The influence of age and sex on the vocal repertoire of the Antillean manatee (*Trichechus manatus manatus*) and their responses to call playback

REBECCA UMEED, Programa de Pós-Graduação em Biologia Animal, Universidade Federal de Pernambuco, Centro de Ciências Biológicas, Departamento de Zoologia, Avenida Prof. Moraes Rego, 1235, Cidade Universitária, Recife, Pernambuco 50670-420, Brazil; FERNANDA LÖFFLER NIEMEYER ATTADEMO, Centro Nacional de Pesquisa e Conservação de Mamíferos Aquáticos, Estrada do Forte Orange, s/n - Pilar, Ilha de Itamaracá, Pernambuco 53900-000; BRUNA BEZERRA,[1] Programa de Pós-Graduação em Biologia Animal, Universidade Federal de Pernambuco, Centro de Ciências Biológicas, Departamento de Zoologia, Avenida Prof. Moraes Rego, 1235, Cidade Universitária, Recife, Pernambuco 50670-420, Brazil.

ABSTRACT

Limited information is available regarding the acoustic communication of Antillean manatees, however, studies have shown that other manatee taxa produce vocalizations as a method of individual recognition and communication. Here, the acoustic signals of 15 Antillean manatees in captivity were recorded, aiming to (1) describe their acoustic repertoire, (2) investigate the influence of sex and age on vocalization, and (3) examine manatee responses to call playback. Six acoustic signals ranging in mean fundamental frequencies from 0.64 kHz to 5.23 kHz were identified: *squeaks* and *screeches* were common to adult males, adult females, and juveniles; *trills* were common to adult males and females; *whines* were specific to males; *creaks* were specific to females; and *rubbing* was specific to juveniles. The structure of *squeak* vocalizations was significantly different between age and sex classes and *screech* structure was significantly different between age classes. *Squeaks* and *screeches* produced by juveniles had higher frequencies of maximum energy when compared to those produced by adult males and females. A significant 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 identifying and noninvasively monitoring manatees in the wild in Brazil.

Key words: Antillean manatee, *Trichechus manatus manatus*, vocalization structure, acoustic signals, call rate, marine mammals.

Maintaining acoustic contact is especially important for species in which individuals 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 among individuals (Miksis-Olds and Tyack 2009). *Trichechus manatus latriostris* and *Trichechus manatus manatus* typically inhabit grazing pastures in shallow coastal waters and adjacent freshwater

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[1]Corresponding author (e-mail: brunamb1234@gmail.com or bruna.bezerra@ufpe.br).

ecosystems and are absent from areas that lack these two habitats (Garcia-Rodriguez 1998). Manatee habitats are often subject to anthropogenic disturbances such as boat traffic. As a result shallow waters may experience high levels of turbidity (Miksis-Olds and Tyack 2009). High levels of turbidity would result in a further decrease of visual capability (Sousa-Lima *et al.* 2002). Overall, olfactory structure and 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 efficiently such as tactile and vocal signals (Sousa-Lima *et al.* 2002). In fact, sound production by manatees has been observed in several studies (Florida Department of Environmental Protection Division of Marine Resources 1996, Nowacek *et al.* 2003, O'Shea and Poche 2006). *Trichechus manatus latirostris* and *Trichechus inunguis*, for instance, produce vocalizations with a fundamental frequency of between 2 kHz and 6 kHz and several higher harmonics (Sousa-Lima *et al.* 2002, Niezrecki *et al.* 2003, Mann *et al.* 2006), with vocalization durations lasting between 0.25 s and 0.5 s (Niezrecki *et al.* 2003).

Early research proposed that manatees were adapted to hearing low frequency sounds (Bullock *et al.* 1982); however, later studies found that manatee auditory frequency detection for tonal stimuli ranged from 0.25 kHz to 90.5 kHz (Gerstein *et al.* 1999, Gaspard *et al.* 2012). Studies have differed in their descriptions of manatee peak hearing sensitivity: Gerstein *et al.* (1999) suggested a narrow range of peak sensitivity falling between 8 kHz and 20 kHz, whereas a later study by Gaspard *et al.* (2012) suggested a broader range of peak sensitivity extending from 8 kHz to 32 kHz (Gaspard *et al.* 2012). It is thought that manatees may present identity information in their vocalizations (Sousa-Lima *et al.* 2008) as this has been found in other species of marine mammals (Caldwell and Caldwell 1965, Sousa-Lima *et al.* 2002, Charrier *et al.* 2009, Gridley *et al.* 2014). Individual vocal recognition has been found between a mother and calf pair of Florida manatees (Reynolds 1981), as have individual recognition and vocal signature information been found in Amazonian manatees (Sousa-Lima *et al.* 2002). Age and sex have been thought to influence manatee vocalization structure. Sousa-Lima *et al.* (2002) found differences between male and female Amazonian manatee vocalizations for mean maximum and mean 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).

