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RESPOSTAS COMPORTAMENTAIS DO BOTO-VERMELHO (*INIA GEOFFRENSIS*) AO TURISMO DE INTERAÇÃO NO BAIXO RIO NEGRO, AMAZONAS

ANGÉLICA CRISTINA GOUVEIA NUNES

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Sinopse:

Estudei respostas comportamentais do boto-vermelho, *Inia geoffrensis* ao turismo de interação na região do baixo Rio Negro, Amazonas, Brasil. Através de observação direta, monitorei três diferentes agregações de botos formadas em flutuantes que oferecem atividades de nado e alimentação. Gravei vocalizações dos diferentes grupos e o comportamento predominante. Aspectos como reações comportamentais a diferentes estímulos, repertório vocal e variações acústicas entre contextos comportamentais foram avaliados.

Palavras- chave: Boto-vermelho, *Inia geoffrensis*, Bioacústica

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RESUMO

Variações acústicas em fonações de animais podem carregar codificações de motivação, as quais em espécies sociais podem melhorar a comunicação dentro e entre grupos. Os botos-vermelhos são considerados animais com baixa atividade vocal, mas capazes de produzir uma série de sons. Os contextos e funções destas vocalizações ainda não estão esclarecidos, assim como a composição do repertório vocal da espécie. O presente estudo teve como objetivos: 1) descrever o repertório vocal do boto-vermelho e verificar se as fonações correspondem a contextos comportamentais específicos; 2) verificar as respostas comportamentais de três agregações de botos-vermelho formadas em locais de interação e relacioná-las com à atividade turística. Gravações acústicas e observações comportamentais foram realizadas em três diferentes flutuantes de interação com botos na região do baixo Rio Negro entre maio e agosto de 2014. Nestes locais, agregações de botos se formam em função do alimento oferecido. Cada lugar possui indivíduos reconhecidos por meio de marcas e padrões individuais de cicatrizes. Parâmetros acústicos simples e robustos foram medidos e analisados. Variáveis acústicas dos diferentes tipos sonoros não diferiram entre as áreas de estudo, mas variaram significativamente entre contextos comportamentais. A frequência dominante na fundamental (PFF) e a frequência máxima (MF) em comportamentos agressivos foram mais baixas que em outros contextos. O repertório vocal da espécie é formado por gradações e transições, sendo difícil a categorização em tipos sonoros discretos. Botos alteraram seu comportamento devido às interações com humanos e o uso de alimentação suplementar. No local mais antigo de interação, a probabilidade dos animais de permanecer em comportamento agressivo foi maior. Animais permanecem próximos ao flutuante boa parte do tempo. Isto deve ocorrer devido ao fato de estarem sensibilizados ao estímulo alimentar e terem sido condicionados ao contato humano devido ao uso de alimentação suplementar como forma de atração. Sugerimos medidas de manejo para garantir a qualidade da atividade turística e o bem-estar dos animais.

ABSTRACT

Behavioral responses of Amazon river dolphins (*Inia geoffrensis*) to touristic interaction in lower Negro river, Amazonas

Acoustic variations in animal vocalizations may carry codifications of motivation, which in social species may improve communication among and between groups. The Amazon river dolphin, or boto, are considered acoustically quiet animals, but are capable of producing may sounds. The behavioral contexts and functions of these vocalizations are still unclear, as well as the species vocal repertoire. The goals of this study were to describe the botos vocal repertoire and verify if vocalizations corresponds to specific behavioral contexts. Acoustic recordings and behavioral observations were made in three different floating-houses were in-water interactions with these animals occur, in the lower Negro river region, between May to August 2014. In these sites, wild boto aggregations are formed due to offer of fish. Each site has different animals, that are recognized by marks and scarring patterns. Simple and robust acoustic parameters were measured and analyzed through acoustic softwares. Acoustic variables did not differ among the three sites, but did significantly varied among behavioral contexts. The peak frequency at the fundamental (PFF) and max frequency (MF) in aggressive behavioral contexts were much lower than in other contexts. The vocal repertoire of this species is compound of gradations and transitions and are occasionally difficult to separate into discrete call types. Botos changed their behavior due to interactions with humans and the use of supplementary feeding. At the oldest location of interaction, the probability of animals to stay in aggressive behavior increased. Animals remain close to the floating most of the time. This might be due to the sensitisation to supplementary feeding. Botos have been conditioned to human contact due to the use of supplementary feeding as a means of attraction. We suggest management measures to ensure the quality of tourism and animal welfare.

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INTRODUÇÃO GERAL

A observação de animais selvagens é uma modalidade popular do ecoturismo e teve início na década de 60 com os "safaris fotográficos" pelo continente africano. Desde então a atividade vem crescendo em número de adeptos e diversificando nas formas de interação com a vida silvestre (Tapper, 2006). O turismo de observação de cetáceos como indústria mundial foi estimado em atrair mais de 9 milhões de participantes por ano em 87 países e territórios, com um valor comercial estimado maior do que US$1 bilhão (Hoyt & Iñíguez, 2008). Com o surgimento de programas de interação com cetáceos, golfinhos e baleias tornaram-se uma atração turística popular e as oportunidades de contato direto com animais de vida livre ocorrem em vários lugares ao redor do mundo (Reeves, Smith, Crespo, & di Sciara, 2003).

No mundo, cerca de 20 espécies são os alvos principais de atividades de interação (Samuels & Bejder, 2004). Alguns exemplos são o golfinho nariz-de-garrafa (*Tursiops sp.*) e o golfinho-rotador (*Stenella longirostris*). Entre os locais conhecidos por proporcionarem a prática de tais atividades destacam-se a Austrália, Nova Zelândia e Estados Unidos (Samuels, Bejder, Constantine, & Heinrich, 2003). No Brasil, várias espécies têm sido o foco deste tipo de atividade, como baleias-jubartes (*Megaptera novaeangliae*) na Bahia, botos-cinzas (*Sotalia guianensis*) em São Paulo e golfinhos-rotadores (*Stenella longirostris*) em Fernando de Noronha (Rocha-Campos, Câmara, & Pretto, 2011).

No estado do Amazonas atividades de nado com e alimentação de botos-vermelhos (*Inia geoffrensis)* têm ocorrido como atração turística em hotéis de selva e restaurantes flutuantes à beira dos rios. Existem ao menos cinco empreendimentos desenvolvendo atividade turística com os botos-vermelhos próximos à capital Manaus. Todos estão localizados dentro ou no entorno de áreas de preservação ambiental (Alves et al. 2011).

O boto-vermelho é também comumente chamado de boto-rosa. Isso se deve ao tom rosado da pele adquirido conforme avança para a idade adulta (A. R. Martin & da Silva, 2006). Botos pertencem à ordem Cetartiodactyla (Price, Bininda-Emonds, & Gittleman, 2005), subordem Odontoceti, família Iniidae, gênero *Inia* (Rice, 1998). Encontram-se amplamente distribuídos nos rios da Amazônia, tendo como principais barreiras biogeográficas as grandes corredeiras, cachoeiras e rios de pequeno porte (Best & da Silva, 1993).

O gênero *Inia* apresenta três espécies: *Inia geoffrensis* está distribuída na bacia Amazônia, enquanto que a subespécie *Inia g. humboldtiana* encontra-se distribuída na bacia do Orinoco (Best & da Silva, 1993). *Inia boliviensis* distribui-se na bacia do rio Madeira e não está restrita à extensão boliviana da bacia (W Gravena, Farias, da Silva, da Silva, & Hrbek, 2014). Uma quarta espécie para o gênero foi recentemente descrita. Acredita-se que *Inia araguaiaensis* esteja restrita à bacia Tocantins-Araguaia (Hrbek et al., 2014).

Os botos são animais geralmente solitários e raramente são vistos em grupos coesos com mais de três indivíduos. Agregações maiores podem se formar em função de alimentação, descanso ou em épocas de corte e acasalamento (Best e da Silva, 1993). Os botos são os maiores golfinhos de rio do mundo e apresentam pronunciado dimorfismo sexual (A. R. Martin & da Silva, 2006), sendo os machos, em média, 16% maiores e 55% mais pesados que as fêmeas. Tamanho corporal avantajado é um indicador de comportamento agressivo entre machos, já que confere vantagem competitiva durante conflitos (A. R. Martin & da Silva, 2006). O elevado grau de dano físico e cicatrizes encontrados em machos adultos demonstra que estes animais provavelmente competem entre si por algum recurso, que pode ser alimento ou fêmea, já que não existe evidência de territorialidade (A. R. Martin & da Silva, 2006). Em cativeiro, não chegam a estabelecer hierarquia social através de comportamento agressivo (Best & da Silva, 1993), mas existe relato de agressão intraespecífica que levou à morte um macho adulto (Sylvestre, 1985)

Os programas de interação com animais selvagens possuem a característica comum de oferecer alimento para que os animais aproximem-se e possam ser vistos mais facilmente (Orams, 2002). Atraídos pela oferta de alimento fácil, agregações de botos estão sendo condicionadas a receber alimento oferecido por turistas, que aproveitam este momento para observar, nadar próximos e tocar os animais (Barezani 2005; Alves et al. 2011). A análise de material genético de uma agregação no Parque Nacional de Anavilhanas confirmou que dez dos indivíduos condicionados eram machos e nenhuma fêmea foi registrada na área durante a pesquisa (Gravena 2007). A presença majoritária de machos em agregações condicionadas a receber alimento das pessoas pode ser um gatilho para que estes indivíduos engajem em comportamento de agressividade que possivelmente conduza a formação de algum nível de hierarquia na competição pelo recurso.

O uso de alimentação suplementar para atrair animais selvagens pode trazer diversas consequências negativas. Para golfinhos, já foram relatadas mudanças na área de vida, no período de atividade e redução do sucesso reprodutivo (Donaldson, Finn, Bejder, Lusseau, & Calver, 2012b; Orams, 2002; Samuels & Bejder, 2004). Um dos principais problemas relatados é o aumento na agressividade, direcionada não só à coespecíficos como também à pessoas (Orams, Hill, & Jr., 1996; Samuels, Bejder, & Heinrich, 2000; Santos, 1997).

Com o crescimento do número de locais de atração de botos no Amazonas (Alves et al. 2011), medidas de manejo e monitoramento da atividade precisam ser tomadas para possibilitar a manutenção e a qualidade dessa atividade, evitando danos aos botos e acidentes com os turistas (Romagnoli, 2010). Um estudo conduzido no Parque Nacional de Anavilhanas, um dos mais antigos e conhecidos locais de interação com botos no Amazonas, demonstrou que os animais que recebem alimentação suplementar incrementam a competição quando não estão sendo alimentados e quando o são, alguns exibem hierarquia de dominância para obter o alimento (L. C. P. de S. Alves, Andriolo, Orams, & Freitas Azevedo, 2013).

O contato com pessoas pode causar reações comportamentais complexas em animais selvagens. Bejder et al. (2009) afirmam que a exposição da vida silvestre ao estímulo humano repetitivo e contínuo pode sensibilizá-los. Neste caso, sensibilização é definida como uma modificação comportamental adaptativa onde os animais apresentam progressiva intensificação de sua resposta comportamental ao estímulo o qual estão expostos, porque aprendem que o estímulo tem consequências significativas para eles (Bejder, Samuels, Whitehead, Finn, & Allen, 2009).

Respostas comportamentais incluem sinais de comunicação de diversas modalidades. Golfinhos dependem fortemente do som para explorar o ambiente, forragear e comunicar-se com outros indivíduos.Sendo assim a comunicação acústica é um elemento importante do comportamento destes animais. Golfinhos produzem basicamente três tipos de sons: tonais, cliques de ecolocalização e *burst pulses* (Richardson, 1995). Por questões pragmáticas, a maioria dos estudos voltados para comunicação dos golfinhos tem focado em um tipo específico de sinal tonal, os assobios (Dudzinski et al., 2009), sinal produzido por grande parte dos golfinhos e geralmente atribuído às espécies mais sociais (Podos, Silva, & Rossi-Santos, 2002).

Os sons pulsados (ou cliques), por sua vez, são geralmente estudados no contexto da ecolocalização (Au & Hastings, 2008) e poucos estudos sugerem seu uso para comunicação em contextos sociais (Blomqvist & Amundin, 2004; Dawson, 1991; Overstrom, 1983; Weilgart & Whitehead, 1997; Weir, Frantzis, Alexiadou, & Goold, 2007). Porém, algumas características dos sons pulsados como a direcionalidade, a relativa facilidade com a qual podem ser localizados, sua variabilidade e intensidade com o qual podem ser produzidos aumentam o seu valor potencial como sinal de comunicação (Dudzinski et al., 2009). Em estudos com o golfinho nariz-de-garrafa, sons pulsados do tipo *burst pulse* caracterizados como *cracks*, *pops*, *squawks* e *squeals* de alta intensidade e ampla largura de banda foram observados em situações de alarme e medo durante encontros agonísticos/agressivos e em situações de confronto (Overstrom, 1983; Herzing, 1996; Blomqvist e Amundin, 2004).

Estudos em cativeiro demonstraram que botos produzem uma variedade de sons, como cliques de ecolocalização, intensos cliques únicos, estalos de mandíbula entre outros tipos sonoros (Caldwell et al., 1966, Caldwell e Caldwell, 1970). A presença de assovios no repertório vocal da espécie foi registrada em alguns estudos (Ding et al., 2001; Diazgranados & Trujillo, 2004; May-Collado and Wartzok, 2007), mas ainda existem controvérsias sobre assovios atribuídos aos botos devido a sobreposição espacial com o tucuxi (*Sotalia fluviatilis*), espécie simpatrica que reconhecidamente assovia e forma grandes grupos (Podos et al., 2002; May-Collado & Wartzok, 2007).

As funções destas fonações e de outros sinais acústicos não vocais não estão totalmente esclarecidas, uma vez que a observação dos animais em ambiente natural é dificultada pela turbidez da água e a associação de sons com comportamentos individuais torna-se mais complicado, pois a localização do emissor é difícil. O estudo do sistema de comunicação vocal dos botos é uma importante ferramenta para estudo do comportamento social da espécie, podendo gerar informações passíveis de serem utilizadas, por exemplo, em projetos de monitoramento acústico passivo. Além disso, a associação de sons com contexto comportamental permite correlação com o estado motivacional dos indivíduos, permitindo a geração de informações diagnósticas para inferir sobre os tipos de interações entre indivíduos monitorados acusticamente.

Portanto, para determinar os efeitos do turismo de interação no comportamento dos botos-vermelho, esta pesquisa teve como objetivos: 1) descrever o repertório vocal da espécie, relacionando os tipos sonoros com o contexto comportamental em que foram emitidos; 2) verificar as respostas comportamentais de três agregações de botos formadas em locais de interação e relacioná-las com à atividade turística.

