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The newly described Araguaian river dolphins, *Inia araguaiaensis* (Cetartyodactyla, Iniidae), produce a diverse repertoire of acoustic signals

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The newly described Araguaian river dolphins, *Inia araguaiaensis* (Cetartyodactyla, Iniidae), produce a diverse repertoire of acoustic signals

Abstract: The recent discovery of the Araguaian river dolphin (*Inia araguaiaensis*) highlights how little we know about the diversity and biology of river dolphins. In this study, we described the acoustic repertoire of this newly discovered species in concert with their behaviour in free-ranging, human-habituated individuals. We analysed 393 signals that we classified into 13 types of tonal sounds (n=15) and 66 types of pulsed calls (n=378). The most common sounds were short two-component calls. Thirty-five percent (n=140) of these calls were emitted by calves as they reunited with their mothers suggesting a key role in mother-calf communication. Our findings show that the acoustic repertoire of river dolphins is far from simple. Furthermore, the calls described here are similar in acoustic structure to those produced by social delphinids, such as orcas and pilot whales. Uncovering the context in which these signals are produced may help understand the social structure of this species and contribute to our understanding of the evolution of acoustic communication in whales.

Introduction

River dolphins of the genus *Inia* - commonly known as botos - are evolutionary relics found exclusively in the Amazon, Orinoco, and Tocantins River Basins of South America (Best & Da Silva, 1989, 1993; Hrbek et al., 2014; May-Collado & Agnarsson, 2011; Pilleri & Gihr, 1977; Santos et al., 2012, 2014). Like the franciscana dolphin (*Pontoporia blainvillei*), the baiji (*Lipotes vexillifer*), and the Ganges and Indus river dolphins (*Platanista* spp.), botos have flexible necks
and backbones, a low and large-based dorsal fin, and a slender rostrum (Best & Da Silva, 1989, 1993; Brownwell & Herald, 1972; Cassens, et al. 2000; Crespo et al., 1998; Da Silva et al., 2009; Hamilton et al., 2001; Leatherwoods & Reeves, 1994; Reeves & Martin, 2009; Zhou 2009). Botos have a preference for habitats with slow currents and high prey concentration such as bays, confluences, small streams, and channels and island margins (Gomez-Salazar et al., 2012a, 2012b; Martin & Da Silva, 2004; Martin, Da Silva & Salmon, 2004; Pavanato et al., 2016). However, residency patterns vary within locations from long-term residency to occasional visitors (Martin & Da Silva, 2004). Although, botos are traditionally considered solitary, with long-term social interactions restricted to mothers and their calves, large aggregations have been documented during foraging and mating events (Best & Da Silva 1989, 1993; Martin, Da Silva & Rothery, 2008).

The acoustic repertoire of botos has traditionally been thought to be limited to a few sounds (Podos et al., 2002), however, studies of free-ranging and captive botos suggest otherwise. Among some of the sounds reported for botos are burst-pulsed sounds, jaw-snaps, low-frequency sounds, pulsed sounds, echolocation clicks, and whistles (Amorin et al., 2016; Cadwell et al., 1966; Diazgranados & Trujillo, 2002; May-Collado & Wartzok, 2007; Ding et al., 1995; Ding, Würsig & Leatherwoods, 2001; Kamminga et al., 1993; Podos, Da Silva & Rossi-Santos., 2002; Penner & Murchison, 1970). Ding, Würsig & Leatherwoods (2001) also described the emission of low-frequency whistles (up to 5 kHz) for Peruvian botos. However, this discovery was disputed (Podos, Da Silva & Rossi-Santos, 2002) due to the presence of sympatric tucuxi dolphins (Sotalia fluviatilis) known to emit whistles. Later, May-Collado & Wartzok (2007) confirmed that botos do emit whistles, but at much higher frequencies (up to 48 kHz) than previously thought. These high frequency whistles were recorded from botos at the Yasuni and Napo rivers in Ecuador. Today,
there is a consensus that, while botos do emit whistles, these sounds are emitted rarely (Diazgranados & Trujillo, 2002; May-Collado & Wartzok, 2007; Ding, Würsig & Leatherwoods, 2001) and likely play a different social role as the one described for delphinids (May-Collado & Wartzok, 2007). Podos, Da Silva & Rossi-Santos (2002) found that the acoustic repertoire of Amazonian botos consisted primarily of pulsed calls with a low emission rate. However, these results were likely limited by the sampling rate of the recorders used by the authors. Amorin et al. (2016) studied the same population using a broadband frequency recording system and described a high emission of a variety of pulsed calls.