In Brazil, *Trichechus manatus manatus* is under threat of extinction[2] and is both geographically isolated by stretches of water (Alicea-Pou 2001) and morphologically and genetically distinct from *T. manatus latirostris* (Rodrigues *et al.* 1998, Hunter *et al.* 2012, Barros *et al.* 2016). *T. manatus manatus* occur in northern and northeastern 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. In fact, very little is known about the acoustic communication of Antillean manatees. The acoustic repertoire of Antillean manatees has not been fully described (Alicea-Pou 2001). Only two call types, "clicks" and "vocalizations," were previously physically described as isolation calls of captive Antillean manatees (Sousa-Lima *et al.* 2008). Research on Antillean manatee acoustic communication is necessary as it may

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[2]MMA (2014). Portaria do Ministério do Meio Ambiente N° - 444 de 17 de Dezembro de 2014 [Ordinance No. 444 of the Brazilian Ministry of the Environment, 17 December 2014].

contribute to the conservation of this threatened species in Brazil. Thus, the aims of this study were to (1) describe the acoustic repertoire of captive *T. manatus manatus*, (2) investigate the influence of age and sex on the signals produced, and (3) study manatee responses to vocalization playbacks. Overall manatees are regarded as solitary, weakly social, or semisocial animals (Hartman 1979, Reynolds 1981, Harper and Schulte 2005, Hénaut *et al.* 2010). If we consider the theory of social and vocal coevolution, where complex vocal repertoires appear to be associated with complex social structures (McCombe and Semple 2005), we would not expect a large acoustic repertoire in our study animals. Nevertheless, we predict that age and sex differences could result in the production of different types of acoustic signals and signals with structural variations. Finally, we expect that vocalizations played back to the manatees will elicit vocal and/or postural responses compared to control sound files. This was a captive study on manatees artificially segregated by age and sex.

METHODS

Animals and Study Site

The study was carried out at the Brazilian Centre for Research and Conservation of Aquatic Mammals (Centro Nacional de Pesquisa e Conservação de Mamíferos Aquáticos e O Instituto Chico Mendes de Conservação da Biodiversidade [CMA/ICMBio]) on Itamaracá Island in Northeastern Brazil, between January 2015 and April 2016. The study included 15 Antillean manatees (Table 1): seven adult females, four adult males, and four juveniles (three males and one female), distributed in three separate pools. It was not possible to record female and male juveniles separately because they were located in the same pool. There was one pool for the adult males and one pool for the adult females. The adult pools were octagonal in shape and had dimensions of 10.1 m wide by 4.15 m deep and the juvenile pool was a rectangle 10 × 5 m and 4 m deep.

Vocalization Recordings

The animals were recorded for a total of 106 h: 38 h of female vocalizations, 34 h of male vocalizations, and 34 h of juvenile vocalizations. A Cetacean Research Technology hydrophone (Model SQ26-H1, Linear frequency response: 0.02–45 kHz, +3/–12 dBs) was used, which was placed inside of a closed pipe 1 m long, with holes drilled in the sides and bottom and connected to a Zoom H1 recorder (linear frequency response: 20 Hz to 20 kHz at a 16-bit quantization and 48 kHz sampling rate; Recording level was set manually to 64). The pipe was necessary to prevent the manatees from chewing the hydrophone and did not appear to affect the quality of vocalizations recorded. The pipe was placed at the side of the pool to minimize manatee access to the pipe.

Headphones were used to allow for the monitoring of vocalizations during recordings. Vocalizations were recorded in noncompressed WAV format, between 0600 and 1800, with the day being divided into three sections: 0600–1000, 1000–1400, and 1400–1800. Within each of these time intervals 2 h of vocalizations were recorded each day, 4 d per week, between January 2015 and April 2015. Vocalizations were recorded as 5 min sessions within these time intervals. A total of 6 h of recordings were conducted per day and each group of manatees was recorded on

Table 1. Structure of the captive manatee population included in the present study at the Centro Nacional de Pesquisa e Conservação de Mamíferos Aquáticos/Instituto Chico Mendes de Conservação da Biodiversidade (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	Sex	Estimated age (yr)	Age category	Parental relation
Xica	Female	53 (deceased 05/15)	Adult	—
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

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).