Sendo assim, este trabalho encontra-se dividido em dois capítulos: Capítulo 1 - “Amazon river dolphin (*Inia geoffrensis*) vocal repertoire and associated behavior”, no qual propomos e descrevemos 14 tipos sonoros que compõem o repertório vocal da espécie e o contexto comportamental em que foram emitidos; Capítulo 2 -“Behavioural effects of tourism on Amazon river dolphins, *Inia geoffrensis*, in the Negro river”, onde analisamos os efeitos comportamentais de curto prazo causados pela alimentação artificial e interação com humanos em três diferentes locais e discutiremos alternativas de manejo da atividade turística de interação com os botos-vermelho.

OBJETIVOS

Objetivo geral:

Analisar o impacto antrópico do turismo no comportamento do boto-vermelho (*Inia geoffrensis*).

Objetivos específicos:

1) Descrever o repertório vocal da espécie, relacionando os tipos sonoros com o contexto comportamental em que foram emitidos;

2) Verificar as respostas comportamentais de três agregações de botos-vermelho formadas em locais de interação e relacioná-las com à atividade turística.

Capítulo 1

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Amazon river dolphin (*Inia geoffrensis*) vocal repertoire and associated behavior

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ABSTRACT

Uncertainty about the repertoire of sounds produced by Amazonian river dolphins stems from the spatial overlap of this species, the boto, *Inia geoffrensis*, and the sympatric tucuxi, *Sotalia fluviatilis.* Amazon river dolphins, or botos, are considered quiet animals, but are capable of producing many sounds. The behavioral contexts and functions of associated vocalizations are still unclear, as well as the species vocal repertoire. Evidence from Peru, Colombia and Ecuador indicate that the boto also produces whistles that were previously attributed only to tucuxis. Acoustic recordings and behavioral observations were made at three different floating-houses in the lower Negro river region, where in-water interactions with these animals occur, between May and August 2014. In these sites, aggregations of wild botos are formed due to fish provisioning. Simple and robust acoustic parameters were measured and analyzed. The vocal repertoire was classified into 14 sound types. Acoustic variables did not differ among the three sites, but varied among behavioral contexts. Low-frequencies were related to hostile contexts. The dominant frequency at the fundamental and maximum frequency in aggressive behavioral contexts was lower than in other contexts. Botos' whistles had flat frequency, short duration and were not produced in bouts.

Keywords**:** *Inia geoffrensis,* Amazon river dolphin, vocal repertoire, communication, behavior

I. INTRODUCTION

The Amazon river dolphin, or boto (*Inia spp.*), is found in most of the Amazon and Orinoco river basins. Large rapids, waterfalls and small tributaries are the only barriers to the animals' movement (Best & da Silva, 1993; W Gravena et al., 2014). They are usually solitary and rarely seen in cohesive groups of more than 3 individuals. Mother and calf pairs comprise most groups of two individuals. Large loose aggregations can be formed for feeding, resting and social purposes (Best & da Silva, 1993).

The genus *Inia* is divided into three species: *Inia geoffrensis geoffrensis* occurs in the Amazon basin, the subspecies Inia g. humboldtiana occurs in the Orinoco river basin (Best & da Silva, 1993) and Inia boliviensis occurs in the Madeira river basin and is not restricted to Bolivian extension (Gravena et al., 2014). *Inia araguaiaensis* was recently described and is believed to be restricted to the Araguaia-Tocantins river basin (Hrbek et al., 2014).

The taxonomic relations among river dolphins remain controversial. Genetic evidence supports the hypothesis that river dolphins are polyphyletic (Cassens et al., 2000; Hamilton et al., 2001; May-Collado & Agnarsson, 2006). The South American river dolphins (franciscana, *Pontoporia blainvillei* and Amazon river dolphin) are a sister group of the Superfamily Delphinoidea; the baiji (*Lipotes vexillifer*) sister species of this clade; and the susu (*Platanista gangetica*) is the most basal species of river dolphins (Cassens et al., 2000).

These phylogenetic relationships are considered when analyzing vocal types across different taxa. Whistles are tonal signals often considered a character that evolved with sociality (Herman & Tavolga, 1980; Podos et al., 2002), especially within Delphinidae. This hypothesis was based on the absence of whistles in river dolphins. However, whistles have been attributed to baiji and boto (Wang et al., 1999; Wang et al., 2006; May-Collado & Wartzok, 2007) and in other social non-delphinid toothed whales (Aguilar de Soto et al., 2012; Panova, Belikov, Agafonov, Bel'kovich, & Bel'kovich, 2012; Shapiro, 2006).

The Amazon river dolphin is known to produce several types of vocalizations such as echolocation clicks, single intense clicks and burst pulses (Caldwell e Caldwell, 1970; Diazgranados & Trujillo 2002; Tregenza et al. 2007; Sasaki 2013). Burst pulses were aurally described as screams, *barks*, *squeaks*, *squawks*, *creaks*, *cracks*, *grate*, *squeaky-squawk*, *whimper* and *yelps* (Caldwell et. al, 1966; Nakasai & Takemura, 1975; Diazgranados & Trujillo, 2002).

Sounds with harmonic structure and multiple notes were reported in different studies (Norris et al., 1972; Nakasai & Takemura, 1975; Podos et al., 2002). Dominant frequencies of *Inia* sounds are bellow all delphinid frequency parameters (Podos et al., 2002), with fundamental frequencies ranging from 1.5 up to 8 kHz (Norris et al., 1972; Nakasai and Takemura, 1975; Podos et al., 2002). Reported values for whistle fundamental frequencies vary from 5.30 up to 48.10 kHz (May-Collado & Wartzok, 2007) and below 13kHz (Ding et al., 2001; Diazgranados and Trujillo, 2004). These whistles have short duration (0.002 to 1s) and were not produced in bouts.

The mechanism of sound production in *Inia* has not been fully understood. We assume that nasal passages (rather than laryngeal components) are the structures used for sound generation (Cranford, 2000). Despite the term “vocalization” refers to the use of vocal cords, these are not primarily used for sound production.

Characterizing the vocal repertoire of a species and understanding the behavioral context of sound production are important to understand the evolution of animal communication (Wiley and Richards, 1978; Endler, 1992; Podos et al., 2004), possible impacts of human activities on animal communication (Richardson & Würsig, 1997) and the possible use of passive acoustic monitoring techniques to answer behavioral and ecological issues such as density and abundance estimates, habitat use and seasonality (Mellinger, Stafford, Moore, Dziak, & Matsumoto, 2007). This paper aims to describe aspects of the

Amazon River dolphin vocal repertoire and the behavioral context in which these sounds were recorded.

II. METHODS

A. Study site

The study area encompasses the lower Negro river region, where touristic dolphin watching and swim-with activities have occurred since 1998 (Alves et al. 2011). These activities take place in wooden floating structures positioned alongside the river and three sites were selected for this study. Data collection occurred in in the vicinity of São Thomé, (3°05'35.4"S 60°28'31.0"W), from 30 may to 11th of June 2014; in Novo Airão city, at the National Park of Anavilhanas (02°37′13.7″S 60°56′45.9″W), from 21 July to 4th August 2014; and in Cachoeira do Castanho (3°5'54.35"S 60°17'10.64"W), from 14th to 29th August 2014. São Thomé and Cachoeira do Castanho are small riverine communities within the Environmental Protected Area of Rio Negro, a conservation state unit (Fig. 1).

Genetic research on the Novo Airão aggregation concluded that all individuals were males and no females were sighted during the study (Gravena 2007). There is no genetic information for the other two sites, but in São Thomé 15 individuals were captured for scientific purposes and were all males (da Silva 2015, pers. comm.). In Cachoeira do Castanho, supposedly there’s in addition a mother-calf pair. The aggregations varied daily from 1-12 individuals (pers. obs.). Each place has different individuals, all of them recognized and distinguished by scars, pigmentation patterns and other marks (e.g. Gravena 2007; Gomez-salazar et al. 2011).

B. Sound and behavior recordings

Behavioral observations and acoustic recordings were made from a fixed point in the floating structure where in-water interactions with botos occur. An omnidirectional HTI SSQ96 hydrophone (-162 dB re 1V/µPa, 20 Hz - 25 kHz) coupled to a Sony PCM-D50 digital recorder (sampling rate 48 kHz, 16 bits resolution) was used for acoustic recordings. Identification of individual call emissions was not possible due to equipment limitation.

To associate behaviors and vocalizations, the recorder was synchronized with a chronometer used to control time sampling. Behavior data were collected using scan sampling technique (Altmann 1974). The scan was made every 2 minutes and the behavioral state/event of each individual in the aggregation was registered. Behavior was also recorded using a hi-definition camera (GoPro Hero 3+). Video recordings were analyzed to describe behaviors not observed at the time of the sample. Behaviors were included in seven categories defined *a posteriori* based on the activity where the majority (>50%) of individuals were engaged. If this criterion was not met, acoustic recordings were discarded.

The behavioral categories defined were: foraging, socialization, aggression, play, rest, milling and human-dolphin interaction. Foraging occurred when the botos were surfacing with rapid changes in direction, long dives and prey could often be observed. Socialization was considered when two or more botos were engaged in activities that include close proximity, body contact and sexual displays. This category does not include agonistic/aggressive and play behaviors. Interactions that involved fighting, threat and submission displays were considered aggression and play was defined as interactions between two or more botos involving chases among individuals and pushing or pulling objects with no apparent intent other than the behavior itself. Resting was characterized by slow swimming with no fixed direction or by remain stationary at the surface or underwater. Milling occurred when the boto was swimming and constantly changing direction while at surface. Human-dolphin interaction comprises behaviors observed during interactions of botos and humans such as approach to feeding area, hand-feeding, touching and begging.

C. Acoustical analyses

Acoustic analyses were performed using the software Raven Pro 1.5 (Cornell Lab of Ornithology), applying Hanning window; FFT=512 and 50% overlap. Sound parameters were measured from spectrograms, oscillograms, and power spectra and used to describe vocalizations. The parameters measured were (CD) call duration, (LF) lowest frequency of the sound, (HF) highest frequency of the sound, (MF) max frequency of the sound, (Q1) 1st quartile frequency, frequency that divides the selection into two frequency intervals containing 25% and 75% of the energy in the selection, (Q3) 3rd quartile frequency, which represents the frequency that divides the selection into two frequency intervals containing 75% and 25% of the energy in the selection, (DF) difference between the highest and the lowest frequency of the entire sound and (FI) initial frequency and (FF) final frequency of the sound. These values are computed automatically by the software. (PFF) peak frequency of the fundamental, (PP) number of pulses of the sound (for pulsed sounds), (IPI) inter-pulse interval, (PF1) peak frequency of the first formant and (PF2) peak frequency of the second formant (only for pulsed sounds) were measured using oscillograms and power spectra. The number of pulses was counted using oscillograms for sounds with regularly spaced pulses. For sounds with a large number (>10) of pulses, we estimated the number of pulses by dividing the duration of pulsed stretches by the inter-pulse interval within that stretch (Leuchtenberger et al., 2014). Only social pulsed signals were considered for analysis. Echolocation clicks were discarded because the recording system could not record the entire pulse..

D. Sound classification

The vocal repertoire of Amazon river dolphin does not fall into obvious discrete categories. Sounds were classified according to visual inspections of spectrograms and were divided into 14 call types (Table I, Figs. 1-2).

To analyze the relationship between behavioral contexts of sounds emission, we considered the predominant behavior, which was determined as the behavioral state in which more than 50% of the botos within the aggregation were involved at the time of sampling.

E. Statistical analyses

All statistical analyses were performed in R 2.15 Software (R Foundation for Statistical Computing, 2011). Most of the statistical procedures were based on Leuchtenberger et al. (2014). A principal-coordinate analysis (PCoA) was carried out to ordinate the 15 acoustic variables of the 14 sound types from the Manhattan-distance matrix. Using the scores of three first axis of PCoA, we tested with a PERMANOVA the statistical association of the behavioral contexts and sounds emitted and also for significant differences among the 14 sound types. A Kruskal-Wallis test with the most important variables of axis one and two of PCoA was used to investigate whether there were differences between behavioral contexts and sound parameters.

A post-hoc test using multiple pairwise comparison procedure with Bonferroni correction (Kutner et al., 2005) was used to identify differences in sound types produced between pairs of behavioral contexts. To illustrate the variation among sound types, we conducted a hierarchical clustering analysis using Manhattan-distance matrix of the median values of the sound variables for each sound type and the average linkage between groups

(UPGMA). This analysis results in a dendrogram that represents similarity between sound clusters (Boisseau, 2005)

III. RESULTS

In total, 783 Amazon river dolphin vocalizations with good signal to noise ratio were manually detected on 61 hours of recordings from the three sites. During rest, only sparse echolocation clicks were recorded and were not considered for analysis. During socialization no social pulsed sounds were registered.

The first three axis of PCoA explained 53.5% of the variance in sound parameters. IPI followed by PF2, PF1 and PU contributed most to the first axis. In the second axis the most important variables were Q1, MF, Q3, FI, FI, LF, HF, respectively. The differences between behavioral contexts and sound parameters are summarized in Table I. The vocal repertoire of Amazon river dolphins was considerably explained by the 14 proposed sound types (PERMANOVA: $F_{12.486}$= 64.2, R^2= 0.50, P<0.001).

Regardless of the level of clustering we applied to the data, there was significant overlap in the principal component scores between sound types and the analysis only considered BP2 and whistles as discrete categories, treating other sound types as subcategories (Fig. 6).

A. Vocal repertoire and behavioral contexts

Example spectrograms of each call type are illustrated in Figures 3 and 4. Descriptive statistics of the main acoustic parameters for each call type are summarized in Table I (Appendix).

1. **BP1.** The burst pulses classified as type 1 ranged from 12 to 397.7 pulses (Fig. 3a). BP1 had minimum frequency of 385 kHz and maximum frequency above 25 kHz. The inter-pulse interval varied from 0.0002 to 0.005 (median = 0.001, Table 2). BP1 was recorded during play (47.6%), human-dolphin interaction (28.5%), aggression (14.12), foraging and milling (both 4.7%).

2. **BP2.** The burst pulses classified as type 2 ranged from 424 to 1663 pulses and presented side bands (Fig. 3b). BP2 ranged from 0.585 kHz up to 25 kHz. The inter-pulse interval varied from 0.0004 to 0.001 (median = 0.0007, Table 2). BP2 was recorded during human-dolphin interaction (80%) and play (20%).

3. **Mixed sounds (MS).** Consists in an abrupt transition from BP1 to a harsh flat frequency harmonic sound (TS4) (Fig. 3c). The pulse stretch had 22 to 95 pulses and PFF of the harmonic component had from 0.747 to 1.997 kHz. It was recorded during play (80%) and human-dolphin interaction (20%).