A major constraint in studying river dolphins is that they usually do not perform conspicuous surface displays combined with the difficulty of identifying individuals in the field (Best & Da Silva, 1989, 1993; Brownwell & Herald, 1972; Cassens, et al. 2000; Crespo, Harris & González, 1998; Da Silva, 2009; Hamilton et al., 2001; Leatherwoods & Reeves, 1994; Reeves & Martin, 2009; Zhou 2009). Here we studied the acoustic repertoire of free-ranging Arauğuaian botos that regularly visit a fish market in Mocajuba in Northern Brazil (Santos et al., 2014). This semi-controlled setting gave us the unique opportunity to combine acoustic technology with underwater behavioural observations.

Material & Methods

Study area

This study took place along the Tocantins River in the town of Mocajuba in Pará State, Brazil (Fig. 1). The Tocantins River is classified as a clearwater river with a small floodplain as the river runs through a narrow valley. There are large sandbanks in the river’s main channel where herbaceous vegetation may occur, there are floating vegetation, and submerged aquatic macrophytes where there is light penetration (Junk et al., 2011). At its lower reaches, water cycles in this region are very dynamic with a greater rainfall from November to April, with the highest
waters on March, rainfall declines from May to October, with lowest waters on September (Ribeiro, Pretere & Juras, 1995). There is also a daily cycle of tide pulses (Goulding et al., 2003; Ribeiro, Petrere & Juras, 1995). Mocajuba has a fish market that serves as the main place to acquire fish products for the city and the riverside communities. The wastes of the market and the provision of fish by locals attracts botos to the pier. This set up together with low turbidity waters during the dry season allows great proximity to botos enabling us to identify individuals and observe their behaviour in detail (Fig. 2).

Figure 1. Location of the Mocajuba fish market at the margins of the Tocantins River
Figure 2. Mocajuba’s fish market, clear and shallow waters allow for detailed observation of Araguaian botos’ behaviour. (A): Taken from drone footage by our team led by Gabriel Melo-Santos. (B) Photo by Rodrigo Tardin.

Data collection

Acoustic and behavioural data was collected in visits that ranged from three to 15 days during October to December 2013, March 2014, June 2015, July, September and December 2016. The presence of botos at the market depends on the market opening hours, which is the time when the animals are fed (Santos et al., 2014). Therefore our observations took place in the morning. Behavioural observations were collected in a continuous all-events sampling (Mann, 1999). For each session, we collected the following data: number of individuals present, age class (adult, juvenile, calf), and sex (based on the presence of mammary slits). In addition, animals were identified based on natural marks on the dorsal and ventral parts of the body, given that the botos in the market frequently swim upside down (Santos et al., 2014). Photographs of their bodies were taken with a Nikon 3200 SLR Camera (Nikon Corp., Tokyo, Japan) and a 70x300mm zoom lens (Nikon Corp., Tokyo, Japan). Underwater video was collected with a GoPro Hero 4 (GoPro Inc.,
San Mateo, USA) held on hand (Fig. 5). Notes and drawings of the marks and their locations were also taken if we were unable to take pictures. We held permits to perform this study issued by SISBio (number 52892) from the Brazilian Ministry of Environment.

Sound recordings were taken continuously in synchrony with behavioural observations. We used three recording systems along the study: (1) an Aquarian hydrophone (Anacortes, Washington, USA) connected to a Tascam DR1 digital recorder (22kHz sampling rate), (2) a CR1 hydrophone (Cetacean Research Technology, Seattle, USA) connected to a pre-amplifier and a Tascam DR-44WL (96kHz sampling rate) and (3) a Soundtrap (Ocean Instruments, New Zealand, 576kHz, sampling rate).

**Data analysis**

All recorded signals were inspected using a spectrogram analysis in Raven Pro 1.5 (Cornell Laboratory of Ornithology, New York, USA). Only whistles and pulsed calls with good signal to noise ratio were selected for further analysis. Signals were assigned visually *posteriori* into categories based on their shape of their contours (Fig.6). Other defining characteristics of the classification of botos sounds were duration - short (<200ms) versus long (>200ms) signals - and the presence of nonlinear phenomena: (a) subharmonics - signals with additional spectral components in the harmonic stack, generally in multiples of ½ or ⅓ of the fundamental frequency- and (b) biphonation - signals with the presence of two independent fundamental frequencies (Tokuda et al., 2002; Wielden et al., 1998). Pulsed calls produced in a succession where considered a single signal if they were separated by less than 200 ms. Using a rarefaction curve (Magurran, 2004), we evaluated how much of the acoustic repertoire was registered during
our sampling period. Using a Whittaker diagram (Magurran, 2004), we assessed the occurrence of
the signals recorded as part of these animal’s repertoire. Analyses were conducted in R.

