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Delving deep into unheard waters: new types of low frequency pulsed sounds described for the boto (*Inia geoffrensis*)

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Abstract
The Amazon river dolphin, or boto (*Inia geoffrensis*), is an endangered species, distributed over an extensive area of the Amazon–Orinoco basin. Here, we analyzed the acoustic behavior of a previously unstudied population of botos at the Juami-Japurá Ecological Station, Amazonas. We categorized call types according to their visual and acoustical parameters. Random forest decision trees were used to assess the validity of our classification. Six types of narrowband pulsed sounds were found, of which four are being described for the first time for the species. The vocalizations analyzed had mean maximum frequency of 25.7 kHz and mean minimum frequency of 1.1 kHz. The narrowband pulsed sounds are complex and different from those emitted by most members of the family Delphinidae. Although the acoustic repertoire of botos is not completely understood in functional terms, these pulsed sounds may play an important role in the social structure of the animals.

Keywords *Inia geoffrensis* · Amazon river dolphin · Boto · Bioacoustics · Narrowband pulsed sounds

Introduction

River dolphins are found only in South America and Southern Asia (Reeves et al. 2009), under great human pressure (Reeves et al. 1994; Trujillo et al. 2010). In South America, at least two river dolphin species (*Inia geoffrensis*–boto—and *Sotalia fluviatilis*—tucuxi) occur in three river basins (Amazon, Orinoco and Tocantins-Araguaia) (Best and da Silva 1989a; Best and da Silva 1989b; Pilleri and Gihr 1977; Rice 1998; Dudgeon et al. 2006), and two new species (*I. boliviensis* and *I. araguaiaensis*) are under consideration to be different from *I. geoffrensis* (Best & da Silva, 1989; Hrbek et al. 2014). Da Silva et al. (2018a) found a decline in the populations of *I. geoffrensis* and *S. fluviatilis* in Central Amazon, which led to the classification of *I. geoffrensis* as "endangered" by the IUCN (da Silva et al. 2018b). The Amazon basin is the main habitat for botos and their behavior are strongly influenced by the annual water cycle. They live close to the riverside and aggregate in confluences, lakes and small channels; during the period of full water, they can also be found in flooded forests (Gomez-Salazar et al. 2012). Cetaceans that inhabit deep or turbid waters such as rivers, estuaries and bays, with low visibility, use acoustic perception to assist with functions related to survival such as foraging and navigation (Parsons and Dolman 2003). In general, studies with Amazon river dolphins have not considered multiple areas of their distribution and not much is known about their acoustic behavior.

Knowing the diversity of acoustical signals of these animals is crucial for understanding the species' biology. Communication mechanisms among animals have evolved to adapt to different abiotic conditions (Morton 1975; Wiley and Richards 1978; Sugiura et al. 1999). The habitat of Amazon river dolphins is characterized by water rich in sediments, which affects the acoustical properties of vocalizations, since the characteristics of the acoustic environment can interfere with the perception of sound (Hamilton 1980; Medwin 2005). In particular, lower frequency sounds can be better transmitted to nearby animals without great losses due to the high attenuation, spreading and scattering process that presents an environment rich in sediment (Hamilton 1980).

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Although botos do not form cohesive groups, they can aggregate in productive environments with high fish densities and low current speeds (Gomez-Salazar et al. 2012). The narrowband pulsed sounds of *Inia* sp. are characterized as low in frequencies (Caldwell and Caldwell 1966; Ding et al. 1995; Diazgranados and Trujillo 2002; Podos et al. 2002; Amorim et al. 2016; Melo-Santos et al. 2019). Since they can potentially reach greater distances without considerable losses, they may have the function of carrying information from the vocalizing animal, thus being important for the social functioning of the species.

