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Rebecca Umeed

Comportamento vocal em *Trichechus manatus manatus*

Recife 2016

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Dissertação apresentada ao Programa de Pós-graduação em Biologia Animal da Universidade Federal de Pernambuco, para a obtenção do título de Mestre em Biologia Animal sob a orientação da Profa. Dra. Bruna Martins Bezerra.

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It always seems impossible until itôs done.

- Nelson Mandela

Resumo

O peixe-boi marinho, *Trichechus manatus manatus*, é uma das subespécies mais ameaçadas no nordeste do Brasil, e foi classificado como em perigo de extinção no país. O conhecimento da comunicação de peixes-boi marinhos ainda está na etapa de infância, porém estudos tem demonstrado o uso de vocalizações como um método de reconhecimento individual e comunicação em outras espécies de peixes-boi. Os estudos incluídos nesta dissertação foram realizados no Centro Nacional de Pesquisa e Conservação de Mamíferos Aquáticos (CMA), localizado na Ilha de Itamaracá, no nordeste do Brasil. Seis tipos de vocalizações foram identificados: duas destas vocalizações, squeaks e screeches foram comuns às fêmeas, machos e juvenis. A estrutura de squeaks foi diferente entre animais de sexos e faixa etárias diferentes e a estrutura de screeches foi diferente apenas entre animais de faixa etária diferente. Experimentos de playback demonstraram um aumento nas vocalizações dos peixes-boi após reprodução de estímulos vocais quando comparado ao estímulo controle. Isso sugere que os peixes-boi marinhos utilizam vocalizações como um método de comunicação. Estes resultados introduzem a possibilidade de utilizar as vocalizações de peixes-boi marinho na identificação e no monitoramento não-invasivo desses animais na natureza, onde a identificação de idade ou sexo é difícil por causa da turbidez da água e a timidez da espécie. O padrão de comportamento, também, foi descrito para os peixes-boi marinhos em cativeiro: sete categorias comportamentais foram identificadas. Diferenças sexuais e etárias foram identificadas no uso dessas categorias. Experimentos de playback foram realizados utilizando estímulos de jangada motorizada, catamarã, lancha e controle de silêncio, para identificar preliminarmente os efeitos que o ruído de barcos tem no comportamento e na vocalização de peixes-boi juvenis. Respostas comportamentais e vocais ocorreram aos estímulos de barcos, tendo a jangada motorizada provocado uma reposta mais intensa nos peixes-boi juvenis. Os resultados deste estudo sugerem a limitação no uso de jangadas motorizadas em áreas de ocorrência de peixes-boi.

Palavras chave: Ameaçado. Estrutura de vocalizações. Comunicação. Poluição. Conservação.

Abstract

The Antillean manatee, *Trichechus manatus manatus*, is one of the most endangered species in North-eastern Brazil and has been classified as under threat of extinction (MMA 2014). Knowledge on the acoustic communication of Antillean manatees is still in its infancy, however studies have demonstrated the use of vocalizations as a method of individual recognition and communication in other manatee taxa. The studies included in this paper were performed at the *Centro Nacional de Pesquisa e Conservação de Mamiferos Aquáticos* (CMA), located on the Itamaracá Island, North-eastern Brazil. Six types of vocalizations were found to be produced by the manatees included in the study, with two of these calls, *squeaks* and *screeches* found to be common to females, males and juveniles. Vocalization structure was found to demonstrate gender and age differences for *squeaks* and only age differences for *screeches*. Playback experiments showed an increase in manatee vocalizations following the playback stimuli, which suggests the use of vocalizations as a method of communication in *T. manatus manatus*. These results may introduce the possibility of using vocalizations in the identification and passive and active non-invasive monitoring of manatees in the wild where sex or age identification can be difficult due to water turbidity and discrete nature of the Antillean manatees. Manatee behavioral patterns were also investigated using scan and focal behavior observations: seven behavior categories were identified for the manatees included in this study. Both gender and age differences were found in the use of these behavior categories. Playback experiments using motorized jangada, catamaran, fishing boat and control stimuli were carried out to preliminarily identify the effects of boat noise on juvenile manatee vocal and postural behavior. Responses to boat noise stimuli were demonstrated in vocal and postural behavior, with the motorized jangada stimuli eliciting the greatest response from juvenile manatees. The results of this study suggest the limitation of the use of motorized jangadas in wild areas of known occurrence of Antillean manatees in Brazil.

Key words: Endangered. Vocalization structure. Communication. Noise pollution. Conservation.

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1 Literature Review: Vocal behavior in manatees: communication, call structure, noise pollution and conservation issues.

1.1. Abstract

Sound production by manatees has been recorded in several studies. In this paper we provide an overview of the vocal behavior of manatees, discussing taxon-specific information, the role of vocalizations in their communication and vocalization structure. Furthermore, we follow with a review on the current evidences of the impacts of underwater noise pollution on manatee communication and general behavior. We summarize strategies to minimize impacts of noise pollution on manatees, identify gaps in our knowledge and suggest key areas for future research and conservation.

Key words: *Trichechus*. Vocalizations. Calling frequency. Anthropogenic impact. Acoustic ecology.

1.2. Introduction

Three species of manatees exist in the world today: the Amazonian manatee, *Trichechus inunguis;* the West African manatee, *Trichechus senegalensis* and the West Indian manatee, *Trichechus manatus.* All manatee species are listed as vulnerable to extinction by the World Conservation Union (MMA, 2014). Within the species *T. manatus,* exist two sub-species: the Florida manatee, *T. manatus latirostris* and the Antillean manatee, *T. manatus manatus*. These two sub-species are separated both geographically and genetically (Alicea-Pou 2001; Barros 2014; Landrau-Giovannetti et al. 2014). Manatees are confined to warm waters since they are generally intolerant of cool water temperatures due to their low metabolic rate and high thermal conductance (Langtimm et al., 1998). Florida manatees, in particular, are known for migrating during the winter months to warmer waters (Langtimm et al. 1998). Manatees generally occupy shallow waters of depths between 3-8m with the maximum depth observed to be 14.8m (Alves et al. 2013). *Trichechus manatus latriostris* and *Trichechus manatus manatus* typically inhabit grazing pastures in shallow coastal waters and adjacent freshwater ecosystems and are absent from areas that lack these two habitats (Garcia-Rodriguez 1998). *T. inunguis* habitat, on the other hand, is restricted to the Amazon basin (Garcia-Rodriguez 1998). Manateesô habitats are often subject to anthropogenic factors such as boat traffic. As a result shallow waters may experience high levels of turbidity particularly during the summer months (Miksis-Olds & Tyack 2009). High levels of turbidity would result in further decrease of visual capability (Sousa-Lima et al. 2002). Overall, the olfactory structure and the underwater visual acuity are poor in manatees (Mackay-Sim et al., 1985; Bauer et al., 2003).

Therefore, they may have evolved other methods of maintaining contact between individuals such as tactile and vocal signals (Sousa-Lima et al., 2002).

Maintaining acoustic contact is especially important for animals that are separated to such an extent that visual contact is no longer possible (Rendall et al., 1996), therefore, selective forces may promote the evolution of acoustic communication between individuals (Miksis-Olds & Tyack., 2009). Communication can be defined as an association between the behavior of a signaller and the behavior of a receiver as a consequence of a signal transmitted between them (Wiley and Richards, 1978). Each signal is associated with a particular referent, which provides information about the signalerôs identity, behavioral tendencies or external circumstances (Wiley and Richards, 1978).

Sound production by *T. inunguis* and *T. manatus latirostris* has been recorded in several studies for example: Florida Department of Environmental Protection Division of Marine Resources, 1996; Nowacek et al., 2003; Oôshea and Poche, 2006. However, the acoustical repertoire of Antillean manatees has not been fully described (Alicea-Pou, 2001). It is believed that manatees may present identity information in their vocalizations, which suggests the use of vocalizations in communication (Sousa-Lima et al., 2008). Wild manatees are often exposed to boat noise and boat traffic. This noise has the potential to mask vocalizations, which results in an increased difficulty in communication (Miksis-Olds & Tyack., 2009). Boat noise at the waterôs surface may also be indistinguishable to manatees and may result in manatee-boat collisions (Gerstein et al., 1999). Research has indicated that acoustic variation may be used as a warning indicator of disturbance when individual fitness has not notably changed (Laiolo 2010) and it is therefore necessary to continue to investigate the effects of noise pollution on manatee acoustic communication.

This paper provides an overview of the vocal behavior of manatees. We discuss the structure of manatee vocalizations and the possible use of vocalizations as a method of manatee communication. Furthermore, we include a review of the current evidence on the impacts on underwater noise pollution on manatee vocal behavior. Possible strategies that may be implemented to minimize the impact of noise pollution on manatee communication are summarized and we identify areas of further research and suggest possible conservation methods.

1.3. The Role of Vocalizations in Communication

Acoustic communication requires both the detection of a signal and the discrimination of differences among signals by the receiver (Wiley and Richards, 1978). Animals present several types of communication: chemical, tactile, electrical, visual and acoustic communication (Tyack, 2000). In aquatic ecosystems, sound is often a more reliable method of communication than visual displays (Dudzinski, 1996). Several species of marine mammals are known to use vocalizations as a method of communication, such as delphinids (Evans & Bastian, 1969; Herman & Tavolga, 1980; Janik, 1998; Sayigh et al., 1995); finback whales, *Balaenoptera physalus*, (Tyack, 2000) and killer whales, *Orcinus orca* (Tyack, 2000; Deeke et al., 2005).

The mechanisms for sound production in terrestrial mammals and marine mammals are similar: in both cases sound is made by passing air under pressure past membranes that vibrate (Tyack, 2000). The main difference between sound production in terrestrial mammals and marine mammals is that terrestrial animals must open their mouths to propagate sound into the surrounding environment due to the different densities of air and water (Tyack, 2000). Whereas, underwater vocalizations of marine mammals generate sound vibrations that have a similar density to that of seawater and the sound, therefore, transfers well into their surrounding environment (Tyack, 2000). Sound travels at a speed of $331.29ms^{-1}$ (Wong, 1986) in air however, underwater sound travels up to $1500ms^{-1}$ (Forrest, 1994; Tyack & Miller, 2002). Unlike air, shallow waters have two boundaries: the substrate and the water surface (Forrest, 1994). Sound, therefore, is constrained by the boundary conditions at the substrate and the surface (Forrest, 1994). As a result only signals above a certain frequency will propagate with minimal attenuation underwater (Forrest, 1994). These two boundaries act as a high pass filter in aquatic systems that is much greater and more predictable than filtering in terrestrial systems (Forrest, 1994).

Acoustic communication may serve for several purposes in marine mammals, such as reproductive advertisement displays in humpback whales and bowhead whales;

recognition systems and contact calls; signals for maintaining parent-offspring contact and for maintaining the coordination of groups (Tyack, 2000; Tyack & Miller, 2002). For many animal species it is thought that vocalizations are used principally as a method of communication between mothers and calves (Rendall et al., 1996). Since it is important for mothers to recognize and maintain contact with their calves, a method of individual recognition is necessary (Halliday, 1983; Rendall et al., 1996; Sousa-Lima et al., 2002). Studies have shown evidence of mother-calf vocal communication in several marine mammal species such as Atlantic walruses, *Odobenus rosmarus rosmarus* (Charrier et al., 2010); Indo-Pacific humpback dolphins *Sousa chinensis* (Van Parijs & Corckeron, 2001); beluga whales, *Delphinapterus leucas* (Vergara & Barrett-Lennard, 2008) and bottlenose dolphins, *Tursiops truncatus* (Tyack & Sayigh, 1997). Sirenians have a long-term investment in parental care, often up to three years, and it is therefore possible to assume that there is selective pressure for a mother to nurse her own calf rather than the calf of another individual (Sousa-Lima et al., 2002). Therefore, a reliable method of individual recognition is necessary, such as the use of vocalizations and contact calls (Sousa-Lima et al., 2002).

Almost all animal species have evolved recognition systems in order to differentiate conspecifics from ñothersò; some species have mechanisms for recognizing individuals, kin and mates (Tyack, 2000). Bengston and Fitzgerald (1985) observed that Florida manatee vocalization rates were dependent on an individualôs behavior: mating and cavorting had the highest vocalization rates (Yan et al. 2005). For Bengston and Fitzgerald (1985), this suggested that vocalizations might have a role in communication. Oôshea et al (2006) found that the highest rate of vocalization occurred between mothers and calves, which suggests mother-calf recognition and the use of vocalizations as a method of communication between them. They also found that the physical structure of Florida manatee vocalizations: duration; fundamental frequency and non-linear elements, suggest adaptations for shallow-water communication in manatees as well as providing information on individual identity (Oôshea & Poche, 2006). The notion of individual recognition in manatees is supported by a study by Sousa-Lima et al (2002) on Amazonian manatees. It was found that each individual had a single type of harmonic isolation call (Sousa-Lima et al., 2002). Signature whistles have also been found to be produced by bottlenose dolphins, and are thought to be important in establishing vocal or physical contact between individuals (Caldwell et al., 1990) and maintaining group cohesion (Janink & Slater, 1998). Barbary macaque mothers, *Macaca sylvanus;* sub-Antarctic fur seal mothers, *Arctocephalus tropicalis* (Charrier et al., 2003) and northern elephant seal mothers, *Mirounga angustirostris* (Insley, 1992) are able to recognize their offspring through the use of individual vocal recognition. In a similar way, mother and calf vocalizations in Amazonian manatees are thought to function as a method of maintaining close contact with each other (Hartman, 1979). Sousa-Lima et al (2002), conclude that the primary function of Amazonian manatee signature vocalizations would be individual recognition and identification as well as location and attraction between specific individuals. Currently, there is very little research focusing on the role of vocalizations in communication by *T. manatus manatus* but the research that exists for other species of manatees indicates a strong possibility that *T. manatus manatus* may also use vocalizations as a method of communication.

1.4. Vocalization Structure

Research on manatee vocalizations has been focused, principally, on *Trichechus manatus latirostis* and *Trichechus inunguis* (Sousa-Lima et al., 2002; Niezrecki et al., 2003; Miksis-Olds & Tyack, 2009). Studies have found that the fundamental frequency of *T. manatus latirostris* vocalizations is between 2kHz and 5kHz (Niezrecki et al., 2003; Yan et al., 2005; Oôshea & Poche, 2006) and the fundamental frequency of *T. inunguis* is between 1.2kHz and 4kHz (Sousa-Lima et al., 2002). Very little research exists on the vocalizations produced by *T. manatus manatus* and it is thought that Sonoda & Takemura (1973) were the first to describe the physical structure of vocalizations produced by this specie (Alicea-Pou, 2001). They found that vocalizations produced by this sub-specie had a fundamental frequency of between 0.2kHz and 7kHz. A recent study by Chavarría et al. (2015) reported tonal calls with fundamental frequencies ranging from 0.7 to 8.1kHz in *T. manatus manatus*. No research has clearly investigated the vocal repertoire of *T. manatus manatus* and it therefore remains to be fully described (Alicea-Pou, 2001). The mechanism of sirenian sound production is generally unknown (Landrau-Giovannetti et al., 2014). Manatees have no true vocal cords in their larynx (Caldwell and Caldwell, 1985), therefore it is believed that other homologous structures could be responsible for generating sounds (Landrau-Giovannetti et al., 2014). Landrau-Giovanetti et al (2014) stated that tissues with a density close to that of water will permit more efficient energy transfer from an individualôs body to the surrounding water. Chapla et al (2007) found that the soft tissues of manatee heads have a density similar to that of seawater, which suggests easy propagation of sound waves from one medium to another. However, the definitive path of the transfer of sound to water has not yet been established for manatees (Landrau-Giovannetti et al., 2014).