4. **TS1.** TS1 is a low frequency sound with flat harmonic structure (Fig. 3d). It is aurally compared to a scream. This sound is usually chaotic and contains at least three visible harmonics. It’s the longest tonal sound with mean duration of 0.88 (SD = 2.23, Table 2) s and frequencies ranging from 0.218 up to 25 kHz. TS1 was heard more during human-dolphin interaction (72.7%), play (18,1%), aggressive and social contexts (both 4.5%).

5. **TS2.** TS2 is a tonal sound with harmonics and chaos (Fig. 3e). It is usually produced in bouts of 2 to 6 notes and had 8 to 12 visible harmonics. TS2 had mean duration of 10s and

ranged from 0.171 up to 25 kHz. It was recorded during human-dolphin interaction (57.8%), socialization (19,6%), aggression (12.7%) and foraging (9.8%).

6. **TS3.** TS3 is an upsweep harmonic sound (Fig. 3f). Mean duration was 0.14s, frequencies ranged from 0.477 up to 25 kHz and the number of visible harmonics varied from 5 to 12. TS3 was recorded during human-dolphin interaction (63.9%), socialization (18%), aggression (11.4%) and milling (6.5%).

7. **TS4.** TS4 is a harsh flat frequency harmonic sound (Fig. 3g). Frequencies ranged from 0.047 up to 25 kHz, the mean duration was 0.09s and the visible harmonics ranged from 8 to 16. TS4 was heard more during human-dolphin interaction (56.5%) followed by play (18.8%), aggression (13.9%), socialization (9.8%), and milling (0.8%).

8. **TS5.** TS5 is a downsweep harmonic sound (Fig. 3h). This sound ranged from 0.692 up to 25 kHz, with mean duration of 0.14s and with 10 to 16 visible harmonics. TS5 was heard more during aggressive context (50%), human-dolphin interaction (33.3%) and play (16.6%).

9. **TS6.** TS6 is frequency modulated harmonic sound (Fig. 3i). The number of visible harmonics ranged from 10 to 18, the mean duration was 0.17s and ranged from 0.520 up to 25 kHz. It was recorded during human-dolphin interaction (56.5%), aggression (30.4%), play (8.7%) and socialization (4.3%).

10. **TS7.** TS7 is convex-shaped harmonic sound (Fig. 4a). TS7 is produced in bouts, with 2 to 16 notes with PFF decreasing in frequency. Mean duration of each note was 0.08s with

PFF ranging from 0.654 to 4.687 kHz. TS7 was recorded during socialization (46.8%), human-dolphin interaction (32.5%) and aggression (20.6%).

11. **TS8.** TS8 are sounds with subharmonics components (Fig. 4b). The mean duration was 0.15s, with 4 to 9 visible harmonics and ranged from 0.585 to 19.142 kHz. TS8 was recorded during foraging (40%), aggression, play and socialization (20% each).

12. **TS9.** TS9 are harmonic concave-shaped sounds (Fig. 4c). The number of visible harmonics ranged from 3 to 10, the mean duration was 0.1s and frequencies ranged from 0.410 up to 25 kHz. TS9 was heard more during human-dolphin interaction (55.7%), milling (15.3%), play (11.5%), aggression (9.6%) and socialization (7.6%).

13. **TS10.** TS10 are harmonic bell-shaped sounds (Fig. 4d). The mean duration was 0.1s and the number of visible harmonic varied from 5 to 10. Frequencies ranged from 0.154 up to 25 kHz. TS10 was heard during human-dolphin interaction (52.1%), socialization (25.7%), aggression (20%), play (1.5%) and foraging (0.5%).

14. **Whistle (WS).** Whistles are narrow-band frequency-modulated tonal sounds (Fig. 4e). The mean duration (0.41± 0.28) was longer than in other harmonic sounds. Frequencies ranged from 1.214 to 22.924 kHz. Whistles were heard during human-dolphin interaction, milling (both 44.4%) and aggression (11.1%).

B. Sound parameters and behavioral contexts

Botos were more vocal more during human-dolphin interaction (n=394) than in other contexts, such as milling (n=18) and foraging (n=14). This might be due to the distance from the animals to the hydrophone, since the device was fixed, it limited the opportunity to record other sound types because the animals were not close (<100m) during all activities (e.g. foraging and milling).

In general way, vocalizations were short in duration and the dominant frequency at the fundamental did not exceed 4 kHz (Table I). A Kruskal-Wallis *post hoc* test with Bonferroni correction indicated that sound parameters varied significantly among behavioral contexts (Table II).

The longest calls were heard during human-dolphin interaction. Vocalizations emitted during aggressive behavior were significantly different from other behavioral contexts (Table III). The calls had the lowest high frequency, delta frequency, 1st quartile frequency, 3rd quartile frequency, max frequency, initial and final frequency and also the lowest dominant frequency at the fundamental. Vocalizations emitted during play had the highest frequency values for high frequency, 3rd quartile frequency, max frequency, initial and final frequencies (all values are summarized in Table III).

IV. DISCUSSION

Amazon river dolphins' vocal repertoire is complex and consists in gradations and transitions between sound types (Fig. 5). Gradations are defined as the gradual transformation of one sound type into another (Wong, Stewart, & Macdonald, 1999). The sounds classified as TS2 to TS10 form a continuum and are occasionally difficult to separate into discrete call types due to the overlap in temporal and spectral characteristics among sound types (Fig. 6).

The sound types TS2, TS7, TS9 and TS10 were characterized in previous studies (e.g. Caldwell e Caldwell 1966) as *grate, squeal, squawk, yelps* and *barks***.** These sounds were classified as pulsed sounds. We observed that these calls are similar to the low frequency narrow-banded sounds described for *Tursiops truncatus* in Schultz et al. (1995) and Simard et al. (2011). These cited sounds types generally had a sinusoidal structure, with no consistent association with pulsed sounds, which have clear clicks. Nonetheless, these sounds sometimes had clicks in its structure (similar to the sound described in Schultz et al. 1995), suggesting that the vocal repertoire of botos is not a compound of discrete categories, but of graded sounds.

Most of the vocalizations are produced in bouts and consists of heterogeneous series of short-duration notes (as noted by Podos et al. 2002) and they sudden change, with a short temporal gap, from one sound type to another. The sound types seem to occupy positions along a continuum that varies from widely spaced pulses (BP1) to a continuous sinusoidal sound (whistle) and vice-versa. The capability to control the inter-pulse interval gives an explanation for the gradation. As the interval between pulses decreases until there is no interval, the vocalizations begin to approximate a constant sine wave, resulting in whistle production (Murray, Mercado, & Roitblat, 1998).

Discreteness seems to be common in calls with specific ecological functions, while the flexibility of gradation may serve to communicate a wider range of information (Keenan, Lemasson, & Zuberbühler, 2013), resulting in an increasing variability of sound combinations.

Nonlinear phenomena, such as biphonations, subharmonics, and determinist chaos were observed in 4 sound types (MS, TS2, TS4 and TS8). These acoustic phenomena originate from nonlinearities in the vocal production system (Fitch, Neubauer, & Herzel, 2002), but may also be produced as a result of systemic infection or diseases in the vocal tract (Riede et al., 1997). These phenomena are considered normal events for many mammalian species (Wilden, Herzel, Peters, & Tembrock, 1998) and may play a role in communication (Tyson, Nowacek, & Miller, 2007) and may indicate motivation (Briefer, 2012).

The presence of transitions, gradations and non-linear phenomena on botos' vocal repertoire represent a good example of the linkage between communication and social complexity and supports the social intelligence hypothesis of Freeberg et al. (2012). The hypothesis supports that species living in more complex social groups present a more sophisticated communication system ("large number of structurally and functionally distinct elements or a high amount of bits of information", Freeberg et al. 2012), which is necessary to deal with a wider range of social interactions in different behavioral contexts. Non-linear phenomena as well as gradations and transitions have been reported to compound the vocal repertoire of several cetaceans species such as short-finned pilot whales (Nemiroff & Whitehead, 2009; Sayigh, Quick, Hastie, & Tyack, 2013), belugas (Karlsen, Bisther, Lydersen, Haug, & Kovacs, 2002; Panova et al., 2012), humpback whales (Mercado, Schneider, Pack, & Herman, 2010) and right whales (Clark, 1982). The fact that botos are considered solitary species (Best & da Silva, 1993) contrasts with the assumption that large and graded repertoires are more likely to occur in highly social species (McComb & Semple, 2005).

Previous studies with captive and wild botos have only described the sound types described in this study as TS7, TS9 and TS10, which are typical *Inia* vocalizations. We attribute this to the fact of the unusual situation of provision/interaction activities. Certain contexts or stimuli may demand greater need to transmit information (Freeberg et al., 2012). When botos were interacting with humans, they were more likely to engage in higher levels of arousal behavior and as a consequence they emitted all sound types described (Fig. 7), thus suggesting that vocal activity is correlated with excitement level.

Sound types TS2, TS7 and TS10 closely resemble the hypothesized chevron-shaped sound presented by Bradbury & Vehrencamp (2011), which they considered equivalent to *chirp* or *bark*. They characterized this sound as pure tone vocalization associated with ambivalence, indecision, curiosity, or mild alert. The cited sound types where emitted only during periods of high social activity (Fig. 7).

Whistles recorded in this study fall into the descriptions of previous studies. Despite the fact that whistles recorded in Negro river had higher frequencies values than reported by Ding et al (2000), they were similar in shape, being flat and with little frequency modulation (Figs. 4e and 5). Due to equipment limitations, no high-frequency whistles were recorded, which limit comparisons with whistles described by May-Collado et al (2007). Despite this, some similarities were observed: whistles were short in duration and not produced in bouts.

When analyzing the sounds emitted in different behavioral contexts, we observed that aggressive contexts differ from the remaining contexts in several acoustic parameters (Table III, Appendix). The presence of low-frequency sounds during aggressive contexts and high-frequency sounds associated with play corroborates with the motivational-structural code scheme proposed by Morton (1977). According to this theory, coupled frequency and bandwidth acoustic features can express the arousal behavioral state of mammals and birds. Aggression is expressed with increasingly lower-frequency, harsh, atonal sounds and fearful or friendly contexts are characterized by high frequency and tonal sounds.

Low-frequency sounds emitted during aggression might serve as an indicator of body size. Individuals with large body size can produce lower-frequency sounds than smaller ones (May-Collado et al. 2007). Boto is one of the most sexually dimorphic of all cetaceans and also among river dolphins with males much larger than females (A. R. Martin & da Silva, 2006). The emission of low-frequency sounds during aggression might serve as a signal of willingness and fight ability, since large individuals have competitive advantage in aggressive contests (Morton, 1977).

The peak frequency at the fundamental did not exceed 4 kHz (except for whistles, Table I, Appendix). The vocalizations are audible and low in frequency, being susceptible of masking effects from boat noise due to the overlap between frequencies used in communication and dominant frequencies of anthropogenic noise.

V. CONCLUSIONS

This research is the first contribution to the understanding of Amazon river dolphin vocal repertoire and associated behavior. Here we present spectrograms and descriptions of diverse sound types. These animals seem to use acoustic signals to mediate social interactions, fact illustrated by the high number of vocalizations during human-dolphin interaction, a situation where animals are competing for artificial food resource. This study identified that botos rarely whistle, consequently until recent it was considered a non-whistling species. Moreover, our investigation showed that botos have a much more complex communication system than previously described and it’s based on gradation and transitions rather than discrete sound types. The variety of sound types and possible combinations, as well as the function of nonlinear phenomena should be considered in future studies. We also suggest the investigation on possible individual discrimination and potential impacts of boat traffic on communication.

VI. AKNOWLEDGEMENTS

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FIGURES

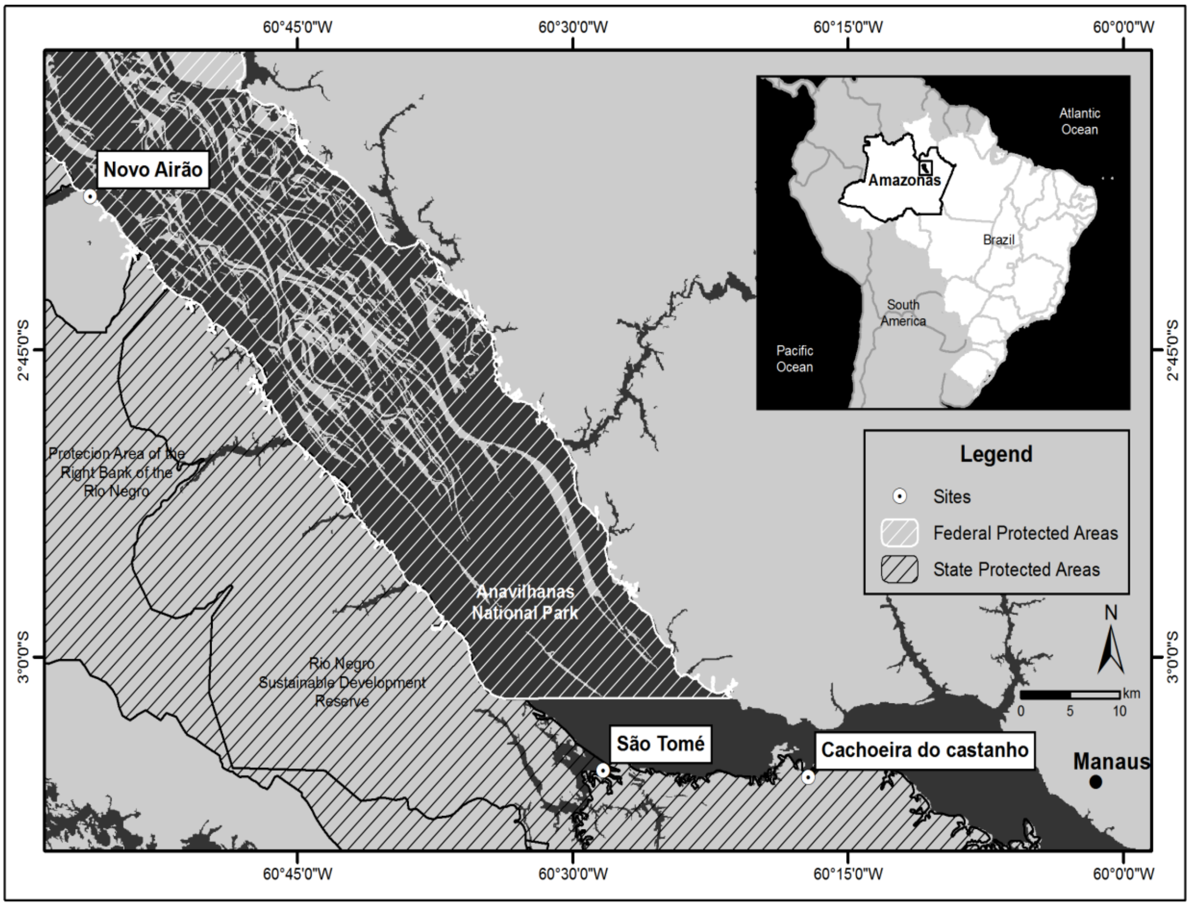

Fig. 1 Map of study area, presenting the three sites of data collection: Cachoeira do Castanho, São Thomé and Novo Airão, located in lower Negro river, Amazonas state, Brazil.