The boto produces broadband pulsed sounds, narrowband pulsed sounds (Caldwell and Caldwell 1966) and whistles (Diazgranado and Trujillo 2001; Ding et al. 2001; May-Collado and Wartzok 2007). The broadband pulsed sounds were divided by Caldwell et al. (1966) and Caldwell and Caldwell (1967) into four categories: single intense clicks, echolocation clicks, jaw-snaps and burst-pulsed signals. The narrowband pulsed sounds were described for the first time by Caldwell and Caldwell (1966) and they are not found in the tucuxi (*S. fluviatilis*). Few attempts have been made to describe and understand the context of these sounds. The majority of studies have described the broadband pulsed sounds of the boto, but not much is known about the narrowband pulsed sounds. Pulsed sounds are associated with close-range interactions among individuals, as in agonistic interactions among males for access to females (Herzing 2000). Burst-pulses sounds in general are not well studied, although they seem to have a predominantly communicative function (Herman and Tavolga 1980). Diazgranados and Trujillo (2002) recorded these calls for two populations in the Orinoco river basin in Colombia, and Podos et al. (2002) described one type of narrowband pulsed sound produced by *Inia geoffrensis,* during foraging behavior, when such pulsed signals possibly occurred. Melo-Santos et al. (2019) analyzed the repertoire of *Inia araguaiaensis*, where some narrowband pulsed sounds were found and described as complex in structure, presenting nonlinear phenomena.

Given the lack of knowledge about the narrowband pulsed sounds produced by the boto, and the plausible social function of these vocalizations, we aimed to describe and categorize these sounds in a population of *I. geoffrensis* at Juami-Japurá Ecological Station, Amazonas, Brazil.

Materials and methods

Study area

The Juami-Japurá Ecological Station (JJES) (01º39'S and 68º02'W) is a Brazilian conservation unit in Amazonas State, with an area of more than 800 thousand hectares (Fig. 1). It covers the entire hydrographic basin of the Juami river and it is located in the interfluvial region of the

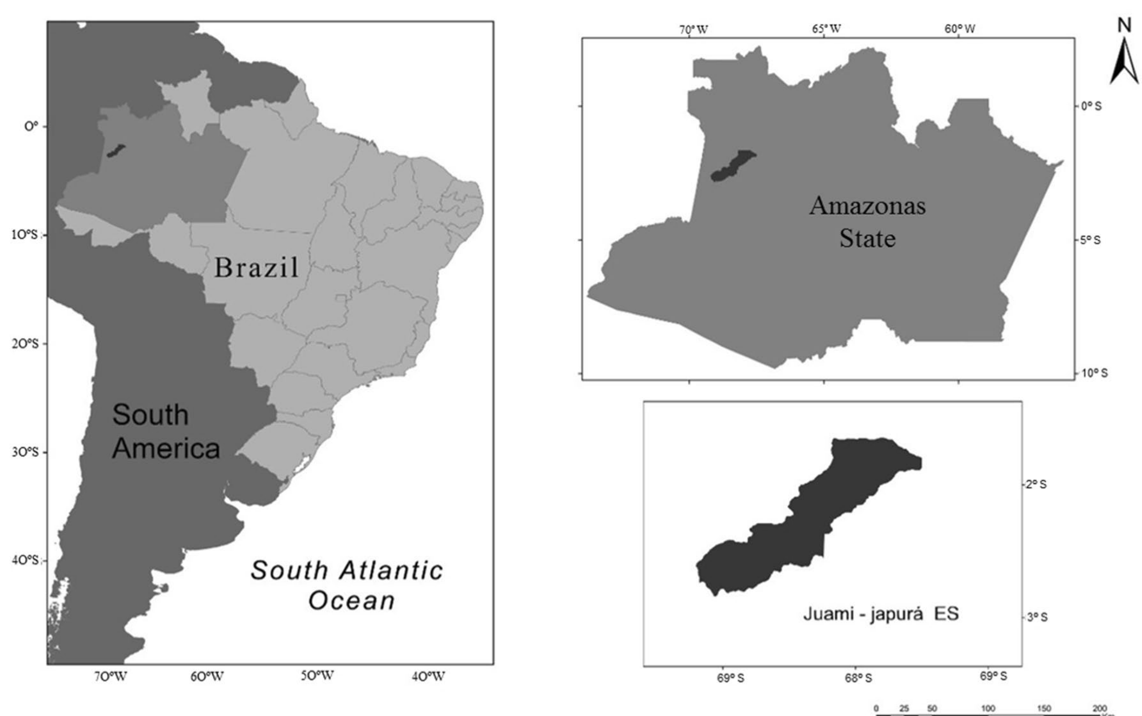

Fig. 1 Study area: Juami-Japurá Ecological Station, in the State of Amazonas, Brazil

Solimões river and the Japurá river. The climate is the rainy tropical type (Af subgroup of the K€ oppen climate classification system) and the dry season occurs from July to November (RadamBrasil 1977).