Several other species of marine mammals have been found to produce vocalizations. Studies have found that the vocalizations of *Erignathus barbatus*, commonly known as bearded seals, have a fundamental frequency of between 0.13kHz and 10.5kHz (Cleator et al., 1989). *Balaenoptera musculus,* blue whales, have been found to produce vocalizations with a fundamental frequency between 0.16kHz and 0.18kHz (Rivers, 1997). Vocalizations of low frequency, such as those of blue whales, are most commonly produced by species that demonstrate long distance communication, the low frequency of the vocalizations allow for longer propagation of the sounds (Tyack, 2000). Echolocation pulses on the other hand have high fundamental frequencies: bottlenose dolphins have been found to produce pulses with a fundamental frequency of between 100kHz and 130kHz (Tyack, 2000). Bottlenose dolphins commonly form social units (Wells et al. 1987; Connor et al. 2001; Lusseau 2007), therefore the high frequency pulses are suited for communication between individuals that are within close proximity to one another. Blue whales are generally solitary animals and therefore, the low frequency of their vocalizations may provide a method of long distance communication that is necessary for individuals to find mates and reproduce (McDonald et al., 2006). Manatee mothers stay with their calves for up to three years (Sousa-Lima et al., 2002), however, manatees in general do not form permanent social units. Therefore, the frequency range of manatee vocalizations may have evolved to allow for both long distance communication as well as communication between mothers and calves in relatively close proximity.

1.5. The Effect of Boat Noise on Manatee Vocal Behavior

The effective range of acoustic communication in aquatic environments depends on several factors: the acoustic propagation loss characteristics of the area, the frequency and amplitude of the vocalizations emitted, the hearing sensitivity of the animals and the ambient noise (Miksis-Olds & Tyack, 2009). The effective range of communication may also be affected by environmental noise: if the frequency of environmental noise overlaps with that of a vocalization, important signal information may be masked and therefore interfere with communication (Gerstein, 2002; Brumm & Slabbekoorn, 2005; Tyack, 2008; Miksis-Olds & Tyack, 2009). Communication theory identifies several ways a sender can modify an acoustic signal to compensate for noise, such as increasing the repetition of a signal, increasing its duration and altering the frequency of a signal (Tyack, 2008; Miksis-Olds & Tyack, 2009). One reaction to noise level is known as the Lombard effect, which has been found in humans as well as other mammalian species (Scheifele et al., 2005). It refers to the unconscious tendency of an individual to raise their vocal amplitude when confronted with a noisy environment (Scheifele et al., 2005). *Delphinapterus leucas,* St. Lawrence River belugas have been shown to exhibit the Lombard effect, by increasing or decreasing their vocalization level in coincidence with the environmental noise level (Scheifele et al., 2005). Studies have demonstrated an increase in the duration of killer whale vocalizations in the presence of boat traffic (Foote et al., 2000), as well as an increase in duration of humpback whale song in the presence of low frequency active sonar (Miller et al., 2000). Pilot whales have been found to increase the number of whistles produced in the presence of mid-frequency sonar (Rendall & Gordon, 1999), similarly bottlenose dolphins were found to increase the number of whistles produced in response to boat approaches (Buckstaff, 2004). A study by Miksis-Olds & Tyack (2009) found that Florida manatees modified their vocalization structure and rate in the presence of ambient noise. Compensation techniques in Florida manatees were found to be dependent on behavioral state and the presence of calves (Miksis-Olds & Tyack, 2009). Research has also shown that Florida manatee foraging in sea grass beds is significantly negatively correlated with the number of boats passing per five minutes during the morning hours (Miksis-Olds et al., 2007). This study by Miksis-Olds et al (2007) suggests that Florida manatees select foraging areas with the least low frequency noise due to anthropogenic activity. The results of these studies suggest that boat noise may affect both the vocal and foraging behavior of Florida manatees (Miksis-Olds et al., 2007; Miksis-Olds & Tyack, 2009).

Boat noise is characteristically different to biological noise: underwater boat noise has two domains, noncavitating and cavitating (Gerstein, 2002). The frequency and power of boat noise is directly related to the speed of the vessel: the faster the propeller rotation, the more cavitation is created (Gerstein, 2002). Small bubbles are created and collapse and as a result, form a broad range of frequencies (Gerstein, 2002). On the other hand, when boat velocity decreases, the amount of turbulence decreases and the frequency and power spectrum of the noise are greatly reduced (Gerstein, 2002). In a study by Gerstein (2002), it was found that manatees were unable to detect the noise from idling boats. These results suggest that a boat travelling with a slowly rotating propeller generates noise that is indistinguishable from the ambient noise until it is extremely close to a manatee (Gerstein, 2002). One of the main strategies of conservation management of Florida manatees has been to implement idling and slow-speed zones, however, this may in fact, increase the number of manatee-boat collisions (Gerstein, 2002). Conversely, despite longer exposure time to boat noise during boat idling, slower vessel speed may offer manatees extra time to assess the threat and to act in order to avoid a collision (Nowacek et al., 2004). Florida manatees have been found to respond to boat presence at between 25m and 58m (Nowacek et al., 2004) and the most common reactions to boat presence have been described as slow submergence and movement to deeper water i.e. a flight response (Nowacek et al., 2004; Miksis-Olds et al., 2007b). Studies have found that Florida manatees were able to hear boats from up to 100m away, however it is not known how accurately they are able to localize the boats (Colbert et al., 1999). Florida manatee hearing thresholds have been reported to fall between 0.4kHz and 46kHz, with the range of best hearing recorded to be between 6kHz and 20kHz and the range of peak frequency sensitivity to be between 16kHz and 18kHz (Ketten et al., 1992; Gerstein et al., 1999). These results suggest that manatees have greater peak sensitivity than most pinnipeds and comparable peak sensitivity with some odontocetes (Gerstein et al., 1999). Gerstein et al (1999) suggest that manatee hearing is poorly suited to detect the low frequency noise of boats, typically between 0.01kHz and 2kHz (Colbert et al., 1999), produced at the water surface: low frequency sounds produced at the surface are significantly attenuated and can become indistinguishable from background noise levels. Manateesô ability to hear low frequency noise is weakest when the manatee is at the surface and therefore, more vulnerable (Gerstein et al., 1999). Studies have demonstrated that some animal species use a combination of two cues in order to localize sounds, for example Indian Elephants that use the time of arrival and noise level differences, whereas other species such as the hedgehog use only one cue: level differences (Colbert et al., 1999). Interaural time distances (the distance that sound travels from one ear to the other divided by the speed of sound) have been shown to be directly correlated with the upper frequency limits in mammals (Ketten et al., 1992). The narrower an individualôs heard, the shorter the interaural time distance and the higher the frequency limit (Ketten et al., 1992). Interaural time distances are small in *T. manatus* and the structure of *T. manatus latriostris* inner ears suggests that they lack sensitivity and directionality compared to most mammals (Ketten et al., 1992). It is, therefore, important to further investigate how manatees perceive their environment to understand how humans may be impacting their environment and to introduce effective conservation management methods (Ketten et al., 1992; Colbert et al., 1999). Due to the high levels of manatee mortality due to collisions with boats, there has been an increased interest in the development of acoustic warning and detection technologies to minimize collisions between manatees and boats (OôShea et al., 2006). These technologies aim to utilize high frequency directional acoustic beacons to provide both directional and distance cues to manatees to warn manatees of approaching vessels (Gerstein et al., 1999). No research exists investigating the effects of boat noise on the vocal behavior of *Trichechus manatus manatus*, it is therefore necessary to study the impact that boat noise has on this threatened species in order to implement conservation and management methods to prevent its extinction in Central and South America.

1.6. Conclusions and Future Directions

All species of manatees are vulnerable to extinction (MMA, 2014). It is believed that some species of manatees utilize vocalizations as a method of communication used principally in individual recognition and to maintain contact between individuals (Halliday, 1983; Rendall et al., 1996; Sousa-Lima et al., 2002). *T. manatus manatus* is one of the least known with regards to their vocal communication. Florida manatee vocalizations have a frequency range of 2kHz-5kHz (Niezrecki et al., 2003; Yan et al., 2005, Oôshea et al., 2006); this sub-specie may have evolved high frequency vocalizations as an adaptation to their shallow water habitat, since higher frequency sounds suffer less attenuation than low frequency sounds (Forrest, 1994). The frequency range of manatee vocalizations may also allow for both close contact communication as well as longer distance communication. Manatees are often exposed to increased ambient noise due to boat traffic: this may have multiple negative effects on manatee communication (Miksis-Olds and Tyack, 2009). The low frequency noise that boats produce at the water surface is often indistinguishable to manatees from ambient noise and therefore, results in manatee-boat collisions (Gerstein et al., 1999). Acoustic alarm systems are being developed to attempt to limit the number of collisions that occur between manatees and boats (OŒShea et al., 2006).

Further research focusing on the effects of boat noise on Antillean manatee vocal behavior and general activity pattern in the wild are necessary in order to implement effective conservation and management methods for the species. There is much still unknown about manatee vocal behavior. The information included in manatee vocalizations is still undetermined: it is possible that different vocalizations communicate different messages such as warning alarms and individual identification and it remains to be determined whether manatees produce specific vocalizations to attract a mate. It is clear that there is still much unknown about manatees and their vocal behavior, and more research is therefore necessary to fully understand manatee vocal behavior.

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2 Influence of sex and age on the vocal repertoire of the Antillean manatee (*Trichechus manatus manatus*).

Nota: *o presente capítulo será submetido para a revista Marine Mammal Science. O mesmo foi colocado nas regras da mesma, entretanto as figuras e tabelas foram inclusas no decorrer do texto para facilitar a leitura. Este trabalho foi feito em colaboração com pesquisadores do Laboratório de Etologia e do Centro de Mamíferos Aquáticos de Itamaracá, os quais serão co-autores do estudo.*

2.1 Abstract

Limited information is available about the acoustic communication of *Trichechus manatus manatus*, however, studies have shown that other manatee taxa produce vocalizations as a method of individual recognition and communication. The acoustic signals of 15 Antillean manatees in captivity were recorded, aiming to i) describe their acoustic repertoire and ii) investigate the influence of sex and age on vocalization structure and responses to vocalization playback. Six acoustic signals ranging in mean fundamental frequencies from 0.64kHz to 5.23 kHz were identified: *squeaks* and *screeches* were common to adult males and females and juveniles; *trills* were common to adult males and females; *whines* were specific to adult males; *creaks* were specific to adult females and *rubbing* was specific to juveniles. The physical structure of *squeak* vocalizations differed between age and sex classes and *screech* structure differed between age classes. *Squeaks* and *screeches* produced by juveniles were found to have higher mean maximum fundamental frequencies compared to those produced by adult males and females. An increase in the vocalization rate following vocalization playbacks was found for all three age/sex groups. Our results introduce the potential of using acoustic signals in the identification and non-invasive monitoring of manatees in the wild in Brazil.

Keywords: Vocalizations, Communication, Monitoring, Conservation, Marine mammals.

2.2 Introduction

The order Sirenia is composed of four living species: *Dugong dugong, Trichechus inunguis, Trichechus senegalensis* and *Trichechus manatus* (Landrau-Giovannetti et al. 2014). Within the species *T. manatus,* exist two subspecies: *T. manatus latirostris,* commonly known as the Florida manatee and *T. manatus manatus*, known as the Antillean manatee. In Brazil, *Trichechus manatus manatus* is under threat of extinction (MMA 2014) and is both geographically isolated by stretches of water (Alicea-Pou 2001) and genetically distinct from *T. manatus latirostris* (Hunter et al. 2012). *T. manatus manatus* occur in Northern and North Eastern Brazil, typically inhabiting shallow coastal waters (Garcia-Rodriguez et al. 1998; Alves et al., 2013). Like other species of manatees, the

Antillean manatee may use vocalizations as a method of communication, however no study has confirmed this. Maintaining acoustic contact is especially important for species that are separated to such an extent that visual contact is not always possible or limited by environmental conditions (Rendall et al. 1996). Therefore, selective forces may promote the evolution of acoustic communication between individuals (Miksis-Olds & Tyack 2009).

The acoustic repertoire of Antillean manatees has not been fully described (Alicea-Pou 2001). However, sound production by other species of manatees has been observed in several studies (Florida Department of Environmental Protection Division of Marine Resources 1996; Nowacek et al. 2003; Oôshea and Poche 2006). It has been found that the fundamental frequency of manatee vocalizations is between 2kHz and 5kHz, nonetheless, manatees are capable of hearing sounds between 0.4kHz and 45kHz (Oôshea and Poche 2006). Signature vocalizations have been found in other species of marine mammals (Caldwell & Caldwell 1965; Sousa-Lima et al. 2002; Charrier et al. 2009; Gridley et al. 2014) and it is thought that manatees too, may present identity information in their vocalizations (Sousa-Lima et al. 2008). Individual vocal recognition has been found between a mother and calf pair of Florida manatees (Reynolds 1981), as has individual recognition and vocal signature information been found in Amazonian manatees (Sousa-Lima et al. 2002). Age and gender has been thought to influence manatee vocalizations structure. For instance, Sousa-Lima et al. (2002) found differences between male and female Amazonian manatee vocalizations for mean maximum and minimum fundamental frequencies as well as mean note duration. They also found differences between age classes for signal duration and fundamental range (Sousa-Lima et al. 2002).

Very little is known about the acoustic communication of Antillean manatees and research in this area is necessary as it may contribute to the conservation of this threatened species in Brazil. Thus, the aims of this study were to: i) investigate the production of acoustic signals in *T. manatus manatus* and to ii) investigate the influence of age and sex on the signals produced and also on the animals' responses to vocalization playback. Overall, manatees are regarded as solitary, weakly social or semi-social animals (Hartman 1979; Reynolds 1981; Haper and Schulte, 2005; Hénaut et al. 2010). If we consider the theory of social and vocal coevolution, where complex vocal repertoire appears to be associated with complex social structures (McComb and Semple, 2005; Freeberg et al. 2012), we would not expect a large acoustic repertoire in our study animals. Furthermore, the restrictions in the captive setting also contribute to this expectation. Nevertheless, we predicted that age and sex differences would result in the production of different types of acoustic signals and signals with structural variations. Finally, we expected that vocalizations played back to the manatees would elicit vocal and/or postural responses (potentially different according to sex/age classes) in contrast to control sound files. We hope the present study will extend our knowledge on the use of acoustic signaling as a method of communication between the elusive Antillean manatees. Despite the marked differences between the captive and wild settings for manatees, we consider signal diversity and structure, and responses to call playback in captivity to estimate behaviors in wild animals. We trust that such basic knowledge could be potentially used as an additional survey method for Antillean manatees in their water-turbid estuarine natural environment in Brazil.