Analysis of Vocalizations

Analysis of the vocalizations recorded was achieved through the use of Batsound 4 to create spectrograms (Pettersson Elektronic, Uppsala, Sweden). We classified the recorded vocalizations into distinct types, first by an acoustic assessment made independently by RU and BMB, then on the basis of shared similarities in the spectrograms (considering temporal and structural properties) (adapted from Bezerra *et al.* 2010 and Seiler *et al.* 2015). Only calls that had no overlapping signals and that had clear and identifiable parameters were analyzed (adapted from Miksis-Olds and Tyack 2009). Signals that were ambiguous were excluded from the final analysis. Twelve variables were taken from the fundamental frequency of vocalizations (*i.e.*, the first harmonic) for each vocalization type, unless specified: syllable duration (SD, ms); frequency of maximum energy (FME1st, kHz, obtained from power spectra); frequency of maximum energy of the second harmonic (FME2nd, kHz, obtained from the power spectra); highest frequency (HF, kHz); lowest frequency (LF, kHz); frequency range (ΔF, kHz, the difference between the high frequency and low frequency); frequency at the start of the vocalization (StartF, kHz); frequency at the end of the vocalization (StopF, kHz); duration from start of vocalization to peak frequency of the fundamental frequency (D.Start-P, ms); duration from the peak frequency of the fundamental frequency to the end of the vocalization (D.P-End, ms); the absolute number of harmonics not including the fundamental frequency (NH) and the interval frequency between the fundamental frequency and the second harmonic (IFH, kHz). To collect the measurements from the spectrograms and power spectra, we used the following settings: FFT size = 512, threshold = 12, and Hanning window.

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 found to be produced by both adult males and females (Table 2). Five repeats of each vocalization type were used in the experiments. The vocalizations used as playback stimuli were taken from the adult recordings collected between January and April 2015. The study manatees were not isolated during the recordings of vocalizations or the playback experiments due to animal welfare practices and logistical reasons. Furthermore, due to water turbidity and lack of isolation of the animals, we could not assign calls to specific individuals. Thus, we cannot guarantee that all the animals contributed equally to the final sample size in the vocal repertoire description and playback stimuli. Calls were chosen based on a high signal-to-noise ratio and we used recordings/stimuli from animals from both adult pools. The effect of sex of playback stimuli on manatee vocal response was not considered in this study. During the experiment, it was not always possible to determine the distances of the study animals in relation to the speaker and hydrophone as the manatees were often moving or they were submerged under turbid water. Nevertheless, considering the size of pools in our study site, the maximum distances that the manatees could have been in relation to the speaker and hydrophone would be 8–10 m. A silent control was used to exclude the possibility that extra noise emitted from

Table 2. Features of vocalizations emitted by captive Antillean manatees at the Centro Nacional de Pesquisa e Conservação de Mamíferos Aquáticos/Instituto Chico Mendes de Conservação da Biodiversidade (CMA/ICMBio) on Itamaracá Island in North Eastern Brazil.

Vocalization	Manatee group	n	SD	FME 1st	FME 2nd	HF	LF	F	Start-F	Stop-F	D.Start-P	D.P-End	NH	IFH
Squeak	Females	10	237.2 ± 17.12	2.19 ± 0.13	4.17 ± 0.09	2.49 ± 0.012	1.78 ± 0.13	0.62 ± 0.09	2.37 ± 0.09	2.09 ± 0.13	48.8 ± 21.57	165.80 ± 22.72	2.72 ± 0.70	1.52 ± 0.28
	Males	10	161.9 ± 23.7	2.67 ± 0.19	5.70 ± 0.51	2.90 ± 0.20	2.22 ± 0.14	0.70 ± 0.19	2.49 ± 0.13	2.53 ± 0.15	24.10 ± 7.89	86.10 ± 19.11	2.5 ± 0.60	21.60 ± 0.62
	Juveniles	10	246.4 ± 18.39	3.39 ± 0.19	6.78 ± 0.39	3.83 ± 0.29	2.59 ± 0.08	1.24 ± 0.29	2.90 ± 0.12	3.52 ± 0.24	76.40 ± 10.4	165.80 ± 21.14	2.7 ± 0.05	1.02 ± 0.29
Screech	Females	10	189.6 ± 21.57	2.53 ± 0.16	11.55 ± 0.29	3.01 ± 0.13	1.97 ± 0.21	1.04 ± 0.16	2.70 ± 0.17	2.35 ± 0.13	37.50 ± 10.34	149.40 ± 24.25	4 ± 0.33	0.60 ± 0.3
	Males	10	140.1 ± 13.32	2.31 ± 0.26	2.82 ± 0.06	2.91 ± 0.25	1.42 ± 0.17	1.47 ± 0.25	2.39 ± 0.24	2.02 ± 0.22	24.30 ± 8.21	89.70 ± 8.0	1	0.90 ± 0.11
	Juveniles	10	242.9 ± 29.71	3.62 ± 0.25	3.86 / N/A	4.34 ± 0.27	2.70 ± 0.23	1.64 ± 0.15	3.79 ± 0.33	3.62 ± 0.25	73.5 ± 17.60	164.4 ± 31.97	2 / N/A	0.3 / N/A
Trill	Females	10	236.6 ± 24.47	3.14 ± 0.72	0 / N/A	3.37 ± 42.30	2.52 ± 0.06	0.79 ± 0.08	3.03 ± 0.10	2.79 ± 0.05	95.3 ± 17.44	142.7 ± 13.33	0 / N/A	0 / N/A
Creak	Males	10	179.3 ± 17.24	3.89 ± 0.44	10.4 ± 0.41	5.10 ± 0.60	2.69 ± 0.39	2.41 ± 0.70	3.79 ± 0.36	3.76 ± 0.39	38.6 ± 9.0	126.2 ± 18.13	2 ± 0	1.95 ± 0.34
	Females	10	211.0 ± 29.78	2.89 ± 0.12	0 / N/A	3.81 ± 0.38	2.13 ± 0.23	1.67 ± 0.52	3.09 ± 0.19	3.10 ± 0.45	115.5 ± 35.53	102.7 ± 24.40	0	0 / N/A
Whine	Males	10	153.3 ± 16.45	4.94 ± 0.48	9.55 ± 0.70	5.23 ± 0.53	4.56 ± 0.45	0.67 ± 0.14	4.94 ± 0.51	49.4 ± 0.49	57.9 ± 11.51	116.4 ± 16.44	2 ± 0.46	3.32 ± 0.33
Rubbing	Juveniles	10	78.2 ± 12.55	1.39 ± 0.1	1.50 ± 0.18	3.38 ± 0.24	0.64 ± 0.09	2.74 ± 0.29	1.86 ± 0.2	2.16 ± 0.25	33.5 ± 10.4	41.4 ± 5.30	1 ± 0	2.55 ± 0.34

