The Effect of Auditory Stimulation on Sleep Disruption in West Indian Manatee (Trichechus manatus latirostris)

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THE EFFECT OF AUDITORY STIMULATION ON SLEEP DISRUPTION IN WEST INDIAN MANATEE (*TRICHECHUS MANATUS LATIROSTRIS*)

by

Natalija Lace

A Dissertation
Submitted to the Graduate School
and the Department of Psychology
at The University of Southern Mississippi
in Partial Fulfillment of the Requirements
for the Degree of Doctor of Philosophy

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August 2016
ABSTRACT

THE EFFECT OF AUDITORY STIMULATION ON SLEEP DISRUPTION IN WEST INDIAN MANATEE (TRICHECHUS MANATUS LATIROSTRIS)

by Natalija Lace

August 2016

Florida manatees inhabit waterways where motorized boats are common. Although manatee mortalities resulting from boat strikes are well documented, the effect of boat noise on some manatee behaviors, including rest, has not been investigated. This study focuses on rest behavior and used a playback experiment with four manatees at the Lowry Park Zoo in Florida. We tested their responses to playback stimuli of either boat noise, silence, or manatee calls. A playback trial was initiated when the focal animal showed behavioral characteristics of rest.

Results showed that rest was interrupted in response to the playback of boat noise for each of the manatees. Distinct reactions to playback stimuli were exhibited where for three of the four, rest was interrupted in response to manatee calls; however, rest was not interrupted for any manatee during playback of silence. Boat noise resulted in manatees moving away from the speaker, and manatee calls resulted in their moving toward the speaker. Manatees showed individual differences in their post-playback behaviors in terms of breathing patterns, rest episode duration, and latency of reactions. However, these differences were not statistically significant and did not reflect a specific type of stimuli (boat noise, manatee calls, or silence).

Results indicate that rest could be interrupted immediately after playback in response to both biologically significant sounds (e.g., manatee calls) and anthropogenic
sounds (e.g., boat noise), though reactions differed according to sound type. Overall, no rest behavior or rest episode was affected in any significantly different way by the type of playback stimuli, which indicates that sleep interruptions at such low levels (85–97 dB re 1 µPa) and of such short duration did not have a significant effect on manatees’ overall rest behavior or rest duration.
ACKNOWLEDGMENTS

I would like to thank Dr. Stan Kuczaj who has been my advisor and a true mentor. A special thank you goes to the Lowry Park Zoo staff, especially Virginia Edmonds, Jennifer Galbraith, Tanya Ward, Molly Lippincott, Lisa Di Jenno, and Jaime Vaccaro, who have been very helpful and accommodating for this project. I would also like to express my gratitude to the Lowry Park Zoo General Curator, Lee Ann Rottman. I would also like to thank Peter Simmard who helped me with sound source measurements. Finally, I would like to thank my family for support and unconditional love.
DEDICATION

I dedicate this dissertation to CC, Teco2, Longo, and countless other manatees who are struggling to survive in Florida. I would also like to thank my family for support and unconditional love.
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CHAPTER I - INTRODUCTION

Sleep Behavior Overview

Sleep behavior is found in nearly every taxon of animals that has been studied (Cirelli & Tononi, 2008; Lima, Rattenborg, Lesku, & Amlaner, 2005). Despite ongoing debate (Siegel, 2008), it is generally accepted that there is no animal on this planet that does not sleep (Cirelli & Tononi, 2008). Although the exact purpose of sleep has not been determined, sleep is currently believed to serve a variety of functions, including energy conservation (Velluti, 2008; Zepelin, Siegel, & Tobler, 2005; Zepelin & Rechtschaffen, 1974), memory consolidation (Axmacher et al., 2008; Siegel, 2008; Velluti, 2008), the renormalization of synapses (Cirelli & Tononi, 2008), the strengthening of the immune system (Opp, 2009), the removal of harmful toxins (Xie et al., 2013), increasing the number of myelin-making cells (oligodendrocytes) (Bellesi et al., 2013), and other functions.

The effects of sleep deprivation vary across species and range from negative effects in rats, roaches, humans, and flies (Cirelli & Tononi, 2008; Rechtschaffen, Everson, Kushida, & Gilliland., 2002) to barely noticeable effects in pigeons (Newman, Paletz, Rattenborg, Obermeyer, & Benca,2008). However, some argue that sleep is a complex phenomenon and that the effects of sleep deprivation should be investigated along the dimensions of duration and intensity/quality (Cirelli & Tononi, 2008). Sleep deprivation often results in sleep rebound, which increases slow-wave sleep (SWS) or rapid eye movement (REM) sleep (Mooncroft, 2003). Sleep deprivation is also associated with cognitive impairments and problems with attention, learning and memory (Drummond & McKenna, 2009), the intrusion of sleep into wakefulness, neurochemical
and hormonal changes, the upregulation of genes in the cerebral cortex and other brain areas (Cirelli, 2006), and the development of cardiovascular disease (Quan, 2009). Insomnia also results in a range of health problems, starting with depression and ending with myocardial infarction (Sivertsen et al., 2014).

Despite at least partial agreement that all animals sleep, sleep is not easy to define behaviorally. In general, the behavioral definition of sleep includes four major components: a) immobility (but it is important to note that immobility can be species-specific with respect to sites, positions and even movements (however, see below for more discussion)), b) reversibility, c) reduced responsiveness, and d) compensation for the loss of sleep (Cirelli & Tononi, 2008; Rattenborg & Amlaner, 2001; Siegel, 2008). An additional state called drowsiness or quiet wakefulness is present in many animals. However, there are a number of opinions concerning whether this intermediate state should be considered to be rest or wakefulness. Siegel (2008) made a specific distinction between rest (including quiet wakefulness) and actual sleep and believed that drowsiness or quiet wakefulness should not be considered sleep. Lima et al. (2006) emphasized that quiet wakefulness and drowsiness serve an important function under conditions when rest is highly desirable yet predator detection and awareness are also needed.

While described in many animals during sleep, immobility is not present in all species. For example, dolphins can continue swimming while asleep (Zepelin et al., 2005), and it has also been suggested that some long-distance migrating bird species (e.g., terns and swifts) might sleep while flying. However, immobility is seen in other marine mammals, such as manatees and seals (Lyamin, Manger, Ridgway, Mukhametov, & Siegel, 2008; Mukhametov, Lyamin, Chetyrbok, Vassilyev, & Diaz, 1992).
Reversibility is another component of the behavioral definition of sleep and distinguishes sleep from hibernation or coma (Zepelin et al., 2005). The reversibility of sleep or sleep offset depends on many factors, including hormones (cortisol), body temperature and environmental stimulation (Moorcroft, 2003). Finally, reduced responsiveness does not mean complete disconnection from the environment because auditory processing continues during sleep. Guinea pigs, for instance, showed activity in the auditory cortex while asleep in response to the playback of conspecific whistles (Velluti, 2008). In some human studies, participants have been able to categorize words while sleeping (Kouider, Andrillon, Barbosa, Goupil, & Bekinschtein, 2014).

Sleep can be interrupted by a variety of sensory stimulants (including tactile, auditory and visual stimulants), and whether awakening occurs depends on the nature of the stimulus. Olfactory stimulation often fails to result in awakening but instead alters the emotional tone of the dream (Schredl, Maurer, Hummel, & Stuck, 2009). Intense stimuli will result in awakening; for example, when a person falls off the bed, he or she will wake up because the fall is a powerful stimulus. Intense pain will result in awakening as well as any other intense and uncomfortable stimulation (Nielsen, McGregor, Zadra, Ilnicki, & Ouellet, 1993; Wittig, Zorick, Blumer & Roth, 1982). Auditory stimulation will trigger awakening in some cases, depending on the nature of the stimulation, such as hearing one's name (Portas, 2005). Although auditory processes are modified during sleep (Velluti, 2008), auditory detection and processing do not cease completely. In fact, the auditory system plays a very important role in sleep and awakening and has been shown to modify sleep itself when severed artificially (Velluti, 2008).
Auditory System in Sleep

What happens to the auditory system during sleep? The auditory channel is open during sleep, and the sleeping brain thus experiences auditory stimuli (Velluti, 2008). The auditory system plays a role in awakening (Velluti, 2008). When the auditory systems of guinea pigs and cats were intentionally severed, sleeping episodes became longer. Similarly, when animals were put under conditions of sensory deprivation, the sleep architecture changed (Cutrera et al., 2000; Velluti, 2008). Auditory processing also occurs during sleep, although this processing is modified and progresses differently during wakefulness (Velluti, 2008). While a significant loss of activity occurs in certain neurons that are responsible for muscle tone and consciousness (Siegel, 2008), nearly 50% of the primary cortex neurons continue to fire during sleep, probably because of the need to monitor the environment (Vallet, 1982). The discrimination of meaningful stimuli from other stimuli and ambient noise also occurs in the sleeping brain (Velluti, 2008). When a complex guinea pig whistle was played to a sleeping guinea pig, the auditory cortex responded (although the response differed from the response to the same whistle while awake). When the same whistle was played backwards, the auditory cortex activity decreased, demonstrating that auditory processing and discrimination did not cease completely in the guinea pig cortex during sleep (Pedemonte, Peña, Torterolo, & Velluti, 1997). In one study, participants were able to categorize words while still asleep, further supporting the notion that the sleeping brain is capable of performing cognitive tasks (Kouider et al., 2014).

The thalamic gatekeeping function – that send messages to the auditory complex – has a dual nature when an organism is asleep (Edeline, Manunta, & Hennevin, 2000).
The activity of thalamic cells and the messages that those cells send to the auditory cortex are greatly reduced during sleep (Edeline et al., 2000). However, this activity does not cease completely, as some functions are preserved, such as frequency selectivity and rate level functions (Edeline et al., 2000). This preservation is probably the reason why many stimuli still make their way through to the auditory cortex.

