Monitoring Marine Mammals in Onslow Bay, North Carolina, Using Passive Acoustics

by

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Department of Environment
Duke University

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Laela Sayigh

Dissertation submitted in partial fulfillment of
the requirements for the degree of Doctor of Philosophy in the Department of
Environment in the Graduate School
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ABSTRACT

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Abstract

Passive acoustic monitoring is being used more frequently to examine the occurrence, distribution, and habitat use of cetaceans. Long-term recordings from passive acoustic recorders allow the examination of diel, seasonal, and inter-annual variation in the occurrence of vocalizing marine mammals. With the increased use of passive acoustics as a tool for studying marine mammals, the ability to classify calls to the species level is becoming more important. While studies have found distinctive vocalizations in some cetacean species, further work is required in order to differentiate the vocalizations of delphinid species. I sought to classify odontocete vocalizations to species and to describe temporal variation and depth-related differences in the occurrence of cetacean vocal events detected in archival passive acoustic recordings in Onslow Bay, North Carolina. To determine if odontocete species in offshore waters of North Carolina could be distinguished by their whistles and clicks, I used a towed hydrophone array to make acoustic recordings of species encountered during concurrent visual and acoustic surveys between 2007 and 2010. I recorded whistles from four species (Atlantic spotted dolphins, bottlenose dolphins, rough-toothed dolphins, and short-finned pilot whales) and clicks from five species (Risso's dolphins in addition to the four species listed above). After running a classification and regression tree (CART) analysis on 22 measured variables from the contours of four species' whistles, I generated an optimal classification tree that had a correct classification rate of 74.2%. My results indicate that species-specificity exists in the four species' whistles. My examination of the spectral structure of clicks showed that only Risso's dolphins produced clicks with distinctive spectral banding patterns, although I found that other click parameters, particularly peak and center frequency, might be useful in differentiating the other species. I then used the distinctive banding pattern that I observed in Risso's dolphin clicks to identify this species in recordings made by archival passive acoustic recorders that were deployed at various times and locations between 2007 and 2010. I used these recordings to determine how vocal events varied temporally and spatially in Onslow Bay. My analysis of vocal events observed in these recordings showed that Risso's dolphins, sperm whales, and other delphinids are present in Onslow Bay throughout the year; *Kogia spp.* occur sporadically; and fin and minke whales produce calls that can be detected only between late fall and early spring. I also detected low-frequency downsweeps and two types of low-frequency pulse trains produced by unknown species. After looking at the occurrence of fin whale 20-Hz pulses in relation to downsweeps, I suggest that the downsweeps are produced by sei whales due to the lack of overlap in occurrence. When I looked for diel patterns in the odontocete vocal events, I found a nocturnal increase in the occurrence of clicks from Risso's dolphins and sperm whales, but no diel variation in *Kogia* clicks. I also found that unidentified delphinids showed either an increase in click events at dawn or at night, depending on the time of year and recording location. Finally, my analysis of acoustic data from five recorders deployed in three different depth ranges revealed that there was greater unidentified delphinid and sperm whale vocal activity on recorders located in deep waters, suggesting a greater diversity and density of animals in deeper waters of Onslow Bay. Together, the results of my dissertation demonstrate the value of passive acoustic monitoring in understanding the distribution and temporal trends in cetacean occurrence, and highlight the importance of classifying sounds to the species level in order to better understand the temporal and spatial patterns found.

Dedication

For my husband and parents, who have always supported me.

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This dissertation would not have been possible without an army of people helping me along the way. First, I would like to thank my advisor, Andy Read. He has made a lasting impression over the past several years. His support and advice have been greatly appreciated. One of his many talents is knowing exactly what to say when it is needed...a blessing for a "high stress" person such as myself. He has also given me the opportunity to participate in several research projects along the way for which I am grateful. I have loved being part of the Read Lab and hope to continue collaborating with this wonderful group way into the future.

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General introduction

Passive acoustics – A useful tool to monitor the distribution of cetaceans

Many factors determine the temporal and spatial distribution of cetaceans, including the distribution of prey, predators, competitors, and oceanographic features that may affect prey availability. Cetaceans spend most of their lives underwater, complicating the use of traditional survey techniques (*i.e.*, visual methods) to determine distribution patterns. However, many cetaceans are quite vocal. Thus, in recent years, researchers have begun to use passive acoustic techniques as another tool to determine the distribution of many cetaceans.

Passive acoustic methods hold several advantages over visual surveys, including the ability to monitor in inclement weather, during poor visibility (including during high sea states and in darkness), and in remote locations. Autonomous passive acoustic recorders now are used frequently to monitor populations of cetaceans over extended periods of time (Mellinger et al. 2004, Philpott et al. 2007, Stafford et al. 2007, Verfuß et al. 2007). These instruments provide a long-term record unmatched by visual surveys and also provide information about patterns of daily and seasonal usage of remote habitats, as long as the animals are vocal. To fully interpret temporal and spatial patterns found in long-term acoustic recordings, calls need to be classified to the species level. Species-specificity has been found in many animals, including cetaceans (Steiner 1981, Ding et al. 1995, Matthews et al. 1999, Rendell et al. 1999, Oswald et al. 2003, Soldevilla et al. 2008).

Cetacean sounds

Cetaceans make a variety of sounds. Mysticetes produce low-frequency (mainly < 1 kHz) vocalizations, ranging from basic downsweeps and upsweeps to complex pulse trains and songs (*e.g.*, Payne and McVay 1971, Clark and Johnson 1984, Watkins et al. 1987, Gedamke et al. 2001, Parks and Tyack 2005, McDonald et al. 2006, Dunlop et al. 2007, Baumgartner et al. 2008). Some, but not all, of these sounds have been attributed to the species level (*e.g.*, Clark 1982, Edds 1982, Mellinger et al. 2000, Gedamke et al. 2001, Oleson et al. 2003, McDonald et al. 2005, Parks and Tyack 2005, Rankin and Barlow 2005, Rankin et al. 2005, Berchok et al. 2006, Boisseau et al. 2008).

Odontocetes produce sounds that generally are separated into three structural categories: narrow-band tonal whistles, broad-band clicks, and broad-band burst-pulsed sounds (Richardson et al. 1995), although Murray et al. (1998) describes the graded nature of odontocete calls, categorizing them into two groups (whistles and clicks) with burst-pulses as intermediate sounds. Odontocete whistles, which function in communication (Caldwell and Caldwell 1965, Tyack 1986, Caldwell et al. 1990, Sayigh et al. 1990, Janik et al. 2006), can have varying degrees of frequency modulation and typically occur below 20 kHz (Richardson et al. 1995) although they can extend to higher frequencies (Oswald et al. 2004, Esch et al. 2009a). Clicks, which can function in echolocation (to navigate through the environment and also to find prey; Au 1993) or possibly in communication (Watkins and Schevill 1977, Dawson 1991, Benoit-Bird and Au 2008), extend into the ultrasonic range, with frequencies ranging anywhere from less than 20 kHz to beyond 140 kHz for different species (Richardson et al. 1995). Burst-pulse sounds, which also are thought to function in communication (Dawson 1991, Norris et al. 1994, Lammers et al. 2006), are trains of rapidly produced clicks with such short inter-click intervals that humans perceive them as more tonal in quality (Watkins 1967).

For odontocetes, most research has focused on classifying whistles to species (Steiner 1981, Ding et al. 1995, Matthews et al. 1999, Rendell et al. 1999, Oswald et al. 2003), primarily due to the ease of recording these vocalizations with commercially available hardware with sampling rates in the human hearing range, in which whistles typically occur and are mostly represented (unlike clicks and many burst-pulsed sounds). However, recent advances in hardware technology have made it easier to record the broadband calls of odontocetes that require higher sampling rates into the ultrasonic ranges. With the full spectrum of an animal's vocalizations now available, researchers are able to explore which attributes of its vocal repertoire may be unique to species and individuals. This has led to an emerging body of research focused on classifying odontocete clicks to the species level (Soldevilla et al. 2008, Baumann-Pickering et al. 2011). Although some vocalizations have been classified to the species level, more work is needed in this area, especially for differentiating delphinids. Also, vocalizations of the same species have been found to vary by geographic location (McDonald et al. 2006, Ansmann et al. 2007, Baron et al. 2008), perhaps due to varying environmental conditions (as suggested by Ding et al. 1995) or perhaps due to recording groups of the same species in different behaviors at different locations. This geographic variation within a species makes classification to the species level more challenging on a global scale. More concurrent visual and acoustic surveys are needed not only to identify unknown vocalizations to species but also to examine geographical and behavioral differences in vocalizations.

Factors influencing vocal rate and occurrence

Classifying sounds to the species level is only one of the steps needed to interpret temporal and spatial patterns on autonomous passive acoustic recorders, though, because studies have found that vocal rates can be correlated with behavioral state, group size, and group composition. For example, some of these studies have found a correlation between click activity and behavioral state, with greater click activity occurring while animals are foraging (Jones and Sayigh 2002, Nowacek 2005). This finding has led to the frequent use of clicks as a proxy for foraging. Like clicks, the degree in occurrence of whistles also has been correlated with different behavioral states, with greater numbers of whistles heard during social activity and fewer during traveling (Jones and Sayigh 2002, Cook et al. 2004, dos Santos et al. 2005, Quick and Janik 2008, Hernandez et al. 2010). In addition, certain call types have been found to be associated with specific behaviors. For example, Janik (2000) found that bray calls of bottlenose dolphins (*Tursiops truncatus*) are associated with feeding. Oleson et al. (2007a) found that AB song in blue whales (*Balaenoptera musculus*) was associated with traveling and D calls with breaks in foraging at depth.

Studies also have found that vocal rates vary with group size. Matthews et al. (2001) found that moan rates (number of calls per aggregation, and not per individual, per hour) of North Atlantic right whales (*Eubalaena glacialis*) increased with increasing group size. Likewise, Jones and Sayigh (2002) and Hernandez et al. (2010) found that bottlenose dolphin (*T. truncatus*) vocal rates vary with group size in some locations, with fewer vocalizations produced by smaller groups. In contrast, however, other studies have found that fewer whistles occurred in larger groups (*T. truncatus*: Quick and Janik

2008; *T. aduncus*: Hawkins and Gartside 2010). Hawkins and Gartside (2010) suggested that this difference might be related to group composition, as they found that groups without calves had higher whistle rates than groups with calves. These authors also suggested that sex and age class may influence vocal rates. Sex has been correlated with specific calls, not just vocal rates, in some species (AB calls only in male blue whales: Oleson et al. 2007a; song only in male humpback whales: Darling and Bérubé 2001; song only in male fin whales (*Balaenoptera physalus*): Croll et al. 2002).

In addition, studies have found that certain species are more vocal at different times of the day or year. For example, Gordon et al. (2000) found that striped dolphins vocalized more at night than during the day in the Ligurian Sea, Goold (2000) found more whistling in short-beaked common dolphins at dawn and dusk off the Welsh coast, and Carlström (2005) found greater echolocation by harbor porpoises at night in Bloody Bay, Scotland. Likewise, studies have found increases in calls during the night for North Atlantic right whales in the Bay of Fundy (Matthews et al. 2001) as well as for blue whales in the eastern tropical Pacific (Stafford et al. 2005) and off of Southern California (Wiggins et al. 2005). Conversely, Baumgartner and Fratantoni (2008) found an increase in calls during the day for sei whales in the southwestern Gulf of Maine. A seasonal difference in vocal occurrence was found for bottlenose dolphins in North

Carolina, with more vocal activity, likely correlated with a seasonal change in behavior, in the fall versus the summer (Jacobs et al. 1993).

Because vocalization rates and occurrence may vary for different groups depending on species, population, behavior, group size, group composition, and/or time of year or day, vocal activity budgets as well as daily behavioral activity budgets need to be estimated for different species. Matthews et al. (2001) found that North Atlantic right whale moans tended to cluster in time. Such clustering could reduce the probability of detection, if, for example, the animal is silent the entire time it is within detection distance of the recorder. This type of information (clustering of vocal events and daily vocal activity budgets) is necessary for the interpretation of acoustic data that do not have concurrent visual data. Dedicated work on animal behavior can help to clarify how vocalization variables (such as frequency variables, duration, number of inflection points, etc.) and vocalization occurrence and rate change with different behaviors, group sizes, and group composition (as seen by Henderson et al. 2011, Henderson et al. *In press*), as well as provide information on daily activity budgets of all species in different geographical locations. Because seasonal differences in vocal activity have been found (Jacobs et al. 1993), the collection of data during different seasons of the year is also important. Information on vocalization rates and occurrence and how and why they might vary is important for interpreting and understanding temporal and spatial patterns observed in acoustic recordings.

Factors affecting acoustic detections

The aforementioned factors that affect vocal rates and occurrence are important considerations when interpreting data from acoustic recordings. However, it is also important to consider factors that might affect acoustic detections on the recording instrument itself. These factors include propagation conditions, source level of the calls, ambient noise levels, and receiver (in this case, instrument) sensitivity (Richardson et al. 1995).

Propagation efficiency is affected by depth, seafloor bottom type, bottom slope, the frequency range of the call, temperature (and thus season), salinity, and pressure. Deep water and shallow water propagation models differ, with spherical spreading as the main theoretical mode of sound transmission for deep water and cylindrical spreading for shallow water (Richardson et al. 1995). The depths of the vocalizing animals and the receiver are important to consider as well as they affect sound transmission and detection. Calls produced in the deep sound channel (sound fixing and ranging, or SOFAR, channel), for example, will propagate great distances because nearly-horizontal rays of sound become trapped within that channel. Thus, propagation losses due to reflections off of the surface and bottom can be minimized (Richardson et al. 1995). Sounds produced at the surface may become trapped in a surface duct which can cause recording instruments deployed at the bottom to be in shadow zones (and therefore those calls would not be detected). The Lloyd mirror effect, which involves the formation of interference patterns, also can come into play when calls are produced very close to the surface.

Certain species, such as some beaked whales, only vocalize at depth (Johnson et al. 2004) and are thus difficult to detect with instruments deployed at the surface (such as towed arrays), especially since clicks are highly directional. For directional calls (such as odontocete clicks), detection distances are dependent upon an animal's orientation and location in relation to the hydrophone. In addition, the source level and frequency of the call can affect its detection distance. While odontocete clicks can have high source levels, they mostly (except for sperm whale clicks) occur at higher frequencies, where absorption, which increases with increasing frequency in seawater, has a greater effect on transmission loss and thus detection ranges.

The slope of the seafloor bottom, especially in shallow water, also affects sound propagation (Richardson et al. 1995). If sound encounters a downward sloping bottom, it can spread out into the increased volume. The deeper water also allows for fewer surface and bottom reflections, though. Thus, the net effect of such slopes is generally lower transmission loss. For sound encountering an upward sloping bottom, the reverse is true and thus the net effect is generally greater attenuation. Lastly, propagation is affected by temperature, salinity, and pressure. Decreasing the water's temperature, salinity, or pressure results in a decrease in the speed of sound. Consequently, sound rays will refract towards these areas of lower sound speed (Richardson et al. 1995).

Ambient noise also affects the ability of sounds to be detected. Examples of ambient noise, which is background noise that includes all noise other than the signals of interest, are noise from waves, wind, rain, animals, shipping, industrial activities, and sonar. With increasing ambient noise, signal-to-noise ratios decrease, which may even result in signals being masked (thereby being undetectable). Gordon et al. (2000) found that acoustic detection rates decreased with increasing sea state, wind speed, and background noise levels. Matthews et al. (2001) mention that high sea states and wind can create near-surface air bubbles which can increase attenuation of calls produced at the surface. Thus, it is important to take all of these factors into consideration when trying to interpret and understand data from passive acoustic recorders.

Study area and species of interest

Until very recently, little was known about the cetacean fauna of Onslow Bay, North Carolina. Onslow Bay is located within the South Atlantic Bight, along the North

Carolina coast between Cape Lookout and the Cape Fear River. The shelf break in this area runs along the 200-m isobath. The Gulf Stream, a fast, warm, northward-flowing western boundary current, meanders through this area along the shelf break and slope.

In October 2005, the U.S. Department of the Navy proposed installing a 500-nmi^2 Undersea Warfare Training Range (USWTR) along the Atlantic coast, for the purpose of anti-submarine warfare training using mid-frequency tactical sonar (1-10 kHz) in shallow waters. Four sites were originally selected as possible locations for this range: (1) Jacksonville, Florida; (2) Charleston, South Carolina; (3) Onslow Bay; and (4) a site off northeastern Virginia. In 2005, the Navy identified Onslow Bay as the preferred site. To determine baseline information on the occurrence and distribution of cetaceans in this area, Duke University, the University of North Carolina Wilmington, and the University of St. Andrews developed a monitoring plan that included traditional aerial and shipboard visual surveys and passive acoustic monitoring.

The field component of the monitoring plan for Onslow Bay began in June 2007, with the University of St. Andrews performing statistical modeling, the University of North Carolina Wilmington performing line-transect aerial surveys, and Duke University performing line-transect boat-based surveys, photo-identification, and passive acoustic monitoring. The survey area is 83 km x 74 km (Figure 1) and extends outside the boundaries of the proposed USWTR by 37 km in each direction. The shipboard and aerial survey teams monitor marine mammals along 10 transect lines, each 74 km long and separated by approximately 9 km. Passive acoustic monitoring consists of recordings made by: (1) a towed hydrophone array during boat-based surveys and (2) autonomous passive acoustic recorders, known as High-frequency Acoustic Recording Packages (HARPs; Wiggins and Hildebrand 2007) and "pop-ups" (Bioacoustics Research Program, Cornell Lab of Ornithology), located within the USWTR survey area. The Navy moved its preferred site for the USWTR to Jacksonville, Florida in 2009, but research is still underway in Onslow Bay as part of a wider Navy monitoring effort.

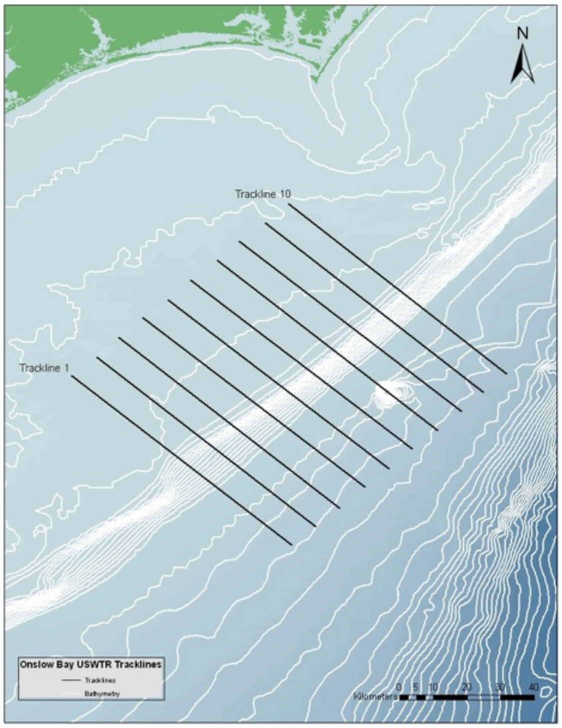

Figure 1: Study area in Onslow Bay, North Carolina. The ten lines running perpendicular to shore represent tracklines used during line-transect visual and acoustic surveys.

In an earlier monitoring effort for the Navy, researchers from the University of North Carolina Wilmington performed aerial surveys in Onslow Bay between September 1998 and October 1999. During these surveys, five species of cetaceans and two unidentified beaked whales were observed. Species included bottlenose dolphins (*T. truncatus*), short-beaked common dolphins (*Delphinus delphis*), pilot whales (*Globicephala spp.*), Risso's dolphins (*Grampus griseus*), and a Cuvier's beaked whale

(*Ziphius cavirostris*). Since June 2007, however, six additional species of cetaceans have been observed during aerial- or vessel-based surveys in the offshore Onslow Bay area. These species include Atlantic spotted dolphins (*Stenella frontalis*), rough-toothed dolphins (*Steno bredanensis*), sperm whales (*Physeter macrocephalus*), humpback whales, fin whales, and beaked whales (*Mesoplodon spp.*).

Due to their distribution ranges, it is likely that the following species may also be present in Onslow Bay: dwarf sperm whales (*Kogia sima*); pygmy sperm whales (*Kogia breviceps*); sei whales (*Balaenoptera borealis*); minke whales (*Balaenoptera acutorostrata*); and possibly North Atlantic right whales. All of the odontocete species make a variety of sounds, including narrow-band whistles, broad-band clicks, and broad-band burst-pulses. All of the mysticete species make a variety of low-frequency (mainly < 1 kHz) calls, including moans, pulse trains, pulses, and downsweeps.

The most common odontocetes observed during visual surveys in Onslow Bay are Atlantic spotted dolphins and bottlenose dolphins. Two ecotypes of bottlenose dolphins are found off the North Carolina coast - the coastal and offshore ecotypes, both of which may be found in the USWTR survey area. In Onslow Bay, both Atlantic spotted dolphins and bottlenose dolphins are commonly sighted over the shelf, although the distribution of the latter extends into deeper waters (Figure 2). Bottlenose dolphins inhabit a range of depths throughout the study area, but larger group sizes are more abundant in deeper waters beyond the 200-m isobath. All of the other species observed during surveys occurred in deeper waters (generally beyond the 200-m isobath). To date, all cetacean groups observed in offshore Onslow Bay have been single-species groups.

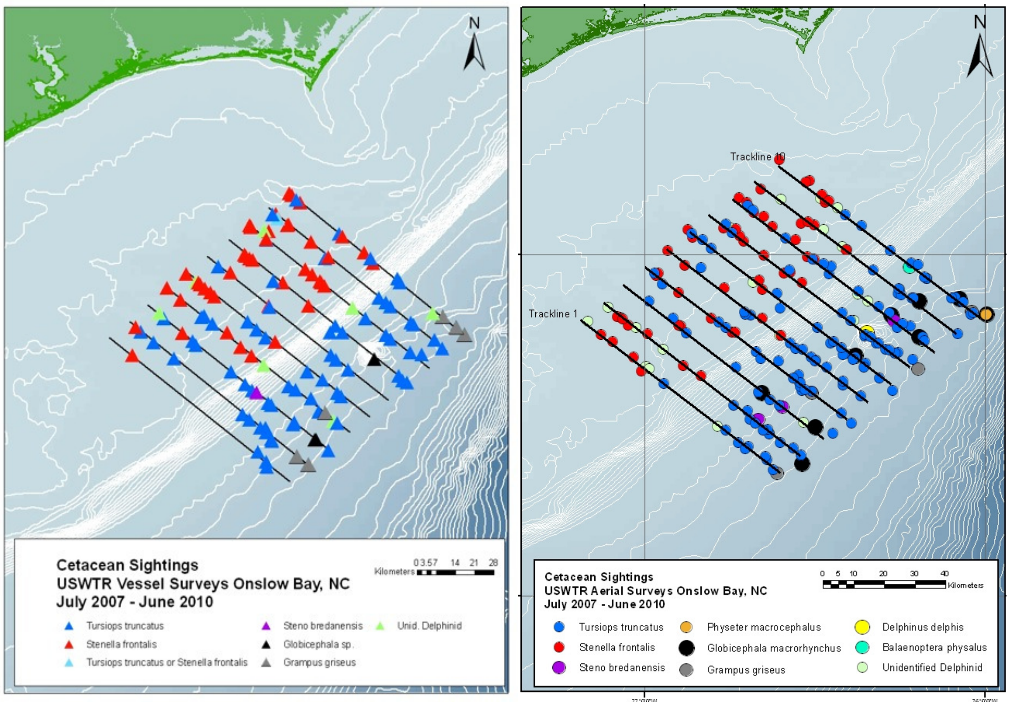

Figure 2: Visual detections of cetaceans from boat-based (left) and aerial (right) surveys in Onslow Bay between July 2007 and June 2010.

Dissertation goals and outline

The overall goal of my dissertation was to use passive acoustic techniques to determine patterns of occurrence and distribution of cetacean species in Onslow Bay. Specifically, I wanted to: determine if species-specific characteristics exist for whistles and clicks of odontocetes; describe the temporal variation of cetacean vocal events; and describe depth-related differences in the occurrence and duration of odontocete vocal events.

In Chapters 1 and 2, I analyze acoustic recordings made between 2007 and 2010 of single-species groups (positively identified by visual observers) of five species, including Atlantic spotted dolphins, bottlenose dolphins, Risso's dolphins, rough-toothed dolphins, and short-finned pilot whales (*Globicephala macrorhynchus*). I use these recordings to look for species-specific patterns in different variables of their whistles (Chapter 1) and clicks (Chapter 2). I then use the findings of distinct peak and notch patterns in the clicks of one species (Risso's dolphins) to identify that species in autonomous recordings that I analyze for Chapter 3.

In Chapter 3, I examine temporal variation in the occurrence of odontocete click events recorded on HARPs between 2007 and 2010. I conduct separate analyses of click events produced by Risso's dolphins, sperm whales, *Kogia spp.*, and other delphinids, describe diel patterns in the occurrence of clicks for each of these groups, and also look generally at seasonal trends.

In Chapter 4, I examine depth-related differences in the occurrence and duration of odontocete vocal events and continue to examine diel patterns in these vocal events. This chapter focuses on approximately one month of continuous recordings, made in 2008, from five "pop-up" acoustic recorders.

In Chapter 5, I look at temporal variation in the occurrence of mysticete vocal events recorded on HARPs between 2007 and 2010. I first describe calls assumed to be produced by mysticetes. Some of these call types have been previously described in the literature and attributed to a certain species. Other call types have not been described in as much detail and have not yet been attributed to a particular species. For all calls described in this chapter, I look generally at seasonal patterns.

I conclude by synthesizing all findings and suggesting ideas for future work that arose while carrying out this research.

Chapter 1: Species-specific whistles of odontocetes in Onslow Bay, North Carolina

1.1 Introduction

Passive acoustics are used frequently as a monitoring tool for cetaceans, so it is important to be able to classify calls to the species level, especially for recordings for which there are no concurrent visual observations. Species-specific vocalizations have been observed in many animals, including birds (Marler 1957), bats (Parsons and Jones 2000, Russo and Jones 2002, Biscardi et al. 2004, Fukui et al. 2004), and cetaceans (Steiner 1981, Ding et al. 1995, Matthews et al. 1999, Rendell et al. 1999, Oswald et al. 2003, Soldevilla et al. 2008).

The vocalizations of odontocete cetaceans are often separated into three main categories - narrow-band tonal whistles, broad-band clicks, and burst-pulsed sounds (Richardson et al. 1995). Most research has focused on using whistles to classify animals to the species level (Steiner 1981, Ding et al. 1995, Matthews et al. 1999, Rendell et al. 1999, Oswald et al. 2003) due to the ease of recording these vocalizations with commercially available hardware with sampling rates in the human hearing range, in which whistles typically occur and are mostly represented (unlike clicks and many burst-pulsed sounds).

In previous studies examining odontocete whistles, the most common variables analyzed included the start and end frequencies, minimum and maximum frequencies, duration, and the number of inflection points (Steiner 1981, Matthews et al. 1999, Rendell et al. 1999, Oswald et al. 2003, Bazúa-Durán 2004, Ansmann et al. 2007, Baron et al. 2008, Azevedo et al. 2010, Gannier et al. 2010, Díaz López 2011). Some studies have included additional variables, such as the number of steps, the frequency range, the central frequency, the mean frequency, the number of harmonics, the start and end slopes of the whistle contour, and the minimum and maximum slopes (Rendell et al. 1999, Oswald et al. 2003, Gannier et al. 2010).

Generally, the results of prior classification work indicate that odontocete whistles contain species-specific information. However, Oswald et al. (2003) suggested that classification rates of odontocete whistles might be biased because such analyses are often performed on whistles recorded from only a few groups (thus possibly over-sampling certain groups or individuals) or because recordings were included from widely separated geographic locations for the different species examined. Classification rates are likely to be biased upwards if over-sampling or pseudo-replication occurs. Bias also could occur if a species only is recorded in the same behavioral state, as Ansmann et al. (2007) and Azevedo et al. (2010) have found intraspecific variation in whistles related to behavior. Recordings from different geographic areas also are likely to introduce bias, as species that are widely separated geographically likely do not share similar acoustic environments and thus may have adapted their vocalizations to background noise (as suggested by Ding et al. 1995). Baron et al. (2008) showed that whistles from the same species may vary with geographic location.

On the other hand, spatial separation of two species prevents them from interacting and thus lowers the need for production of highly distinctive whistles. Steiner (1981) found greater differences in measured whistle variables among sympatric species than among allopatric species. Steiner (1981) reasoned that selection pressures might lead to the development of highly distinctive whistles in sympatric species if whistles are used for species-specific communication.

The present study was conducted in a relatively small geographic area in which most, if not all, odontocete species come into acoustic contact with each other. Therefore, it is reasonable to hypothesize that these species' whistles, assuming they are used in species-specific communication, are distinctive in some way. The goal of my study was to determine if odontocete species inhabiting Onslow Bay, North Carolina, could be distinguished by their whistles. I used recordings of single-species odontocete groups collected during concurrent visual and acoustic line-transect surveys off North Carolina between 2007 and 2010 to examine the species-specificity of whistles produced by Atlantic spotted dolphins (*Stenella frontalis*), bottlenose dolphins (*Tursiops truncatus*), rough-toothed dolphins (*Steno bredanensis*), and short-finned pilot whales (*Globicephala macrorhynchus*). I measured 22 whistle variables, 10 of which have not been commonly reported, to determine which, if any, were useful in classifying these vocalizations to species found in Onslow Bay.

1.2 Methods

1.2.1 Study area and survey platforms

The main survey area consisted of an 83 km x 74 km rectangular region in Onslow Bay, North Carolina (Figure 3), which contained 10 line-transects laid out perpendicular to shore. Onslow Bay is located within the South Atlantic Bight, along the North Carolina coast between Cape Lookout and the Cape Fear River. We conducted boat-based line-transect visual and acoustic surveys from either a 16.2-m fishing vessel (the *F/V Sensation*) or an 11.6-m research vessel (the *R/V Cetus*). A second survey area was located north of Onslow Bay, off Cape Hatteras, North Carolina (Figure 4). This second survey area was chosen due to its proximity to Onslow Bay (approximately 200 km away) and comparatively high abundance of odontocete species. We conducted boat-based line-transect visual and acoustic surveys in this location from the *F/V Sensation*.

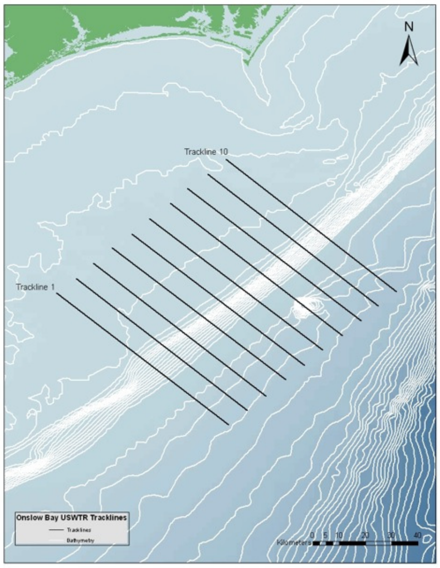

Figure 3: Main study area in Onslow Bay, North Carolina. The ten lines running perpendicular to shore represent tracklines used during line-transect visual and acoustic surveys.

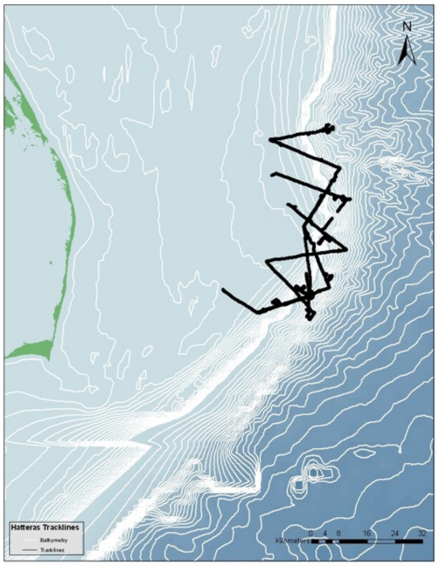

Figure 4: Second study area in Hatteras, North Carolina. The black zigzagging lines represent the survey effort during visual and acoustic surveys.

1.2.2 Data collection

We conducted visual and acoustic line-transect surveys from September 2007 through August 2010. During surveys, high-frequency acoustic recordings (192-kHz sampling rate) were made with a system consisting of a laptop running *Ishmael* software (Mellinger 2001), a MOTU Traveler audio interface (Mark of the Unicorn, Cambridge, MA, USA), and a hydrophone array (Seiche Instruments, UK) with 300 m of tow cable. The array consisted of four potted elements, spaced 1.2 m apart, with a flat (+/- 3 dB) frequency response between 2 and 100 kHz and a sensitivity of -165 dB re 1V/µPa. The hydrophone array was towed 150 m behind the vessel at a speed of approximately 16.7 km/h. Incoming acoustic signals were monitored constantly both visually (via spectrograms in *Ishmael*, with the gain set to -96 dB) and aurally by a trained acoustician. Recordings were made directly to an external USB-connected hard drive using *Ishmael* whenever marine mammal sounds were detected, at which point time and location were noted. Species identification and group size were determined visually by a team of at least three independent observers. Acoustic recordings were analyzed only for single-species groups positively identified in this manner. If another species was sighted within 5.6 km of the focal group, the recording was not included in the analysis to prevent ambiguity of the species being recorded (following Oswald et al. 2007).

1.2.3 Signal and statistical analyses

I used the sound analysis software program *Raven 1.3* (Bioacoustics Research Program of the Cornell Lab of Ornithology, Ithaca, NY) to locate whistles in spectrograms derived from the towed array recordings. This program allows the user to make individual sound files for each detection. As a result, individual whistles were saved as separate files for further analysis. To help minimize over-sampling of individuals, only one whistle was chosen when whistles with similar contours occurred close together in time as a series. Such whistles were likely produced by the same individual. From each recording session, I randomly selected up to 35 good quality whistles (following Oswald et al. 2007 to avoid over-sampling individuals and groups) but no more than twice the estimated group size for further spectral analysis. Contours were extracted from individual files using a Matlab-based program called *Beluga* (written by Volker Deecke and Vincent Janik). To extract whistles in this program, spectrograms were made using a Fast Fourier Transform (FFT) length of 2048, frame length of 2048, and 50% overlap and then filtered by subtracting the average noise spectrum. The frequency contour was then extracted, with frequency measurements made for every time step of 5.33 milliseconds, using the "peaks" method in *Beluga*. The "peaks" method finds the frequency with the highest amplitude at each time step between user-specified frequency boundaries. Whistles with minimum frequencies below 2 kHz, the lower end of the recording system, were not extracted. Portions of the contour that did not extract properly were corrected manually by limiting the frequency range and selecting a smaller portion of the whistle to extract. The resulting whistle contour, consisting of frequency points and duration, was saved.

I measured twenty-two variables for each whistle contour in Matlab (MathWorks, Natick, MA), including: the maximum, minimum, start, end, first quartile, second quartile, third quartile, and mean frequencies (kHz) and slopes (kHz/s); the frequency and slope range; the start and end slope sign; the duration (s); and the number of inflection points (Figure 5). Except for duration, prior to taking measurements, all whistle contours were smoothed using the Loess method (quadratic fit) with an eight-point moving average. The slope between consecutive frequency contour points was determined by taking the difference between their frequencies and dividing by the time step (in this case, 5.33 milliseconds). The first quartile measurements were calculated by taking the average of five points centered around 1/4 of the total whistle length; the second quartile measurements were calculated by taking the average of five points centered around 1/2 of the total whistle length; the third quartile measurements were calculated by taking the average of five points centered around 3/4 of the total whistle length. For whistles that had short durations (< 13 measured points), the first quartile, second quartile, third quartile, start, and end slopes and start and end slope signs were not measured. The frequency range was calculated by subtracting the minimum from the maximum frequency; the slope range was calculated by subtracting the minimum from the maximum slope. The mean frequency was the average frequency of all whistle contour points. The mean slope was determined by taking the average of all slopes calculated between consecutive frequency contour points. The start and end slope signs were either positive (noted as +1), negative (-1), or zero (0) depending on the direction of the slope for 10 points at the beginning (for start slope) or end (for end slope) of the whistle contour. The number of inflection points was the number of times the slope changed from either positive to negative or negative to positive. The start, end, minimum, and maximum frequencies; duration; and number of inflection points are the most commonly reported variables measured for odontocete whistles. The quartile frequencies and slopes; the mean slope; the slope range; and the start and end slope sign have rarely if ever been reported and are thus considered new variables. These new variables were included in this study to determine if they were helpful in species-level classification of odontocete whistles.