Note: Descriptive analysis = average values of the vocalizations ± standard errors; frequency measurements are in kHz and time in ms; n = number of calls included in our descriptive analysis; Females = adult females; Males = adult males; Juveniles = juveniles of both sex. SD = syllable duration ms; FME 1st = frequency of maximum energy of the fundamental frequency kHz; FME 2nd = frequency of maximum energy of the second harmonic kHz; HF = high frequency; LF = low frequency; F = difference between the high frequency and low frequency kHz; Start-F = frequency at the start of a vocalization kHz; Stop-F = frequency at the end of a vocalization kHz; D.Start-P = duration ms from the start of a vocalization to the point of peak frequency of a vocalization; D.P-End = duration ms from the point of peak frequency of a vocalization to the end of a vocalization; NH = number of harmonics not including the fundamental frequency; IFH = interval frequency of harmonics, the difference between the minimum frequency of the second harmonic and the maximum frequency of the fundamental frequency.

the equipment was eliciting a response from the manatees (adapted from Miksis-Olds *et al.* 2007). The silent control could not be performed in the adult male pool because the animals had to be moved due to unforeseen captive management requirements. During the playback experiments vocalizations and manatee behavior were recorded 15 min before, during (the duration of a single vocalization, approximately 3 s), and 15 min 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: 0.2–23 kHz, ±10 dB), connected to a 14 watt battery. The speaker was positioned 1 m below the water surface. The intensity of the playback stimuli was standardized by detecting the sound broadcasted, with the speaker located at a distance of 2 m from the hydrophone. Signals broadcasted as playback stimuli in our experiment were of similar loudness to calls emitted naturally by the study manatees at a comparable distance. Power spectra analysis of rerecorded stimuli showed similar dBu values when compared to calls recorded directly from the animals (*e.g.*, *squeaks*, $n_{1,2} = 5$ (mean ± SE) = -44.10 dBu ± 2.2 and -45.60 dBu ± 3.3, respectively). Thus, we believe that the sound pressure levels of the calls used in our experiments were similar to those emitted naturally by the study manatees. During the playback experiments, the sound was monitored with a headphone attached to the Zoom H1 recorder. The stimulus was played for the duration of one vocalization. The postural behaviors (Table 3) of each animal were observed using *All occurrence* sampling (Altmann 1974) throughout the entire experiment. Postural changes were defined as a change in an individual's behavior when comparing behaviors observed before the vocalization playbacks and after: for example, an individual may have been resting before the playback experiment but immediately after the playback the individual dived, swam towards the speaker, submerged, *etc*. The playback experiment sessions were carried out once in the morning and once in the afternoon. The time interval between day-trials was at least 48 h (*i.e.*, experiments were conducted 3 d per week) to avoid the habituation of the animals to the playback stimuli.

Statistical Analysis

To verify whether manatee adult vocalizations could be separated into three distinct call types, we used permutational analysis of variance (PERMANOVA) on Bray-Curtis dissimilarities, using Primer 6 software. Furthermore, permutational *t*-tests were used for *post hoc* pairwise comparisons. We considered the following variables in our above-mentioned analysis: start frequency, highest frequency, syllable duration, frequency of maximum energy, and number of harmonics. Before PERMANOVA analysis, each variable was normalized. A discriminant function analysis (DFA) with leave-one-out cross validation was used to investigate if it was possible to predict manatee age and sex based on the acoustic properties of certain call types, considering that we knew the age and sex groups *a priori*. We used SPSS 23 software to conduct the DFA. About 1/3 of the acoustic variables were used for the DFA (*i.e.* four variables: start frequency, syllable duration, frequency of maximum energy, and number of harmonics) to minimize the chance of misleading the classification of the calls (Lachenbruch and Goldstein 1979). We randomly selected the variables for the analysis; however, we considered variables common to all call types and those that were commonly used in the literature to discriminate individuals based on age and sex. Due to the turbidity of the water and lack of isolation of the subjects, we could not

Table 3. An ethogram illustrating the behaviors of the Antillean manatees observed during the playback experiments. The ethogram was based on the CMA/ICMBio, Gomes *et al.* (2008) and Mercadillo-Elguero *et al.* (2008) descriptions of Antillean manatee activities.

