



Vocal complexity in Antillean manatees (*Trichechus manatus manatus*)

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Abstract

Vocal complexity can be expressed through variations in repertoire size, structure, and individual manatee repertoires. Here we aimed to assess the complexity of the vocal behaviour of Antillean manatees living in captivity (i.e., artificial pools) and in reintroduction enclosures (i.e., natural enclosures placed in an estuarine area). Specifically, we evaluated: (i) the structure of vocalisations to assess whether they had variants; (ii) the variation in call production (rate and pattern) between groups with different configurations; (iii) whether individuality occurred in vocalisation structure. We found four categories of vocalisations, of which two had different variants. Not all study groups produced all call categories and variants. Older and younger males in the reintroduction enclosures had the highest call rates compared to captive females and captive males. The vocal and behavioural patterns differed between groups. Squeak call structure differed between individuals. Such vocal complexity may aid manatees in adapting to their dynamic social and structural environment, facilitating communication.

Keywords

acoustic signals, vocalisation pattern, vocal complexity, call features, call frequency.

Resumo

A complexidade vocal pode ser expressa através de variações no tamanho do repertório vocal, na sua estrutura e individualidade. Aqui objetivamos avaliar a complexidade no comportamento vocal de peixes-boi-marinheiros vivendo em cativeiro (i.e., piscinas artificiais) e em recintos de reintrodução (i.e., recintos naturais em uma área estuarina). Especificamente, investigamos: (i) a estrutura das vocalizações para avaliar se apresentavam variantes; (ii) a variação na produção de vocalizações (taxa e padrão) entre grupos com diferentes configurações; (iii) se a individualidade ocorre na estrutura de vocalização. Encontramos quatro categorias de vocalizações, das quais duas possuíam variantes. Nem todos os grupos de estudo produziram todas as categorias de vocalizações e suas variantes. Os machos mais velhos e os mais jovens nos recintos de reintrodução tiveram as maiores taxas de vocalizações em comparação com as fêmeas e machos cativos. Os padrões vocais e comportamentais diferiram entre os grupos. A estrutura física da vocalização Squeak diferiu entre os indivíduos. Essa complexidade vocal pode ajudar os peixes-bois-marinheiros a se adaptarem ao seu ambiente social e estrutural dinâmico, facilitando a comunicação.

1. Introduction

Complexity in vocal behaviour can be expressed through the size of a vocal repertoire, the combination of different vocalisation types (Crockford & Boesch, 2003; Ouattara et al., 2009; Krams et al., 2012; Bouchet et al., 2013), the alteration of their physical structure (Mitani & Brandt, 1994; Slocombe et al., 2009), their use in different behavioural or ecological contexts and the varied use and production of vocalisations by different individuals (Gustison et al., 2012; Krams et al., 2012; Gustison & Bergman, 2016; Cheney & Seyfarth, 2018). Variations in vocal behaviour have been linked to the social complexity hypothesis for communication. This predicts that complex social systems can lead to complex communication systems, with the majority of studies focusing on vocal behavioural but not restricted to this mode of communication (Freeberg, 2006; Freeberg et al., 2012; Peckre et al., 2019). Nevertheless, life history traits have also been suggested as drivers of vocal complexity, as can be observed with forest elephants (*Loxodonta cyclotis*), where extensive parental care and slow reproductive rate may have led to vocal complexity (Hedwig et al., 2021).

The ability of animals to modify the structure and production of their calls may influence the success of their survival. For instance, an individual's ability to modify their vocalisations in differing natural environmental scenarios (e.g., fluctuating water turbidity, pH and tide regimes) and anthropogenically-induced scenarios (e.g., presence of anthropogenic noise),

could optimise intraspecific communication — particularly between mothers and calves and between mating individuals — by improving call propagation and delivery (Okabe et al., 2012; Vasconcelos et al., 2012; Caorsi et al., 2017; Charlton et al., 2018; Nunez & Rubenstein, 2020). Despite potentially optimizing communication, the ability of animals to modify their vocalisations may involve behavioural and ecological costs. Altering vocalisations can have metabolic costs that can vary at an individual and species level (Ophir et al., 2010). For bottlenose dolphins, *Tursiops truncatus* (Holt et al., 2013; Noren et al., 2013; Holt et al., 2015), bulldog bats, *Noctilio albiventris* (Dechmann et al., 2013) songbirds (Oberwerger & Goller, 2001) and chimpanzees, *Pan troglodytes schweinfurthii* (Fedurek et al., 2017), metabolic costs have been associated with vocal production to some degree; for example, oxygen consumption increases during call production (Bucher et al., 1982; Ophir et al., 2010; Holt et al., 2015, 2016; Chaverri et al., 2021), which could ultimately affect an animal's growth, reproduction, and anti-predation strategies (Brown et al., 2004).

The production of different call types and differences in call structure have been observed for various marine mammals. For instance, cetaceans use different types of vocalisations in different behavioural scenarios, i.e., they produce echolocation clicks when foraging and whistles in order to communicate with conspecifics (Tyack, 1986; De Souto et al., 2012). Sperm whales, *Physeter microcephalus*, produce clicks with different structures, where longer clicks, lower click repetition rates, and intensity peaks at 1.8 and 2.8 kHz correlate with the presence of mature males (Weilgart & Whitehead, 1988). Captive Antillean manatees, *Trichechus manatus manatus*, produce vocalisations that differ in call length between age groups and demonstrate differences in fundamental frequency parameters between sexes (Sousa-Lima et al., 2008; Umeed et al., 2018). Furthermore, some marine mammal species produce signature vocalisations such as bottlenose dolphins, which produce whistles that have distinctive structural characteristics, indicating the production and use of individual signature whistles (Caldwell & Caldwell, 1965; Caldwell & Caldwell, 1979). Additionally, Amazonian manatees, *Trichechus inunguis*, produce individually stereotyped vocalisations, believed to contain individual signature information (Sousa-Lima et al., 2002) and captive Antillean manatees produce isolation calls thought to contain individual identity information (Sousa-Lima et al., 2008).

Sex-related and intraspecific variabilities in vocalisations are widespread across social animals and have been linked to social complexity (Tibbetts & Dale, 2007; Krams et al., 2012). Across marine mammal species, where parental care is involved, the parent that invests the most care will produce more vocal signals, often up to ten times their average vocalisation rate (Fripp & Tyack, 2008; Dunn et al., 2017). Usually, there are specific vocalisations associated with parental care (Marcoux et al., 2006). For many marine mammal species, including manatees (Sousa-Lima et al., 2002; Sousa-Lima et al., 2008), dugongs, *Dugong dugon* (Fuentes et al., 2016), cetaceans (Rendell et al., 2019) and pinnipeds (Boness & Bowen, 1996), mothers are primarily responsible for the survival of offspring, including gestation, lactation, weaning and post-weaning care (Rendell et al., 2019). Therefore, one could expect that females responsible for parental care would produce vocal repertoires constructed of high vocal signal repetitions of a few base vocalisation types, i.e., simple and repetitive repertoires. Pinniped mothers and calves demonstrate individual call distinctiveness, and evidence indicates that these calls facilitate recognition between lactating mothers and calves (see Insley et al., 2003 for a review). Narwhal mothers, *Monodon monoceros*, also produce highly stereotyped and individually specific contact calls when separated from their calves, which are thought to contain identity information (Ames et al., 2021). Atlantic walrus, *Odobenus rosmarus rosmarus*, barks propagate at greater distances over water, with certain acoustic features being highly resistant to degradation, suggesting that these features may be key for individual identification (Charrier et al., 2010). For captive belugas, *Delphinapterus leucas*, individual differences have been found for pulse patterning, suggesting the use of these calls for individual recognition (Morisaka et al., 2013). On the other hand, Indeck (2020) found that mother and calf humpback whales, *Megaptera novaeangliae*, produce vocalisations that are more difficult to detect — i.e., they have lower call rates with reduced acoustic level and limited active space, and they can also modify their behaviour to avoid unwanted interactions and to maintain acoustic contact.

Captive Antillean manatees produce single-note vocalisations often with multiple harmonics, with the fundamental frequencies falling between 0.64 to 5.23 kHz (Umeed et al., 2018; Merchan et al., 2019) and maximum frequencies potentially reaching up to 150 kHz (Ramos et al., 2020). The fundamental frequencies, contour and frequency range of captive Antillean

manatee vocalisations vary significantly between individuals, sex and age groups (O’Shea & Poché, 2006; Sousa-Lima et al., 2008; Umeed et al., 2018; Merchan et al., 2019). Umeed et al. (2018) and Sousa-Lima et al. (2008) demonstrated that captive adult females produce vocalisations with longer mean durations and lower mean maximum frequencies compared to adult males. Furthermore, studies have demonstrated that captive juvenile male Antillean manatees produce vocalisations with higher mean maximum frequencies compared to captive adult manatees (Umeed et al., 2018) and that calves produce vocalisations with higher frequency parameters compared to adult manatees (Sousa-Lima et al., 2008). Antillean manatees can spend time solitarily, as mother-calf pairs, in mating pairs or as small and large groups (Alves et al., 2013; von Fersen & Walb, 2018; Favero et al., 2020; Gomez-Carrasco et al., 2020). Manatees also have a low reproductive rate and extended maternal care, with a high investment in gestation and nursing after birth (Rathbun et al., 1995; Attademo et al., 2022). Therefore, the possibility of having different group dynamics in the wild (as well as in captivity, due to artificially induced group configurations) and having such life history traits, may suggest some level of vocal complexity in the species in order for individuals to coordinate communication effectively.

