. Spectrograms from the 17 October at hydrophone five. The top image starts at time 14:30 and the bottom image starts at 14:15. Vessel activity is present throughout both spectrograms and no fish noises could be identified.

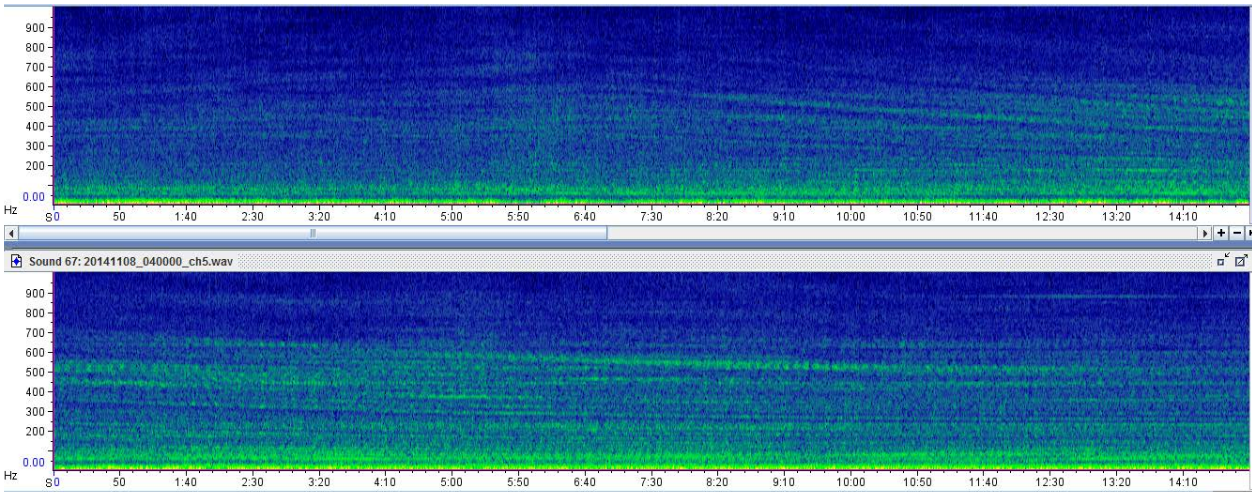

Figure 28. Spectrograms from the 8 November at hydrophone five. The top image starts at time 03:45 and the bottom image starts at time 04:00. Vessel activity is seen throughout both spectrograms and no fish noises could be observed.