Field sampling and spectrographic analysis

We used a drifting boat to find the animals and record the sounds. Once an individual or a group were sighted, the engine of the boat was turned off and the recordings was made until the animals left the area. Data collection was carried out from August 29th to 31th 2012, between 6 am and 6 pm. The individuals or groups were followed along the river, and a distance of approximately five to seven meters was estimated between the hydrophone and the animals to ensure that the sound attenuation did not significantly modify the records. The number of individuals, presence of calves, behavior state and presence of the other species of river dolphin, *Sotalia fluviatilis*, were noted during the recordings.

Vocalizations were recorded with a Cetacean Research™ C54XRS hydrophone positioned between 2 and 4 m depth (+3/– 20 dB, −185 dB re 1 V/μPa, frequency response of 48 kHz/24bits), coupled to a digital Fostex® FR-2 LE recorder. Raven Pro 1.4 software (Cornell Laboratory of Ornithology, Cornell University, New York), configured with a Hamming window, and FFT of 1024 points with 60% of overlap, was used to analyze the calls.

Visual aspects of each vocalization in the spectrogram were assessed to separate them into different types. Vocalizations with similar visual patterns in the spectogram such as format, frequency, presence and duration of syllables, harmonics, in addition to acoustic similarity were used for the categorization of sounds. Initially, one person was responsible for analyzing each vocalization and looking for similarities and differences, to later categorize them. After this first stage, two other people evaluated each type of vocalization visually and acoustically, confirming whether they belonged to the same category previously determined, or whether the same category of sound could be subdivided into additional categories. Then, through Raven software, each vocalization was examined, and the following parameters were extracted for the analysis: minimum frequency, maximum frequency, peak frequency and center frequency, bandwidth (maximum–minimum frequency) and duration. Calls presenting SNR (signal-to-noise ratio) lower than 10 dB (the energy of the sound that is captured by the hydrophone) were disregarded so that time and frequency parameters could be clearly measured. As the analyzed vocalizations contains low frequencies, background noise sometimes interfered with the reliability of minimum frequency measurements. However, as each vocalization was analyzed by a person, and not automatically, greater care was taken to distinguish background noise from boto calls, minimizing errors and obtaining better reliability in measurements. Descriptive statistics–mean, standard deviation, minimum and maximum values–were calculated for each parameter.

To assess the validity of the visual categorization, Random forest models were created using R 3.5 (package randomForest) (version 3.4.3, R Core Team 2015) to classify the pulsed sounds. Random forest models are a series of unpruned classification trees, with 5000 bootstrap samples taken from the original data set. The data not selected to build a tree were referred to as "out-of-bag" (OOB) and were used to assess the validity of classification accuracy of the forest, estimating the error rate (Brieman 2001; Liaw and Wiener 2002). Next, the importance of each variable was tested with the Gini variable importance measure. This metric is based on a weighted mean of the improvement of individual trees based on the inclusion of each variable as a predictor. We used 70% of the data for training and 30% were for testing while the "area under the curve" (AUC) was measured as an evaluation of the classifier.

Results

Eighteen recording sessions, with a total of 8 h and 27 min, were analyzed, in which 1,447 signals were selected. The number of individuals varied from 1 to 6 (2.42 ± 1.5) during the recordings (Table 1). The other river dolphin *Sotalia fluviatilis* was present in 50% of the recording sessions. The observed behavior states of the botos were feeding, traveling, and socializing. However it was not possible to identify the behavior state of the animals during six recording sessions.

Different types of narrowband pulsed sounds were identified and divided into six distinct types according to their visual and acoustic characteristics in the spectrogram. They were: type A ($n = 74$), type B ($n = 1181$), type C ($n = 27$), type D ($n = 134$), type E ($n = 7$) and type F ($n = 24$) (Fig. 2). Types A and B were already described in the literature (Melo-Santos et al. 2019; Podos et al. 2002; Diazgranado and Trujillo 2002; Amorim et al. 2016), while types C, D, E, and F have never been reported before for *Inia geoffrensis*. The descriptive statistics of acoustical parameters for each type are presented in Table 2. The vocalization classified as Type A is characterized by containing single or multiple units and harmonics structures. Type B presented single and multiple units with harmonic structures. The vocalization characterized as type C is composed of a single unit with a harmonic structure. It presented a low mean frequency (Fmin: 1.0 ± 0.7 kHz, Fmax 4.7 ± 1.6 kHz) when compared with the other types. Type D has short duration when compared with the others (0.04 ± 0.02) and may have single or multiple units. Type E presented a mean minimum frequency (12.0 ± 0.8 kHz)