2.3 Methods

2.3.1 Animals and Study Site

The study was carried out in the *Centro Nacional de Pesquisa e Conservação de Mamíferos Aquáticos* and *Chico Mendes Institute of Biodiversity* (CMA/ICMBio) on Itamaracá Island in North Eastern Brazil, between January 2015 and April 2016. The study included 15 Antillean manatees (Table 2.1): seven adult females, four adult males and four juveniles (three males and one female), distributed in separate pools. The male and female pools were octagonal in shape and had dimensions of 10.1m wide by 4.15m deep and the juvenile pool was rectangle 8m wide by 5m deep.

Table 2.1 Structure of the captive manatee population included in the present study at the *Centro Nacional de Pesquisa e Conservação de Mamíferos Aquáticos and Chico Mendes Institute of Biodiversity* (CMA/ICMbio) at Itamaracá Island, Pernambuco, Brazil. Estimated ages for the manatees provided in the table are from June 2016 and the ages at time of death for Xica and Sereia.

Individual	**Gender**	**Estimated Age (years)**	**Age Category**	**Parental Relation**
Xica	Female	53 (Deceased	Adult	-

		05/15)		
Sereia	Female	26 (Deceased 11/15)	Adult	-
Carla	Female	19	Adult	Born in captivity (daughter of Sereia and Netuno, twin sister of Sheila)
Sheila	Female	19	Adult	Born in captivity (daughter of Sereia and Netuno, twin sister of Carla)
Marbela	Female	22	Adult	-
Ivi	Female	4	Juvenile	-
Xuxa	Female	29	Adults	-
Bela	Female	5	Juvenile (Included with adults because of mother)	Born in captivity (daughter of Carla, possible fathers: Netuno and Poque)
Arati	Male	6	Juvenile (Included with adults)	-
Daniel	Male	6	Juvenile	-

Luiz Gonzaga	Male	4	Juvenile	Born in captivity (son of Xuxa, possible fathers: Netuno and Poque)
Netuno	Male	25	Adult	-
Poque	Male	24	Adult	Hybrid Amazonian-Antillean
Zoé	Male	11	Adult	-
Diogo	Male	5	Juvenile	Calf beached in Rio Grande do Norte, no parental connections

2.3.2.Recording Vocalizations

The animals were recorded for a total of 106 hours: 38 hours of female vocalizations; 34 hours of male vocalizations and 34 hours of juvenile vocalizations. We used a Cetacean Research Technology hydrophone (Model SQ26-H1, Linear frequency response: 0.02kHz to 45kHz, +3/-12 dBs), which was placed inside of a closed pipe one meter long, with holes drilled into the sides and bottom and connected to a Zoom H1 recorder (Linear frequency response: 20Hz to 20 kHz). The pipe was necessary to prevent the manatees from chewing the hydrophone and did not affect the quality of vocalizations recorded. The pipe was placed at the side of the pool in order to control manatee access to the pipe. Headphones were used to allow monitoring of vocalizations while recording them. Vocalizations were recorded in non-compressed WAV format, between 6am and 6pm, with the day being divided into three sections: 6am-10am, 10am-2pm and 2pm-6pm. Within each of these time intervals two hours of vocalizations were randomly recorded each day. Vocalizations were recorded as five-minute sessions within these time intervals.

A total of six hours of recordings were conducted per day and each group of animals was recorded on alternating days. No recordings were conducted when keepers were interacting with the animals (e.g. when cleaning the pools or providing food to the animals).

2.3.3. Analysis of Vocalizations

Analysis of the vocalizations recorded was achieved through the use of Batsound 4 (Pettersson Elektronic, Uppsala, Sweden). Four vocalization variables were used for the statistical analysis: start frequency, duration, frequency of maximal energy and number of harmonics. Only four variables were included in the statistical analysis due to the use of a leave-one-out cross validation of a random sample of each vocalization type, to determine whether the suggested vocalization types were separated reliably in multivariate space (Bezerra et al., 2010). In the leave one out cross validation, one variable is left out of the data set. 48kHz and 16bits were used as the sampling rate for input, editing and output of sound files. Spectrogram and power spectra settings were: FFT size=512, threshold=12 and Hanning window.

2.3.4.Vocalization Playback

The playback experiments were carried out in all three pools. Three vocalizations and a silent control were used for the playback experiments: *squeaks*, *screeches* and *trills* since these vocalizations were produced by both adult males and females (Table 2.3). Five repeats of each vocalization type were used in the experiments. A silent control was used to exclude the possibility that extra noise emitted from the equipment was eliciting a response from the manatees (Miksis-Olds et al. 2007b). The silent control could not be performed in the adult male pool because the animals had to be transferred to the *Associação Peixe-boi* in Porto de Pedras - Alagoas, North Eastern Brazil, due to unforeseen captive management requirements. During the playback experiments the vocalizations and the behavior of the manatees were recorded before, during and after the vocalization stimuli was played underwater, using the same equipment mentioned for the recording of manatee vocalizations. The sound was played underwater using a LL916 Underwater Speaker with AC203E Circuit Master (linear frequency response: 200Hz -

23kHz, +/-10dB), connected to a 14watt battery. The stimulus was played subjectively at the same volume as the vocalizations naturally produced by the study manatees and was played for the duration of one vocalization (approximately three seconds). The behavior of each animal was observed throughout the entire experiment. The playback experiments lasted 30 minutes and were carried once in the morning and once in the afternoon every 48 hours to avoid the habituation of the animals to the playback stimulus.

2.3.5. Statistical Analysis

A Discriminant Functional Analysis (DFA) with leave-one-out cross validation was used to determine if our subjective classification of calls were objective and to investigate for sexual and age differences in the calls. Chi-squared tests with Yates correction were used on the playback data to determine the significance, $p<0.05$, of differences in vocalization rates before and after the playback experiments. Cochranôs Q tests were used to determine whether there was a change in behaviors in response to the different stimuli. Furthermore, Friedman Tests with Dunn's post hoc were carried out to check whether there was a difference in the number of animals that altered their behaviors in response to the playback stimuli.

2.4 Results

2.4.1. Vocal Repertoire in Captivity

Six vocalizations types were identified in our study animals (Table 2.1, Table 2.2, Table 2.3, Figure 2.1 and Figure 2.3). Three types of vocalizations were found to be common to adult males and adult females: *squeaks*, *screeches* and *trills*. Two of these vocalizations were common to all animal groups: *squeaks* and *screeches*. One vocalization was exclusive to males: *whines*, one vocalization was exclusive to females: *creaks*, and one vocalization was exclusive to juveniles: *rubbing*.

The structure of the three vocalizations produced exclusively by adults was significantly different (DFA: 66.7% of correct call classification (Fig. 2.1); Function 1 accounted for 80.9% of the variance between calls, *Wilks Lambda=0.35, df=8,* $p<0.05$; Figure 2.1).

2.4.2. Age and Sex Influence on Vocalization Structure

For *Squeaks* and *Screeches* common to adult males, adult females and juveniles, there were both age and sex related differences in call structure: *Squeaks* differed between age, *Wilks Lambda*$=0.48$, $df=4$, $p<0.05$, and sex classes, *Wilks Lambda*$=0.37$, $df=4$, $p<0.05$. Whereas *Screeches* differed only between age classes, *Wilks Lambda*$=0.47$, $df=4$, $p<0.05$, and not between sex class, *Wilks Lambda*$=0.63$, $df=4$, $p>0.05$.

2.4.3. Playback Experiments

Significant differences between the number of vocalizations produced 30 seconds before and 30 seconds after the playback were found for female *squeak*, $X^2=1.12$, $d.f.=1$, $p<0.05$, and juvenile *squeak*, $X^2=7.26$, $d.f.=1$, $p<0.01$, stimuli; male *screech* stimuli, $X^2=13.63$, $d.f.=1$, $p<0.01$, and female *trill*, $X^2=4.57$, $d.f.=1$, $p<0.05$, male *trill*, $X^2=4.17$, $d.f.=1$, $p<0.05$, and juvenile *trill* stimuli , $X^2=4.37$, $d.f.=1$, $p<0.05$, for Chi squared tests with Yates correction (Fig. 2.2). The number of vocalizations produced after the playback was found to be significantly different between the playback stimuli for males, $X^2=5.66$, $d.f.=2$, $p=0.05$, females, $X^2=6.69$, $d.f.=3$, $p=0.05$, and juveniles, $X^2=7.50$, $d.f.=3$, $p=0.05$ (Fig. 2.2) for Chi squared tests.

There was a significant change in animal behavior when comparing the vocalization with the control playbacks for females, $Q=798.75$, $df=3$, $p<0.05$, and juveniles, $Q=798.75$, $df=3$, $p<0.05$. Significant differences were also found in the number of animals that altered their postural behaviors before and after the playback experiments between vocalization stimuli for both females, $X^2=11.11$, $d.f.=3$, $p<0.005$, and juveniles, $X^2=11.52$, $d.f.=3$, $p<0.005$ for Friedman tests with Dunnôs post-hoc (Fig. 2.4). Dunnôs post-hoc tests detected a significant difference between the trill and control trials for females, $p=0.019$ and between the screech and control trials for juveniles, $p=0.013$.

Table 2.2 Average values of the vocalizations variables used to classify each vocalization type. in captive Antillean manatees at the *Centro Nacional de Pesquisa e Conservação de Mamíferos Aquáticos and Chico Mendes Institute of Biodiversity* (CMA/ICMBio) on Itamaracá Island in North Eastern Brazil. FME = frequency of maximal energy (kHz). Dur = duration.

Vocalization	**Manatee Group**	**Syllable Duration (ms)**	**FME 1st harmonic (kHz)**	**FME 2nd harmonic (kHz)**	**High Frequency (kHz)**	**Low Frequency (kHz)**	**æ Frequency (kHz)**	**Start Frequency (kHz)**	**Stop Frequency (kHz)**	**Dur start-peak (ms)**	**Dur peak-end**	**No. Harmonics**	**Interval Frequency Harmonics (kHz)**
Squeak	Females	237.2	2.19	4.171	2.49	1.78	0.62	2.37	2.09	76.4	165.8	27	15.22
	Males	161.9	2.67	5.698	2.90	2.22	0.70	2.49	2.53	37.5	149.4	25	21.60
	Juveniles	246.4	3.39	6.776	3.83	2.59	1.24	2.90	3.52	24.3	89.7	27	1.02
Screech	Females	189.6	2.53	11.55	2.01	1.97	1.04	2.70	2.35	73.5	164.4	4	0.18
	Males	140.1	2.31	5.64	2.91	1.42	1.47	2.39	2.02	95.3	142.7	2	0.19
	Juveniles	242.9	3.62	3.86	4.34	2.70	1.64	3.79	3.62	38.6	126.2	2	0.03
Trill	Females	236.6	3.14	0	3.37	2.52	0.79	3.03	2.79	115.5	102.7	0	0
	Males	179.3	3.89	2.08	5.10	2.69	2.41	3.79	3.76	57.9	116.4	2	0.39
Creak	Females	211	2.89	0	3.81	2.13	1.67	3.09	3.10	33.5	41.4	0	0
Whine	Males	153.3	4.94	9.55	5.23	4.56	0.67	4940	4940	57.9	116.4	2	3.32
Rubbing	Juveniles	78.2	1.39	1.50	3.38	0.64	2.74	1860	2160	33.5	41.4	2	0.55

Table 2.3 A description of the six types of vocalizations identified in the study manatees living at the *Centro Nacional de Pesquisa e Coservação de Mamíferos Aquáticos and Chico Mendes Institute of Biodiversity* (CMA/ICMBio) at Itamaracá Island, Pernambuco, Brazil.

Vocalization	**Age/Sex classes of manatees that produced the vocalization (n)**	**Description of Vocalization**
Squeak	– Females (7) – Males (4) – Juvenile (4)	High pitched squeaking noise, usually short vocalizations, high number of harmonics, often responded to with screeches, squeaks or trills.
Screech	– Females (7) – Males (4) – Juveniles (4)	Bird-like sound, often loud, often heard as a response to squeaks, screeches or trills
Trill	– Females (7) – Males (4)	Similar to the sound of a whistle being blown, often loud, generally produced as a response to another type of vocalization.
Creak	– Females (7)	Low pitched sound, similar to the sound of a door creaking open, generally not repeated in a sequence.
Whine	– Males (4)	Faint, quiet sound. Very fine, high pitched, generally not repeated in a sequence.
Rubbing	– Juveniles (4)	Sound resulting from a mechanical movement of lips rubbing on teeth, almost always repeated more than once, often in sequences of up to 20 repetitions.

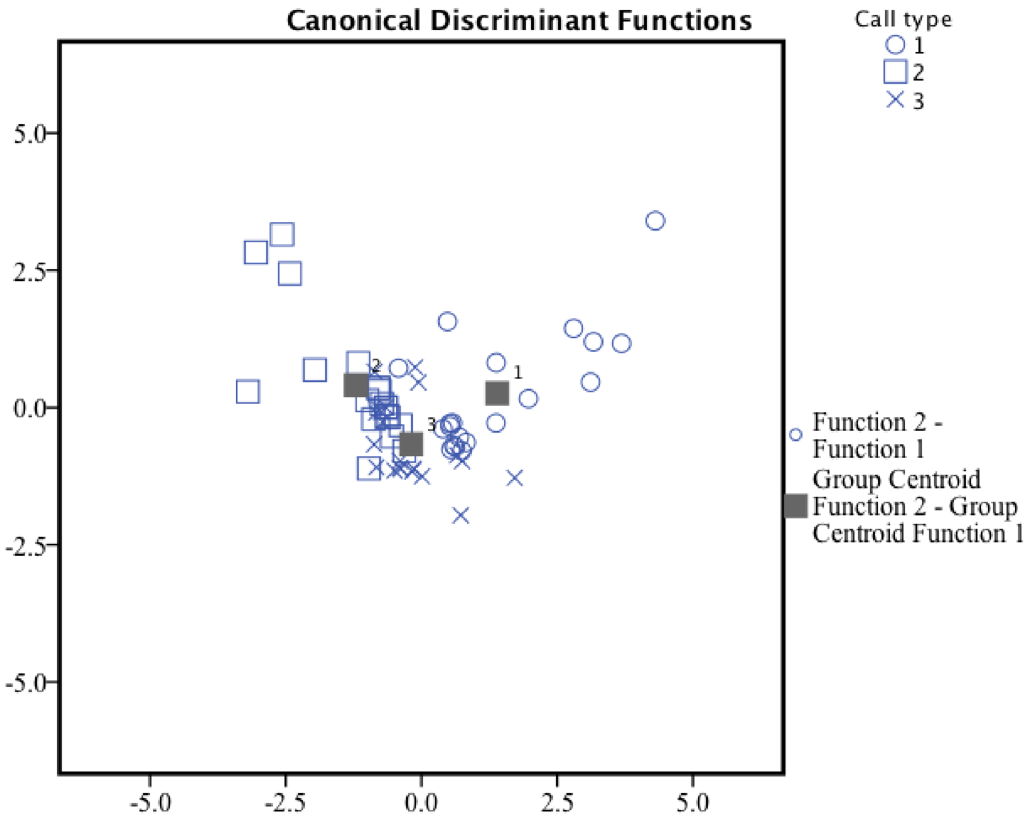

Figure 2.1 Canonical Discriminant Function illustrating the correct classification of the vocalizations into three groups. The classification was 66.7% correct using leave-one-out cross-validation.