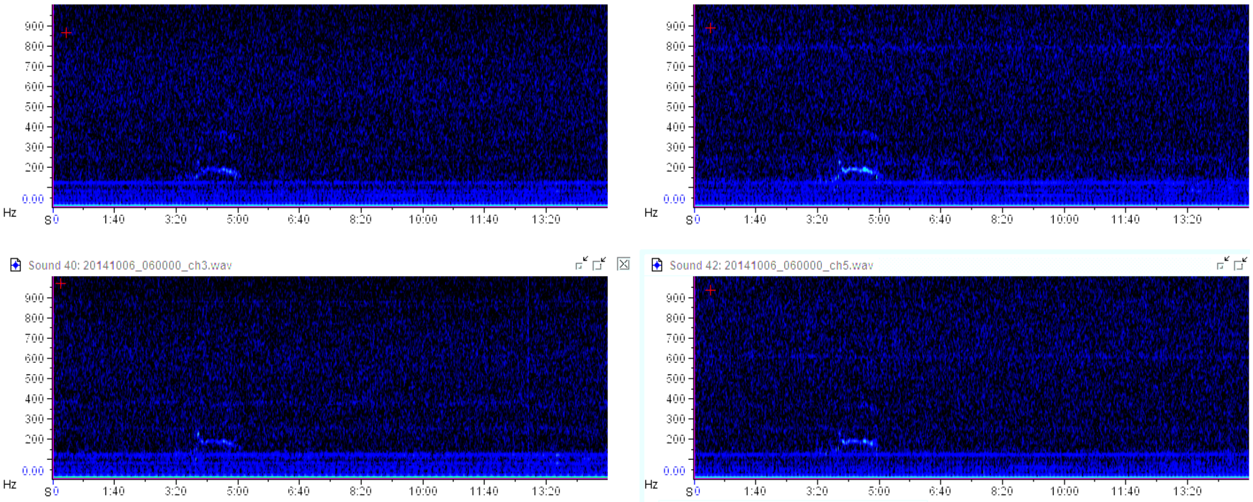

Figure 29. Spectrograms from the same point in time (06:00 on 4 October 2014) at four of the five hydrophones. As shown, the same noise, mostly likely from one of the whale species present in the study site, can be observed at the same time in the four hydrophones.

4. Discussion

Analysis of the sound files collected at this study site showed no obvious seasonal trend in acoustic complexity, but statistical analysis showed at least one month for each location varied significantly from the rest of the year (Figure 2). Also, analysis of the difference between locations for each month showed that at least one location varied significantly for each month (Figure 2). Because these data were collected originally to study North Atlantic right whale migration patterns, data were not collected from July to September so no pattern for the summer season could be obtained. Also, the months of October, June and February were not complete months as retrieval or deployment of the hydrophones typically occurred around these times so a few days at the beginning or end of the month had to be removed from this analysis. This reduced the overall information available to decipher seasonal or spatial trends in biodiversity in this area.

Biodiversity estimates were unable to be collected through on-site visual surveys for this project, but past surveys of marine mammals and fish in the area were collected from previous marine mammal surveys, stranding records, cetacean density models and fishing records available online (Garrison et. al. 2003, Marine Mammal Stranding Network of the North Carolina Central Coast 2016, Daniel 2014, Roberts et. al. 2016). The North Atlantic right whale, fin whale, minke whale, and humpback whale are all migratory species that travel south in the winter and north in the summer, and based on density maps created by the Marine Geospatial Ecology Laboratory at Duke University, these species would be expected to pass through this area at the end of October and November when they are travelling to their summer breeding grounds, and sometime between May and June when they begin their migration up north to the winter feeding grounds (Roberts et. al. 2016).

Where they are located within these coastal waters as they are migrating is still unknown, however, as studies have primarily focused on when the whales are travelling. Modelled data for the North Atlantic right whale have shown that the likely migration corridor for these whales remains close to shore, but no information is provided on exactly how close to the shore they go (Firestone et. al. 2008, Roberts et. al. 2016). Data on the other whale species examined in this project is also provides little information on how shallow these animals may travel, so for the purposes of this project is was assumed that they would not venture into very shallow waters and would likely be detected more clearly by hydrophones four and five (see Figure 1 for hydrophone locations).

Given what is known about when and where whale species would be in the context of this study area, an increase in ACI values starting in October and November resulting from migrating whale species was expected. While whale vocalizations were not the primary cause of the peaks in ACI values, the average ACI values increased for months in the fall which may be caused by whale migrations. This is further verified by the fact that this pattern was observed at the hydrophones located in deeper waters, and fish species that reside primarily offshore spawn in the spring and summer so they would not be expected to increase their acoustic activity during this season (Daniel 2014).

For fish species, seasonality of the fish in this region are well documented for fishing purposes, but given the number of different species present, no single time during the year could be identified where fish activity was expected to be greater (Daniel 2014). Because fish vocalizations have been correlated with spawning activity, this was used as the most likely explanation for any seasonal variability in fish acoustic activity for this project (Mann 2012, Staaterman et. al. 2014, Slabbekoorn et. al. 2010). Most fish species have large spawning events in the summer, but some species' spawning events can reach their peak in the fall (Daniel 2014). Atlantic Spadefish and Channel bass were two species present during most of the months and locations analyzed that spawn primarily in the spring and summer, so as a result the ACI values for these months would be expected to increase (Daniel 2014). With this information, acoustic complexity levels were expected to increase beginning in April, a pattern that can be strongly observed at hydrophone two and is also present at hydrophones four and five (Figure 2). The data gaps at hydrophones one and three are the mostly likely explanations for this pattern not being observed in the remaining locations, as data for the months expected to experience this increase were not available (Figure 2). There is also no information on acoustic activity in the summer, which reduced the ability to identify a pattern.