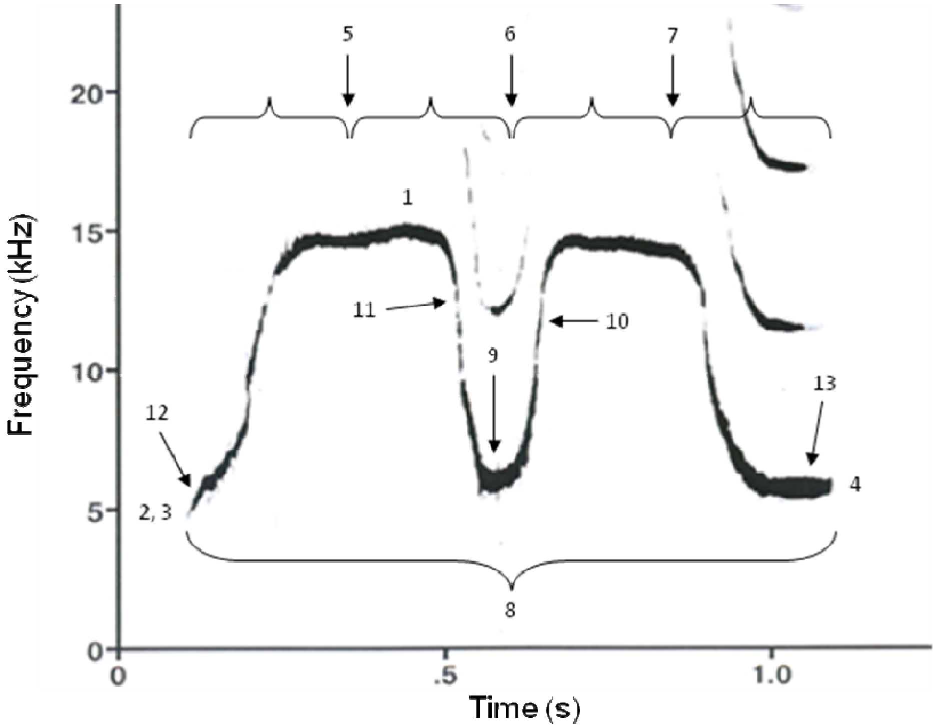

Figure 5: Spectrogram of a whistle showing several of the extracted variables, including: (1) maximum frequency, (2) minimum frequency, (3) start frequency, (4) end frequency, (5) location of 1st quartile measurements, (6) location of 2nd quartile measurements, (7) location of 3rd quartile measurements, (8) duration, (9) example of an inflection point, (10) maximum slope, (11) minimum slope, (12) start slope, and (13) end slope.

I examined species-specificity in whistles of Atlantic spotted dolphins, bottlenose dolphins, rough-toothed dolphins, and short-finned pilot whales. I performed comparisons of each whistle variable for all species using Kruskal-Wallis tests (Zar 1999). I performed multiple comparison tests using Bonferroni corrections on the significant results to determine which species had significantly different whistle variables and to determine the species classification potential for each of the extracted variables. Whistles for which there were missing values (those with short durations (< 13 measured points) that prevented proper slope measurements from begin made) were not included. These non-parametric comparisons were made using JMP software. In addition, I constructed Classification And Regression Trees (CARTs) in Matlab using the 22 measured variables for all four species. The frequency range and slope range are second order variables, but I included these variables in the CARTs because they provide information on the overall bandwidth of a whistle, which is not described by any other value alone. As previously mentioned, some whistles had variables with missing data, but the CART analysis allowed these whistles to be included. Because Atlantic spotted and bottlenose dolphins are the most commonly sighted species during visual surveys in Onslow Bay (*unpublished data*), I also conducted a CART analysis using all 22 variables for just these two species.

CART analysis (Breiman et al. 1984) is a non-parametric technique that uses binary recursive partitioning to split the data into groups, thereby "growing" a decision tree. The largest binary decision tree with all of the measured variables was grown first. Then, 50 sets of 10-fold cross-validations were performed (as suggested by De'ath and Fabricius 2000). These 10-fold cross-validations randomly divided the entire dataset into 10 subsets and used these subsets as the cross validation units. For each cross-validation, the 'best tree' was chosen based on the 1-SE rule, where the smallest-sized tree that has an error rate within one standard error of the minimum cross-validation costs is chosen (Breiman et al. 1984, De'ath and Fabricius 2000). The optimal tree was chosen by taking the most frequently occurring tree size of these 50 'best trees' (indicating the smallest tree with the highest predictive accuracy) and a misclassification rate was obtained. This tree was used to determine the most important variables in classifying whistles to the correct species. This analysis provides the percentage of total whistles assigned to the correct species (the correct classification rate). To determine if these correct classification rates for individual species were greater than expected by chance (calculated by dividing 100% by the number of species: 25% for four species), I performed chi-square tests (Zar 1999) with $\alpha = 0.05$.

Following the cross-validation method described above, I conducted a CART analysis using the same 22 variables on whistles from the same species recorded on different occasions to determine if there was group or intraspecific variation in whistles. This variation is possibly due to factors such as behavioral state (Azevedo et al. 2010) and group composition (as suggested by Rendell et al. 1999). I defined a group as a number of animals of the same species recorded at the same time and location.

To determine if there was any variation between short-finned pilot whale whistles recorded in Onslow Bay and Hatteras, I performed a Wilcoxon rank-sum test (Zar 1999) to look at differences between variables. I also performed a CART analysis. I recorded only two groups of this species in Onslow Bay and four groups in Hatteras and did not obtain information on the behavioral state of each group, so it was not possible to determine if variation in whistle variables was due to geographic variation, behavioral differences, or over-sampling.

1.3 Results

Between September 2007 and August 2010, I used the towed array to make single-species recordings of five species: Atlantic spotted dolphins, bottlenose dolphins, Risso's dolphins (*Grampus griseus*), rough-toothed dolphins, and short-finned pilot whales. No good-quality whistles were recorded in the presence of Risso's dolphins, so this species was omitted from the analysis. I analyzed 624 whistles from 48 recording sessions (Table 1). Recordings from more than one recording session were used for each species to examine species-specificity except for rough-toothed dolphins, which were sighted only once (Table 1).

Table 1: Number of recording sessions and whistles analyzed for each species.

Species	# Recording Sessions	# Whistles Analyzed
Atlantic spotted dolphins	14	162
Bottlenose dolphins	27	338
Rough-toothed dolphins	1	35
Short-finned pilot whales	6	89

1.3.1 Descriptive statistics

Table 2 shows descriptive statistics for the 22 whistle variables measured. Bottlenose dolphins produced whistles that were longer in duration, greater in frequency range, higher in minimum and maximum frequency, and more numerous in inflection points (indicating greater modulation in contour) than the other three species. Rough-toothed dolphins produced the shortest whistles with the smallest frequency range and smallest number of inflection points. Short-finned pilot whales produced whistles with the lowest minimum frequency. Interestingly, Atlantic spotted dolphins produced whistles that were relatively short, but their whistles had the second highest maximum frequency, frequency range, *and* number of inflection points.

Overall, the slope variables and number of inflection points had the highest coefficients of variation for all species and the frequency variables had the lowest (Table 2). Of the four species, short-finned pilot whales had the highest coefficients of variation for all frequency variables, while Atlantic spotted dolphins had the highest coefficient of variation for duration.

1.3.2 Species comparisons of variables

Table 3 summarizes the results of the species comparisons for each of the measured whistle contour variables. For two variables (minimum frequency and end frequency), the differences were statistically significant for every species pair-wise comparison (Table 3), indicating that these variables could be useful for classifying the four species. In addition, nine additional variables (maximum frequency, frequency range, start frequency, first quartile frequency, second quartile frequency, mean frequency, duration, number of inflection points, and minimum slope) exhibited statistically significant differences in all but one pair-wise comparison.

Whistles of short-finned pilot whales and rough-toothed dolphins did not differ in 14 of 22 variables, indicating that these two species produced whistles with similar frequency and slope components. Rough-toothed dolphins and Atlantic spotted dolphins produced whistles that differed significantly in all frequency variables but not in many slope variables. When comparing whistles of short-finned pilot whales to those of Atlantic spotted dolphins and bottlenose dolphins, all frequency variables were significantly different. Whistles of bottlenose dolphins were distinctive from those of rough-toothed dolphins and Atlantic spotted dolphins in all but three or four cases (out of 22), respectively, indicating a high degree of distinctiveness in general.

Finally, when comparing short-finned pilot whales recorded in Onslow Bay to those recorded off of Cape Hatteras (Table 4), nine variables were statistically significantly different (Table 5). Of the nine significantly different variables, eight were frequency variables and all eight of those were higher for pilot whales recorded in Onslow Bay (Tables 4 and 5).

1.3.3 CART analysis

The optimal classification tree for interspecific comparisons examining all four species was chosen as the most frequently occurring optimal tree from the 50 sets of 10-fold cross-validations performed; this represented the smallest tree with the highest predictive accuracy. This tree included seven of the 22 variables: duration, third quartile frequency, maximum frequency, third quartile slope, end slope, first quartile slope, and mean frequency. This optimal tree also consisted of eight terminal nodes and resulted in a correct classification rate of 74.2% (n = 624; Figure 6). All correct classification rates for individual species were significantly greater than the 25% expected by chance (χ^2 test, $p< 0.001$) and ranged from 40.0% for rough-toothed dolphins to 92.3% for bottlenose dolphins (Table 6).

The optimal classification tree constructed using all 22 variables of the whistles of Atlantic spotted and bottlenose dolphins included only the variable duration (n = 500, Figure 7). This optimal tree resulted in a correct classification rate of 82.2%, with Atlantic spotted dolphins correctly classified 74.7% of the time and bottlenose dolphins 87.3% of the time (Table 7). The correct classification rates for each individual species were greater than the 50% expected by chance.

Table 8 shows the results of the CARTs used to examine intraspecific variation. For each of the three species examined (rough-toothed dolphins were not included because only one sample of whistles was available), the percentage of whistles correctly classified to recording session was greater than expected by chance (Table 8). Table 9 shows the results of the additional set of cross-validated CARTs to examine differences between pilot whale whistles recorded in Onslow Bay versus those recorded in waters off of Cape Hatteras. The percentage of whistles correctly classified to geographic location was greater than expected by chance for Hatteras but not for Onslow Bay (Table 9).

Table 2: Means, standard deviations, and coefficients of variation (C.V.) for all 22 measured whistle variables. Q stands for quartile.

	Max Freq (kHz)	Min Freq (kHz)	Freq Range (kHz)	Start Freq (kHz)	End Freq (kHz)	1st Q Freq (kHz)	2nd Q Freq (kHz)	3rd Q Freq (kHz)	Mean Freq (kHz)	Duration (s)	# of Inflec Pts
Atlantic spotted dolphins											
Mean	14.06	8.40	5.65	9.68	12.38	9.76	10.55	11.56	10.69	0.34	1.23
St. Dev.	3.32	2.26	3.22	2.74	3.93	2.27	2.48	2.95	2.11	0.33	1.70
C.V. (%)	23.6	26.9	57.0	28.3	31.7	23.3	23.5	25.5	19.8	95.5	138.3
Bottlenose dolphins											
Mean	17.58	7.62	9.95	9.70	11.02	13.16	12.86	12.45	12.42	1.15	2.49
St. Dev.	3.40	2.07	3.55	3.46	4.53	3.68	3.73	3.79	2.35	0.65	2.18
C.V. (%)	19.3	27.1	35.7	35.6	41.1	28.0	29.0	30.4	18.9	56.5	87.5
Rough-toothed dolphins											
Mean	7.71	5.50	2.21	5.67	7.51	6.24	6.50	6.89	6.60	0.19	0.43
St. Dev.	1.61	1.36	1.43	1.53	1.61	1.46	1.16	1.15	1.16	0.16	0.85
C.V. (%)	20.8	24.7	64.4	27.0	21.5	23.4	17.8	16.7	17.7	85.3	198.4
Short-finned pilot whales											
Mean	7.16	4.19	2.97	4.86	5.73	5.86	5.95	5.76	5.74	0.49	0.74
St. Dev.	3.43	2.30	2.48	2.52	3.40	2.76	2.83	2.93	2.65	0.26	0.97
C.V. (%)	47.9	54.8	83.5	51.7	59.4	47.1	47.6	50.8	46.2	52.5	131.0

	Max Slope (kHz/s)	Min Slope (kHz/s)	Slope Range (kHz/s)	Start Slope (kHz/s)	End Slope (kHz/s)	1st Q Slope (kHz/s)	2nd Q Slope (kHz/s)	3rd Q Slope (kHz/s)	Mean Slope (kHz/s)	Start Slope Sign	End Slope Sign
Atlantic spotted dolphins											
Mean	97.76	-64.18	161.94	16.67	13.52	7.96	18.96	21.84	18.89	0.46	0.35
St. Dev.	87.11	64.41	110.97	35.17	44.59	33.99	34.52	51.94	35.10	0.89	0.94
C.V. (%)	89.1	100.4	68.5	210.9	329.7	427.2	182.0	237.8	185.8	194.0	267.4
Bottlenose dolphins											
Mean	93.41	-79.26	172.67	31.04	-8.09	7.33	2.47	-7.37	2.99	0.77	-0.20
St. Dev.	70.12	63.83	106.79	36.90	40.33	34.47	39.92	42.05	13.56	0.64	0.98
C.V. (%)	75.1	80.5	61.8	118.8	498.7	470.1	1616.8	570.5	453.7	83.6	499.9
Rough-toothed dolphins											
Mean	54.31	-10.39	64.70	12.65	12.76	10.65	8.89	16.31	16.00	0.64	0.64
St. Dev.	51.16	23.03	65.10	19.11	16.63	18.37	18.31	20.77	14.16	0.78	0.78
C.V. (%)	94.2	221.8	100.6	151.1	130.3	172.5	205.9	127.3	88.5	121.3	121.3
Short-finned pilot whales											
Mean	38.99	-28.83	67.82	11.49	-0.98	4.90	0.21	-1.53	2.44	0.48	0.15
St. Dev.	36.40	36.50	61.38	21.02	16.08	14.24	14.65	18.21	6.20	0.88	0.99
C.V. (%)	93.4	126.6	90.5	182.9	1642.9	290.4	6840.7	1187.9	254.5	182.2	681.1

Table 3: Results of Kruskal-Wallis tests and comparisons of 22 measured whistle variables for all six pair-wise species combinations. In this table, Gm = short-finned pilot whales, Sf = Atlantic spotted dolphins, Sb = rough-toothed dolphins, Tt = bottlenose dolphins, and Q = quartile. * Indicates significant differences for the Kruskal-Wallis tests. Shading indicates significant differences of the multiple comparison tests with Bonferroni corrections (family-wise error rate $\alpha = 0.05$). Whistles for which there were missing values were not included.

	Kruskal-Wallis Results	Multiple Comparison Test Results with Bonferroni Corrections					
		Gm/Sf	Gm/Sb	Gm/Tt	Sf/Sb	Sf/Tt	Sb/Tt
Max Freq	p<0.001*	▨		▨	▨	▨	▨
Min Freq	p<0.001*	▨	▨	▨	▨	▨	▨
Freq Range	p<0.001*	▨		▨	▨	▨	▨
Start Freq	p<0.001*	▨	▨	▨	▨		▨
End Freq	p<0.001*	▨	▨	▨	▨	▨	▨
1st Q Freq	p<0.001*	▨		▨	▨	▨	▨
2nd Q Freq	p<0.001*	▨		▨	▨	▨	▨
3rd Q Freq	p<0.001*	▨		▨	▨		▨
Mean Freq	p<0.001*	▨		▨	▨	▨	▨
Duration	p<0.001*	▨	▨	▨		▨	▨
# Inflection Points	p<0.001*	▨		▨	▨	▨	▨
Max Slope	p<0.001*	▨		▨	▨		▨
Min Slope	p<0.001*	▨		▨	▨	▨	▨
Slope Range	p<0.001*	▨		▨	▨		▨
Start Slope	p<0.001*			▨		▨	
End Slope	p<0.001*	▨	▨			▨	▨
1st Q Slope	p=0.300	N/A	N/A	N/A	N/A	N/A	N/A
2nd Q Slope	p<0.001*	▨	▨			▨	
3rd Q Slope	p<0.001*	▨	▨			▨	▨
Mean Slope	p<0.001*	▨	▨			▨	▨
Start Slope Sign	p<0.001*			▨		▨	
End Slope Sign	p<0.001*			▨		▨	▨

Table 4: Means, standard deviations, and coefficients of variation (C.V.) for all 22 measured whistles variables for short-finned pilot whales recorded in waters off of Cape Hatteras and in Onslow Bay. Q stands for quartile.

	Max Freq (kHz)	Min Freq (kHz)	Freq Range (kHz)	Start Freq (kHz)	End Freq (kHz)	1st Q Freq (kHz)	2nd Q Freq (kHz)	3rd Q Freq (kHz)	Mean Freq (kHz)	Duration (s)	# of Inflec Pts
Hatteras											
Mean	6.31	3.64	2.67	4.14	5.03	4.97	5.27	5.11	5.00	0.48	0.75
St. Dev.	3.47	1.95	2.44	1.92	3.21	2.59	2.98	3.07	2.66	0.28	1.10
C.V. (%)	55.0	53.6	91.3	46.4	63.8	52.1	56.6	60.0	53.3	58.2	146.8
Onslow Bay											
Mean	8.15	4.82	3.32	5.71	6.54	6.89	6.74	6.52	6.62	0.50	0.73
St. Dev.	3.13	2.52	2.51	2.87	3.48	2.61	2.44	2.59	2.39	0.23	0.81
C.V. (%)	38.4	52.2	75.6	50.3	53.2	37.9	36.2	39.7	36.1	46.2	110.3

	Max Slope (kHz/s)	Min Slope (kHz/s)	Slope Range (kHz/s)	Start Slope (kHz/s)	End Slope (kHz/s)	1st Q Slope (kHz/s)	2nd Q Slope (kHz/s)	3rd Q Slope (kHz/s)	Mean Slope (kHz/s)	Start Slope Sign	End Slope Sign
Hatteras											
Mean	35.74	-29.28	65.02	9.28	-1.41	6.30	1.79	-1.87	2.74	0.42	0.17
St. Dev.	35.92	37.48	64.63	16.28	17.98	17.01	17.70	23.89	6.77	0.92	1.00
C.V. (%)	100.5	128.0	99.4	175.5	1277.4	269.9	986.3	1280.2	247.1	220.5	597.9
Onslow Bay											
Mean	42.79	-28.31	71.09	14.09	-0.48	3.27	-1.64	-1.14	2.08	0.56	0.12
St. Dev.	37.03	35.79	57.96	25.45	13.74	10.06	9.89	7.66	5.53	0.84	1.00
C.V. (%)	86.6	126.4	81.5	180.7	2878.6	307.8	604.5	670.0	265.2	149.4	824.0

Table 5: Comparisons of 22 measured whistle variables for short-finned pilot whales recorded in Onslow Bay versus those recorded off of Cape Hatteras. Q = quartile. Shading indicates significant differences of the Wilcoxon rank-sum tests (α = 0.05). Whistles for which there were missing values were not included.

	Wilcoxon rank-sum results
Max Freq	▨
Min Freq	▨
Freq Range	
Start Freq	▨
End Freq	▨
1st Q Freq	▨
2nd Q Freq	▨
3rd Q Freq	▨
Mean Freq	▨
Duration	
# Inflection Points	
Max Slope	
Min Slope	
Slope Range	
Start Slope	
End Slope	
1st Q Slope	
2nd Q Slope	
3rd Q Slope	
Mean Slope	
Start Slope Sign	
End Slope Sign	▨

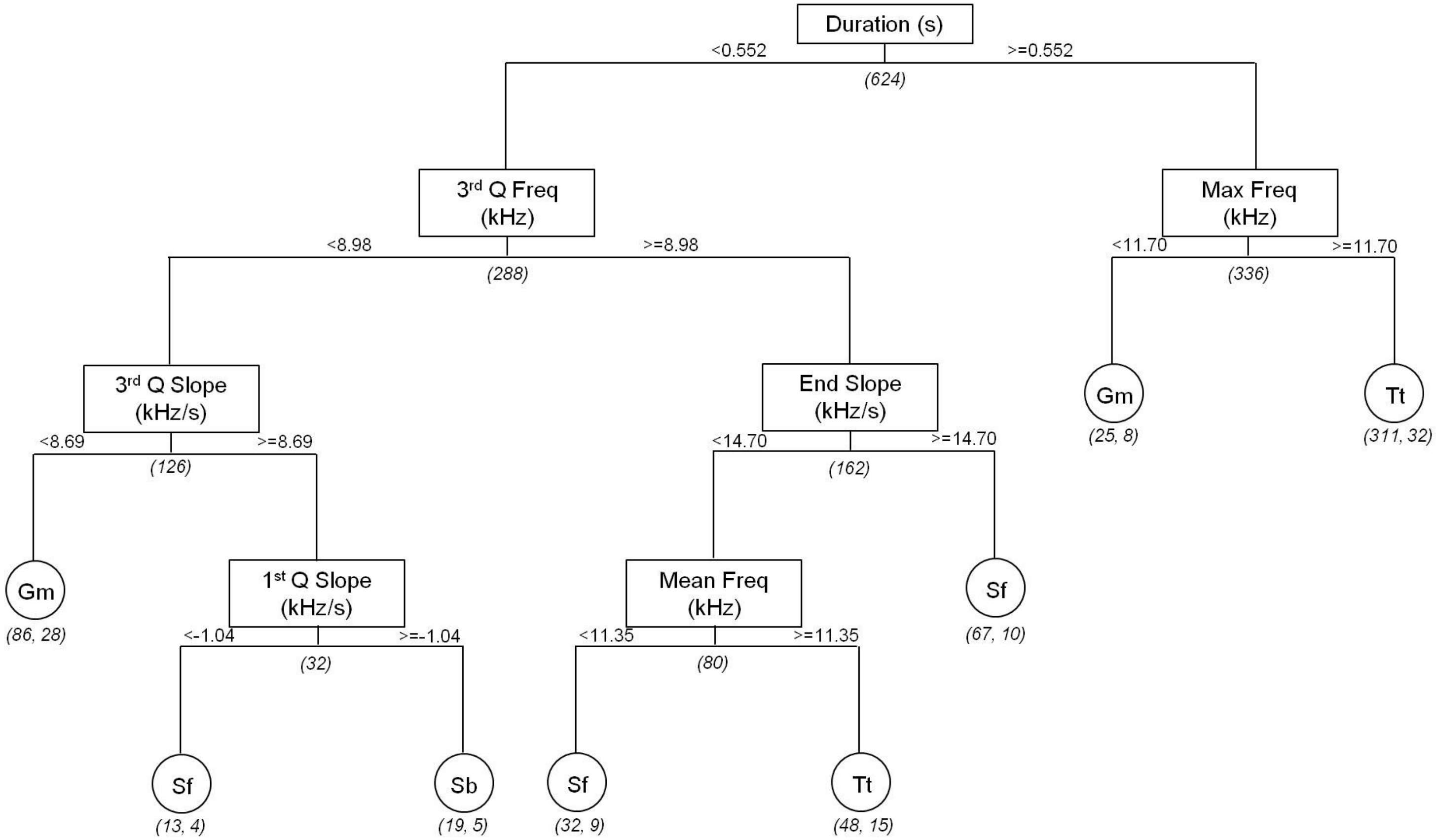

Figure 6: Seven variable CART classification tree examining classification performance of four species. The eight terminal nodes are represented by circles. Gm = short-finned pilot whales, Sf = Atlantic spotted dolphins, Sb = rough-toothed dolphins, and Tt = bottlenose dolphins. For non-terminal nodes, the number in parentheses represents the total number of whistles in each group. For the eight terminal nodes, the first number in parentheses represents the total number of whistles in each group, and the second number represents the total number of whistles that were incorrectly classified.

Table 6: Results of the eight terminal node classification tree examining interspecific differences in whistles of four species. The optimal tree was grown using seven variables (duration, third quartile frequency, maximum frequency, third quartile slope, end slope, first quartile slope, and mean frequency). The overall correct classification was 74.2%, n = 624 whistles. Individual correct classification rates are shown in bold. The percentage of correct classifications expected by chance is 25% for each species.

	% Classified as			
Actual Species	Atlantic spotted dolphins	Bottlenose dolphins	Rough-toothed dolphins	Short-finned pilot whales
Atlantic spotted dolphins	**63.0**	25.9	0.6	10.5
Bottlenose dolphins	4.7	**92.3**	0.3	2.7
Rough-toothed dolphins	8.6	0	**40.0**	51.4
Short-finned pilot whales	6.7	5.6	3.4	**84.3**

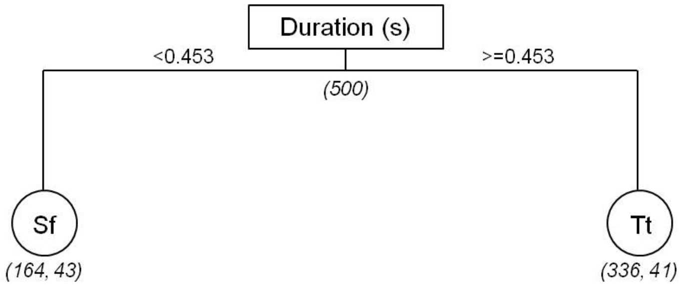

Figure 7: One variable CART classification tree examining classification performance of two species. The two terminal nodes are represented by circles. Sf = Atlantic spotted dolphins and Tt = bottlenose dolphins. For the non-terminal node, the number in parentheses represents the total number of whistles in that group. For the two terminal nodes, the first number in parentheses represents the total number of whistles in each group, and the second number represents the total number of whistles that were incorrectly classified.

Table 7: Results of the classification tree examining interspecific differences in whistles of two species. The optimal tree was grown using only one variable - duration. The overall correct classification was 82.2%, n = 500 whistles. Correct classification rates are shown in bold. The percentage of correct classifications expected by chance is 50% for each species.

Actual Species	% Classified as Atlantic spotted dolphins	 Bottlenose dolphins
Atlantic spotted dolphins	**74.7**	25.3
Bottlenose dolphins	12.7	**87.3**

Table 8: Results of within species CART analyses showing percent correct classification rates and percent expected by chance. Percent correct classification rates are overall percent correct classification rates. Percent expected by chance is calculated by dividing 100% by the number of recording sessions for each species.

Species	# Recording Sessions	# Whistles	% Correct Classification	% Expected by Chance
Atlantic spotted dolphins	11	157	26.8	9.1
Bottlenose dolphins	21	330	14.6	4.8
Short-finned pilot whales	6	89	41.6	16.7

Table 9: Results of CART analysis examining differences in short-finned pilot whale whistles recorded in Onslow Bay (n = 41) versus Hatteras (n = 48). The optimal tree had only one terminal node. Correct classification rates are shown in bold. The percentage of correct classifications expected by chance is 50% for each location.

Location	% Classified as Hatteras	 Onslow Bay
Hatteras	**100**	0
Onslow Bay	100	**0**

1.4 Discussion

The CART analysis performed when using whistles of all four species resulted in an optimal classification tree with an overall correct classification rate of 74.2%. Individual correct classification rates for species ranged from 40.0% to 92.3%, greater than expected by chance alone, although the whistles of rough-toothed dolphins were misclassified as short-finned pilot whale whistles more often than being correctly classified (possibly from the small sample size) indicating poor discriminating performance of the model for that species. Nevertheless, from these results, I conclude that the four species examined do produce whistles with species-specific characteristics and that bottlenose dolphins and short-finned pilot whales produce the most distinctive whistles of the four species examined.

1.4.1 Descriptive statistics

Values of the basic whistle variables (start and end frequency, minimum and maximum frequency, and duration) measured for Atlantic spotted dolphins, bottlenose dolphins, rough-toothed dolphins, and short-finned pilot whales in this study agree reasonably well with those reported in the literature (see Table 10). I should note, however, that I only recorded one group of rough-toothed dolphins. Such a small sample size will not represent the full range of vocalizations exhibited by this species. It is therefore not surprising that the values I found differed from the only other measures reported for rough-toothed dolphins (even when potential geographic variation is not taken into account).

The five variables for short-finned pilot whale whistles measured in this study all fell within the published ranges of means (Table 10), but my recordings contained some whistles with minimum frequency components that fell below 2 kHz (the lower limit of my recording system). I did not extract the contours from these whistles (as this could result in shorter durations, inaccurate minimum frequencies, and possibly incomplete whistles) and thus my estimate of minimum frequency is likely biased upwards and, consequently, the frequency range is likely biased downwards for this species.

Table 10: Descriptive statistics from the literature and from the present study for whistles of species visually observed in Onslow Bay.

Species (Geographic location)	Mean Start Freq (Hz)	Mean End Freq (Hz)	Mean Min Freq (Hz)	Mean Max Freq (Hz)	Mean Duration (s)	Study
Delphinus delphis (Eastern Tropical Pacific)	9800	11400	7400	13600	0.8	Oswald *et al.* 2003
G. macrorhynchus (Caribbean & Tenerife)	7480	8160	5430	9600	0.56	Rendell *et al.* 1999
G. macrorhynchus (Eastern Tropical Pacific)	4400	5500	3600	6100	0.4	Oswald *et al.* 2003
G. macrorhynchus (Gulf of Mexico)	4360	5280	3230	6680	0.48	Baron *et al.* 2008
Globicephala spp. (western North Atlantic)	4350	5160	3430	6260	0.54	Baron *et al.* 2008
G. macrorhynchus (western North Atlantic)	4860	5730	4190	7160	0.49	Present study
G. griseus (Azores & Stornoway)	12100	10830	8830	13440	0.53	Rendell *et al.* 1999
S. frontalis (western North Atlantic continental shelf)	9280	12260	7500	14170	0.43	Baron *et al.* 2008
S. frontalis (western North Atlantic offshore)	9080	13130	7510	15840	0.65	Baron *et al.* 2008
S. frontalis (South Atlantic)	8850	12760	8040	13580	0.36	Azevedo *et al.* 2010
S. frontalis (western North Atlantic)	9680	12380	8400	14060	0.34	Present study
S. bredanensis (Eastern Tropical Pacific)	6800	8500	6300	9100	0.6	Oswald *et al.* 2003
S. bredanensis (western North Atlantic)	5670	7510	5500	7710	0.19	Present study
T. truncatus (western North Atlantic)	11264	10225	7332	16235	1.3	Steiner 1981
T. truncatus (Eastern Tropical Pacific)	11200	9000	7400	17200	1.4	Oswald *et al.* 2003
T. truncatus (Sado Estuary, Portugal)	5800	12100	5400	15000	0.859	dos Santos *et al.* 2005
T. truncatus (northern Gulf of Mexico)	10820	11170	7870	16190	0.88	Baron *et al.* 2008
T. truncatus (western North Atlantic)	10640	12400	8240	15030	0.62	Baron *et al.* 2008
T. truncatus (western North Atlantic)	9700	11020	7620	17580	1.15	Present study

1.4.2 Species classification

The CART analysis examining interspecies differences for all four species produced an optimal classification tree using seven of the 22 extracted whistle variables, resulting in an overall classification rate of 74.2%. Individual species correct classification rates ranged from 40.0% to 92.3%, all of which were significantly greater than expected by chance (25%). Of the seven variables in the optimal classification tree, three showed significant differences in all but one species pair-wise comparison, and three showed significant differences in all but two comparisons (Table 3). Bottlenose dolphins and short-finned pilot whales had the most distinctive whistles; their whistles were classified with the least error. It is not surprising that bottlenose dolphins had the highest correct classification rates, as the whistles of this species were distinguishable from all other species based on several measured variables (Table 2), including duration, which was the variable at the first node in the optimal classification tree made using whistles from all four species (Figure 6).

Significant intraspecific variation was found in the frequency variables between the two sampled locations for short-finned pilot whales (Table 5), but their mean frequency variables were lower than bottlenose and Atlantic spotted dolphins, although these frequencies were similar to those of rough-toothed dolphins (Table 2). Short-finned pilot whales also had the second highest duration, which in combination with the lower frequency whistles and third quartile slope (which was a variable in the optimal classification tree) led to a relatively high correct classification rate.

Atlantic spotted dolphins and rough-toothed dolphins had lower correct classification rates. The lower correct classification rate for Atlantic spotted dolphins (and the misclassifications split between the other three species) was not surprising, given the similarity in measured variables to those of the other species' whistles (Table 2). Lastly, despite having the shortest duration, rough-toothed dolphins also had a low correct classification rate and actually were misclassified as short-finned pilot whales more often than being correctly classified (Table 6). Closer inspection of the variables measured shows many similarities of this species to both short-finned pilot whales and Atlantic spotted dolphins (Tables 2 and 3). The high misclassification rate may have been due to the fact that only one recording session was used for this species and so the inclusion of additional recordings could affect these results. Nevertheless, the optimal classification tree did a poor job at differentiating this species.

Individual classification rates were better than chance, but the coefficients of variation were quite high (Table 2), indicating a high degree of intraspecific variation in these variables. For whistles to be distinguished at the species level, certain variables (or combinations of variables) should exhibit low variation at the intraspecific level, while simultaneously exhibiting high variation between species. According to Hutchison et al. (1968) and Wunderle (1979), a low degree of intraspecific variation in specific call variables may indicate they are used by the animals to determine species identity. Both Steiner (1981) and Ding et al. (1995) found that frequency variables had a relatively low degree of intraspecific variation (with maximum frequency usually exhibiting the lowest variation). Likewise, Rendell et al. (1999) and Gannier et al. (2010) found that frequency variables of whistles were useful in species classification. In general, the results presented here agree with previous findings that frequency variables are important in whistle classification; the frequency variables had the lowest coefficients of variation (Table 2). Overall, the results of the species comparisons of variables in the present study (as well as the study by Gannier et al. (2010)) indicate the importance of frequency variables as discriminators.

In contrast to these relatively stable frequency variables, Steiner (1981), Ding et al. (1995), and Oswald et al. (2003) found that the duration and number of inflection points had a relatively high degree of intraspecific variation. Steiner (1981) suggested that these variables may be used to differentiate individuals, as suggested by Hutchison et al. (1968) and Wunderle (1979). Other studies have found that behavioral state affects these two whistle variables (Ansmann et al. 2007, Esch et al. 2009b, Azevedo et al. 2010). In the present study, when only looking at the variables commonly measured and reported by other researchers (the frequency variables, duration, and number of inflection points), the duration and number of inflection points exhibited the highest coefficients of variation, indicating a high degree of intraspecific variability. Despite this, however, duration proved to be an important variable for discriminating bottlenose dolphins from Atlantic spotted dolphins and rough-toothed dolphins in the present study. This is demonstrated in the optimal classification tree made when using whistles from all four species, in which duration was the variable at the first node (Figure 6), as well as the optimal tree made when using only the two most commonly observed species in Onslow Bay (Atlantic spotted and bottlenose dolphins), in which duration was the only variable included (Figure 7). A combination of duration and maximum frequency separated bottlenose dolphins from short-finned pilot whales. Bottlenose dolphins, of which I recorded 27 groups, produced whistles with much longer durations than any other species (Table 2). These longer durations were partly due to the multi-looped nature of many of their whistles. In any case, it seems that at least in my study area, bottlenose dolphin whistles can be distinguished from those of Atlantic spotted dolphins and rough-toothed dolphins based on this variable.

As suggested by Ding et al. (1995), frequency variables may not be the only variables that can be used to indicate species identity. Gannier et al. (2010) explored the importance of several slope variables in whistle classification but concluded that they were not as useful in discriminating among species because they had high variance, and therefore were possibly linked more closely with behavior. Both Oswald et al. (2003) and Gannier et al. (2010) suggested that it would be worthwhile to explore additional variables to determine if they might improve classification rates. I measured several novel variables in the present study, and although most of these variables had high coefficients of variation, the seven-variable optimal classification tree included three of these new variables (third quartile frequency, third quartile slope, and first quartile slope). Future studies should investigate additional variables, including statistical variables such as frequency and slope variables describing the mode, median, kurtosis, skewness, and standard deviation of whistle contours.

Oswald et al. (2003) and Gannier et al. (2010) both suggested that whistles should be recorded during different behavioral states, as this may lead to more robust classification models with possibly even higher predictive power. Behavior was not recorded while acoustic recordings were being made in the present study because of the limitations of the line-transect survey method. However, like Oswald et al. (2003), I tried to include several recording sessions for each species to help to account for this shortcoming, although this was not possible for rough-toothed dolphins.

The overall correct classification rate for the CART analysis (74.2%) was high, but likely overestimated. The correct classification rates of the intraspecific comparisons were greater than expected by chance for all three species examined (Table 8), indicating that there was some effect of the recording session (perhaps due to individually distinctive whistles, behavior, or group composition). The cross-validation technique did not remove entire recording sessions for each run, as noted by Oswald et al. (2003), so the correct classification rates were likely high because the optimal tree was built on whistles from all recording sessions for which there was greater than one whistle. Also, the CART analysis only included one recording session of rough-toothed dolphins. Therefore, it is unlikely that the entire repertoire of this species was included. Additional recordings of this species would likely affect the outcome of the model.

I was unable to include recordings of all odontocete species present in Onslow Bay. Short-beaked common dolphins (*Delphinus delphis*) were seen only during aerial surveys and a second species (Risso's dolphins) did not produce any good-quality whistles when recorded on two occasions. Including recordings from all species would have created a more complicated classification task but would have been useful, given the ultimate goal of differentiating species from archival recordings. Table 10 includes five of the most commonly measured variables for the whistles of both Risso's dolphins and short-beaked common dolphins. Both of these species produce whistles that are similar to those of Atlantic spotted dolphins and bottlenose dolphins. Thus, I would expect the overall correct classification rate and the individual correct classification rates of Atlantic spotted dolphins and bottlenose dolphins to decrease with the addition of whistles from these species. As suggested in Oswald et al. (2003), including information on seasonality of occurrence, species distribution, and perhaps even how common each species is might help in the classification process. For example, in Onslow Bay, Atlantic spotted dolphins are encountered only in relatively shallow depths (< 200 m) and rough-toothed dolphins are rarely observed (*unpublished data*).

In conclusion, I used a statistical analysis to determine if whistles of Atlantic spotted dolphins, bottlenose dolphins, rough-toothed dolphins, and short-finned pilot whales recorded in Onslow Bay, North Carolina, could be classified to species. The results of the analysis were promising, giving an overall correct classification rate of 74.2% and individual species' correct classification rates ranging from 40.0% to 92.3%.