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 individual's snout breaks the water's surface and they breathe
	Tail above water surface	An individual's 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 individual's head is out of the water and their mouth is observed to be moving in a chewing movement

ascribe calls to distinct individuals. Isolating the animals would have been stressful for them and it would not have been logistically viable in our study. Even though we cannot guarantee that all individuals from the three pools (*i.e.*, seven females, four males, and four juveniles) contributed evenly to the final sample size, we believe that the chances that the same individual contributed alone is fairly small.

After conducting Shapiro-Wilks tests to determine the normality of the playback data, we conducted paired *t*-tests to compare vocalization rate before and after each playback stimuli. Data collected from each manatee pool during the playback experiments were grouped together for each playback stimulus (*i.e.*, data from different age and sex classes). 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.

<div align="center">Results</div>

Acoustic Repertoire in Captivity

Six vocalizations types were identified in our study animals (Tables 2, 4, Fig. 1). Three were found to be common to adult males and adult females: *squeak*, *screeches,* and *trills* (Table 4). Two of the six vocalizations were common to all animal groups: *squeaks* and *screeches* (Table 4). One vocalization was exclusive to males: *whines*, one vocalization was exclusive to females: *creaks*, and one vocalization was exclusive to juveniles: *rubbing* (Table 4).

The structure of the three vocalizations produced exclusively by adults was significantly different from one another (PERMANOVA: pseudo-F = 20.222; df = 2, 57; P = 0.001). Permutational *t*-tests showed that *squeaks* differed from *trills* (t = 5.8799, P = 0.001), *squeaks* differed from *screeches* (t = 4.0097, P = 0.001) and *trills* differed from *screeches* (t = 2.9509, P = 0.001).

Table 4. A description of the six types of vocalizations identified in the study manatees living at the Centro Nacional de Pesquisa e Conservação de Mamíferos Aquáticos e O Instituto Chico Mendes de Conservação da Biodiversidade (CMA/ICMBio) at Itamaracá Island, Pernambuco, Brazil.

| Vocalization	Age/sex classes of manatees that produced the vocalization	Description of vocalization
Squeak	Adult females Adult males Juveniles	High pitched squeaking noise, usually short vocalizations, high number of harmonics, often responded to with screeches, squeaks or trills
Screech	Adult females Adult males Juveniles	Bird-like sound, often loud, often heard as a response to squeaks, screeches or trills
Trill	Adult females Adult males	Similar to the sound of a whistle being blown, often loud, generally produced as a response to another type of vocalization
Creak	Adult females	Low pitched sound, similar to the sound of a door creaking open, generally not repeated in a sequence
Whine	Adult males	Faint, quiet sound. Very fine, high pitched, generally not repeated in a sequence
Rubbing	Juveniles	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
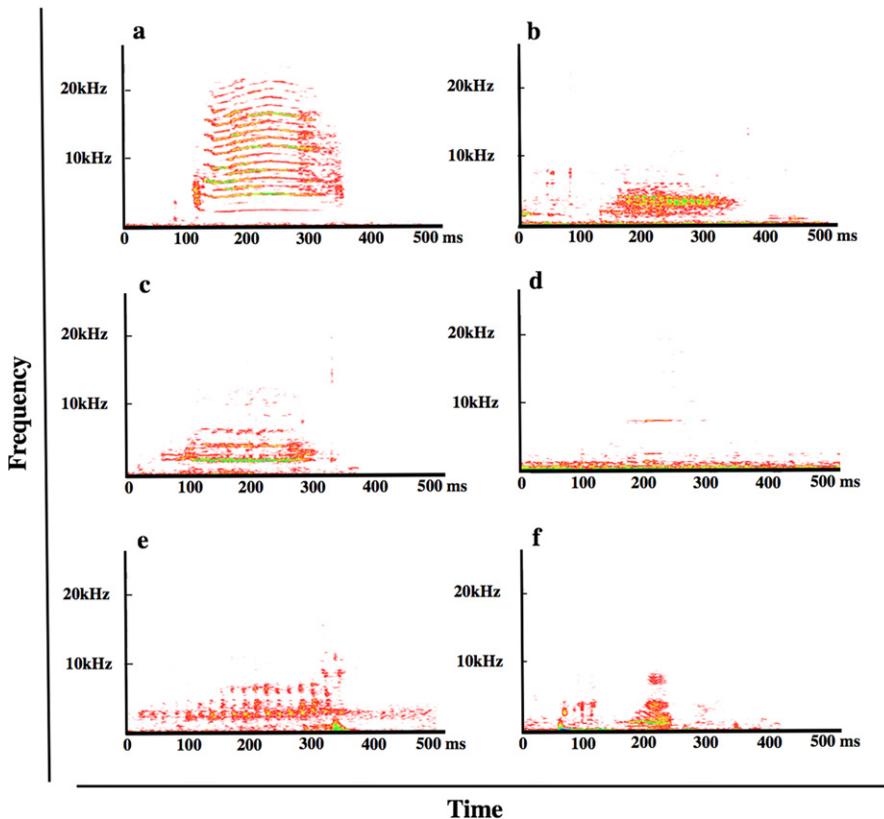
Figure 1. Spectrograms of vocalization types: (a) *squeak*, (b) *screech*, (c) *trill*, (d) *whine*, (e) *creak* and (f) *rubbing* produced by the study manatees in CMA/ICMBio.