In the present study, we aimed to assess vocal complexity in Antillean manatees by evaluating the variation in the structure and production of vocalisations of animals living in captivity (i.e., artificial pools) and in reintroduction enclosures (i.e., readaptation enclosures in a natural estuarine area) in North-eastern Brazil. Here we aimed to (i) categorise calls for individuals living in captive and natural reintroduction enclosures to assess whether variants of each call category exist; (ii) investigate whether call production (i.e., call types, call rate, and the proportion of each call category produced) varied between the study groups, and (iii) investigate whether individuality could be identified when analysing the structure of Squeak vocalisations. We tested the following predictions: (a) Female manatees produce a higher number of vocalisations with a more stereotypical repertoire, i.e., a repertoire composed of a limited number of call types, produced repetitively due to the presence of related individuals in the captive pool; (b) The number of vocalisations differs depending on social context — the greater the number of animals in an enclosure (i.e., the more complex social scenario in the present study), the greater the number of vocalisations produced (group call rate) (i.e., the more complex the vocal behaviour) — suggesting there

is a relationship between animal abundance and call rate; (c) the structure of Antillean manatee vocalisations differs between individuals. This study will improve our understanding of whether Antillean manatees conform to the predictions of the social complexity hypothesis for communication (i.e., “social unities that occur at high densities result in individuals that interact with one another at higher rates”; see Freeberg et al., 2012 for a review).

2. Material and methods

2.1. Study sites and animals

We investigated captive Antillean manatees housed at the Instituto Chico Mendes de Conservação da Biodiversidade/Centro Nacional de Pesquisa e Conservação de Mamíferos Aquáticos (ICMBio/CMA) on Itamaracá Island, Pernambuco, Brazil. We also investigated manatees in a reintroduction centre in the Área de Proteção Ambiental (APA) Costa dos Corais/ICMBio, Porto de Pedras, Alagoas, Brazil (Table 1).

This study was comprised of two parts. Firstly, we investigated the overall call structure and production in four manatee study groups. We then specifically investigated the production of signature vocalisations through the analysis of Squeak vocalisations (see call description below) from four individually recorded manatees. The recording and observation methods for each part of the study are detailed below.

2.2. Observations and recordings — Part 1

When investigating call structure and production, between February 2019 and May 2019, we observed and recorded six captive females (range: 4–32 years old); four captive males (range: 6–27 years old) and four males in two reintroduction enclosures (range: 8–28 years old) (Table 1).

The six captive females were all kept in the same pool with dimensions of 10 × 5 m, and 4 m deep (Figure A1a in the Appendix). The four captive males were kept in the same pool with dimensions of 8 × 5 m and 4 m deep (Figure A1b in the Appendix).

The males in the reintroduction enclosures were kept in two separate enclosures. Assu and Netuno were the older males kept in reintroduction enclosure 1, which measured 27 × 13 m. Aratí and Raimundo were the younger males kept in reintroduction enclosure 2, which measured 31 × 17 m. The depths of the reintroduction enclosures varied with tide regimes

Table 1.

Characteristics of the studied Antillean manatees.

Individual	Sex	Estimated age (yr.)	Age Category	Weight (kg)	Length (cm)	Housing condition	Location
Bela**	♀	8	Adult	585	287	Captive	PE
Canoa	♀	14	Adult	358*	271*	Captive	PE
Carla**	♀	22	Adult	554	295	Captive	PE
Paty***	♀	4–6	Subadult/ Adult	310	248	Captive and Reintroduction 2	PE
Sheila**	♀	22	Adult	572	307	Captive	PE
Xuxa**	♀	32	Adult	828	305	Captive	PE
Daniel	♂	9	Adult	356	275	Captive	PE
Parajuru	♂	6	Adult	325	251	Captive	PE
Poque	♂	27	Adult	387	256	Captive	PE
Zoé	♂	14	Adult	290	251	Captive	PE
Assu	♂	20	Adult	392*	280*	Reintroduction 1	AL
Netuno	♂	28	Adult	476*	315*	Reintroduction 1	AL
Araí	♂	9	Adult	301	263.5	Reintroduction 2	AL
Raimundo	♂	8	Adult	350	277	Reintroduction 2	AL

Estimated ages of each manatee housed in captive or reintroduction conditions (reintroduction enclosure 1 or 2) that were included in the study at the time of data collection in 2019; weight and length measurements were obtained from the closest month (January–June 2019) before or after the study was carried out. The ages were estimated by the staff at the ICMBio/CMA based on the date of birth (for the manatees that were born in captivity i.e., Bela, Carla, Sheila) or based on the physical condition (size, appearance) of manatees at the date of rescue (for the animals that were stranded and subsequently rescued). PE: State of Pernambuco, Brazil; AL: State of Alagoas, Brazil. *Asterisks indicate an extensive time-lapse (>6 months) between the measurements and the study period. **Xuxa is Sheila and Carla's mother (twins), and Sheila is Bela's mother. Thus, these four adult female manatees are related in our study. *** Paty was 4 years old when in captivity and she was just over 6 years old when in Reintroduction enclosure 2 (i.e., sexually mature at both stages). We follow the age category proposed by Hostetler et al. (2021).

as the enclosures are situated in the mangroves of the Tatuamunha River. The reintroduction enclosure walls are constructed from wooden poles with spaces between each pole (Figure A1c and A1d in the Appendix), with silt and clay sandy sediment. Therefore, the manatees in the reintroduction enclosures experienced changes in tide level, water temperature and turbidity. The average daily water temperature during our recordings ranged from 29.2–32.2°C and the average daily water pH ranged from 6.83–7.78.

We recorded vocalisations of the animals housed in captive and reintroduction enclosures during daylight hours, from 7 am until 5 pm between

February 2019 and May 2019. We recorded the vocalisations of the animals during focal group recording sessions of 10-min duration. We did not isolate the animals during these recordings. Thus, the call counts used to calculate call proportions and rates were obtained for each social group (i.e., captive males, captive females, males in reintroduction enclosures 1 and 2) and not individual animals. We conducted a total of 216 focal group sessions (36.3 h total), where 3701 vocalisations were recorded and considered in our analysis. To record the vocalisations, we conducted 74 focal group sessions in the captive female enclosure (12.7 h of recordings), 47 focal group sessions in the captive male enclosure (7.8 h of recordings) and 42 (7 h of recordings) and 53 (8.8 h of recordings) focal group sessions in reintroduction enclosures 1 and 2, respectively. During these recordings, we used the scan observational method (Altmann, 1974) to register the postural behaviours of the animals at 5-min intervals (Table 2). A total of 100 h of scan observations were conducted (Table 2). We calculated the proportion (%) of each postural behaviour produced by the study animals during these observations in order to compare the behavioural patterns of the study groups and relate them to the recorded call patterns.

We recorded the Antillean manatee vocalisations using a Wildlife Acoustics SM4 recorder (sampling frequency: 48 kHz/16 bit; linear frequency response 20 Hz–48 kHz) connected to an SM3 Hydrophonex hydrophone, which was placed inside a PVC pipe (a common pipe made of plastic and vinyl), following the methodology described by Umeed et al. (2018) in order to protect the hydrophone from the manatees. Previous tests performed by Umeed et al. (2018) showed no evidence of echoes or reflection of the vocalisations recorded with the captive manatees. However, we acknowledge that the concrete material used to construct the oceanariums may have affected the sound propagation of the recorded vocalisations, in terms of call amplitude and frequency attenuation, for instance, especially depending on the position of the animal in relation to the hydrophone.

2.3. Observations and recordings — Part 2

To assess whether Antillean manatee call structure reveals caller identity and/or suggests the production of signature vocalisations, we individually recorded four manatees for approximately 30 min each, using a Cetacean Research SQ26-H1 hydrophone, connected to a Zoom H1 recorder (sampling frequency: 48 kHz/16 bit; linear frequency response 20–20 000 Hz).

Table 2.
Ethogram used during the behavioural observations of the study manatees.

Behaviour category	Behaviour	Description
Locomotion	Drift alone	The animal performs a slow movement alone, with no use of flippers
	Social drift	Two or more animals perform a slow movement, with no use of flippers, side by side
	Swimming	The animal performs a fast movement using flippers and/or tail
	Swimming inverted	The animal performs a fast movement using flippers and/or tail, stomach facing upwards
	Diving	The whole body of the animal moves towards the bottom of the pool, back breaking surface of water
Rest	Rest alone	The animal remains still, alone
	Group rest	Two or more individuals remain still together, side by side
	Rest inverted	The animal remains still, stomach facing upwards
	Rest at the side of the pool	The animal position its flipper holding the side of the pool wall, lying against side of pool, and remaining still
Investigate	Rest on side	The animal remains still, lying on side of body
	Interacting with wall/gate	The animals chews the wall/gate, keeping the head out of the water
	Body out of water	The animal keeps the upper part of their body out of water at the side of the pool, supporting itself with flippers
	Head out of water	The animal's head is out of the water
Social	Embrace	The animal uses their 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	The animal places their mouth on food items, followed by chewing and ingestion
	Attempting to feed	Observed mainly in juveniles: juveniles who are no longer fed from bottles by the keepers, try to feed from a bottle placed at the edge of the pool
	Drinking	The animal swallows fresh water from a tap placed at the edge of the pool

Table 2.
(Continued.)

Behaviour category	Behaviour	Description
Submersed	Submersed	Individual is underwater, but it is not possible to clearly observe their behaviour 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 through the nose
	Tail above water surface	An individual's tail is above the 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

The ethogram was adapted from Umeed (2016), Umeed et al. (2018) and Lucchini et al. (2021).