Table 1 Overview of data used in the analysis including the number of individuals and calves of *Inia geoffrensis*, presence of *Sotalia fluviatilis* during the recording session and the behavior state of *I. geoffrensis* in the Juami-Japurá Ecological Station

Recording session	Individuals of *I. geoffrensis*	Number of calves	Presence of *S. fluviatilis*	Behavior state
1	1	0	Yes	–
2	3	0	No	Feeding
3	4	0	No	Feeding
4	2	0	No	Feeding
5	1	0	Yes	–
6	1	0	Yes	–
7	3	0	No	–
8	2	0	Yes	Feeding
9	2	0	No	Traveling
10	2	0	No	–
11	1	0	Yes	Feeding
12	2	1	Yes	Feeding/Traveling
13	2	0	Yes	Feeding
14	6	1	Yes	–
15	2	0	No	Feeding
16	6	0	Yes	Feeding/Socializing
17	3	0	No	Feeding
18	2	0	No	Traveling

and mean maximum frequency (18.6 ± 3.1 kHz) higher than the other types. Finally, type F is similar to type A in all parameters, but was visually and acoustically different, without well-defined harmonics as seen in type A (Fig. 2).

Random forest decision trees (Fig. 3) showed the separation of each type through the measured acoustical parameters (frequencies and duration of the sounds). Given that visual characteristics were distinct, the Random Forest results reinforced such types. Types C and B had more misclassifications (45%), indicating overlaps in the measured acoustical parameters. However the visual characteristics indicated distinct pulse structures, they were thus kept in different categories. The confusion matrix is represented in relative numbers, and the balanced accuracy (i.e., a metric that evaluates the classification of each category independently) was 80% with 95% of confidence intervals (0.72, 0.86) (Table 3). The correct classification rates (80% of total rate) for the vocalizations were: type E (100%), type B (91.8%), type D (90.5%), type F (85.7%), type A (57.9%), and type C (22.2%). The Gini variable importance measure was implemented to estimate the importance of each variable (Fig. 4). The duration of the sound was the most important parameter for classification of each type according to the model.

The "area under the curve" (AUC) values can be visualized in Fig. 5. It is an indicator of the goodness of fit and, according to the test, type E was the best classified, with an AUC of 100%, whereas type C was the worst classified with an AUC of 92.8%.

Discussion

Narrowband pulsed sounds have already been described for other species, e.g., killer whales (*Orcinus orca*), which seems to be the most basal species within the Delphinidae (McGowen et al. 2009; Steeman et al. 2009). Simon et al. (2007) describes the pulsed calls of killer whales as signals consisting of several short pulses emitted at such a high repetition rate that they sound tonal to humans. For killer whales, pulsed sounds have specific functions, such as maintaining the spatial geometry of traveling groups (Miller 2002) or herding prey (Simon et al. 2006).

We did not find whistles in the recordings when only the boto was present. As the delphinid *Sotalia fluviatilis* coexists with *Inia geoffrensis* in the sampled location, we cannot attribute the whistles to *Inia*. Even so, the boto produces whistles at a much lower rate than other delphinids (May-Collado and Wartzok 2007), which lead us to believe that the intraspecific communication for boto is mostly through pulsed sounds described here. The narrowband and low-frequency pulsed sounds have already been described for the boto (Caldwell and Caldwell 1970; Podos et al. 2002, Diezgranados and Trujilo 2002; Rocha 2009; Amorim et al. 2016; Melo-Santos et al. 2019; Melo-Santos et al. 2020), while for the tucuxi only broadband pulsed sounds were described (Norris et al. 1972; Kamminga et al. 1993; May-Collado and Wartzok 2010). Thus, the pulsed sounds described here have been assuredly attributed to the botos.

Six different types of narrowband pulsed sounds were found in this work. Type A was also described by Caldwell and Caldwell (1970), which they termed as "squeal" and presented a minimum frequency of 1 kHz. Rocha (2009) also describes this sound, with a minimum frequency of 1.3 ± 1.1 kHz and a maximum frequency of 17.0 ± 7.5 kHz. Here, the minimum frequency of type A was 1.0 ± 0.8 kHz and the maximum frequency was 12.7 ± 5.9 kHz. This vocalization thus presents visual and frequency parameters consistent with the previous studies.