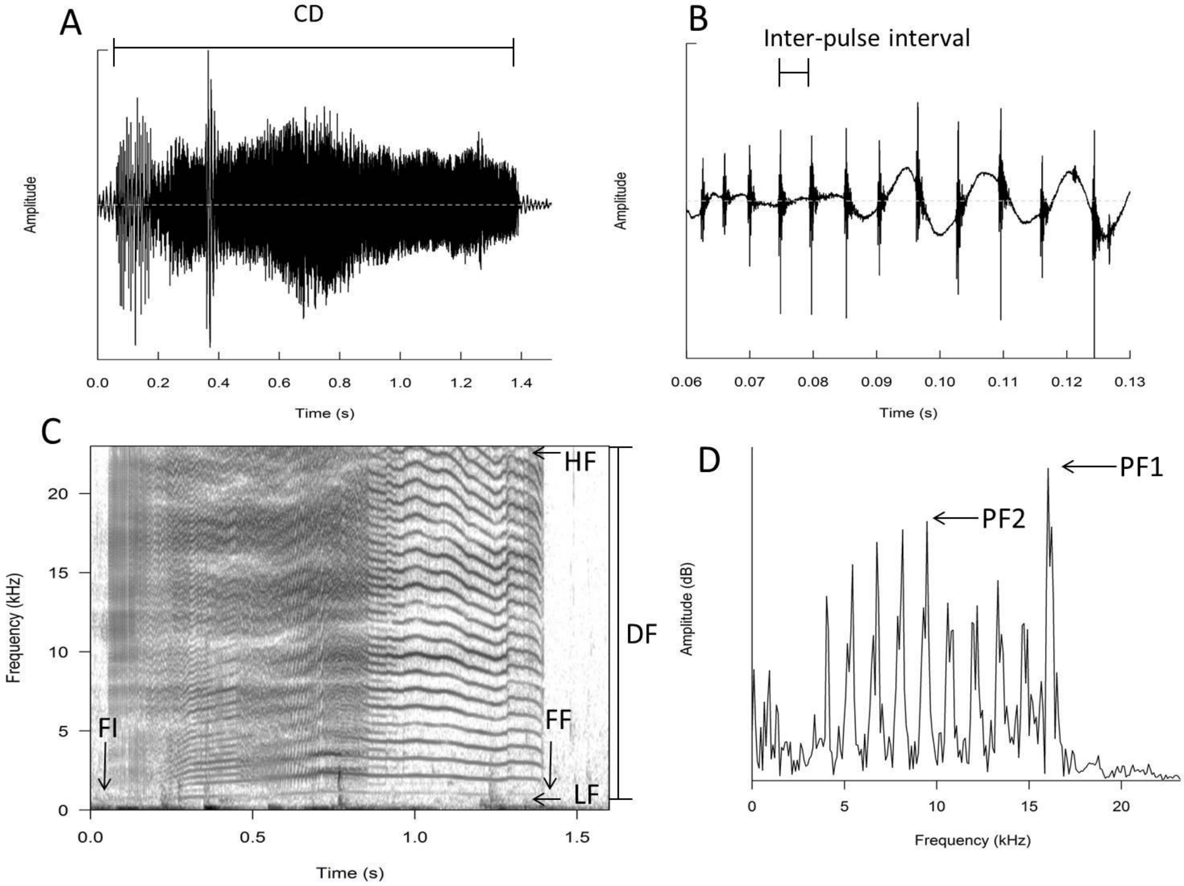

Fig. 2 Measurements of acoustic parameters of an Amazon river dolphin BP2 call: A) Oscillogram used to measure the total duration call (CD). B) Zoom view of oscillogram stretch showing the measurement of the inter-pulse interval used to estimate the number of pulses of the sound (IPI). C) Spectrogram (window size 512) used to measure the lowest frequency (LF) of the sound; the highest frequency (HF) of the sound; the difference (FD) between HF and LF; the initial frequency (LF) and final frequency (FF) of the sound. D) Power spectrum used to measure the peak frequency at the 1st formant (PF1) and at the 2nd formant (PF2).

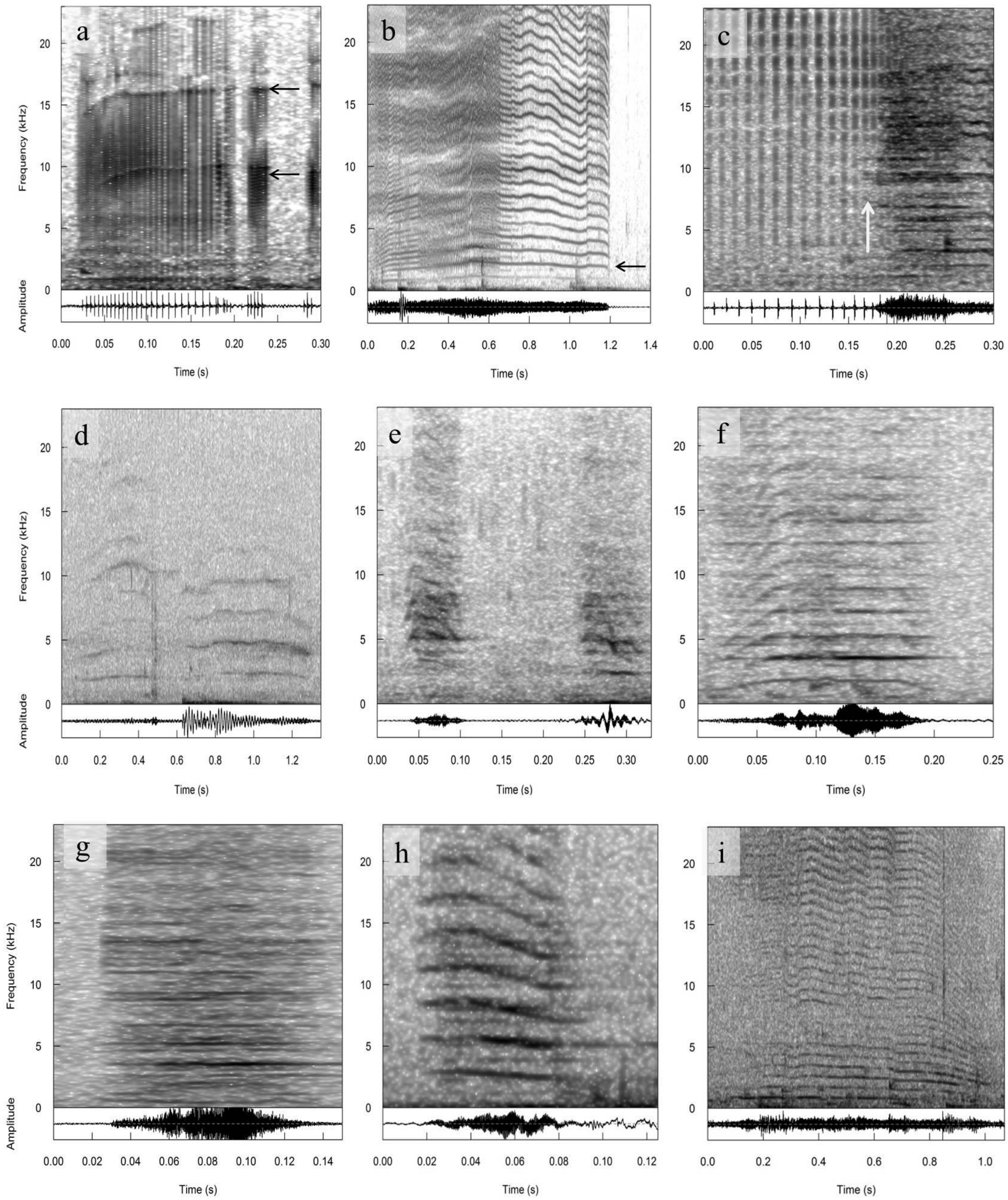

Fig. 3 Spectrograms and oscillograms (bottom) of vocalizations emitted by Amazon river dolphins in lower Negro river, Amazonas, Brazil: a) BP1 (arrows indicate the 1st and 2nd formant); b) BP2 (arrows indicate side bands); c) mixed sounds - BP1 with abrupt transition to TS4 (arrow indicates the transition); d) TS1; e) TS2; f) TS3; g) TS4; h) TS5; i) TS6. All figures in Hanning window, FFT=512.

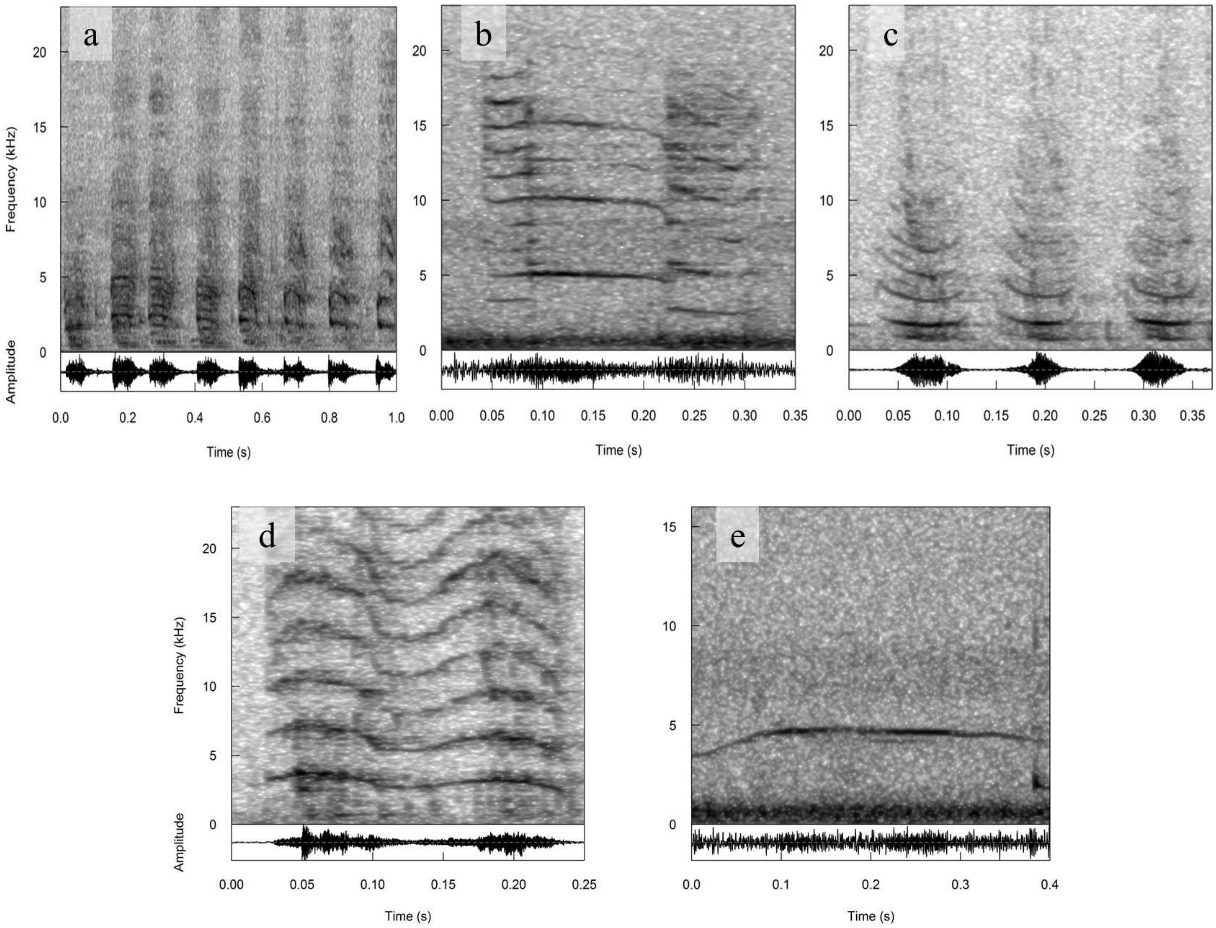

Fig. 4 Spectrograms and oscillograms (bottom) of vocalizations emitted by Amazon river dolphins in lower Negro river, Amazonas, Brazil: a) TS7; b) TS8; c) TS9; d) TS10; e) whistle. All figures in Hanning window, FFT=512.

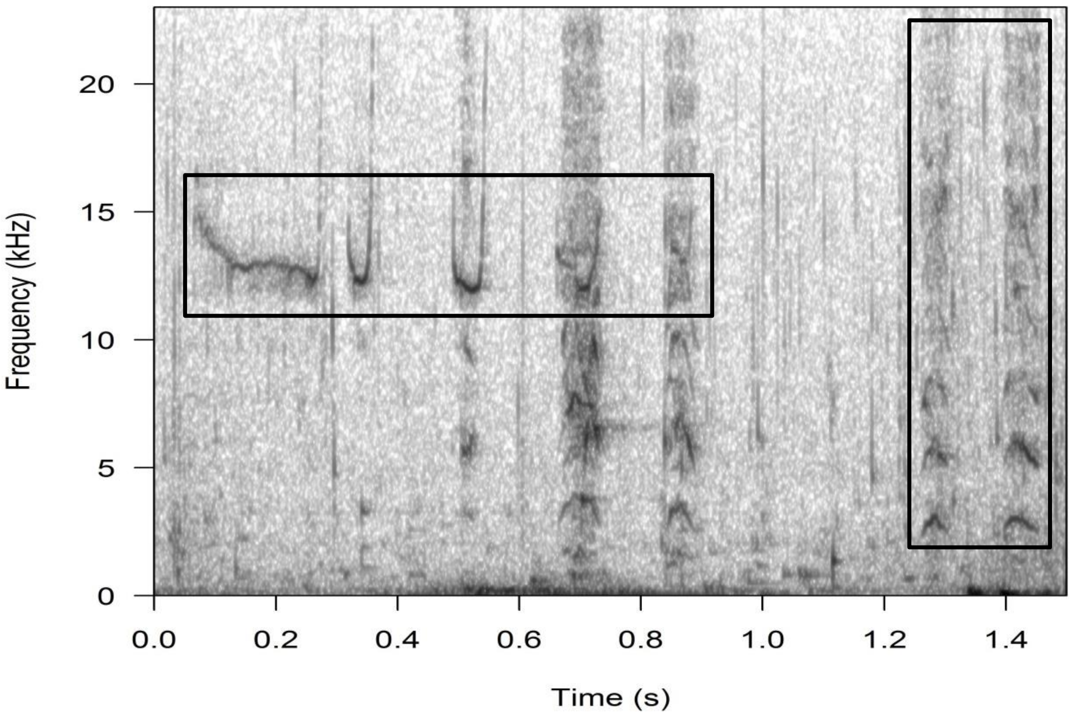

Fig. 5 Spectrogram with an example of gradation and transition in Amazon river dolphin vocal repertoire. The horizontal box shows a gradation between whistles to TS7, a typical Inia vocalization. Transition to TS7 is detached by the vertical box. Hanning window, FFT=512.