The recordings were taken in the reintroduction enclosures at the APA Costa dos Corais/ICMBio, Porto de Pedras, Alagoas, during a routine veterinary health check-up from 30 November to 2 December 2020. During the health check-up, the veterinarians removed each animal from the pool at low tide. This allowed us to record the remaining animal in its respective pool, individually. We observed the animals *ad libitum* (Altmann, 1974) while the vocalisations were recorded. It is important to note that the recorded manatees may have been able to hear free-living manatees in the river and the other manatees in the nearby reintroduction enclosure. Since we held the hydrophone close to the individual study manatees, we would expect their vocalisations to be louder and more clearly defined on the spectrograms and any calls from manatees outside the enclosures would be significantly quieter and less clearly visible. However, in our recordings calls such as these were not observed, thus we trust that we recorded and analysed only the individual study manatee calls. We recorded one juvenile female called Paty (an individual previously included in the captive female recordings in part 1 of the present study), and three adult males called Raimundo, Assu and Netuno (individuals from the reintroduction enclosures also previously included in part 1 of this study). Paty was transferred from the captive enclosure in Pernambuco (ICMBio/CMA) to reintroduction enclosure 2 in Alagoas (APA Costa dos Corais) in July 2019. Paty and Raimundo inhabited reintroduction enclosure 2, and Assu and Netuno were housed in reintroduction enclosure 1 during the recording period.

A period of 10 min was allocated before recording each animal separately, to allow the individual to resume normal behaviours following the removal of their conspecific from the enclosure. Recordings were conducted at a maximum distance of 5 m from the animals at low tide. In these enclosures water depth varies from 3.5 m to 1 m, depending on the tide. We recorded the animals with the help of a kayak on the water or from a platform, which provided access to the reintroduction enclosures. We selected the Squeak call type (Figure 1) as it was concurrent with the isolation vocalisations described by Sousa-Lima et al. (2008) for captive Antillean manatees. It was also the most tonal call type observed in our study, which is described in the literature as being a common isolation call in mammals (Zeifman, 2001; Newman, 2004, 2007; Lingle et al., 2012). Only Assu produced one other vocalisation type (Trill, see Figure 4), thus we only included Squeak calls in our analyses.

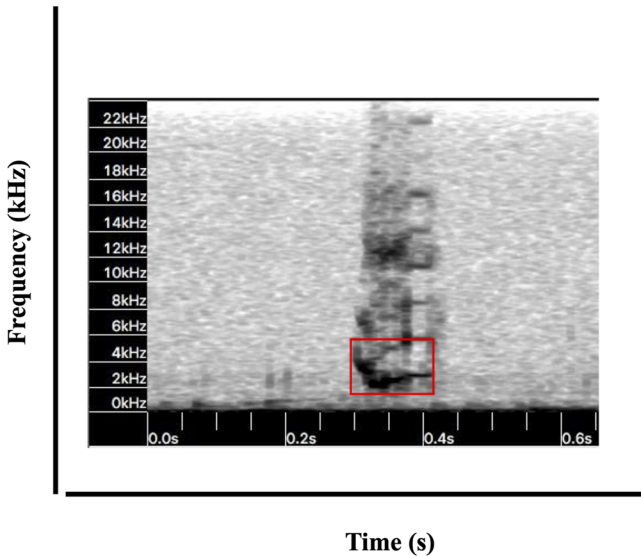


Figure 1. Example of a Chirp call. The red rectangle shows where the structural variables were measured from for each call. The spectrogram was generated using Kaleidoscope Pro5 Software, Wildlife Acoustics (512 samples for FFT, time resolution of 5.33 ms, 50% overlap). Chirps were produced exclusively by captive males.

2.4. Acoustic analysis and call categorisation

We generated spectrograms to analyse the vocalisations using Kaleidoscope Pro5 Software, Wildlife Acoustics (512 samples for FFT, time resolution of 5.33 ms, 50% overlap).

We calculated the call rates for each group of recorded animals (i.e., captive females, captive males, males in reintroduction enclosure 1 and males in reintroduction enclosure 2). Call rates were calculated by dividing the total number of calls by the number of animals in each group and then per focal recording session (number of calls/group size/number of focal sessions), in order to compare call rates between groups. Additionally, we calculated the proportion (%) of each call type produced by the study animals.

We also measured the structural variables of the calls using spectrograms and waveforms generated in Kaleidoscope Pro 5. We manually extracted 11 different structural variables from the spectrograms and waveform of the vocalisations (Table 3).

Table 3.

Definition of acoustic variables used in our analysis.

Structural variable	Measurement description
Call duration (ms)	Time from the start of the vocalisation to the end of the vocalisation
Frequency of maximum energy (kHz)	Frequency at which the greatest amount of energy is found, derived from the power spectra.
Maximum and minimum frequency (kHz)	Maximum and minimum frequency of the call
Start and end frequency (kHz)	Start and end frequency of the call
Bandwidth (kHz)	Maximum minus minimum frequency
Distance from start to maximum frequency (ms)	Time from the start of the call to the point of the maximum frequency obtained
Distance from maximum frequency to the end of the call (ms)	Time from the point of maximum frequency to the end of the call
Number of harmonics	The total number of harmonics (other than the fundamental) observed for each call, including subharmonics.
Inter-harmonic frequency interval (kHz)	The minimum frequency of the second harmonic minus the maximum frequency of the fundamental harmonic, to determine the frequency interval between the first and second harmonics.

The measurement description followed Umeed et al. (2018). All measurements were taken from the fundamental harmonic from spectrograms and waveforms (temporal parameters) generated using Kaleidoscope Pro5 software, Wildlife Acoustics.

Calls were initially classified into four categories: Squeaks, Trills, Chirps and Pulse calls (Table 4) and their variants. One single researcher (RU) categorized the calls. Call categorisation was based on visual spectrogram inspections and by using previously published descriptions of manatee calls. Variants of each call category were defined here as vocalisations that met the overall visual criteria for inclusion in the main call category but demonstrated specific and consistent subtly varying physical structures resulting in their classification as different variants of the main call category.

2.5. Statistical analysis

2.5.1. Call categories and variants

We performed Discriminant Function Analyses (DFA) using the leave-one-out cross validation method to confirm if our classification of call categories could separate the calls into Squeak, Trill and Chirp categories. We used the

Table 4.

Classification of call categories based on call structure.

Call Category	Description
Chirps	Chirps were classified as short (<100 ms) harmonic vocalisations following the same classification as Brady et al. (2020) in order to aid potential comparisons. (Figure 1).
Pulse calls	Pulse calls often demonstrated a “cross-hatching” pattern during the pulse phonation, with the distance between harmonics approaching the effective frequency resolution of the spectrogram (based on descriptions by Buder et al., 2008). (Figure 2).
Squeaks	<p>Squeaks had a tonal harmonic structure either with or without the presence of subharmonics and/or loft regimes and were defined following Umeed et al. (2018) and Brady et al. (2020) where we grouped “Squeaks, High Squeaks, Squeak-Squeals and Squeals” defined by Brady et al. (2020) into a single category of “Squeaks” (Figure 3).</p> <p>Squeak 1, tonal vocalisation comprised of a single harmonic.</p> <p>Squeak 2, tonal vocalisations comprised of two to three harmonics, with a downward frequency contour at the end of the call, often with some temporal smearing.</p> <p>Squeak 3, tonal vocalisation with many harmonics, subharmonics and temporal smearing</p> <p>Squeak 4, tonal vocalisation comprised of two to three harmonics with a loft regime at the start and end of the call.</p> <p>Squeak 5, hill-shaped tonal vocalisation, comprised of many harmonics.</p>
Trills	<p>The Trill category was adapted following Umeed et al.’s (2018) classification, where we categorised trills, screeches and creaks into a single category i.e., noisy calls with some degree of deterministic chaos, often with the absence of a harmonic structure and a predominance of low frequency energy (Buder et al., 2008). We initially grouped four trill variants as described below, but statistical analysis failed to recognise all trill variants (see result section). (Figure 4).</p> <p>Trill 1, harmonic structure, with temporal smearing towards the end of the call and an elevated frequency contour towards the start of the call.</p> <p>Trill 2, harmonic, flat structure, with elements of temporal smearing at the centre of the call.</p> <p>Trill 3, harmonic, flat structure with elements of temporal smearing at the beginning of the call.</p> <p>Trill 4, chaotic, non-harmonic call.</p>

software SPSS v 28 (IBM Statistics) to perform the DFAs in this study. We included four variables in the analyses: duration (ms), frequency of maximum duration (kHz), maximum frequency (kHz) and the number of harmonics. These four variables were selected due to their inclusion in previous studies investigating the vocal structure of manatee vocalisations (Sousa-Lima et al., 2008; Umeed et al., 2018). Pulse calls were not included in the DFA due to the small sample size ($N = 8$) and the fact that only two variables (duration (ms) and frequency of maximum energy (kHz)) were analysed for this call type. Despite the level of chaos and no defined harmonics other than the fundamental, trill variant 4 was included in the analysis because the parameters used could be extracted from the call variant (i.e., Call duration, Frequency of Maximum Energy and Maximum Frequency) and the number of harmonics was considered as 0. Permutational Multivariate ANOVAs (PERMANOVA), using Primer 6 software, were then conducted to test the statistical validity of the groups that we considered in the DFA, in order to determine the accuracy of call categorisation. The same analysis sequence (i.e., DFA followed by PERMANOVA) was performed to test for the differences between the variants of each call category. Whenever possible, we considered at least 10 sample vocalisations from each animal group (i.e., captive females, captive males, reintroduction males 1, and reintroduction males 2) for each call category variant. At least four different individuals contributed to the final sample in the DFA, however it is likely that more individuals contributed due to the number of individuals in each enclosure. Ten vocalisations were selected as this was the minimum number of calls produced for each variant, with the exception of Pulse calls.