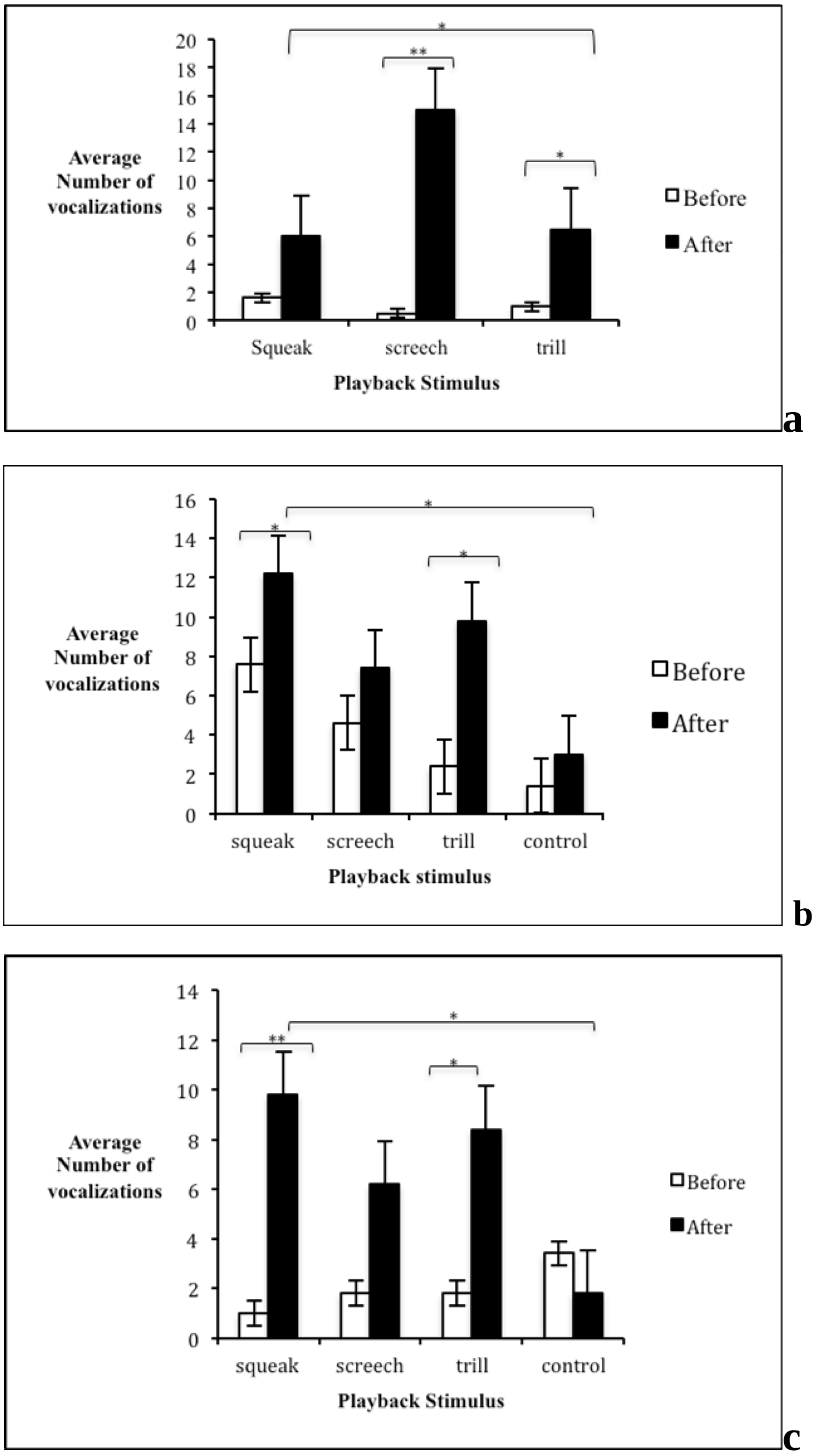

Figure 2.2 Average number of vocalizations produced 30 seconds before and 30 seconds after the playback experiments for a) males, b) females and c) juveniles with. * symbolizes a significant differences where p≤0.05, ** symbolizes a significant difference where p<0.01 for Chi-squared tests. A silent control was not performed in the male pool because the animals were moved away to another aquatic mammal centre in North-Eastern Brazil.

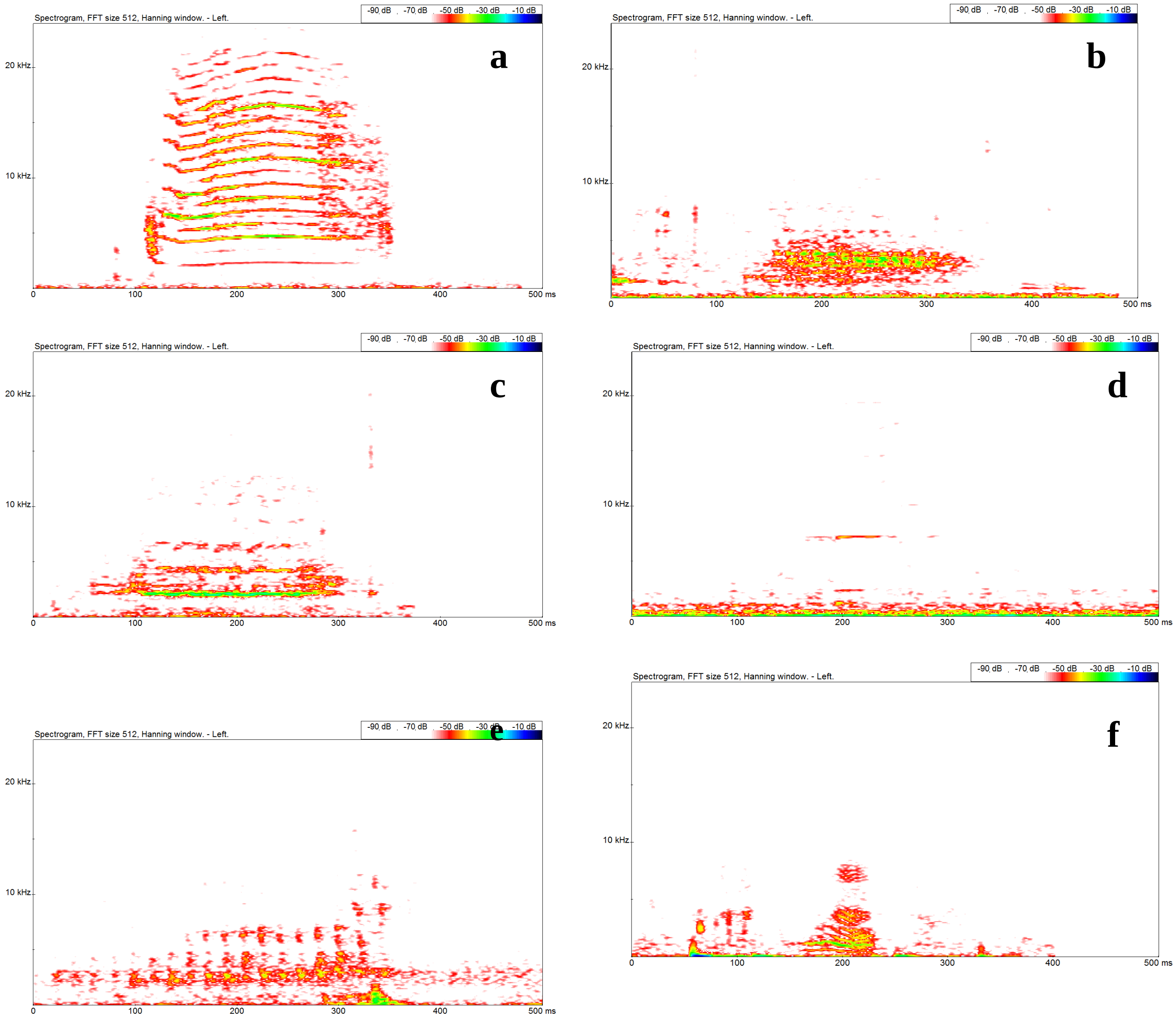

Figure 2.3 Spectrograms of vocalization types: a) squeak, b) screech, c) trill, d) whine, e) creak and f) rubbing produced by the study manatees in CMA/ICMBio.

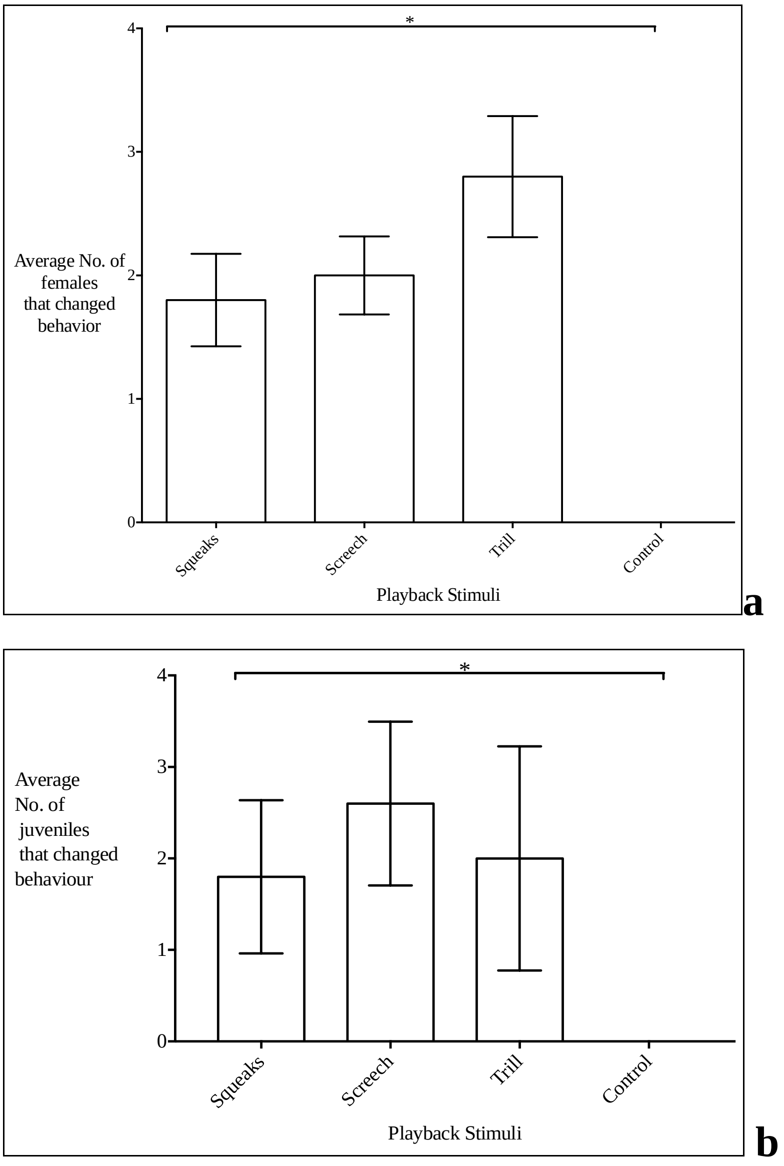

Figure 2.4 The average number of individuals that demonstrated a change in behavior after the playback stimulus was played. Significant differences were found in the number of animals that changed behavior after vocalization stimuli for both a) females and b) juveniles. No manatees were found to change their behavior after the control stimulus was played back. * Demonstrates a differences of $p<0.005$ for Friedman tests with Dunnôs post-hoc.

2.5 Discussion

The captive study Antillean manatees produced a small vocal repertoire, as predicted by the social complexity hypothesis for vocal communication (McCombe and Semple, 2005; Freeberg et al., 2012). Six acoustic signals were recorded and found to be distinguishable through spectrogram analysis and observer hearing. The mean fundamental frequencies of the signals identified in this study ranged from 0.64kHz to 5.23 kHz. Some of these signals had similar duration and peak frequencies to those found by Miksis-Olds et al. (2009) in Florida manatees. Miksis-Olds et al. (2009) identified two vocalization types *chirps* and *squeaks*: the average duration of *chirps* was found to be 222.8ms and the average duration of *squeaks* was found to be 198.4ms. *Squeaks, screeches* and *trills* identified in this study had similar average durations and were common to all age and sex classes. Miksis-Olds et al. (2009) also found that the average peak frequency of *chirps* was 5.097 kHz with the average peak frequency of *squeaks* being recorded as 3.341kHz. The mean maximum frequencies of vocalizations identified in this study were generally lower than the values found for Florida manatees (Miksis-Olds et al. 2009). We found that *whine* vocalizations were specific to adult male manatees, *creak* vocalizations were specific to adult females and *rubbing* sounds were specific to juveniles. These findings together with the differences in vocalization structure could allow for gender identification of manatees through the recording of manatee vocalizations in the wild and would aid in the monitoring of this species.

Gender differences were detected in the vocalization structure of the manatees included in this study: females exhibited higher values for syllable duration and lower values of mean maximum and minimum fundamental frequencies compared to vocalizations produced by the males in this study. Gender differences in vocalization structure in captive killer whales have also been found (Dahlheim & Awbrey 1982). Male bottlenose dolphin calves, *Tursiops truncatus,* have also been found to be more likely to produce signature whistles that were similar to their mothersô signature whistles than female calves (Sayigh et al. 1995). Age differences in vocalization structure were observed in our study animals, where vocalizations produced by juveniles were found to have longer syllable duration and higher mean maximum and minimum fundamental frequencies compared to adult males and females. This corresponds to Sousa-Lima et al.ôs (2008) findings that calves produced higher values for all acoustic variables except the number of harmonics. Nevertheless, Sousa-Lima et al. (2002) found that the vocalizations of female Amazonian manatees had greater values of mean maximum and minimum fundamental frequencies compared to vocalizations produced by males. They also found that Amazonian calves had lower values of mean note duration and greater values of fundamental range compared to the vocalizations produced by males and females. These differences between Amazonian and Antillean manatees gives some evidence of interspecific variation in vocalization structure in manatees.