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. The accuracy of predicting manatee age based on *squeak* acoustic properties was 86.7% (and 83.3% by leave-one-out-cross-validation DFA, Wilks' lambda = 0.515, χ^2 = 17.238, df = 4, P = 0.002). Adults produced *squeaks* with lower average frequency of maximum energy and lower average start frequencies when compared to *squeaks* produced by juveniles. The DFA differentiated significantly between *squeaks* from adults and juveniles using FME (Wilks' lambda = 0.600, F = 18.665, df_1 = 1, df_2 = 28, P < 0.0001) and Start-F (Wilks' lambda = 0.722, F = 10.767, df_1 = 1, df_2 = 28, P = 0.003). The variables SD (Wilks' lambda = 0.903, F = 3.019, df_1 = 1, df_2 = 28, P = 0.093) and NH (Wilks' lambda = 0.999, F = 0.019, df_1 = 1, df_2 = 28, P = 0.891) did not contribute significantly to differentiating *squeaks* according to age class.

The accuracy of predicting sex based on the acoustic properties of *squeaks* was 78.9% (and 68.4% by leave-one-out-cross-validation DFA, Wilks' lambda = 0.475, χ^2 = 11.166, df = 4, P = 0.025). *Squeaks* recorded in the adult female pool had longer mean syllable duration, lower mean frequency of maximum energy, and lower mean

start frequencies compared to *squeaks* recorded in the adult male pool. Nevertheless, the DFA differentiated significantly between *squeaks* from adult males and adult females using only SD (Wilks' lambda = 0.600, F = 11.330, df_1 = 1, df_2 = 17, P = 0.004). The variables FME (Wilks' lambda = 0.853, F = 2.929, df_1 = 1, df_2 = 17, P = 0.105), Start-F (Wilks' lambda = 0.986, F = 0.239, df_1 = 1, df_2 = 28, P = 0.631) and NH (Wilks' lambda = 0.975, F = 0.443, df_1 = 1, df_2 = 17, P = 0.514) did not contribute significantly to differentiating *squeaks* according to sex class.

The accuracy of predicting age based on acoustic properties of *screeches* was 83.3% (and 80.0% by leave-one-out-cross-validation DFA, Wilks' lambda = 0.512, χ^2 = 17.430, df = 4, P = 0.002). *Screeches* produced by adults had lower mean syllable duration, lower mean frequencies of maximum energy and lower mean start frequencies compared with *screeches* produced by juveniles. The DFA differentiated significantly between *screeches* from adults and juveniles using SD (Wilks' lambda = 0.786, F = 7.604, df_1 = 1, df_2 = 28, P = 0.01), FME (Wilks' lambda = 0.597, F = 18.935, df_1 = 1, df_2 = 28, P < 0.0001) and Start-F (Wilks' lambda = 0.636, F = 16.005, df_1 = 1, df_2 = 28, P < 0.0001). The variable NH did not contribute significantly to differentiating *screeches* between adults and juveniles (Wilks' lambda = 0.993, F = 0.190, df_1 = 1, df_2 = 28, P = 0.666).

Screeches did not differ significantly between sex classes (Wilks' lambda = 0.631, χ^2 = 6.899, df = 4, P = 0.141). The variables SD (Wilks' lambda = 0.849, f = 3.029, df_1 = 1, df_2 = 17, P = 0.100), FME (Wilks' lambda = 0.967, F = 0.576, df_1 = 1, df_2 = 17, P = 0.458), Start-F (Wilks' lambda = 0.901, F = 1.878, df_1 = 1, df_2 = 28, P = 0.188) and NH (Wilks' lambda = 0.975, F = 0.427, df_1 = 1, df_2 = 17, P = 0.522) did not contribute significantly to differentiating *screeches* between adult males and females.