As mentioned earlier, the discrimination between meaningful and irrelevant stimuli occurs during sleep. Therefore, when stimuli are significant and have special meaning, they are more likely to result in waking, as LeVere, Davis, Mills, and Berger (1976) have shown. In humans, an individual’s name is an important stimulus, and uttering or even whispering a person’s name will awaken him or her (Portas, 2005). Mothers have been shown to wake at the cries of their babies (Portas, 2005), and in other species, including some prey species, a predator’s roar might also result in waking.

At the same time, some stimuli are sometimes incorporated into dreams (in humans), and sleep is preserved (Koulack, 1969). Stimuli such as water spraying and percutaneous electric shock were incorporated directly into dreams (dreaming about electric shock and cold rain) (Koulack, 1969). However, though cold water sprays were incorporated into dreams in 42% of cases, they still resulted in sleep offset in 30% of all trials (Dement & Wolpert, 1958).

The reason that some stimuli become incorporated into dreams is not easy to determine; in general, such stimuli should have at least some influence upon the sleeping individual without being strong enough to elicit awakening (Koulack, 1969). The stimuli that have shown some incorporation into dreams are sinus tone, neutral stimuli, non-significant words, rocking of the sleeper’s bed, mild pain and mild electric stimulation,
Unihemispheric Sleep

Unihemispheric sleep is defined by distinct EEG patterns that occur one hemisphere engages in activity corresponding to EEG patterns, as when an individual is awake, whereas another hemisphere shows diminished activity—slow waves in particular—as seen on EEGs when an individual is asleep (Rattenborg et al., 2000). Unihemispheric sleep has been recorded in cetaceans, seals, sea lions, manatees, birds, and even reptiles such as crocodiles (Rattenborg, Amlaner, & Lima, 2000), although unihemispheric sleep in manatees has not been studied extensively (Mukhametov et al., 1992). A proposed function of unihemispheric sleep is that it aids in the detection of predators and danger as well as supports heightened vigilance (Lima et al., 2005).

Predator species and species that live in low danger environments sleep more soundly, experience more REM sleep and awaken less easily (Siegel, 2009). Prey species and species that live in high danger environments sometimes have fragmented sleep, awaken easily and engage in unihemispheric sleep (Lima et al., 2005; Siegel, 2009). For example, birds have unihemispheric sleep in which one brain hemisphere shows activity while another is at rest (Rattenborg & Amlaner, 2001). The proposed function of unihemispheric slow-wave sleep (USWS) in birds is to aid in predator detection and to support rapid awakening in the case of eminent danger.

Another potential function involves improved efficiency, for which USWS can be useful in some situations unconducive to rest, including long migrations (Rattenborg & Amlaner, 2001; Rattenborg, Lima, & Amlaner, 1999). However, unihemispheric sleep is
less efficient (Rattenborg & Amlaner, 2001), meaning that when birds engage in such sleep, they most likely do so at great risk of predation (Lima et al., 2005). Rattenborg et al. (1999) tested sleep in mallard ducks and concluded that ducks sleeping on the edge of the group had a higher proportion of unihemispheric sleep in comparison to ducks sleeping safely in the middle of the group. In addition, ducks regulate which hemisphere will be awake and which hemisphere will be asleep depending on the eye that remains open and faces outside the group for predator detection. Ducks also showed rapid arousal and escape when presented with threatening visual stimulation that resembled a flying hawk.

Birds are not the only species that have unihemispheric sleep. Dolphins, whales, sea lions, and manatees also have unihemispheric sleep (Lyamin et al., 2008). Small delphinids that sleep in groups behave differently from mallard ducks and usually direct the open eye not outside the group but inside the group, most likely to monitor group members (Goley, 1999). Dolphins, whales, and manatees rely on sound processing as well as vision to assess danger. Thus, auditory vigilance can be as important as visual vigilance. Indeed, dolphins showed remarkable auditory vigilance when they were forced to respond to auditory stimuli during a 72-hour period. Not only did they respond correctly over the 72 hours of the study, but they also showed hardly any serious impairment (with the exception of having some latency in their responses at some points during the 24-hour cycle). This study did not measure the physiological effects of maintaining vigilance for 72 hours, so it is impossible to know the full extent of the possible effects. In addition, the presence of USWS was not confirmed as the study did
not measure EEG directly. Thus, it is possible that the dolphins were able to maintain 72 straight hours of auditory vigilance by using USWS sleep (Ridgway et al., 2009).

In general, the function of unihemispheric sleep in cetaceans, sirenians and pinnipeds is a matter of debate (Lyamin et al., 2008; Rattenborg et al., 2000). Three major functions have been proposed, including aiding in breathing, thermoregulation and maintaining vigilance. Eared seals sleep in water and on land and exhibit USWS, SWS and REM (Lyamin et al., 2008). When fur seals sleep in water, they have more USWS. True seals do not have USWS; when in the water, these seals sleep while holding their breath and must awaken to get more air (Rattenborg & Amlaner, 2001). Elephant seals are thought to sleep while performing deep dives for their prey. They sleep during the descent, then wake up to catch the food and may sleep again on their way back to the surface (Mitany, Andrews, Sato, Kato, & Naito, 2010).

The function of USWS in manatees may differ from what is currently proposed for dolphins and birds (Rattenborg & Amlaner, 2001). Manatees do not have particularly good vision, nor do they sleep, move, or breathe in the same way as dolphins (Rattenborg & Amlaner, 2001). It is possible that USWS allows manatees to monitor the environment and helps them to awaken rapidly when potential danger is detected (Rattenborg et al., 2000). This could be a useful evolutionary adaptation as, for example, in Florida, manatees were hunted by native Seminole Indians (Husar, 1977). Later, manatees were poached, but overall, Florida manatees were not targeted specifically for commercial harvest like manatees in other areas of the Caribbean (Domning, 1982).

Although several centuries of predation is not long enough to for manatees to develop evolutionary adaptations for increased vigilance, the way in which manatees
currently behave in Cuba indicates that they have a certain plasticity and can adapt their behavior depending on ecological and predatory pressures. In areas where manatees are still hunted, including Cuba, they tend to avoid humans (Gonzalez–Socoloske, Olivera-Gomez, & Ford, 2009), even to the point that approaching them in a boat is impossible. In that light, those manatees might have benefited from being vigilant. Today, Florida manatees do not have any natural predators apart from humans and have not been hunted for a long time (Reep & Bonde, 2006), so it is not clear why they would need to stay vigilant in the way that birds do when they are in dangerous conditions.

Manatee Sleep

Manatee sleep has not been studied extensively. The only physiological study of manatee sleep was performed in 1992 by Mukhametov et al. Several electrodes were implanted in a manatee’s head, and she was observed for five days. It must be noted that the electrode implants could have affected the results by stressing the animal and introducing pain and discomfort. The manatee slept in two favorite spots, and its respiration pauses increased significantly during sleep episodes. The manatee had SWS sleep, including USWS sleep. Over a period of 24 hours, approximately 25% of all SWS sleep was USWS sleep. The manatee also experienced REM sleep that constituted only 1% of the total observation time. The manatee slept during respiration pauses and awakened briefly for each breath, as observed for seals.

Manatees have two distinct respiration patterns: regular, rhythmic single breaths and ventilative breathing in which a manatee holds its breath for a long period of time and then takes a series of short breaths while remaining near the surface (Gallivan, Kanwisher, & Best, 1986; Hartman, 1979). Ventilative breathing appears to be associated
with only two specific activities: resting and bottom feeding. The duration of respiratory pauses during these activities is much longer than the respiratory pauses observed during other activities, such as milling, feeding, cavorting and travelling (Hartman, 1979; Reynolds, 1981). In other species, such as seals, respirations become prolonged and less regular during rest. Thus, breathing patterns can be also used in the behavioral definition of sleep (Rattenborg & Amlaner, 2001).

Behavioral rest in manatees was described by Hartman (1979) and Reynolds (1981). Reynolds (1981) noted increased respiratory pauses during rest, and Hartman (1979) reported two major positions of manatees at rest: bottom rest, when the manatee was lying at the bottom of streams or bays, and a suspended floating posture just below the surface. Hartman (1979) also noted that bottom rest was accompanied by ventilation breathing (see below) and that disturbed manatees interrupted their rest and assumed a position that would support take off if needed (see definition of sleep interruption in the Methods section).

Manatees’ rest patterns are polyphasic, meaning that sleep occurs during the day and at night, at no particular time (Hartman, 1979). However, a more recent study involving captive manatees documented a more complicated activity pattern that included both ultradian (i.e., cycles repeatable within the typical 24-h circadian day; Holguín–Medina, Fontenele-Araujo, Romero, Cortes, & Muñoz-Delgado2015) and circadian components, in which rest was associated with 24-h circadian rhythms. In that study, the manatees tended to be more active during the day than at night and also had more rest in the afternoon than during the morning hours (Holguín-Medina et al., 2015). Manatees in Crystal River rested throughout the day with no particular time preferences, and rest
episodes lasted from two to twelve hours. Hartman (1979) proposed that manatees get six to ten hours of rest distributed over a period of 24 hours. However, manatees at Blue Lagoon and St. Jones River in Florida exhibited patterns of day/night activities (Reynolds, 1981). Another sirenian, the dugong, was more active during the night, supposedly due to the lower risk of being hunted (Jonklaas, 1961).