2.5.2. Call production/usage

We performed a Permutational ANOVA to test for differences in the call rates produced during the focal group sessions and to evaluate differences in call production between the study groups. We used chi-square tests with contingency tables to compare both the vocal and the behavioural patterns of the study groups, in order to evaluate differences in call usage. Behaviours were classified according to Umeed (2016), Umeed et al. (2018) and Lucchini et al. (2021) (see Table A1 in the Appendix for the ethogram used for our observations).

2.5.3. Individuality in call structure

To determine if there was individuality in the structure of Squeak calls, we performed a DFA using the leave-one-out cross validation method to

compare the physical structures of the vocalisations produced by the four individually recorded animals. We included 10 vocalisation samples from each animal and four acoustic variables in this DFA (i.e., duration, frequency of maximum energy, maximum frequency and number of harmonics). A total of 10 vocalisations were selected as this was the minimum number produced by all individuals. Additionally, we performed a PERMANOVA and a PERMDISP analysis to test for the homogeneity of dispersions using Primer 6 software. We used this sequence of statistical tests (i.e., DFA–PERMANOVA–PERMDISP) because the DFA is a multivariate statistical technique used to analyse (eventually weighing) variables that distinguish particular groups. The DFA identifies which variables discriminate between categorical groups however, it does not statistically evaluate which categorical groups are significantly different among them. The latter analysis was performed here using a PERMANOVA (i.e., to verify whether the Squeak calls of the individual manatees differed statistically). The PERMDISP analysis was used here to confirm that the differences between groups were related to their position in multivariate space and not to dispersion (thus, PERMDISP tested for multivariate homocedasticity). We also performed a Spearman's correlation on the BMIs and the average highest frequencies of Squeak calls from the reintroduction animals recorded individually, to check whether body size (BMI) could be influencing individual call structure.

3. Results

3.1. Call categories

We grouped vocalisations into four categories: Squeak, Trill, Chirp and Pulse calls (Table 4). Statistical analyses comparing the physical structure (Table 5) of Squeak, Trill and Chirp confirmed our call categories. We correctly classified 62.4% of the calls that we assigned to the different call categories, using a leave-one-out cross-validated DFA. We found that all variables used in the DFA contributed to differentiate Squeak, Trill and Chirp call categories, except frequency of maximum energy (duration: Wilks' Lambda = 0.838; $F = 23.928$; $df1 = 2$; $df2 = 247$ $p < 0.001$; frequency of maximum energy: Wilks' Lambda = 0.990; $F = 1.238$; $df1 = 2$; $df2 = 247$ $p = 0.292$; maximum frequency: Wilks' Lambda = 0.948; $F = 6.802$; $df1 = 2$; $df2 = 247$; $p = 0.001$; number of harmonics: Wilks' Lambda = 0.820; $F = 27.068$; $df1 = 2$; $df2 = 247$; $p < 0.001$). Function 1 explained 61.8%

Table 5.
Structure of Antillean manatee calls and their variants for all the recorded study manatees.

Vocal signal	Call duration (ms)	Frequency of maximum energy (kHz)	Maximum frequency (kHz)	Minimum frequency (kHz)	Bandwidth (kHz)	Start frequency (kHz)	End frequency (kHz)	Duration from start to maximum frequency (ms)	Duration from maximum frequency to end (ms)	Number of harmonics	Frequency interval between harmonics (kHz)
Squeak 1 (N = 30)	274.113 ± 16.033	3.224 ± 0.126	3.723 ± 0.124	2.977 ± 0.125	0.746 ± 0.038	3.607 ± 0.128	3.577 ± 0.130	32.713 ± 10.974	241.400 ± 17.084	0.700 ± 0.153	1.100 ± 0.256
Squeak 2 (N = 40)	261.831 ± 9.505	3.017 ± 0.141	3.860 ± 0.172	2.599 ± 0.120	1.260 ± 0.100	3.659 ± 0.169	3.555 ± 0.176	30.968 ± 6.401	230.863 ± 10.415	2.275 ± 0.245	1.727 ± 0.176
Squeak 3 (N = 20)	212.513 ± 7.471	1.002 ± 0.092	1.2721 ± 0.095	0.860 ± 0.080	0.412 ± 0.029	1.256 ± 0.095	1.195 ± 0.086	2.739 ± 1.910	209.774 ± 7.106	8.850 ± 0.059	0.426 ± 0.071
Squeak 4 (N = 10)	264.565 ± 16.794	3.926 ± 0.076	4.677 ± 0.141	2.467 ± 0.149	2.210 ± 0.174	3.304 ± 0.139	4.432 ± 0.100	50.198 ± 5.570	214.647 ± 17.642	4.600 ± 0.636	1.294 ± 0.296
Squeak 5 (N = 30)	317.908 ± 20.203	2.385 ± 0.137	2.825 ± 0.130	1.982 ± 0.144	0.843 ± 0.054	2.559 ± 0.142	2.493 ± 0.131	52.349 ± 5.683	265.560 ± 18.839	6.500 ± 0.540	1.040 ± 0.129
Chirp (N = 10)	95.132 ± 6.326	2.450 ± 0.301	3.073 ± 0.357	2.273 ± 0.264	0.800 ± 0.060	2.912 ± 0.265	2.995 ± 0.237	20.228 ± 8.693	72.905 ± 10.740	2.700 ± 0.637	0.861 ± 0.095
Trill 1 (N = 30)	260.430 ± 6.653	3.226 ± 0.106	4.367 ± 0.157	2.746 ± 0.083	1.621 ± 0.150	4.309 ± 0.166	3.386 ± 0.189	8.491 ± 4.057	251.939 ± 7.499	2.267 ± 0.235	1.705 ± 0.164
Trill 2 (N = 20)	293.024 ± 13.353	2.809 ± 0.209	3.295 ± 0.226	2.475 ± 0.198	0.920 ± 0.055	3.166 ± 0.215	3.156 ± 0.234	46.187 ± 6.767	246.837 ± 13.283	2.350 ± 0.466	1.435 ± 0.284
Trill 3 (N = 20)	283.064 ± 19.241	2.499 ± 0.219	3.286 ± 0.256	2.202 ± 0.176	1.084 ± 0.125	3.207 ± 0.259	3.135 ± 0.264	31.863 ± 11.187	251.201 ± 22.046	1.750 ± 0.270	1.002 ± 0.250
Trill 4 (N = 20)	247.978 ± 16.981	2.496 ± 0.101	3.287 ± 0.125	1.195 ± 0.108	1.449 ± 0.136	3.243 ± 0.131	3.059 ± 0.141	5.541 ± 3.998	242.438 ± 18.351	0	NA

Average values with standard error of the 11 structural variables analysed. Frequency measurements in kHz and time measurements in ms. NA, not applicable.

of the variance and Function 2 explained 38.2%. The PERMANOVA tested the statistical validity of the call categories that we considered to perform the DFA and confirmed the accuracy of our call categorisation ($df = 2$; $SS = 100.65$; $MS = 50.323$; $pseudo-F = 13.883$; $p(\text{perm}) = 0.0001$; unique permutations = 9948). Pair-wise tests confirmed that Squeaks were different from Trills ($t = 3.9371$; $p(\text{perm}) = 0.0001$; unique permutations = 9954), Squeaks were different from Chirps ($t = 3.0403$; $p(\text{perm}) = 0.0001$; unique permutations = 9953; and Trills were different from Chirps ($t = 4.3932$; $p(\text{perm}) = 0.0001$; unique permutations = 9948).

We found that Squeaks and Trills were produced by all the study groups (captive females, captive males and males in both reintroduction enclosures). We found that Chirps were only produced by captive males (Figure 1). We found that Pulse calls were only produced by males and had an average duration (ms) ($N = 8$) of 493.04 ± 159.09 and an average frequency of maximum energy (kHz) of 0.99 ± 0.21 . Spectrograms illustrating the structure of pulse calls can be found in Figure 2.

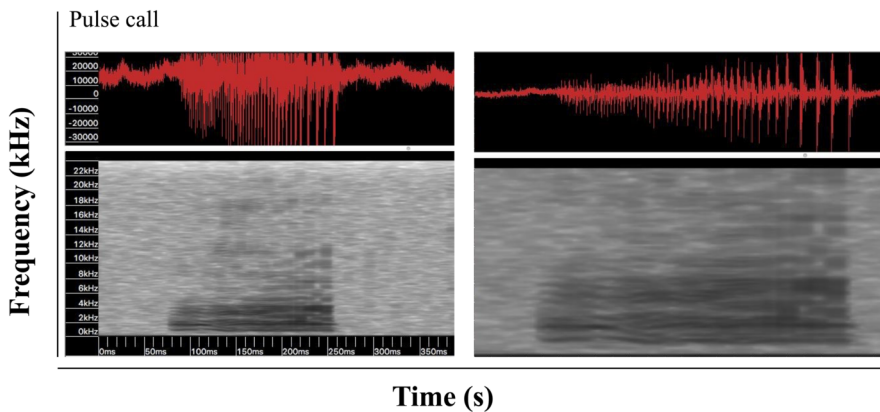


Figure 2. An example of a Pulse call, which were produced by the captive males and the males in reintroduction enclosure 1. Spectrograms were generated using Kaleidoscope Pro5 Software, Wildlife Acoustics (512 samples for FFT, time resolution of 5.33 ms, 50% overlap). Pulse calls were not included in the DFA analysis as they did not meet the established criteria for the minimum number of calls ($N = 10$) and only two variables were measured for this call type: duration (ms) and frequency of maximum energy (kHz). Here we present the preliminary analysis of this call, which should be further investigated in future studies.