Just as seasonality of the spawning activity varies between fish species, the spatial positioning of the fish throughout the year also varies (Daniel 2014). Fishing records in North Carolina have identified where fish spend most of their time and when they are likely to move inshore or offshore for spawning, or migrate out of the area, but the exact depth range for some of these species is still unknown (Daniel 2014). For this project, it was assumed that coastal species wouldn't go past the continental shelf break, making it feasible for these species to be detected at all hydrophone locations since they are all within the coastal shelf (Figure 1). Most of the fish identified in this project, however, eat primarily benthic invertebrates so it is likely they would stay in shallower waters closer to the coast and less likely that they would be detected by the hydrophone in 90 meters of water (Daniel 2014). This could explain the peak in acoustic complexity observed at hydrophone two in April that was not observed to the same extent in the deeper water hydrophones (Figure 2).

There was no substantial difference between the species identified at each location. One fish species, Sheepshead, was only detected by hydrophones located in shallow waters below approximately 30 meters, which is consistent with what is known of their life history traits. These are primarily coastal fish that prefer to live around structured habitats in shallow waters (Daniel 2014). There was also much less fish activity present at hydrophone five which was located in approximately 90 meters of water. The increased ACI values observed were potentially sounds produced by Gag grouper or Amber jack, but the intensity of these sounds and how often they were observed was much less than recordings from other locations making them difficult to identify. There was also more vessel activity observed at this location which resulted in the masking of most fish vocalizations present.

Species identification of fish based on vocalizations was done through comparison to recording available through DOSITS and Macaulay Library, online databases that contain numerous recordings of fish sounds (The Cornell Lab of Ornithology 2017b, Discovery of Sound in the Sea 2016). These collections of animal audio clips are very helpful for identifying acoustic signatures for many species, but this method is not 100% accurate. Many fish produce similar noises in similar frequency bandwidths, so it is not always easy to distinguish between species. Fish may also produce multiple vocalizations used for different purposes which can interfere with the accuracy of the aural identification. Many of the sound clips used for identification recorded fish in captivity where the hydrophone was positioned very close to the animal of interest and there was no background noise to alter the sound in anyway (The Cornell Lab of Ornithology 2017b).

The use of acoustic indices could also introduce some bias in the results. While efficient, indices compress a vast amount of soundscape information into a single number, which increases the risk of missing short duration acoustic events (Parsons et. al. 2016). In this project, the ACI was calculated for a full 15 minute file, which was appropriate for analyzing temporal changes over a full year or month, but also potentially removes information in the process. Most of the sharp peaks in acoustic complexity examined in this paper were the result of some sort of disturbance, either from vessel activity or possibly predation, but these trends were only observed when data were examined at a much smaller temporal scale.

Results of this study showed significant differences between months and hydrophone depths, but it is possible that some sounds observed were not isolated to one hydrophone, as apparent in Figure 29. Sound, especially in this low-frequency bandwidth below 1000 Hz, travels efficiently underwater and has the potential to carry over great distances in deeper waters (NRC 1994, Lammers et. al. 2008, Hatch et. al. 2008). While this effect is not seen in very shallow waters, there were sounds detected by multiple hydrophones at the same point in time at the hydrophones further away from the coast (Figure 29). This could impact the quantification of acoustic diversity and ambient sound levels detected by certain hydrophones, and could cause misinterpretations of the biological behavior being predicted in this habitat based on these recordings.