Classification was particularly good for bottlenose dolphins and short-finned pilot whales. The whistles of Atlantic spotted dolphins had a high correct classification rate although they were also misclassified as bottlenose dolphin whistles 26% of the time (Table 6). The whistles of rough-toothed dolphins had the lowest correct classification rate and were misclassified as short-finned pilot whale whistles more often than being correctly classified.

Despite the poor ability of the model to discriminate rough-toothed dolphins, I conclude that species-specificity in the whistles of these four species recorded in Onslow Bay and Hatteras does exist, with the most distinctive whistles belonging to bottlenose dolphins and short-finned pilot whales. Future work should focus on obtaining more recordings of rough-toothed dolphins as well as recordings of other species. In addition, some whistles of Atlantic spotted dolphins and short-finned pilot whales included nonlinear components (subharmonics). I did not extract whistles that possessed these features, but future studies could explore the addition of these sounds in building classification models. Finally, future research also should experiment with building a classifier using all vocalizations (whistles, clicks, and burst-pulses) as described by Roch et al. (2007), which might improve classification performance.

Chapter 2: Examination of the species-specificity in the clicks of odontocetes in Onslow Bay, North Carolina

2.1 Introduction

With the increased use of passive acoustics as a monitoring tool, the importance of classifying calls to the species level is becoming more important, especially for recordings for which there are no concurrent visual observations. Species specificity has been found in the vocalizations of birds (Marler 1957), bats (Parsons and Jones 2000, Russo and Jones 2002, Biscardi et al. 2004, Fukui et al. 2004), and cetaceans (Steiner 1981, Ding et al. 1995, Matthews et al. 1999, Rendell et al. 1999, Oswald et al. 2003, Soldevilla et al. 2008). The toothed cetaceans, or odontocetes, produce sounds that often are categorized into three groups: narrow-band tonal whistles, broad-band clicks, and burst-pulsed sounds (Richardson et al. 1995), although Murray et al. (1998) describes the graded nature of odontocete calls, categorizing them into two groups (whistles and clicks) with burst-pulses as intermediate sounds. Of these categories, whistles have been the focus of a considerable body of research for odontocetes because they typically occur at relatively low frequencies compared to other odontocete vocalizations (thus being mostly represented with lower sampling rates of recording equipment). However, recent advances in hardware technology have made it easier to record the broadband calls of odontocetes that require higher sampling rates into the ultrasonic ranges. This has led to an emerging body of research focused on classifying odontocete clicks to the species level.

Recent studies have found species-specific characteristics in the clicks of several odontocetes. For example, sperm whales (*Physeter macrocephalus*) have been successfully identified from their clicks (Goold and Jones 1995, Barlow and Taylor 2005). Beaked whales can be distinguished from other odontocetes by the upsweep nature of their clicks. Their clicks also are distinctive at the species level, as their frequency and inter-click intervals vary considerably (Johnson et al. 2004, Gillespie et al. 2009, Baumann-Pickering et al. 2011). More specifically, Baumann-Pickering et al. (2011) found that duration, inter-click interval, bandwidth, peak frequency, and center frequency were all important in species discrimination. Other studies have differentiated the clicks of four porpoise species at the subfamily level using duration (Kamminga et al. 1996) and distinguished harbor porpoise (*Phocoena phocoena*) clicks from those of dolphins and false killer whales (*Pseudorca crassidens*) based on the frequency ranges and waveforms of the clicks (Nakamura and Akamatsu 2004). Nakamura and Akamatsu (2004) also showed that false killer whale clicks could be distinguished from those of common dolphins (*Delphinus delphis*), bottlenose dolphins (*Tursiops truncatus*), Pacific white-sided dolphins (*Lagenorhynchus obliquidens*), and baiji (*Lipotes vexillifer*) based on duration and peak frequency, but they were unable to differentiate the other four dolphin species. More recently, Baumann-Pickering et al. (2010) found that the temporal parameters duration and inter-click interval were not robust click parameters for delphinids (as they were affected by recording and analysis conditions) and were thus not as useful in species classification. These authors did find, however, that the spectral parameters median peak and center frequencies were useful in differentiating between three delphinids: melon-headed whales (*Peponocephala electra*), bottlenose dolphins, and Gray's spinner dolphins (*Stenella longirostris longirostris*). In addition, Soldevilla et al. (2008) found distinct peak and notch spectral patterns (indicated by high and low amplitudes, respectively) for Pacific white-sided dolphins (*Lagenorhynchus obliquidens*) and Risso’s dolphins (*Grampus griseus*). Together, these studies suggest that peak frequency characteristics (including the dominant peak frequency and other minor peak and notch spectral patterns throughout the entire click bandwidth), click duration, and even inter-click intervals can be species-specific; however, for discriminating among delphinids, the spectral features seem to be more useful.

For vocalizations to be distinguished at the species level, certain call variables (or combinations of variables) should exhibit low variation within a species, while simultaneously exhibiting high distinctiveness between species. When a species exhibits high variability in its call variables, classifying calls to a species becomes more challenging for researchers. For whistles, studies have found that frequency variables typically have a low degree of intraspecific variation (Steiner 1981, Ding et al. 1995), which may be a result of physical constraints of sound production or an adaptation to their environment (Ding et al. 1995). Similarly, delphinids may be limited in their ability to vary the spectral features of their clicks (perhaps because of their morphology as suggested by Soldevilla et al. (2008) for Risso's and Pacific white-sided dolphins), which may be why studies have found such features to be more useful in discrimination.

The present study was conducted in a relatively small geographic area in which most, if not all, odontocete species come into acoustic contact with each other. The goal of the present study was to determine if delphinid species inhabiting Onslow Bay, North Carolina, could be distinguished by their clicks. I used recordings of single-species groups collected during concurrent visual and acoustic line-transect surveys off North Carolina between September 2007 and August 2010 to examine the species-specificity of clicks produced by Atlantic spotted dolphins (*Stenella frontalis*), bottlenose dolphins, Risso's dolphins, rough-toothed dolphins (*Steno bredanensis*), and short-finned pilot whales (*Globicephala macrorhynchus*). Similar to Soldevilla et al. (2008), I examined the spectral peak and notch structure of delphinid clicks, looking for consistent peaks and notches that were unique to a species that possibly could be used in species differentiation. I also examined other spectral features (including peak frequency, center frequency, and -3 dB bandwidth) and the temporal feature duration to determine if these parameters could be used in species differentiation.

2.2 Methods

2.2.1 Study area and survey platforms

The main survey area consisted of an 83 km x 74 km rectangular region in Onslow Bay, North Carolina (Figure 8), which contained 10 line-transects laid out perpendicular to shore. Onslow Bay is located within the South Atlantic Bight, along the North Carolina coast between Cape Lookout and the Cape Fear River. We conducted boat-based line-transect visual and acoustic surveys from either a 16.2-m fishing vessel (the *F/V Sensation*) or an 11.6-m research vessel (the *R/V Cetus*). A second survey area was located north of Onslow Bay, off Cape Hatteras, North Carolina (Figure 9). This second survey area was chosen due to its proximity to Onslow Bay (approximately 200 km away) and comparatively high abundance of odontocete species. We conducted boat-based line-transect visual and acoustic surveys in this location from the *F/V Sensation*.

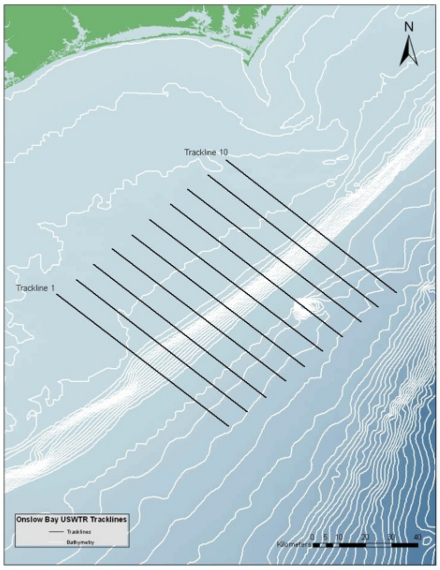

Figure 8: Main study area in Onslow Bay, North Carolina. The ten lines running perpendicular to shore represent tracklines used during line-transect visual and acoustic surveys.

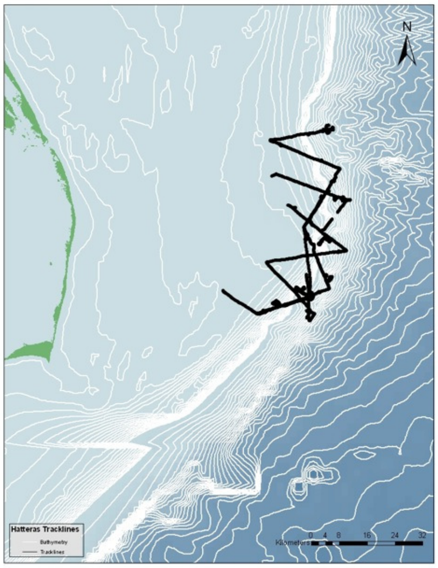

Figure 9: Second study area in Hatteras, North Carolina. The black zigzagging lines represent the survey effort during visual and acoustic surveys.

2.2.2 Data collection

We conducted visual and acoustic line-transect surveys from September 2007 through August 2010. During surveys, high-frequency acoustic recordings (192-kHz sampling rate) were made with a system consisting of a laptop running *Ishmael* software (Mellinger 2001), a MOTU Traveler audio interface (Mark of the Unicorn, Cambridge, MA, USA), and a hydrophone array (Seiche Instruments, UK) with 300 m of tow cable. The array consisted of four potted elements, spaced 1.2 m apart, with a flat (+/- 3 dB) frequency response between 2 and 100 kHz and a sensitivity of -165 dB re 1V/μPa. The hydrophone array was towed 150 m behind the vessel at a speed of approximately 16.7 km/h. A 5-m rope tail was attached to the end of the hydrophone array for stabilization. Incoming acoustic signals were monitored constantly both visually (via spectrograms in

Ishmael, with the gain set to -96 dB) and aurally by a trained acoustician. Recordings were made directly to an external USB-connected hard drive using *Ishmael* whenever marine mammal sounds were detected, at which point time and location were noted. Species identification and group size were determined visually by a team of at least three independent observers. Acoustic recordings were analyzed only for single-species groups positively identified in this manner. If another species was sighted within 5.6 km of the focal group, the recording was not included in the analysis to prevent ambiguity of the species being recorded (following Oswald et al. 2007).

2.2.3 Signal and statistical analyses

I used customized routines in Matlab (The MathWorks Inc., Natick, MA) to select clicks from the towed array recordings. As in Soldevilla et al. (2008), to find the start and end times of clicks, a detector algorithm that operated in the frequency domain was applied first. Spectra were calculated using 5.33 ms of data and a Fast Fourier Transform (FFT) length of 1024, 50% overlap, and a Hann window. For each spectrum, the mean of the surrounding 3 s of data was subtracted. After this spectral mean subtraction, the algorithm picked spectra as possible clicks when a minimum percentage of frequency bins in their spectra (defined as 12.5% in the present study) exceeded a threshold (11 dB) within a specified frequency bandwidth (5-95 kHz). For each possible click, 7.5 ms of sound before and after the detection initially was labeled as the start and end of the possible click, respectively.

Once the possible clicks were selected, a finer resolution click detector that operated in the time domain and used a Teager energy operator (Kaiser 1990) was applied. The complete click with reverberations was obtained by calculating the Teager energy with a 10-point running average. The start and end points of the clicks were defined as the first and last points that exceeded the noise floor (the 40th percentile of energy for each click) by a factor of three. Spectra of the selected clicks were calculated using 1.33 ms of data following this defined start of the signal and a 256-point FFT with a Hann window. The remaining data were used in calculating a mean noise spectrum.

For each recording session, a mean noise spectrum was calculated by averaging the noise spectra for each individual click. This mean noise spectrum was subtracted from each click spectrum associated with the same recording session. After this spectral mean subtraction, spectral amplitudes were normalized between 0 and 1.

From each recording session, click trains were selected randomly such that click trains were chosen at most one time and the total number of click trains was no greater than two times the estimated group size (following Soldevilla et al. 2008). Clicks that were separated by less than 0.5 s were considered part of the same click train for the purposes of the present study. From the selected click trains, up to five clicks were selected randomly. I chose to include up to five clicks per click train because trains could include clicks from multiple animals that were clicking at the same time (and thus had overlapping trains). Also, Au (1993) and Madsen et al. (2004) showed that individual variation exists in the spectral structure of clicks depending on the animal's orientation to the hydrophone. I wanted to include such individual variation in my analysis to help account for the unknown orientation of wild, free-ranging dolphins. Figure 10 shows an example of overlapping click trains recorded in the presence of Risso's dolphins. This figure also shows the individual variation between clicks (note the "wiggle") in one train, likely a result of the animal changing its orientation to the hydrophone as it scans the environment.

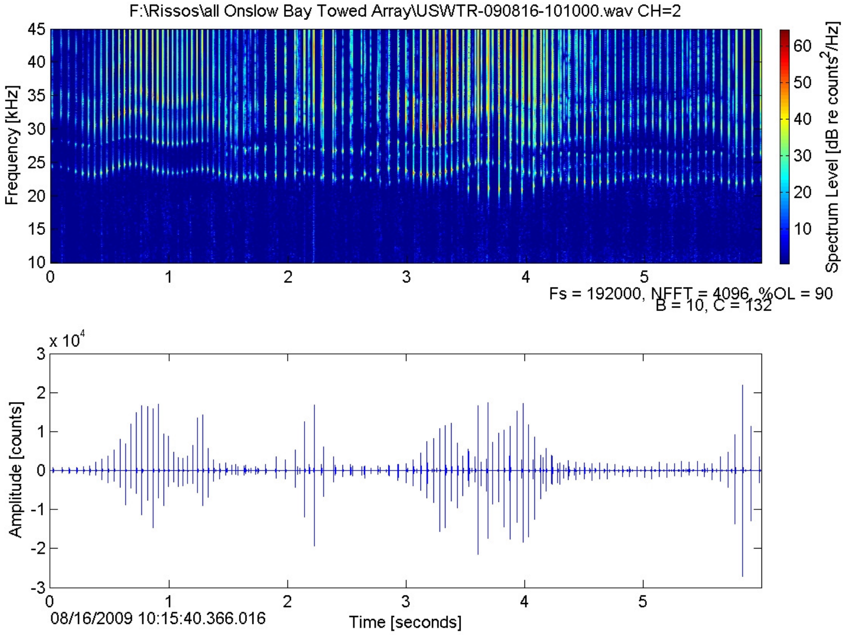

Figure 10: Spectrogram (top) and waveform (bottom) of overlapping click trains recorded in the presence of Risso's dolphins. Note the "wiggle" in the click trains, likely a result of the animal changing its orientation to the hydrophone as it scans the environment.

Once the clicks were selected, they were analyzed for species-specificity by determining the frequency values of consistent spectral peaks and notches in the frequency domain. As in Soldevilla et al. (2008), the frequency ranges for consistent peaks and notches were established by implementing a first-order regression-based algorithm that pulled frequency values from the normalized click spectra. For each species, histograms consisting of 750 Hz-wide bins were made showing the number of clicks with peaks or notches at each frequency value. These bins were compared to a random uniform distribution (the expected distribution if there were no consistent peak or notch values) using a one-tailed z-test ($\alpha = 0.05$; Zar 1999). Bins that rose significantly above this uniform distribution indicated they occurred more often than expected by chance. Frequency bins that were significantly greater than the random uniform distribution and that had at least one adjacent frequency bin that was also significantly greater were considered consistent. Because clicks were selected by an automated process and not manually reviewed, it was possible that whistles and sudden changes in boat or flow noise occurred with the individual clicks. Such noise was most likely to occur below 15 kHz. Thus, I only considered peaks and notches to be consistent if they were above 15 kHz (also similar to Soldevilla et al. 2008). Finally, to obtain the means and ranges for the frequency values of consistent peaks and notches, a set of Gaussian curves was fit to each histogram that had consistent bands using Gaussian mixture models. The dominant Gaussian curve fit to each consistent peak or notch was used to obtain the mean and standard deviation for each peak or notch frequency value.

Also using the sub-sampled data that I analyzed for consistent peaks and notches, I made standard measurements for each click to determine if they differed among species. These standard measurements included duration, peak frequency (the frequency of maximum amplitude in the spectrum), center frequency (the frequency that divides the spectrum into two frequency intervals of equal energy), and the -3 dB bandwidth (calculated for the peak frequency). I chose not to examine inter-click interval because I was only making measurements on sub-sampled clicks (up to five per train) and click trains may have contained overlapping trains. Also, Baumann-Pickering et al. (2010) found that temporal parameters are not robust when trying to differentiate delphinid species (as recording situation - equipment, depth, sea state, animal location in relation to the hydrophone, etc. - can affect the results of duration and inter-click interval). I ran Kruskal-Wallis tests (Zar 1999) on each click parameter to determine the potential of using them for species differentiation. I performed multiple comparison tests using Bonferroni corrections on the significant results to determine which species had parameters that were significantly different. These statistical analyses were performed using JMP (SAS, Cary, NC).

2.3 Results

Between 2007 and 2010, I used the towed array to make single-species recordings of five species: Atlantic spotted dolphins, bottlenose dolphins, Risso's dolphins, rough-toothed dolphins, and short-finned pilot whales. From 46 recordings, the number of click trains I analyzed was no more than twice the group size, and the number of clicks per click train did not exceed five (Table 11). Multiple recording sessions were included for each species to examine species-specificity except for rough-toothed dolphins, which were sighted only once (Table 11). Group sizes ranged from 1 to 143 animals, and the total number of click trains during a recording session ranged from 1 to 74 trains.

Table 11: Number of recording sessions, group size, and number of clicks analyzed for each species.

Species	# Recording Sessions	Mean Group Size	Total # Click Trains	# Click Trains Selected	# Clicks Selected
Atlantic spotted dolphins	14	28.6	2168	524	2620
Bottlenose dolphins	24	10.8	3114	464	2320
Rough-toothed dolphins	1	27	496	54	270
Risso's dolphins	3	25.3	392	102	510
Short-finned pilot whales	4	28.5	590	134	670

Table 11 gives the total number of recording sessions and total number of clicks analyzed for consistent peaks and notches for each species. I found that only Risso's dolphins produced clicks with frequency values that consistently alternated between high (peaks) and low (notches) amplitudes (Figures 11c and 12c). The number of clicks that had peaks and notches at these frequency bands was greater than expected by chance. No other species had such consistent peaks and notches in their clicks (Figures 11 and 12).

I determined the means and standard deviations of consistent peaks and notches by fitting univariate Gaussian mixture models to the peak and notch histograms from

Risso's dolphin clicks. Peaks in this species' clicks appeared at 22.4 (± 0.9), 26.0 (± 0.9), and 32.6 (± 1.5) kHz, while notches occurred at 20.4 (± 1.6), 24.0 (± 1.0), 26.8 (± 1.4), 29.2 (± 2.1), and 34.2 (± 2.0) kHz (Figure 13).

Figure 11: Concatenated spectrograms (after spectral mean subtraction, left) and mean normalized spectral plots (right) of clicks using Hann-windowed data for (a) Atlantic spotted dolphins, (b) bottlenose dolphins, (c) Risso's dolphins, (d) rough-toothed dolphins, and (e) short-finned pilot whales. For the figures on the left, frequency is plotted on the y-axis and click number is plotted on the x-axis. Oranges and yellows represent greater magnitudes. Frequency bands that alternate between high and low amplitudes are apparent between 20 and 35 kHz for Risso's dolphins. Breaks between recording sessions are indicated by black vertical lines. For the figures on the right, the solid line represents the mean normalized amplitude and the dotted lines represent one standard deviation.

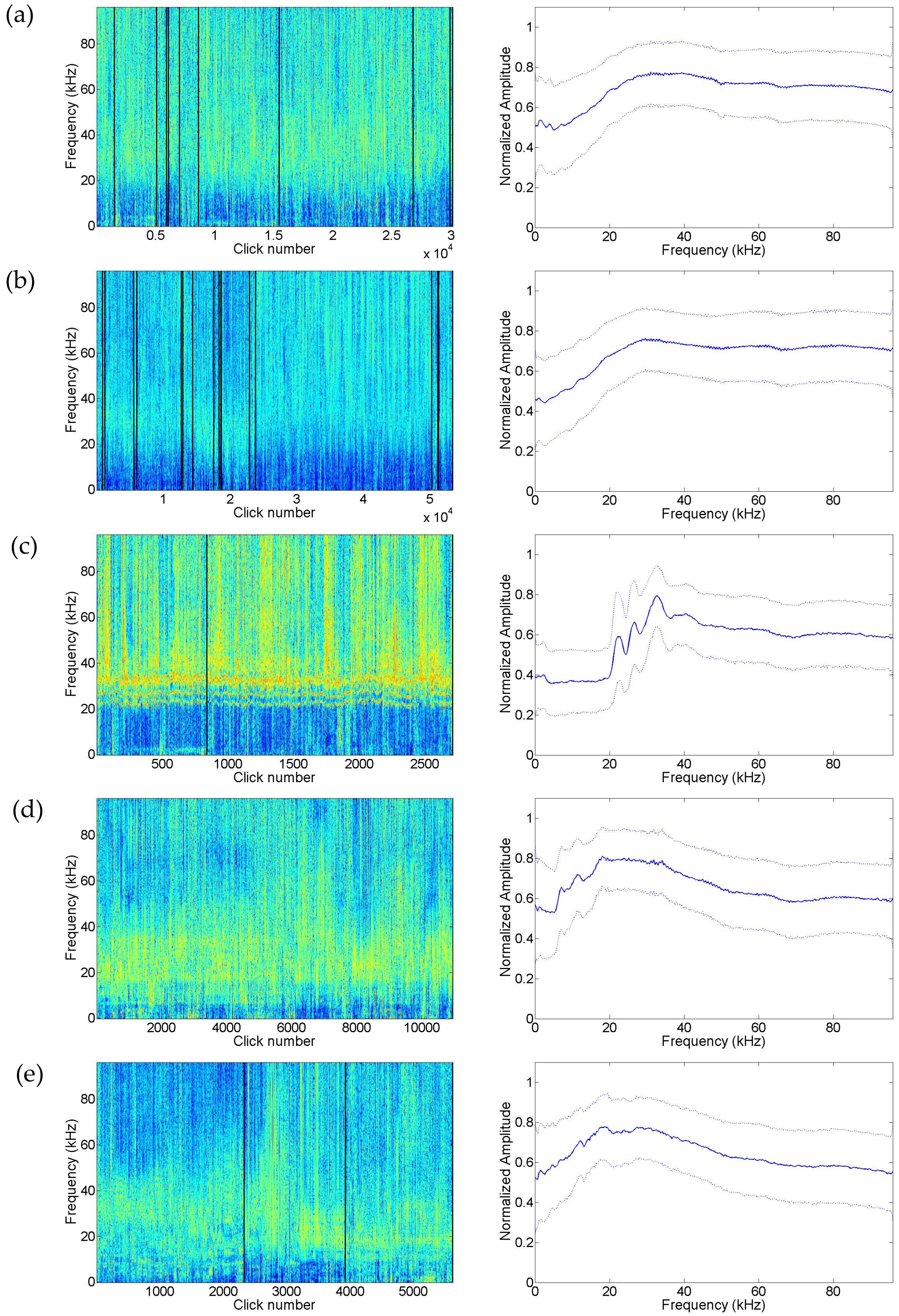

Figure 11 (Continued)

Figure 12: Histograms showing frequencies (kHz) of spectral peaks (left) and notches (right) for (a) Atlantic spotted dolphins, (b) bottlenose dolphins, (c) Risso's dolphins, (d) rough-toothed dolphins, and (e) short-finned pilot whales. The red line represents the mean of the expected random uniform distribution. Black diamonds near the top indicate frequency bars that had counts that were significantly greater than the random uniform distribution (one-tailed z-test, $\alpha = 0.05$). Groups (≥ 2) of consecutive black diamonds indicate frequencies that were considered to have consistent peaks and notches. Only peaks and notches with frequencies between 15-96 kHz were considered consistent.

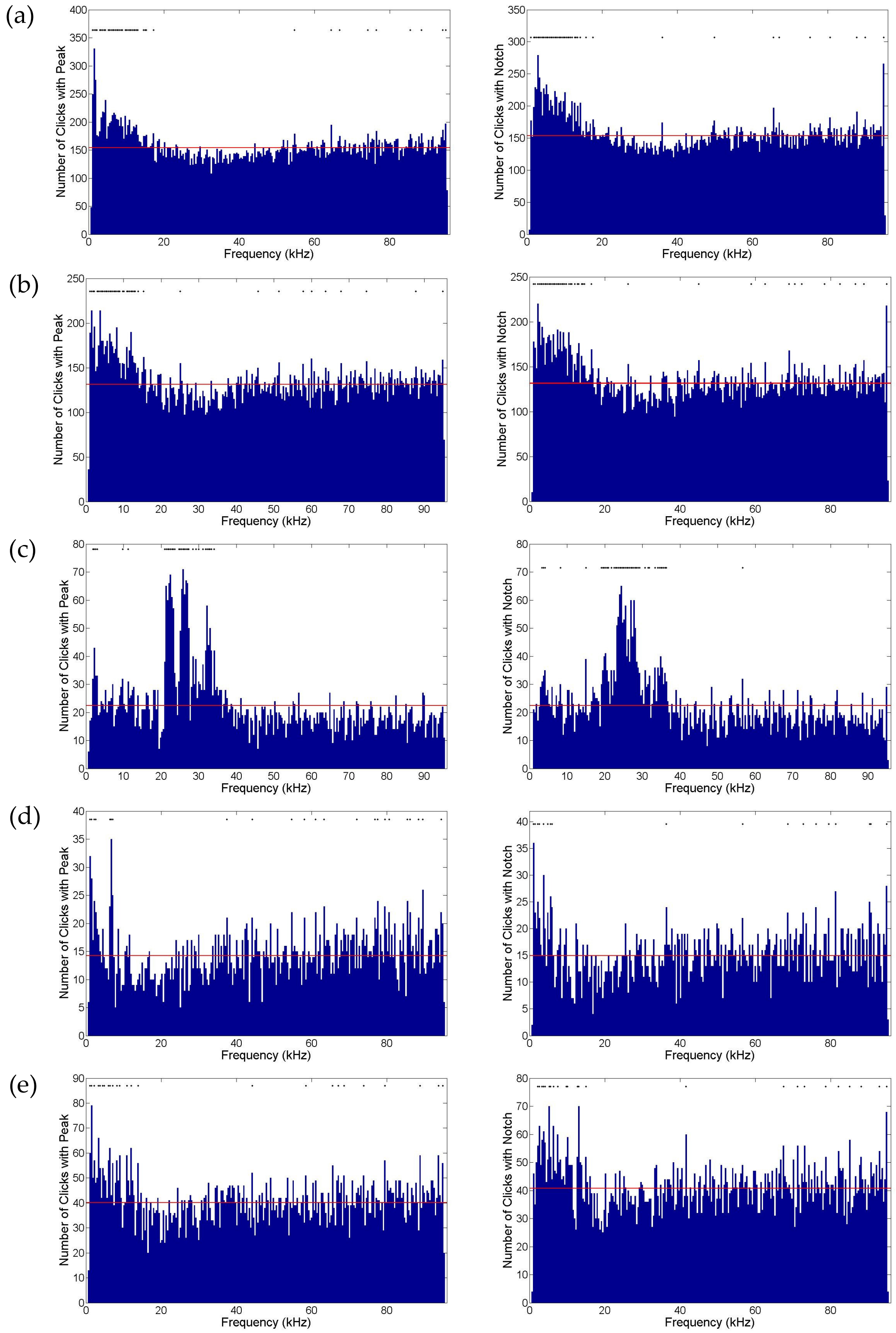

Figure 12 (Continued)

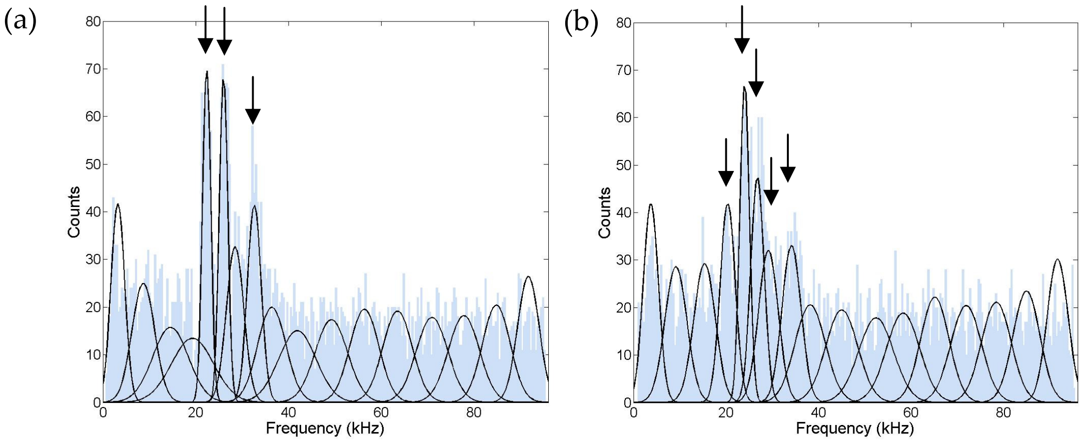

Figure 13: Curves of Gaussian mixture model fit to the (a) peak and (b) notch histograms for Risso's dolphins. For (a), arrows indicate consistent peaks (μ (± σ): 22.4 (± 0.9), 26.0 (± 0.9), and 32.6 (± 1.5) kHz). For (b), arrows indicate consistent notches (μ (± σ): 20.4 (± 1.6), 24.0 (± 1.0), 26.8 (± 1.4), 29.2 (± 2.1), and 34.2 (± 2.0) kHz).

Table 12 gives the mean and standard deviation of four standard click measurements (duration, peak frequency, center frequency, and -3 dB bandwidth) for clicks with reverberations of each species. I found significant differences between species in each of the parameters I measured: duration ($p < 0.001$, Kruskal-Wallis, $\alpha = 0.05$); peak frequency ($p < 0.001$, Kruskal-Wallis, $\alpha = 0.05$); center frequency ($p < 0.001$, Kruskal-Wallis, $\alpha = 0.05$); and -3 dB bandwidth ($p < 0.001$, Kruskal-Wallis, $\alpha = 0.05$). Table 13 shows the results of the post-hoc multiple comparison tests with Bonferroni corrections.

Table 12: Mean (± standard deviation) of four standard measurements for clicks (with reverberations) of each species.

	Duration (ms)	Peak Frequency (kHz)	Center Frequency (kHz)	-3 dB Bandwidth (kHz)
Atlantic spotted dolphins	0.44 (± 0.33)	45.9 (± 23.5)	48.5 (± 11.5)	2.0 (± 2.8)
Bottlenose dolphins	0.43 (± 0.33)	41.8 (± 24.0)	46.7 (± 11.8)	2.0 (± 3.4)
Risso's dolphins	1.06 (± 0.65)	32.6 (± 12.3)	42.0 (± 10.0)	1.2 (± 1.3)
Rough-toothed dolphins	0.43 (± 0.23)	28.1 (± 15.3)	34.5 (± 9.3)	2.1 (± 1.8)
Short-finned pilot whales	0.62 (± 0.47)	28.9 (± 15.5)	35.1 (± 8.8)	1.7 (± 2.6)

Table 13: Results of post-hoc tests with Bonferroni corrections comparing the four standard click measurements between each species. Gg = Risso's dolphins, Gm = short-finned pilot whales, Sf = Atlantic spotted dolphins, Sb = rough-toothed dolphins, and Tt = bottlenose dolphins. Shading indicates significant differences, with family-wise error rate = 0.05.

	Gg/Gm	Gg/Sb	Gg/Sf	Gg/Tt	Gm/Sb	Gm/Sf	Gm/Tt	Sb/Sf	Sb/Tt	Sf/Tt
Duration	▨	▨	▨	▨	▨	▨	▨			
Peak Freq	▨	▨	▨	▨		▨	▨	▨	▨	▨
Center Freq	▨	▨	▨	▨		▨	▨	▨	▨	▨
-3 dB Bandwidth	▨	▨	▨	▨	▨			▨	▨	

2.4 Discussion

Of the five species examined in the present study, I found that Risso's dolphins were the only species to produce clicks with distinctive spectral peak and notch structure between 15-96 kHz that could be used in species classification. Specifically, I found alternating frequency values of high (peaks) and low (notches) amplitude that were consistent among this species' clicks. Although differences do exist, these peak and notch frequencies are similar to the findings of Soldevilla et al. (2008), who examined the clicks of Risso's dolphins recorded offshore of southern California (peaks: 22.4, 25.5, 30.5, and 38.8 kHz; notches: 19.6, 27.7, and 35.9 kHz).

The main differences between the click patterns described by Soldevilla et al. (2008) and what I describe here are one less peak frequency and two additional notch frequencies. These differences may have been the result of the smaller sample size and/or the inclusion of up to five clicks per click train in the present study. Whereas Soldevilla et al. (2008) used data from six recording sessions and analyzed thousands of clicks, I only had data from three recording sessions and only analyzed 510 clicks. Soldevilla et al. (2008) also only included a single click from each randomly selected click train whereas I included up to five clicks per randomly selected click train. The inclusion of more than one click per train could have led to oversampling of individuals, which may have affected the results. However, because click trains were defined as clicks separated by < 0.5 s, more than one animal's clicks could be found in a click train (as in the case of overlapping trains). In addition, because spectral features are affected by the orientation of the animal to the hydrophone (Au 1993, Madsen et al. 2004), some degree of variation within individuals was expected in the peaks and notches of clicks, and I thought this should be incorporated if possible. For these reasons, and also for the reason of increasing the sample size, I included up to five clicks per click train in my analysis. Nevertheless, including more clicks from each train likely introduced more variation in the degree to which clicks were off-axis, which may have increased the number of notches in my results, as Madsen et al. (2004) found increasing notches the farther off-axis a Risso's dolphin's clicks were recorded.

Geographic variation may be another explanation for the differences (not only the different numbers of peaks and notches but also the slight differences in actual peak and notch frequency values) seen between the present study and that by Soldevilla et al. (2008). Preliminary examination of Risso's dolphin clicks recorded off Hatteras, NC, and Jacksonville, FL, reveal similar patterning to that described here, with slight differences (*unpublished data*). I believe that the overall similarities in click spectral features of the Risso's dolphins recorded in these different locations indicate that while some geographic variation exists, the presence of distinct peak and notch spectral structure is potentially a species differentiating characteristic for Risso's dolphins that can be used in other geographic areas as well. Once again, I did expect some degree of variation to occur in the peaks and notches of clicks not only within an individual as they scan the environment, but also within a species (as individual variation in vocalizations is commonly reported within a species). As long as such variation is less than the variation found between species, though, the distinctive features still can be used as species-specific characteristics. For example, while Soldevilla et al. (2008) found what they hypothesized to be population-specific patterns in Pacific white-sided dolphins, the species as a whole was still distinct from Risso's dolphins.

The lack of spectral peak and notch patterns in the other four species was not surprising. Soldevilla et al. (2008) analyzed the clicks of five species and found consistent peaks and notches in only two species (Risso's dolphins and Pacific white-sided dolphins), both of which have similar sound-production pathways that are different from the other species they examined, as outlined by these authors. One of the three species they examined that did not have spectral peak and notch patterns was bottlenose dolphins, for which I also did not find spectral patterns.

I did find significant differences between the five species' clicks in the standard measurements (Table 13), but these results should be viewed with caution for several reasons. First, I did not record the entire bandwidth of clicks with the recording equipment used in the present study. Secondly, clicks were selected via an automated process and were not manually reviewed. Thus, whistles, engine and flow noise, and noise from the survey vessel's echosounder may be included with the clicks. This extra noise would bias the results toward the frequencies in which they occur. Nevertheless, the results indicate that these spectral and temporal parameters may be useful in species differentiation. Peak and center frequency may be the most useful of these parameters, as found by Baumann-Pickering et al. (2010), except for the case of rough-toothed dolphins and short-finned pilot whales (Table 13). Interestingly, I found significant differences in all standard measurements when comparing Risso's dolphins to all other species, which highlights the differences in this species' clicks compared to the other four species. All of these parameters paired with other click characteristics may be helpful in differentiating clicks among species. In addition, Baumann-Pickering et al. (2010) found reduced variability and increased discrimination performance when using mean values of groups of clicks instead of single click values. Thus, future work should consider averaging clicks when looking at these other click features.

Since the ultimate goal of the present work is to differentiate species from archival recordings in Onslow Bay, future work also should focus on obtaining more recordings of short-finned pilot whales, Risso's dolphins, and especially rough-toothed dolphins, as well as recordings of other species not included in the analyses but present in the study area. Short-beaked common dolphins (*Delphinus delphis*) is one such species. Soldevilla et al. (2008) analyzed the clicks of this species from recordings offshore of southern California. These authors found no consistent spectral banding patterns so it would be interesting to see how the clicks of animals recorded in the Atlantic might compare.

I also believe that there are two species that inhabit deeper waters in Onslow Bay during the winter that have not been recorded in conjunction with visual sightings. While reviewing recordings from a High-frequency Acoustic Recording Package (HARP; Wiggins and Hildebrand 2007) located at 335-m depth in Onslow Bay, I found two new spectral banding patterns in clicks (Figure 14). Such clicks have never been recorded on the towed array in Onslow Bay and thus likely belong to other species than the ones I analyzed here. Common dolphins have been sighted in this region during our aerial surveys, but have not been acoustically recorded. Future work may link them to these recordings if North Atlantic populations exhibit spectral banding in their clicks. Other possible (although perhaps less likely) odontocetes that might inhabit the area include Clymene dolphins (*Stenella clymene*), false killer whales (*Pseudorca crassidens*), killer whales (*Orcinus orca*), Pantropical spotted dolphins (*Stenella attenuata*), pygmy killer whales (*Feresa attenuata*), spinner dolphins (*Stenella longirostris*), and striped dolphins (*Stenella coeruleoalba*). In addition, beaked whales (*Mesoplodon spp.* and *Ziphius cavirostris*) have been seen in Onslow Bay; however, because these click types are not upswept in frequency, it is less likely that they are produced by beaked whales.