3.2. Call variants

We statistically found five Squeak variants, but our analysis failed to confirm the four Trill variants initially grouped. The call variants Squeak 2, Squeak 3, Squeak 4, Trill 1 and the calls Chirp and Pulse have not been previously described for Antillean manatees. The variants Squeak 1, Squeak 5, Trill 2, Trill 3, Trill 4 were previously described by Umeed et al. (2018), where the trill variants were named trills, screeches and creaks.

3.2.1. Squeak calls

When we considered Squeak variants, we were able to differentiate five variants (Figure 1) using the discriminant functional analysis (DFA) and the PERMANOVA. We correctly classified 74.6% of Squeak vocalisations using a leave-one-out cross-validated DFA. All variables contributed to the differentiation between the variants (duration: Wilks' Lambda = 0.794; $F = 8.097$; $df1 = 4$; $df2 = 125$; $p < 0.001$; frequency of maximum energy: Wilks' Lambda = 0.364; $F = 54.537$; $df1 = 4$; $df2 = 125$; $p = 0.001$; maximum frequency: Wilks' Lambda = 0.276; $F = 82.014$; $df1 = 4$; $df2 = 125$; $p < 0.001$; number of harmonics: Wilks' Lambda = 320; $F = 66.354$; $df1 = 4$; $df2 = 125$; $p < 0.001$). Function 1 explained 78.4% of the variance, Function 2 explained 15.3%, Function 3 explained 6.1%. The PERMANOVA confirmed the accuracy of our Squeak variant categorisation ($df = 4$; $SS = 289.66$; $MS = 72.415$; pseudo- $F = 39.993$; $p(\text{perm}) = 0.0001$; unique permutations = 9944) and showed that we could differentiate all variants using a pairwise comparison (Table A1 in the Appendix).

3.2.2. Trill calls

When considering the Trill variants, we could not differentiate all four variants (Figure 2) using the DFA and the PERMANOVA. We correctly classified 53.8% of Trill variants using a leave-one-out cross-validated DFA. Three out of the four variables were important for differentiating the variants (duration: Wilks' Lambda = 0.969; $F = 0.943$; $df1 = 3$; $df2 = 89$; $p = 0.423$; frequency of maximum energy: Wilks' Lambda = 0.809; $F = 7.013$; $df1 = 3$; $df2 = 89$; $p < 0.001$; maximum frequency: Wilks' Lambda = 0.829; $F = 6.140$; $df1 = 3$; $df2 = 89$; $p < 0.001$; number of harmonics: Wilks' Lambda = 0.660; $F = 15.252$; $df1 = 3$; $df2 = 89$; $p < 0.001$). Function 1 explained 89.6% of the variance, Function 2 explained 8.7% and function 3 explained 1.7%. However, although the PERMANOVA confirmed differences between Trill variants ($df = 3$; $SS = 72.25$; $MS = 24.083$;

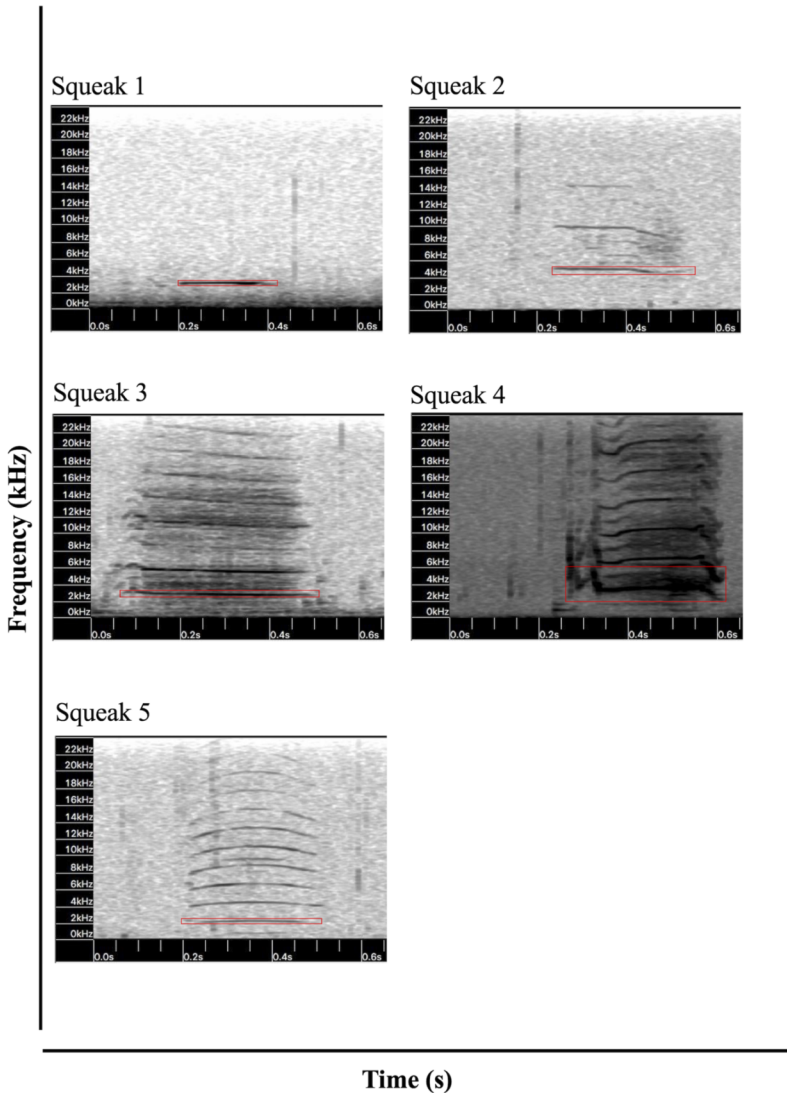


Figure 3. Spectrograms showing the Squeak call category and its variants, Squeaks 1–5. The red rectangles show where the structural variables were measured from for each call. Spectrograms were generated using Kaleidoscope Pro5 Software, Wildlife Acoustics (512 samples for FFT, time resolution of 5.33 ms, 50% overlap). Squeak 1 was produced by captive males and females and the younger males in reintroduction enclosure 2. Squeak 2 was produced by all the study animals. Squeak 3 was produced by the males in the reintroduction enclosures. Squeak 4 was produced exclusively by the older males in reintroduction enclosure 1. Squeak 5 was produced by captive males and females and the younger males in reintroduction enclosure 2.

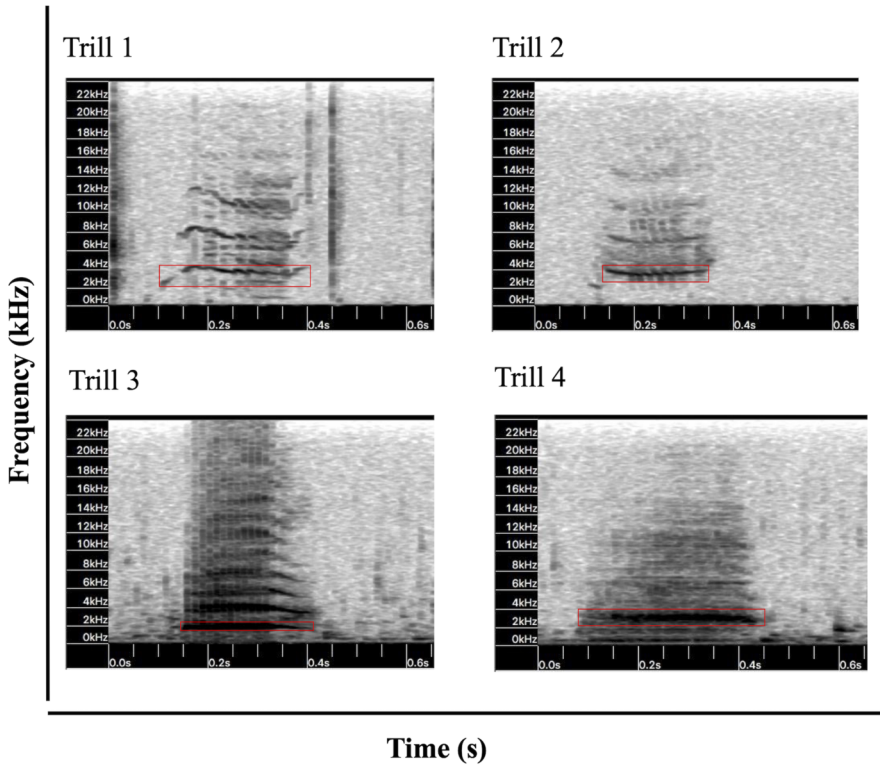


Figure 4. Spectrograms showing Trill calls and its variants, Trills 1–4. Red rectangles show where the structural variables were measured from for each call. Spectrograms were generated using Kaleidoscope Pro5 Software, Wildlife Acoustics (512 samples for FFT, time resolution of 5.33 ms, 50% overlap). Trill 4 was excluded from the DFA analysis as this variant presents a greater level of chaos and no clearly defined harmonics. Trill 1 was produced by captive females and the males in both reintroduction enclosures. Trill 2 was produced by all the study animals. Trill 3 was produced by the captive males and females. Trill 4 was produced by captive males and females.

pseudo- $F = 7.0181$; $p(\text{perm}) = 0.0001$; Unique permutations = 9931), it failed to differentiate all trill variants from one another (Table A2 in the Appendix).