The study males, females and juvenile Antillean manatees all responded, with vocal and postural responses, to their own vocalizations when artificially played underwater, indicating that Antillean manatee vocalizations may not be used exclusively for contact between mothers and calves as previously suggested (Hartman 1979). Acoustic signals associated with different types of behaviors other than mother-calf contacts have been observed in other marine mammals. For instance, vocalizations have been associated with courtship, foraging, excitement and distress in bottlenose dolphins and Atlantic spotted dolphins, *Stenella frontalis* (Herzing 1996). Signature whistles have also been found to be produced by female bottlenose dolphins as well as by mature males and male calves (Sayigh et al. 1995) and are thought to be involved in individual recognition (Caldwell et al. 1990). Vocalizations are also used as reproductive advertisement displays in humpback whales, *Megaptera novaeangliae,* and bowhead whales, *Balaena mysticetus* (Tyack, 2000). The reactions of the Antillean manatees in this study to the playback experiments (i.e. an increase in vocalization rate and a change in their postural behavior) also introduce the possibility of playing manatee vocalizations in the wild to survey the species. This is based on the fact that vocalization types differed between individuals of different ages and sexes and that the study manatees often exhibited investigatory behavior after the playback experiments. Such findings may help to estimate the structure of the manatee population in wild areas where it is difficult to see the animals due to high water turbidity, for example, commonly observed in estuarine areas inhabited by Antillean manatees in Northeast Brazil.

Since *squeaks* appeared to induce a large response they therefore, may be a good candidate when attempting to monitor species numbers via vocalization playback. Automated recording systems provide the ability to sample areas for extended periods of time and allow for sampling to continue throughout the night and in poor weather conditions, thereby increasing the probability of detecting a given species (Bridges & Dorcas 2000; Mellinger et al. 2007). This system allows for decreased disturbance to the animals and therefore decreases the likelihood of missing an easily disturbed (Bridges & Dorcas 2000) and discrete species such as *Trichechus manatus manatus*. Acoustic surveys have already been used to monitor species of marine mammals (MacDonald & Moore 2002; Moore et al. 2006) and have been found to detect one to ten times more cetacean groups than visual surveys (MacDonald & Moore 2002; Mellinger et al. 2007). Thus they provide an alternative method to surveying marine species, especially those that are difficult to observe such as manatees.

The description of the different vocalizations produced by the manatees in this study introduce the possibility of using vocalizations together with the vocalization playback as a lower cost and more precise identification and monitoring method of wild Antillean manatees when compared with other techniques such as aerial surveys (e.g. Alves et al. 2013). Acoustic surveillance and monitoring of Antillean manatees in the wild may facilitate systematic surveys and aid to our understanding of wild populations (Lefebvre et al. 2001). However, further comparisons of the vocalizations produced by the manatees in captivity to those produced by manatees in the wild would be necessary before an effective implementation of such monitoring method. Further studies investigating whether manatees use specific vocalizations in specific behavioral situations such as stimulating or distressful situations would also provide valuable insight into the vocal behavior and social communication of Antillean manatees.

2.6 Acknowledgements

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3 Activity budget in captive Antillean manatees (*Trichechus manatus manatus*) and preliminary effects of boat noise on juvenile vocal and postural behaviors.

***Nota:** o presente capítulo será submetido para a revista Marine Mammal Science. O mesmo foi colocado nas regras da mesma, entretanto as figuras e tabelas foram inclusas no decorrer do texto para facilitar a leitura. Este trabalho foi feito em colaboração com pesquisadores do Laboratório de Etologia e do Centro de Mamíferos Aquáticos de Itamaracá, os quais serão co-autores do estudo.*

3.1 Abstract

Although advances have been made on the effects of boat noise on Florida manatees (*Trichechus manatus latriostris),* to our knowledge, studies on such effects on the geographically and genetically isolated Antillean manatees (*T. manatus manatus*) are still scarce. Thus, the aims of the present study were i) to report Antillean manatee behavioral patterns, ii) to identify the effects of boat noise on their vocal and postural behaviors in captivity and iii) to investigate the extent of overlapping between Antillean manatee vocalizations and boat noise. Behaviors were recorded using scan and focal observations. Seven behavioral categories were identified for the study animals (7 females, 4 males and 4 juveniles). Gender and age differences were found in the use of these behaviors: females spent most of their time *resting* on the water surface, while males stayed *submerged* and juveniles were mostly engaged in *locomotion* and *social* behaviors. Playback experiments using motorized jangada, catamaran, fishing boat and control stimuli were carried out for the juveniles. Vocalizations and behaviors were recorded throughout the playback experiments. No vocal or postural responses were apparent for the control stimuli, whereas changes in vocal and postural behaviors were observed in response to the boat noise stimuli. The immediate response of the animals was generally a flee-response and a reduction in vocalization production. Motorized jangada stimuli were found to have the greatest impact on Antillean manatee vocalizations and behavior, with the largest number of individuals altering their behaviors. Boat noise frequencies were also found to overlap with manatee vocalization frequencies. The results of the present study brings evidence on the negative effects of boat noise on captive Antillean manatee behavior, suggesting that there should be a limit in the use of motorized jangadas commonly observed in wild areas of known occurrence and reintroduction of Antillean manatees in Brazil.

Keywords: Vocalizations, Flight response, Noise pollution, Conservation**.**

3.2 Introduction

Hartman (1979), described manatees as mildly social but essentially solitary animals. Most manatee associations are temporary, with the exception of mother-calf relationships (Van Meter 1989). The strongest associations are thought to be formed between individuals of the same sex, with males generally being more social than females (Van Meter 1989; Koelsch 1997). Dominance hierarchies are not thought to exist in manatee communities, however individuals are often seen imitating the behaviors of others (Van Meter 1989). Manatees spend between two and twelve hours resting per day and spend between six and eight hours feeding (Van Meter 1989; Reynolds & Odell 1991). Studies have demonstrated that manatees exhibit behaviors such as social, mill, travel, rest and feed (Miksis-Olds & Tyack 2009) and that they may select behaviors depending on efficiency and nutrient intake (Horikoshi-beckett & Schulte 2006).

The effective range of acoustic communication depends on several factors such as ambient noise and the frequency and amplitude of individual vocalizations (Miksis-Olds & Tyack 2009). When the frequency of environmental noise overlaps the frequency of vocalizations, important signal information may be lost and communication becomes limited (Miksis-Olds & Tyack 2009). This lack of communication may result in the loss of contact between manatee mothers and calves, which could potentially affect calf survival (Bengston & Fitzgerald 1985). Communication theory proposes several ways in which a sender can modify an acoustic signal to compensate for environmental noise, such as increasing the repetition of a signal, increasing its duration and altering the frequency of a signal (Miksis-Olds & Tyack 2009). Studies have demonstrated that some species of marine mammals, such as bottlenose dolphins, *Tursiops truncatus*, and Indo-Pacific humpback dolphins, *Sousa chinensis*, often alter their vocal behavior in the presence of boats (Scarpaci et al. 2001; Van Parijs & Corkeron, 2001). Florida manatees have also been found to modify their vocalization structure and rate of emission in the presence of environmental noise (Miksis-Olds & Tyack 2009). However, there is limited knowledge on the effects of environmental noise on the vocal and postural behavior of the Antillean manatee, a subspecies under threat of extinction (MMA 2014) in Brazil, which is both geographically and genetically isolated from Florida Manatees (Alicea-Pou 2001; Hunter et al. 2012).

Boat noise may directly affect manatee behaviors such as feeding and nursing (OŝShea 1995). Nowacek et al. (2004) found that *T. manatus latriostris* demonstrated a flight response as a result of the detection of boat presence. Research has also indicated that the time Florida manatees spend foraging in sea grass beds is negatively correlated to the number of boats passing per five minutes in the morning hours (Miksis-Olds et al. 2007). These results suggest that Florida manatees select foraging areas with the least ambient noise (Miksis-Olds et al. 2007). Boats may also directly affect manatee mortality: the most common cause of manatee mortality is collisions with watercrafts (Van Meter 1989;

Niezrecki et al. 2003; Nowacek et al. 2003; Phillips et al. 2004), accounting for between 26% and 31% of mortalities annually (Miksis-Olds et al. 2007). Collisions with boats often do not instantly kill manatees, however, they may cause wounds that affect manatee long-term survival and/or their ability to reproduce (Van Meter 1989; OôShea 1995). In 2001, the number of registered boats in Florida increased to over 900,000 (United States Coast Guard 2002). As a result a renewed interest in manatee acoustic detection technology has arisen (Oôshea & Poche 2006) in an attempt to reduce the number of manatee-boat collisions.

Although there have been advances on how boat noise affects Florida manatees' general behaviors and vocal behavior, to our knowledge, this is the first study to investigate the impact of boat noise on Antillean manatees, *Trichechus manatus manatus*. The present study aims to: i) report Antillean manatee behavioral pattern, ii) identify the effects of boat noise on their vocal and postural behaviors as well as, iii) investigate the extent of overlapping between Antillean manatee vocalizations and boat noise. It is expected that Antillean manatees will reduce vocalization rate in the presence of boat noise and that they will demonstrate an immediate flight response in the presence of boat noise. It is also expected that the fundamental frequency of some manatee vocalization types will overlap with the frequency of boat noise.

3.3 Methodology

3.3.1. Animals and Study site

The study was carried out in the *Centro Nacional de Pesquisa e Conservação de Mamíferos Aquáticos and Chico Mendes Institute of Conservation and Biodiversity* (CMA/ICMBio) on Itamaracá Island in North Eastern Brazil. The temporal behavioral study was carried out between January 2015 and April 2015 and included seven adult females, four juveniles (three males and one female) and four adult males: each group was in a separate pool. The male and female pools were 10.1m wide x 4.15m deep and the juvenile pool was 8m wide x 5m deep. The boat noise playback experiments were carried out during May-June 2016 and included the juvenile pool, which at the time of the experiment had an adult male placed in it too, i.e. the experiment included four juveniles and one adult male. Females were not included in this study as two females had recently died and it was decided that the playback experiments could cause them further stress in case of a negative response to stimuli. The adult males were not included in the study since they had been previously transferred to the *Associação Peixe-boi* in Porto de Pedras - Alagoas, Brazil.

3.3.2. Temporal Behavior Study

Two methods of behavior analysis were used: scan and focal sampling (Altmann 1974). Scan sampling involved observing the behavior of all animals in each pool every five-minutes: a total of 100 hours were recorded for seven females, four males and four juveniles. Focal sampling included 20, five-minute sessions for each individual included in the study: eight females and seven males. A total of 428 sessions were recorded. Male juveniles were mixed with adult males, as was the female juvenile included in the adult female pool, during the focal observations. There was therefore, eight females in one pool, three adult-juvenile mixed males in another pool and five adult-juvenile mixed males in a third pool. Behavior was observed between 6am and 6pm for all behavioral sampling and each group was observed on alternating days. A full list of behaviors included in the study can be found in Table 9.1.

3.3.3. Boat Noise Playback

Three different boat recordings and one silent control recording were used in the playback experiments: motorized jangada, fishing boat and catamaran (see Fig. 3.1 for spectrograms of each stimuli and Appendix iii for images of boats). The boat noises were obtained in Itamaracá Island estuarine area (7.8105° S, 34.8391° W) from a 20m distance from the three boats types using a SQ26-H1 hydrophone model (linear frequency response: 0.02kHz to 45kHz, +3/-12 dBs) which was placed inside of a closed pipe one meter long, with holes drilled into the sides and bottom and connected to a Zoom H1 recorder (linear frequency response: 20Hz to 20 kHz). Antillean manatees that have previously been released by the CMA/ICMBio are known to visit this estuarine area (Attademo et al. 2015). Three repeats lasting six seconds were used for each boat type and control stimuli. This length of time was used to avoid greatly stressing the animals while still exposing them to the playback stimuli. A different sound file from each of the different stimuli was used in each repeat. A silent control was selected to expose the animals to any extra noise that may have been emitted through the speaker and that could potentially elicit a response (adapted from Miksis-Olds et al. 2007b). A control of underwater ambient noise was not used since the manatees were in captivity and the ambient noise was consistent for all playback experiments. During the playback experiments manatee vocalizations were recorded using the same recording equipment described above to record the boat noises. Video recordings of manatee behavior were also captured during the playback experiments using a Cannon Legria HFR38 camera. The stimuli were played underwater using a LL916 Underwater Speaker with AC203E Circuit Master (linear frequency response of 200Hz - 23kHz, +/-10dB), connected to a 14watt battery. The speaker was placed 50cm under the water surface, suspended by rope attached to the top of the dividing gates, to simulate the position of a boat in water and the sound was played at a volume subjectively simulating a distance of 20m from the boat in relation to the manatees. The playback sessions lasted 20 minutes (i.e. 10 min monitoring the animals before and after the playback of the 6s stimulus) and were performed once in the morning and once in the afternoon, every 48 hours to avoid the habituation of the manatees to the playback stimulus and to avoid additional stress to the animals' routines at CMA/ICMBio.

3.3.4. Data Analysis

The vocalizations produced by manatees were analyzed using Batsound 4 (Pettersson Elektronic, Uppsala, Sweden). 48kHz and 16bits were used as the sampling rate for input, editing and output of sound files. Spectrogram and power spectra settings were: FFT size=512, threshold=12 and Hanning window. The video recordings were analyzed and behaviors scored using Iphoto video player.

3.3.5. Statistical Analysis

Chi-squared tests were used to determine whether performance of behaviors followed an even distribution within each manatee group. Furthermore, a binomial Z test was used to determine differences between groups for each behavior recorded during scan sampling observations. Kruskal Wallis tests with Dunnôs post-hoc were used to determine whether there were differences between groups for behaviors recorded during focal sampling observations for the three animal groups. A Cochranős Q test was used to compare manatee change in behavior during the boat noise and control playback trials. Friedman tests with Dunnős post-hoc were used to determine significance between the number of individuals that immediately dived (i.e. had a flight response) when playback stimuli were played and the number of individuals that swam towards the noise source after each playback stimuli stopped playing.

Table 3.1 An ethogram constructed by the authors involved in the behavioral observations during this study, using the ethogram provided by the CMA/ICMBio as a reference, illustrating the behaviors included in the behavioral analysis. Previous ethograms including manatees in captivity at the CMA/ICMBio, for example Anzolin et al. 2014, were also used for behavior references.