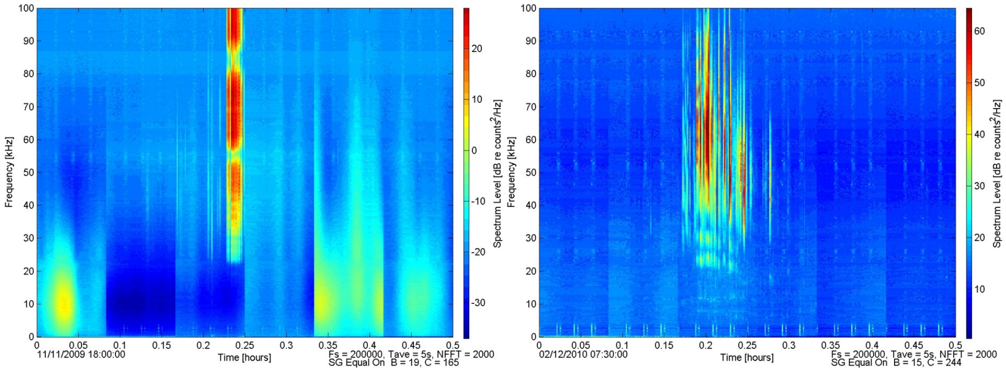

Figure 14: Two new click spectral patterns found in archival recordings from a HARP located in Onslow Bay at 335-m depth between November 2009 and April 2010.

In any case, because no spectral patterns were found for four of the five species examined in the present study and these five are the most commonly sighted species in Onslow Bay, it seems likely that data collected on archival recorders in Onslow Bay that have clicks with the peak and notch pattern I described here for Risso's dolphins are produced by Risso's dolphins. However, without recording all of the other species that inhabit Onslow Bay, it is impossible to say if the consistent peaks and notches found are truly unique.

2.5 Conclusion

My examination of spectral peak and notch features of clicks produced by Atlantic spotted dolphins, bottlenose dolphins, Risso's dolphins, rough-toothed dolphins, and short-finned pilot whales revealed promising results in terms of species-specificity for only one species - Risso's dolphins. However, I found that other spectral and temporal parameters, particularly peak frequency and center frequency, might yield positive results in differentiating the other four species, especially when combined with parameters not measured in the present study. More work needs to be done before this can occur, though, such as manual inspection of the clicks to remove those with whistles and those with engine, flow, or echosounder noise. Given the ultimate goal of differentiating species from archival recordings, future work also should include adding recordings from all local odontocete species and running discriminant function, classification and regression tree, and/or random forest analyses on the data to determine the classification performances of these models. Finally, combining data from both clicks and whistles into a model might help improve classification performance.

Chapter 3: Temporal variation in the occurrence of odontocete click events in Onslow Bay, North Carolina

3.1 Introduction

Passive acoustic techniques are being used with increasing frequency to monitor populations of marine mammals (Mellinger et al. 2007, Zimmer 2011). These techniques provide a long-term record unmatched by visual surveys and allow researchers to examine diel, seasonal, and inter-annual trends in the occurrence of vocalizing marine mammals.

Several previous studies have used stationary instruments to examine seasonal and inter-annual trends in cetacean occurrence and vocalization rates (Mellinger et al. 2004, Oleson et al. 2007b, Verfuß et al. 2007, Soldevilla et al. 2010a, Soldevilla et al. 2010b). Seasonal patterns in vocalizations may reflect migratory movements or seasonal variation in the production of calls. For example, passive acoustic monitoring has documented seasonal variation that may be related to migratory movements of several odontocete species, including: sperm whales (*Physeter macrocephalus*) in the Gulf of Alaska (Mellinger et al. 2004); harbor porpoises (*Phocoena phocoena*) in the Baltic Sea (Verfuß et al. 2007); and Pacific white-sided dolphins (*Lagenorhynchus obliquidens*) in the Southern California Bight (Soldevilla et al. 2010b). In contrast, Jacobs et al. (1993) documented seasonal variation in the occurrence of vocalizations of bottlenose dolphins (*Tursiops truncatus*), which were associated with seasonal changes in behavior.

On shorter time scales, many odontocetes exhibit diel variation in vocalization rate associated with concomitant changes in behavioral state. Examples of such diel variation include: Hawaiian spinner dolphins (*Stenella longirostris*), which produce fewer whistles when resting during the day than while foraging at night (Norris et al. 1994); striped dolphins (*Stenella coeruleoalba*), which show an increase in vocal activity at night (Gannier 1999, Gordon et al. 2000); short-beaked common dolphins (*Delphinus delphis*), which exhibit a crepuscular pattern in whistle production (Goold 2000); harbor porpoises, which increase their echolocation rate at night (Carlström 2005); and Risso's dolphins (*Grampus griseus*), which demonstrate a nocturnal increase in the occurrence of echolocation (Soldevilla et al. 2010a). Most of these diel trends in vocal activity are likely driven by circadian patterns of foraging activity (Norris et al. 1994, Gannier 1999, Gordon et al. 2000, Goold 2000, Soldevilla et al. 2010a), some of which are associated with diel changes in the availability of prey, such as the vertical migration of mesopelagic fish and squid.

Many odontocetes that exhibit nocturnal increases in vocalization rate feed on prey associated with the deep scattering layer (DSL). Specifically, Hawaiian spinner dolphins (Norris et al. 1994), striped dolphins (Blanco et al. 1995), common dolphins (Overhotlz and Waring 1991, Evans 1994, Pusineri et al. 2007), and Risso's dolphins (Clarke 1996) all feed on prey items that occur within the DSL. Studies suggest that these odontocetes forage at dusk or at night (Hawaiian spinner dolphin: Benoit-Bird and

Au 2003; striped dolphin: Gannier 1999, Gordon et al. 2000; common dolphin: Evans 1994, Pusineri et al. 2007; Risso's dolphin: Shane 1995).

Species that feed throughout the day, however, are not expected to show diel vocal patterns in echolocation clicks (which often are used as a proxy for foraging). Such species include: sperm whales (Watkins et al. 2002, Amano and Yoshioka 2003, Watwood et al. 2006); beaked whales (*Ziphius cavirostris* and *Mesoplodon densirostris*; Johnson et al. 2004, Tyack et al. 2006); and short-finned pilot whales (*Globicephala macrorhynchus*; Aguilar Soto et al. 2008).

I used High-frequency Acoustic Recording Packages (HARPs; Wiggins and Hildebrand 2007) to determine how odontocete click events varied temporally in Onslow Bay, North Carolina. I analyzed data from five HARP deployments, which occurred at various times throughout the year and at various locations, to determine if the overall occurrence of odontocete click events showed diel or seasonal patterns.

In addition, I examined diel patterns in vocal events for two species and one genus that can be identified by clicks: Risso's dolphins, sperm whales, and *Kogia spp*. I hypothesized that: (1) Risso's dolphins would show a nocturnal increase in click occurrence, as found by Soldevilla et al. (2010a); (2) sperm whales would not show any diel pattern in click production, because they forage throughout the day; and (3) *Kogia* would not show any diel pattern in click occurrence, because some of their cephalopod prey do not undergo vertical migrations (West et al. 2009).

3.2 Methods

3.2.1 Study area

The study area is located in Onslow Bay, North Carolina (Figure 15), within the South Atlantic Bight. The Gulf Stream, a fast, northward-flowing western boundary current, runs through a portion of this area along the shelf break and slope.

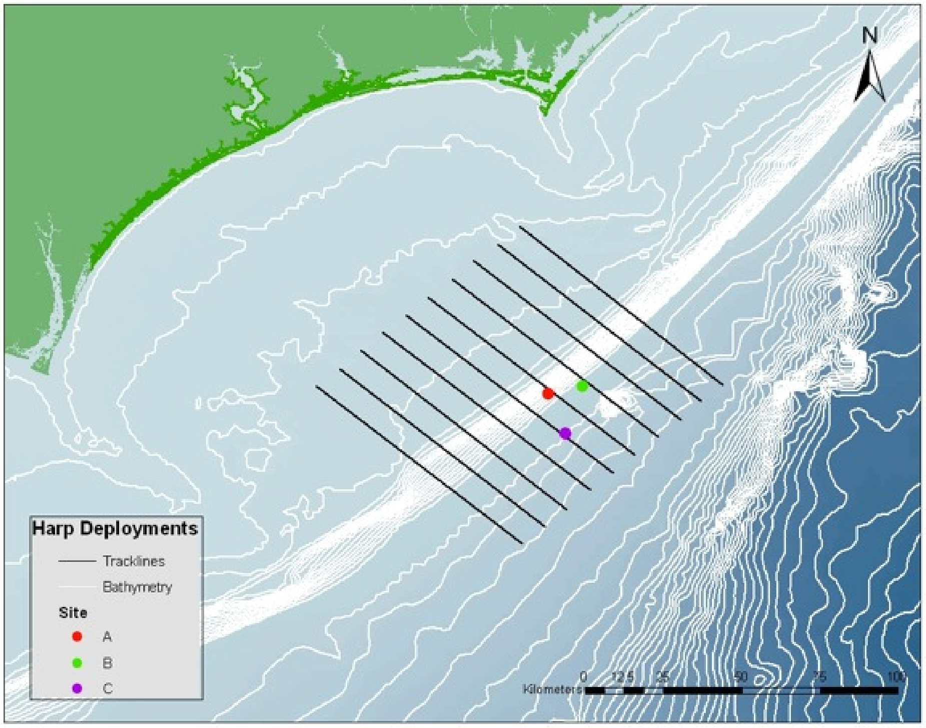

Figure 15: Study area in Onslow Bay, North Carolina, showing the three sites where HARPs were deployed. The ten lines running perpendicular to shore represent tracklines used during line-transect visual and acoustic surveys.

3.2.2 Instruments

I deployed HARPs in Onslow Bay, NC, over five periods between October 2007 and April 2010 (Figure 15; Table 14). All instruments sampled at 200 kHz. In the first deployment, the instrument recorded every other five minutes, but at the beginning of 2008, it began to record continuously. For the second and third deployments, the instruments recorded every other five minutes. For the fourth and fifth deployments, the HARPs recorded for five minutes and then were off for 10 minutes. Figure 15 shows the locations of these deployments. Table 14 and Figure 16 provide details on the timing of the deployments, the duty cycle, and depth of each deployment.

Table 14: HARP deployment sites, times, depths, and duty cycles. *Represents the initial duty cycle but instrument recorded continuously starting January 1, 2008.

Deployment	Site	Start Date	End Date	# Days Recorded	Depth (m)	Duty Cycle
1	A	10-Oct-07	16-Jan-08	99	162	5 min on/5 min off*
2	B	30-May-08	10-Sep-08	104	232	5 min on/5 min off
3	A	24-Apr-09	9-Aug-09	108	174	5 min on/5 min off
4	A	8-Nov-09	24-Feb-10	109	171	5 min on/10 min off
5	C	8-Nov-09	20-Apr-10	164	335	5 min on/10 min off

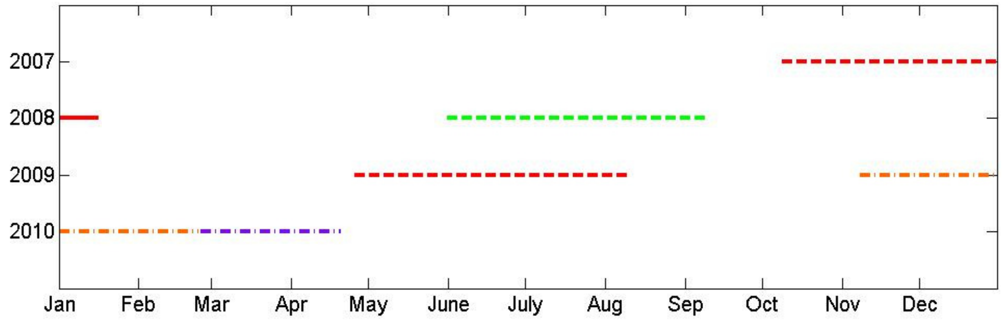

Figure 16: HARP sampling effort for each deployment and site, including duty cycle information. Site A = red, Site B = green, Site C = purple, Site A and C overlap = orange. For duty cycles, continuous line = recorded continuously, dashed line = recorded for every other five minutes, and dotted and dashed line = recorded for five minutes and then was off for 10 minutes.

3.2.3 Analysis

I detected vocal events by examining Long-Term Spectral Averages (LTSAs; Wiggins and Hildebrand 2007) generated in Triton (Scripps Institution of Oceanography). I visually inspected 30-minute LTSAs (with 100 Hz and 5 s resolution) for high-energy vocal events created by odontocete clicks. For each click event, I noted the start and end day and time.

Once all of the click events were detected, I sorted them into the one of four groups - Risso's dolphins, sperm whales, *Kogia spp.*, and other delphinids - using the following criteria. Soldevilla et al. (2008) first described unique patterns in the clicks of Risso's dolphins off Southern California, characterized by consistent peaks of energy at 22.4, 25.5, 30.5, and 38.8 kHz. I found similar click patterns in the HARP datasets analyzed here and in towed array recordings of Risso's dolphins in Onslow Bay, with consistent peaks at 22.4, 26.0, and 32.6 kHz (see Chapter 2). Thus, I classified clicks showing these peaks as Risso's dolphins. Sperm whales produce highly distinctive clicks, lower in frequency than those of other odontocetes (Backus and Schevill 1966, Watkins and Schevill 1977, Weilgart and Whitehead 1988). *Kogia spp.* produce high-frequency narrow-band clicks from approximately 60 kHz to 200 kHz, with peak frequencies around 125 kHz and an inter-click interval between 40-70 ms (Marten 2000, Madsen et al. 2005). The HARPs, sampling at 200 kHz with a 0.01-100 kHz bandwidth, were not able to capture the full frequency range of *Kogia spp.* clicks, but clicks with minimum frequencies and inter-click intervals resembling those published for *Kogia spp.* were detected in the recordings and labeled as *Kogia spp.* clicks. All other click events were assigned to the other delphinids group and likely included clicks from Atlantic spotted dolphins, bottlenose dolphins, common dolphins, rough-toothed dolphins, short-finned pilot whales, and perhaps other species.

After I binned these click events into the four groups, I examined them for diel patterns in occurrence by dividing the recordings into one-minute bins. Bins with clicks present were assigned a score of 1 and those with clicks absent were assigned a score of 0. Photoperiod status (dawn, day, dusk, and night) was assigned to each one-minute bin, based on data from the U.S. Naval Observatory (http://aa.usno.navy.mil). Dawn was defined as the time between the beginning of nautical twilight[1] and sunrise, day

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[1] Nautical twilight is the time of day when the sun is at -12°. The beginning of nautical twilight occurs shortly prior to sunrise and the end of nautical twilight occurs shortly after sunset.

was between sunrise and sunset[2], dusk was between sunset and the end of nautical twilight, and night was between the end of nautical twilight and the beginning of nautical twilight. For each calendar date within each photoperiod category (dawn, day, dusk, and night), I summed bins with clicks to give the overall duration of click events. Also for each date and within each photoperiod, I calculated sampling effort by summing the total number of one-minute bins in which recordings were made. Finally, I corrected the overall duration of click events for effort by dividing by each photoperiod's recording effort for each date. I examined diel variation in this effort-corrected overall duration of click events (or occurrence) for each individual group (Risso's dolphins, sperm whales, *Kogia spp.*, and other delphinids) for each deployment and also for all deployments combined (for all groups except other delphinids)[3] using a Kruskal-Wallis test (Zar 1999). I performed multiple comparison tests using Bonferroni corrections on the significant results to determine which photoperiods were significantly different in terms of click event occurrence. I only included days with detections for these analyses. If the recorders did not collect data during all photoperiods for a day (such as on the start or end date of the deployment recording period), that day was excluded from the analyses. All statistical analyses were performed using JMP (SAS, Cary, NC).

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[2] Sunrise and sunset are defined as the time of day when the sun is at 0°.

[3] I performed statistical analyses on the data by deployment to account for possible seasonal and inter-annual differences in the data as well as possible site differences. I performed statistical analyses on the data combined across all deployments to look for overall diel trends for each group (except for other delphinids).

3.3 Results

The daily occurrence and duration of the click events for each group and for each recorder are shown in Appendix A. It was not possible to measure accurate durations of click events given the duty cycles employed on the HARPs; however, estimates of click event durations are provided for each group below.

3.3.1 Risso's dolphins

Risso's dolphin clicks were detected during all deployments during all months except for March and September (although there was not much recording effort in the latter (Figure 16)). The duration of click events ranged from approximately 1 minute to 2 hours and 55 minutes[4]. With the exception of the fourth deployment (which had a small sample size and could not be examined statistically), there was significant diel variation in the occurrence of Risso's dolphin click events for all deployments (Figure 17 and Table 15). For the first (fall-winter at site A), second (summer at site B), and third (spring-summer at site A) deployments, clicks occurred more frequently during night than during dawn or day. During the fifth deployment (fall-spring at site C), a greater number of minutes with clicks was detected during night than during dawn. Finally, when all Risso's click events were combined together, there were significantly more

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[4] As mentioned previously, duration of click events was estimated because click events often spanned an "off period" when the instrument was not recording.

clicks during night than during dawn, day, or dusk and more clicks during dusk than during dawn (Table 15).

Figure 17: Time of Risso's click events (left) and diel pattern of Risso's click events (right) for (a) the first deployment located at Site A, (b) the second deployment located at Site B, (c) the third deployment located at Site A, (d) the fourth deployment located at Site A, and (e) the fifth deployment located at Site C. (f) Diel pattern of Risso's click events for all deployments combined. In figures on the left, black bars represent duration of click events and shading indicates periods of darkness, determined from the U.S. Naval Observatory (http://aa.usno.navy.mil). In figures on the right, the blue vertical bars represent the percentage of days with click events present by time of day (GMT), and the horizontal bar indicates periods of light (white), periods of darkness (black), and periods that may be light or dark depending on the time of year (gray).

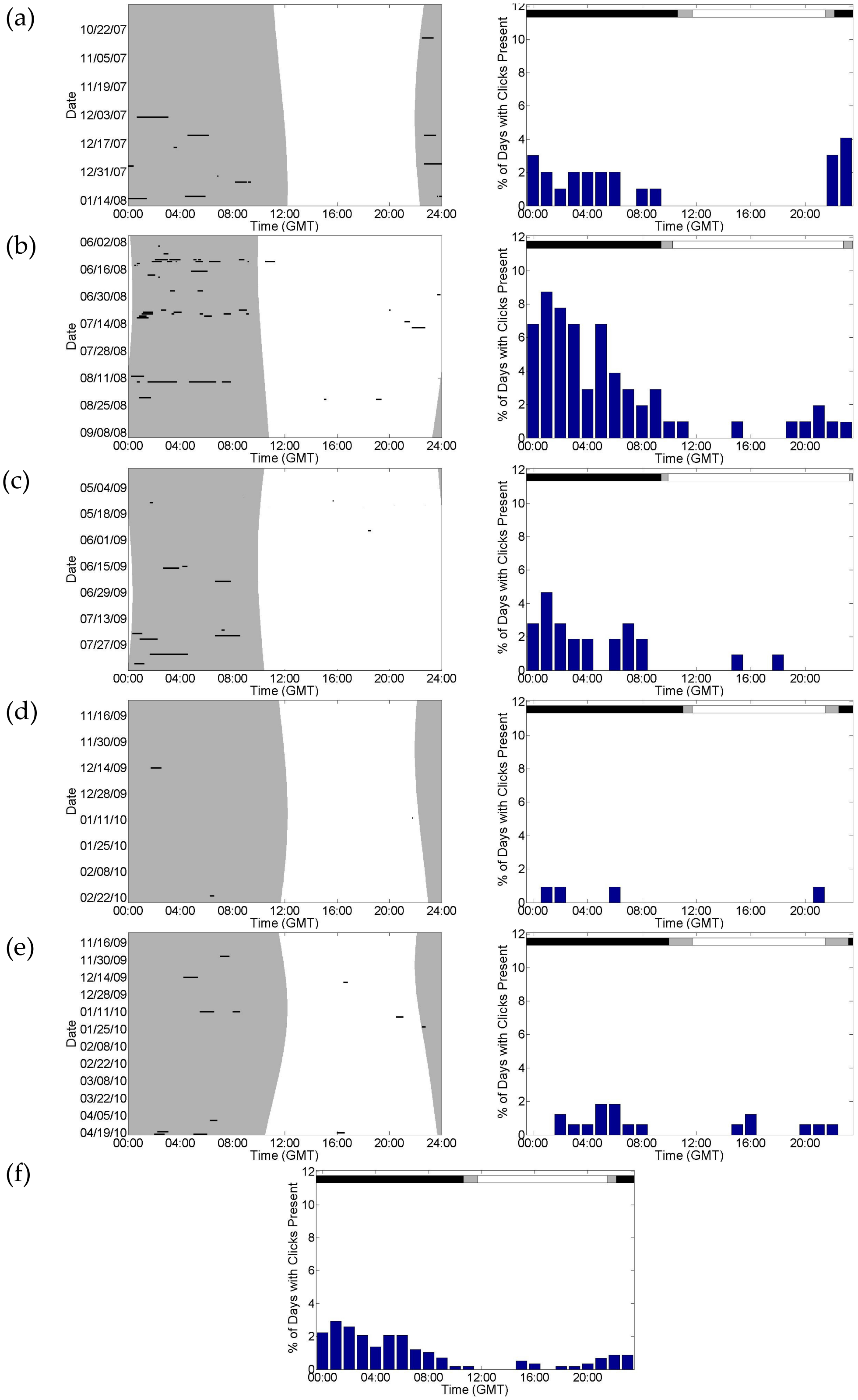

Figure 17 (Continued)

Table 15: Diel variation in the occurrence of Risso's dolphin clicks from each deployment. Mean (± standard deviation) is percentage of daily minutes with clicks present corrected by sampling effort. * Indicates significant differences. † Indicates sample size was not sufficient for statistical analysis.

Recording Dates	Deployment and Site	# Days with detections		Mean (± standard deviation)			Kruskal-Wallis results	Significant multiple comparison test results
			Dawn	Day	Dusk	Night		
10/10/07 - 1/16/08	1A	10	0.0 (± 0.0)	0.0 (± 0.0)	8.3 (± 15.0)	9.2 (± 7.2)	p<0.001*	night > dawn & day
5/30/08 - 9/10/08	2B	22	2.6 (± 7.2)	1.0 (± 2.3)	8.1 (± 11.7)	11.7 (± 15.6)	p<0.001*	night > dawn & day
4/24/09 - 8/9/09	3A	13	0.0 (± 0.0)	0.3 (± 0.6)	6.7 (± 13.7)	9.7 (± 11.6)	p<0.001*	night > dawn & day
11/8/09 - 2/24/10	4A	3	0.0 (± 0.0)	0.0 (± 0.0)	3.3 (± 5.6)	3.9 (± 3.7)	†	N/A
11/8/09 - 4/20/10	5C	10	0.0 (± 0.0)	2.0 (± 3.3)	2.0 (± 6.2)	7.0 (± 7.6)	p=0.012*	night > dawn
11/8/09 - 4/20/10	Combined	58	1.0 (± 4.5)	0.8 (± 2.0)	6.5 (± 11.8)	9.6 (± 11.9)	p<0.001*	night > dawn, day, & dusk; dusk > dawn

3.3.2 Sperm whales

Sperm whale clicks were detected during all deployments in all months except for September (which had little recording effort), with the duration of click events ranging from approximately 1 minute to 2 hours and 50 minutes. Significant diel variation in sperm whale click occurrence was found only for the first (fall-winter at site A) and fifth (fall-spring at site C) deployments, during which sperm whale clicks occurred significantly more during night than during dawn for the first deployment and significantly more during night than during dawn and day for the fifth deployment (Figure 18 and Table 16). No significant differences in sperm whale click occurrence were found among photoperiods for the other deployments although the sample size of the third deployment was insufficient for statistical analysis. However, when all of the sperm whale click events were combined, a significant difference was found among photoperiods, with more clicks during night than during dawn, day, or dusk (Table 16).

Figure 18: Time of sperm whale click events (left) and diel pattern of sperm whale click events (right) for (a) the first deployment located at Site A, (b) the second deployment located at Site B, (c) the third deployment located at Site A, (d) the fourth deployment located at Site A, and (e) the fifth deployment located at Site C. (f) Diel pattern of sperm whale click events for all deployments combined. In figures on the left, black bars represent duration of click events and shading indicates periods of darkness, determined from the U.S. Naval Observatory (http://aa.usno.navy.mil). In figures on the right, the blue vertical bars represent the percentage of days with click events present by time of day (GMT), and the horizontal bar indicates periods of light (white), periods of darkness (black), and periods that may be light or dark depending on the time of year (gray).

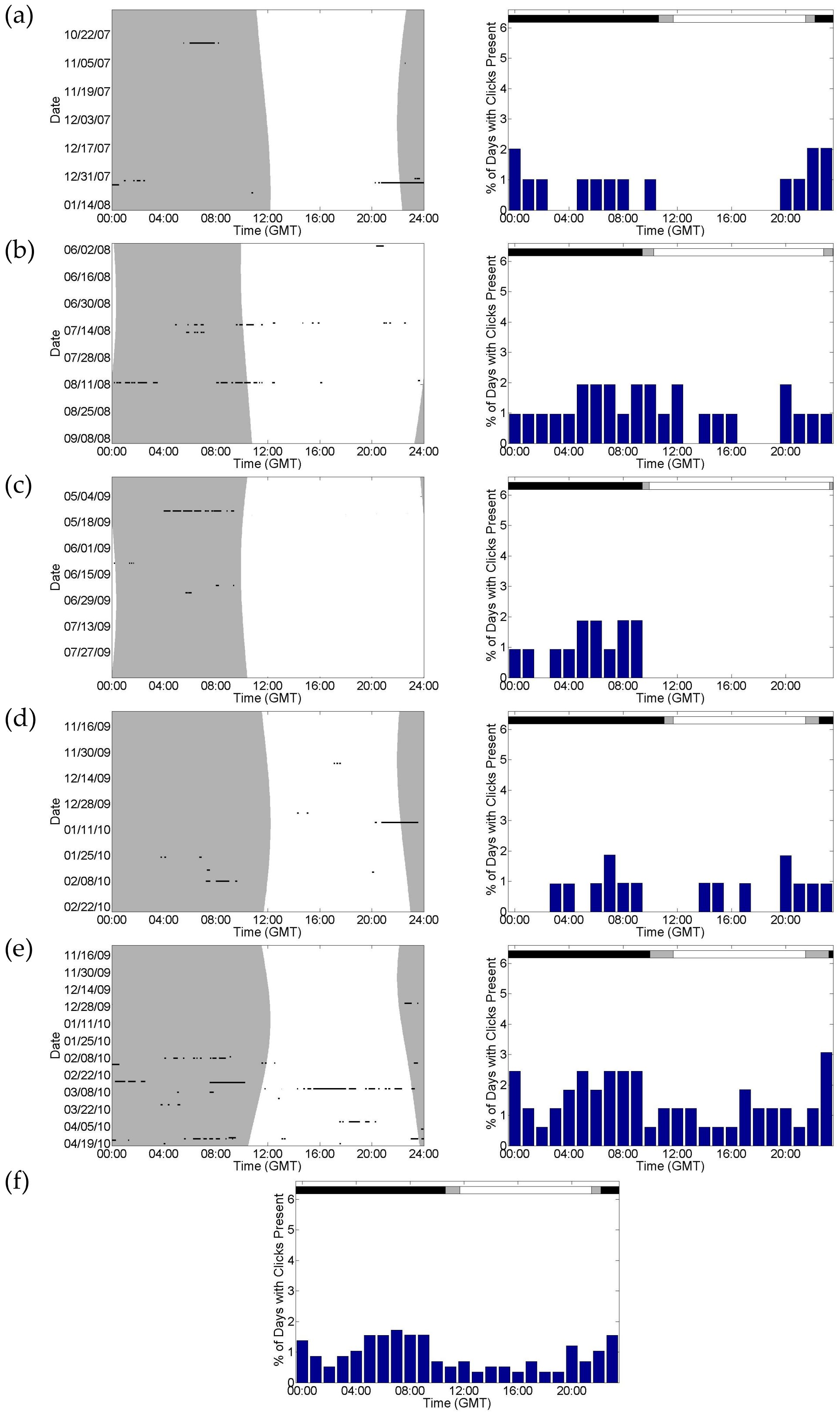

Figure 18 (Continued)

Table 16: Diel variation in the occurrence of sperm whale clicks from each deployment. Mean (± standard deviation) is percentage of daily minutes with clicks present corrected by sampling effort. * Indicates significant differences. † Indicates sample size was not sufficient for statistical analysis.

Recording Dates	Deployment and Site	# Days with detections	Mean (± standard deviation)				Kruskal-Wallis results	Significant multiple comparison test results
			Dawn	Day	Dusk	Night		
10/10/07 - 1/16/08	1A	7	0.0 (± 0.0)	1.0 (± 2.6)	14.7 (± 37.6)	5.4 (± 6.7)	p=0.010*	night > dawn
5/30/08 - 9/10/08	2B	6	20.3 (± 33.3)	2.3 (± 2.6)	4.0 (± 8.5)	7.0 (± 11.1)	p=0.945	N/A
4/24/09 - 8/9/09	3A	4	4.4 (± 5.8)	0.0 (± 0.0)	1.4 (± 2.7)	13.6 (± 19.6)	†	N/A
11/8/09 - 2/24/10	4A	7	0.0 (± 0.0)	2.4 (± 3.0)	14.3 (± 37.8)	3.2 (± 4.9)	p=0.082	N/A
11/8/09 - 4/20/10	5C	18	1.4 (± 4.2)	4.3 (± 13.7)	8.3 (± 13.9)	6.5 (± 8.9)	p=0.005*	night > dawn & day
11/8/09 - 4/20/10	Combined	42	3.9 (± 13.9)	2.7 (± 9.1)	9.1 (± 22.9)	6.5 (± 9.6)	p<0.001*	night > dawn, day, & dusk

3.3.3 Kogia spp.

Kogia spp. clicks were detected during all deployments, with the duration of click events ranging from approximately 1 to 5 minutes. *Kogia* clicks were detected in the months of February, May, June, July, August, November, and December. Small sample sizes prevented statistical analysis in all cases except for the fifth deployment (fall-spring at site C), in which no significant diel variation in *Kogia spp.* click occurrence was found (Figure 19 and Table 17). When all *Kogia spp.* click events were combined together for the Onslow Bay deployments, no significant variation was found among photoperiods (Table 17).

Figure 19: Time of *Kogia* click events (left) and diel pattern of *Kogia* click events (right) for (a) the first deployment located at Site A, (b) the second deployment located at Site B, (c) the third deployment located at Site A, (d) the fourth deployment located at Site A, and (e) the fifth deployment located at Site C. (f) Diel pattern of *Kogia* click events for all deployments combined. In figures on the left, black bars represent duration of click events and shading indicates periods of darkness, determined from the U.S. Naval Observatory (http://aa.usno.navy.mil). In figures on the right, the blue vertical bars represent the percentage of days with click events present by time of day (GMT), and the horizontal bar indicates periods of light (white), periods of darkness (black), and periods that may be light or dark depending on the time of year (gray).

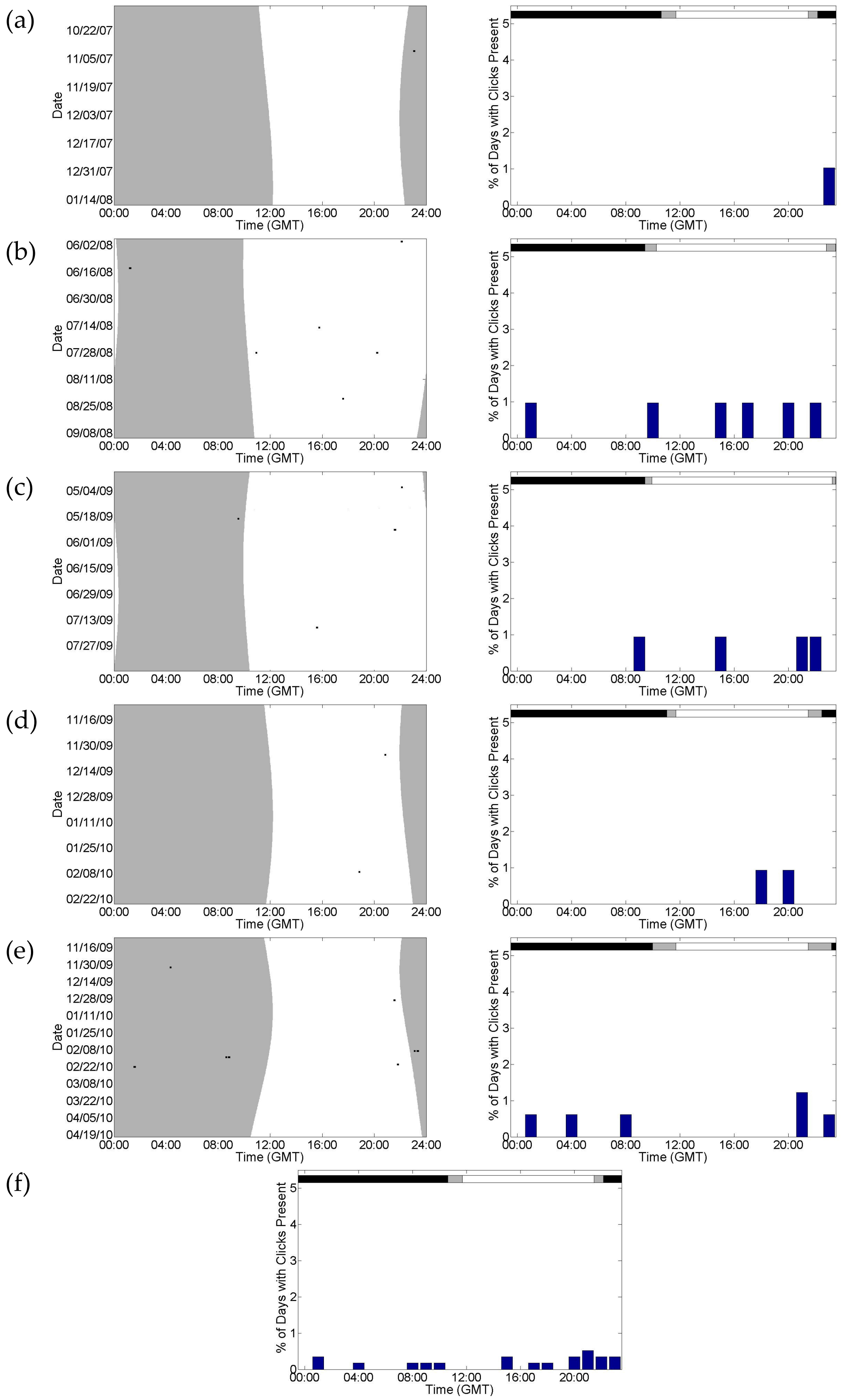

Figure 19 (Continued)

Table 17: Diel variation in the occurrence of *Kogia* clicks from each deployment. Mean (± standard deviation) is percentage of daily minutes with clicks present corrected by sampling effort. * Indicates significant differences. † Indicates sample size was not sufficient for statistical analysis.

Recording Dates	Deployment and Site	# Days with detections	Mean (± standard deviation)				Kruskal-Wallis results	Significant multiple comparison test results
			Dawn	Day	Dusk	Night		
10/10/07 - 1/16/08	1A	1	0.0 (N/A)	0.0 (N/A)	3.3 (N/A)	0.0 (N/A)	†	N/A
5/30/08 - 9/10/08	2B	5	0.9 (± 1.9)	0.6 (± 0.3)	1.1 (± 2.5)	0.0 (± 0.0)	†	N/A
4/24/09 - 8/9/09	3A	4	0.4 (± 0.7)	0.5 (± 0.5)	0.0 (± 0.0)	0.0 (± 0.0)	†	N/A
11/8/09 - 2/24/10	4A	2	0.0 (± 0.0)	0.8 (± 0.3)	0.0 (± 0.0)	0.0 (± 0.0)	†	N/A
11/8/09 - 4/20/10	5C	6	0.0 (± 0.0)	0.3 (± 0.6)	2.1 (± 4.2)	0.8 (± 1.0)	p=0.246	N/A
11/8/09 - 4/20/10	Combined	18	0.3 (± 1.1)	0.4 (± 0.5)	1.2 (± 2.8)	0.3 (± 0.7)	p=0.075	N/A

3.3.4 Other delphinids

I detected hundreds of click events from other delphinid species (likely including Atlantic spotted dolphins, bottlenose dolphins, common dolphins, rough-toothed dolphins, and short-finned pilot whales). These click events were detected during all deployments in every month the instruments were recording. The duration of these unidentified click events ranged from approximately 1 minute to 10 hours and 24 minutes.

Significant variation in the occurrence of other delphinid click events was found for all deployments (Figure 20 and Table 18). In the first deployment (fall-winter at site A), more click events occurred during dawn than during day, dusk, or night and more during night than during dusk. During the second deployment (summer at site B), more click events were found during night than during dawn or day. This pattern was also seen during deployments 3 (spring-summer at site A) and 5 (fall-spring at site C). The fourth deployment (fall-winter at site A) had more click events during night than day.