3.3. Call and behavioural patterns

All manatee groups produced Squeaks and Trills. Nevertheless, there was a variation in the number of call variants produced according to sex and enclosure type. Captive females produced six variants of two vocalisation types: three Squeak variants and three Trill variants. Captive males produced all

call types, including three Squeak variants, two Trill variants, Chirps and Pulse calls. The males in reintroduction enclosure 1 produced four variants from two vocalisation categories: three Squeak variants, and one Trill variant. The males in reintroduction enclosure 2 produced five types of variants and three vocalisation categories: four Squeak variants, one Trill variant and Pulse calls.

The call rates (i.e., number of calls divided by the number of animals in the enclosure divided by the observational session) differed between captive females, captive males, males in reintroduction enclosure 1 and reintroduction enclosure 2 ($df = 3$; $SS = 2795.8$; $MS = 931.93$; pseudo- $F = 38.721$; $p(\text{perm}) = 0.0001$; unique permutations = 9955). Pair-wise tests indicated that the call rates of each group varied in relation to each other (Table A3 in the Appendix). The call rates for captive females, captive males, males in reintroduction enclosure 1 and males in reintroduction enclosure 2 were 3.87, 1.99, 12.19, and 6.83 calls per animal per observational session, respectively.

We found significant differences in the call patterns of the different study groups when considering the proportion of each call category produced (Squeaks, Trills, Chirps, Pulses) (Chi square test: 1027.35; $gl: 9$; $p < 0.001$; Figure 5). The behavioural patterns (i.e., the proportion of the different behavioural categories performed) also differed between the groups (Chi square test: 456.79; $gl: 24$; $p < 0.001$; Figure 6).

3.4. Individual differences in Squeak structure

We found that each animal produced a Squeak vocalisation that consistently had the same basic structure (Figure 7), but that often varied in terms of the number of harmonics and duration (Table 6).

We correctly classified 80.0% of individual manatee squeak vocalisations using a leave-one-out cross-validated DFA (Figure 8). We found that all squeak call variables contributed to the differentiation between the individual manatees (duration: Wilks' Lambda = 0.416; $F = 16.879$; $df1 = 3$; $df2 = 36$; $p < 0.001$; frequency of maximum energy: Wilks' Lambda = 0.437; $F = 15.444$; $df1 = 3$; $df2 = 36$; $p < 0.001$; maximum frequency: Wilks' Lambda = 0.317; $F = 25.898$; $df1 = 3$; $df2 = 36$; $p < 0.001$; number of harmonics: Wilks' Lambda = 0.671; $F = 5.894$; $df1 = 3$; $df2 = 36$; $p < 0.002$). Function 1 explained 63.5% of the variance, Function 2 explained 27.7%, and Function 3 8.8%. The PERMANOVA indicated a highly significant difference among the animal vocalisations ($df = 3$; $SS = 84.24$; $MS = 28.08$;

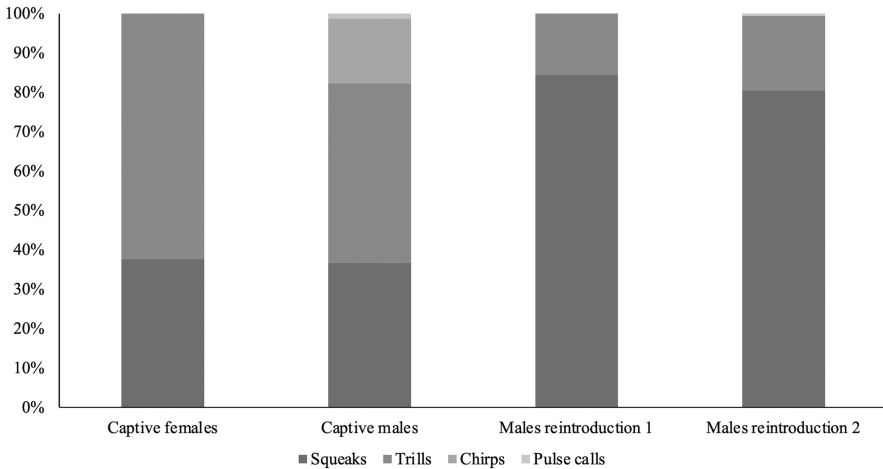


Figure 5. Proportion (%) of vocalisations produced by each study group, captive females ($N = 6$), captive males ($N = 4$), males in reintroduction enclosure 1 ($N = 2$) and males in reintroduction enclosure 2 ($N = 2$). The proportion of vocalisations produced by each group were significantly different for each call type recorded.

Pseudo- $F = 14.087$; $p = 0.0001$, Unique permutations = 9943) and pairwise tests could differentiate between all four individuals (Table A4 in the Appendix). The PERMDISP analysis indicated no significant difference of multivariate dispersion among the animals ($F = 3.25$; $df1 = 3$; $df2 = 36$; $p = 0.108$). Furthermore, there was no correlation between maximum frequency of Squeak calls and the BMI of the four manatees recorded individually (Spearman's rho -0.600 ; $N = 4$; $p = 0.40$).

4. Discussion

Here we demonstrate complexity in different aspects of Antillean manatee vocal behaviour. We described four call categories and their variants produced by Antillean manatees living in captive and reintroduction enclosures and experiencing different artificial social contexts established by enclosure type. Two of the call categories are newly described for Antillean manatees (i.e., Chirps and Pulse calls), which were produced exclusively by the captive and reintroduction males in our study. Chirp calls have been previously described by Brady et al. (2020) for Florida manatees, *Trichechus manatus latirostris*, however, no information on sex-specificity was provided in the study. Two of the call categories (Squeaks and Trills) presented distin-

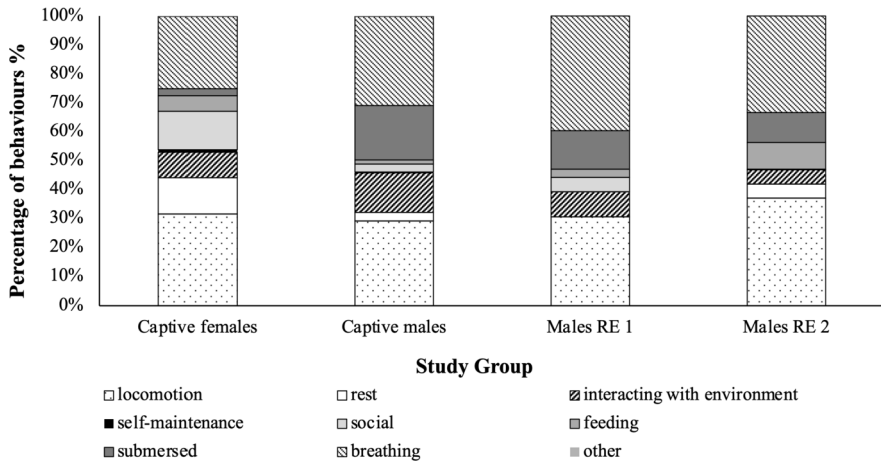


Figure 6. The observed focal behaviours during the recordings for each study group (captive females ($N = 6$), captive males ($N = 4$), males in reintroduction enclosure 1 (Males RE1) ($N = 2$) and males in reintroduction enclosure 2 (Males RE 2) ($N = 2$)). The ethogram used here followed behavioural definitions by Umeed et al. (2018) (locomotion, rest, social, feeding, submersed and other) and Lucchini et al. (2021) (interaction with environment, self-maintenance) (Table 2). The proportion of behaviours demonstrated for each study group were significantly different.

guishable variants, some of which were also newly described for Antillean manatees. However, not all call variants were produced by all social groups in our study.

Overall, the study manatees presented different call repertoires, call structures and call production according to their social context. The ability to adjust vocalisations depending on environments and social contexts can be useful for optimising communication (Hanna et al., 2014; Gill et al., 2015). This ability may be particularly useful for manatees since they often move between areas of mangroves, open sea and estuaries and therefore, experience physical changes in their environments (Favero et al., 2020; Medeiros et al., 2021; Santos et al., 2022) and can have varied group configurations (Ramirez-Jimenez et al., 2017). The ability to vary vocal production and structure may be especially useful when trying to avoid the degradation of physical call attributes during propagation due to variations in water temperature, pH (Ilyina et al., 2009) and shallow depths (Ramos et al., 2020) or masking of vocalisations by ambient noise (Miksis-Olds & Tyack, 2009). Many species are known to alter their vocalisations (rate and physical struc-