Manatees at Lowry Park Zoo appear to have certain patterns in their resting behavior, especially animals that have been at the zoo for some time. It is likely that the zoo routine influences the time of the day when the manatees rest. New animals have slightly different rest patterns upon arrival, but they are also usually unwell and very stressed. One orphaned baby manatee, which was bottle fed every two hours, had several short periods of rest during the day.

Sleep can be extremely important for manatees. For example, Amazonian manatees fast for as long as seven months and live off of their fat reserves (Best, 1983). Sleep can help manatees to slow down their metabolism, can help with thermoregulation and can aid in conserving energy (Holguin-Medina et al., 2015; Horikoshi-Beckett & Schulte, 2006; Zepelin & Rechtschaffen, 1974). One of the proposed functions of sleep is memory consolidation, which is also extremely important for manatees, especially juvenile animals that must learn a lot. Manatees must remember travel routes, particularly routes to hot springs, power plants and other warm water areas in winter (Reep & Bonde, 2006). Remembering these routes is crucial for manatees because they are highly susceptible to cold stress. If manatees cannot find a warm body of water, they usually die very quickly. During the past two winters, manatees unfortunately endured unprecedented losses because of cold stress. Memorizing feeding areas is also extremely
important because high quality food yields significant fat reserves and can save a manatee’s life. During baseline observations for the current study, one non-focal pregnant female rested more than other animals; thus, sleep can also be very important for pregnant manatees (personal observation).

Manatees inhabit areas where recreational and commercial traffic is common and are vulnerable not only to direct injuries from watercraft but also to the noise produced by watercraft. For that reason, manatee hearing and the effects of noise on manatees have received some interest, along with attempts to determine why manatees are susceptible to getting hit by boats. Manatee hearing has been studied for some time; however, many questions remain. Based on anatomical studies, Ketten, Odell, and Domning, (1992) concluded that manatees have unimpressive ears with low acuity and a lack of directional hearing. Manatees have no external pinnae, and their external auditory channel is probably non-functional, as in cetaceans. Newborn manatees have a fully developed auditory system, indicating that the auditory system is highly important from birth. The tympano-periotic complex (TPC) is not attached to any bones in the skull, and the tympanic membrane closely resembles the general tympanic membrane found in terrestrial animals.

However, two features of the manatee hearing system differ significantly from those of other marine mammals. Manatees have massive ossicles (the inner ear bones), which are not found in any other marine mammal; the function of this enlargement is not completely understood. Manatees also have a unique zygomatic process (part of the skull) that consists of zygomatic, squamisal and periodic bones. This process is highly enlarged, and its structure and density are unlike anything previously found in any marine
mammal. The process has cartilaginous labyrinths that more closely resemble a sponge than a bone (Ketten et al., 1992). The process is also saturated with fats that consist primarily of triacylglycerols (Ames, Van Vleet, & Reynolds, 2002). All of the above observations indicate that the zygomatic process may play a role in hearing, specifically low frequency hearing. However, because the fat content is so different from that observed in a dolphin’s jaws, the process probably works differently than previously described for other animals (Ames et al., 2002).

Long before any studies of manatee hearing, ancient tribes in South America, which have been hunting manatees for hundreds of years, noticed that manatees have acute hearing and even had the saying “hears like a manatee,” which means that one has exceptional hearing (O’Shea et al., 1988). Indeed, in contrast to the conclusions of Ketten et al. (1992), subsequent behavioral and physiological studies revealed that manatees have not only acute ears but also good localization abilities. Behavioral audiogram and evoked potential studies revealed that the manatee’s hearing range is between 0.4 kHz and 46 kHz (Gerstein, Gerstein, Forsythe, & Blue, 1999).

The upper limit has not been settled because at least one study described the upper limit as 60 kHz (Klishin, Diaz, Popov, & Supin, 1990), while another study reported a much lower limit of 35 kHz (Popov & Supin, 1990). The best range of hearing lies between 6 and 20 kHz, with peak sensitivity at 16 to 18 kHz (Gerstein et al., 1999). These range and sensitivity values are expected because manatee vocalizations range from 2.5 up to 5 kHz with average source levels of 112.5 dB (Phillips, Niezrecki, & Beusse, 2004). In contrast to predictions based on the anatomy of the ear, manatees have very good sound localization abilities in a tested range of 0.2-20 kHz, especially for
broadband stimuli (Colbert, Gaspard, Reep, Mann, & Bauer, 2009). Chapla, Nowacek, Rommel, & Sadler (2007) proposed that airspaces in the middle air can explain manatees’ sound localization abilities. The manatee’s hearing system also has a relatively high temporal resolution that was tested experimentally by Mann et al. (2005), who found the resolution to be at least 10 times that described for humans and approximately 50% of that found in dolphins.

Despite those anatomical and audiogram studies, the path that sound takes to reach the TPC complex remains unclear. Three distinct models were discussed by Chapla et al. (2007). The first model describes the zygomatic process, which includes low-density oil-saturated bones with the ability to influence the tympanic membrane. The second model simply states that sound enters the skull and reaches the tympanic membrane in a direct way through the fatty tissues in the head. The third model suggests that the lungs and even the skeletal system are involved in delivering some vibrations to the ears.

Manatees have ears similar to those of elephants (Fischer, 1990), and it was recently discovered that elephants have infrasonic communication (Payne, Langbauer, & Thomas, 1986). Manatees’ low frequency hearing acuity was questioned by Gerstein et al. (1999), who stated that manatees would have problems detecting boats at the dominant low frequencies that are produced by boats at the surface (it turned out that manatees are very good at detecting boats; see below). One participant in Gerstein et al.’s (1999) study was able to detect stimuli below 0.4 kHz, but only after some training, so it is possible that this manatee switched from using its hearing system to using vibrotactile detection. However, the same author later argued that manatees have no use for
infrasonic detection because their calls do not have a low frequency component (Gerstein, Gerstein, Forsythe, & Blue, 2004). In contrast, elephants use infrasonic communication and thus benefit from vibrotactile detection (O’Connell-Rodwell et al., 2007).

The manatee’s brain shows certain specializations that indicate the importance of the auditory system (Verhaart, 1972). Compared to the vision-related regions of the brain, which are small (the lateral geniculate nucleus, superior colliculus, and thalamus), massive auditory nuclei are found in the brainstem and the thalamus. The auditory area of the cerebral cortex is enlarged, and hypertrophied areas exist in the somatosensory parts of the brain (Verhaart, 1972).

Boats and Boat Noise

Despite the suggestion by Gerstein et al. (1999) that manatees would have problems detecting boats due to their behavioral audiogram and anatomy, some playback studies have reported otherwise. Boat strikes account for 30% (Reynolds, 1999) of annual manatee mortalities, and that rate has been steadily increasing since 1976, when boat strikes only constituted 11.4% of annual manatee mortalities (Calleson & Frohlich, 2007). Today, 97% of all manatees in Florida have one or more scars from a boat strike (Nowacek et al., 2004). Thus, it is imperative to understand whether manatees hear boats and how manatees behave in a boat’s presence, especially at rest. The earliest study reporting the reaction of manatees to boat noise was performed by Moore (1951). Weigle, Wright, and Huff (1994) performed a pilot playback study in which manatees reacted to boat noise by slowly submerging and moving toward deeper waters. This reaction occurred when the boat was 50-58 meters away from the focal animals. Recreational
boats pose a major threat to manatees (Calleson & Frohlich, 2007), and boat noise frequencies (10-20,000 Hz) fall within manatees’ hearing range.

Manatees should be able to hear boats when the major sound energy is concentrated just below 2 kHz, as the estimated source level of a small boat at one meter is 120-160 dB re 1 μPa (Richardson, Green, Malme, & Thompson, 1995). Recent studies also confirmed that manatees can not only detect boats but also react to boats and even discriminate among various types of vessels (Miksis-Olds, Donaghay, Miller, Tyack, & Reynolds, 2007; Nowacek et al., 2004). Nowacek et al. (2004) showed that manatees respond primarily with a flight response, and 49% of animals showed a reaction that included turning and/or moving to deeper waters and changing the swimming speed. Manatees reacted when the boat was an average of 25-50 meters away, and some even reacted when the boat was 68 meters away.

In a later study, Miksis-Olds et al. (2007) performed a playback study with three major boat sound categories: idle, a planning approach and a personal watercraft (PWC) (jet ski) approach. The playback was initiated when the manatees were feeding or resting, although the authors did not specify responses based on the behavioral state. The manatees responded and attempted to move to deeper waters. However, in at least one study, bottom resting manatees did not show any reaction to boat approaches (Weigle et al., 1994). Manatees also showed very good discrimination abilities and some variability in their responses; only 37% of manatees responded to the idle approach, 63% responded to the planned approach, and 100% responded to the PWC approach. The authors also proposed that the response to PWC playback could be a startle response without any cognitive processing or recognition. Individual differences were also observed. For
example, in Nowacek et al.’s (2004) study, one manatee did not react at distances of up to one meter. These differences could occur because of age, prior history with boats, hearing, activity, and even reproductive state (Nowacek et al., 2004). It is clear that manatees not only are able to detect boats at long distances but also react to them with a clear flight response (increasing swimming speed and moving to deeper waters away from the source).

Rycyk (2013) investigated how Florida manatees react to approaching boats and found that their reactions depended on their position and their behavior pre-exposure; resting manatees were less likely to change their behavior in response to approaching boats than socializing or travelling manatees (Rycyk, 2013). Manatees that were exposed to boat noise in shallows tended to react faster and to move to deeper waters (Rycyk, 2013). Detectable changes in behavior were dependent on sound levels, with louder boat sounds resulting in more detectable changes in behavior.