Due to the apparent change in diel patterns of click events for the fourth deployment, the data also were divided into two parts - one part (4a) included data from November and December while the other part (4b) included data from January and February (Table 18). When I divided the data into these two parts, two different patterns appeared: the November-December data had significantly more click events during dawn than during day, dusk, or night, and the January-February data had significantly more click events during night than during dawn or day.

Figure 20: Time of other delphinid click events (left) and diel pattern of other delphinid click events (right) for (a) the first deployment located at Site A, (b) the second deployment located at Site B, (c) the third deployment located at Site A, (d) the fourth deployment located at Site A, and (e) the fifth deployment located at Site C. In figures on the left, black bars represent duration of click events and shading indicates periods of darkness, determined from the U.S. Naval Observatory (http://aa.usno.navy.mil). In figures on the right, the blue vertical bars represent the percentage of days with click events present by time of day (GMT), and the horizontal bar indicates periods of light (white), periods of darkness (black), and periods that may be light or dark depending on the time of year (gray).

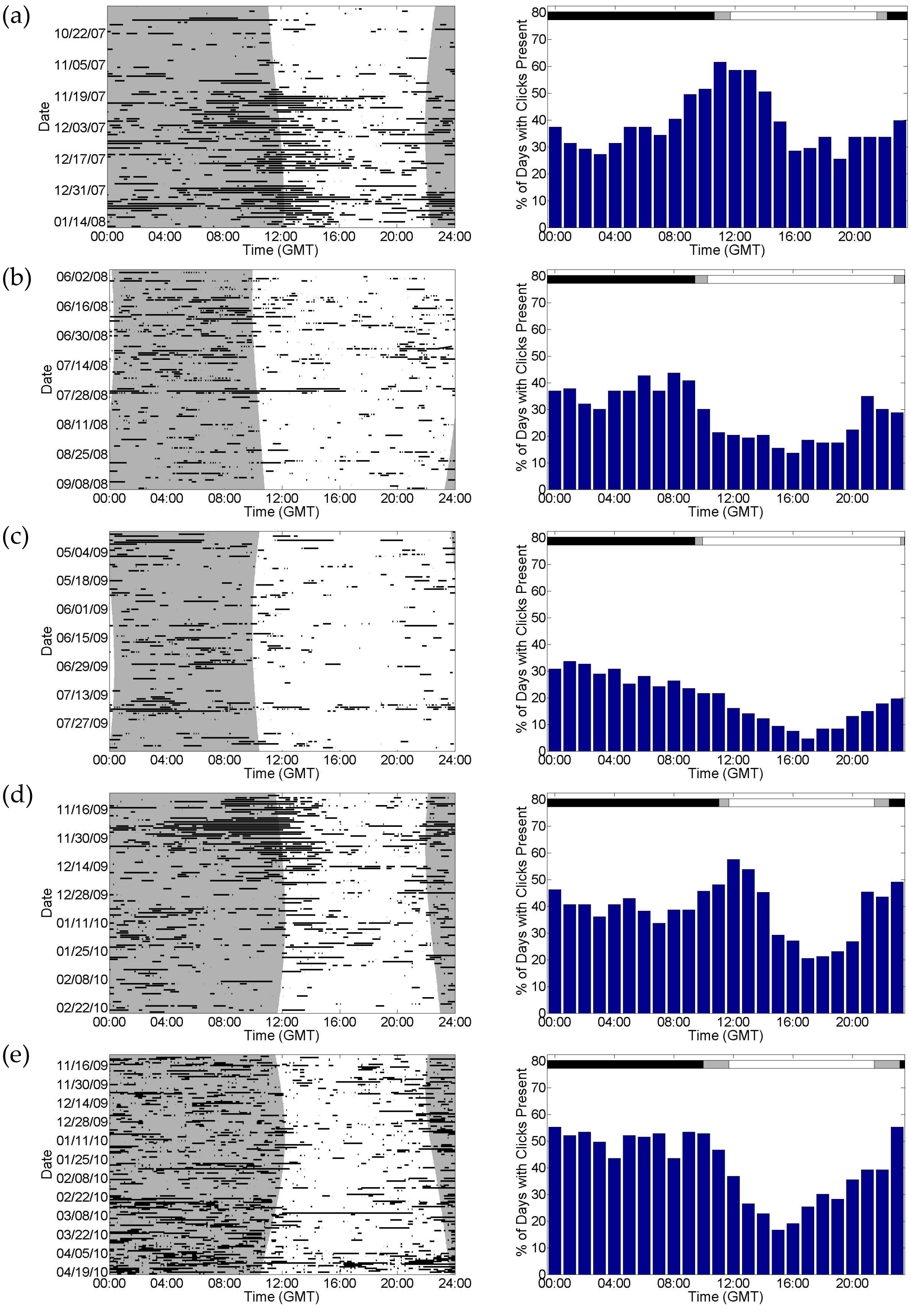

Figure 20 (Continued)

Table 18: Diel variation in the occurrence of other delphinid click events from each deployment. Mean (± standard deviation) is percentage of daily minutes with clicks present corrected by sampling effort. Due to the apparent change in timing of click events for Deployment 4, the data also were divided into two parts - one part (Part a) included data from November and December while the other part (Part b) included data from January and February. * Indicates significant differences.

Recording	Deployment	# Days with		Mean (± standard deviation)			Kruskal-Wallis	Significant multiple comparison
Dates	and Site	detections	Dawn	Day	Dusk	Night	results	test results
10/10/07 - 1/16/08	1A	93	45.9 (± 39.5)	19.1 (± 19.5)	19.9 (± 29.0)	22.0 (± 19.9)	p<0.001*	dawn > day, dusk, & night; night > dusk
5/30/08 - 9/10/08	2B	97	14.9 (± 23.2)	8.1 (± 10.0)	14.1 (± 18.3)	17.9 (± 18.6)	p<0.001*	night > dawn & day
4/24/09 - 8/9/09	3A	89	10.5 (± 18.9)	5.9 (± 8.9)	14.2 (± 20.0)	17.5 (± 19.5)	p<0.001*	night > dawn & day
11/8/09 - 2/24/10	4A	108	35.4 (± 38.1)	14.5 (± 16.0)	20.8 (± 25.1)	21.9 (± 20.1)	p=0.017*	night > day
11/8/09 - 12/31/09	4A a	54	57.0 (± 38.8)	18.1 (± 16.7)	26.2 (± 28.8)	27.5 (± 23.5)	p<0.001*	dawn > day, dusk, & night
1/1/10 - 2/24/10	4A b	54	13.9 (± 21.9)	10.8 (± 14.5)	15.4 (± 19.5)	16.3 (± 14.1)	p=0.003*	night > dawn & day
11/8/09 - 4/20/10	5C	162	20.1 (± 24.4)	11.0 (± 14.3)	25.1 (± 29.1)	25.8 (± 18.2)	p<0.001*	night > dawn, day, & dusk

3.4 Discussion

Clicks of Risso's dolphins, sperm whales, *Kogia spp.*, and other delphinids all were recorded during each HARP deployment in Onslow Bay. In general, my predictions of diel variation in the production of these click events were supported by the recordings. As predicted, for example, I found an increase in the occurrence of Risso's dolphin clicks at night but no diel pattern in *Kogia spp.* clicks. However, contrary to my prediction, I found a nocturnal increase in the occurrence of sperm whale clicks.

3.4.1 Risso's dolphins

My analysis of the HARP recordings revealed that Risso's dolphins occur year-round in Onslow Bay. Passive acoustic monitoring provided a more complete picture of the occurrence of Risso's dolphins in Onslow Bay than year-round visual aerial- and vessel-based surveys, which have detected Risso's dolphins primarily in May, June, and

August, with sporadic sightings in October and a single sighting in January (*unpublished data*). The paucity of visual detections during the non-summer months reflects the difficulty of conducting visual surveys during the winter months when weather conditions are poor.

As hypothesized, Risso's dolphins showed a nocturnal increase in the occurrence of click events (Table 15). Overall, my results agree with those of Soldevilla et al. (2010a) and lend more support to the idea that Risso's dolphins forage at night, as originally suggested by Shane (1995). Further support for this idea comes from the results of deployments 1, 2, 3, and 5, which showed no significant differences in click occurrence between night and dusk. Prey in the DSL start vertically migrating at dusk (Herring 2002). An increase in the occurrence of click events at dusk with a high rate of click occurrence throughout the night strongly suggests a nocturnal pattern of foraging in this species.

3.4.2 Sperm whales

My analysis also found that sperm whales are present throughout the year in Onslow Bay. Once again this is in contrast to conclusions drawn from year-round visual aerial- and vessel-based surveys. These surveys yielded only a single sperm whale sighting in Onslow Bay (in October 2009 at the end of a survey line in deep water). Sperm whale clicks also were acoustically detected on a towed array during these visual surveys, although there was no visual confirmation of these detections (*unpublished data*).

The lack of sightings of sperm whales may be due to long dive durations of this species. In contrast, the large number of acoustic detections may reflect the fact that clicks are produced frequently while diving and may be detected at distances up to 10-40 km away, as seen by Barlow and Taylor (2005) in the North Pacific. The most likely explanation for this discrepancy is that the sperm whales producing these clicks occurred in waters deeper than those surveyed by the boat and aerial vessels.

The hypothesis that sperm whale clicks would not exhibit a diel pattern in click production was not supported by the results (Table 16). I found a significant nocturnal increase in the occurrence of sperm whale click bouts when all deployments were combined. Tagged sperm whales have been observed making deep dives (suggestive of foraging) throughout the day and night (Watkins et al. 2002, Amano and Yoshioka 2003, Watwood et al. 2006), so it was surprising to find a trend of increased click production at night. However, it is possible that the HARPs did not record many sperm whale click events during the day due to the relatively shallow locations of the instruments, especially in relation to the likely location of vocalizing sperm whales. Watwood et al. (2006) found that diving sperm whales in the western Atlantic Ocean started clicking at depths of approximately 223 m and stopped clicking at deeper depths while ascending. Thus, if sperm whales located in deep waters (> 600 m) in Onslow Bay follow this pattern, much of their clicking activity would not have been recorded due to the transmission paths of the clicks. During the night, however, when squid occur higher up in the water column (many of the cephalopod species eaten by sperm whales (Fiscus et al. 1989, Clarke et al. 1993, Clarke 1996, Evans and Hindell 2004) exhibit diel vertical migrations (Roper and Young 1975)), the transmission path of sperm whale clicks may have coincided with the recorders. An alternative scenario is that sperm whales moved into and out of the recording area in a diel pattern that made their clicks detectable by the recorders only at night. For example, sperm whales may have moved closer to the shelf break at night to forage and then retreated to deeper waters during the day. At present, it is not possible to distinguish between these two alternative explanations.

3.4.3 Kogia spp.

Kogia spp. are present sporadically in Onslow Bay throughout the year, although individuals of this genus have never been visually detected in Onslow Bay. Unlike sperm whales which produce clicks that can travel tens of kilometers, *Kogia* produce high frequency clicks that do not propagate very far, indicating that the animals producing these clicks were close to the recorders. The greater number of detections at the deeper sites (Sites B and C) provides support for a pelagic distribution of this species, which has been reported elsewhere (Willis and Baird 1998, Baird 2005).

The recordings only contained a few *Kogia spp.* click events, although these clicks were present in every deployment. The sample sizes were small for the first four deployments preventing statistical analysis, but I found no significant diel variation in the occurrence of *Kogia spp*. clicks for the fifth deployment or the combined results (Table 17).

3.4.4 Other delphinids

Delphinid cetaceans are present year-round in Onslow Bay. The click events of the other delphinids group showed significant diel trends during every deployment (Table 18). In general, the occurrence of click events was greatest at dawn and at night. The first basic pattern (an increase at dawn) was seen in the first deployment and first part of the fourth deployment which occurred at the same site (Site A) during similar months (late fall-winter), while the second basic pattern (a nocturnal increase) was seen for the second (summer at site B), third (spring-summer at site A), and fifth (fall-spring at site C) deployments as well as the second part of the fourth deployment (winter at site A) (Table 18).

Thus, the first deployment and the first part of the fourth deployment, which recorded at the same site (Site A; Figure 15) during similar months but in different years (Figure 16), showed a very similar pattern (Figures 20a and 20d). Beginning in mid-November, a strong pulse of longer-duration and clustered click events is evident in the late night-dawn-early morning period. This strong pulse was not seen in any of the other datasets, including the recordings from the fifth deployment at Site C (Figure 20e) that was made during the same time period as the fourth deployment (Table 14). This absence of a crepuscular pulse at Site C suggests that perhaps animals moved toward the shelf break area (which runs along the 200-m isobath in Onslow Bay) at that time. In addition, the longer duration click events detected during this period at this shallower site suggests that either animals were moving toward the shelf break area, were staying in this shallow area for longer periods of time, were composed of larger group sizes, and/or were from a migratory species. If the longer events were due to animals staying in the area for longer periods, it seems likely that these extended click events are indicative of a behavioral change, either reflecting movement into the area or a change in vocal behavior, such as an increase in foraging. The question then remains as to whether this pulse resulted from a certain species moving into this area to feed, or reflects animals staying longer and foraging on prey aggregated during that time of the year. Similar horizontal movements have been described for Hawaiian spinner dolphins (Benoit-Bird and Au 2003), dusky dolphins in the south Atlantic (*Lagenorhynchus obscurus*, Würsig and Würsig 1980), and striped dolphins in the northwestern Mediterranean Sea (Gannier 1999).

Year-round visual surveys (aerial- and vessel-based) indicate that the two most common cetacean species in Onslow Bay are Atlantic spotted dolphins and bottlenose dolphins. Although the other delphinid click events could not be attributed to a species, it is likely that most are from Atlantic spotted and bottlenose dolphins. Spotted dolphins in the study area only have been sighted over the continental shelf (*unpublished data*), so click events on the deeper HARPs (Sites B and C) are likely to have been made by bottlenose dolphins. Atlantic spotted dolphins feed on a variety of fish, small cephalopods, and benthic invertebrates (Perrin 2009) and perhaps would not be expected to show a diel pattern in their click activity. Not much is known about the prey preferences or foraging activity of offshore bottlenose dolphins, although Barros and Odell (1990) did find that the stomach contents of one offshore bottlenose dolphin primarily contained ommastrephid squids, which exhibit diel vertical migrations. A nocturnal trend in click occurrence (seen in each season although at different sites) could reflect diel foraging patterns of offshore bottlenose dolphins similar to those of Risso's dolphins, with animals waiting for the DSL to rise in the water column before commencing foraging.

Overall, though, it is difficult to interpret the differences in diel trends because of the potentially confounding effect of seasonal variation in click occurrence as well as potential differences at each site. Future studies should examine whether a seasonal pattern exists and, if so, determine possible reasons for such seasonal differences, including seasonal changes in occurrence, in click behavior, and/or in activity. More work is required to determine which, if any of these, or combination of these, is driving these changes in diel trends.

3.5 Conclusions

These data provided a more complete picture of the occurrence of odontocete cetaceans in Onslow Bay and indicated that several species are present throughout the year, contrary to the conclusions drawn from visual surveys. Additionally, I have discovered the sporadic presence of a cryptic genus never detected during visual surveys in Onslow Bay - *Kogia*.

Overall, these data provided support for the hypotheses that Risso's dolphins increase their click occurrence during night while *Kogia* show no diel pattern in click occurrence. Using clicks as a proxy for foraging, these results are consistent with what is known about these species' foraging behaviors. In contrast, my study did not find support for the hypothesis that sperm whales exhibit no diel pattern in click occurrence and instead found evidence of a nocturnal pattern. These results are not consistent with what is known about this species' foraging behavior in other locations, as tagged individuals have been found to dive deeply (suggestive of foraging) during both day and night (Watkins et al. 2002, Amano and Yoshioka 2003, Watwood et al. 2006). However, I believe that this nocturnal pattern can be explained if sperm whales approached the shelf break to forage at night or if they remained in deeper waters throughout the day but foraged at shallower depths as their prey rose in the water column thereby making their clicks become more detectable by the HARPs at that time.

Interesting patterns emerged from the data for other delphinid click events, but without being able to separate click events to the species level, it is hard to draw conclusions from this group. Future studies should focus effort on discriminating these clicks. Most of the detected clicks were produced by this group of species and likely consist primarily of Atlantic spotted and bottlenose dolphins. Teasing apart the clicks of this group would allow further examination into the diel click patterns of other species, and perhaps provide insight into when foraging occurs. Such information could add to our understanding of the foraging behavior of offshore bottlenose dolphins. In addition, future studies should include dedicated work on animal behavior, clarifying how clicks and click occurrence change with different behaviors, as well as providing information on daily activity budgets of different species in Onslow Bay. The collection of data using DTAGs paired with focal follows during different seasons of the year may shed light on the possible causes of the diel patterns found in this study.

Chapter 4: Habitat and time of day influence the occurrence of odontocete vocalizations in Onslow Bay, North Carolina

4.1 Introduction

Distribution patterns of many odontocete cetaceans have been linked to habitat parameters that likely reflect the availability and distribution of prey (Baumgartner 1997, Davis et al. 1998, Cañadas et al. 2002). Such oceanographic and physiographic parameters can be used to differentiate habitats of species that occur in the same geographic region. To discriminate species-specific habitat preferences, researchers typically analyze data collected on visual surveys with synoptic information on habitat parameters, such as depth, sea surface temperature, etc., to create mathematical descriptions of the physical environment used by each species.

In the Gulf of Mexico, for example, Davis et al. (1998) found that it was possible to discriminate the distribution of 13 cetacean species using a single habitat parameter - depth. Atlantic spotted dolphins (*Stenella frontalis*) occurred in the shallow waters of the continental shelf and along the shelf break, in contrast to bottlenose dolphins (*Tursiops truncatus*), which most commonly inhabited deeper waters of the upper slope. All other species were found in pelagic waters, with short-finned pilot whales (*Globicephala macrorhynchus*) and Risso's dolphins (*Grampus griseus*) occurring predominantly along the mid-to-upper portion of the slope and rough-toothed dolphins (*Steno bredanensis*) and sperm whales (*Physeter macrocephalus*) being found in even deeper waters.

In a similar fashion, Cañadas et al. (2002) found that depth had the strongest influence on the distribution of seven cetacean species in the Alboran Sea, but also determined that slope was an important factor. More specifically, they observed bottlenose dolphins over the continental shelf and shelf break area but with a preference for areas with steep slopes, particularly in depths between 200-400 m.

Hamazaki (2002) used a similar approach to classify 13 cetacean species to habitat type using visual survey data collected between Cape Hatteras, North Carolina, and Nova Scotia, Canada. Sperm whales occurred seaward of the continental slope in waters greater than 1500 m. Bottlenose dolphins, common dolphins, Risso's dolphins, and pilot whales were all found in waters less than 1500 m, but the distribution of these species was discriminated by a combination of depth and temperature (Hamazaki 2002). More specifically, bottlenose dolphins were sighted in warm, shallow waters; common dolphins in cool, shallow waters; Risso's dolphins in warm, deep waters; and pilot whales in relatively cool, deep waters.

Time of day also can affect a species' distribution, although at a finer scale. For example, Hawaiian spinner dolphins (*Stenella longirostris*) move offshore late in the day to feed on the deep scattering layer (DSL) as it rises towards the surface at dusk (Norris et al. 1994, Benoit-Bird and Au 2003). Würsig and Würsig (1980) found that dusky dolphins (*Lagenorhynchus obscurus*) in the south Atlantic occurred in shallow waters while resting, perhaps as an anti-predator strategy, and moved to deeper waters to feed. Gannier (1999) found that striped dolphins (*Stenella coeruleoalba*) in the northwestern

Mediterranean Sea moved inshore to feed at the shelf break at night and returned offshore during the day.

In Onslow Bay, North Carolina, a consortium of academic institutions has conducted year-round aerial- and boat-based line-transect visual surveys since 2007. These surveys were designed to provide baseline information on the occurrence, distribution, and density of marine mammals to the U.S. Navy. In general, the density of cetaceans is quite low in Onslow Bay. The most common odontocetes observed during these surveys are Atlantic spotted dolphins and bottlenose dolphins. The former species occurs exclusively over the shelf, and the distribution of the latter extends into deeper waters (*unpublished data*). Thus, bottlenose dolphins inhabit a range of depths throughout the study area, but larger group sizes are more abundant in deeper waters beyond the 200-m isobath. A more diverse assemblage of odontocetes occurs in deeper waters, including bottlenose dolphins, Risso's dolphins, rough-toothed dolphins, short-beaked common dolphins (*Delphinus delphis*), short-finned pilot whales, and sperm whales (*unpublished data*).

Insights into the occurrence and distribution of cetaceans from visual surveys are, of course, limited by weather and visibility. As a result, there is little information about the distribution of these animals during the winter, when weather conditions are poor, and about how their patterns of distribution may change over diel cycles, which may reflect variation in behavioral state or time-specific habitat preferences. Marine mammals vocalize frequently, however, and researchers have used passive acoustic techniques to monitor occurrence, distribution, and temporal patterns in the vocal activity of cetaceans (Stafford et al. 2001, Burtenshaw et al. 2004, Clark and Clapham 2004, Mellinger et al. 2004, Wiggins et al. 2005, Munger et al. 2008, Soldevilla et al. 2010a). Remote, autonomous passive acoustic recorders are being used with increasing frequency to monitor populations of marine mammals over extended periods (Mellinger et al. 2004, Oleson et al. 2007b, Philpott et al. 2007, Stafford et al. 2007, Verfuß et al. 2007). Acoustic recorders provide long-term temporal records unmatched by visual surveys and can collect data in any type of weather or visibility.

Thus, during July 2008, five passive acoustic recorders were deployed in Onslow Bay to monitor the presence of vocalizing cetaceans during a Naval training exercise. I analyzed data from these recorders to determine: (1) if there were differences in the occurrence and duration of odontocete vocal events at different depths; and (2) if the occurrence of odontocete vocal events showed diel patterns at any of the sites or depths.

4.2 Methods

4.2.1 Study area

The study area for this research project was located in Onslow Bay, North Carolina (Figure 21), within the South Atlantic Bight. The shelf break in this area runs along the 200-m isobath. The Gulf Stream, a fast, northward-flowing western boundary current made up of warm waters, runs through a portion of this area along the shelf break and slope.

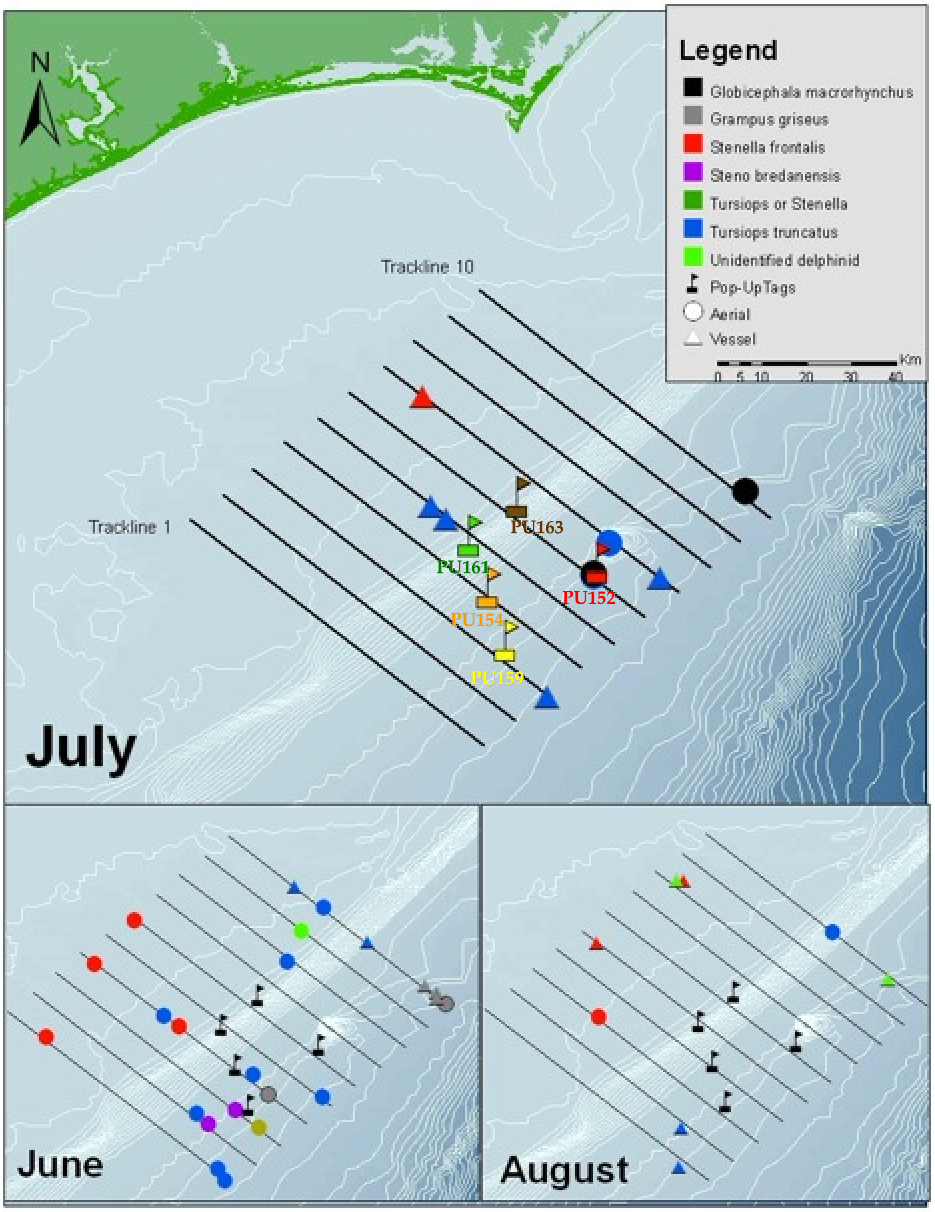

Figure 21: Onslow Bay study area showing visual survey (aerial and boat) sightings of odontocetes in June, July, and August 2008. The ten black lines represent the survey tracklines. Locations of the five acoustic recorders are shown in each figure but were deployed only in July 2008.

4.2.2 Instruments

Five archival passive acoustic recorders, informally known as "pop-ups," designed and manufactured by the Bioacoustics Research Program, Cornell Lab of Ornithology, were deployed in Onslow Bay (Figure 21). Two (PU161 and PU163) were deployed in shallow water (64-73 m; "shallow" recorders), one (PU154) in medium-depth water (236 m; "medium-depth" recorder), and two (PU152 and PU159) in deeper water (~366 m; "deep" recorders). Pop-ups are positively buoyant, but anchored to the ocean floor. The recorders are released from the sea floor via an acoustically activated burn wire release mechanism and "pop up" to the surface. These units are capable of recording up to 120 GB of acoustic data on an internal hard drive and can be programmed to record continuously or on a duty cycle. The units used in this study sampled continuously at 32 kHz from July 6 – July 27, 2008. Once the units were retrieved, the hard drives were removed, and the data were downloaded and converted from raw bin files to .wav files using software made by The Bioacoustics Research Program.

4.2.3 Analysis

I detected vocal events by viewing Long-Term Spectral Averages (LTSAs; Wiggins and Hildebrand 2007) generated in a Matlab-based program (The MathWorks, Inc., Natick, MA) called Triton (Scripps Whale Acoustic Lab, Scripps Institution of Oceanography). LTSAs provide a way to visualize several minutes to weeks of data concisely in the same figure, thereby allowing for rapid review of large data sets. I manually inspected 30-minute LTSAs for high-energy locations denoting whistles, clicks, and burst-pulses (Richardson et al. 1995). For each vocal event, I noted the start and end day and time. Within each vocal event, I also noted the start and end times of: (1) click events; and (2) whistle and burst-pulse events. I then sorted the vocal events into two groups: delphinids and sperm whales. Sperm whales produce highly distinctive clicks that are lower in frequency than those of other odontocetes and have been well-described in the literature (Backus and Schevill 1966, Watkins and Schevill 1977, Weilgart and Whitehead 1988).

For the delphinid vocal events detected on each instrument, I calculated: (1) the number of vocal events per day, (2) the *daily vocal activity*, and (3) the duration of individual vocal events (referred to as *vocal event duration*). *Daily vocal activity* was calculated by summing the duration of all vocal events for each day. *Vocal event duration* was calculated by subtracting the start day and time of a given vocal event by the end day and time of that same vocal event. I used Wilcoxon rank-sum tests (Zar 1999) to determine if there were significant differences between the two shallow recorders and the two deep recorders for each of these parameters. I used the results of this analysis to determine if the two recorders in the same depth category should be examined individually (if results indicated they were significantly different) or together (if results indicated they were not significantly different). Once this was determined, I used a Kruskal-Wallis test (Zar 1999) to determine if there were significant differences between:

(1) the shallow and medium-depth recorders; (2) the shallow and deep recorders; and (3) the medium-depth and deep recorders. I performed multiple comparison tests using Bonferroni corrections to determine how these three parameters varied with depth. The recorders collected data for differing amounts of time on July 6 (when they were deployed) and on July 27, 2008 (when they were retrieved), so data from these two days were not used for the analyses of the number of vocal events per day or *daily vocal activity*.

I examined diel patterns in the occurrence of vocal events (separated into (1) click events and (2) whistle and burst-pulse events) for each recorder by dividing the recordings into one-minute bins. Bins with vocalizations were assigned a score of 1 and those without vocalizations were assigned a score of 0. Photoperiod (day versus night) was assigned to each one-minute bin, based on data obtained from the U.S. Naval Observatory website (http://aa.usno.navy.mil). Day was defined as between sunrise and sunset[1] and night was defined as between sunset and sunrise. For each calendar date within each photoperiod category (day versus night), I summed bins with vocalizations to give the overall number of one-minute bins with vocal activity present per photoperiod. Also for each date and within each photoperiod, I calculated sampling effort by summing the total number of one-minute bins in which recordings were made. Finally, I corrected the overall duration of vocal events for effort (since photoperiod

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[1] Sunrise and sunset are defined as the time of day when the sun is at 0°.

duration varies by day) by dividing by each photoperiod's sampling effort for each date. I examined diel variation in this effort-corrected overall duration of vocal events (or occurrence) for delphinid click events, delphinid whistle and burst-pulse events, and sperm whale click events using a Wilcoxon rank-sum test. I only included days with detections in the analysis. Since the recorders did not collect data during all photoperiods on the start date (July 6th) or the end date (July 27th), I excluded these days from the analyses. All statistical analyses were performed using JMP software (SAS Institute Inc., Cary, NC).

4.3 Results

4.3.1 Delphinids

Whistles, clicks, and burst-pulses of delphinid species were detected on all recorders (Table 19). The daily occurrence and duration of these vocal events for each recorder are shown in Figure 22. Table 20 reports the mean (± standard deviation) of the number of vocal events, *daily vocal activity*, and *vocal event duration* for each depth.

No significant difference in the number of vocal events per day (all call types combined) was found on recorders at the same depth (Wilcoxon rank-sum, shallow x shallow: p=0.408; deep x deep: p=0.290). Significant differences in the number of vocal events were found at different depths (Kruskal-Wallis, p=0.004), with a significantly greater number of vocal events per day on the deep versus shallow and deep versus medium-depth recorders, but no significant difference between the medium-depth and shallow recorders.

There was no significant difference in *daily vocal activity* (all call types combined) on recorders at the same depth (Wilcoxon rank-sum, shallow x shallow: p=0.913; deep x deep: p=0.617). Significant differences in *daily vocal activity* were found among the depths (Kruskal-Wallis, p<0.001), with significantly more vocal activity per day on the deep versus shallow recorders and on the deep versus medium-depth recorders, but no significant difference between the medium-depth and shallow recorders.

There was a significant difference in *vocal event duration* (all call types combined) between the two shallow sites (Wilcoxon rank-sum, p=0.037), with significantly longer vocal events found in the PU161 recordings. No significant difference in vocal event duration was found between the two deep recorders (Wilcoxon rank-sum, p=0.325). I did not combine the shallow recorders for the comparisons of *vocal event duration* between different depths because they were significantly different. Significant differences were found in *vocal event duration* at different depths (Kruskal-Wallis, p=0.019). The post-hoc tests revealed significantly longer vocal events on (1) the medium recorder versus PU163 (a shallow recorder), and (2) the deep recorders versus PU163. No significant difference was found between (1) the medium-depth and deep recorders, (2) the medium-depth recorder and PU161 (a shallow recorder), or (3) the deep recorders and PU161.

As shown in Figure 23 (left side) and Table 21, both deep recorders and one shallow recorder (PU163) showed significant nocturnal increases in delphinid click activity. The medium-depth recorder showed no significant difference in the occurrence of click events during day versus night. The sample size for the other shallow recorder (PU161) was too small for statistical analysis.

Analysis of diel variation in whistle and burst-pulse events (Figure 23 (right side); Table 22) revealed that only one deep recorder (PU159) showed a significant nocturnal increase in whistle and burst-pulse activity. No other recorder showed significant differences in the occurrence of such events during day versus night.

Table 19: The number of vocal events and the minimum, maximum, and mean duration (min) for the vocal events for each recorder. "DS" refers to vocal events detected of delphinid species; "Pm" refers to click events of sperm whales.

	Shallow Recorders		Medium Recorder		Deep Recorders		
	PU161	PU163	PU154		PU159	PU152	
	DS	DS	DS	Pm	DS	DS	Pm
# of Vocal Events	49	79	81	174	158	135	6
Minimum Duration	1	1	1	1	1	1	4
Maximum Duration	74	102	370	532	493	564	51
Mean Duration	16	13	25	44	27	34	17

Figure 22: Daily occurrence (left) and duration (right) of delphinid vocal events for (a) PU161 (shallow recorder), (b) PU163 (shallow recorder), (c) PU154 (medium-depth recorder), (d) PU159 (deep recorder), and (e) PU152 (deep recorder). Shading in figures on the left indicates periods of darkness, determined from the U.S. Naval Observatory website (http://aa.usno.navy.mil).

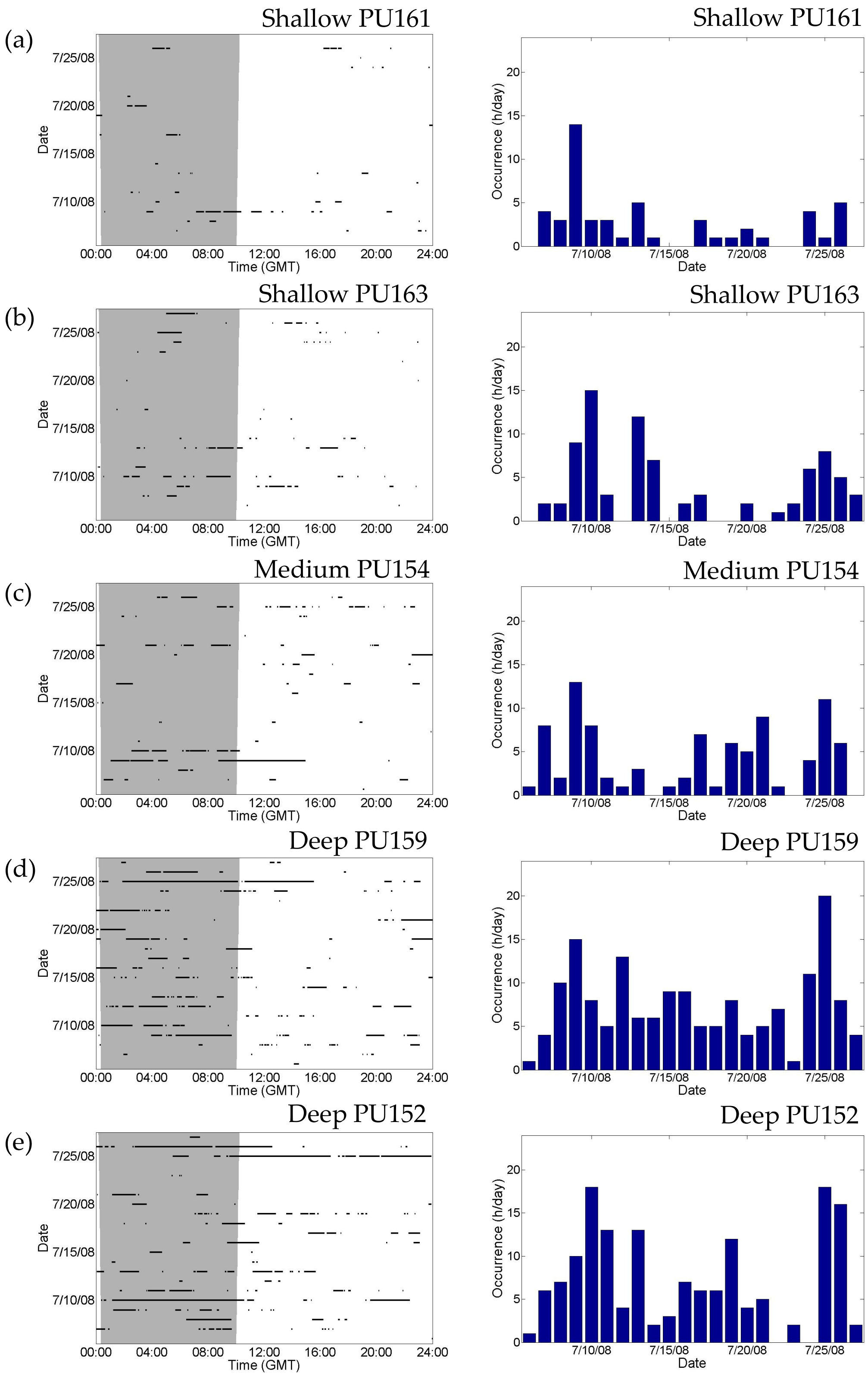

Figure 22 (Continued)

Table 20: Mean (± standard deviation) for number of vocal events per day, *daily vocal activity*, and *vocal event duration* for delphinids.