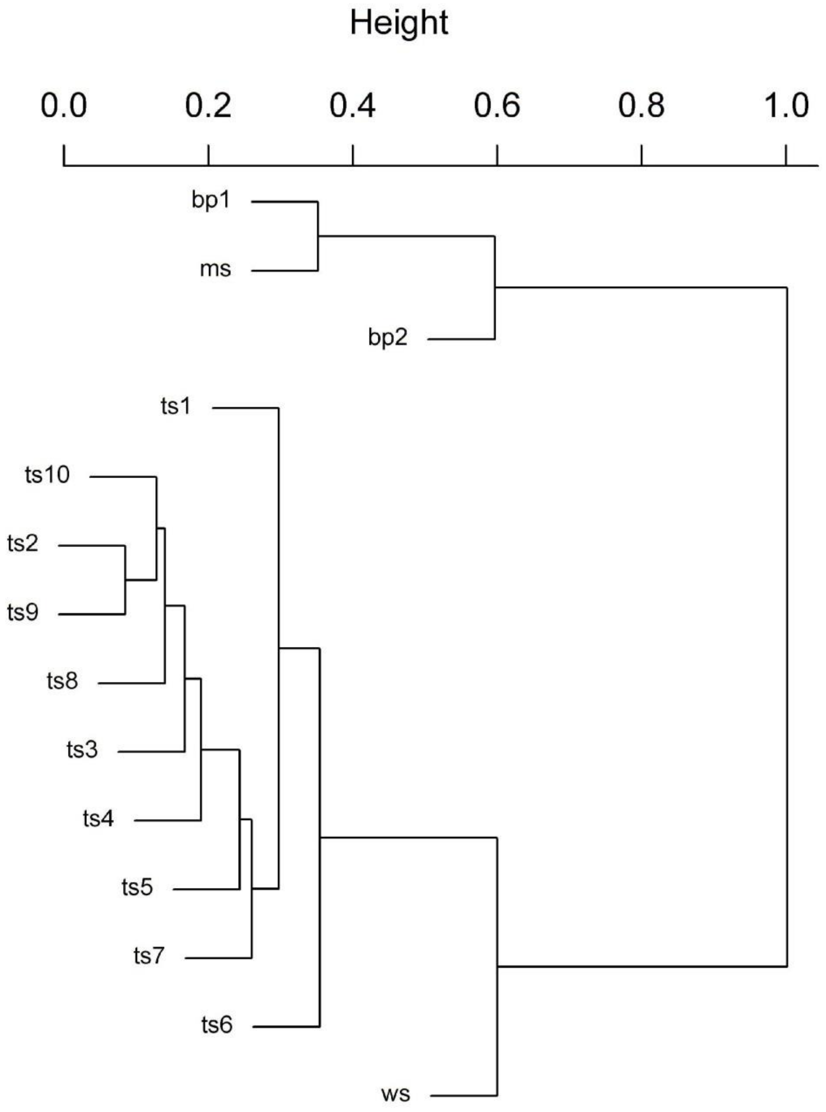

Fig. 6 Dendrogram of hierarchical-cluster relationship of the 14 sound types (n=783 vocalizations) emitted by Amazon river dolphins distributed in three sites in lower Negro river, Amazonas, Brazil, from May to August 2013. *Height represents a vector of the distances between clusters at successive stages, shorter end branches indicates similarity of sound types.

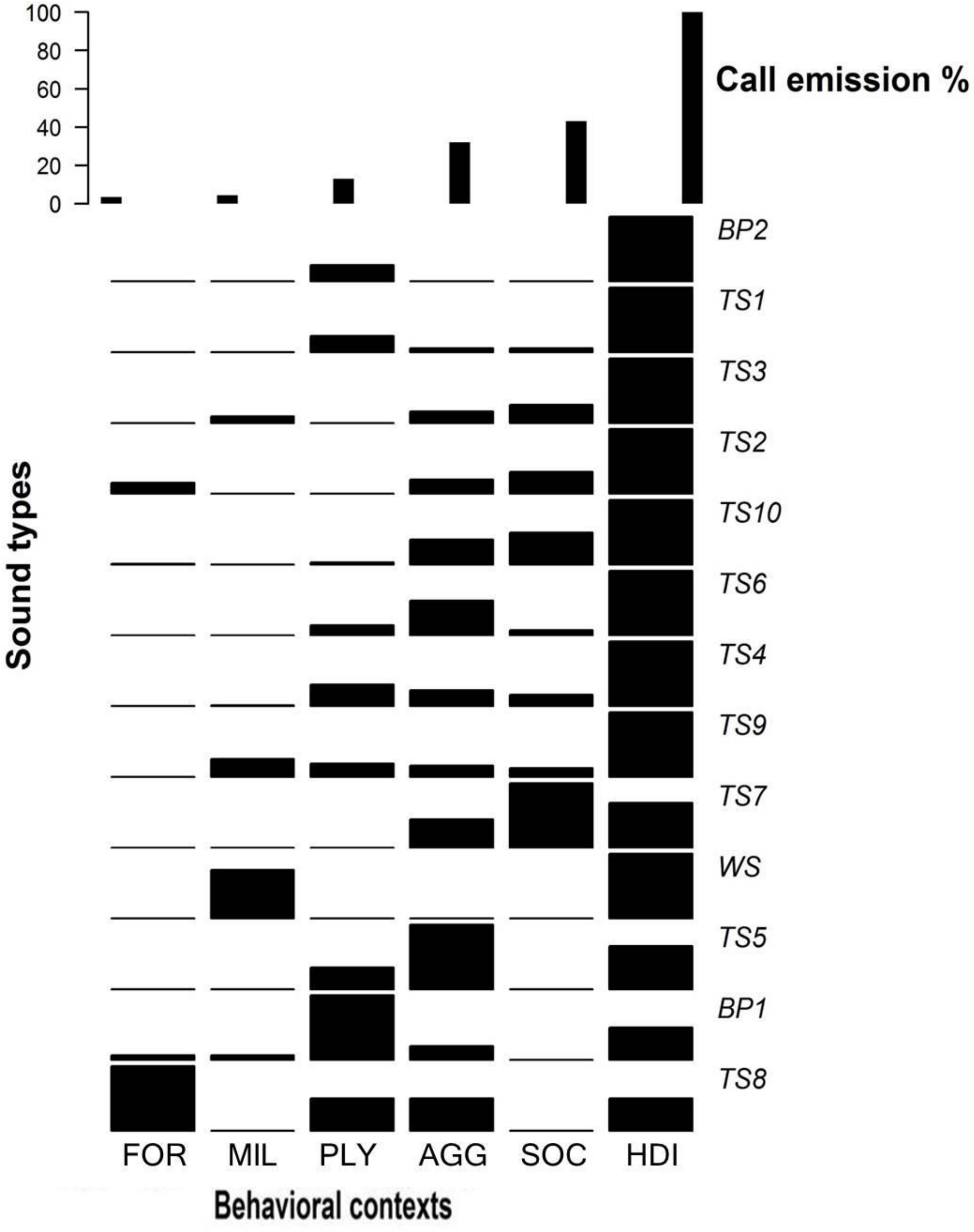

Fig. 7 Proportions of Amazon river dolphin vocalizations given in different behavioral contexts (AGG, aggression; FOR, foraging; HDI, human-dolphin interaction; MIL, milling; PLY, play; SOC, socialization. Sound types are ordered according to the behavioral contexts where each vocalization was emitted the most.

TABLES

Table I Descriptive statistics [X ± SD or median (minimum–maximum ranges)] for all proposed sound types emitted by Amazon river dolphins from 3 distinct sites monitored from May to August 2014 in the lower Negro river, Brazil. N represents the number of sounds used for acoustic measurements. Variables: CD: call duration, LF: lowest and HF: highest frequencies of the sound, Q1: 1st quartile frequency, Q3: 3rd quartile frequency, MF: max frequency of the entire sound, DF: difference between the highest and the lowest frequency of the entire sound, FI: Initial frequency and FF: final frequency of the entire sound, PFF: peak frequency at the fundamental, PP: number of pulses of the sound, IPI: inter-pulse interval, PF1: peak frequency at the first formant (for pulsed sounds), PF2: peak frequency at the second formant (for pulsed sounds). *temporal units in seconds(s), frequency units in kHz.

Sound type	N	CD	LF	HF	Q1	Q3	MF	DF	FI	FF	PFF	PP	IPI	PF1	PF2
BP1	21	0.11± 0.12	2.455± 2.499	20.877 ± 6.878	5.008± 3.806	8.933± 6.092	5.258± 4.180	23.479± 11.033	6.771± 5.444	4.798± 4.828	1±0	106± 95	0.001± 0.001	5.662± 5.079	6.456± 5.960
BP2	5	0.65± 0.46	1.850± 1.025	4.742± 0.575	7.125± 4.389	14.737 ± 6.500	3.618± 2.662	37.481± 9.736	7.274± 6.613	8.830± 8.079	1±0	873± 491	0.0007± 0.0003	10.728 ± 8.685	12.897 ±6.484
MS	5	0.12± 0.07	1528± 0.827	17.756 ±5.924	3.812± 1.954	5.296± 2.595	4.936± 3.322	19.372± 13.073	5.296± 2.190	4.390± 1.860	1.268± 0.507	57±33	0.002± 0.002	4.826± 2.566	4.733± 2.549
TS1	22	0.91± 2.27	2.479± 1.453	16.391 ±6.127	4.022± 1.725	7.614± 3.224	5.424± 4.057	15.866± 9.603	4.599± 2.362	5.711± 3.661	3.701± 2.418	1±0	1±0	1±0	1±0
TS2	102	0.10± 0.06	1.763± 0.992	14.101 ±7.523	2.958± 1.566	4.613± 2.251	3.376± 1.896	12.452± 7.567	3.850± 2.581	3.508± 2.111	2.305± 1.320	1±0	1±0	1±0	1±0
TS3	61	0.14±0.1	2.386± 1.526	15.747 ±7.088	3.939± 1.928	6.933± 3.399	4.511± 2.311	13.432± 6.825	4.768± 3.189	4.639± 2.860	3.149± 1.672	1±0	1±0	1±0	1±0
TS4	122	0.09± 0.04	1.859± 1.351	16.844 ±7.679	3.609± 2.291	6.278± 3.888	4.235± 2.805	14.987± 7.172	5.014± 3.841	3.794± 3.084	2.231± 1.424	1±0	1±0	1±0	1±0
TS5	6	0.14± 0.05	1.539± 1.051	14.494 ±10.550	2.750± 1.353	4.015± 2.353	4.452± 3.858	12.955± 10.105	3.663± 4.159	3.202± 3.013	1.892± 1.065	1±0	1±0	1±0	1±0
TS6	23	0.17± 0.13	1.766± 1.206	17.866 ±7.516	2.506± 1.618	6.289± 4.324	2.394± 1.561	16.100± 7.305	4.469± 4.632	3.107± 2.508	2.094± 1.305	1±0	1±0	1±0	1±0

TS7	160	0.08±0.03	0.974±0.461	11.581±7.288	1.705±1.191	2.892±1.918	2.184±1.650	10.612±7.173	2.274±1.658	2.266±1.775	1.496±0.771	1±0	1±0	1±0	1±0
TS8	5	0.15±0.07	1.955±0.844	14.386±4.849	3.262±1.572	5.681±3.085	3.299±1.576	12.431±4.168	5.718±5.195	3.449±2.345	2.535±0.159	1±0	1±0	1±0	1±0
TS9	52	0.10±0.05	1.862±1.417	13.366±7.705	2.956±2.246	5.373±3.706	3.510±2.381	11.505±7.140	3.760±3.042	4.015±2.656	2.277±1.489	1±0	1±0	1±0	1±0
TS10	190	0.10±0.06	1.631±1.366	13.386±8.232	2.628±1.915	4.704±3.522	3.095±2.374	11.783±7.883	3.254±2.643	3.185±2.695	2.142±1.485	1±0	1±0	1±0	1±0
WS	9	0.41±0.28	7.450±3.937	10.972±5.462	8.286±3.458	8.593±3.415	8.390±3.404	3.522±3.084	8.593±4.284	8.697±5.350	8.351±3.416	1±0	1±0	1±0	1±0

Table II Significant differences for each sound parameter of Amazon river dolphins vocalizations among behavioral contexts. χ^2 and p-value are results of a Kruskal-Wallis test. Variables: LF: lowest and HF: highest frequencies of the sound, Q1: 1st quartile frequency, Q3: 3rd quartile frequency, MF: max frequency of the entire sound, FI: Initial value and FF: final value of the entire sound, IPI: inter-pulse interval.

Variable	**Behavioral contexts**	χ^2	**p-value**
LF	AGG- HDI; AGG-MIL; AGG-PLY; SOC-HDI; SOC-MIL, SOC-PLY	125.2	< 0.001
HF	AGG-HDI;AGG-MIL;AGG-PLY;AGG-SOC;PLY-FOR;PLY-HDI;PLY-MIL; PLY-SOC	160	< 0.001
Q1	AGG-PLY; AGG-HDI;PLY-HDI; PLY-MIL; PLY-SOC; SOC-MIL; SOC-HDI	82.8	< 0.001
Q3	AGG-HDI; AGG-MIL; AGG-PLY; AGG-SOC; PLY-FOR; PLY-HDI;SOC-HDI	181.1	< 0.001
MF	AGG-HDI; AGG-MIL; AGG-SOC; PLY-FOR; PLY-HDI; PLY-SOC; MIL-SOC	72.2	< 0.001
FI	AGG-HDI; AGG-MIL; AGG-PLY; AGG-SOC; PLY-FOR;PLY-SOC;PLY-HDI; SOC-HDI	151.8	< 0.001
FF	AGG-HDI;AGG-MIL; AGG-PLY; SOC-HDI;SOC-MIL;SOC-PLY	124.7	< 0.001
IPI	AGG-PLY; PLY-HDI; PLY-SOC; PLY-FOR	89.5	< 0.001

Table III Descriptive statistics [X ± SD or median (minimum–maximum ranges)] of the acoustic parameters of all vocalizations emitted in three different aggregations of Amazon river dolphins among different behavioral contexts. N represents the number of sounds used in acoustic analysis. Variables: CD: call duration, LF: Minimum frequency and HF: higher frequency of sound, Q1: 1st quartile frequency Q3: 3rd quartile frequency MF: maximum frequency, DF: difference between the highest and lowest frequencies , FI: Initial frequency and FF: Final frequency of sound PFF: dominant frequency of the fundamental, PP: number of pulses of the sound, IPI: interval between pulses, PF1: dominant frequency of the first formant (for pulsed sounds), PF2 : dominant frequency of the second formant (for pulsed sounds). * time unit in seconds (s), frequency in kHz units. [a] Only one pulsed sound was recorded during this behavior.