Behavior Category	**Behavior**	**Description**
Locomotion	Drift alone	Slow movement, no use of flippers, animal is alone
	Group drift	Slow movement, no use of flippers, animal is with one or more individuals
	Swimming	Faster movement using flippers and/or tail
	Swimming inverted	Faster movement using flippers and/or tail, stomach facing upwards
	Diving	Whole body moves towards the bottom of the pool, back breaking surface of water
Rest	Rest alone	Still, alone
	Group rest	Still, one or more individuals together
	Rest inverted	Still, lying on back
	Rest at the side of the pool	Flipper holding side of wall, lying against side of pool, still
	Rest on side	Still, lying on side of body
Investigate	Interacting with wall/gate	Chewing the wall/gate, head is out of the water
	Body out of water	Upper part of body is out of water at the side of the pool, supporting itself with flippers
	Head out of water	Head of the animal is out of the water
Social	Embrace	Use of flippers to embrace another individual

	Chewing another individual	One individual chews the body of another
	Snout touch	The snout of one individual touches the snout of another
Feeding	Feeding	Ingesting food items
	Attempting to feed	Observed mainly with juveniles: juveniles who no longer feed from bottles try to feed from a bottle placed at the edge of the pool
	Drinking	Drinking fresh water from a tap placed at the edge of the pool
Submersed	Submersed	Individual is underwater and it is not possible to clearly observe their behavior due to water turbidity
Other	Chewing flippers	An individual is observed chewing their own flippers
	Breathing	An individualôs snout breaks the waterôs surface and they breathe
	Tail above water surface	An individualôs tail is above water and the rest of the body is submerged and out of sight
	Slapping water	The individual slaps the water using its tail or body
	Moving mouth	An individualôs head is out of the water and their mouth is observed to be moving in a chewing movement

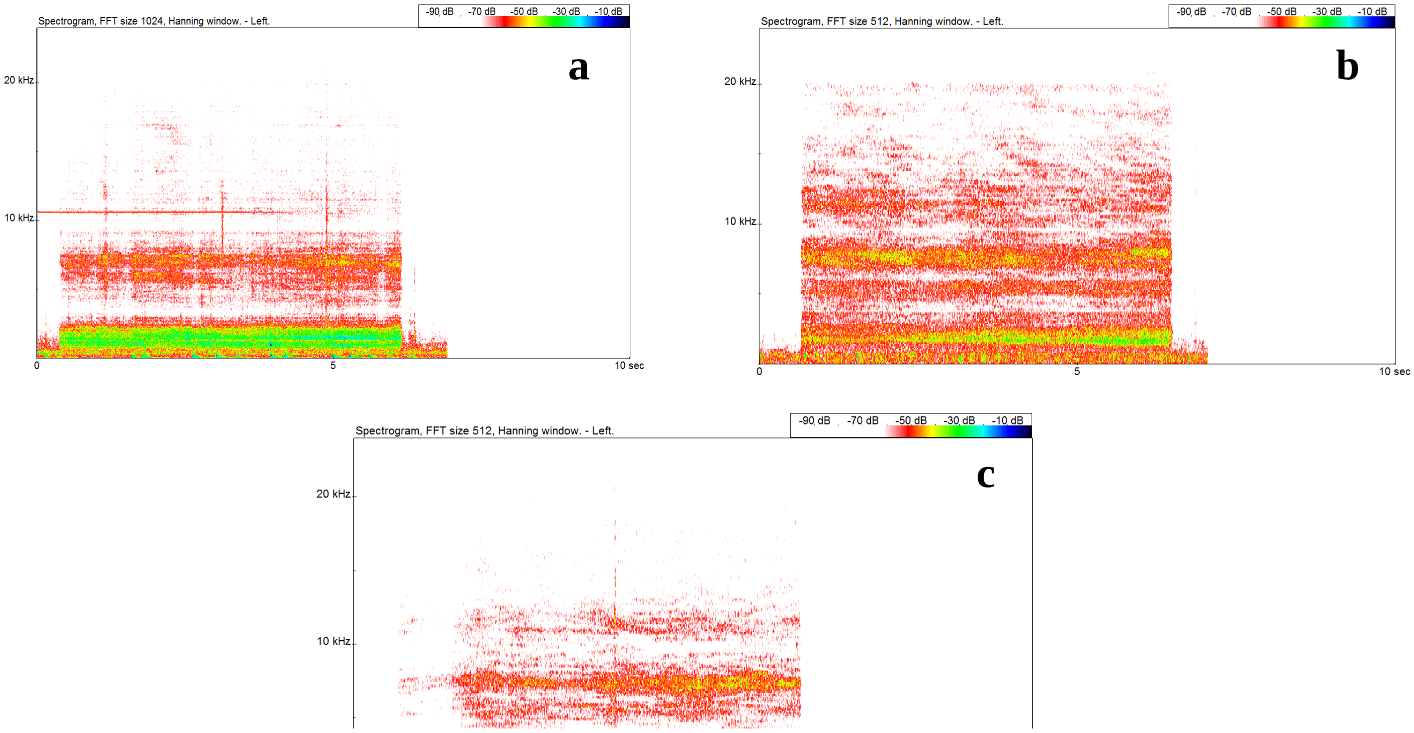


Figure 3.1 Spectrograms of a) catamaran b) motorized jangada and c) fishing boat playback stimuli

3.4 Results

9.4.1. Activity Budget

Manatees were observed to demonstrate behaviors that could be divided into seven categories: locomotion, rest, investigate, social, feeding, submerged and other (Table 3.1). Significant differences were found between the number of times each behavior was observed during scan sampling within the female, $X^2=512.07$, $d.f.=6$, $p<0.001$, male, $X^2=77.82$, $d.f.=6$, $p<0.001$ and juvenile groups, $X^2=42.40$, $d.f.=6$, $p<0.001$. (Fig. 9.2), showing that the behaviors were not evenly performed by manatees in captivity.

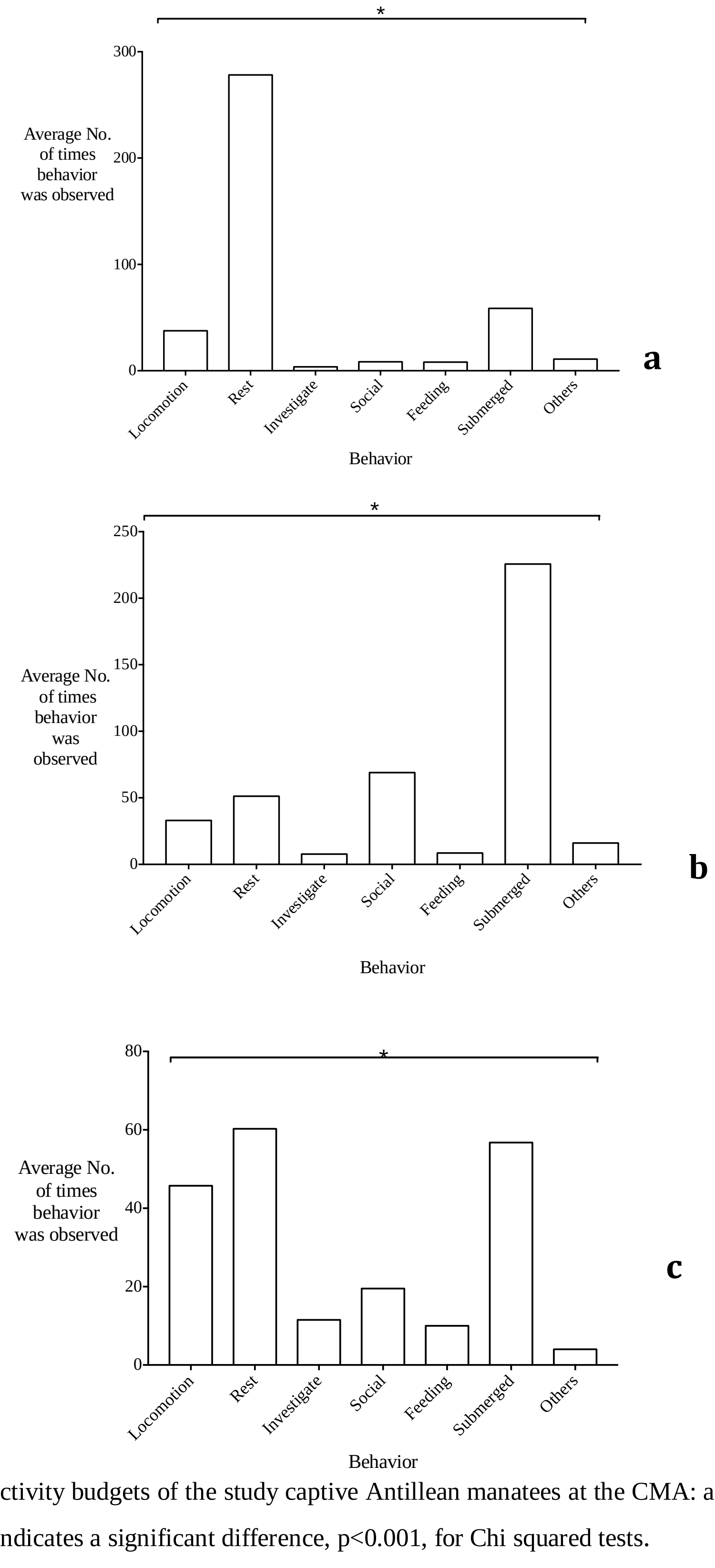


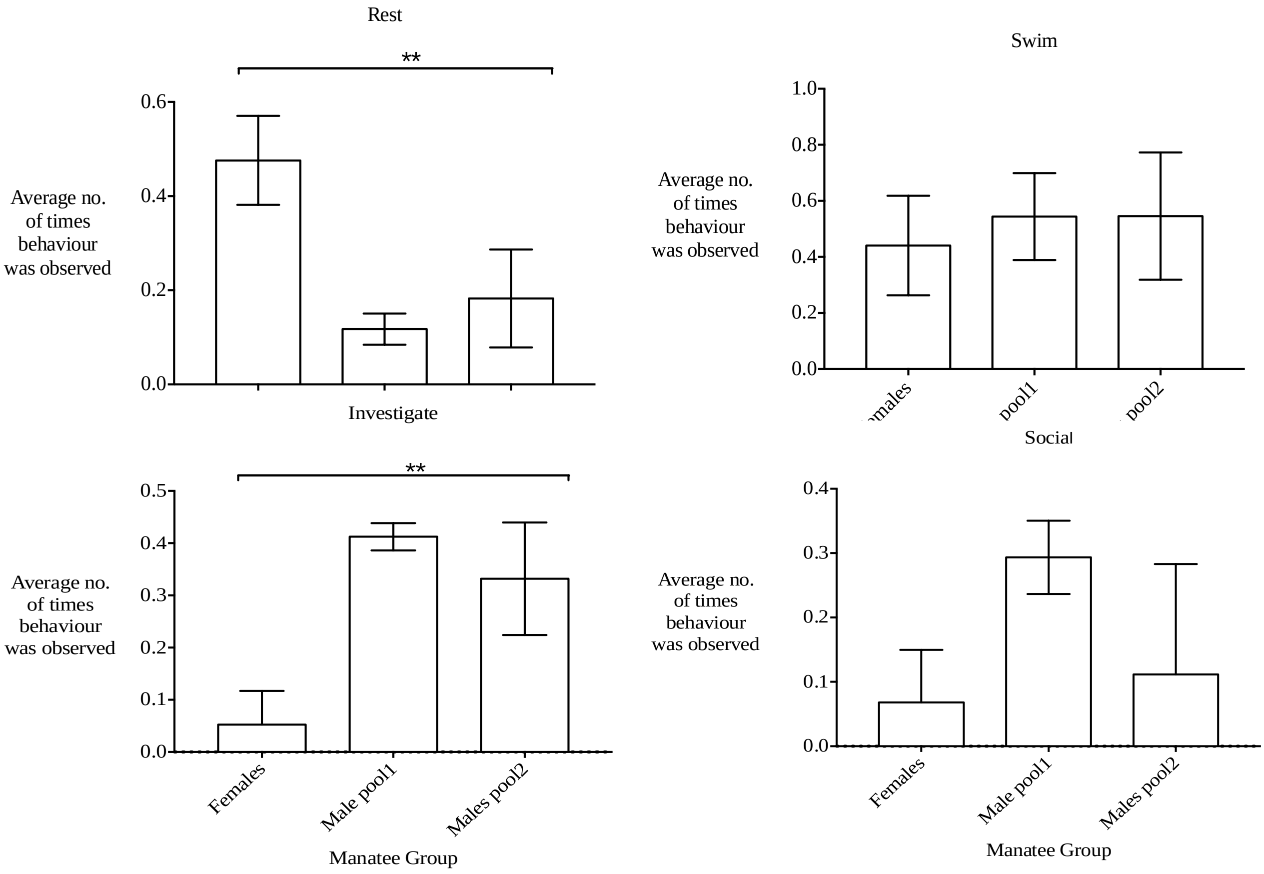
Figure 3.2 Activity budgets of the study captive Antillean manatees at the CMA: a) females, b) males and c) juveniles. * indicates a significant difference, p<0.001, for Chi squared tests.

Significant differences were also found for the number of times a behavior was observed during scan sampling between manatees groups (Table 3.2).

Table 3.2 Results of binomial Z tests of the number of behaviors recorded during scan observations. Significant differences were found between females, males and juveniles for all behaviors.

Behavior	**Z^2**	**Critical Z score**	**Degrees of freedom**	**p**
Locomotion	45.3	2.58	2	p<0.001
Rest	2486.7	2.58	2	p<0.001
Investigate	6.3	2.58	2	p<0.05
Social	209.8	2.58	2	p<0.001
Feeding	6.5	2.58	2	p<0.05
Submerged	475.6	2.58	2	p<0.001
Other	38.8	2.58	2	p<0.001

Kruskal Wallis tests with Dunnôs post-hoc demonstrated a significance between the number of times *rest, H=10.76, d.f=2, p=0.0002, investigate, H=10.96, d.f=2, p=0.0001 submerged, H= 8.42, d.f.=2, p=0.0043* and *other, H=8.77, d.f.=2, p=0.0028,* behaviors were observed during focal sampling between females, males in pool 1 and males in pool 2 (Fig. 9.3).