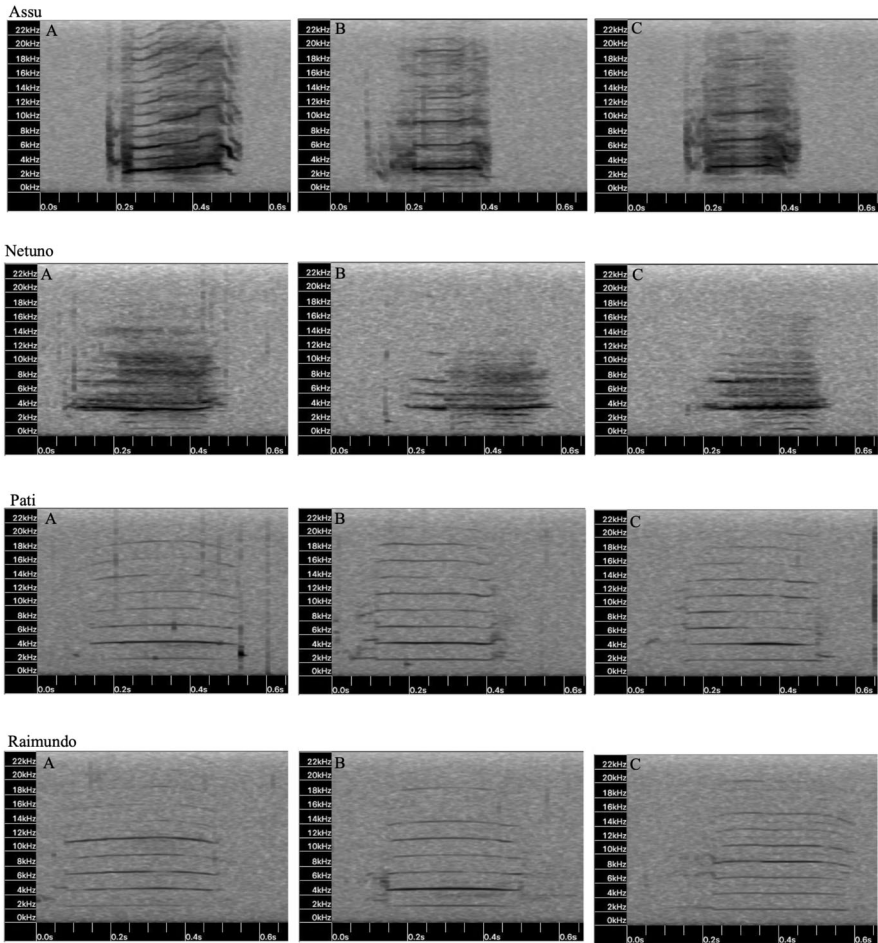


Figure 7. Inter and intraindividual differences (A, B, C) in the squeak calls produced by Assu, Netuno, Raimundo and Paty. Spectrograms were generated using Kaleidoscope Pro5, Wildlife Acoustics, (512 samples for FFT, time resolution of 5.33 ms, 50% overlap) to visually illustrate the individuality observed for the individual-specific vocalisations.

ture) to compensate for cue-masking effects in noise polluted environments (Berger-Tal et al., 2019).

On the other hand, some level of vocal stability could be potentially beneficial for communicating in different social contexts (Rekdahl et al., 2013). Some factors that were not accounted for in our study, such as pH, salinity, turbidity, tide level, as well as enclosure depth and wall material, could have affected the physical structure of the vocalisations recorded. For exam-

Table 6. The average and standard error values of the variables analysed to identify 10 signature Squeak vocalisations produced by each of the four study animals.

	Animal			
	Assu	Netuno	Raimundo	Paty
Vocalisations (<i>N</i>)	10	10	10	10
Call duration (ms)	302.806 ± 10.156	387.083 ± 9.648	446.836 ± 12.792	372.735 ± 21.672
Frequency of maximum energy (kHz)	3.593 ± 0.114	1.627 ± 0.399	2.337 ± 0.014	2.362 ± 0.012
Maximum frequency (kHz)	4.811 ± 0.204	1.954 ± 0.410	3.259 ± 0.137	2.645 ± 0.029
Minimum frequency (kHz)	2.858 ± 0.119	1.485 ± 0.386	2.222 ± 0.020	2.277 ± 0.018
Bandwidth (kHz)	1.954 ± 0.207	0.469 ± 0.040	1.038 ± 0.131	0.368 ± 0.029
Start frequency (kHz)	4.005 ± 0.247	1.858 ± 0.400	3.260 ± 0.137	2.645 ± 0.029
End frequency (kHz)	3.237 ± 0.224	1.858 ± 0.400	2.612 ± 0.120	2.489 ± 0.017
Duration from start to maximum frequency (ms)	67.158 ± 29.07	0	0	0
Duration from maximum frequency to end (ms)	235.648 ± 27.867	387.083 ± 9.648	446.836 ± 12.792	372.735 ± 21.672
Number of harmonics	7.80 ± 1.009	11 ± 0.537	8.3 ± 0.448	7.7 ± 0.367
Frequency interval between harmonics (kHz)	0.361 ± 0.344	0.136 ± 0.196	1.157 ± 0.162	1.590 ± 0.115

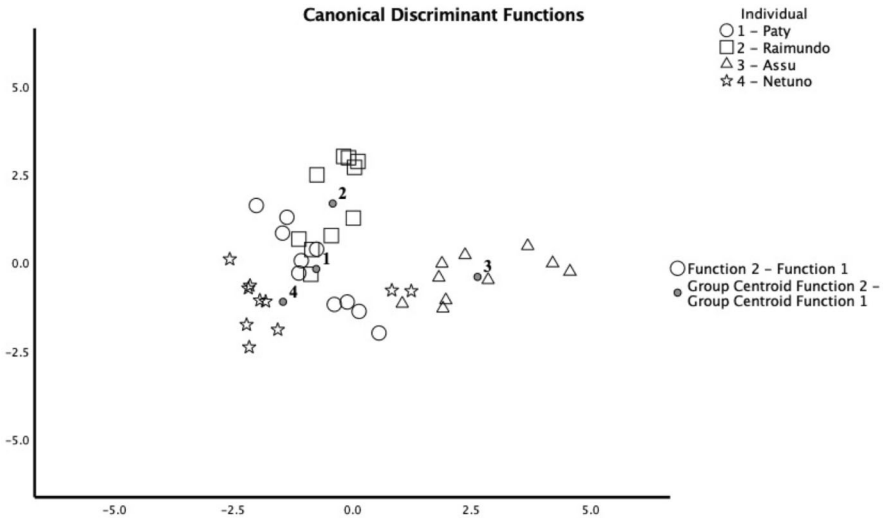


Figure 8. Distribution of the discriminant scores for the individual calls produced by the four study manatees in the reintroduction enclosures along two canonical discriminant functions established to discriminate among the vocalisations. The scatterplot demonstrates the classification of the vocalisations produced by individuals in the reintroduction enclosures.

ple, the physical structures of the calls produced by captive animals may be structurally different to the same calls produced by the manatees in the reintroduction centres, as a result of propagation effects caused by abiotic variables, or reflection from the captive enclosure walls. Chavarría et al. (2015) found that the sound transmission of Antillean manatee calls is influenced by estuarine processes, as well as estuarine sedimentary cumulative effects and concluded that frequency transmission is mainly dependent on river depth and bottom characteristics. Additionally, studies in marine environments have demonstrated that sound absorption decreases with increasing temperatures, as well as with decreasing pH, facilitating underwater sound propagation (Ilyina et al., 2009). As such, natural, as well as anthropogenic noises, will travel further, resulting in an increase in underwater noise levels (Kloepper & Simmonds, 2014; Gazioglu et al., 2015). Therefore, it is important to consider the effects of abiotic water features on manatee vocalisation structure in future studies. Behavioural context may also affect call structure, however, since we did not control for behavioural context in this study, future studies are required to further elaborate this notion.

All call categories and the squeak variants could be statistically distinguished. Nevertheless, the low correct call classification for Trill variants

in the DFA was reflected in the PERMANOVA which failed to distinguish between some of the Trill variants we previously grouped. This result may have been influenced by some factors that were not considered in our analysis — i.e., manatee group, sex or individuals—since it was not feasible to isolate the animals to record them individually. Green (1981) notes that specific morphologies in the vocal repertoire of one sex may be absent in the other. These differences may be due to anatomical dissimilarities, which result in the production of a specific acoustic shape by only one of the sexes (Green, 1981). Green (1981) further notes that such differences would be expected to be seen in animals that have reached maturity. However, if these differences are associated with control of the phonatory apparatus rather than anatomical dissimilarities, they would be observed in early puberty or even before (Green, 1981). Such anatomical differences have not yet been observed for West Indian manatees, *Trichechus manatus*, however research on the topic is limited (Murie, 1872; Landrau-Giovanetti et al., 2014). Currently, research has shown that the larynx of manatees is structurally similar to that of terrestrial mammals and that vocal folds appear to be the primary mechanism for sound production in manatees, however sexual dimorphism in manatee vocal mechanisms has not yet been addressed (Landrau-Giovanetti et al., 2014).

The repertoire of call variants varied between the animal groups, where the captive males had the most varied repertoire, producing all call category types (Squeaks, Trills, Chirps and Pulse calls), including a total of five statistically distinguishable call variants. Followed by the younger adult males in reintroduction enclosure 2, who produced three call types (Squeaks, Trills, Pulse calls), including five statistically distinguishable call variants too. This result supports the idea that age may influence call production in Antillean manatees since, from the perspective that call variants may be indicative of individuality, the greater variance in younger males may suggest that manatee individuality is defined at a later stage in life (Sousa-Lima et al., 2008; Umeed et al., 2018). Our study captive females had the least varied repertoire producing only two types of vocalisations, thus supporting our first prediction that females would produce more stereotypical (i.e., simpler) repertoires. Furthermore, since several Squeak and Trill variants and two call categories (i.e., Chirp and Pulses) were found to be exclusively produced by males, we reinforce the findings of previous studies that have demonstrated that sex influences vocalisation production in Antillean manatees (Umeed et al., 2018).

The overall call rate varied between the study groups. We found that older males in reintroduction enclosure 1 had the highest call rate followed by the younger males in reintroduction enclosure 2, the captive females and the captive males. This was contrary to our first and second predictions, where we expected females to produce the greatest number of vocalisations, since the captive females comprised the study group with the greatest number of individuals. Thus, Antillean manatees may not conform to the entirety of the social complexity hypothesis for communication for the vocal modality (see Freeberg et al., 2012 for a review of the different predictions of this hypothesis). It is possible that it was not energetically beneficial nor necessary for females to maintain frequent vocal contact with one another as they were constantly in close proximity to one another in their captive enclosure. We suggest that other modalities of communication, such as, tactile (see Lucchini et al., 2021 for the variety of tactile signals exhibited in Antillean manatees), should be considered in the future when testing the social complexity hypothesis for manatee communication. On the other hand, males in the reintroduction enclosures had a greater amount of space and a lower number of individuals in the enclosure, potentially creating a more complex scenario, i.e., the males were able to separate and come together more freely, likely requiring greater vocal production in order to maintain contact. Studies in captivity have also demonstrated that manatees exhibit more social behaviour and vocalise more during the night compared to during the day (Hénaut et al., 2010; Costa et al., 2018). Therefore, the fact that this study was conducted during the day may also help to explain the lower call rates recorded for the captive females.