Type A was found in six recordings, when there were 2—6 dolphins in the area, indicating that this vocalization may have a social function. In two recordings, we identified the presence of calves. Melo-Santos et al. (2019) also described this type of sound for *Inia araguaiaensis*, where they called "short-two component calls". In their study, the sounds were present in 74% of the repertoire ($n = 538$) analyzed and only when calves were in the group. However,

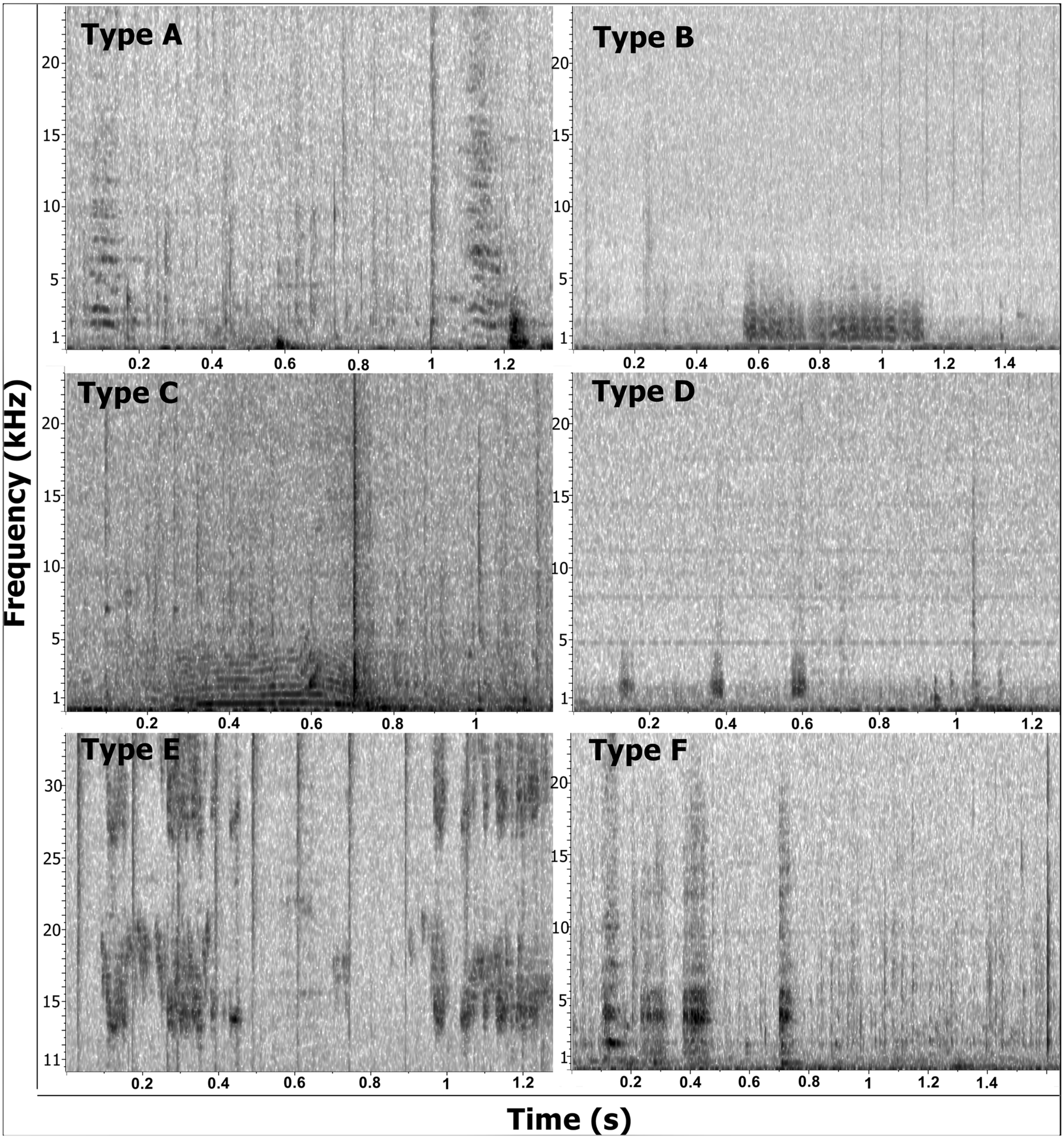

Fig. 2 Types of narrowband pulsed sounds produced by *Inia geoffrensis*. The images are on the same frequency and time scale

they analyzed human-habituated animals, which can behave acoustically different from non-habituated animals analyzed here.