Statement of the Problem

The lethal effects of boats have been well investigated, and boats are an important cause of manatee mortality in Florida. However, the non-lethal effects of boat noise have not been investigated explicitly. While numerous studies attempted to determine the effect of boat noise on manatee behaviors, no study has investigated how boat noise specifically affected resting behavior in Florida manatees and the impact of boat noise on rest episodes. The auditory stimuli (e.g., boat noise) that the organism finds important in his waking life are more likely to result in awakening than other, non-significant stimuli (Portas, 2005). When an organism sleeps under the risk of predation or in a situation
perceived as dangerous or stressful, the sleep architecture changes: sleep becomes lighter and the organism sleeps less and awakens more often (Lima et al., 2005).

If the species has unihemispheric sleep, this type of sleep becomes more prevalent during rest episodes (compared to bihemispheric sleep), probably because one of the proposed functions of unihemispheric sleep is better vigilance and a lower arousal threshold (Lima et al., 2005). Boat noise might represent a much bigger problem than currently realized. If manatees demonstrate the flight response in their waking lives, as has been documented by Miksis-Olds et al. (2007), Nowacek et al. (2004), and Rycyk (2013), boat noise would not only awaken resting manatees more easily but would also change their sleeping architecture, forcing more USWS sleep, shorter overall rest episodes and less REM sleep, especially if the manatees are stressed and/or perceive their surroundings to be dangerous. Manatees sometimes sleep during the day (previous studies and baseline observations), and boating usually takes place during the day, so boats could affect manatee sleep.

Thus, the goal of the current study was to investigate the effect of certain forms of auditory stimulation (boat noise) on sleeping manatees. To address this question, an auditory playback experiment was used. Manatee sleep was defined behaviorally using a combination of three major components: immobility, prolonged/variable respiration pauses, and staying in the same area. The current study focused on two effects of playback: the immediate reaction to playback and the impact of playback on the overall rest episode. We hypothesized that playback trials with boat noise and positive control noise (i.e., manatee calls) would result in an immediate reaction and interrupt sleep episodes. We also hypothesized that boat noise, but not manatee calls or dummy trials,
would affect manatee sleep parameters, as suggested by research reporting the flight response to boat noise exposure in waking life, including rest episode duration, breathing pattern, changes in behavioral states, time to resume rest, and time spent in the resting area.
CHAPTER II - METHODS

Study Site

Playback experiment trials were conducted at the Lowry Park Zoo's Manatee Rehabilitation Center in Tampa, Florida between September 2010 and January 2013 under Federal Fish and Wildlife Permit #MA226641-0 and in accordance with the University of Southern Mississippi Institutional Animal Care and Use Protocols #09051405 and #11092213. The facility has three medical pools and two large exhibit pools where the playback trials were conducted. The two exhibit pools have an irregular shape, with a larger pool capacity of approximately 130,000 gallons and a smaller pool capacity of 100,000 gallons. Both pools have large underwater viewing windows in the area accessible to the public. The exhibit pools have an irregular bottom with logs, rocks, and platforms and have irregular depth profiles, ranging from 10 to 4 feet in some areas. The facility has special channels and gates that connect all of the exhibit pools and medical pools, and the manatees are often switched between pools for medical procedures, for pool cleaning or for enrichment purposes. The manatees are fed several times a day, and their pools are cleaned daily by a diver. In addition, the manatees are not trained and typically have minimal interactions with zoo staff, as all manatees are eventually released and should not be habituated to close human contact.

Study Subject

Lowry Park Zoo's Manatee Rehabilitation Center is a manatee rescue and rehabilitation facility that receives manatees for rehabilitation, treatment and release. The most common causes of admission are cold stress, boat strikes and injuries, and being an orphan. Animals stay in the facility for various periods of time that range from months to
years, depending on their situation and progress. All manatees are eventually released, and the facility does not have permanent captive residents. The Lowry Park Zoo's Manatee Rehabilitation Center separates males and females to prevent breeding, but manatees can communicate acoustically and interact through gates with members of the opposite sex.

Table 1

*Characteristics of the Study Participants*

<table>
<thead>
<tr>
<th></th>
<th>Teco2</th>
<th>Longo</th>
<th>Epac</th>
<th>Little Joe</th>
<th>CC</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sex</strong></td>
<td>male</td>
<td>male</td>
<td>male</td>
<td>male</td>
<td>male</td>
</tr>
<tr>
<td><strong>Age at Release</strong></td>
<td>2 y.o., juvenile</td>
<td>~ 2 y.o., juvenile</td>
<td>~2 y.o., juvenile</td>
<td>20+ y.o., adult</td>
<td>5+ y.o., young adult</td>
</tr>
<tr>
<td><strong>Condition at admission</strong></td>
<td>Orphan</td>
<td>Cold stress</td>
<td>Cold stress</td>
<td>Orphan</td>
<td>Cold stress</td>
</tr>
<tr>
<td><strong>Weight at admission</strong></td>
<td>271 lbs</td>
<td>271 lbs</td>
<td>385 lbs</td>
<td>42 lbs</td>
<td>271 lbs</td>
</tr>
<tr>
<td><strong>Length at admission</strong></td>
<td>190 cm</td>
<td>183 cm</td>
<td>213 cm</td>
<td>104 cm</td>
<td>183 cm</td>
</tr>
<tr>
<td><strong>Weight at release</strong></td>
<td>665 lbs</td>
<td>790 lbs</td>
<td>820 lbs</td>
<td>1649 lbs</td>
<td>790 lbs</td>
</tr>
<tr>
<td><strong>Length at release</strong></td>
<td>232 cm</td>
<td>252 cm</td>
<td>254 cm</td>
<td>332 cm</td>
<td>252 cm</td>
</tr>
</tbody>
</table>
All animals used in the study were males, for the facility tends to receive more males than females, although also because both sexes are housed separately to avoid breeding. Each animal was subject to three playback trials—boat noise, manatee call, and dummy trial—at a rate of one trial per day. The order of trials and type of stimuli were both determined randomly. One animal, Longo, was subject to only two playback trials (i.e., manatee call and boat noise) and was therefore excluded from analysis; descriptive data from his trials are nevertheless reported in the Results section. The study subject characteristics are summarized in Table 1.

Playback Stimuli

The stimuli used for playback included experimental stimuli (boat noise), positive control stimuli (manatee calls) and dummy stimuli (a file with 20 seconds of silence). Within each category, 4 auditory recordings were prepared to avoid pseudoreplication (Deecke, 2006; McGregor et al., 1992; McGregor, 2000). For example, the experimental category included four playback stimuli: B1, B2, etc. The same procedure was used for the control stimuli (M1, M2, etc.). Each animal was played one control, one experimental, and one dummy stimulus, which were assigned randomly. The playback file contained 15 seconds of actual sound preceded by five seconds of silence and one second of fade in and followed by one second of fade out and five seconds of silence. Thus, the whole file was 27 seconds long (see Figures 1 and 2). The files were prepared in Adobe Audition using 700 Hz HP and 14,000 LP filters.
Figure 1. Spectrogram and waveform of an example positive control stimulus for playback (manatee calls).

Figure 2. Spectrogram and waveform of an example experimental stimulus for playback (boat noise).

Boat Sound Stimuli

The experimental stimuli were actual boat noise recordings that were recorded from approaching boats in Tampa Bay where Florida manatees are often found. The
stimuli were recorded using an omnidirectional hydrophone Reson TC4033 with a sensitivity of $-203\,\text{dB re}\,1\,\text{V/μPa}$, a bandpass filter of 0.5-100 kHz with 50 dB gain and a DAQ 12-bit analog-to-digital card (M series 6062) with a Toshiba laptop. In previous studies, manatees have shown strong behavioral responses (avoidance, swimming away, etc.) to boat noise playback (Miksis-Olds et al., 2007; Nowacek et al., 2004). Boat sounds were selected as experimental stimuli because a) manatees react to boat noise while awake and change their behavior; b) boat noise is expected to increase in manatee habitats; and c) we do not know the effects, especially non-lethal effects, of boat noise on manatee sleep (for example, disruption).

**Manatee Sound Stimuli**

Manatee sound stimuli were recorded at the Lowry Park Zoo Manatee Rehabilitation Center in Tampa, Florida using a Wilcoxon pre-amplified hydrophone with a sensitivity of $-188\,\text{dB re}\,1\,\text{V/μPa}$, a bandpass filter of 0.5-20 kHz with 50 dB gain and a SONY DAT digital audio recorder. Because the rehabilitation center typically receives more males than females, the positive control stimuli were recorded from all female groups. Positive control stimuli were selected based on the recommendations outlined by Deecke (2006) not only because such stimuli are capable of evoking responses but also because it was unknown whether boat noise would interrupt rest. At the Lowry Park Zoo Hospital, males and females are often housed separately. Naturally, both sexes are very interested in each other during shifting procedures, especially when a new manatee is introduced into the pool. Manatees appear to be aware of which animals are in the pool with them at all times, and the addition of any new manatee always elicits visible interest and an exploratory approach.
In addition, to make the positive control stimuli even more noticeable, I used recordings from the baseline observations in which the manatees were restless and agitated and increased their calling rates. Each positive control stimulus file contained between seven and ten manatees’ calls and was prepared in the same way as the experimental stimuli.