Depth & PU#	# Vocal Events	Daily Vocal Activity (min)	Vocal Event Duration (min)
Shallow PU161	2.5 (± 2.5)	39.9 (± 67.3)	16.3 (± 17.6)
Shallow PU163	4.0 (± 4.3)	51.7 (± 74.4)	14.3 (± 24.3)
Shallow Combined	3.2 (± 3.5)	45.8 (± 70.3)	N/A
Medium PU154	4.1 (± 3.7)	103.5 (± 146.7)	24.7 (± 45.6)
Deep PU152	6.5 (± 5.6)	228.4 (± 255.8)	33.4 (± 74.6)
Deep PU159	7.8 (± 4.8)	216.2 (± 189.8)	27.1 (± 57.7)
Deep Combined	7.2 (± 5.2)	222.3 (± 222.4)	30.0 (± 66.0)

Figure 23: Percentage of time with delphinid click events (left) and whistle and burst-pulse events (right) by time of day (GMT) for (a) PU161 (shallow recorder), (b) PU163 (shallow recorder), (c) PU154 (medium-depth recorder), (d) PU159 (deep recorder), and (e) PU152 (deep recorder). The black bars at the top of the figure represent times of darkness, the white bars represent times of light, and the gray bars represent times that could be light or dark depending on the time of year, as determined from the U.S. Naval Observatory website (http://aa.usno.navy.mil).

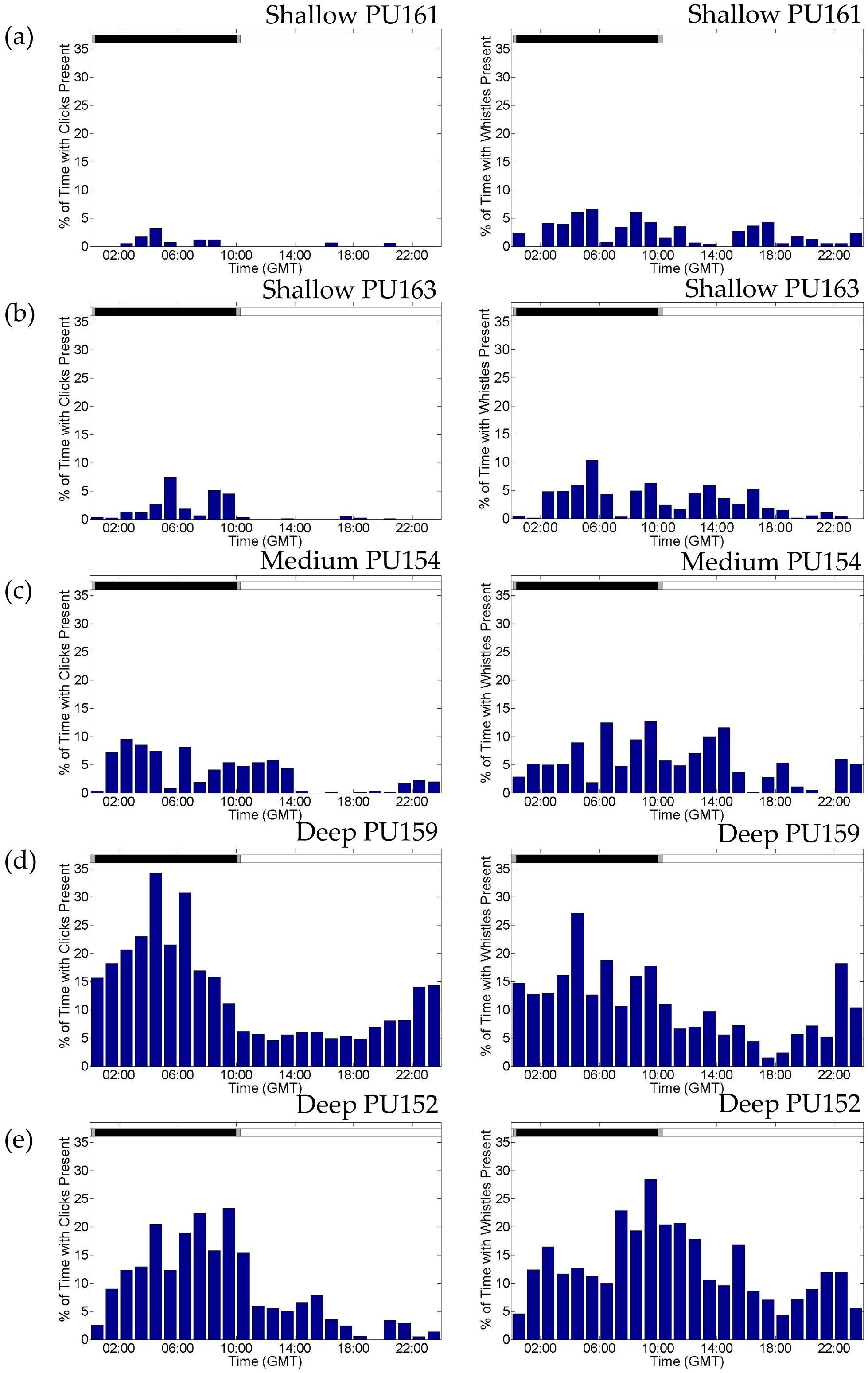

Figure 23 (Continued)

Table 21: Diel activity of delphinid clicks by depth. Mean (± standard deviation) is percentage of daily minutes with clicks present. * Indicates significant differences. † Indicates sample size was not sufficient for statistical analysis.

Depth & Pop-Up ID	# days with detections	Mean (± Standard Deviation) Day	Night	Wilcoxon rank-sum results	Significant multiple comparison test results
Shallow PU161	5	0.4 (± 0.4)	3.7 (± 4.4)	†	N/A
Shallow PU163	11	0.2 (± 0.4)	4.8 (± 6.9)	p=0.014*	Night > Day
Medium PU154	14	2.9 (± 6.7)	8.2 (± 13.0)	p=0.341	N/A
Deep PU159	19	8.1 (± 22.3)	22.9 (± 24.5)	p=0.007*	Night > Day
Deep PU152	15	5.8 (± 13.2)	21.7 (± 23.9)	p=0.001*	Night > Day

Table 22: Diel activity of delphinid whistles and burst-pulses by depth. Mean (± standard deviation) is percentage of daily minutes with whistles and burst-pulses present. * Indicates significant differences.

Depth & Pop-Up ID	# days with detections	Mean (± Standard Deviation) Day	Night	Wilcoxon rank-sum results	Significant multiple comparison test results
Shallow PU161	16	2.3 (± 3.5)	4.9 (± 7.5)	p=0.393	N/A
Shallow PU163	15	3.0 (± 4.9)	6.0 (± 7.8)	p=0.261	N/A
Medium PU154	15	6.5 (± 9.3)	9.5 (± 13.6)	p=0.868	N/A
Deep PU159	19	8.1 (± 10.5)	17.7 (± 20.4)	p=0.034*	Night > Day
Deep PU152	18	13.1 (± 21.1)	17.9 (± 22.0)	p=0.350	N/A

4.3.2 Sperm whales

Sperm whale clicks were detected only on the medium-depth recorder (PU154) and one of the deep recorders (PU152; Figures 24 and 25; Table 19).

Analysis of diel variation in sperm whale clicks for the medium recorder (PU154), which had numerous detections, showed a significant nocturnal increase in sperm whale clicks (Wilcoxon rank-sum, $p<0.001$; % of minutes during the day with clicks: 0.05 (± 0.1); % of minutes during the night with clicks: 61.9 (± 25.7); Figure 25a). Sperm whale clicks were detected during the night on a regular basis on this recorder

(Figure 24a). Many of these click bouts appeared to have been made by a single animal. The deep recorder (PU152) only had detections on two days (Figure 24b), so the sample size was not sufficient for statistical analysis.

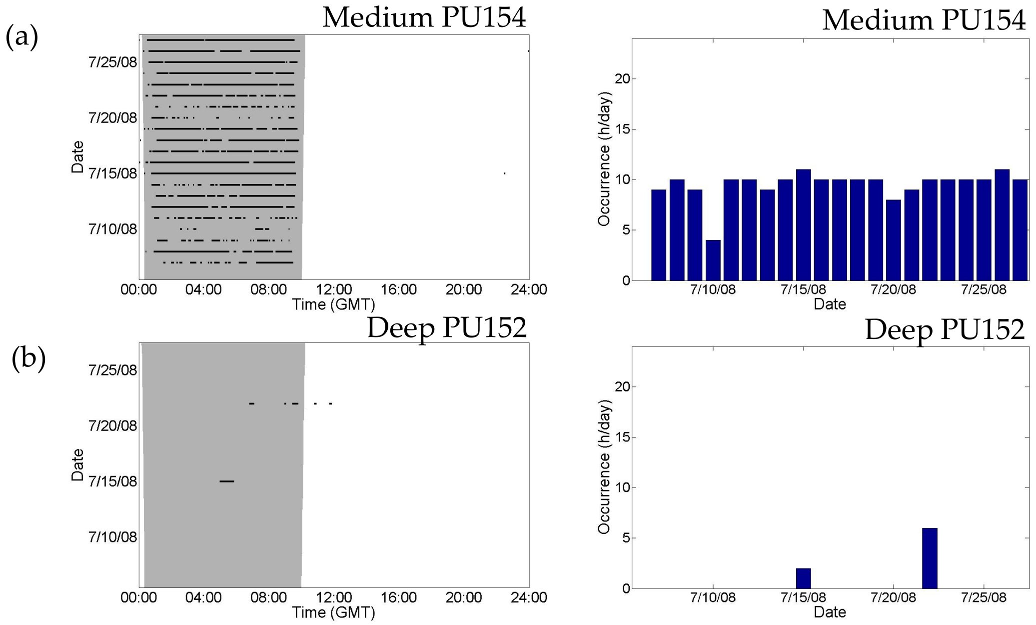

Figure 24: Daily occurrence (left) and duration (right) of sperm whale click events for (a) PU154 (medium-depth recorder) and (b) PU152 (deep recorder). Shading in figures on the left indicates periods of darkness, determined from the U.S. Naval Observatory website (http://aa.usno.navy.mil).

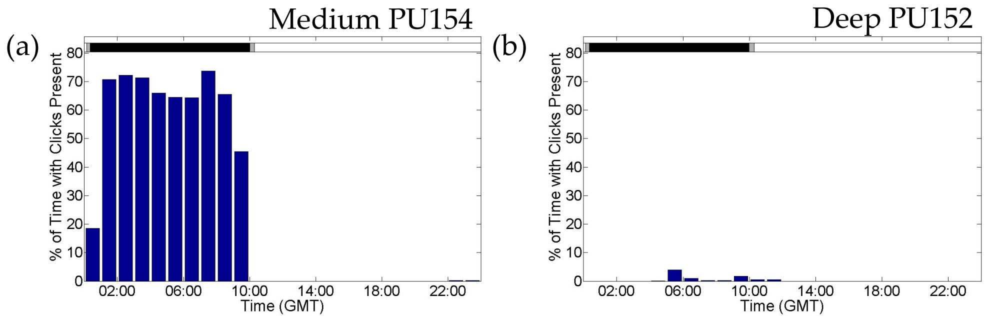

Figure 25: Percentage of time with sperm whale click events by time of day (GMT) for (a) PU154 (medium-depth recorder) and (b) PU152 (deep recorder). The black bars at the top of the figure represent times of darkness, the white bars represent times of light, and the gray bars represent times that could be light or dark depending on the time of year.

4.4 Discussion

Odontocete vocal activity varied with depth in Onslow Bay, with significantly more vocal events and significantly greater *daily vocal activity* occurring in deeper water. Diel trends in delphinid clicks also were found to vary with depth, with nocturnal trends observed at some, but not all, sites. A diel trend in delphinid whistles and burst-pulses was only found at one site (a deep site, PU159). Sperm whale clicks were detected every night on the medium-depth recorder, suggesting that one or more sperm whales moved onto the shelf break to forage during the recording period in July 2008.

4.4.1 Delphinids

Delphinid vocal events were detected on each of the passive acoustic recorders (Table 19). Most of these delphinid vocal events likely were produced by Atlantic spotted or bottlenose dolphins, as these two species are by far the most commonly sighted cetaceans in boat-based and aerial surveys in the area (*unpublished data*).

When comparing recorders at different depths, I found that recorders deployed in deeper waters recorded significantly more vocal events (both in terms of the total number of vocal events and the number of vocal events per day) and longer amounts of *daily vocal activity* than recorders at any other depth. The greater number of vocal events on the deep recorders drove the greater *daily vocal activity*, because *vocal event duration* was similar for all sites but one. This increased number of vocal events could be due to greater vocal activity in delphinid groups in deeper waters, more groups of delphinids in deeper waters, or a combination of the two. I will discuss each of these possibilities below.

The shallow recorders were located at depths of 64-73 m. Atlantic spotted dolphins and bottlenose dolphins are the only two species that have been sighted in these depths in Onslow Bay (*unpublished data*). The finding of significantly shorter *vocal event durations* on one of the shallow recorders was somewhat surprising. One explanation for these shorter *vocal event durations*, however, may come from the observation of smaller group sizes of bottlenose dolphins in shallower waters (*unpublished data*, Figure 26). Other studies have found that bottlenose dolphin vocal rates vary with group size in some locations, with fewer vocalizations produced by smaller groups (Jones and Sayigh 2002, Hernandez et al. 2010). Another explanation for these shorter *vocal event durations* could result from the behavioral state of groups, which, if different at this shallow site, may play a role; some studies have found that vocal rate and occurrence are correlated with behavioral state, generally finding more whistles during social activity and fewer during traveling and more echolocation clicks during foraging (Jones and Sayigh 2002, Cook et al. 2004). Vocal activity budgets may vary for different groups as well depending on species, population, or group composition (such as the presence and/or number of calves). Thus, it is possible that groups at this shallow site may be silent for a greater proportion of time due to either group size, behavioral state, or species or group composition. I found no significant difference in the number of vocal events per day between the two shallow recorders (Table 20), but I did find a greater number of total vocal events (1.6 times as many) on the shallow recorder that had significantly shorter *vocal event durations* (PU163, Table 19). Some of the shorter vocal events detected on PU163 occurred close together in time to other short vocal events (see Figure 22b). It is possible that such events were counted as multiple vocal events when in fact they were produced by the same group that either was silent much of the time that it was within range of the recorder or was moving into and out of detection range of the instrument.

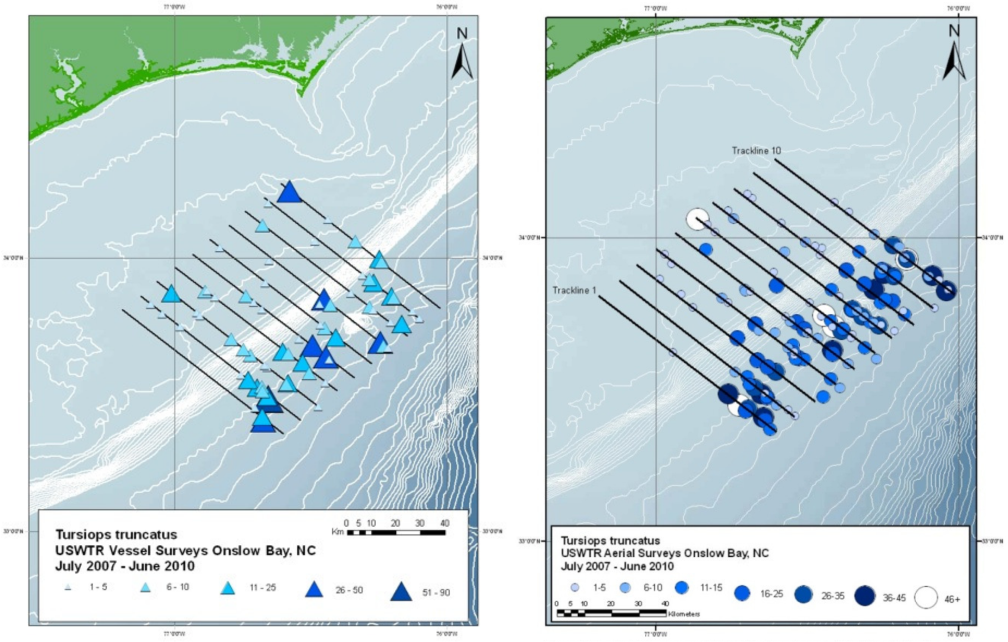

Figure 26: Visual detections and group sizes of bottlenose dolphins in the Onslow Bay survey area from boat-based (left) and aerial (right) surveys.

The medium-depth recorder had 1.6 times more vocal events than the shallow recorder that had similar *vocal event durations* (PU161, Table 19). This medium-depth recorder was similar to both of the shallow recorders in terms of *daily vocal activity*. The medium-depth recorder was located along the shelf break, an area often associated with increased biological activity (Mann and Lazier 1996). This recorder also likely was influenced by the Gulf Stream, which runs through a portion of the survey area. The front created by the western edge of the Gulf Stream flows near the shelf break area in the survey area, but meanders inshore and offshore throughout the year.

Lastly, the deep recorders, which had the most *daily vocal activity* and the greatest number of vocal events (both in terms of the total number of vocal events and the number of vocal events per day), were located on the continental slope, in approximately 366 m. All species recorded during visual surveys in Onslow Bay, with the single exception of Atlantic spotted dolphins, have been seen in waters of this depth (*unpublished data*). Sperm whale clicks were the only type of vocalization identified to species in this study, but calls similar to those produced by pilot whales (included in the delphinid species category here) were present in the recordings. Such calls were only detected on the deep recorders, which agrees with the sighting data and the findings of deep water prey in the stomachs of short-finned pilot whales stranded in North Carolina (Mintzer et al. 2008). The presence of these calls observed only on the deep instruments and the presence of sperm whale clicks only at the medium and deep sites suggest the existence of a more diverse cetacean assemblage at these depths in comparison to the shallow sites. This is in agreement with the results of aerial and shipboard surveys in this area (*unpublished data*), and the findings of Davis et al. (1998) from the Gulf of Mexico. A more diverse assemblage of species at these deeper depths also could explain the significantly greater number of vocal events observed on the deeper recorders if some of these species are more vocal than Atlantic spotted dolphins and small groups of bottlenose dolphins, or if there is a higher density of cetaceans in these waters.

Significantly more click activity was observed at night than during the day on both deep recorders and on one shallow recorder (PU163, Table 21). Click activity of the other shallow recorder (PU161) could not be examined statistically due to the small sample size (n = 5 days with clicks present), but Figure 23a (left) does suggest that more click activity occurred at night than during the day. Also, while no significant pattern emerged for the medium-depth recorder, there was more click activity at night and during the morning hours than during the middle and end of the day (Figure 23c left). Odontocetes use clicks in echolocation to navigate and find prey (Au 1993) and possibly in communication (Watkins and Schevill 1977, Dawson 1991, Benoit-Bird and Au 2008). Possible explanations for a nocturnal increase in click activity include diel changes in behavior, such as an increase in foraging activity at night, or an increase in the number of animals in the area.

Some studies have found a correlation between click activity and behavioral state, with greater click activity occurring while animals are foraging (Jones and Sayigh 2002, Nowacek 2005). At least two of the species detected in deeper waters during visual surveys forage on prey in the DSL (common dolphin: Overhotlz and Waring 1991, Evans 1994, Pusineri et al. 2007; Risso's dolphin: Clarke 1996). Studies suggest that both of these species forage at dusk or at night when their prey are undertaking vertical migrations (common dolphin: Evans 1994, Pusineri et al. 2007; Risso's dolphin: Shane 1995). In fact, Soldevilla et al. (2010a) and Chapter 3 showed that Risso’s dolphins, a deep-water species, show a nocturnal trend in their clicking behavior that likely is associated with their prey preference for squid found in the DSL. Unfortunately, though, given the sampling rate employed for the present study, only clipped clicks of Risso's dolphins would be detected on these pop-ups.

Other species whose prey preferences are less known also may be responsible for the observed increase in click activity at night. The nocturnal increase in click activity was noted at both the deep and shallow recorders, which suggests an increase in foraging at night by Atlantic spotted and/or bottlenose dolphins. Atlantic spotted dolphins feed on a variety of fish, small cephalopods, and benthic invertebrates (Perrin 2009) and, thus, might not be expected to exhibit such a diel pattern, although very little is known about the foraging behavior of this species. Less is known about the foraging activity of offshore bottlenose dolphins, although Barros and Odell (1990) found ommastrephid squids in the stomach contents of one individual. These squid inhabit the epipelagic zone and exhibit diel vertical migrations. Thus, if offshore bottlenose dolphins exhibit diel feeding patterns and wait for their prey to move towards the surface before foraging, a nocturnal trend in click activity would be expected.

An alternate hypothesis to explain the nocturnal increase in click production is that delphinids are found within detection distance of the instruments more frequently at night than during the day due to fine-scale daily movements. As noted previously, some species make daily horizontal movements as a result of their foraging strategies (*e.g.*, actively following prey or moving from a resting location to a foraging location). Such horizontal movements have been described for Hawaiian spinner dolphins (Norris et al. 1994, Benoit-Bird and Au 2003), dusky dolphins (Würsig and Würsig 1980), and striped dolphins (Gannier 1999). Thus, it is possible that animals move into the area from either shallower or deeper waters during the night. The significant nocturnal increase in whistles and burst-pulses on one of the deep recorders (PU159, Table 22), which corresponds to the nocturnal increase in the occurrence of clicks, may be evidence of group movement into those depths at night.

The lack of a diel trend in click activity on the medium-depth recorder was surprising given that all other recorders with a large enough sample size had a nocturnal trend. A low period of click activity was evident during the middle of the day on this instrument (Figure 23c left) although whistles and burst-pulses continued. At present, it is not possible to interpret this finding without more information on the specific identity of cetaceans recorded on this pop-up.

4.4.2 Sperm whales

Sperm whale click events were recorded only on the medium-depth and one deep recorder (PU152). Sperm whale clicks were detected during the night on the medium-depth recorder for long periods of time (Figure 24a). Sperm whales have not been observed in such shallow waters in Onslow Bay, so it was surprising to find such a regular pattern of sperm whale clicks each night on this instrument. It is possible that one or more sperm whales moved to the shelf break in Onslow Bay to feed at night, similar to striped dolphins in the northwestern Mediterranean Sea (Gannier 1999). In fact, many of the clicks detected on the medium-depth recorder appeared to have been produced by a single animal at a time, suggesting that the same individual may have been consistently foraging at night near the waters of the steep upper slope during that month of observations.

4.5 Conclusion

I found that depth and time of day influence the occurrence of odontocete vocalizations in Onslow Bay. Specifically, I found that delphinid vocal events occurred most often in deeper waters, likely due to a greater diversity and density of animals. The finding that click occurrence increased at night at the shallow and deep sites is likely indicative of a behavioral change, with animals beginning to forage as the DSL rises to the surface. Not much is known about the foraging activity of Atlantic spotted or offshore bottlenose dolphins, which are the two most common species found in the area, but the nocturnal trend seems to indicate that at least one of these species, if not both, exhibit diel feeding patterns. The regular nocturnal occurrence of low-frequency clicks on the recorder near the shelf break suggests that one or more sperm whales moved into that area to feed at night throughout the present study. Despite these patterns in the occurrence of vocal events, it is important to remember that this study gives a conservative representation of actual odontocete presence, as groups of animals that are present but remain silent go undetected using this survey method.

Chapter 5: Temporal patterns in the occurrence of mysticete calls in Onslow Bay, North Carolina

5.1 Introduction

Baleen whales produce a number of low-frequency (mainly < 1 kHz) vocalizations, ranging from basic downsweeps and upsweeps to complex pulse trains and songs (*e.g.*, Payne and McVay 1971, Clark and Johnson 1984, Watkins et al. 1987, Gedamke et al. 2001, Parks and Tyack 2005, McDonald et al. 2006, Dunlop et al. 2007, Baumgartner et al. 2008). Some, but not all, of these sounds have been attributed to the species level (*e.g.*, Clark 1982, Edds 1982, Mellinger et al. 2000, Gedamke et al. 2001, Oleson et al. 2003, McDonald et al. 2005, Parks and Tyack 2005, Rankin and Barlow 2005, Rankin et al. 2005, Berchok et al. 2006, Boisseau et al. 2008). A species' call type characteristics can vary with geographic location (blue whales (*Balaenoptera musculus*): McDonald et al. 1995, Stafford et al. 2001, Mellinger and Clark 2003, McDonald et al. 2006; minke whales (*Balaenoptera acutorostrata*): Mellinger et al. 2000, Gedamke et al. 2001, Rankin and Barlow 2005; sei whales (*Balaenoptera borealis*): McDonald et al. 2005, Rankin and Barlow 2007, Baumgartner et al. 2008).

The species-specific nature of many vocalizations has allowed researchers to examine temporal variation in baleen whale call rates using remote, autonomous passive acoustic recorders (Stafford et al. 2001, Mellinger and Clark 2003, Nieukirk et al. 2004, Stafford et al. 2005, Wiggins et al. 2005). On a long-term scale, seasonal and inter-annual trends in calls have been described for several baleen whale species (Watkins et al. 1987, Stafford et al. 1999, Stafford et al. 2001, Nieukirk et al. 2004, Heimlich et al. 2005, Wiggins et al. 2005). Seasonal variation in vocalization occurrence has been found for fin whales (*Balaenoptera physalus*) off of Bermuda (Watkins et al. 1987) and in the mid-Atlantic Ocean (Nieukirk et al. 2004); for blue whales in the North Pacific (Stafford et al. 2001), in the mid-Atlantic Ocean (Nieukirk et al. 2004), in the eastern tropical Pacific (Stafford et al. 2005), and off of Southern California (Wiggins et al. 2005); for minke whales in the mid-Atlantic Ocean (Nieukirk et al. 2004); and for Bryde's whales (*Balaenoptera edeni*) in the eastern tropical Pacific (Heimlich et al. 2005). Seasonal variation in vocalization occurrence may reflect migratory movement in and out of the monitored area or inherent variation in the production of calls.

Despite a considerable amount of research, several types of sounds have not yet been attributed to a specific species, either because no animals were sighted during concurrent visual and acoustic surveys or because no concurrent visual survey was conducted. There are also cases in which multiple species produce similar calls, which can lead to ambiguity in identifying the vocalizing species. For example, fin whales and sei whales produce similar downsweeps (compare Boisseau et al. 2008 Type II fin whale call to the sei whale call described by Baumgartner et al. 2008), and humpback whales (*Megaptera novaeangliae*) have been known to produce sounds similar to the North Atlantic right whale (*Eubalaena glacialis*) up-call (Van Parijs et al. 2009). Even when the species responsible for producing a particular sound cannot be identified, temporal trends can still be determined for the call type in question, with the hope that the vocalizing species will be identified in the future. Previous studies have taken this approach (Stafford et al. 1999, Nieukirk et al. 2004).

In the present study, I deployed High-frequency Acoustic Recording Packages (HARPs; Wiggins and Hildebrand 2007) in Onslow Bay, North Carolina. Year round aerial- and boat-based visual surveys also have been conducted in this area since 2007. During these visual surveys, two species of mysticetes were visually detected: fin and humpback whales. Other mysticetes with distribution ranges encompassing Onslow Bay include sei, minke, and North Atlantic right whales. In the North Atlantic, humpback, minke, sei, and North Atlantic right whales migrate to more northerly feeding grounds during spring and early summer, and return to southerly breeding grounds during winter (Clapham 2009, Horwood 2009, Kenney 2009, Perrin and Brownell 2009), although the location of breeding areas is unknown for some of these species. Fin whales in the Southern Hemisphere are known to migrate between feeding and breeding grounds. Less is known about this species in the North Atlantic, but it is thought that a similar latitudinal migration occurs (Aguilar 2009). The HARPs in Onslow Bay were located in the corridor between mysticete breeding and feeding grounds for at least humpback whales and North Atlantic right whales, so acoustic recordings could contain vocalizations from these species during migration. As not all breeding ground locations are known, it is possible that sounds during the breeding season could be recorded if such grounds are farther offshore of my study site. The types of sounds known to be produced by these species in the North Atlantic are described below.

Fin whales produce 20-Hz 1-second pulses that sweep downward from approximately 23 Hz to approximately 18 Hz, with peak energy often occurring at 20 Hz (Watkins et al. 1987). These pulses occur singularly, in irregular series, and in repetitive sequences (Watkins et al. 1987). Watkins et al. (1987) found inter-pulse intervals in the repetitive sequences occurring either consistently at a single pulse rate or at two alternating pulse intervals ("doublet").

Humpback whales produce a variety of sounds. Until recently, most research on their vocalizations has focused on song (first described by Payne and McVay 1971), which is comprised of units with frequencies ranging from as low as 30 Hz to as high as 4 kHz and perhaps even higher (Payne and Payne 1985, Tyack and Clark 2000). In addition to song, humpback whales produce a variety of social vocalizations that range from approximately 30 Hz to 2.5 kHz (Dunlop et al. 2007), and may extend as high as 12 kHz (Stimpert et al. 2011).

North Atlantic right whales also produce a variety of sounds, with call types including up-calls, screams, gunshots, warbles, downcalls, and blows (Parks and Tyack 2005). These calls, depending on the type, occur at frequencies from less than 10 Hz to greater than 8.5 kHz, and they range in duration from 10 milliseconds to greater than 1.5 seconds (Parks and Tyack 2005).

Sei whales, recorded in the northwestern Atlantic Ocean off of Cape Cod, MA, produce low-frequency calls that last one to two seconds and sweep down from an average of 82 to 34 Hz (Baumgartner et al. 2008). These calls are usually produced singularly but also occur in pairs and occasionally in triplets (Baumgartner et al. 2008). Calls occurring in pairs or triplets had average inter-call intervals of 3.5 seconds, as measured from the start of one call to the start of the successive call (Baumgartner et al. 2008).

Finally, minke whales in the North Atlantic Ocean produce two kinds of pulse trains - a "speed-up" version and a "slow-down" version (Mellinger et al. 2000). The speed-up pulse trains begin with average pulse rates of 1.5 pulses/second and end with average pulse rates of 2.8 pulses/second (Mellinger et al. 2000). In contrast, the slow-down pulse trains begin with average pulse rates of 4.5 pulses/second and end with average pulse rates of 2.9 pulses/second (Mellinger et al. 2000). Mellinger et al. (2000) reported that the speed-up pulse trains found in the Caribbean ranged in frequency from 199 Hz to 366 Hz while the slow-down pulse trains ranged from 267 Hz to 348 Hz. Mellinger et al. (2000) found that some pulse trains had an additional pulse in the 500-750 Hz range.

I used HARPs to determine how mysticete vocal events varied temporally in Onslow Bay. I analyzed data from five HARPs, deployed at various times and multiple locations throughout the year, to examine the presence of mysticete vocal events, as well as to determine if the occurrence of such vocal events showed seasonal patterns. In addition, I described the characteristics of the calls found in the recordings and, when possible, classified them to species based on previously reported descriptions.

5.2 Methods

5.2.1 Study area

The study area for this research is located in Onslow Bay, North Carolina (Figure 27), within the South Atlantic Bight. The Gulf Stream, a fast, northward-flowing western boundary current made up of warm waters, runs through a portion of this area along the shelf break and slope.

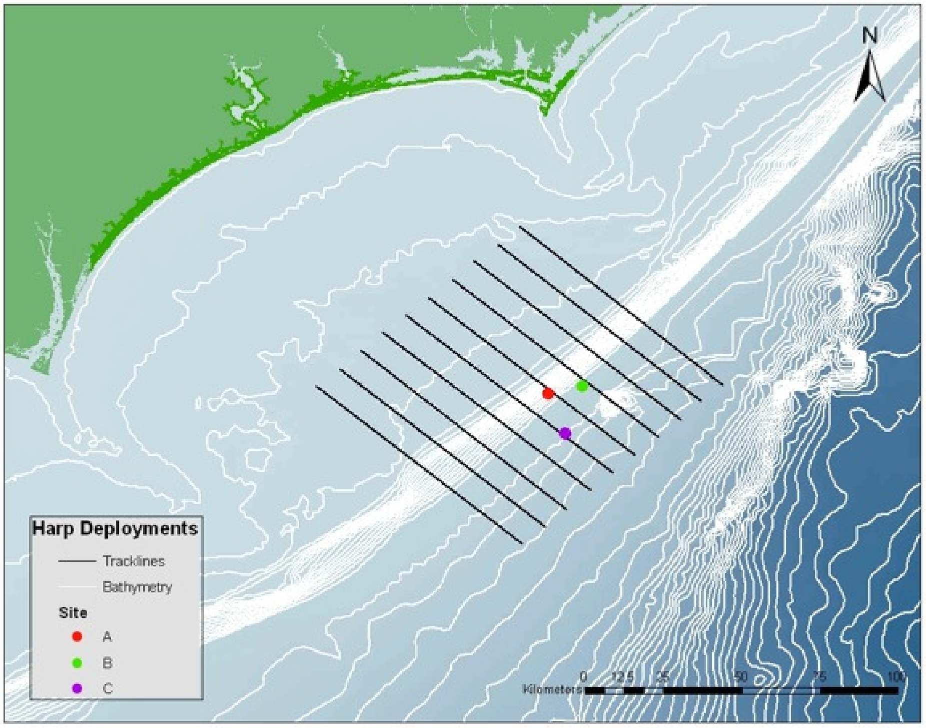

Figure 27: Study area in Onslow Bay, North Carolina, showing the three sites where HARPs were deployed. The ten lines running perpendicular to shore represent tracklines used during line-transect visual and acoustic surveys.

5.2.2 Instruments

I deployed five HARPs in Onslow Bay, NC, between October 2007 and April 2010 (Figure 27; Table 23). All instruments sampled at 200 kHz. In the first deployment, the instrument recorded every other five minutes, but at the beginning of 2008, it began to record continuously. For the second and third deployments, the instruments only recorded every other five minutes. For the fourth and fifth deployments, the HARPs recorded for five minutes and then did not record for the next 10 minute period.

Table 23: HARP deployment sites, times, depths, and duty cycles. *Represents the initial duty cycle but instrument recorded continuously starting January 1, 2008.

Deployment	Site	Start Date	End Date	# Days Recorded	Depth (m)	Duty Cycle
1	A	10-Oct-07	16-Jan-08	99	162	5 min on/5 min off*
2	B	30-May-08	10-Sep-08	104	232	5 min on/5 min off
3	A	24-Apr-09	9-Aug-09	108	174	5 min on/5 min off
4	A	8-Nov-09	24-Feb-10	109	171	5 min on/10 min off
5	C	8-Nov-09	20-Apr-10	164	335	5 min on/10 min off

5.2.3 Analysis

The acoustic data were decimated 100 times to achieve a 2-kHz sampling rate. Baleen whale calls (vocal events) were detected in these decimated data by examining 30-minute Long-Term Spectral Averages (LTSAs; Wiggins and Hildebrand 2007) generated in Triton (Scripps Institution of Oceanography) with a 1-Hz frequency bin and 5-second resolution. For each detected vocal event, the start and end day and time were noted. Daily vocal durations were calculated from this information for each dataset and seasonal trends were examined during each deployment period.

Once all of the vocal events were identified, they were sorted by call type and assigned to a species (when possible) using the characteristics of published call types. Several variables were measured for calls with high signal-to-noise ratios using Raven Pro 1.4 (Bioacoustics Research Program, Cornell Lab of Ornithology) as described below. The mean and standard deviation are reported for all call characteristics.

For the 20-Hz pulses that I identified as fin whale vocalizations, spectrograms were computed (FFT size 512 samples, 75% overlap, Hann window) and maximum, minimum, center (the frequency that divides the spectrum into two frequency intervals of equal energy), and peak (the frequency of maximum amplitude) frequency as well as duration were measured and averaged. Only a few examples were chosen for measurement since these calls are well described in the literature. Inter-pulse interval, defined as the duration from the start of one pulse to the start of the next consecutive pulse, was measured for four consecutive pulses from 13 high quality bouts (FFT size 1024 samples, 75% overlap, Hann window). Patterns of variation in these inter-pulse intervals were examined by performing an analysis of variance (ANOVA; Zar 1999).

For downsweeps, spectrograms were computed (FFT size 512 samples, 75% overlap, Hann window) and start, end, maximum, minimum, center, and peak frequency as well as duration were measured and then averaged. For downsweeps with different start and maximum frequencies, a Student's t-test was conducted to determine if these frequencies were significantly different. When downsweeps occurred as doublets and triplets, the inter-call interval was measured from the start of one call to the start of the next consecutive call. For those calls occurring as triplets, the two measured inter-call intervals were compared for similarity using a Student's t-test.

For pulse trains, spectrograms were computed (FFT size 256 samples, 75% overlap, Hann window) and trains were divided into three main groups: minke, consistent, and short. Minke pulse trains included two subgroups: slow-down pulse trains and speed-up pulse trains, both of which are described in detail by Mellinger et al. (2000) who concluded that they were most likely produced by minke whales. Due to the similarities to the Mellinger et al. (2000) minke whale calls, I combined the slow-down and speed-up pulse trains together ("minke whale calls") to look at seasonal trends. Slow-down pulse trains have decreasing pulse rates (pulses/second) throughout the train whereas speed-up pulse trains have increasing pulse rates throughout the train (Mellinger et al. 2000). Consistent pulse trains were those with a lower peak frequency than minke pulse trains and consistent pulse rates and inter-pulse intervals. Short pulse trains included pulse trains that were of shorter overall duration than minke pulse trains but had pulses of similar frequency ranges. Consistent pulse trains and short pulse trains have not been described in the literature previously and therefore could not be assigned to a species.