Type B was the most commonly produced sound (81%), found in 14 out of 18 recording sessions. Unlike type A, type B was found in both solitary individuals and in groups of 2–6 animals, and most of the time the dolphins were feeding, behavior that is characterized by deep dives (Layne 1958, Best and da Silva 1989). Type B was rarely emitted when the animals were traveling and socializing. We suggest that this vocalization is associated with feeding behavior, but it may also play a social function. Podos et al. (2002) also reported this vocalization when animals were feeding. For the former authors and for Amorim et al.

Table 2 Descriptive statistics of the six types of boto's (*Inia geoffrensis*) narrowband pulsed sounds in the Juami-Japurá Ecological Station

	Type A	Type B	Type C	Type D	Type E	Type F
Minimum freq. (kHz)	1.0 ± 0.8 [1.2–3.4]	0.5 ± 0.2 [0.08–1.7]	1.0 ± 0.7 [0.3–2.5]	0.8 ± 0.2 [0.2–1.6]	12.0 ± 0.8 [11.5–12.9]	1.9 ± 0.8 [0.4–2.7]
Maximum freq. (kHz)	12.3 ± 5.9 [3.4–25.7]	3.4 ± 2.9 [1.1–10.8]	4.7 ± 1.6 [2.2–8.3]	4.1 ± 1.2 [2.2–12.8]	18.6 ± 3.1 [15.1–23.1]	11.5 ± 5.8 [3.6–20.2]
Center freq. (kHz)	2.5 ± 1.8 [0.4–9.7]	1.3 ± 0.3 [0.4–2.5]	2.2 ± 1.1 [0.7–4.1]	1.7 ± 0.3 [0.4–1.6]	14.1 ± 0.4 [13.4–14.7]	3.5 ± 0.7 [1.5–4.1]
Bandwidth (kHz)	11.7 ± 5.6 [2.9–24.9]	2.9 ± 1.5 [0.5–10.3]	3.7 ± 1.4 [1.6–7.4]	3.3–1.1 [1.4–11.3]	6.5 ± 2.5 [3.9–10.1]	9.6 ± 6.1 [3.1–19.2]
Peak freq. (kHz)	2.2 ± 1.8 [0.4–10.1]	1.2 ± 0.4 [0.1–2.6]	2.1 ± 1.5 [0.4–6.0]	1.6 ± 0.4 [0.4–2.9]	14.1 ± 0.4 [13.4–14.8]	3.3 ± 0.8 [1.1–3.7]
Duration (s)	0.57 ± 0.61 [0.06–2.9]	0.3 ± 0.2 [0.03–1.5]	0.46 ± 0.4 [0.1–1.6]	0.04–0.02 [0.01–0.12]	0.28 ± 0.13 [0.1–0.5]	0.2 ± 0.1 [0.1–0.6]

Values presented as mean ± standard deviation and maximum and minimum values in brackets

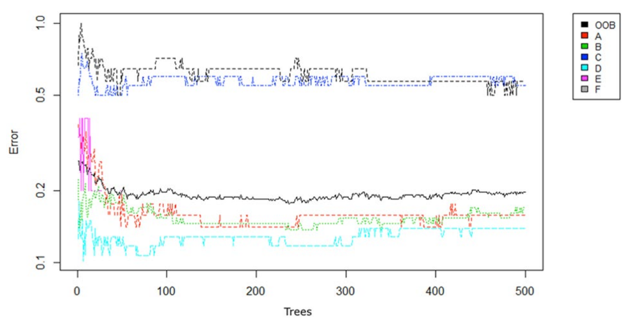

Fig. 3 Learning curve of Random Forest classification for narrowband pulsed sounds (A, B, C, D, E, and F) produced by *Inia geoffrensis*. These curves show the learning process during the computation of the classification model for each type of the training dataset. Each tree had its error designated along with the out of bag error (OOB)

(2016), the maximum frequency of this sound was around 5 kHz, similar to what we found in this study, with a maximum frequency of 4.9 ± 1.9 kHz. Diazgranado and Trujillo (2002) also described these vocalizations for two populations of the subspecies *Inia geoffrensis humboldtiana*, on the Orinoco River, Colombia.