CONTEXT	N	CD	LF	HF	DF	Q1	Q3	MF	FI	FF	PFF	PU	IPI	PF1	PF2
AGG	129	0.8±0.9	1.251±1.231	7.587±7.994	6.495±7.783	1.817±1.693	3.057±3.084	2.094±1.982	2.273±2.619	2.204±2.343	1.549±1.396	304±95	0.0002±0.00005	8.200±0.384	8.944±6.453
FOR	14	0.14±0.09	1.597±0.547	9.409±4.496	7.811±4.066	2.538±1.402	3.770±2.770	2.715±1.658	2.627±1.373	3.726±2.789	1.706±0.737	67.5[a]	0.002[a]	4.220[a]	5.678[a]
HDI	394	3.5±6.7	2.060±1.687	15.512±7.115	13.845±7.627	3.426±2.264	5.926±3.527	3.980±2.637	4.458±3.404	4.144±3.027	2.582±1.788	360±513	0.00006±0.0004	0.179±1.633	0.209±1.662
MIL	18	0.2±0.2	3.214±2.508	12.995±7.695	9.786±8.101	4.729±3.046	6.979±4.477	5.036±2.859	4.551±2.949	4.520±2.799	3.194±2.717	159.7[a]	0.003[a]	2.164[a]	14.536[a]
PLY	55	0.09±0.1	1.960±0.853	20.557±5.211	20.194±8.137	4.668±2.278	8.524±4.469	5.347±3.313	6.328±3.891	4.685±3.506	2.208±1.055	120±209	0.0008±0.0005	6.640±4.725	6.589±5.676
SOC	173	0.11±0.09	1.178±0.805	15.218±7.054	14.039±6.922	2.094±1.326	3.764±2.637	2.574±1.776	2.873±2.2129	2.622±2.260	1.697±1.034	1±0	1±0	1±0	1±0

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Capítulo 2

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Behavioral effects of tourism on Amazon river dolphins, *Inia geoffrensis*, in the Negro river

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ABSTRACT

Tourism based on in-water encounters with cetaceans can be considered beneficial for humans and dolphins. However, there has been an increasing amount of studies showing negative short- and long-term effects of tourism on cetaceans' behavior. The state of Amazonas, Brazil, is the most popular place to have in-water encounters with wild Amazon river dolphins, or boto. We aimed to quantify the impact of human interactions on the behavior of botos that participated in swim-with activities. We collected information on behavioral categories of botos in three different interaction sites: Cachoeira do Castanho, São Thomé and Novo Airão. We calculated the probabilities of transition from one behavior to another using a first-order, time-discrete Markov chain model. Behavioral transitions during which human-dolphin interaction occurred were compiled in an "impact" chain. All other transitions were tallied in a control chain. We than quantified the effect of interactions during behavioral transitions by comparing the behavioral transition probabilities of both chains. Socialization, foraging, milling and resting were disrupted by interactions. The probability of occurrence of aggression during and after human-dolphin interaction increased. In Novo Airão, the probability of staying in aggressive behavior increased due to human-dolphin interaction. We observed that botos of the three sites remained in close proximity to the floating even under control. We concluded that animals from the three locations are conditioned to human contact due to food reinforcement. Conditioned individuals tend to stay in the same location, diminishing its home range area. We recommend management measures to decrease risky interactions with dolphins and ensure animals welfare.

KEY WORDS Amazon river dolphins. Behavioral reaction. Feeding. Tourism impact.

INTRODUCTION

Wildlife tourism is uncritically perceived to be inherently beneficial, and so trends to be considered “ecotourism”. Wildlife watching has been described as non-consumptive in contrast to the immediate and lethal outcomes of hunting (Duffus and Dearden, 1990).

The proliferation of in-water interactions with cetaceans in the wild has prompted concern of scientists and several organisations due to both short- and long-term negative effects (Mann et al. 1999; Orams 2002; Samuels and Bejder 2004; Smith et al. 2008; Donaldson et al. 2012; Foroughirad and Mann 2013). Several negative impact have been reported, such as reduced avoidance responses to hazardous situations, reduced overall behavioral repertoires and conditioning to food provisioning, which can compromise “wildness”(Donaldson et al., 2012a).

In Amazonas State, this activity has grown in the last decade (L. C. P. S. Alves, Andriolo, et al., 2011). In-water interactions and feeding of Amazon river dolphins (or boto) occurs in the lower Negro river since 1998 (Barezani, 2005) and became a very popular touristic activity. The growth of the activity was not accompanied by quality, resulting in a poorly planned activity, with disorganized infrastructure, exposing animals to high intensities of observation and feeding (7 days a week, during all daylight hours). It has been reported that botos tend to engage more often in aggressive behaviors due to food provisioning (L. C. P. de S. Alves et al., 2013), but no information about other behavior alterations are available.

Studies on the impact imposed on wildlife by touristic activities allow the establishment of base lines for the regulation of commercial dolphin watching. This study aims to quantify the impact of human interactions on the behavior of botos (*Inia* *geoffrensis*) in lower Negro river, Amazonas state. Our goals were to understand how in-water interactions and the use of food-reinforcement affect the frequency of behavioural categories and behavioral transitions of botos in three different locations. We hypothesize that botos that are longer exposed to human contact are more likely to engage in aggressive behavior and the use of food-provisioning to attract the animals makes them remain close to the feeding area.

STUDY AREA

The study area encompasses the lower Negro river region, where commercial dolphin watching and swim-with activities occur. These activities take place in wooden floating structures positioned alongside the river. All data collection occurred in 2014, in three selected places: the vicinity of São Thomé, (3°05'35.4"S 60°28'31.0"W), from May 30th to June 11th, where tourism activities with botos deliberated started in 2005 (owners, pers. comm.), a small riverside community; in Novo Airão city, at the National Park of Anavilhanas (02°37′13.7″S 60°56′45.9″W) from July 21st to August 4th - since 1998 this site offers swim-with and feeding of botos, being the first place that started this activity in Amazonas state (Barezani 2005; Alves et al. 2011); and in Cachoeira do Castanho (3°5'54.35"S 60°17'10.64"W) from August 14th to 29th. São Thomé and Cachoeira do Castanho are small riverine communities placed at Environmental Protected Area of Rio Negro, a conservation state unit.

Study populations

Genetic research on Novo Airão aggregation concluded that all individuals are males (Gravena 2007). There is no genetic information for the other two sites, but in

São Thomé 15 individuals were captured for scientific purposes and were all males (da Silva, pers. comm.). In Cachoeira do Castanho, supposedly there is also a mother-calf pair. The aggregations varied daily from 1-12 individuals (pers. obs). Every place has different individuals that, until the end of the study, were not sighted moving between the three locations. Individuals could be recognized and distinguished by scars, pigmentation patterns and other marks (e.g. Gomez-salazar et al 2011).

METHODS

Observations were made from a fixed point in the floating structure where interactions with botos occur. The area was divided into three sections around the floating: 1) less than 10m; 2) from 10m to 30m; and 3) from 30m to 50m, which is the limit of reliable observation without binoculars. Observations were conducted daily in the morning (from 09:00 h to 12:00 h) and in the afternoon (from 13:00 h to 16:00 h), when, generally, the water visibility was favorable.

Behavioral data was collected using scan sampling technique (Altmann, 1974) at every 2 minutes. At each scan, the behaviors (events and states) of each individual in the aggregation were recorded (Appendix). The behavior was also recorded using a hi-definition camera (GoPro Hero 3+). Video recordings were analyzed to help describing the behaviors.

Behavioral categories definitions

Individual behaviors were included in seven behavioral categories defined *a posteriori* for each boto aggregation observed, considering the activities in which the majority (>50%) of individuals were engaged. If this criterion was not met, the sample was discarded. This approach focus on the proportion of individuals estimated to be engaging in a behavior (Janet Mann, 1999).

The seven behavioral categories observed during the study were: foraging, socialization, aggression, play, rest, milling and human-dolphin interaction. Foraging occurred when the botos were active surfacing with rapid changes in direction, long dives and prey could often be observed. Socialization occurred when two or more botos were engaged in activities that include close proximity, body contact and sexual displays. Interactions that involved fighting, threat and submission displays were considered as aggression and play was defined as interactions between two or more botos involving chases among individuals and pushing or pulling objects with no apparent intent other than the behavior itself. Resting was characterized by slow swimming with no determined direction or by remain stationary at the surface or underwater. Milling occurred when the boto was swimming without engaging in other activities. Human-dolphin interaction reunites behaviors observed during interactions of botos and humans such as feeding area approach, hand-feeding, touching and begging (Appendix).

Statistical analysis

We assessed the effect of human interactions in behavioral transitions developing a first-order Markov chains. The chains quantify the probability that a behaviour will follow the immediately preceding one (Lusseau, 2003). We calculated probabilities of transitions from preceding to succeeding behavioral categories using a conservative approach, eliminating any transition followed or affected by the beginning/ending of a interaction session (Meissner et al., 2015). These analyses compare the behaviour of the dolphins both when in the presence and in the absence of in-water interactions while simultaneously taking into account the temporal dependence between behavioural states.

Two two-way contingency tables (preceding *versus* succeeding behavioral categories) were developed for the three sites and the number of transitions between the behavioural categories in both control and interaction conditions were calculated (Lusseau, 2003; Meissner et al., 2015). Transition probabilities from the immediately preceding to the succeeding behavioural category were calculated for the control and interaction chains separately by (Lusseau, 2003):

$$P_{ij} = \frac{a_{ij}}{\sum_{j=1}^{7} a_{ij}}, \sum_{j=1}^{7} P_{ij} = 1,$$

where *i* is the preceding behavior, *j* is the succeeding behavior (*i* and *j* range from 1 to 7 because of the defined seven behavioral categories), a_{ij} is the number of transitions observed from behavior *i* to *j*, and P_{ij} is the transition probability from *i* to *j* in the Markov chain.

We tested the effect of human-dolphin interactions on transition probability by comparing control and impact matrices with a chi-squared test. Furthermore, each control transition was compared to its impact counterpart with a Z test for proportions, using rates (frequency/unit of time) of behavioral categories for both control and impact conditions. All statistical analyses were performed in R 2.15 Software (R Foundation for Statistical Computing, 2011).

RESULTS

In Cachoeira do Castanho, the amount of observation during control was 392 min and in impact was 506 min, during 11 days and a total of 37 hours. Animals were absent during 9% of the time. Botos spent 43% under control observations (39 min, SD=36 min) and 56% under impact (56 min, SD=40 min). The mean number of individuals was 2.3 (SD=0.9, max=5).

The total amount of control observation in São Thomé was 656 min and in impact was 450 min, during 15 days of observation and 46 hours. Botos were absent 19% of the time. Animals stayed 59% of the time in control (medium 43 min, SD=23 min) and 41% in impact observation (46 min, SD=13 min). The mean number of individuals was 2.3 (SD=1.3, max=12).

In a total of 54 hours and 12 days of observation, control was observed in Novo Airão during 46% of the time (604 min) and impact during 53% (706 min). Botos were absent only 7% of the time. Control bouts lasted a medium of 54 min (SD=29 min) and impact had a medium of 58 min (SD=32 min). The mean number of individuals was 2.3 (SD=1.15, max=6).

As expected, individuals at Cachoeira do Castanho were registered 90% in area 1 during impact, 60% in area 2 and 40% in area 3. During control, animals stayed 80% in area 3, approximately 60% in area 2 and almost 20% in area 1 (Fig. 1). These values are considering error bars.

Comparing the frequency of occurrence of botos at São Thomé in three distance scales and considering the error bars, we observed that animals visited area 1 almost 40% during control, and areas 2 and 3 were visited 60% and 40% respectively. During impact, individuals tended to stay in area 1 (80%) and area 2 (50%). Botos occurred in area 3 only 20% during impact (Fig. 1). Considering error bars, botos occupied area 1 at

Novo Airão about 80% during impact, and remained in areas 2 and 3 about 30% and 35%, respectively. While in control, botos were sighted 40% in area 1, 35% in area 2 and about 65% in area 3 (Fig. 2).

There was no difference between the observed control and impact rates of behavioral categories in São Thomé ($Z = 1.5$, $p = 0.12$) and Cachoeira do Castanho ($Z= 0.33$, $p = 0.73$). However, it is possible to observe that individuals at São Thomé frequently mill, play, rest and socialize more during control bouts, in contrast, aggressive behaviors are more likely to occur during impact (Fig. 2). At Cachoeira do Castanho botos were more likely to forage, socialize and mill during control, while aggressive exhibitions occurred more during impact (Fig. 2). And at Novo Airão, botos milled and socialized more during control, and similar to other sites, aggressive behaviors occurred at higher rates during impact and tourism did have an effect on rates of behavioral state of the animals in Novo Airão ($Z = 2.48$, $p= 0.01$).

There were 326 behavioral transitions classified as control at Cachoeira do Castanho and 219 as impact. At São Thomé. 330 behavioral transitions were classified as control and 224 as impact. And in Novo Airão, 298 were registered as control and 347 as impact. Behavioral transitions were affected by tourism interactions in the three locations (Cachoeira do Castanho $\chi2=287.08$, $df=169$, $p<0.01$; São Thomé χ $2=657.11$, $df=36$, $p<0.01$; Novo Airão $\chi2=284.38$, $df=156$, $p<0.01$) but the effect was not observed over all transitions.

At Cachoeira do Castanho, São Thomé and Novo Airão, transition of aggression to human-dolphin interaction and the inverse, human-dolphin interaction to aggression increased; also in Novo Airão, the probability of staying in aggressive behavior was significantly.

In the three locations, the probability of staying foraging, playing (except Novo Airão), milling, rest and socialization decreased. At Cachoeira do Castanho, the probability of transition of play to milling and socialization to milling decreased. At São Thomé, the transitions of milling to play, milling to socialization and the inverse (socialization to milling) decreased. And at Novo Airão, the probability of botos to transit from milling to human-dolphin interaction and socialization to human-dolphin interaction has also decreased.

DISCUSSION

In-water interactions with free-ranging dolphins is very popular, however, deleterious effects of commercial tourism activities on marine mammals have been reported in several studies (Bejder et al., 2009; Constantine, 2001; Samuels & Bejder, 2004). Our study provides evidence that botos does alter its behavior due to food-reinforcement and in-water interactions with humans.

The probability of socialization, forage, mill and rest decreased while the probability of occurring aggression after interaction with humans increased in all sites. Foraging is a critical component for any predator and disruption to this behavior due to food-reinforcement can potentially result in dependency of human provisioning (Orams 2002; Cunningham-Smith et al. 2006; Hawkins et al. 2008; Foroughirad and Mann 2013). The probability of stay playing in São Thomé and Cachoeira do Castanho decreased. In Novo Airão, individuals did not engage very often in playing (Fig. 2), so its effect in transition probabilities was not statistically significant.