Playback Experiments

There were significant increases in call production after all call playback stimuli (*i.e.*, *squeak*, *screeches*, and *trill* calls), whereas, no significant increase in vocalization

Table 5. Comparison between number of calls uttered before and after the playback stimuli considering all age sex classes.

|		n	Number of calls before playback stimuli Mean ± SD	Number of calls after playback stimuli Mean ± SD	t	df	P
Squeak	Adult females, males an d juveniles	12	2.00 ± 2.26	10.17 ± 6.87	–4.05	11	0.002
Screech	Adult females, males and juveniles	12	2.75 ± 3.19	8.17 ± 6.48	–2.21	11	0.049
Trill	Adult females, males and juveniles	12	1.92 ± 1.51	8.50 ± 5.96	–3.60	11	0.04
Silence control	Adult females, and juveniles	10	2.40 ± 2.95	2.10 ± 1.60	0.39	9	0.71

Note: n = number of playback trials.
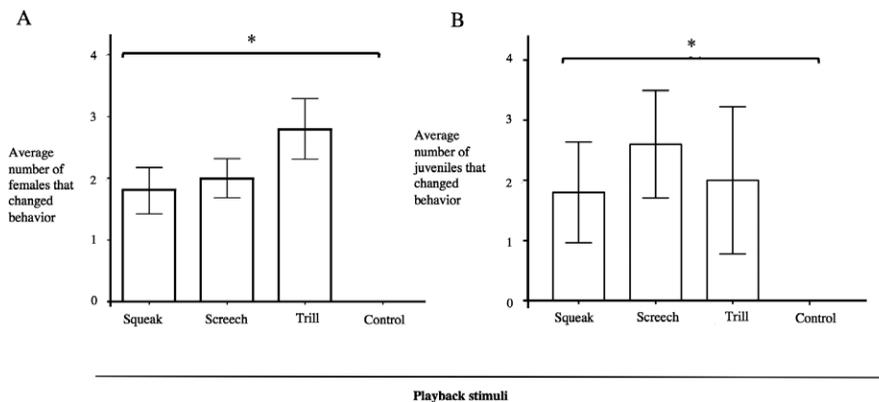
Figure 2. The average number of individuals that demonstrated a change in behavior after the playback stimulus was played. Significant differences were found in the number of animals that changed behavior after vocalization stimuli for both (A) females and (B) juveniles. No manatees were found to change their behavior after the control stimulus was played back. An asterisk (*) demonstrates a difference of $P \leq 0.005$ for Friedman tests with Dunn's *post hoc*.

production was observed after the control playback stimulus (*i.e.*, silent control) (Table 5).

Significant differences were also found in the number of animals that altered their postural behaviors when comparing behaviors immediately before and after the playback stimuli for both females (Friedman test = 11.11, df = 3, P = 0.005) and juveniles (Friedman test = 11.52, df = 3, P = 0.005) (Fig 2). Dunn's *post hoc* tests detected a significant difference between the *trill* and control trials for females, P = 0.02 and between the *screech* and control trials for juveniles, P = 0.01. There were no observed changes in postural behavior after the control stimuli.

DISCUSSION

The captive Antillean manatees included in this study 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 vocalizations identified in this study ranged from 0.64 kHz to 5.23 kHz. These frequencies overlap manatee best-hearing sensitivity, which is suggested to be an adaptation to facilitate the detection of conspecific vocalizations (Gaspard *et al.* 2012). Sousa-Lima *et al.* (2008) found that Antillean manatees produced two types of isolation vocalizations, *clicks* and *vocalizations*. They found that *clicks* had dominant frequencies of between 1.0 kHz and 4.0 kHz and that *vocalizations* had mean fundamental frequencies of between 0.5 kHz and 2.45 kHz. In our study, isolation calls were not recorded since the animals included in the study were at no point "isolated"; however, this could suggest that future studies on both wild and captive Antillean manatees may reveal more acoustic signal types. Furthermore, considering that manatee hearing extends well into the ultrasonic range (Gerstein *et al.* 1999, Gaspard *et al.* 2012), the potential for the production of

ultrasonic signals should be further investigated. The low critical ratios reported by Gaspard *et al.* (2012) (8–32 kHz) also suggest that manatees are able to perceive the harmonics of some of the vocalizations reported in this manuscript. Some of the vocalizations identified in this study had similar durations 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 mean duration of *chirps* was found to be 222.8 ms and the mean duration of squeaks was found to be 198.4 ms. *Squeaks, screeches*, and *trills* identified in this study had similar mean durations and were common to all age and sex classes. Miksis-Olds *et al.* (2009) also found that the mean peak frequency of *chirps* was 5.097 kHz with the mean peak frequency of *squeaks* being recorded as 3.341 kHz. 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 males, *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 the identification of the sex of manatees through the recording of manatee vocalizations in the wild and could aid in the monitoring of this subspecies.