The narrowband pulsed sounds characterized here as types C, D, E and F are being described for the first time in this work. They differ from one another in visual and acoustic characteristics. Type C is a pulsed sound with a low frequency and a single unit. When compared with the other types, it has a long duration. Type D was found in only two recording sessions—132 sounds were found in a session of 12 min during the morning with four *Inia* in cohesive formation and with a presence of one *Sotalia fluviatilis*; and only 2 type D sounds were found in another recording, during the evening, with two *Inia* and no *Sotalia*. This type is visually similar to type B, however it differs in the parameters analyzed.

Inia's vocalizations of types B, C and D have low frequencies that are comparable to social vocalizations of other dolphins. The other species of *Inia*, the Araguaian boto (*Inia araguaiaensis*) produces social sound frequencies lower than those of other delphinids (Melo-Santos et al. 2019). The authors hypothesize that this characteristic allows animals to communicate more efficiently in the complex habitat in which they live, such as rivers. Signals emitted in sediment-rich water have more attenuation and geometric spreading along their propagation (Stoll 1985; Kibblewhite 1989;

Table 3 Confusion matrix showing the correct classification of each narrowband pulsed sound type of *Inia geoffrensis*

	Type A	Type B	Type C	Type D	Type E	Type F	Correct classification (%)	Balanced accuracy
Type A	58	26	8	0	0	8	57.7	0.89
Type B	2	92	2	4	0	0	91.8	0.85
Type C	11	45	22	0	0	22	22.2	0.61
Type D	0	7	2	91	0	0	90.5	0.95
Type E	0	0	0	0	100	0	100	1.00
Type F	0	0	14	0	0	86	85.7	0.80

Values are in percentages. Expected classification scores are based on the proportion of all events composed of each type. Global accuracy = 0.8 (95% CI: 0.72, 0.86)

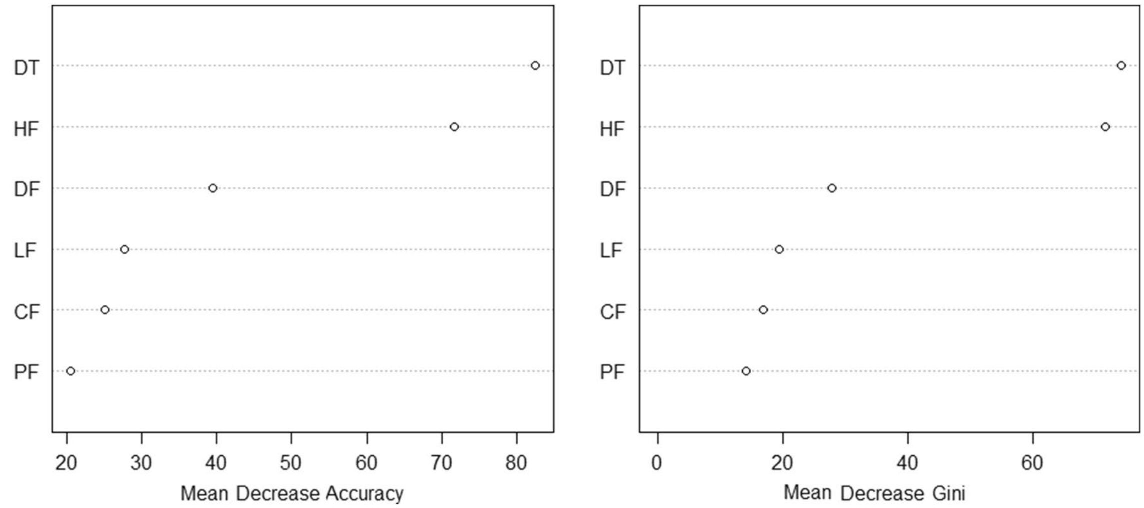

Fig. 4 Mean decrease accuracy and Gini variable importance measure showing the importance of each analyzed parameter of the vocalizations for the model. From the least important to the most important: *PF* peak frequency; *CF* central frequency; LF = lower frequency; *DF* delta frequency (maximum frequency–minimum frequency); *HF* highest frequency; *DT* duration