We found that the proportion of use of the different call categories varied between the four groups, which reflects the different behavioural patterns observed for each group. Squeaks were produced significantly more by the males in the readaptation enclosures, compared to the animals in the captive enclosures and Trills were produced significantly more by captive animals. Brady et al. (2022) found that animals under greater levels of stress almost exclusively produced Squeak vocalisations, whereas animals produced “Squeals”, described here as Trills, during cavorting behaviours. This may explain the differences in vocal call type production observed here. Sex, social context and enclosure limitation may explain this result. Green (1981) organised sex-specific differences in animal acoustic signals into three main categories: (1) vocalisations that are produced by one sex and not the other;

(2) vocalisations that are produced by both sexes, however, differ in their usage; and (3) vocalisations produced by both sexes yet, have different structures that result from sexual dimorphism in the structure and size of sound-producing organs (Sayigh et al., 1995). The first and the third differences described by Green (1981), were demonstrated by Umeed et al. (2018) for Antillean manatees, in terms of vocal production since, as of yet, no anatomical studies have been performed on sexual dimorphism in sound-producing organs. However, here we provide indirect evidence of Green's second category of differences for Antillean manatees for the first time. Our results suggest that the vocalisations that are produced by both sexes (i.e., Squeaks and Trills) differ in their usage — i.e., animals of different sexes with different behavioural patterns present different call patterns. Nevertheless, studies focusing on recording specific behaviours and vocalisations from specific focal individuals (rather than a focal group) would be the ideal scenario to test this theory of sex-related differences, suggested by Green (1981). Such ideal observational conditions, however, may not be easily obtained (with Antillean manatees housed in captive, natural reintroduction or free-living scenarios) in Brazil without causing potential isolation-related stress in the animals.

We found that the four individuals recorded separately in the reintroduction enclosures in the year 2020, produced calls with unique physical structures, thus supporting our final prediction that Antillean manatee vocalisations differ structurally between individuals. Although studies have demonstrated that body size and call frequency are negatively correlated (Matthews et al., 1999; May-Collado et al., 2007; Sousa-Lima et al., 2008; Dunn et al., 2017), we found no correlation between the BMI and maximum frequency of squeak calls. Here, Netuno's calls had the lowest average maximum frequencies and the lowest frequency range, and he was the largest and oldest of the individually recorded animals. However, Paty was the smallest individual and also produced calls with low average maximum frequencies. Additionally, the calls produced by Raimundo and Paty had highly similar structures which suggests that manatee signature vocalisations may be learned as calves or may be defined during the sub-adult development stage and likely simulate the acoustic signals of related and/or unrelated individuals (Green, 1981; Sousa-Lima et al., 2008). This phenomenon has been recorded for bottlenose dolphins, *Tursiops truncatus* (Fripp et al., 2005), as well as for Blainville's beaked whales, *Mesoplodon densirostris* (Dunn et

al., 2017) and killer whales, *Orcinus orca* (Nousek et al., 2006). Paty was raised in isolation at the ICMBio/CMA for three years and was then moved to the female oceanarium. She was then translocated to reintroduction enclosure 2 when she was four years old and was placed together with Raimundo. Thus, Paty and Raimundo spent a period of 1 year together at the time of our recordings. However, further studies are required to investigate at what age signature vocalisations in manatees become defined, if at all.

Individuality in vocalisations has been identified for several other species of marine mammals, such as bottlenose dolphins, *Tursiops truncatus* (Caldwell & Caldwell, 1979); free-ranging common dolphins, *Delphinus delphis* (Fearey et al., 2019); Indo-Pacific bottlenose dolphins, *Tursiops aduncus* (Gridley et al., 2014); captive beluga whales, *Delphinapterus leucas* (Morisaka et al., 2013) and narwhals, *Monodon monoceros* (Shapiro, 2006). The widespread occurrence of signature vocalisations highlights their importance for individual survival. Across mammalian species, the most common infant isolation call is a long, tonal call which effectively recruits help from mothers (Zeifman, 2001; Newman, 2004, 2007; Lingle et al., 2012). This apparent requirement of some level of vocal stability may introduce the idea of signature vocalisations for mammalian species, i.e., vocalisations that remain the same independent of social and physical contexts (Rekdahl et al., 2013). This could suggest the use of Squeaks as potential signature vocalisations in manatees, as they generally have longer durations and are more tonal, compared to the other types of vocalisations described here. Our findings introduce the possibility of creating an individual vocalisation database for manatees in north-eastern Brazil, where reintroduced and wild manatee vocalisations could be recorded and archived for monitoring purposes. This would facilitate governmental and independent agencies in identifying and monitoring individual animals and may be a potentially important tool for the conservation of North-eastern manatee populations.

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Appendix A

Table A1.

Results of pair-wise tests indicating that all Squeak variants differ in relation to each other.

Pair-wise comparison	<i>t</i>	<i>p</i> (perm)	Number of unique permutations
Squeak 1, squeak 2	2.9561	0.0002	9935
Squeak 1, squeak 3	11.023	0.0001	9946
Squeak 1, squeak 4	3.3279	0.0001	9949
Squeak 1, squeak 5	5.0565	0.0001	9955
Squeak 2, squeak 3	11.709	0.0001	9941
Squeak 2, squeak 4	2.6844	0.0003	9948
Squeak 2, squeak 5	5.4343	0.0001	9949
Squeak 3, squeak 4	11.463	0.0001	9943
Squeak 3, squeak 5	5.2657	0.0001	9939
Squeak 4, squeak 5	3.7923	0.0001	9956

The structural variables of the calls used in the comparison were duration (ms), frequency of maximum energy (kHz), maximum frequency (kHz) and number of harmonics.

Table A2.

Results of pair-wise tests indicating that Trill variants differ in relation to each other.

Pair wise comparison	<i>t</i>	<i>p</i> (perm)	Number of unique permutations
Trill 1, trill 2	1.2379	<u>0.1933</u>	9945
Trill 1, trill 3	2.7378	0.0008	9944
Trill 1, trill 4	4.8409	0.0001	9937
Trill 2, trill 3	1.6627	<u>0.0551</u>	9955
Trill 2, trill 4	3.4013	0.0001	9948
Trill 3, trill 4	2.1509	0.0073	9955

The structural variables of the calls used in the comparison were duration (ms), frequency of maximum energy (kHz), maximum frequency (kHz) and number of harmonics. Underlined values indicate non-significant results.

Table A3.

Pair-wise test results for the call rates (number of calls/number of animals/number of observational sessions) of each study group in relation to each other.

Pair-wise comparison	<i>t</i>	<i>p</i> (perm)	Number of unique permutations
Captive females, captive males	2.8641	0.0034	1149
Captive females, reintroduction males enclosure 1	8.5683	0.0001	1068
Captive females, reintroduction males enclosure 2	3.2107	0.0015	952
Captive males, reintroduction males enclosure 1	10.581	0.0001	718
Captive males, reintroduction males enclosure 2	5.1524	0.0001	575
Reintroduction males 1, reintroduction males 2	4.2161	0.0001	394

Table A4.

Pair-wise test results indicating differences between the Squeaks produced by four individual manatees.

Group	<i>t</i>	<i>p</i> (perm)	Number of unique permutations
Paty, Raimundo	2.6629	0.0064	9443
Paty, Assu	3.8761	0.0002	9420
Paty, Netuno	2.4546	0.004	9479
Raimundo, Assu	4.8776	0.0001	9425
Raimundo, Netuno	2.9119	0.0014	9483
Assu, Netuno	4.6051	0.0003	9446

The structural variables of the calls used in the comparison were duration (ms), frequency of maximum energy (kHz), maximum frequency (kHz) and number of harmonics.

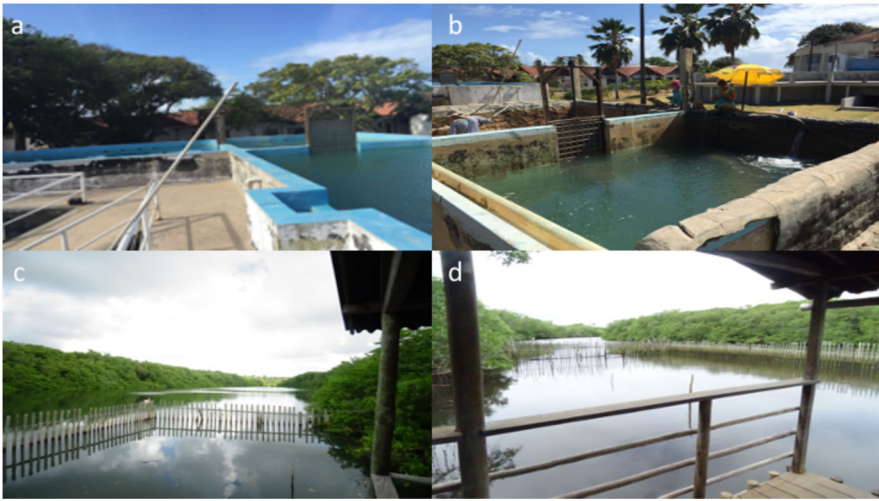


Figure A1. Images of the (a) captive female pool, (b) captive male pool, (c) reintroduction male enclosure 1 and (d) reintroduction male enclosure 2. Photo sources: RU, KL.