In Novo Airão, the probability of staying in aggressive state increased due to human-dolphin interaction. The use of food-reinforcement to make wildlife visible has led to increase aggressiveness in several species (e.g. Hamadryas baboons, Kamal et al. 1997; bottlenose dolphins, Orams et al. 1996).

Interestingly, Barezani (2005) reported that botos in Novo Airão did not engage frequently in contests due to food offer, which contrasts with the findings in this research. This change in response might be indicating that individuals in Novo Airão are sensitized to human stimuli. Sensitisation refers to a process of behavioral response where individuals increasingly respond to a repeated stimulus that has significant consequences for the animal (Richardson et al. 1995). Individuals that are sensitized exhibit a gradual intensification of their response to stimuli and reflects an individual’s cumulative experience with humans (Bejder et al., 2009). Because individuals continuously visit the floatings and accept food from humans, we can affirm that these animals are conditioned to human contact through food reinforcement.

If we consider the standard error line on Figure 1, we observe that botos of the three sites remain in close proximity to the floating even under control, when there were no people interacting. These results might support the affirmation of Barezani (2005) that animals prefer these areas due to diminished stream in relation to the river channel. Botos are frequently seen in areas with such characteristics (a. R. R. Martin, da Silva, & Salmon, 2004), however, the possible explanation for this result is that botos that remain close to the floating have advantage because they increase the chance of being fed when an interaction session starts. Thus, conditioned dolphins tend to stay in the same location, diminishing its range area (Samuels & Bejder, 2004).

MANAGEMENT IMPLICATIONS

In the three locations, botos were vulnerable to inappropriate human behaviors such as hugging, kissing, mounting, excessive touching and teasing. We recommend an enforcement regime to control the number of persons that interact in the water with botos and to limit their approach and the amount of time spend with the animals. These measures along with the control of food amount that could be given to dolphins and the period that they could be fed has led to reduced amount of time spent in provisioning area in a group of food-provisioned bottlenose dolphins in Australia (Foroughirad & Mann, 2013). The quality of fish storage and preparation should also be a concern in order to avoid the ingestion of contaminated or inappropriate food by the dolphins.

As supported by Romagnoli (2010), the establishments that promote in-water interactions with botos need to fulfill some infrastructure criteria like organization and cleaning, safety for tourists and promote short lectures to arise environmental awareness. We highlight that all cited measures need to overlap with education of everyone involved in tourism activity with botos, such as boat drivers, floating managers and touristic guides. These suggested steps intend to decrease risky interactions with dolphins and ensure the achievement of a responsible balance between dolphins' welfare and human interests.

ACKNOWLEDGEMENTS

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FIGURES

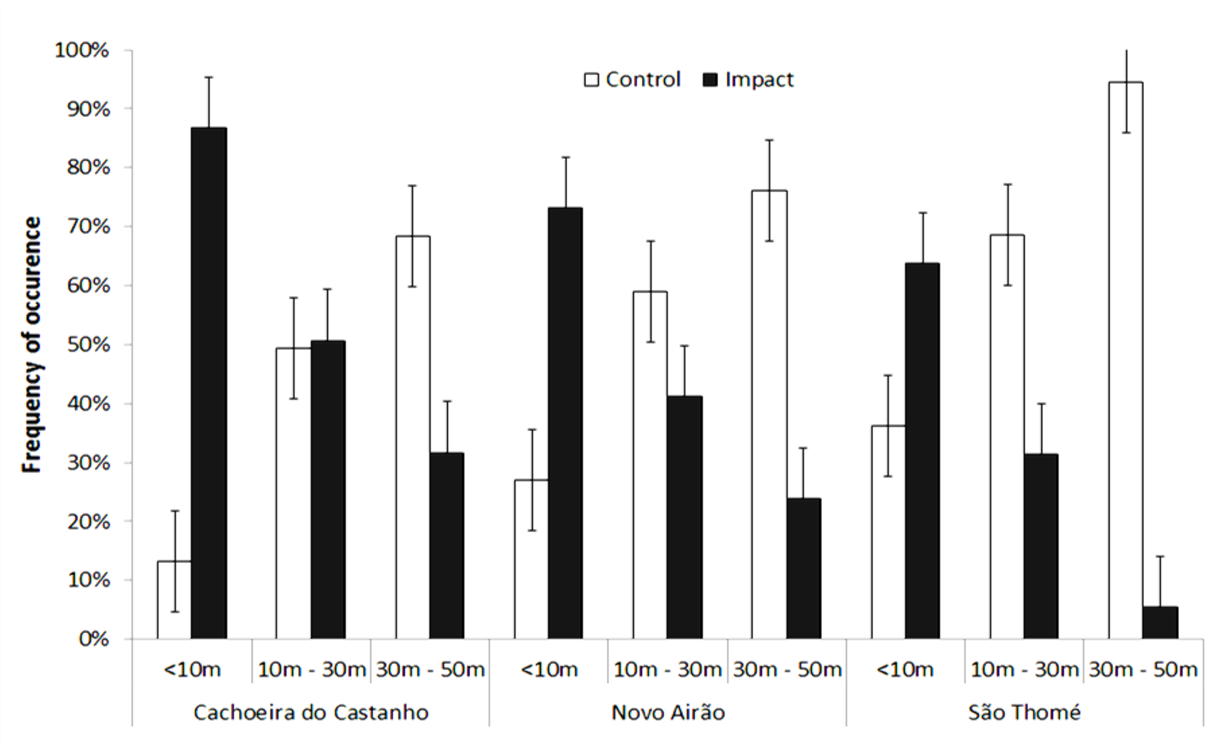

Figure 1 Frequency of occurrence of Amazon river dolphins in three different distance classes around three different feeding sites, lower Negro river, Brazil. Observations were made with and without interaction with humans (impact and control, respectively).

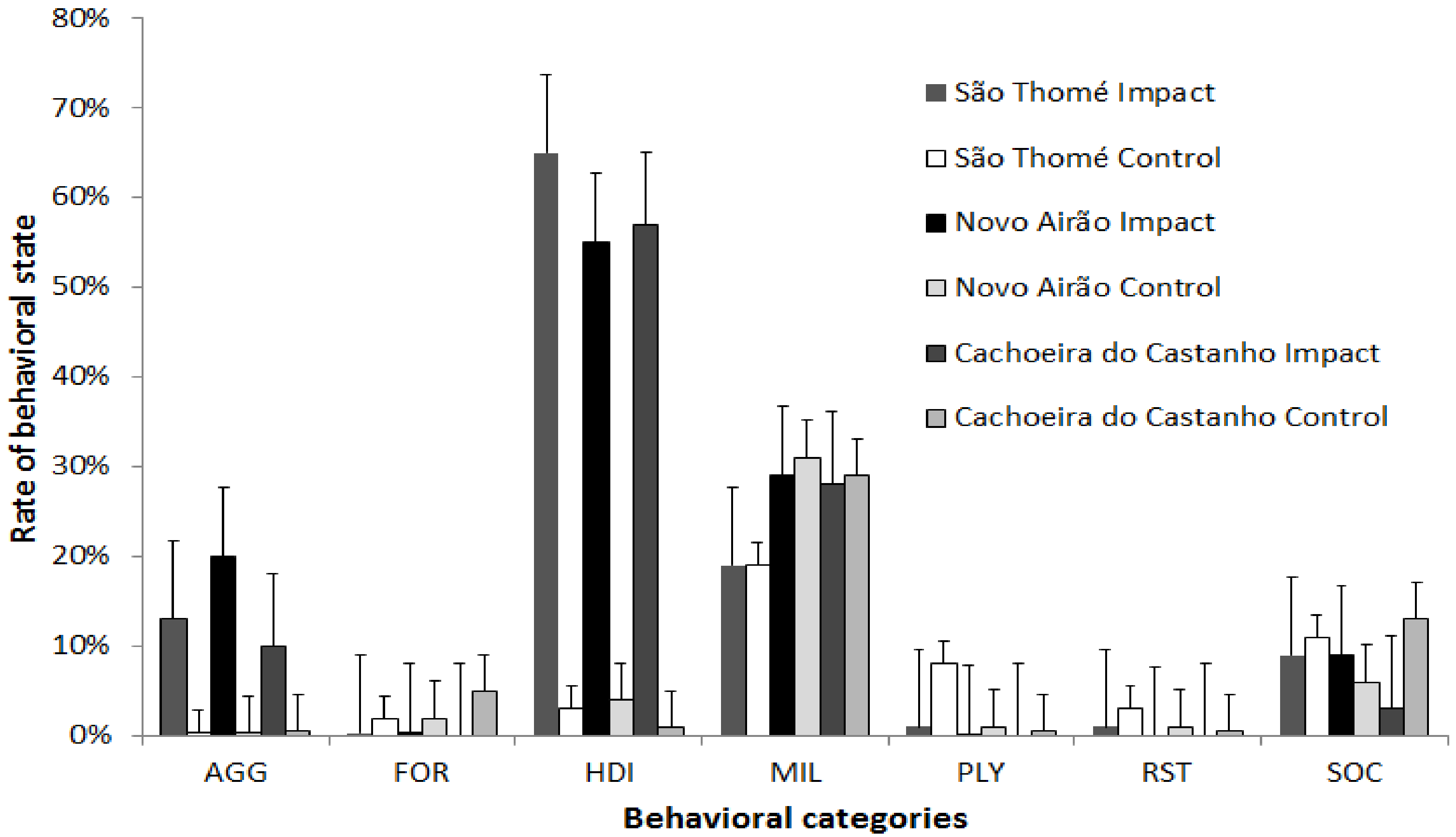

Figure 2 Effect of touristic interaction on the rates of behavioral state of Amazon river dolphin in three different locations (see legend). Graph shows the rate (frequency/total amount of time in control or impact) of each behavioral state (AGG: aggression; FOR: foraging; HDI: human-dolphin interaction; MIL: milling; PLY: play; RST: rest; SOC: socialization) in the presence and absence of tourists. Error bars represents 95% confidence intervals.

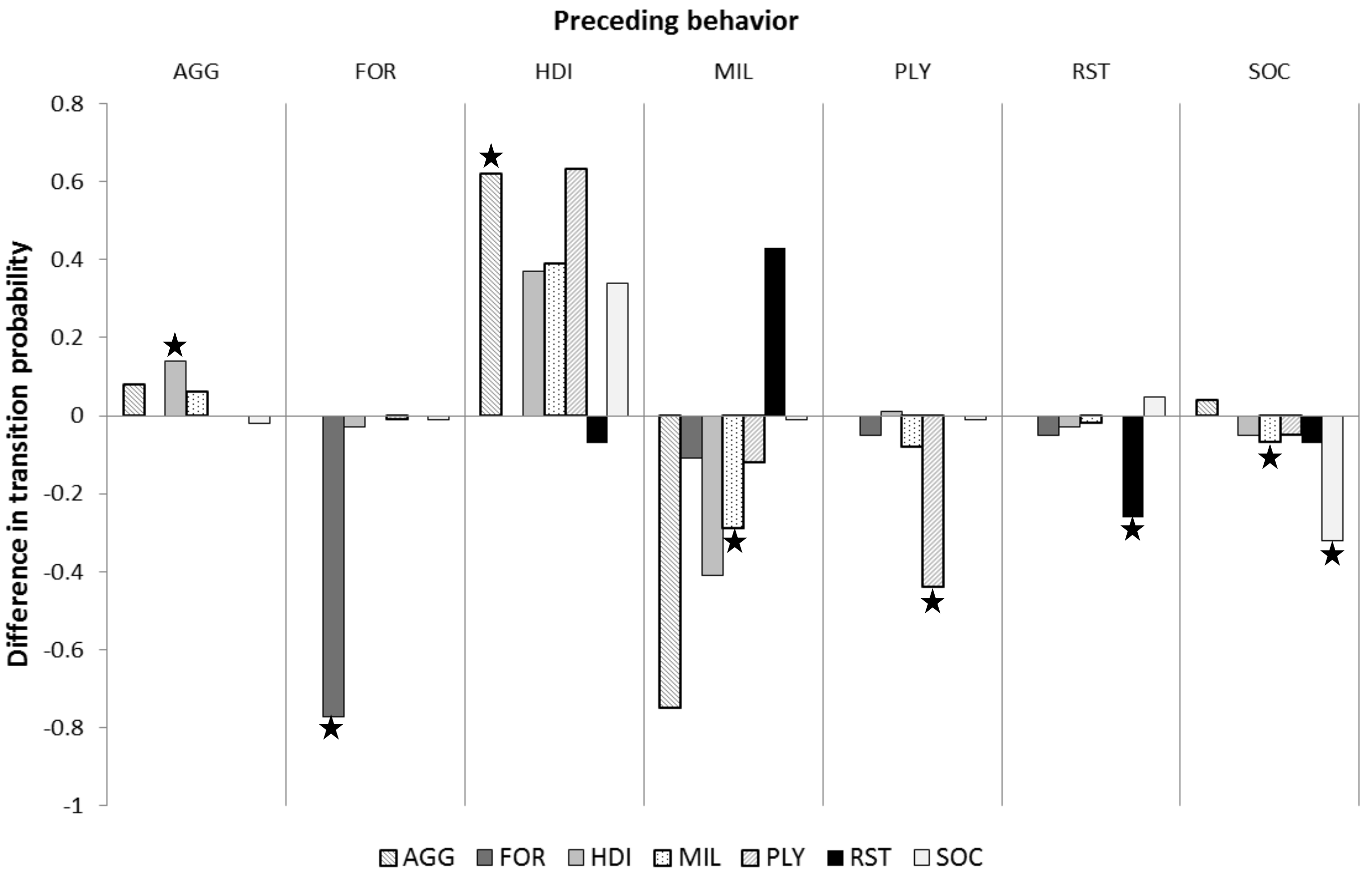

Figure 3 Effect of tourism in Cachoeira do Castanho. Graph shows the differences in behavioral transitions probabilities (Pij(impact) - Pij(control)) of Amazon river dolphins. Negative values means that he behavioral transitions of the control chain is superior to the one of the impact chain. The graph is composed of seven parts, one for each preceding state, separated by vertical lines. In each part, bars correspond to succeeding behavioral state (see legend). The seven transitions with a significant difference ($p<0.01$) are marked with a star.