After the pulse trains were divided into these three groups (minke, consistent, and short) and subgroups (slow-down and speed-up), the peak frequency and train duration were measured and averaged. For all but the short pulse trains, pulse rate, defined as the number of pulses per second and calculated by the same method as in Mellinger et al. (2000), and peak frequency were measured at the beginning and end of each train. For the slow-down pulse trains, pulse rate and peak frequency also were measured at two locations in the middle of the train: just prior to and just after the obvious change in inter-pulse interval.

Sounds likely produced by humpback whales only were detected on a single day and thus were not examined in detail. However, for likely humpback whale calls, spectrograms were computed and maximum and minimum frequency and duration were measured and averaged for all calls with good signal-to-noise ratios. Since humpback whales have a diverse vocal repertoire, downsweeps detected on the same day that humpback whale calls occurred were not included in either the analysis of downsweeps or humpback whale calls as the overlap created more ambiguity in the actual calling species.

All statistical analyses were performed using JMP software (SAS Institute Inc., Cary, NC) and Excel (Microsoft).

5.3 Results

5.3.1 Fin whale 20-Hz pulses

Series of 20-Hz pulses (Figure 28; see Table 24 for call descriptive statistics) were detected on the three HARP deployments that occurred between the months of November and April (Figure 29), but not on the two deployments that occurred between May and September. These sounds are known to be associated with fin whales (Watkins et al. 1987, Thompson et al. 1992). In this study, the mean inter-pulse interval for these sounds was 14.56 ± 1.30 seconds (n = 39). There was no significant variation in the inter-pulse intervals between four consecutive calls on 13 occasions (ANOVA, F = 0.2603, p = 0.772).

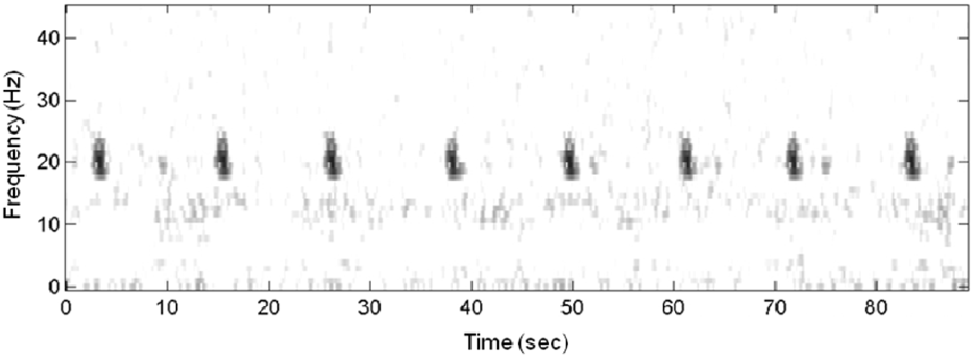

Figure 28: Spectrogram of eight 20-Hz pulses (FFT size 2048 samples, 90% overlap, Hann window).

Table 24: Measured variables of fin whale 20-Hz pulses based on 26 calls (14 calls from the fourth deployment and 12 from the fifth deployment). C.V. stands for coefficient of variation. Maximum frequency is equivalent to start frequency, and minimum frequency is equivalent to end frequency for these calls.

	Duration (s)	Max Freq (Hz)	Min Freq (Hz)	Center Freq (Hz)	Peak Freq (Hz)
Mean	1.48	28.5	10.4	19.5	19.5
St. Dev.	0.27	1.6	1.0	0.0	0.0
C.V. (%)	18.4	5.6	9.5	0	0

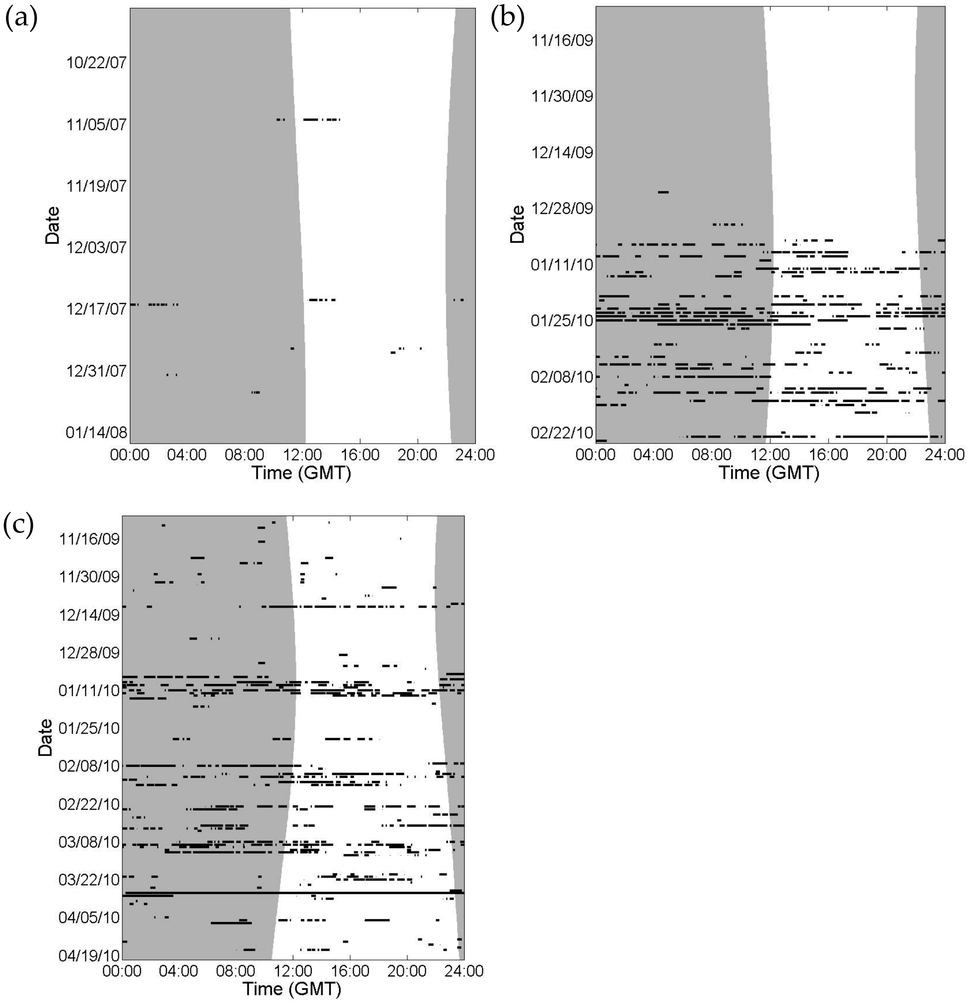

Figure 29: Occurrence of fin whale 20-Hz pulses for (a) the first deployment located at Site A, (b) the fourth deployment located at Site A, and (c) the fifth deployment located at Site C. Black bars represent duration of vocal events and shading indicates periods of darkness, determined from the U.S. Naval Observatory (http://aa.usno.navy.mil).

5.3.2 Downsweeps

Low frequency downsweeps, occurring mostly as singles and pairs but also occasionally as triplets, and sweeping from an average of 106.1 to 33.1 Hz (Figure 30, Table 25), also were detected on the three HARP deployments between the months of

November and April (Figure 31). Most downsweeps started at a maximum frequency and then swept downward, but a few first swept upward briefly before sweeping downward (Figure 32). These "hooked" downsweeps had significantly lower start frequencies when compared to maximum frequencies (Student's t-test, $p = 0.033$; Table 26). The mean inter-call interval for paired downsweeps was 3.59 (± 0.72) seconds (n = 119: 24 calls from the first deployment, 74 from the fourth deployment, and 21 from the fifth deployment). When comparing the inter-call intervals between the first and second (3.30 ± 0.86 seconds) and second and third downsweeps (3.71 ± 1.15 seconds) for those occurring in triplets, the difference was not statistically significant (Student's t-test, $p = 0.318$; n = 13: 1 from the first deployment and 12 from the fourth deployment).

During the first deployment, downsweeps were detected between December 26, 2007 – January 14, 2008. In the fourth and fifth deployments, downsweeps were first detected mid-November 2009 and continued until the end of December 2009, started again mid-January 2010 and ended mid-February 2010. For the fifth deployment, these calls were detected during two days in mid-March 2010. Figure 33 shows the occurrence of downsweeps in relation to fin whale 20-Hz pulses.

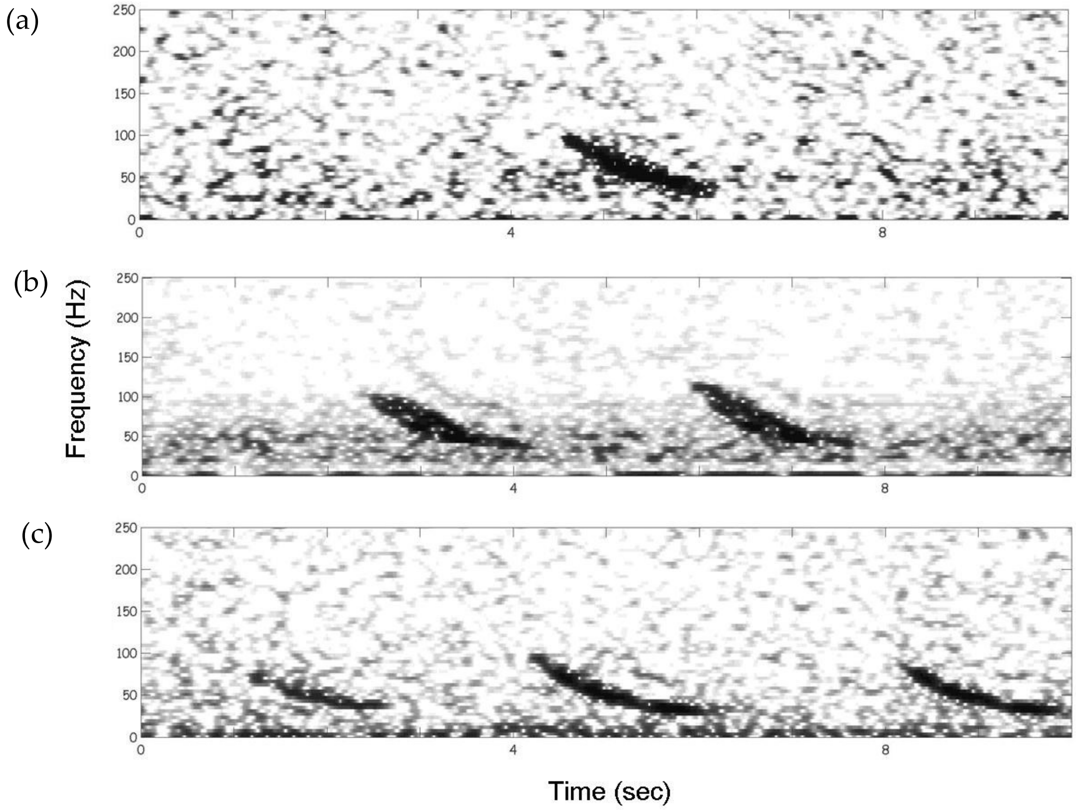

Figure 30: Spectrograms of downsweeps occurring as a (a) single, (b) pair, and (c) triplet (FFT size 512 samples, 75% overlap, Hann window).

Table 25: Measured variables of downsweeps (non-hooked) based on 396 calls (158 calls from the first deployment, 186 from the fourth deployment, and 52 from the fifth deployment). C.V. stands for coefficient of variation. In all cases for non-hooked downsweeps (as reported here), the maximum frequency was equivalent to the start frequency, and the minimum frequency was equivalent to the end frequency.

	Duration (s)	Max Freq (Hz)	Min Freq (Hz)	Start Freq (Hz)	End Freq (Hz)	Center Freq (Hz)	Peak Freq (Hz)
Mean	1.56	106.1	33.1	105.8	33.1	55.3	53.1
St. Dev.	0.29	18.0	7.0	17.8	7.0	8.5	10.0
C.V. (%)	18.7	17.0	21.0	16.8	21.0	15.3	18.9

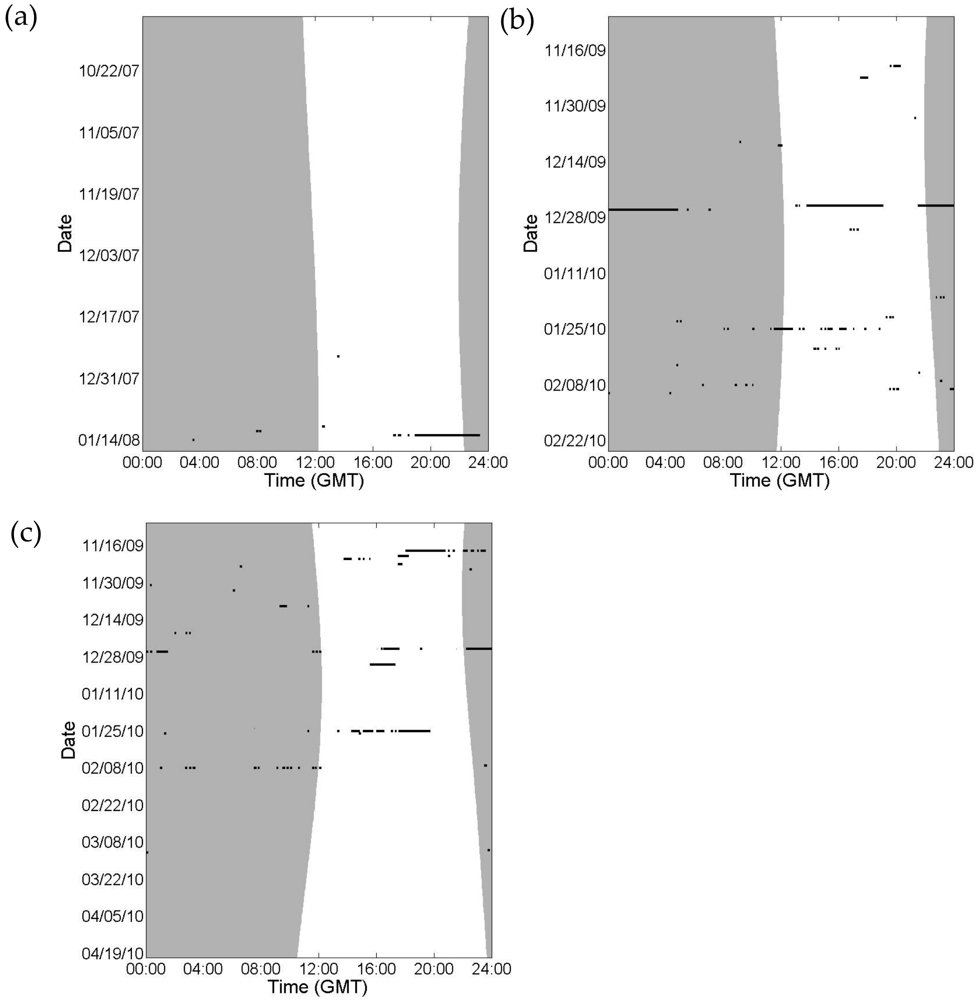

Figure 31: Occurrence of downsweeps for (a) the first deployment located at Site A, (b) the fourth deployment located at Site A, and (c) the fifth deployment located at Site C. Black bars represent duration of vocal events and shading indicates periods of darkness, determined from the U.S. Naval Observatory (http://aa.usno.navy.mil).

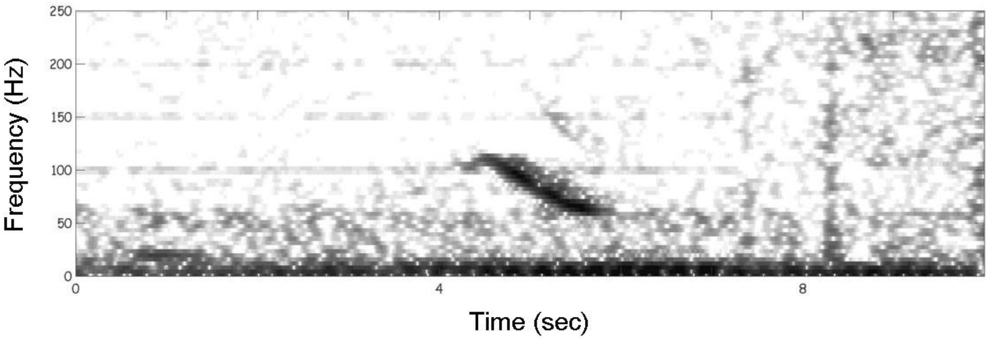

Figure 32: Spectrogram of "hooked" downsweep (FFT size 512 samples, 75% overlap, Hann window).

Table 26: Measured variables of "hooked" downsweeps based on five calls (1 call from the first deployment, 3 from the fourth deployment, and 1 from the fifth deployment). C.V. stands for coefficient of variation. In all cases, the minimum frequency was equivalent to the end frequency.

	Duration (s)	Max Freq (Hz)	Min Freq (Hz)	Start Freq (Hz)	End Freq (Hz)	Center Freq (Hz)	Peak Freq (Hz)
Mean	1.75	123.7	40.6	103.9	40.6	64.1	60.9
St. Dev.	0.30	20.6	9.9	10.1	9.9	8.5	6.5
C.V. (%)	16.9	16.7	24.3	9.7	24.3	13.3	10.7

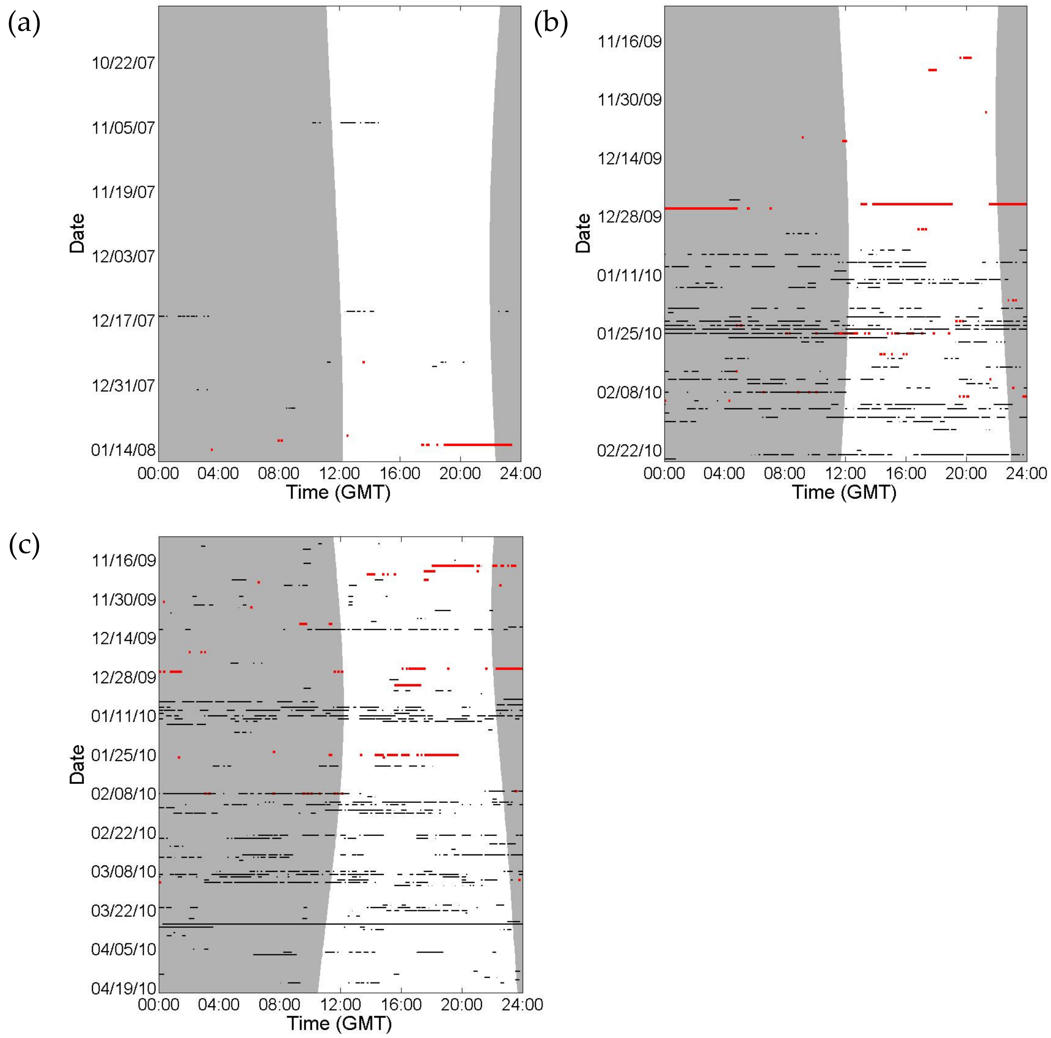

Figure 33: Time of fin whale 20-Hz pulses (black bars) and downsweeps (red bars) for (a) the first deployment located at Site A, (b) the fourth deployment located at Site A, and (c) the fifth deployment located at Site C. Black and red bars represent duration of vocal events and shading indicates periods of darkness, determined from the U.S. Naval Observatory (http://aa.usno.navy.mil).

5.3.3 Pulse trains

As with the 20-Hz pulses and downsweeps, low frequency pulse trains were detected on the three HARP deployments between the months of November and April

(Figures 34-36). Four types of pulse trains were found: slow-down pulse trains, speed-up pulse trains, consistent pulse trains, and short pulse trains (Table 27, Figure 37). The slow-down pulse trains and speed-up pulse trains are similar to those described by Mellinger et al. (2000) for minke whales recorded near Puerto Rico, although those reported here have lower fundamental frequencies. The slow-down pulse trains, however, did occasionally have higher-frequency components (Figure 38). Generally, minke pulse trains were first detected in mid- to late-November. For the first and fourth deployments, these calls occurred until the end of the deployment recording period. For the fifth deployment, the calls stopped at the end of March and then resumed mid-April for several days just prior to the end of the recording period.

Consistent pulse trains had pulses with peak frequencies at 51.5 ± 6.8 Hz (Table 27) and with regular and consistent inter-pulse intervals. On very few occasions, high-frequency clicks with peak frequency at approximately 20 kHz were found to be associated with the consistent pulse trains (Figure 39).

Short pulse trains were short in duration overall in comparison to other pulse trains. These had durations on the order of 8.5 seconds in comparison to 58-80 seconds found in the other pulse trains (Table 27).

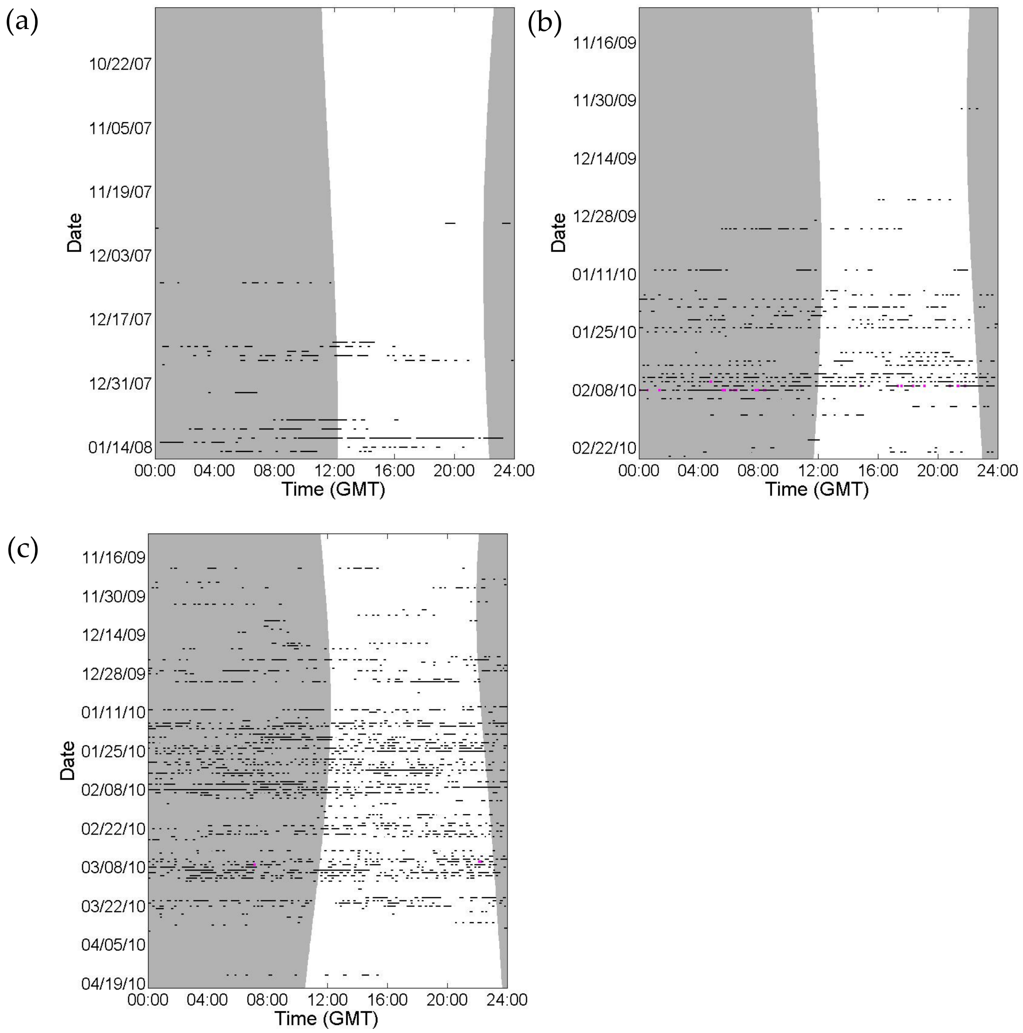

Figure 34: Occurrence of minke pulse trains for (a) the first deployment located at Site A, (b) the fourth deployment located at Site A, and (c) the fifth deployment located at Site C. Black bars represent duration of minke pulse trains (mainly "slow-down") and pink bars represent "speed-up" pulse trains. Shading indicates periods of darkness, determined from the U.S. Naval Observatory (http://aa.usno.navy.mil).

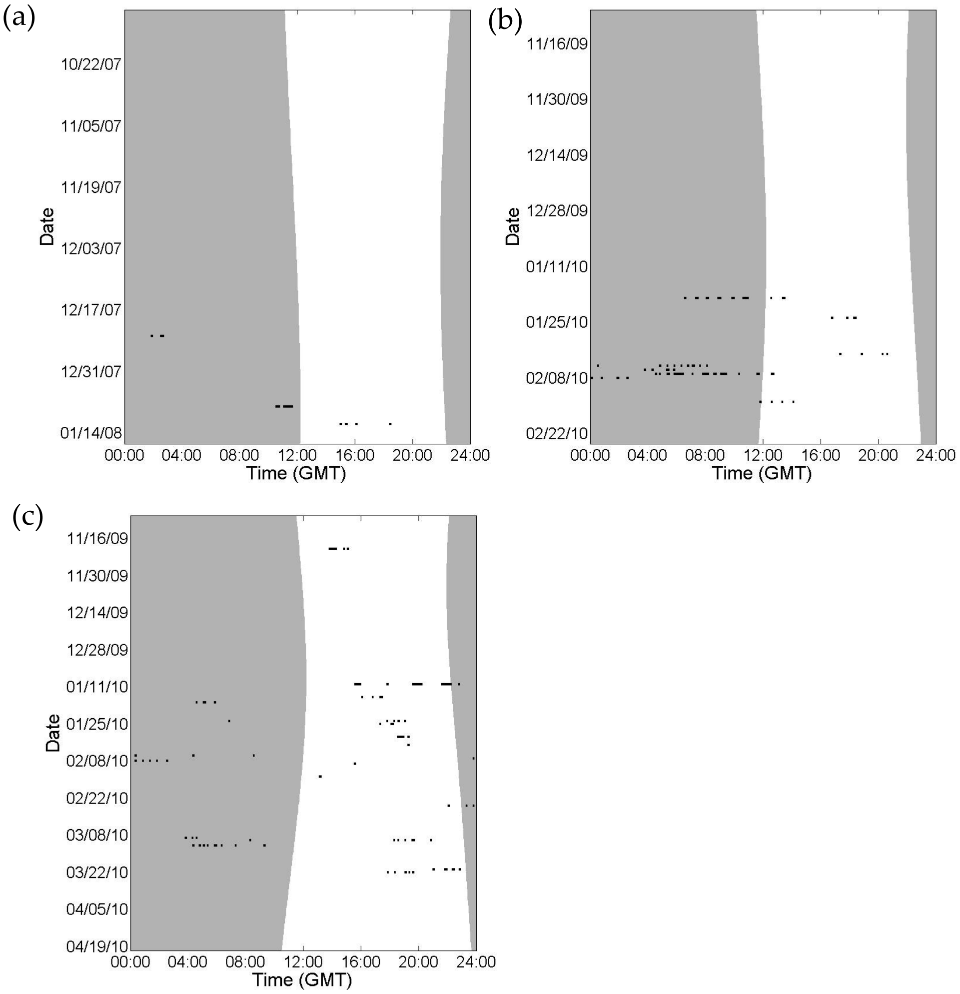

Figure 35: Occurrence of consistent pulse trains for (a) the first deployment located at Site A, (b) the fourth deployment located at Site A, and (c) the fifth deployment located at Site C. Black bars represent duration of vocal events and shading indicates periods of darkness, determined from the U.S. Naval Observatory (http://aa.usno.navy.mil).

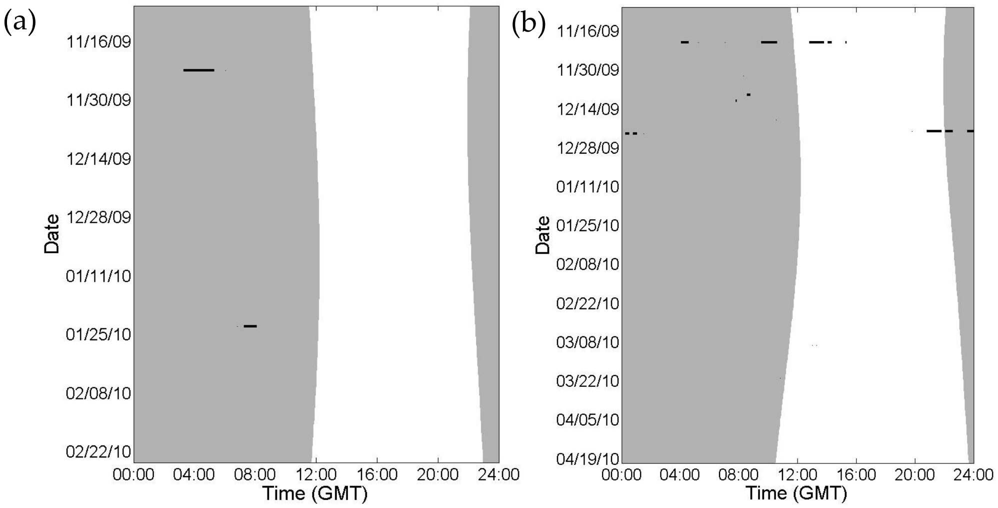

Figure 36: Occurrence of short pulse trains for (a) the fourth deployment located at Site A and (b) the fifth deployment located at Site C. Black bars represent duration of vocal events and shading indicates periods of darkness, determined from the U.S. Naval Observatory (http://aa.usno.navy.mil).

Table 27: Measured variables of four types of pulse trains. Values are mean ± standard deviation.

	Slow-down pulse trains	Speed-up pulse trains	Consistent pulse trains	Short pulse trains
# of calls measured	25	5	12	30
Duration (s)	80.25 ± 16.17	62.64 ± 5.93	58.66 ± 8.99	8.50 ± 7.18
Peak Frequency (Hz)	165.2 ± 5.0	117.2 ± 40.2	51.5 ± 6.8	152.1 ± 45.5
# of calls measured	10	5	10	0
Pulse rate (pulses/s):				
start of pulse train	4.77 ± 0.70	1.63 ± 0.05	1.12 ± 0.13	N/A
middle of pulse train before change	3.02 ± 0.08	N/A	N/A	N/A
middle of pulse train after change	2.04 ± 0.13	N/A	N/A	N/A
end of pulse train	1.83 ± 0.05	3.11 ± 0.34	1.11 ± 0.14	N/A
Peak Frequency (Hz):				
start of pulse train	139.8 ± 17.9	112.5 ± 11.8	46.9 ± 14.7	N/A
middle of pulse train before change	158.2 ± 5.0	N/A	N/A	N/A
middle of pulse train after change	162.9 ± 2.7	N/A	N/A	N/A
end of pulse train	157.4 ± 4.6	170.3 ± 84.6	52.4 ± 10.4	N/A

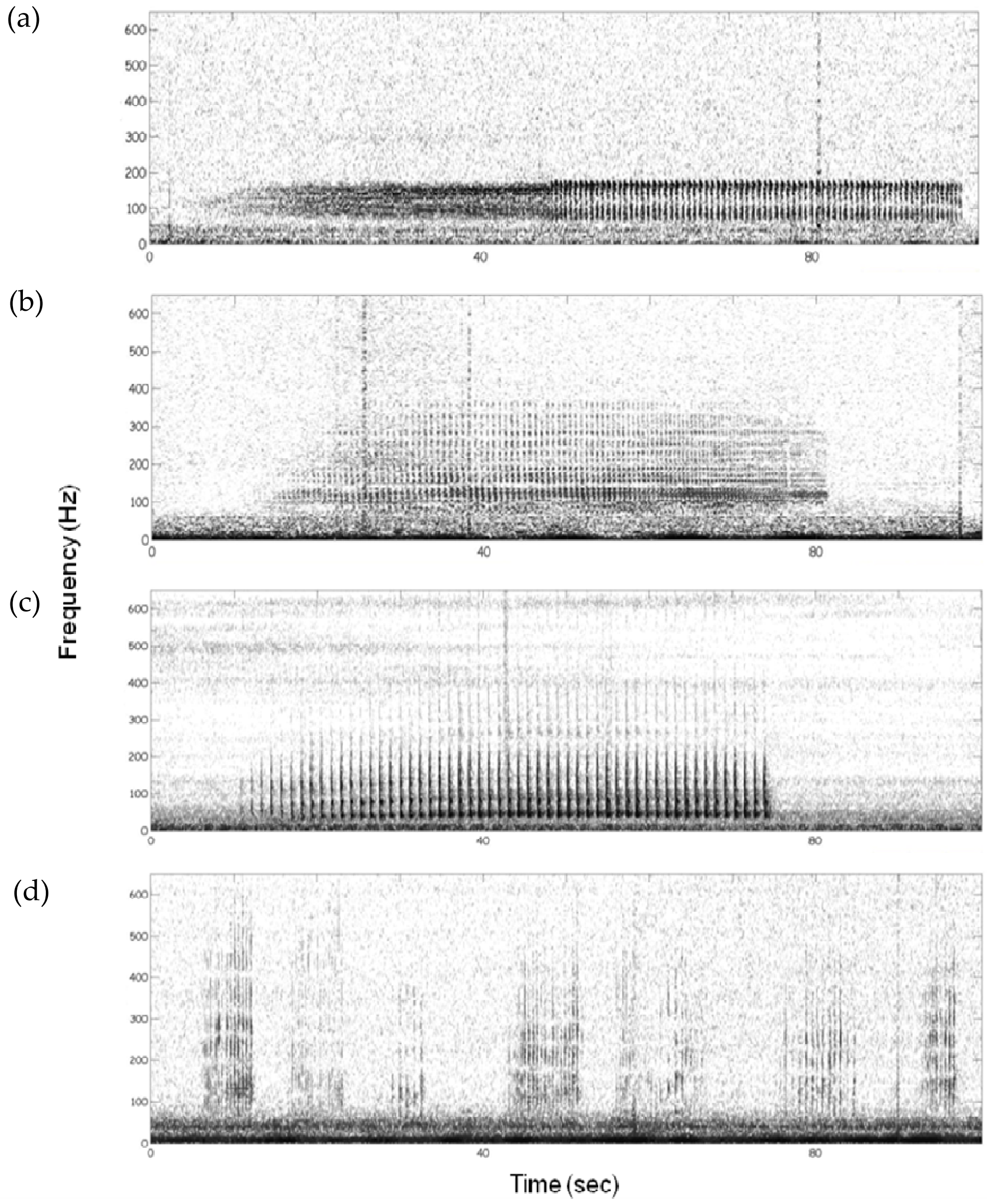

Figure 37: Spectrograms of different types of pulse trains showing (a) slow-down, (b) speed-up, (c) consistent, and (d) short pulse trains (FFT size 512 samples, 75% overlap, Hann window).