5. Management Implications and Future Work

Results of this study have shown the usefulness of the Acoustic Complexity Index in monitoring acoustic activity over time, and what these values could indicate about biological activity. The ACI is a good metric for between site comparisons of biological activity in acoustic environments, and can be used to show acoustic diversity versus just species diversity (Harris 2014, Bertucci et. al. 2016, Harris 2016). This could be a useful tool for managing habitats because knowing how organisms use their habitat and when they are most acoustically active can provide valuable information for regulating anthropogenic impacts. Especially as noise pollution becomes a greater concern, knowing the times throughout the year when organisms are utilizing sound for reproduction, foraging or social communication can help reduce the effects of masking on these organisms. Some mitigation measures used to reduce the impacts of noise pollution include time/area closures, so information regarding seasonal and spatial uses of the environment can be used to determe when and where this is appropriate (Gedamke et. al. 2016). Recent work has also shown that North Atlantic right whales in particular may use North Carolina as more than just a migration corridor, so if this region were to be designated as critical habitat for these species, knowing how they are using this habitat and when they are most acoustically active is extremely important for the protection of this species (Johnson et. al. 2017).

The ACI has also demonstrated the potential impacts of masking. An example of masking of biological activity by anthropogenic noise pollution was demonstrated in Figures 27 and 28, where the complexity values decreased substantially, and aural inspection of the audio files revealed that the only distinguishable noise was that of the vessel passing through the area. The fact that this phenomenon was observed multiple times at this location highlights how important it is to monitor anthropogenic noise inputs and the effect they have on the marine organisms in this area. Vessel activity was observed at all locations throughout all times of the year, most likely originating from the local fishing ports where charters can be rented throughout the year for inshore and offshore fishing (National Parks Service 2016, Big Tahuna Sport Fishing 2017, Oregon Inlet Fishing Center 2017). The two major commercial ports in North Carolina are located in Morehead City and Wilmington, two locations further south than the study site, but there are a number of ships that pass by Cape Hatteras on their way up and down the East Coast, so particularly in deeper waters, this activity would still likely have an effect on the organisms in the region (North Carolina Ports 2017).

Future work in this area would benefit from a closer examination of ambient noise levels and sound propagation throughout the region. Recordings of the acoustic environment as well as information about the physical characteristics of the environment would increase knowledge of how sound is travelling through these waters and what sound sources are of the greatest concern (Hatch et. al. 2012). Collecting long term data and analyzing it with either the equivalent continuous sound levels (LEQs) or Sound Pressure Levels (SPLs) is an efficient way to characterize changes in ambient sound levels over time (Barber et. al. 2011, Heenehan 2016, Harris 2014). LEQs can be used to calculate a single value which represents the sound level over a given time period that is equivalent to the unsteady sound levels of the original recording over the same time period (Barber et. al. 2011, Heenehan 2016). SPLs are a representation of the root-mean-square sound level within a particular time period and frequency bin that are commonly used to express the amplitude of sound (Merchant et. al. 2015, Staaterman et. al. 2014, Harris 2014). These metrics allow easy examination of sound levels over relatively large temporal scales and can be used to identify higher sound levels that typically correspond with anthropogenic activity (Heenehan 2016).

To fully examine anthropogenic impacts, more information is needed to determine the how noise can impact fish. Studies have shown altered behavior in response to increased noise, and results examined in this project verify these conclusions, as there was increased fish activity observed after a boat drove through the site (Slabbekoorn et. al. 2010). This could be coincidence or response to something else not detected by the acoustic recordings, but regardless more information is needed to determine what is causing this response, and if this increased vocalization intensity and frequency has any consequences for organism health. Little information is known about the ability of organisms to detect, classify and localize sounds, and this has been studied somewhat in marine mammals, it remains unknown for other species and could help explain why this response was observed (Thomas 2012).

Acknowledgements

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