Results

Botos were observed on 32 days (sightings happened everyday) and we collected 20.2
hours of acoustic recordings. Group sizes ranged between 3 to 12 individuals. These animals
repeatedly visited the market allowing us to photo-identify the animals and often following specific
individuals. We were able to identify nine individuals by their natural marks, five adult females,
one adult male, one juvenile female, one female calf and one male calf. Only two behaviours were
observed: socialization and feeding (Fig.3). Social interactions consisted of animals having
physical contact with one another and swimming alongside each other. Although, occasionally
animals would bite the neck of another when waiting to be fed. While we did not specifically test
for associations between individuals, the most stable associations appeared to be between mothers
and their calves. Feeding behaviour consisted of animals soliciting food with the head out of the
water and open-mouthed or poking humans with their snout. However, with the help of underwater
cameras we were able to match some of the observations to the vocalizing animals (see below).
Figure 3. The two behaviours observed during this study were socialization and feeding. (A) and (B): botos engaged in social activity, slow-swimming and physical contact, (C): boto feeding on a fish and (D): waiting to be fed. Photos (A), (B) and (C) by Gabriel Melo-Santos and (D) by Luiza Pereira.

Based on signal quality we selected a total 393 acoustic signals and classify them into six major sound types and 78 sub-categories. The six sound types were: long-two-component calls, long calls with subharmonics, short calls with biphonation (short-two-component calls), short calls without non-linear phenomena, short-calls with subharmonics, and tonal sounds (Fig. 4, Table 1). While we found a diversity of calls the rarefaction curve indicates that 20 hours of acoustic sampling was not sufficient to capture most of the acoustic repertoire of these animals (Fig. 5).
However, botos do seem to produce some signals more abundantly than others (Fig. 6). The long-calls with subharmonics (n=11) and whistles (n=15) were rarest and the short-two-component calls were the most commonly produced (n=246). Interestingly, 35% (n=140) of these were short two-component calls. Some of these calls were produced by calves as observed by the bubble emission from the blowhole, and their emission was followed by physical contact with their mothers (Fig. 7).

Figure 4. Examples of acoustic signals recorded during our study: (A) - Long call with subharmonics followed by two short two-component calls; (B) - Long two-component call; (C) - Narrow-banded frequency modulated whistle and the longest sound registered in this study; (D) - Short call with no non-linear phenomena. E – A short call with subharmonics.
Table 1. Major classification categories of signals recorded from the Araguaian river dolphin at the Mocajuba fish market.

<table>
<thead>
<tr>
<th>Vocalization type</th>
<th>Number of sub-categories</th>
<th>Number of sounds produced</th>
</tr>
</thead>
<tbody>
<tr>
<td>Long two-component calls - pulsed calls longer than 0.200s, with biphonation as the most marking characteristic, may exhibit subharmonics</td>
<td>9</td>
<td>18</td>
</tr>
<tr>
<td>Long calls with subharmonics - calls longer than 0.200s, may exhibit biphonation</td>
<td>5</td>
<td>11</td>
</tr>
<tr>
<td>Short two-component calls - pulsed calls shorter than 0.200s, with biphonation as the most marking characteristic, may exhibit subharmonics</td>
<td>29</td>
<td>246</td>
</tr>
<tr>
<td>Short calls - signals shorter than 0.200s with no non-linear phenomena</td>
<td>7</td>
<td>29</td>
</tr>
<tr>
<td>Short calls with subharmonics - calls shorter than 0.200s, may exhibit biphonation</td>
<td>15</td>
<td>74</td>
</tr>
<tr>
<td>Whistles - tonal narrow-banded signals longer than 0.100s, may exhibit harmonics</td>
<td>13</td>
<td>15</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>78</strong></td>
<td><strong>393</strong></td>
</tr>
</tbody>
</table>
Figure 5. Rarefaction curve showing the cumulative number of call types detected with increasing sampling time (hours of recordings analysed). The curve suggests that 20 hours of acoustic sampling is not enough to capture most of the acoustic repertoire of the Araguaian river dolphin.
Figure 6. Whittaker diagram displaying the occurrence of calls emitted by Araguaian botos with most calls produced rarely.
Figure 7. (A): Vocalizing calf as indicated by the bubbles blowing from its blowhole and associated short-two-component call, taken from video footage by our team. (B) Spectrogram of calls produced by a calf of Araguaian boto, followed by a bubble stream.

Discussion

Our results show that the Araguaian river dolphin has a more diverse acoustic repertoire than previously documented for the genus *Inia* (Amorin et al., 2016; Cadwell et al., 1966; Diazgranados & Trujillo, 2002; May-Collado & Wartzok, 2007; Ding et al., 1995, 2001; Kamminga et al., 1993; Podos, Da Silva & Rossi-Santos, 2002). Early studies described members of the genus *Inia* as silent animals (Podos et al., 2002) or with a limited acoustic repertoire (Amorin et al., 2016; Cadwell et al., 1966; Diazgranados & Trujillo, 2002; May-Collado & Wartzok, 2007; Ding et al., 1995; Ding, Würsig & Leatherwoods, 2001; Kamminga et al., 1993; Podos, Da Silva & Rossi-Santos, 2002). However, here we show that the acoustic repertoire of *Inia* dolphins is diverse and likely as complex as the acoustic repertoire of delphinids.