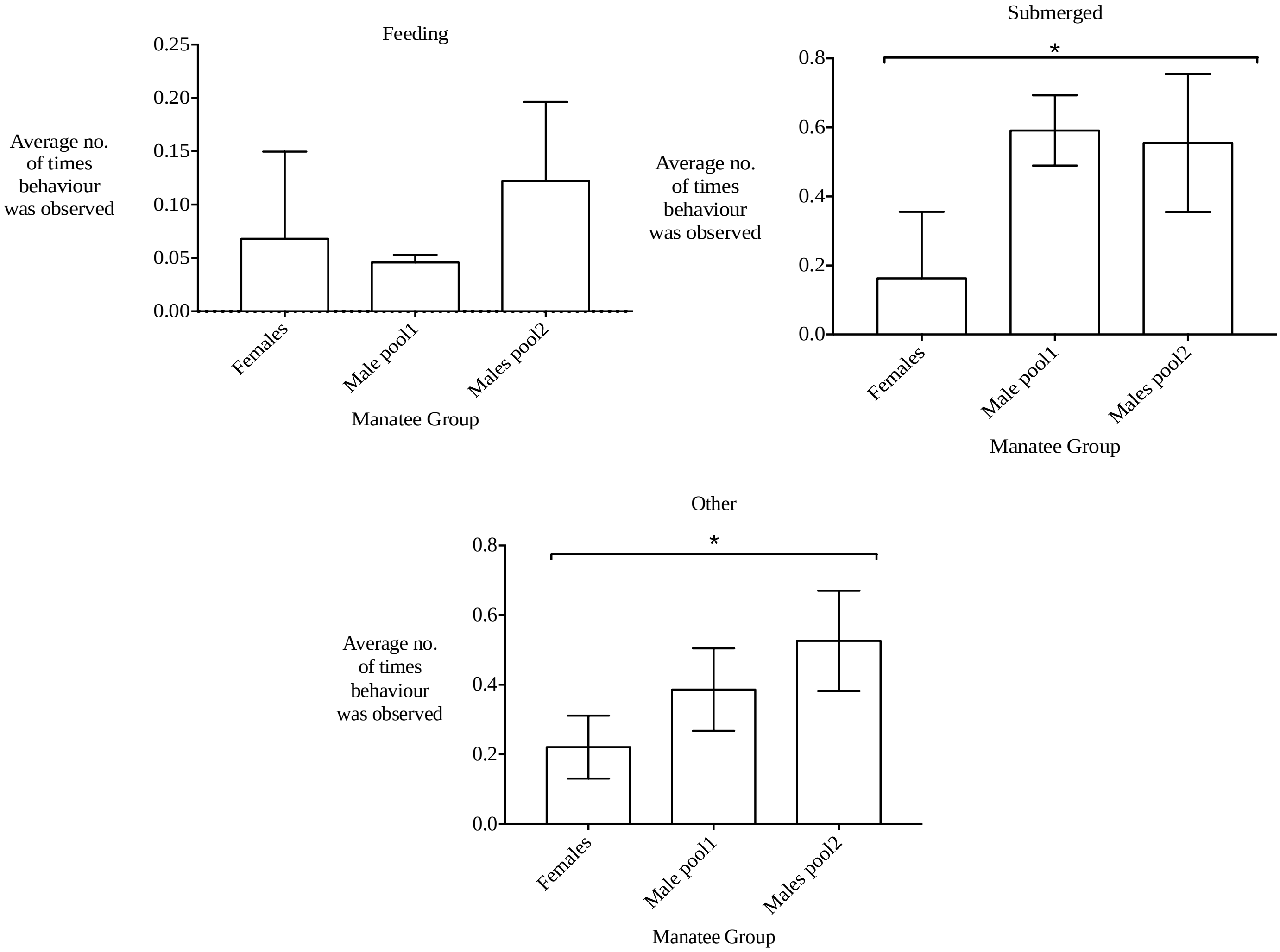


Figure 3.3 Average number of times a behavior was demonstrated for females, males in pool 1 and males in pool 2 during focal behavioral observations for all behaviors. * indicates a significance of p<0.005, and ** indicates a significant of p<0.0005.

3.4.2. Boat Noise Playback

There was a significant change in manatee behaviors when comparing the boat noise with the control playbacks for juveniles, $Q=155.25$, $df=3$, $p<0.05$. A Friedman test with Dunnõs post-hoc demonstrated no significant difference between the average number of animals that dived during playback experiments, $X^2=6.21, d.f.=3$ $p=0.1$ (Fig. 9.4).

A significant difference was found for the number of juveniles that swam in the direction of the noise when each playback stimuli stopped playing, $X^2=7.88$, *d.f.=3* $p<0.05$ (Fig. 9.5).

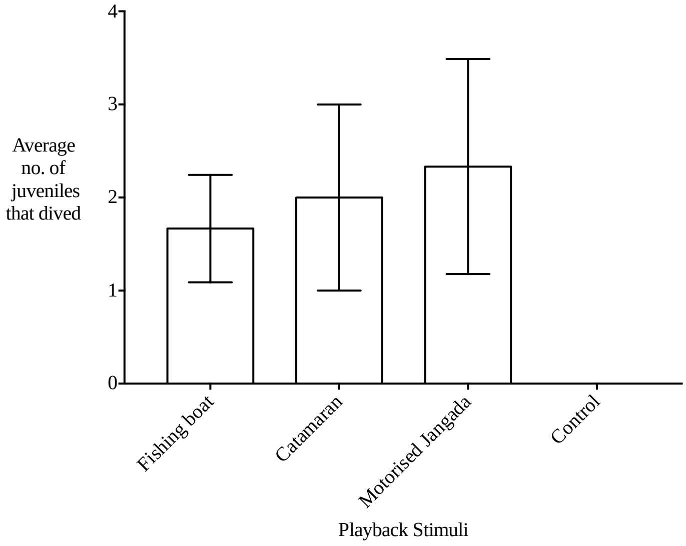


Figure 3.4 Average number of juveniles that dived when playback stimuli were played.

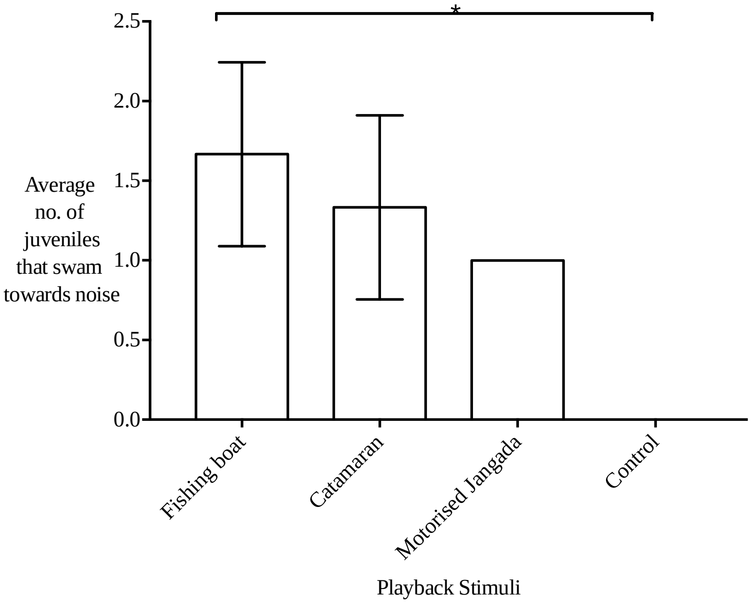


Figure 3.5 Average number of juveniles that swam towards the noise source when the playback stimuli stopped. * indicates a significant difference of $p<0.05$ for a Friedman test with Dunnôs post-hoc.

Chi-squared tests were used to determine if there was a significant difference between the number of juveniles that dived and swam towards the noise source during the first experiment and last experiment. For animals that dived, Chi-squared tests demonstrated no significant differences for fishing boat, $X^2=1.07, p=0.3$, catamaran, $X^2=1.07, p=0.3$ and motorized jangada, $X^2=1.85, p>0.1$. For animals that swam towards the noise sources after the playback stimuli stopped playing, Chi-squared tests demonstrated no significant difference for fishing boat, $X^2=1.07, p=0.3$, catamaran, $X^2=1.60, p>0.2$ and motorized jangada, $X^2=1.72, p>0.2$.

The average number of vocalizations produced one minute before, during and one minute after the playback stimulus was played was found to be significantly different for juveniles for all playback stimuli: motorized jangada, $X^2=7.71, d.f.=2, p<0.05$, catamaran, $X^2=8.11, d.f.=2, p<0.05$, fishing boat, $X^2=4.86, d.f.=2, p<0.05$ and control, $X^2=6.38, d.f.=2, p<0.05$ (Fig. 9.6).

3.4.3.Vocalization overlapping

High and low frequencies of all three types of boat noise were found to mask the average high and low frequencies of each vocalization type found to be produced by manatees (Tables 3.3 & 3.4).

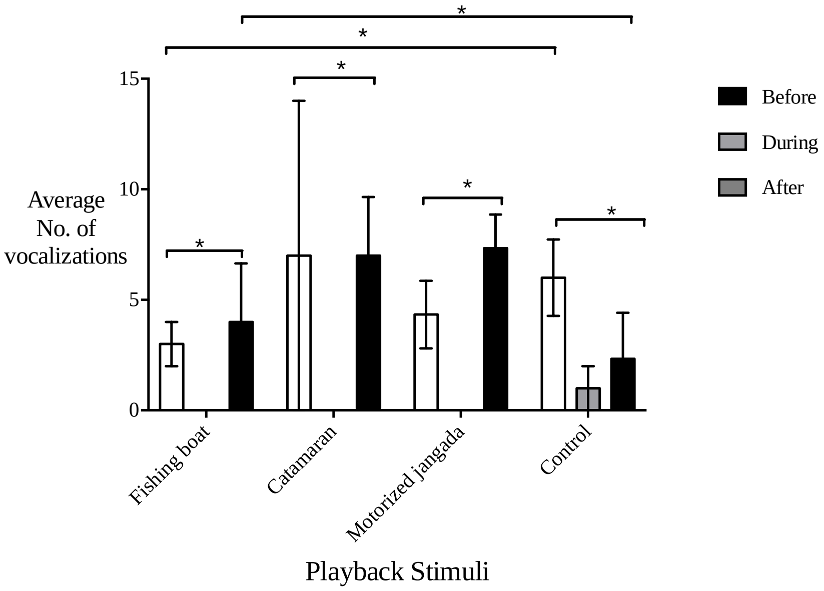


Figure 3.6 The average number of vocalizations produced one minute before, during and one minute after playback experiments. * indicates a significant difference of $p<0.05$

Table 3.3 Average high and low frequencies for each type of vocalization found to be produced by Antillean manatees (results of vocalization analysis in Chapter 1).

Vocalization	**Manatee Group**	**Average High Frequency (kHz)**	**Average Low Frequency (kHz)**
Squeak	Females	2.49	1.78
	Males	2.90	2.22
	Juveniles	3.83	2.59
Screech	Females	2.01	1.97
	Males	2.91	1.42
	Juveniles	4.34	2.70
Trill	Females	3.37	2.52
	Males	5.10	2.69
Creak	Females	3.81	2.13

Whine	Males	5.23	4.56
Rubbing	Juveniles	3.38	0.64l

Table 3.4 High and low frequency values for each type of boat noise used in playback experiments (n=3 for each stimuli).

Boat Type	**Average High Frequency (kHz)**	**Standard Error**	**Average Low Frequency (kHz)**	**Average Frequency of Maximum Energy (kHz)**	**Standard Error**
Catamaran	8.1	0.17	<0.1	1.3	0.24
Motorized jangada	19.7	0.15	<0.1	1.9	0.05
Fishing boat	19.3	0.79	<0.1	1.1	0.26

3.5 Discussion

Seven behavioral categories were identified in this study. The number of times each behavior was demonstrated was found to be significantly different within each manatee group. Females demonstrated *rest* behavior significantly more than any other behavior, males demonstrated *submerged* behavior significantly more than any other behavior and juveniles demonstrated *locomotion, rest* and *submerged* behavior more than the other behaviors. It has been suggested that manatees in captivity spend significantly more time resting than manatees in the wild (Horikoshi-Beckett & Shulte 2006). This is thought to be an energy saving method, since manatees in captivity receive food once a day, they may remain stationary to conserve energy (Horikoshi-Beckett & Shulte 2006). This could explain the high frequency of resting behavior observed for females since they are generally larger than males (Odell 1982) and therefore, the need to conserve energy should be greater. The high frequency of *resting* behavior demonstrated by females could also be due to the high number of manatees in a single pool of dimensions 10.1m x 4.15m. Seven females occupied a pool the same size as a pool containing four males and therefore, locomotion may not have been facilitated for females. Males in pools 1 and 2 demonstrated significantly more *investigatory* behavior than females, this may be partly due to the fact that males were generally more active than females. Males in pools 1 and 2 were often observed to support their upper bodies out of the water on the side of the pool, a behavior that was not commonly observed in females. This may be another example of females conserving energy and not participating in high-energy cost activities. Males generally demonstrated more *social* behavior than females, a finding that is supported by existing studies for Florida manatees (Van Meter 1989; Koelsch 1997). There was no significant difference between the occurrence of *feeding* behavior in males and females since they received food at the same controlled time every day. Males in pool 2 demonstrated a higher frequency of feeding, which may be due to the fact that two of the individuals in pool 2 were juveniles and still received bottle feeds at controlled times twice a day as well as receiving other food at a controlled time each day. The results demonstrate a significant difference between the number of times *submerged* behavior was observed for males and females. In the wild manatees may rest on the sea floor, however, they have also been found to feed and swim slowly while submerged (King & Heinen 2004). It was not always possible to observe behaviors when manatees were submerged due to water clarity however, since females demonstrated less submerged behavior than males this may suggest that submerged behavior could be associated with *locomotion* behaviors. Future studies using movement and energy expenditure loggers (e.g. Wilson et al. 2015) would help to clarify the animals' behaviors when submerged in both captive and wild individuals

It was expected that in the presences of boat noise, manatees would reduce vocalization rate and that they would exhibit an immediate flight response. The results of this experiment allow for the null hypothesis to be rejected: no vocalizations were recorded during the time when each boat stimulus was played and at least one individual dived for each boat stimuli. The number of vocalizations was found to increase after the playback stopped for motorized jangada and fishing boat stimuli. The greater vocal response after the playback suggests that manatees may communicate with each other once a potential threat has passed, possibly, to determine whether another individual has been harmed. The motorized jangada stimuli induced the greatest vocal response after the playback stopped, it also caused the greatest number of animals to dive during the playback and the least number of animals to swim towards the noise source after the playback stopped. Combining all three factors (i.e. avoidance behaviors), the results suggest that the motorized jangada stimulus was the most distressful of the four stimuli. These results confirm the results found by Nowacek et al. (2004) and Miksis-Olds et al. (2007b), who found that personal water-crafts, <4.9m long, using an inboard motor that powers a jet pump, induced the greatest flight responses in Florida manatees. These results may be of great importance since motorized jangadas are one of the most common boat types used in manatee habitats in North-eastern Brazil and are often used in eco-tourism in areas such as Barra de Mamamguape -Paraíba, Itamaracá ï Pernambuco and Porto de Pedras, Alagoas - (pers. obs.). This may introduce the possibility of conservation methods aimed at limiting the use of motorized jangadas in areas where manatees are known to frequent. The fishing boat stimuli appeared to be the least distressful, but most threatening boat noise stimulus, with the lowest number of individuals diving during the playback and the highest number of individuals swimming towards the noise source after the playback. The reaction of the manatees in this study may explain why manatees most commonly collide with fishing boats: the lack of a flight response may suggest that manatees have more difficulty in hearing fishing boats approaching and therefore do not dive until it is too late. Thus qualifying these boats as the most threatening to manatees. Notably, the individuals involved in this study were either born in captivity or were taken to the center very early in their lives (~7 days). This study demonstrates that manatees that have had no previous encounters with boat noise, exhibit the expected flight response, which has only been recorded in wild manatees (Miksis-Olds et al. 2007b). This suggests that the flight response in manatees is not learned but is an instinctive response-behavior to unknown or stressful noise. Since playback experiments were not carried out during feeding times it is not possible to confirm the results of Miksis-Olds et al. (2007b), that manatees spend less time feeding in the presence of boat noise. It was also not always possible to determine the behaviors of the manatees that were already submerged when the playback stimuli were played.