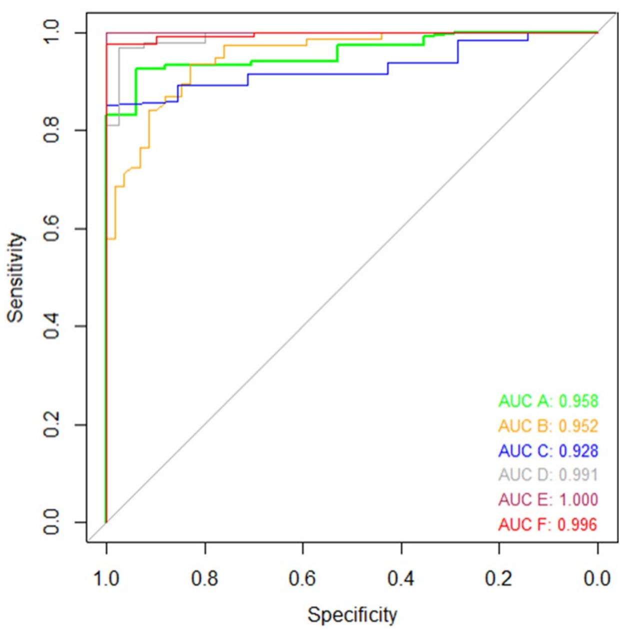

Fig. 5 Receiver operating characteristic (ROC) curves: each curve represents the sorting of the efficiency of the model for the sound types A, B, C, D, E, and F, and the area under the curve (AUC) is the indicator of the goodness of fit

Bradley and Stern 2008; Amorim et al. 2016); thus lower frequencies can be better transmitted to nearby animals (Hamilton 1980). Types B and C were also emitted by solitary individuals. May-Collado and Wartzok (2007) found whistles for *I. geoffrensis geoffrensis* in solitary animals, but suggest that individuals could still be in acoustic contact with other animals. The authors hypothesized that the boto uses whistles in the context of maintaining distances among them, rather than to promote group cohesion. Territorial birds also use sounds to maintain distances among animals (usually males), suggesting acoustical sexual selection (Catchpole and Slater 1995; Morisaka 2012). As we know that botos have sexual dimorphism (males are bigger) and use sexual displays (e.g., object carrying behavior, Martin et al. 2008), there is the possibility that *Inia* use whistles and narrowband pulsed sounds for reproductive displays.

Type E was found in only one recording session, when there were three *Inia* and no *Sotalia*. The animals were feeding in cohesive formation. Type F was found in the same recording as type E and in one more, when there were six *Inia* feeding and socializing. We observed that these sounds are emitted in a social context, and, therefore, we suggest that as with the other types they have a social function.

The categories of sound described here were first differentiated through visual analysis. Some sounds are clearly distinguished by their visual and aural characteristics, but others raised questions and it was necessary to further analyze categories and confirm the types of sounds. In addition to having other researchers participating in the categorizations, statistical analysis was also crucial to confirm the types found. The models used different variables, such as the frequencies and duration of the sounds to find similarities and differences between them. Therefore, in addition to the manual analysis based on the visual and aural characteristic of the sound, we also had a statistical confirmation based on the parameters of the vocalizations. Random forest models confirmed the categorization of the pulsed sounds in six different types. According to the model, sound type C had the highest error rate and was poorly classified (22.2%). This could be explained because type C and B present similarities in the frequency parameters (classification rate of type C with type $B=44.4\%$). Although they have similar values, they are visually and acoustically distinct from each other. As shown in Fig. 2, while type C has one single unit during approximately 0.5 s, type B has ten units. Values from type E differed most from the other vocalizations since it has higher frequency and it does not show defined harmonics and units. This type was emitted eight times in a single recording, which was performed as soon as the dolphins were sighted.

Our knowledge of river dolphins has been growing lately and gaining more attention among researchers. Even so, several aspects about the species remain a mystery, such as a greater understanding of their bioacoustics. In this work, we describe sounds emitted by botos that have never been reported before, which is important to direct our efforts in search of understanding the context of vocalizations, their functions and importance for the species. With just a few days of data collection in the field due to logistical restrictions, we already managed to find four new types of sounds produced by these animals. We still have many questions to answer and we hope that the sound categories described here will serve as a basis for future research on this species.

Conclusions

The species *Inia geoffrensis* produces at least six types of narrowband pulsed sounds. Four types are being described for the first time in this work. Although the acoustic repertoire of the boto is not completely understood in functional terms, the use of the various sound types generally depends on an animals' activity. This study serves as a basis for future research that aims to understand the context of the species' vocalizations and the evolution of the acoustic behavior of Odontoceti, as well as for comparisons with other populations and among species of the genus *Inia*.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s42991-021-00134-1.

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Data availability Available data.

Code availability Not applicable.

Declarations

Conflict of interest On behalf of all authors, the corresponding author states that there is no conflict of interest.

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