**Stimulus source levels and received levels measurement test**

To determine the actual loudness of the playback stimuli, a test was performed to measure both the source level and the received level. Sound measurements were made at one of the Lowry Park Zoo rehabilitation pools at 1:30 pm ET on May 29, 2012, with a water temperature of 82.6°F. An underwater speaker was placed at a depth of 4.5 feet, and the receiving hydrophone was first placed at 1 meter (for the source level measurement) and then placed 11 feet away from the speaker at a depth of 5 feet (for the received level measurement). Sound was recorded using a calibrated recording system and a calibrated hydrophone. The M Audio Microtrack II, s/n 7261, recording system was calibrated in 2011 and showed the following response: Flat (+/- < 3 dB) 100 Hz - 45 kHz. The Hydrophone HTI 96-MIN was also calibrated in 2011 and showed the following response: Flat (+/- 3 dB) up to 30 kHz. For the source level measurements, sounds were played at 1 meter, with the gain set at 0. For the received level, sounds were played 11 feet from the receiver with a gain setting of 1. The audio files were examined, and the loudest parts of the files were cut (duration was between 0.5 and 1 second), including only the loudest bandwidth of the sound. The measured RMS voltage was then converted to dB re 1 μPa (Table 2). This was done to measure the source level (loudness) of the playback files.
Table 2

*Playback Stimuli and Their Characteristics*

<table>
<thead>
<tr>
<th>Type</th>
<th>Code</th>
<th>Duration, sec</th>
<th>SL, dB re 1 µPa</th>
<th>RL, dB re 1 µPa</th>
<th>SSSO, sec</th>
<th>SSLBPPB, sec</th>
<th>Subject's Name</th>
</tr>
</thead>
<tbody>
<tr>
<td>Manatee</td>
<td>M1</td>
<td>15</td>
<td>119.37</td>
<td>97.57</td>
<td>1270</td>
<td>171</td>
<td>CC</td>
</tr>
<tr>
<td>Manatee</td>
<td>M2</td>
<td>15</td>
<td>114.93</td>
<td>89.79</td>
<td>1960</td>
<td>175</td>
<td>Epac</td>
</tr>
<tr>
<td>Manatee</td>
<td>M3</td>
<td>15</td>
<td>113.34</td>
<td>87.52</td>
<td>1402</td>
<td>140</td>
<td>Teco2</td>
</tr>
<tr>
<td>Manatee</td>
<td>M4</td>
<td>15</td>
<td>106</td>
<td>87.21</td>
<td>1564</td>
<td>90</td>
<td>Little Joe</td>
</tr>
<tr>
<td>Boat</td>
<td>B1</td>
<td>15</td>
<td>116.27</td>
<td>89</td>
<td>1035</td>
<td>160</td>
<td>CC</td>
</tr>
<tr>
<td>Boat</td>
<td>B2</td>
<td>15</td>
<td>113.34</td>
<td>88.72</td>
<td>1476</td>
<td>185</td>
<td>Teco2</td>
</tr>
<tr>
<td>Boat</td>
<td>B3</td>
<td>15</td>
<td>113.91</td>
<td>85.55</td>
<td>1663</td>
<td>153</td>
<td>Epac</td>
</tr>
<tr>
<td>Boat</td>
<td>B4</td>
<td>15</td>
<td>114.93</td>
<td>96.89</td>
<td>1130</td>
<td>196</td>
<td>Little Joe</td>
</tr>
<tr>
<td>Dummy</td>
<td>S1</td>
<td>15</td>
<td>0</td>
<td>0</td>
<td>1942</td>
<td>190</td>
<td>Epac</td>
</tr>
<tr>
<td>Dummy</td>
<td>S2</td>
<td>15</td>
<td>0</td>
<td>0</td>
<td>1330</td>
<td>150</td>
<td>CC</td>
</tr>
<tr>
<td>Dummy</td>
<td>S3</td>
<td>15</td>
<td>0</td>
<td>0</td>
<td>1313</td>
<td>162</td>
<td>Little Joe</td>
</tr>
<tr>
<td>Dummy</td>
<td>S4</td>
<td>15</td>
<td>0</td>
<td>0</td>
<td>285</td>
<td>191</td>
<td>Teco2</td>
</tr>
<tr>
<td>Manatee</td>
<td>M5</td>
<td>15</td>
<td>107.88</td>
<td>87.67</td>
<td>1360</td>
<td>134</td>
<td>Longo</td>
</tr>
<tr>
<td>Boat</td>
<td>B5</td>
<td>15</td>
<td>116.67</td>
<td>87.13</td>
<td>1320</td>
<td>145</td>
<td>Longo</td>
</tr>
</tbody>
</table>

Note: SSSO is the abbreviation for Seconds Since Sleep Onset (playback introduced); SSLBPPB is the abbreviation for Seconds Since Last Breath Prior to Playback Introduced.
Playback Experimental Design and Data Analysis

Playback Equipment

The playback experiment was performed using a Lubell UW-30 Underwater speaker system, a frequency response of 100 Hz-10 kHz, and maximum output levels of 153 dB at 150 Hz, 30 watts, and 8 ohm. The underwater speaker was connected to a PAT-20TB amplifier with an additional output power of 20 watts (8 ohm). The stimuli were played via a Trio V218 mp3 audio player, and a portable powerpack battery supplied power for the whole playback system. A pre-amplified hydrophone (-188 dB re 1 V/μPa) and a custom-made bandpass filter (500 Hz-20 kHz), and pre-amplifier (50 dB) were used for underwater sound monitoring. The hydrophone was connected to a SONY-DAT digital audio recorder. Video recordings were made using a Panasonic PV-GS39 camcorder, a FUJI AV180 digital camera, and a Vivitar DVR 410 digital camera.

Playback Experiment Design

The playback trials took place between 1:30 pm and 4:14 pm because this is typically a time when the manatees tend to rest as no pool cleaning is done during this time; the manatees are not fed during this time, and limited zoo staff are present. The type of stimuli were selected randomly one day prior to the trial (as described above). To facilitate a successful playback trial, several conditions had to be satisfied:

1. The manatee should be completely immobile between respiratory pauses and should stay in the same spot for at least 15-20 minutes.

2. If the manatee moves during respiration, the movement should not exceed a distance of one body length.
3. For 15-20 minutes prior to the playback trial, respiration should have a ventilation breathing pattern or a prolonged breathing pattern.

The speaker was slowly lowered into the pool a few minutes prior to a playback trial at the depth of approximately one foot and was removed after playback. The reason for the shallow deployment of the speaker was to mimic the source of boat noise which is normally emitted near the surface of the water. The playback was initiated when between 90 and 196 seconds had passed since the manatee took a breath and when the manatee was between 285 and 1960 seconds into a rest episode. Three video cameras placed at various angles recorded the behavioral reaction to the playback and the post-playback behaviors.

*Manatee Response Measurement and Data Analysis*

The manatees’ reactions to the playback were coded in several ways. With respect to sleep interruption, the manatees’ reactions were coded 1 for sleep interrupted and 2 for sleep not interrupted. Sleep interruption was defined as a manatee’s ceasing to stay immobile, changing position, and starting to move. The behavioral responses were scored 1 for move away from the speaker, 2 for no reaction, and 3 for move towards the speaker. The latency of response (time in seconds between the initiation of playback and the start of movement) was also recorded.

Several variables were used to determine the effect of playback on sleep behavior. These variables included: the number of minutes milling (see Appendix B for definitions of behavioral states) within 3 minutes post-playback, the number of minutes sleeping within 20 minutes post-playback, the time in seconds between playback stimulus exposure and the next breath, the overall rest episode duration, time to immobility,
changes in behavioral states within 20-min post-playback, and time in seconds spent in the resting area post-playback; for detailed definitions of variables, see Appendix C.

To determine how the rest episode was affected by the playback, several pre-post variables were used: pre-post playback average respirations, pre-post playback index of respiration variability and pre-post playback vocalizations per minute (Appendix C). Behavioral observations included continuous behavioral sampling of the focal animal with simultaneous video recordings of behaviors and acoustical recordings of vocalizations (behavioral states are defined in Appendix B).

Data Analysis

The data were analyzed using Friedman’s test to test for differences with respect to stimulus categories, and latency of response. Pre-post playback differences were analyzed using the Wilcoxon Signed Ranks test. All tests had predetermined p=0.05 significance levels and were two-tailed. All statistical analyses were performed using the SPSS Statistics 17 software.
CHAPTER III - RESULTS

Reaction to the Playback

A total of five animals were tested, though one was excluded from analysis because he was not subject to a dummy playback trial. Descriptive data from his two trials are nevertheless reported below. During all trials, manatees were bottom resting. The four remaining subjects interrupted their sleep in 7 out of 8 playback trials, and none of the subjects interrupted their sleep during the dummy trials. The sleep interruption scores (sleep interrupted, sleep not interrupted) differed significantly based on the type of playback stimulus (Friedman test, $k = 3, n = 4, \chi^2 = 6.5, p = 0.039$) (Figure 3). Post-hoc analysis indicated significant differences between dummy trials and boat noise trials (Wilcoxon signed-rank test, $n = 4, Z=-2.0, p = 0.046$).

The post-hoc analysis employed a Least Significant Difference comparison, and no Bonferroni correction was used due to the small sample size. The differences observed between the manatee call trials and the dummy trials and between manatee call trials and the boat noise trials were not significant. The behavioral response scores (move away from the speaker, no reaction, move towards the speaker) differed significantly depending on the type of playback stimulus (Friedman test, $k = 3, n = 4, \chi^2 = 7.6, p = 0.022$) (Figure 4). Post-hoc analysis indicated a significant difference in behavior responses between the dummy trials and the boat noise trials (Wilcoxon signed-rank test, $n = 4, Z=-2.0, p = 0.046$) and a marginally significant difference (due to high variability in responses) in behavioral responses between the manatee call playback trials and the boat noise trials (Wilcoxon signed-rank test, $n = 4, Z=-1.890, p = 0.059$). The post-hoc analysis employed a Least Significant
Difference comparison, and no Bonferroni correction was used due to the small sample size.