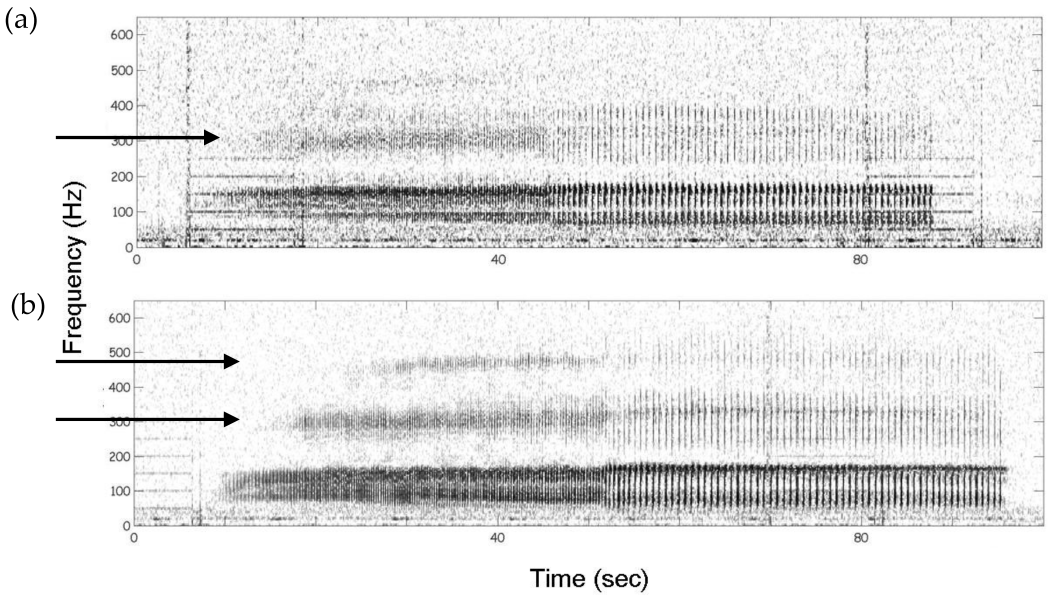

Figure 38: Spectrograms of slow-down pulse trains showing (a) one set of higher-frequency components and (b) two sets of higher-frequency components (FFT size 512 samples, 75% overlap, Hann window). The arrows indicate the higher-frequency components.

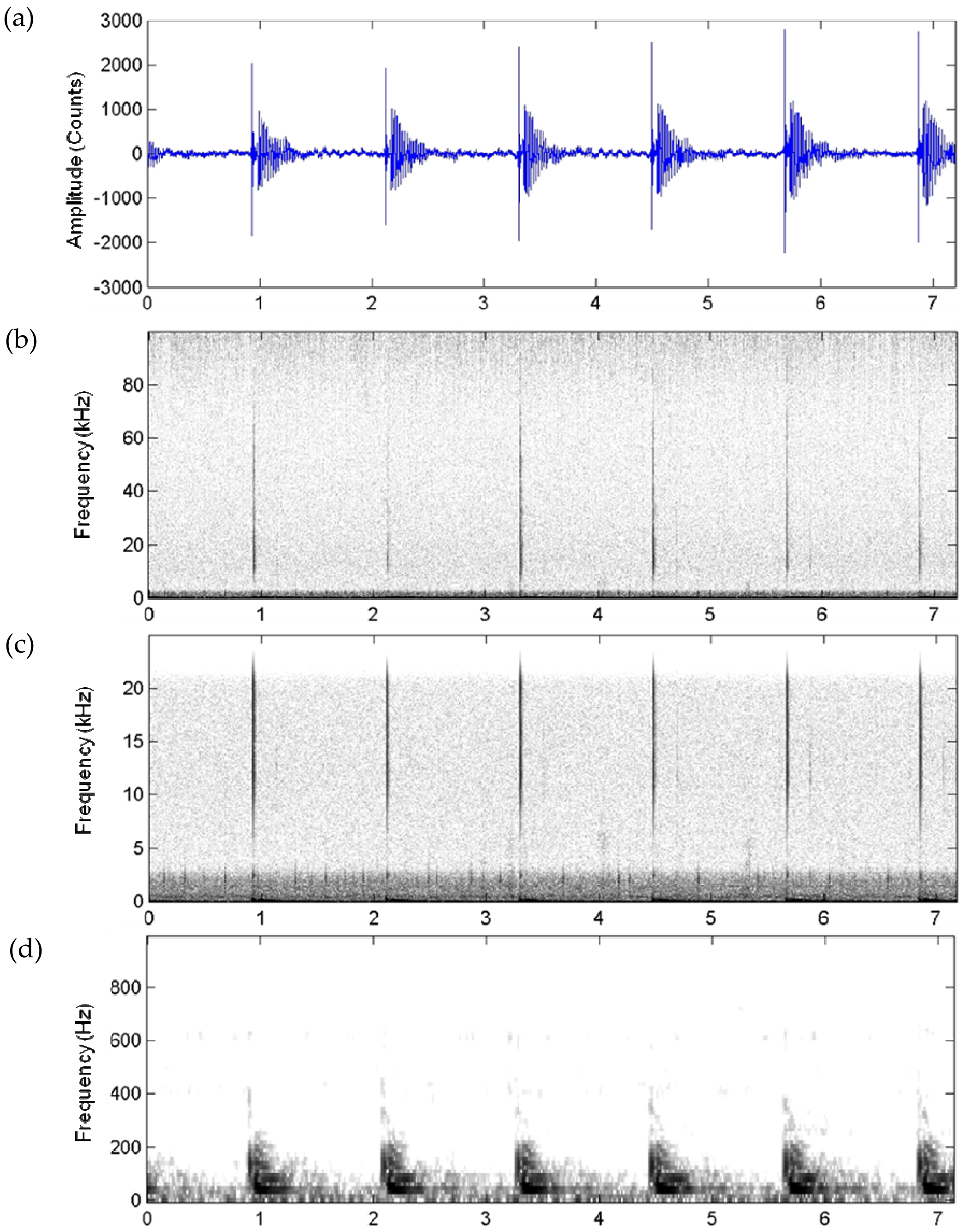

Figure 39: Example of part of a consistent pulse train showing higher-frequency components. (a) Waveform, (b) spectrogram showing non-decimated data (sampling rate 200 kHz, FFT size 1024 samples, 75% overlap, Hann window), (c) spectrogram showing decimated data (sampling rate 50 kHz, FFT size 1024 samples, 75% overlap, Hann window), and (d) spectrogram showing decimated data (sampling rate 2 kHz, FFT size 128 samples, 75% overlap, Hann window).

5.3.4 Humpback whale calls

Calls likely produced by humpback whales were detected only during the fifth HARP deployment on April 18, 2010 (Figure 40). With duty-cycled data, it is impossible to say how long bouts lasted; however, in most cases, calls were seen on either side of a break in recording. These calls occurred between 81 and 549 Hz and ranged in duration from 0.3 to 2.2 seconds (Table 28). The frequency ranges (maximum - minimum frequency) of these sounds varied from 11.7 to 103.9 Hz. Up-call-like sounds were heard in a single bout. In addition, downsweeps were heard at the same time as some of the humpback whale calls. Due to ambiguity of the species producing the calls, these downsweeps were not included in the analysis.

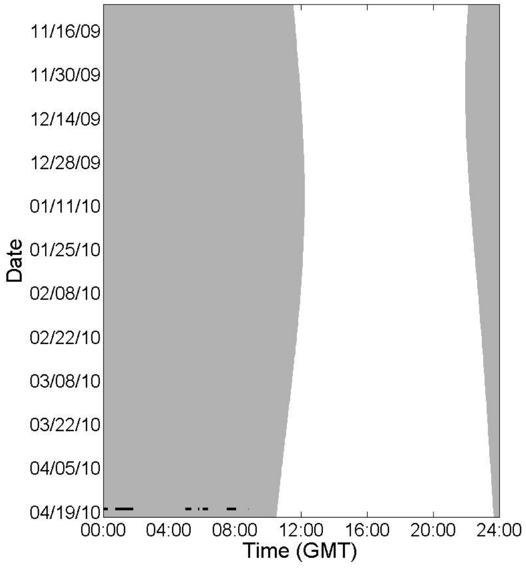

Figure 40: Occurrence of humpback whale calls detected in the fifth deployment located at Site C. Black bars represent duration of vocal events and shading indicates periods of darkness, determined from the U.S. Naval Observatory (http://aa.usno.navy.mil).

Table 28: Measured variables of calls likely from humpback whales based on 155 calls. C.V. stands for coefficient of variation.

	Duration (s)	Max Freq (Hz)	Min Freq (Hz)
Mean	0.92	275.9	241.6
St. Dev.	0.39	102.9	100.5
C.V. (%)	42.0	37.3	41.6

5.4 Discussion

Sounds from fin, minke, and humpback whales were recorded on three HARPs deployed in Onslow Bay between November 2007 and April 2010. In addition, downsweeps and two types of pulse trains produced by unidentified species were recorded during the same time period. Except for the humpback whale calls, these sounds were produced throughout the winter when these mysticetes are expected to be on breeding grounds.

5.4.1 Fin whale 20-Hz pulses

Series of fin whale 20-Hz pulses were detected on all three winter HARP deployments (Figure 29). These 20-Hz pulses were not as common during the first deployment, but this may be a result of noise, likely from the mooring design of the instrument, in the frequency range where these pulses occur. The first deployment in particular had considerable low-frequency noise so it is impractical to look at differences between that deployment and the fourth deployment.

The earliest detections of 20-Hz pulses began in November, indicating fin whales are migrating within detection distance of Onslow Bay at this time. The acoustic recorders in this study were located at a similar latitude as the northeastern instrument along the mid-Atlantic Ridge in the Nieukirk et al. (2004) study, which found detections starting in October.

For the fourth and fifth deployments, which began at the same time (November 2009) but at different sites, it is interesting to note that fin whales were detected much earlier on the deeper instrument (fifth deployment) than on the shallower one (fourth deployment; Figure 29). The most likely explanations for this finding are either that fin whales migrating through the area at this time are located in deeper waters or that fin whales migrating through at this time are only vocal in deeper waters. Starting in January 2010, however, 20-Hz pulses were regularly detected on both the fourth and fifth HARP deployments and continued at least through mid-April (Figure 29). These calls were not detected after April. Once again, my findings agree with Nieukirk et al. (2004), who found far fewer detections starting in May along the mid-Atlantic Ridge.

The inter-pulse intervals of pulses in regular, repetitive sequences found in this study (14.56 ± 1.30 seconds) were slightly shorter than the inter-pulse intervals of 17.5 ± 0.4 seconds reported by Nieukirk et al. (2004) from the mid-Atlantic and of 19 seconds reported by McDonald et al. (1995) from the Northeast Pacific. The pulse durations found in this study (1.48 ± 0.27 seconds) were longer than those from the North Atlantic (0.50 ± 0.14 seconds) reported by Boisseau et al. (2008) and slightly longer than those from the mid-Atlantic (0.9 ± 0.1 seconds) reported by Nieukirk et al. (2004). One explanation for the longer durations reported here versus those reported by Boisseau et al. (2008) is the difference in end frequency. The start frequencies are similar, but Boisseau et al. (2008) reported end frequencies of 18 ± 1 Hz whereas I found end frequencies of 10.4 ± 1.0 Hz. Boisseau et al. (2008) also report a higher peak frequency (22.8 ± 1.5 Hz) than what I found in this study (19.5 ± 0.0 Hz).

5.4.2 Downsweeps

Fin whales and sei whales produce similar downsweeps. Boisseau et al. (2008) recorded downsweeps in the presence of fin whales in the North Atlantic. These calls ("Type II") swept from 72 to 34 kHz and were usually produced prior to 20-Hz pulses, although they also were produced alone. The figure showing these Type II downsweeps shows a pair, each of which appears to last approximately 1 second (see Figure 4 in Boisseau et al. 2008). Baumgartner et al. (2008) described downsweeps produced by sei whales in the western North Atlantic as sweeping down from 82.3 ± 15.2 Hz to 34.0 ± 6.2 Hz over 1.38 ± 0.37 seconds. Baumgartner et al. (2008) state that these downsweeps mainly occurred as single calls, but pairs also were detected, as well as triplets on a few occasions. For pairs, Baumgartner et al. (2008) found the inter-call interval to be 3.5 ± 0.36 seconds; they found similar intervals for triplets.

Support for the idea that the downsweeps may have been produced by sei whales comes from similarities in call characteristics and inter-call intervals found in this study and in the study by Baumgartner et al. (2008). The inter-call interval for pairs (and triplets) and the ending frequency for the downsweeps were very similar to those found by Baumgartner et al. (2008). The start frequency in this study was higher and the duration of the call longer, but these differences may be explained if a greater portion of the call was detected in this study. As Baumgartner et al. (2008) stated, lower starting frequencies and consequently shorter durations were found (and expected) for calls farther away from the recorders (attributed to attenuation of those higher frequencies) whereas the ending frequency was more stable. Thus, if more of the call was detected, I would expect to find higher start and maximum frequencies as well as longer duration calls.

I did not find a great amount of daily overlap between downsweeps and 20-Hz pulses (Figure 33). The fourth and fifth deployments overlapped in time through February 20th, 2010. Taking this into account, there were 34 days with downsweeps and 102 days with 20-Hz pulses out of 164 days of recordings in total. Of the 34 days that downsweeps were detected in Onslow Bay, 20 days had both downsweeps and 20-Hz pulses. I would have expected to find much more daily overlap if fin whales produced the downsweeps detected in this study, so it seems likely that a different species, perhaps a sei whale, produced these calls. Nevertheless, I cannot rule out the possibility that fin whales produce these downsweeps individually, without producing 20-Hz pulses. I also cannot rule out the possibility that a different species produced these downsweeps until more species-specific information from concurrent visual and acoustic surveys is obtained for the area.

5.4.3 Pulse trains

Of the four types of pulse trains detected in the acoustic data, only two have been described previously. Many of the minke pulse trains I detected were faint, but of the ones that had good signal-to-noise ratios, most were slow-down pulse trains. This finding is in contrast to the findings of Mellinger et al. (2000), who found mostly speed-up pulse trains near Puerto Rico. Geographic variation in call type production may explain this difference.

Another difference between the findings of the present study and those of Mellinger et al. (2000) is the frequency range of these pulse trains, with those reported here lower in frequency. A possible explanation for this difference is that only the higher-frequency components were detected by Mellinger et al. (2000) and not the fundamental frequencies. The acoustic data analyzed in Mellinger et al. (2000) were high-pass filtered mainly at 100 Hz (but sometimes at 200 Hz). As the fundamental frequency range in the slow-down pulse trains without the higher-frequency components is below 200 Hz, these would not be detected with a 200-Hz high pass filter. Perhaps a combination of high-pass filtering and fewer higher-frequency components in slow-down trains near Puerto Rico could lead to higher detections of speed-up pulse trains. Mellinger et al. (2000) proposed an alternative explanation for the finding of more speed-up trains, stating that slow-down trains may have been equally abundant but detected less often. These authors reasoned that this could be due to lower received levels of the slow-down trains (if they were produced near the ocean surface which resulted in shadow zones) or lower source levels (Mellinger et al. 2000). Nonetheless, I detected very few speed-up pulse trains with good signal-to-noise ratios so it does seem reasonable that geographic variation in call type production is involved at least to some extent.

Pulse rates reported here and by Mellinger et al. (2000) are comparable for speed-up and slow-down pulse trains. The one main difference was the pulse rate at the end of slow-down trains, found to be 1.8 ± 0.1 seconds here and 2.9 ± 0.9 seconds in Mellinger et al. (2000). This difference may be a result of the shorter train durations reported in Mellinger et al. (2000) (60.9 ± 5.8 seconds versus 80.3 ± 16.2 seconds), although another reason for the shorter durations could be from less of the entire call appearing in the higher-frequency components. As seen in Figure 38, the higher-frequency components often did not start at the actual beginning of the slow-down pulse trains. Of course, truncation at the beginning of these trains would not explain a difference in pulse rate at the end of the trains, and the beginning pulse rates between this study and the one by Mellinger et al. (2000) were similar.

Consistent pulse trains were found intermingled with minke pulse trains (compare Figures 35 and 34). Therefore it seems reasonable to suggest that these also are produced by minke whales, but this cannot be definitely confirmed until concurrent visual and acoustic surveys occur in the presence of these sounds. The high-frequency clicks with peak frequencies at approximately 20 kHz found in the present study associated with the consistent pulse trains (Figure 39) are similar to ones described in the presence of minke whales by Winn and Perkins (1976); however, these authors did not mention an association with lower pulse trains. It is possible that these high-frequency clicks are preparatory sounds for the lower-frequency pulses. Nevertheless, because of the very consistent and repetitive nature of these lower pulse trains, I cannot rule out the possibility that these sounds (the consistent pulse trains) were produced by fish or that the source is not biologic but instead anthropogenic.

The short pulse trains were an interesting finding in this study. These trains were highly variable, as seen by the high standard deviation (Table 27). What is most interesting is the timing of these events in relation to the minke pulse trains (Figures 36 versus 34). Most of these short pulse trains, although somewhat rare and appearing in clusters, occur early in the period when baleen whale calls were detected in Onslow Bay. If these pulse trains are produced by minke whales, it is possible that they represent a transitional stage as minke whales are migrating to the breeding grounds. Most pulse trains reported in the literature that are attributed to minke whales have been recorded during the breeding season (Winn and Perkins 1976, Mellinger et al. 2000, Gedamke et al. 2001, Rankin and Barlow 2005). Although Rankin and Barlow (2005) report that boings, the minke whale pulse train found in the North Pacific, are “seasonally common,” they report that February is during peak boing season. As with the consistent pulse trains, I also cannot rule out the possibility that these sounds were produced by fish and not baleen whales.

5.4.4 Humpback whale calls

Humpback whale calls were detected on only one day - April 18, 2010. These calls were repetitive in nature, occurred in frequency bands between 81 and 549 Hz, and had durations from 0.27 to 2.21 seconds. At times, it appeared that there was more than one caller. Because of the repetitive nature of these calls and because they continued for several minutes, it is less likely these sounds are social sounds (A. Stimpert, personal communication, April 20, 2011). Only fundamental frequencies were detected for these calls, so it is likely the caller(s) was not close to the acoustic recorder.

5.5 Conclusions

Sounds from fin, minke, and humpback whales were recorded on three HARPs deployed in Onslow Bay between November 2007 and April 2010. In addition, downsweeps and two types of pulse trains produced by unidentified species were recorded during the same time period. Due to the lack of overlap in downsweeps and fin whale 20-Hz pulses, I find it unlikely that the downsweeps were produced by fin whales and suggest they might be produced by sei whales instead. Due to the overlap in consistent pulse trains with minke whale (mainly slow-down) pulse trains, I hypothesize that these sounds also are produced by minke whales. The species that produces the short pulse trains remains more of a mystery; however, the timing of these sounds in relation to other pulse trains is interesting to note. More work involving concurrent visual and acoustic surveys, specifically in the winter months, needs to be done in the Onslow Bay area to verify the source of these call types.

General conclusion

Passive acoustics as a monitoring tool for marine mammals

Passive acoustics is being used with increasing frequency to monitor vocalizing marine mammals (Thompson and Friedl 1982, Mellinger et al. 2004, Mellinger et al. 2007, Stafford et al. 2007, Verfuß et al. 2007, Zimmer 2011). Many line-transect surveys now routinely incorporate passive acoustic monitoring using towed hydrophone arrays (Leaper et al. 2000, Barlow and Taylor 2005). Such acoustic monitoring is particularly useful for species that are difficult to detect visually (*i.e.*, those that remain underwater for extended periods of time, such as sperm or beaked whales). Many researchers also are using remote, autonomous passive acoustic recorders to monitor populations of marine mammals over extended periods of time (Mellinger et al. 2004, Oleson et al. 2007b, Philpott et al. 2007, Stafford et al. 2007, Verfuß et al. 2007). These instruments not only provide a long-term record unmatched by visual surveys, but also can provide information about patterns of daily and seasonal usage of remote habitats (as long as the animals are vocal).

My use of passive acoustic techniques in Onslow Bay, North Carolina, has shown that this area has a surprising amount of odontocete vocal activity given the low number of sightings from boat-based surveys. Visual surveys in Onslow Bay average one or two sightings per 74-km trackline at most, and thus, the number and duration of vocal events detected on the High-frequency Acoustic Recording Packages (HARPs, Chapter

3) and "pop-up" acoustic recorders (Chapter 4) throughout the year were greater than expected. The nocturnal patterns seen for some groups of cetaceans (Risso's dolphins, sperm whales, and other delphinids) provided new information that visual surveys could not. The pulse of increased longer duration click events seen at dawn on the HARPs at the site near the shelf break in mid-November to early December is intriguing, although difficult to evaluate until either delphinid vocalizations can be classified to the species level or more visual surveys take place in winter. Since winter is a season that is very windy for offshore waters of North Carolina, it seems that classifying vocalizations to species holds greater promise for understanding patterns of cetacean habitat use.

I found that patterns of delphinid vocal events differ with depth and season, which highlights the need to classify vocal events to the species level. This is the only way to truly understand which species are responsible for the observed spatial and temporal patterns. My results from Chapter 1 show that species-specificity does exist in at least four species found in Onslow Bay (Atlantic spotted dolphins, bottlenose dolphins, rough-toothed dolphins, and short-finned pilot whales), although more work still needs to be done to increase the correct classification rates of Atlantic spotted dolphins and, particularly, rough-toothed dolphins. The inclusion of whistles from additional species inhabiting Onslow Bay into the classification model also is necessary but likely will decrease the correct classification rates. Thus, the use of additional whistle variables, particularly frequency variables which seem to have the highest potential for discriminating species, should be considered. Fortunately, whistling odontocetes also produce clicks that can be used separately or in conjunction with whistles in differentiating sounds among species.

In Chapter 2, I examined clicks to determine if they could be used to distinguish species. My examination of this vocalization type showed that the clicks of Risso's dolphins recorded in Onslow Bay contained spectral peak and notch features similar to those described by Soldevilla et al. (2008). These spectral structure values are consistent and seem to occur in Risso's dolphin clicks from other geographic areas as well (such as Jacksonville, Florida), indicating that these features might be a distinguishing characteristic for Risso's dolphins world-wide. In agreement with the results of Baumann-Pickering et al. (2010), I found that of the three spectral and one temporal parameter I measured for clicks, peak and center frequency might be the most useful for differentiating clicks of other delphinid species (Chapter 2). Creating a model that contains both whistle and click variables might improve the classification performance and should be considered in the future. Also, including additional information as suggested by Oswald et al. (2003), such as information on seasonality of occurrence, species distribution, and the prevalence of each species in the area of interest, might strengthen the model further. In addition, I found several vocal events in the HARP recordings that contained low-frequency narrow-band and low-frequency burst-pulse sounds. Similar sounds have been described by Schultz et al. (1995) for bottlenose dolphins. If these sounds are produced by only one species (which should be investigated in future studies), it would be useful to add such vocalizations into a model for species differentiation.

Identifying species that are producing clicks can provide insight into their foraging activity, as clicks can be, and often are, used as a proxy for foraging. Since little is known about such activity for the two main species seen in Onslow Bay (Atlantic spotted and offshore bottlenose dolphins), this information could provide insight into when foraging occurs.

My use of passive acoustics has allowed *Kogia spp.* (Chapter 3), minke whales (Chapter 5), and possibly sei whales (Chapter 5) to be added to the list of animals present in Onslow Bay, species which have yet to be visually detected in this area. The detection of fin and minke whale calls as well as downsweeps throughout the breeding season (Chapter 5) highlights the possibility that breeding grounds for these species are located somewhere offshore of Onslow Bay. Alternatively, the breeding grounds might be located farther south but some individuals may not undertake the full migration to such grounds, as seen with juvenile humpback whales which winter off of Virginia.

Future Work

The results of my dissertation demonstrate the value of passive acoustic monitoring and highlight the importance of classifying sounds to the species level in improving our understanding of the different temporal and spatial patterns of cetacean occurrence and habitat use (Chapters 3 and 4). Future work thus should focus on building classifiers using whistles and/or clicks to differentiate delphinid species. Given the ultimate goal of differentiating species from archival recordings, future work also should include adding recordings from all local odontocete species and running discriminant function, classification and regression tree, and/or random forest analyses on the data to determine and compare the classification performances of these models. If these models prove to be successful, the deployment of several passive acoustic recorders in Onslow Bay at the same time and at specific sites throughout the year can help elucidate the possible daily fine-scale movements of species that might be occurring and might be causing the nocturnal patterns that I found in Chapters 3 and 4.

Future work also needs to look at animal behavior, specifically clarifying how vocalization type and occurrence change with different behaviors, as well as providing information on daily activity budgets of different species in Onslow Bay. Data collected using DTAGs paired with focal follows during different seasons of the year may shed light on the possible causes of the diel patterns found in the present studies. The use of satellite tags on different species in and around Onslow Bay also may provide information on daily fine-scale movements. The possibility that the increased number of vocal events during winter at the site along the shelf break could be caused by an influx of animals into the area also could be examined using satellite telemetry or additional visual surveys during the winter and during the early morning hours. The use of active acoustics could provide information on the prey dynamics along the shelf break area, potentially indicating why more vocal events are observed in that area during certain times of the year (such as from the aggregation of prey).

In order to determine the source of calls that have been neither identified to species nor recorded on the towed hydrophone array, more work involving concurrent visual and acoustic surveys in Onslow Bay and surrounding areas (especially in deeper waters than normally surveyed) is required. These unidentified calls include: (1) two click types with different spectral banding patterns than any of the species recorded on the towed hydrophone array (Chapter 2), (2) low-frequency downsweeps (Chapter 5), (3) consistent pulse trains with peak energy around 50 Hz (Chapter 5), and (4) short pulse trains (Chapter 5). Thus, towed hydrophone arrays with the capability of recording baleen whales, and possibly recording at higher sampling rates than in the present studies (to determine if features of some clicks occur at higher frequencies), should be used during these surveys. Finally, the deployment of several recorders in Onslow Bay in an arrangement in which baleen whale calls can be localized could shed light on the location of the calling whales during the breeding season.

Appendix A: Daily occurrence and duration of click events for Risso's dolphins, sperm whales, *Kogia spp.*, and other delphinids

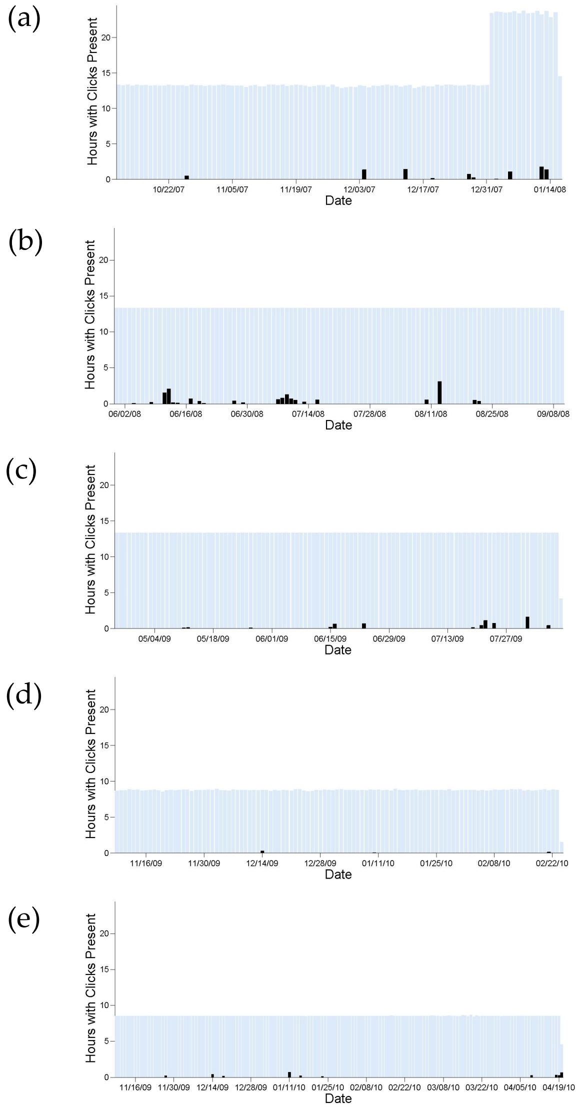

Figure 41: Daily occurrence of Risso's clicks for (a) the first deployment located at Site A, (b) the second deployment located at Site B, (c) the third deployment located at Site A, (d) the fourth deployment located at Site A, and (e) the fifth deployment located at Site C. Black bars represent the number of hours with clicks present each day. Blue bars represent the number of hours of recording effort each day.

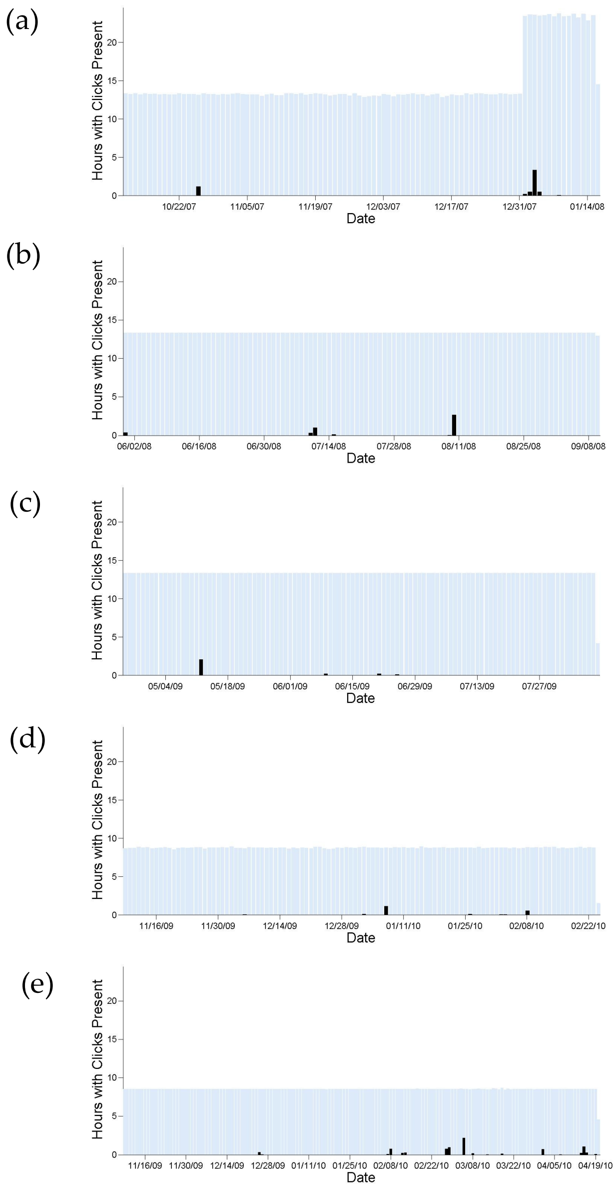

Figure 42: Daily occurrence of sperm whale clicks for (a) the first deployment located at Site A, (b) the second deployment located at Site B, (c) the third deployment located at Site A, (d) the fourth deployment located at Site A, and (e) the fifth deployment located at Site C. Black bars represent the number of hours with clicks present each day. Blue bars represent the number of hours of recording effort each day.

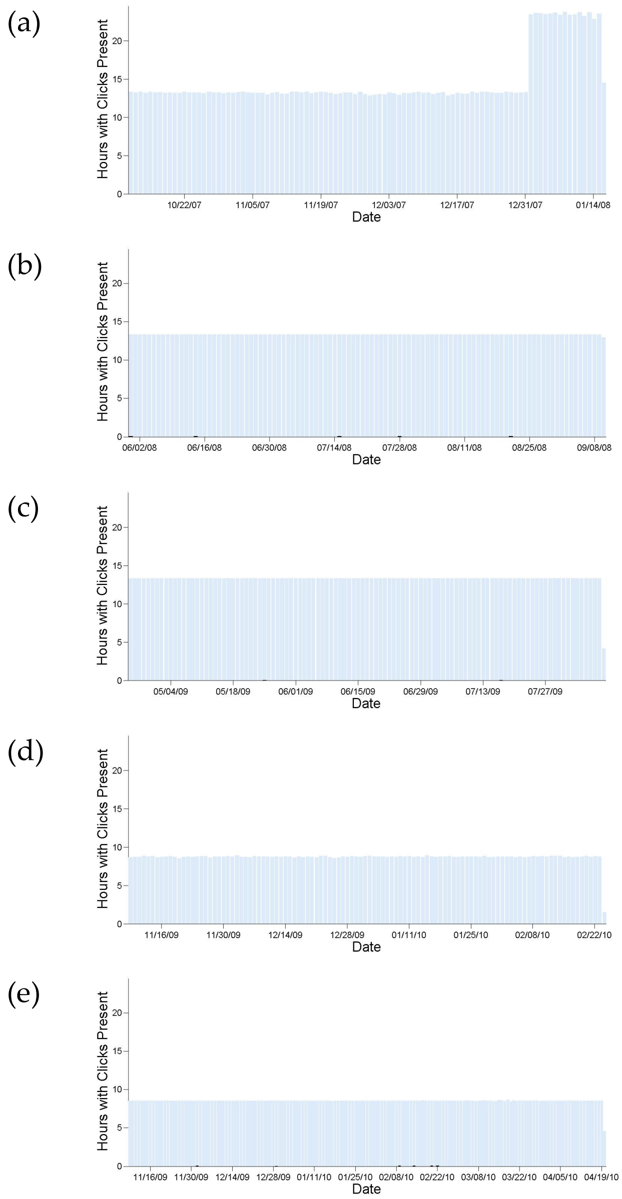

Figure 43: Daily occurrence of *Kogia* clicks for (a) the first deployment located at Site A, (b) the second deployment located at Site B, (c) the third deployment located at Site A, (d) the fourth deployment located at Site A, and (e) the fifth deployment located at Site C. Black bars represent the number of hours with clicks present each day. Blue bars represent the number of hours of recording effort each day.

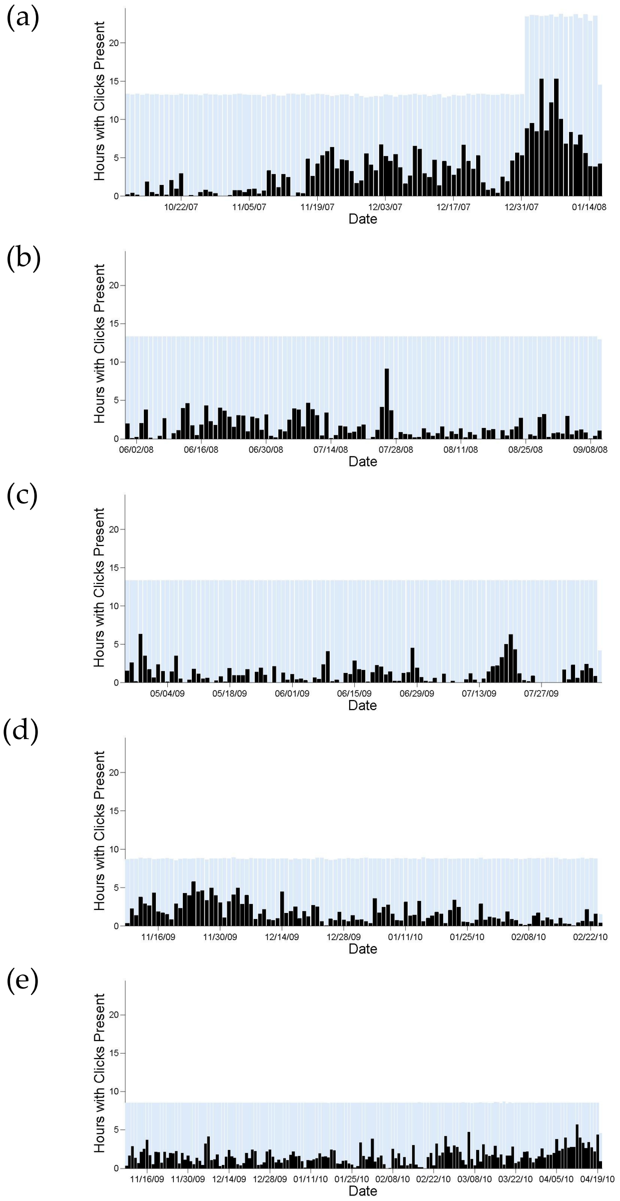

Figure 44: Daily occurrence of other delphinid clicks for (a) the first deployment located at Site A, (b) the second deployment located at Site B, (c) the third deployment located at Site A, (d) the fourth deployment located at Site A, and (e) the fifth deployment located at Site C. Black bars represent the number of hours with clicks present each day. Blue bars represent the number of hours of recording effort each day.

Table 29: Minimum, maximum, and average duration of Risso's click events for each HARP deployment.

Deployment	Site	# Vocal Events	Min Duration (h:mm)	Max Duration (h:mm)	Avg Duration (h:mm)
1	A	12	0:04	2:25	1:01
2	B	46	0:02	2:15	0:33
3	A	13	0:01	2:55	0:52
4	A	3	0:04	0:48	0:24
5	C	12	0:17	1:05	0:42

Table 30: Minimum, maximum, and average duration of sperm whale click events for each HARP deployment.

Deployment	Site	# Vocal Events	Min Duration (h:mm)	Max Duration (h:mm)	Avg Duration (h:mm)
1	A	14	0:01	3:50	0:29
2	B	43	0:01	0:42	0:09
3	A	20	0:01	0:41	0:11
4	A	15	0:01	2:50	0:19
5	C	65	0:01	2:44	0:16

Table 31: Minimum, maximum, and average duration of *Kogia* click events for each HARP deployment.

Deployment	Site	# Vocal Events	Min Duration (h:mm)	Max Duration (h:mm)	Avg Duration (h:mm)
1	A	1	0:03	0:03	0:03
2	B	6	0:02	0:04	0:02
3	A	4	0:01	0:04	0:02
4	A	2	0:02	0:02	0:02
5	C	9	0:01	0:05	0:02

Table 32: Minimum, maximum, and average duration of other delphinid click events for each HARP deployment.

Deployment	Site	# Vocal Events	Min Duration (hh:mm)	Max Duration (hh:mm)	Avg Duration (hh:mm)
1	A	616	0:01	8:09	0:48
2	B	954	0:01	10:24	0:16
3	A	525	0:01	6:35	0:24
4	A	784	0:01	10:16	0:35
5	C	1493	0:01	9:16	0:25

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Publications

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Honors and Awards

1st Place Award for Best PhD Student Poster, SEAMAMMS, April 2009
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2nd Place Award for Best Master's Student Oral Presentation, SEAMAMMS, March 2005
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