All boat stimuli frequencies appear to mask the frequencies of manatee vocalizations. A study by Miksis-Olds et al. (2009) found that Florida manatees decreased vocalization rate with increasing noise levels during socializing and feeding behaviors. They suggest that this pattern demonstrates that manatees wait until it is quiet to begin vocalizing while socializing and feeding (Miksis-Olds et al. 2009). The manatees included in this study were also found to wait until the noise stopped to begin vocalizing again. Communication theory suggests that manatees should increase their vocalization effort when the benefits of effective communication outweigh the costs of increased energy expenditure (Tyack 2008; Miksis-Olds et al. 2009). This may explain the lack of manatee vocalizations when the stimuli were played: it was not beneficial for the manatees to increase vocalization source level and maintain communication while the noise was playing. Miksis-Olds et al. (2009), also found that under high noise levels, Florida manatees increased vocalization source levels during milling behavior. They suggest that this is due to the fact that animals are further apart and it is therefore, beneficial for an individual to increase the source level under high noise levels when separated from the group in order to maintain acoustic contact with other individuals (Miksis-Olds et al. 2009). The manatees in this study however, were in a pool of diameter 8m and separation of great distances was not possible. This reinforces the idea of communication theory: that it was not energetically beneficial for the manatees in this study to increase vocalization source levels during playback experiments since they were constantly in close proximity to each other. Nonetheless, further research is necessary to investigate the effects of overlapping between boat noise and manatee vocalizations, especially for females and calves.

No significant differences were found between the number of manatees that dived in the first and last trials for all playback stimuli, nor were significant differences found between the number of animals that swam towards the noise source in the first and last trial for all playback stimuli. This suggests that the individuals involved in the study did not become habituated to the playback stimuli. Behavioral studies investigating the effect of environmental noise on Florida manatee behavior found that in all seven cases of boats approaching within 25m of manatees, a behavioral change in travelling or milling was demonstrated, with animals leaving the area in two of the cases and increasing swimming speed in one case (Miksis-Olds 2006). This suggests that manatees in the wild also do not become accustomed to boat noise and that the captive manatees included in this study were reacting in a manner which has also been noted in wild manatees. Manatee behavioral response to boat noise appears to depend on the distance of the watercraft in relation to the individual (Miksis-Olds 2006). In a study by Miksis-Olds (2006), Florida manatees were not found to increase swimming speed nor were they observed to leave the area when boats passed at a distance of 100m and they were observed to demonstrate a behavioral change only in one instance. The playback stimuli were subjectively played at a volume that simulated a 20m distance from the manatees: this may have resulted in the marked *dive* reaction of manatees during the playback experiments. If the noise had been played at a lower volume, simulating a distance of >50m, the results of this study may have been notably different as is suggested by studies involving wild manatees (Miksis-Olds 2006).

Seven behavior categories were used in this study. Significant differences were found between the number of behaviors demonstrated within groups as well as between groups. Significant differences were found between the number of vocalizations produced before, during and after playback experiments. The number of animals that dived during playback experiments was not found to be significant, however, the number of animals that swam in the direction of the noise source after playback experiments was found to be significant. The results of this study suggest that manatees find motorized jangadas the most distressful of the three boat types used in this study. This may have a great impact on eco-tourism, since this boat type is commonly used to take tourists to known manatee habitats (pers.obs). It could therefore, be suggested that conservation programs should limit the number of motorized jangadas in areas where manatees are known to frequent, in order to minimize stress to manatees. Manatee reactions to fishing boat noise suggest that they find this boat type the least distressing. Nevertheless, it may be the most threatening of all three boat types. As previously mentioned above, it is possible that manatees are unable to hear this type of boat noise and this may be the reason behind the high rates of manatee-boat collisions. The results of this study, therefore, reinforce the importance of research in the area of manatee alarm systems, since such alarms could greatly reduce the number of collisions between manatees and boats. Further studies are necessary to investigate the effects of boat noise on wild Antillean manatees and to determine whether wild manatees demonstrated the same flee response as captive manatees when exposed to the common motorized jangada.

3.6 Acknowledgements

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4 Final Considerations

The results of this research introduce the possibility of using vocalization types and structure as a method of monitoring *Trichechus manatus manatus* populations in the wild, as well as using vocalizations to identify individual manatee age and sex where this may not be visually possible. Based on the results presented in this dissertation, it may also be possible to use vocalization playbacks in the wild to stimulate a manatee response, together with the analysis of vocalization structure and type to determine population composition. This suggested method of population monitoring might prove to be more accurate than visual population surveys as it is not often possible to determine sex and age visually. However, it is necessary to investigate the vocal repertoire and to test this monitoring technique with wild Antillean manatees, as it is possible that they do not produce the same vocal repertoire as captive manatees.

The activity budget results indicate that females spend a significantly larger amount of time resting that males and juveniles. The *CMA/*iCMBio has recently (December 2015) changed the organization of manatee pools, allowing for females to swim between two pools of diameter 10.1m x 4.15m. This is expected to provide more space for females to travel and to improve the female manateesô health. The results presented in this dissertation also indicate that motorized jangadas elicit the greatest flee-response in Antillean manatees. This may have a large impact on the eco-tourism industry in North-eastern Brazil, since this is the most common boat type used to transport tourists. Studies have demonstrated that wild Florida manatees show a similar response to this boat type and it could therefore, be suggested that conservation organizations such as *CMA/iCMbio* attempt to limit the number of motorized jangadas in areas where manatees are known to frequent. It is also necessary to educate local human populations on the impact that boat noise may have on Antillean manatees. *Trichechus manatus manatus* is a species under threat of extinction and it is therefore, necessary that all conservation methods available be implemented in order to protect this endangered species.

5 Appendix A: The submission guidelines for each journal can be found in the websites listed in the table below.

Journal	Website
Mammalian Biology	https://www.elsevier.com/journals/mammalian-biology/1616-5047/guide-for-authors#68000
Marine Mammal Science	http://onlinelibrary.wiley.com/journal/10.1111/(ISSN)1748-7692/homepage/ForAuthors.html

6 Appendix B: Table illustrating previous studies on manatee vocalizations and behavior.

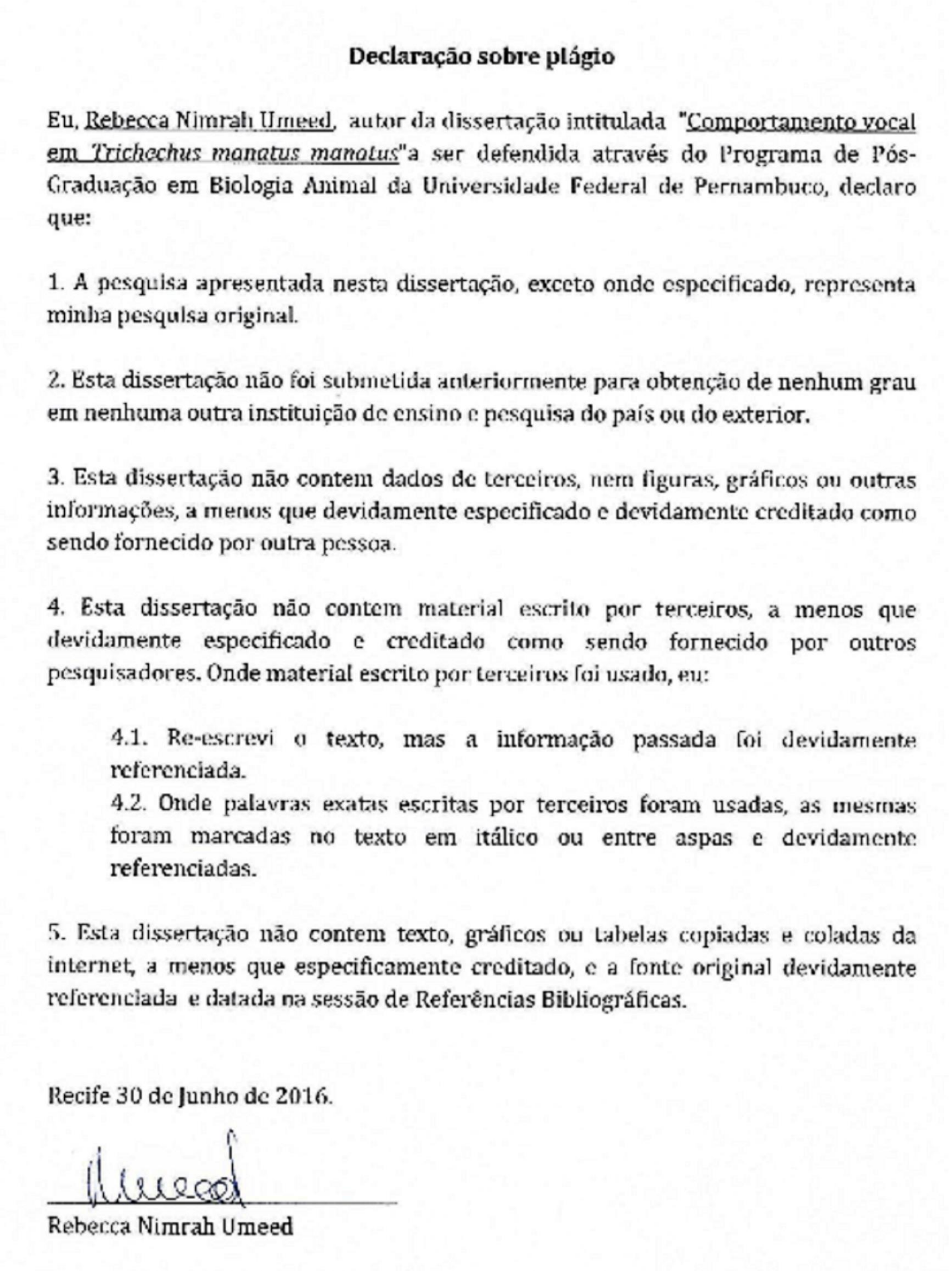
Author	Title	Finding
Bengston and Fitzgerald 1985	Potential Role of Vocalizations in West Indian Manatee.	Florida manatee vocalization rates are dependent on an individualôs behaviour. Potential role of vocalizations in communication.
Caldwell and Caldwell 1985	Handbook of marine mammals.	Manatees have no true vocal cords.
Chapla et al. 2007	CT Scans and 3D Reconstructions of Florida Manatee (Trichechus	Soft tissue of manatee heads has a similar density to that of seawater.

	manatus latirostris) heads and ear bones.	
Chavarría et al. 2015	The relationship between acoustic habitat, hearing and vocalizations in the Antillean manatee (*Trichechus manatus manatus*, Linnaeus, 1758).	Florida manatee produce tonal calls with fundamental frequencies of 0.7kHz-8.1kHz.
Gerstein et al. 1999	The underwater audiogram of the West Indian manatee (*Trichechus manatus*)	Manatee hearing is poorly suited to detect low frequency noise of boats.
Gerstein 2002	Manatees, bioacoustics and boats: hearing tests, environmental measurements and acoustic phenomena may explain together why boats and animals collide	Florida manatees are unable to detect noise from idling boats.
Ketten et al. 1992	Structure, function and adaptation of the manatee ear.	Florida manatee hearing thresholds are between 0.4kHz and 46kHz. Range of best hearing is between 6kHz and 20kHz. Range of peak frequency of hearing is between 16kHz and 18kHz. Interaural time distances in Florida manatees suggest

		that they lack sensitivity and directionality to sound.
Miksis-Olds et al. 2007	Noise level correlates with manatee use of foraging habitats.	Florida manatee foraging is negatively correlated with the number of boats passing per 5 minutes.
Miksis-Olds and Tyack 2009	Manatee (*Trichechus manatus*) vocalization usage in relation to environmental noise levels.	Florida manatees modify structure and rate of vocalizations in the presence of environmental noise.
Niezrecki et al. 2005 (Yan et al. 2005; O'Shea and Poche 2006)	Acoustic detection of manatee vocalizations.	Fundamental frequency of Florida manatee vocalizations between 2kHz and 5kHz.
Nowacek et al. 2004	Florida manatees, *Trichechus manatus latirostris,* respond to approaching vessels.	Florida manatees found to respond to boat presence at 25m and 50m. Most common reaction of manatees to the presence of boat noise is a flight response.
O'Shea et al. 2006	Aspects of underwater sound communication in Florida manatees (*Trichechus manatus latirostris*).	Highest rate of vocalizations in Florida manatees was between mothers and calves. Physical structure of Florida manatee vocalizations suggest

		adaptations for shallow water communication and identity information.
Sonoda and Takemura 1973	Underwater sounds of manatees, *Trichechus manatus manatus* and *Trichechus manatus inunguis* (Trichechidae).	First to describe *Trichechus manatus manatus* vocalization structure. Fundamental frequencies found to be between 0.2kHz and 7kHz.
Sousa-Lima et al. 2002	Signature information and individual recognition in the isolation calls of Amazonian manatees, *Trichechus inunguis* (Mammalia: Sirenia).	Signature vocalizations in Amazonian manatees. Vocalizations of Amazonian manatees have fundamental frequencies of between 1.2kHz and 4kHz.

7 Appendix C: Plagiarism Declaration



Declaração sobre plágio

Eu, Rebecca Nimrah Umeed, autor da dissertação intitulada "Comportamento vocal em *Trichechus manatus manatus*" a ser defendida através do Programa de Pós-Graduação em Biologia Animal da Universidade Federal de Pernambuco, declaro que:

1. A pesquisa apresentada nesta dissertação, exceto onde especificado, representa minha pesquisa original.

2. Esta dissertação não foi submetida anteriormente para obtenção de nenhum grau em nenhuma outra instituição de ensino e pesquisa do país ou do exterior.

3. Esta dissertação não contem dados de terceiros, nem figuras, gráficos ou outras informações, a menos que devidamente especificado e devidamente creditado como sendo fornecido por outra pessoa.

4. Esta dissertação não contem material escrito por terceiros, a menos que devidamente especificado e creditado como sendo fornecido por outros pesquisadores. Onde material escrito por terceiros foi usado, eu:

    4.1. Re-escrevi o texto, mas a informação passada foi devidamente referenciada.
    4.2. Onde palavras exatas escritas por terceiros foram usadas, as mesmas foram marcadas no texto em itálico ou entre aspas e devidamente referenciadas.

5. Esta dissertação não contem texto, gráficos ou tabelas copiadas e coladas da internet, a menos que especificamente creditado, e a fonte original devidamente referenciada e datada na sessão de Referências Bibliográficas.

Recife 30 de Junho de 2016.

Rebecca Nimrah Umeed

8 Appendix D: Images of the three swimming pools included in the study, a) female pool 10.1m x 4.15m; b) male pool 10.1m x 4.15m and c) juvenile pool 8m x 5m.

a

Source: Rebecca Umeed 2016

b

Source: Rebecca Umeed 2016

Source: Rebecca Umeed 2016

9 Appendix E: Images of each boat type included in this study, a) catamaran, b) fishing boat and c) motorized jangada.

a

Source: http://ecoviagem.uol.com.br/brasil/pernambuco/recife/agencia-turismo/eloim-viagens-e-turismo/. Accessed: 2016

b

Source: Rebecca Umeed 2016

c

Source: Rebecca Umeed 2016