Figure 3. Sleep interruption in response to playback stimuli.

Figure 4. Behavioral reaction to playback stimuli according to stimulus category.
The latency of the response to boat noise playback stimuli was slightly shorter \((M=9.25, SD=2.06, n=4)\) than the latency of the response to manatee call playback stimuli \((M=12.6, SD=2.88, n=3)\), but this difference was not statistically significant (Wilcoxon signed-rank test, \(n=3, Z=-1.604, p=0.109\)).

Effect on Sleep Behavior and Sleep Episodes

Although sleep was interrupted, short-term (minutes milling within 3 minutes post-playback, Friedman test, \(k=3, n=4, \chi^2 = 5.2, p = 0.074\)) and long-term (minutes sleeping within 20 minutes post-playback, Friedman test, \(k=3, n=4, \chi^2 = 3.5, p = 0.174\)) behavioral states did not differ significantly for boat noise, manatee calls and dummy trials (even though individual variations in post-playback behavior were observed) (Figure 5). As Figure 5 demonstrates, great variability in responses affected the significance of the results.

![Figure 5. Number of minutes of milling behavior during 3 minutes post-playback](image-url)
Although the manatees interrupted their sleep in response to playback stimuli and continued to rest during dummy trials, this sleep interruption did not appear to significantly affect the manatees’ overall sleep behavior. Immediately following the playback, the manatees did not rush to take a breath, and the time between playback stimulus exposure and the next breath did not differ significantly for dummy, manatee call and boat noise trials (Friedman test, $k = 3$, $n = 4$, $\chi^2 = 0.5$, $p = 0.779$). Time to immobility did not differ significantly for the dummy, manatee call, or boat noise trials (Friedman test, $k = 3$, $n = 4$, $\chi^2 = 5.2$, $p = 0.074$). Similarly, the overall rest episode duration did not differ significantly depending on the type of playback stimulus (Friedman test, $k = 3$, $n = 4$, $\chi^2 = 0.133$, $p = 0.936$).

The results showed that manatees spent the shortest amount of time (in seconds) in the resting area post-exposure to boat noise playback stimuli ($M=84.5$, $SD=150.34$, $n=4$) in comparison to post-exposure to manatee calls ($M=583$, $SD=1104$, $n=4$) or to silence during the dummy trials ($M=1066$, $SD=101$, $n=4$), but this difference was not statistically significant (Friedman test, $k = 3$, $n = 4$, $\chi^2 = 3.5$, $p = 0.174$) (Figure 6). Lastly, the number of behavioral states that changed within 20 min post-playback did not differ significantly for the dummy, manatee call, or boat noise trials, as a Wilcoxon signed-rank test indicated ($n = 4$, $Z=-0.552$, $p = .581$). Table 3 summarizes data for all variables discussed above.
To investigate the effects of playback within a sleep episode, three variables were tested: pre-post playback average respirations, pre-post playback index of respiration variability, and pre-post playback vocalizations per minute. None of these variables differed significantly for any of the playback stimuli (Table 3). A detailed graphic representation of the playback trials is included in Figure 6 and Appendix A.

*Figure 6.* Number of seconds spent in the resting area within 20 minutes post-playback.
Table 3

*Summary of playback data variables*

<table>
<thead>
<tr>
<th>Variable</th>
<th>Boat Noise</th>
<th></th>
<th></th>
<th>Manatee Call</th>
<th></th>
<th></th>
<th>Dummy Trial</th>
<th></th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
<td>Min/Max</td>
<td>Mean</td>
<td>SD</td>
<td>Min/max</td>
<td>Mean</td>
<td>SD</td>
<td>Min/max</td>
</tr>
<tr>
<td><strong>Reaction</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Type of reaction*</td>
<td>1</td>
<td>0</td>
<td>1/1</td>
<td>2.75</td>
<td>0.5</td>
<td>2/3</td>
<td>2</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Latency, sec</td>
<td>9.25</td>
<td>2.06</td>
<td>7/12</td>
<td>12.66</td>
<td>2.88</td>
<td>11/16</td>
<td>n/a</td>
<td>n/a</td>
<td>n/a</td>
</tr>
<tr>
<td>Milling 3 minutes post-PB, min</td>
<td>1.75</td>
<td>0.95</td>
<td>1/3</td>
<td>2.00</td>
<td>1.41</td>
<td>0/3</td>
<td>0</td>
<td>0</td>
<td>0/0</td>
</tr>
<tr>
<td>Seconds from playback to breath, sec</td>
<td>108.25</td>
<td>64.50</td>
<td>28/177</td>
<td>137.75</td>
<td>46.29</td>
<td>103/204</td>
<td>147.25</td>
<td>145.78</td>
<td>11/347</td>
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<td><strong>Effect on Sleep Behavior</strong></td>
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<tr>
<td>Time spent in resting area after playback</td>
<td>84.5</td>
<td>150.34</td>
<td>7/310</td>
<td>583.25</td>
<td>1104.77</td>
<td>11/2240</td>
<td>1066</td>
<td>101.9</td>
<td>960/1200</td>
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<td></td>
<td>220.5</td>
<td>132.22</td>
<td>135/417</td>
<td>459.25</td>
<td>547.18</td>
<td>0/1250</td>
<td>n/a</td>
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<tr>
<td>Time to immobility, sec</td>
<td>5.5</td>
<td>3.31</td>
<td>2/10</td>
<td>3.25</td>
<td>2.87</td>
<td>0/7</td>
<td>2.5</td>
<td>2.51</td>
<td>0/6</td>
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<td>Changes in behavioral</td>
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<td>states within 20 minutes</td>
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<td>post-playback</td>
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<tr>
<td>Minutes sleeping within</td>
<td>11.5</td>
<td>7.72</td>
<td>3/19</td>
<td>8.25</td>
<td>8.88</td>
<td>0/20</td>
<td>16.5</td>
<td>3.41</td>
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Note: p<0.05 significance is marked with *. 
Figure 7. Changes in behavioral states in response to playback
Figure 8. Changes in respirations in response to playback
CHAPTER IV – DISCUSSION

In the current study, manatees interrupted their sleep in response to both boat noise and manatee calls and continued to rest during the dummy trials. Manatees also demonstrated differential responses to the playback stimuli, with boat noise eliciting movement away from the speaker and manatee call playback eliciting movement towards the speaker. However, this sleep interruption did not appear to affect sleeping behavior and sleeping episodes in a way that produced statistically significant differences, despite the fact that manatees showed individual variations in their post-playback behaviors. None of the playback stimuli elicited statistically significant differences in behaviors immediately after playback or within 20 minutes after the playback. Sleep episode duration and changes in respirations did not differ significantly among the playback stimuli types.

Even within the rest episode, pre-playback and post-playback parameters, such as average respirations, index of respiration variability or average vocalizations per minute, did not differ significantly for any type of playback stimulus played. In sum, the research hypothesis was only partially supported. Although manatees interrupted their rest and reacted differently depending on the playback stimulus, none of the playback stimuli had a statistically significant effect on sleeping behavior in comparison to the variations caused by chance alone.

In general, the lack of response differences among the playback stimuli in the playback experiment could be attributed to three main factors (McGregor, 2000). First, the selected response measures might not be sensitive enough to detect subtle changes in
responses. However, the current study utilized numerous measures that had the potential to detect changes in sleep behaviors that included activity states, respirations, variability in respirations, staying in the resting area, and vocalization rates. Additionally, the pre- and post-playback analysis also had the potential to detect changes within sleep episodes that were elicited by the playback. In addition, it could be argued that the manatees did react to stimuli and did interrupt their sleep, and these reactions were due to the stimuli and not the noise the equipment made, as the animals did not react to the silent dummy trials performed using the same equipment.

A second explanation could be that the manatees did not perceive the differences between the boat noise stimuli and the manatee call stimuli (McGregor, 2000). This possibility could be rejected because the manatees showed different reactions to the playback stimuli, indicating that they did indeed perceive the differences between the playback stimuli, as the manatee calls elicited approach behaviors (or no reaction), while all boat noise trials elicited movement away from the speaker.

The third possibility is that we observed no differences in rest behaviors due to the "floor effect," whereby a threshold for eliciting changes in rest behaviors was not achieved (McGregor, 2000; Rosenblatt, Beer, Busnel, & Slater, 1985). This explanation warrants some consideration, given that the sleep behavior parameters did not differ for the manatee call, boat noise and silent dummy trials. Additionally, the playback stimulus intensities were relatively low (between 85 and 97 dB re 1 μPa); although the stimuli were loud enough to interrupt sleep, they were either not loud enough or long enough to significantly affect sleep behavior.
Only three manatees interrupted their sleep in response to manatee calls, and this response did not differ significantly from the lack of response seen in the dummy trials. Because the playback stimuli included female manatee calls, it is possible that the calls will only attract the attention of males. Manatees reach sexual maturity between 3 and 5 years of age (Hartman, 1979), and the animal that did not interrupt his sleep in response to the manatee calls was a juvenile. Another possibility is that the three manatees that responded to the manatee calls actually recognized these calls because they were housed with these females at some point and could definitely hear them vocalizing. Hartman (1979) argued that manatees are very vocal during sexual courtship and play behavior and proposed that manatees could recognize vocalizations based on individual characteristics. The animal that did not respond arrived at the Lowry Park Zoo later. That manatee was not housed with female manatees and did not interact with them through the gate like the three other subjects; thus, that manatee might not have heard the calls of the females. The manatee calls elicited approach behavior from three manatees, indicating that those manatees did not perceive the stimulus as threatening and moved closer to investigate the source of the sound.