While pulsed calls were the most commonly produced sounds, tonal sounds were also produced. These findings agree with previous descriptions of whistle emission from Peruvian
(Ding, Würsig & Leatherwoods, 2001) and Ecuadorian botos (May-Collado & Wartzok, 2007) and other river dolphins like the Franciscana and the baiji (Cremer et al., 2017; Wang, Wang & Akamatsu, 1999; Wang et al., 2006). In these studies, the function of emitted whistles was unclear. May-Collado & Wartzok (2007) found that botos emitted whistles in a different social context than in delphinids, keeping distance between each other, rather than promoting social interactions as in marine dolphins.

Among pulsed calls, the short-two-component call was the most commonly produced sound. These calls were emitted in what appear to be mother-calf interactions. Our video footage and some underwater follows show bubbles emanating from calves’ blowholes while they emitted these calls as they approached their mothers after a short separation (see Supplementary Material). Bubble streams are often used as a cue to identify vocalizing animals (Bebus & Herzing, 2015; Fripp, 2005; Jones, 2014) and in this case the bubble stream revealed that the calves were producing the calls and did so in a repetitive fashion. These vocal patterns are similar to what has been described for calves of bottlenose dolphins, which use signature whistle as contact calls, where calves increase whistle emission as they approach their mothers (Smolker, Mann & Smuts, 1993). Given the strength of mother-calf associations in botos (Best & Da Silva, 1989, 1993) and the characteristics of their habitat, a shared signal that enhances mother-calf recognition may be key as they move through murky waters and complex underwater vegetation. The complex structure of botos’ might also have led to evolution towards signals with short duration, longer signals might suffer interference of echoes caused by obstacles (sandbanks, underwater vegetation, riverbed, even the water surface). Notwithstanding, social signals produced by *Inia* sister taxa *Pontoporia* who also evolved in riverine environments are also short (Cremer et al., 2017).
Several species of toothed whales emit calls of similar acoustic nature as the ones described here for botos (Filatova et al., 2012; Fitch et al., 2002; Ford, 1989; Deecke et al., 1999, 2010, 2011; Garland et al., 2015; Marcoux et al., 2012; Miller & Bain, 2000; Papale et al., 2015; Pérez et al., 2017; Sjare & Smith, 1986; Vergara & Barrett-Lennard, 2008; Vergara, Michaud & Barrett-Lennard, 2010; Yurk et al., 2002; Zwamborn & Whitehead, 2017). For example, the calls of killer (Orcinus orca), pilot (Globicephala spp.) whales have been shown to contain non-linear features suggesting the may carry information on group identity and maintaining social cohesion (Deecke et al., 2010; Pérez et al., 2017; Yurk et al., 2002; Zwamborn & Whitehead, 2017). Similarly, Marcoux, Auger-Méthé & Humphries (2012) show evidence that narwhal (Monodon Monoceros) calls might be related to specific groups or individuals. Non-linear calls have also been reported to convey individuals’ identity and/or emotional state (Fitch, Neubauer & Hetzel, 2002; Papale et al., 2015). Given these similarities we propose these two-component signals evolved early in the evolutionary history of toothed whales as social contact signals, likely for mother-calf interactions and later in the lineage leading to delphinids it evolved into a group recognition signal.

Conclusions

We show that the acoustic repertoire of botos is far from been simple. The Araguaian river dolphins studied at Mocajuba fish market produce a diverse acoustic repertoire, as we found 78 sound types, mostly pulsed calls, and our analysis indicate that there is more to discover. Notwithstanding, these sounds are mostly complex in structure presenting nonlinear phenomena. The animals we studied are habituated to humans, which provided a unique opportunity to shed light on the acoustic and social behaviour of this understudied species. Under relatively controlled
conditions we identified more than half of the studied animals and recorded their acoustic and underwater behaviour. When possible, we matched recordings with video footage of calves as they reunited with their mothers. During this reunions calves appeared to use the two-component calls as contact calls, nevertheless further investigation is needed to understand the importance of these calls for mother-calf interactions. Furthermore, given that Araguaian river dolphin calls are similar in acoustic structure to those of delphinids, we proposed that these signals evolved early in the evolutionary history of toothed whales as social calls, likely as mother-calf contact calls, and that later in the lineage leading to dolphins its function evolved to group/family call recognition.

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