Sex differences were detected in the physical structure of the vocalizations produced by the manatees included in this study: females exhibited higher values for syllable duration and lower values of mean maximum and mean minimum fundamental frequencies compared to vocalizations produced by the males in this study. Sousa-Lima *et al.* (2002) found that the vocalizations of female Amazonian manatees had greater values of mean maximum and mean minimum fundamental frequencies compared to vocalizations produced by males. Furthermore, Sousa-Lima *et al.* (2008) found that isolation vocalizations produced by captive female Antillean manatees had higher values for signal duration, mean maximum, and mean minimum fundamental frequencies compared to vocalizations produced by captive male Antillean manatees. Sex differences in the vocalization structure of other captive marine mammals such as killer whales have also been found (Dalheim and Awbrey 1982). Male common bottlenose dolphin calves, *Tursiops truncatus*, were found to be more likely to produce signature whistles that were similar to their mothers' signature whistles than female calves (Sayigh *et al.* 1995). It is possible that sex differences in vocalization structure may be used as method of mating partner recognition, which may be useful during reproductive seasons. However, further research in this area would be necessary to confirm this possibility.

Age-class differences in call structure were also observed in our study animals, where vocalizations produced by juveniles were found to have longer syllable durations and higher mean maximum and mean minimum fundamental frequencies compared to adult males and females. This is similar to Sousa-Lima *et al.*'s (2008) findings that calves produced higher values for all acoustic variables except the number of harmonics. It would therefore appear that the younger the Antillean manatee, the longer the syllable duration and the higher the mean fundamental frequencies. However, Sousa-Lima *et al.* (2002) 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. Differences between Amazonian and Antillean manatee vocalizations such as these, indicate interspecific variation in manatee vocalization structure. Age differences in vocalization structure together with sex differences may therefore, provide valuable information about the signal producer, which may not be possible to determine visually due to the turbid habitat of manatees in Brazil.

Male, female, and juvenile Antillean manatees responded with vocal and postural responses, to their own vocalizations when artificially played underwater. These results support findings from previous studies that showed an increase in Florida manatee vocalization rate following vocalization playback experiments in the wild (Philips *et al.* 2004). The results of the present study could indicate that Antillean manatee vocalizations may not be used exclusively for contact between mothers and calves as previously suggested (Hartman 1979). Acoustic signals associated with behaviors other than mother-calf interactions 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). Manatee vocalizations may therefore, be associated with different types of behavior as well as mother-calf contact. Future research should focus on associating certain types of vocalizations with specific behaviors as well as defining specific types of vocalizations used in different social contexts. The reactions of the study manatees 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 this species. Such findings may help to elucidate the structure of manatee populations in wild areas, as they are often difficult to observe due to the high turbidity of the water common in estuarine habitats of Antillean manatees found in northeastern Brazil.

Acoustic surveys have already been used to monitor species of marine mammals (MacDonald and Moore 2002, Ichikawa *et al.* 2006, Moore *et al.* 2006, Tsutsumi *et al.* 2006) and have been found to detect 1–10 times more cetacean groups than visual surveys (MacDonald and Moore 2002, Mellinger *et al.* 2007). In the present study *squeaks* appeared to induce a more intense vocal response in captive manatees, they therefore may be a good candidate when attempting to monitor Antillean manatee numbers in Brazil *via* vocalization playback. Nocturnal surveys in the wild should also be considered since the captive manatees were also fairly vocal during the night (RU, personal observation). Playback surveys have proven efficient in locating other species of marine mammals. For instance, the detection of vocalizing dugongs has been achieved using the playback of conspecific calls (Ichikawa *et al.* 2009). Detection probability of individuals was found to increase from 12.5% visually to 19.2% acoustically (Ichikawa *et al.* 2009). However, it is possible that background noise levels may affect the success of acoustic monitoring and the distance at which manatee vocalizations can be detected (Niezrecki *et al.* 2003; Phillips *et al.* 2004, 2006). As a result background noise cancellation technologies have been developed in order to minimize the masking of manatee vocalizations (Yan *et al.* 2005, Gur and Niezrecki 2007).

The quantitative and qualitative description of the vocal repertoire produced by the manatees in this study introduce the possibility of using information on their acoustic repertoire together with vocalization playbacks as a low cost (compared to aerial surveys, *e.g.*, Alves *et al.* 2013) identification and monitoring method of wild Antillean manatees. Acoustic surveillance and monitoring of Antillean manatees in the wild may facilitate systematic surveys and aid in 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 the effective implementation of such a monitoring method. Acoustic properties of calls uttered by animals in captivity and the wild should be tested due to the potential interference of pool structure on call propagation. Further studies investigating whether manatees use specific vocalizations in specific behavioral situations such as stimulating or distressing situations would also provide valuable insight into the vocal behavior and social communication of Antillean manatees. The present study extends our knowledge on the use of acoustic signaling as a method of communication between the elusive Antillean manatees. Despite the marked differences between captive and wild settings for manatees, we consider signal diversity and structure as well as responses to playback vocalizations in captivity to estimate behaviors in wild manatees. We trust that such basic knowledge could potentially be used as an additional survey method for Antillean manatees in their water-turbid, estuarine, natural environment in Brazil.

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