All four manatees interrupted their sleep in response to boat noise, and this response differed significantly from the dummy trials, in which the animals continued to sleep. These results indicate that boat noise could interrupt sleep at even very low received levels between 85 and 97 dB re 1 μPa. Previous studies found that manatees reacted to boat noise at various levels: 150 dB in a study performed by Miksis-Olds et al. (2007) and 118 dB re 1 μPa in a study performed by Rycyk (2013). The current study
results indicated that boat noise could be disruptive even at very low levels and short durations (15 seconds of sound). Previous studies indicated that manatees can indeed detect distant boat sounds; distances of up to 1 km away were mentioned by Nowacek (2004). However, manatees start showing an actual reaction and behavioral changes when the boat is 25-50 m away (Miksis-Olds et al., 2007). This observation indirectly supports the previously discussed notion that the playback sounds must pass some sort of intensity threshold for the manatees to start showing detectable behavioral reactions and changes in sleep behaviors.

It is possible that the boat noise and manatee call stimuli interrupted sleep simply because of their novelty, as novel sounds were introduced in an otherwise relatively quiet pool. This explanation is unlikely because of the differential reactions to manatee calls and boat noise, which indicated that the manatees perceived these stimuli differently. In addition, although manatees are rarely housed alone and are routinely exposed to other manatees’ calls while resting, the animals do not interrupt their rest in response to those calls. Because research shows that the discrimination of meaningful stimuli from other stimuli and ambient noise also takes place in the sleeping brain (Velluti, 2008), it could be argued that all four manatees perceived the boat noise stimulus as meaningful enough to warrant awakening, and at least 3 manatees also perceived the manatee calls as meaningful enough to warrant awakening.

This finding is especially remarkable because the playback stimuli were played at relatively low sound levels. While awakening tends to occur when auditory stimuli are intense (Haynes et al, 1985), it was also documented that significant and meaningful
stimuli will evoke awakening at lower sound levels (LeVere et al., 1976). For example, whispering one’s name will awaken a person because one's name is a meaningful and significant auditory stimulus (Portas, 2005).

The manatee calls were selected as a positive stimulus with the assumption that calls of opposite gender manatees that are not housed with the study subjects would elicit responses. It could be argued that in the context of auditory stimulation during rest, boat noise elicits reactions that are similar to those elicited by biologically significant and meaningful calls (opposite sex calls) (i.e., arousal and short-term sleep interruption). The fact that one manatee did not react when exposed to manatee calls but all manatees interrupted their sleep in response to boat noise indicates that boat noise could be an even more potent auditory stimulus that affects sleep even at low sound levels and short sound durations.

Sleep behavior is important and is seen in all aquatic mammals, so sleep interruptions could be costly for a manatee, as manatees use daytime sleep to "conserve their energetic demands" (Horikoshi-Beckett & Schulte, 2006, p. 297). Thus, manatees might attempt to minimize rest interruptions. This theory could explain why even though rest was interrupted, the overall sleep behavior and rest episode were unaffected. If a manatee awakens to assess the situation, because the boat noise only played for 15 seconds, the animal could go back to sleep because the stimulus was no longer present. Even though milling with three minutes post-playback did not differ significantly depending on the playback stimuli, the data showed that some manatees milled between 1.75 and 2 minutes post-playback and did not go back to sleep immediately (see
Appendix A for individual responses). Similarly, it is possible that short sleep interruptions are not as detrimental for manatees as they are for humans because manatees have to wake up frequently to breathe. In contrast, humans and other animals do not hold their breath while asleep in the same way as manatees, and their sleep interruptions are more detrimental (Cirelli, 2006; Quan, 2009). Mukhametov et al. (1992) performed the only physiological sleep study on manatees and found that electrocorticogram indicated that manatees have a short arousal from sleep when taking a breath and then exhibit a "rapid return to the interrupted stage of sleep" (Mukhametov et al., 1992, p. 418). Hence, due to the very nature of manatee sleep, short sleep interruptions might not be very detrimental, unless the manatee does not feel safe and has to move to a different area.

At the same time, recent research shows that at least in humans, napping could be very beneficial and could in fact mitigate or at least lessen the effect of poor quality sleep at night, as naps aid in the recovery of both the immune and neuroendocrine systems (Faraut et al., 2015). Manatee sleep patterns have been thought to be polyphasic, with some activities, such as feeding, present at night (Hartman, 1979). Another study by Kikuchi et al. (2011) found no difference in inactivity patterns during the day versus during the night, indicating that manatees were active both during the day and at night. A newer study argued that manatees showed a pronounced circadian pattern of rest at night and activity during the day. This study was performed with captive manatees who might have adapted to captive life and a feeding schedule (Holguin-Medina et al., 2015). Nonetheless, day time sleep in manatees could be important not only because it is needed
to conserve energy but also because it could help manatees to recover from poor night time sleep when they have to feed or travel at night.

The current study did not collect any physiological data concerning sleep behavior and is unable to make conclusions about whether the quality of sleep or the sleep architecture could be affected by the playback. For example, Lima et al. (2005) described how birds (who, like manatees, have unihemispheric sleep) tend to change their sleep architecture and engage in a lighter state of sleep if they perceive the situation as threatening. When the risk of predation was high, birds also spent more time in unihemispheric sleep and changed the time of the day when they slept. Lima et al. (2005) noted that unihemispheric sleep is typically considered to be a high vigilance state in which the arousal threshold is lowered in comparison to REM or SWS sleep. Birds also showed variations in sleep site selection because safety was very important during sleep. The manatees in the current study also showed some changes in resting area preferences post-playback, even though the differences were not statistically significant. The current study could not determine the amount of unihemispheric sleep that the manatees had pre- or post-playback.

Even though the current study did not find a significant difference in how much time manatees spent in their resting areas post-playback, the raw data showed that the manatees spent less time in their resting areas post-boat noise playback ($M=84.5$, $SD=150.34$, $n=4$) compared to post-exposure to manatee calls ($M=583$, $SD=1104$, $n=4$) or to silence during dummy trials ($M=1060$, $SD=101$, $n=4$). The current study used a small sample and nonparametric tests that are generally less powerful for detecting meaningful
differences; this design could explain why the observed differences were not large enough to be significant. This topic could be an area of investigation for future studies. Horikoshi-Beckett and Schulte (2006) noted that manatees select certain areas for resting and tend to use these areas repeatedly. Hence, displacement could be costly and could interrupt the manatees’ natural movements because manatees tend to select resting and feeding areas and then use the same routes to move between these areas for an extended period of time until they no longer have enough to feed on or find another, more suitable area (Horikoshi-Beckett & Schulte, 2006).

One important question is how manatees perceive boat noise in terms of its threat level. The current study results show that the manatees tried to move away from the sound source (as opposed to moving towards the speaker in the manatee calls trials). Previous studies showed similar tendencies, with manatees typically trying to swim to deeper waters when exposed to boat noise (Miksis-Olds et al., 2007; Reep & Bonde, 2006), changing swimming speed (Nowacek, 2004) and showing differential reactions depending on their position (Rycyk, 2013). Manatees exposed to boat noise in shallows tended to react faster and to move to deeper waters (Rycyk, 2013). Interestingly, Rycyk (2013) reported that resting manatees were less likely to change their behavior in response to an approaching boat in comparison to socializing or travelling manatees. Rycyk (2013) also reported that detectable changes in behavior were dependent on sound levels, with louder boat sounds resulting in more detectable changes in behavior. In the current study, there was no significant difference in the latency of response to playback
stimuli; in other words, the manatees did not react faster in response to boat noise than in response to manatee calls, although there were some notable individual differences.

Study Limitations and Future Directions

Several limitations of this study must be considered. The sample size was very small, and nonparametric tests might not have been powerful enough to detect significant differences in such a small sample. Alternatively, small samples must demonstrate very large differences between categories to yield statistically significant results. Two variables showed differences between playback stimuli categories (minutes milling within 3 minutes post-playback and the amount of time (in seconds) spent in the resting area post-exposure to playback stimuli), but these differences were not large enough to be statistically significant. Future studies could investigate these two variables in a larger sample to determine whether statistically significant differences could be detected.

Although the participants were selected randomly from the available manatees, all of the subjects ended up being males. Females could have an entirely different response to playback stimuli due to gender differences or even reproductive status; for example, pregnant females, nursing females, or females with non-nursing but not yet independent calves might be more sensitive to boat noise sounds due to their vulnerability. Future studies could include females in the sample to determine whether such a design would produce different results.

One of the findings of this study was that the manatees interrupted their sleep at very low sound levels, but the overall effect on sleep was not significant. Future research could determine what levels of boat sound will not only interrupt sleep but also result in
significant alterations of sleeping/resting behaviors. Similarly, sounds of longer duration, with continuous or intermittent playback stimuli durations, could also be studied to determine the sound duration at which manatees start to manifest significant changes in sleep behaviors.

Finally, physiological studies could investigate the effects of sleep interruption on the quality of sleep and sleep architecture. It is possible that sleep interruptions could result in an increase in unihemispheric sleep, as observed in birds. Unihemispheric sleep aids in faster awakening under conditions of perceived danger (Rattenborg et al., 2000), but in the current study, although the manatees had a slightly shorter awakening latency in response to boat noise, that difference was not statistically significant.

**Conclusion**

The current study demonstrated that manatees can interrupt their rest in response to boat noise and can do so at even very low noise levels and short noise durations. Even though manatee calls also resulted in sleep interruption, the reaction to the playback stimuli differed. The manatees’ overall rest behavior and rest episodes were not affected differently by the different types of playback stimuli, indicating that at least at these low sound levels and short sound durations, sleep interruptions do not necessarily have significant effects on overall rest behavior or duration.
Figure A1. Teco: Manatee Call, M3

Number of manatees in the pool: 3
Figure A2. CC: Manatee call, M1

Number of manatees in the pool: 3.
Figure A3. CC, Boat noise, S1

Number of manatees in the pool: 3
Figure A4. Little Joe, Boat noise, B7

Number of manatees in the pool: 3
Figure A5. Teco, Boat noise, B4.

Number of manatees in the pool: 3
Figure A6. Little Joe, Manatee call, M4

Number of manatees in the pool: 3
Figure A7. Teco, Silence

Number of manatees in the pool: 3
Figure A8. Little Joe, Silence

Number of manatees in the pool: 3
Figure A9. CC, Silence

Number of manatees in the pool: 3
Figure A10. Epac, Manatee call, M2

Number of manatees in the pool: 3.
Figure A11. Epac, Boat noise, S5

Number of manatees in the pool: 3.
Figure A12. Epac, Silence

Number of manatees in the pool: 3.
Figure A13. Longo, Boat noise, S3.

Number of manatees in the pool: 3.
Figure A14. Longo, Manatee call, M6.

Number of manatees in the pool: 4.
APPENDIX B - Definitions and Results

Table A1.

*Definition of behavioral states.*

<table>
<thead>
<tr>
<th>State</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mill</td>
<td>Focal animal moves in various directions, no physical contact with any other manatees</td>
</tr>
<tr>
<td>Travel</td>
<td>Focal animal moving steadily in one direction, often in a big circle</td>
</tr>
<tr>
<td>Social</td>
<td>Focal animal is engaged in physical contact with other manatees, including touching, hugging, nuzzling, breathing in unison</td>
</tr>
<tr>
<td>Surface feeding</td>
<td>Focal manatee consumes food that floats on the surface of the pool</td>
</tr>
<tr>
<td>Bottom feeding</td>
<td>Focal manatee consumes food that is offered in special feeding tubes that placed on the bottom, or consumes any matter from the bottom of the pool</td>
</tr>
<tr>
<td>Quiet/wakefulness/relaxed</td>
<td>One or more behavioral criteria for sleep is violated, i.e. respirations are not that prolonged; moves more than one body length, not completely immobile between respirations.</td>
</tr>
<tr>
<td>Sleep</td>
<td>1. Complete immobility during respirations interrupted only by movements to get air.</td>
</tr>
</tbody>
</table>
2. Remaining in the same area over the duration of whole rest episode/ changing position no more than one body length.

3. If present, loss of body tone (in some animals).

4. The presence of ventilation breathing or prolonged respirations.

5. Reduced vocalization rates

Note. Behavioral states were coded based on each animal’s predominant state per minute
Table A2.

Definitions of variables.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Definition</th>
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<tbody>
<tr>
<td>Seconds from sleep to playback initiation</td>
<td>The interval between the onset of sleep and initiation of the playback sequence, measured in seconds</td>
</tr>
<tr>
<td>Seconds from last breath to playback initiation</td>
<td>The interval between the most recent breath and initiation of the playback sequence, measured in seconds</td>
</tr>
<tr>
<td>Seconds from playback to breath</td>
<td>The interval between the initiation of the playback sequence and the manatee’s next breath, measured in seconds</td>
</tr>
<tr>
<td>Interrupted sleep</td>
<td>Categorical variable, coded 1 for interrupted sleep and 2 for uninterrupted sleep</td>
</tr>
<tr>
<td>Latency of reaction</td>
<td>The interval between the initiation of the playback sequence and any visible movement, measured in seconds</td>
</tr>
<tr>
<td>Type of reaction</td>
<td>Categorical variable, coded 1 for moved away from the speaker, 2 for no reaction, and 3 for moved toward the speaker</td>
</tr>
<tr>
<td>Overall rest episode</td>
<td>The interval between the onset and end of sleep, often extending beyond 20 minutes post-playback, measured in minutes</td>
</tr>
<tr>
<td>----------------------</td>
<td>--------------------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Average pre-playback respiration</td>
<td>Mean of respiration pauses pre-playback, measured in seconds</td>
</tr>
<tr>
<td>Average post-playback respiration</td>
<td>Mean of respiration pauses post-playback, measured in seconds</td>
</tr>
<tr>
<td>Index of pre-playback ventilation variability</td>
<td>Calculated based on the method of Miksis–Olds et al. (2007, p.640), measured in seconds and calculated for pre-playback respiratory pauses; the greater the value, the greater the manatee’s ventilation variability</td>
</tr>
<tr>
<td>Index of post-playback ventilation variability</td>
<td>Calculated based on the method of Miksis–Olds et al. (2007, p.640), measured in seconds and calculated for post-playback respiratory pauses; the greater the value, the greater the manatee’s ventilation variability</td>
</tr>
<tr>
<td>Time spent in resting area after playback started</td>
<td>The interval that the animal remained (or moved no more than its body length) in the resting area since the initiation of playback, seconds</td>
</tr>
<tr>
<td>Average pre-playback vocalizations</td>
<td>Mean vocalizations pre-playback per minute based on the number of active animals; 10 vocalizations per minute with two active animals present showing any behavior state</td>
</tr>
</tbody>
</table>
Average vocalizations 20 minutes post-playback: Mean vocalizations post-playback per minute based on the number of active animals; 10 vocalizations per minute with two active animals present showing any behavior state except sleep yielded five vocalization per minute per active animal.

Time to immobility: Time period in seconds between manatee's interrupting its rest episode and resuming rest once again.

Changes in behavioral states within 20 minutes post-playback: The number of behavioral states changes within 20 minutes post-playback.
Table A3.

*Results of pre- and post-playback data analysis*

<table>
<thead>
<tr>
<th>Variable</th>
<th>Manatee call</th>
<th>Boat</th>
<th>Dummy</th>
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</thead>
<tbody>
<tr>
<td>Average pre-playback respiration</td>
<td>203.74</td>
<td>234.36</td>
<td>195.7</td>
</tr>
<tr>
<td>Average post-playback respiration</td>
<td>116.30</td>
<td>167.24</td>
<td>172.66</td>
</tr>
<tr>
<td>Index of pre-playback ventilation variability</td>
<td>156.66</td>
<td>275.2</td>
<td>184.62</td>
</tr>
<tr>
<td>Index of post-playback ventilation variability</td>
<td>118.56</td>
<td>137.76</td>
<td>134.34</td>
</tr>
</tbody>
</table>

*p* values:

- Average pre-playback respiration: 0.068, 0.465, 0.144
- Index of pre-playback ventilation variability: 0.144, 0.144, 0.273
- Average pre-playback vocalizations: 0.49, 1.48, 1.27
- Average vocalizations 20 minutes post-playback: 0.70, 1.59, 2.58
- *p* values: 0.180, 0.715, 0.180
APPENDIX C – IACUC Approval Letters

INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE
NOTICE OF COMMITTEE ACTION

The proposal noted below was reviewed and approved by The University of Southern Mississippi Institutional Animal Care and Use Committee (IACUC) in accordance with regulations by the United States Department of Agriculture and the Public Health Service Office of Laboratory Animal Welfare. The project expiration date is noted below. If for some reason the project is not completed by the end of the three year approval period, your protocol must be reactivated (a new protocol must be submitted and approved) before further work involving the use of animals can be done.

Any significant changes (see attached) should be brought to the attention of the committee at the earliest possible time. If you should have any questions, please contact me.

PROTOCOL NUMBER: 09051405
PROJECT TITLE: The Effect of Auditory Stimulation on Sleep Disruption in the Beluga Whale (Delphinapterus leucas)
PROPOSED PROJECT DATES: 06/15/2009 to 08/15/2010
PROJECT TYPE: New Project
PRINCIPAL INVESTIGATOR(S): Stan Kuczaj, Ph.D.
COLLEGE/DIVISION: College of Education & Psychology
DEPARTMENT: Psychology
FUNDING AGENCY/SPONSOR: Departmental
IACUC COMMITTEE ACTION: Full Committee Review Approval
PROTOCOL EXPIRATION DATE: 09/30/2011

[Signature]
Robert C. Bateman, Jr., Ph.D.
IACUC Chair

6-11-09
Date
INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE
NOTICE OF COMMITTEE ACTION

The proposal noted below was reviewed and approved by The University of Southern Mississippi Institutional Animal Care and Use Committee (IACUC) in accordance with regulations by the United States Department of Agriculture and the Public Health Service Office of Laboratory Animal Welfare. The project expiration date is noted below. If for some reason the project is not completed by the end of the three year approval period, your protocol must be reactivated (a new protocol must be submitted and approved) before further work involving the use of animals can be done.

Any significant changes (see attached) should be brought to the attention of the committee at the earliest possible time. If you should have any questions, please contact me.

PROTOCOL NUMBER: 11092213
PROJECT TITLE: The Effect of Auditory Stimulation on Sleep Disruption in the West Indian Manatee (Trichechus manatus latirostris)
PROPOSED PROJECT DATES: 10/01/2011 to 09/30/2014
PROJECT TYPE: Renewal/Continuation of a Previously Approved Project
PRINCIPAL INVESTIGATOR(S): Stan A. Kuczaj, Ph.D.
COLLEGE/DIVISION: College of Education & Psychology
DEPARTMENT: Psychology
FUNDING AGENCY/SPONSOR: Departmental
IACUC COMMITTEE ACTION: Full Committee Review Approval
PROTOCOL EXPIRATION DATE: 09/30/2014

Jodie Jawor, Ph. D.
IACUC Chair

Date
REFERENCES


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