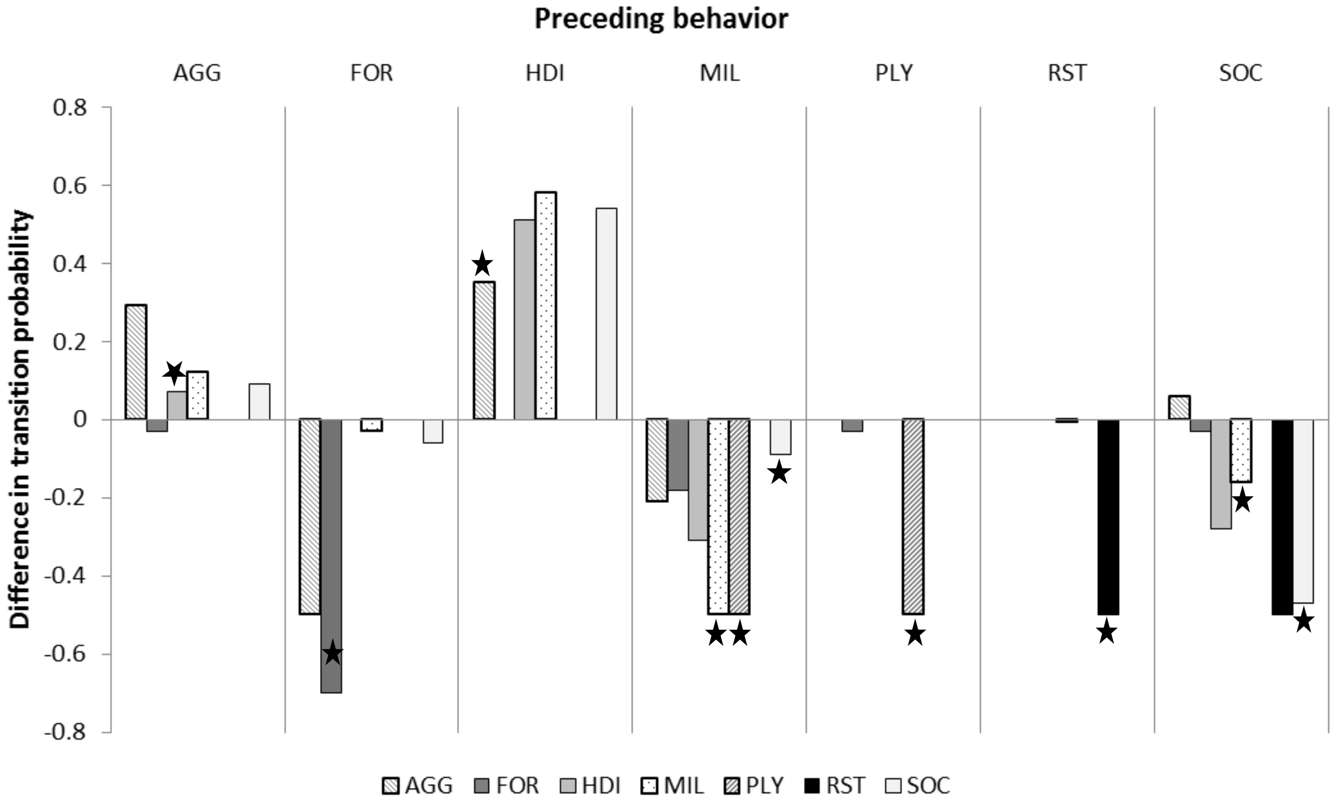

Figure 4 Effect of tourism in São Thomé. Graph shows the differences in behavioral transitions probabilities (Pij(impact) - Pij(control)) of Amazon river dolphins. Negative values means that he behavioral transitions of the control chain is superior to the one of the impact chain. The graph is composed of seven parts, one for each preceding state, separated by vertical lines. In each part, bars correspond to succeeding behavioral state (see legend). The seven transitions with a significant difference (p<0.01) are marked with a star.

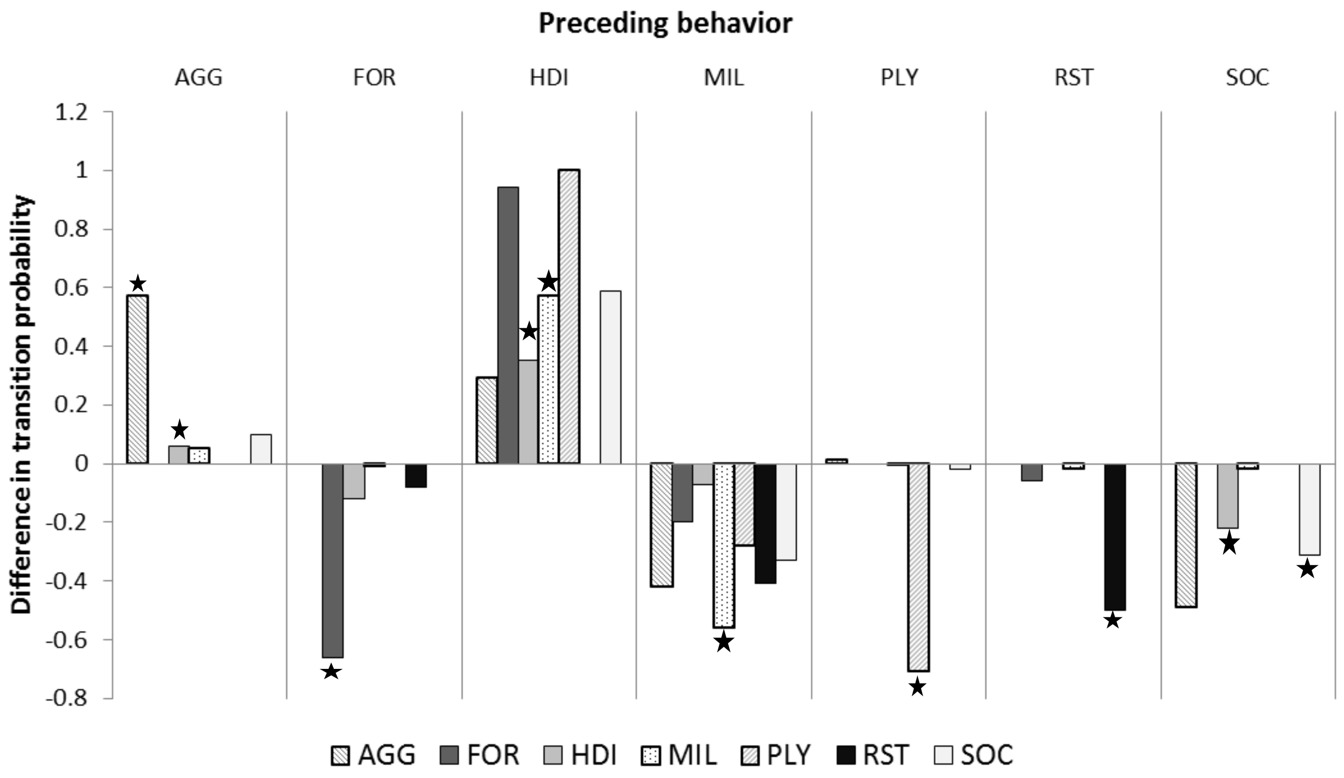

Figure 5 Effect of tourism in Novo Airão. Graph shows the differences in behavioral transitions probabilities (Pij(impact) - Pij(control)) of Amazon river dolphins. Negative values means that he behavioral transitions of the control chain is superior to the one of the impact chain. The graph is composed of seven parts, one for each preceding state, separated by vertical lines. In each part, bars correspond to succeeding behavioral state (see legend). The seven transitions with a significant difference ($p<0.01$) are marked with a star.

APPENDIX

Ethogram of *Inia geoffrensis.*

Social Interaction: Any interaction where two or more individuals are engaged in activities that include close proximity, body contact and sexual displays. This category does not include agonistic/aggressive and play behaviors		
ABR	Abreast	Two or more individuals swim parallel and synchronized in the same orientation, maintaining body proximity
AHD	Ahead	Three or more individuals swim in the same orientation and synchronized. One stands ahead, while others remain behind.
BDC	Body contact	Two or more individuals swim in parallel, diagonal or above one another while maintaining body contact.
ECC	Encircling	Individual swims around a person in circles once or several times in a small radius
NXT	Next to	Two or more individuals swims in close proximity of each other in any direction except parallel or perpendicular
PAR	Parallel	Individuals swims in close proximity and in the same orientation without body contact
PET	Petting	One dolphin moves pectoral fin along another's body part
OCA	Object carrying	Individual carry objects in mouth in a ritualized manner, languidly spinning on their own axis with the head above water, mouth agape
SYB	Synch breath	Two or more individual synchronously surface and breath while keeping close proximity
Agonistic/aggressive: Interactions that involves fighting, threat and submission displays		
BTE	Bite	Individual deliberately bites a person or another individual
CHA	Chase	Two individuals actively follow another
ATK	Chase and bite	Individual actively follows and bites another
FGT	Fight	Stalking, biting and jostling in the string between two or more dolphins

FLS	Fluke (tail) slap	Individual movements its flukes in order to hit another dolphin or a person
GRA	Grab	Individual holds with mouth another individual or a person in order to force a movement
JCP	Jaw clap	Dolphin quickly open and close the jaw producing a loud snap
OPM	Open mouth	Individual remains stationary with mouth open in front of another dolphin or person
POK	Poke	Individual uses its rostrum to forcibly touch another dolphin or person
PUS	Pushing	Individual uses its body to forcibly push other dolphin or person
RAM	Ram	Individual uses its body to hit another (in any part of the body) while swimming fast
FLE	Flee	Individual approaches the feeding platform and flees.
Play: Swift movements, fast swimming, chases among individuals and pushing or pulling objects with no apparent intent other than the behavior itself. Individual behaviors were repeated, intermixed with other behaviors, or increased in complexity		
BUP	Belly up	The ventral part of the individual facing up. Activity observed during social interactions as play and rest.
LOB	Lobtail	Individual hits its caudal fin on the water surface producing a loud sound
PRA	Poke rostrum in air	Individual stays a few seconds shaking its head above the surface. Behavior observed during play
SFR	Surface rolls	Individual rotates the body (360 degrees/stationary)
TOU	Tail out	Individual clearly raise and shake its flukes above the water and sinks slowly
ULT	Up-side down lobtail	Individual on the surface, horizontally, with the ventral part facing up. Animal uses its flukes to hit the water
PLY	Play	Fast swimming and interaction with other botos. The animals were actively swimming and manipulating objects such as leafs and branches.
Rest: Slow swimming with no determined direction, floating in the surface or remain still underwater		
RST	Rest	Horizontally, individual remain still on the surface or right below. Slow movements with no defined direction

SUS	Suspended swim	Individual remains in vertical position, suspended in water
Human-dolphin interaction: Behaviors observed during interaction of botos and humans.		
AVT	Avoid touch	Animal avoids physical contact attempts initiated by tourists
DAP	Direction adaptation	One or more individuals change its swim trajectory and move toward the feeding area
FAA	Feeding area approach	Individual or groups approach the feeding area and remains for few minutes. This behavior occurs even without tourists
HFE	Hand feeding	Individual receives fish from the hand of the feeder
BEG	Beg	Individual remains with its head out of water and with mouth open
HSW	Horizontal swim	Individual remains stationary on feeding area (below the surface), moving only its pectoral fins.
HUP	Head up	Individual raises the head above water, mouth closed, in the direction of potential feeder
OTC	Object touching	Individual tolerate the touch by objects handled by a person
PCT	Physical contact	Individual initiates physical contact with a person in non-aggressive manner, touching the person with its rostrum
PUL	Pull	Individual use its teeth for handling person’s objects (vest, bathing suit...)
RNF	Remain near feeder	Individual or group stay next to the feeder
RNS	Remain near swimmer	Individual or group stay next to the tourist
SCK	Snacking	Individual hold fish on its mouth without eating it
SWF	Swim under the floating	Individual regularly approach the feeding area and remains under the floating dock. This behavior occurs even without tourists
TTC	Tolerate touching	Individual accepts the touch of a person
Foraging: High surface activity, rapid changes in direction and long dives. Prey can often be observed at the surface.		

DIV	Long dive	Individual remains > 1 min diving until resurfacing
FOR	Foraging	Individuals are actively moving on the surface. LOB and FBO frequent. Fish might be seen jumping in the same location
LOB	Lobtail	Individual hit its caudal fin on the water surface producing a loud sound
SHA	Sharking	Individual swims on the surface showing only its dorsal fin
Milling: Boto moved through the water without engaging in other activities		
ASF	Active surfacing	Individual actively swims and dives, when on surface, its dorse and head remains visible
SBR	Surface breath	Individual returns to the surface and breathe without audible noise. Only melon and dorsal fin are visible
SDO	Sink down	Individual sinks while positioned vertically
TOD	Tail out dive	On the surface, individual curves its dorse and animal increases angle of re-entry. The tail is lifted out of the water and the individual dive vertically
RGD	Regular dive	Individual swims slowly returning to the surface to breathe and dips without leaving the caudal fin visible
SSW	Slow swim	Individual slowly swims on the surface, returning regularly to breathe and dive in head first, leaving the flow at shows under the surface

SÍNTESE

Atraídos pela oferta de alimento fácil, agregações de botos-vermelho (*Inia geoffrensis*) estão sendo condicionadas a receber alimento oferecido por turistas, que aproveitam este momento para observar, nadar próximos e tocar os animais. Com o crescimento do número de locais de atração destes animais no estado do Amazonas, medidas de manejo e monitoramento da atividade precisam ser tomadas para possibilitar a manutenção e a qualidade da atividade, evitando danos aos botos e acidentes com os turistas. Com isso, o objetivo deste trabalho foi analisar o impacto antrópico do turismo no comportamento do boto-vermelho. Para isso, buscou-se verificar as respostas comportamentais de três agregações de botos-vermelho formadas em locais de interação e relacioná-las com à atividade turística, além disso, buscamos relacioanar os diferentes sons emitidos pelos botos com os contextos comportamentais em que foram registrados. Variáveis acústicas dos diferentes tipos sonoros não diferiram entre as áreas de estudo, mas variaram significativamente entre contextos comportamentais. As frequências dominante na fundamental e máxima em comportamento agressivos foram mais baixas que em outros contextos, corroborando com a hipótese de motivação estrutural do som. O repertório vocal da espécie é formado por gradações e transições, sendo difícil a categorização em tipos sonoros discretos. Os animais vocalizaram mais quando estavam agitados, o que coincide com os momentos em que uma interação com humanos estava ocorrendo. Os botos são suscetíveis a estímulos e podem alterar seu comportamento devido às interações com humanos e o uso de alimentação suplementar. Os animais respondem ao alimento oferecido por turistas aumentando sua agressão interespecífica, sendo que a probabilidade dos animais de permanecer em comportamento agressivo após interação com humanos foi maior no local onde a atividade turística acontece há mais de 17 anos. Além disso, os animais permanecem nos arredores dos flutuantes de interação e na maior parte do tempo, estão a menos de 10 metros. Isto deve ocorrer devido ao fato de estarem sensibilizados ao estímulo alimentar e terem sido condicionados ao contato humano devido ao uso de alimentação suplementar como forma de atração. Sugerimos medidas de manejo para garantir a qualidade da atividade turística e o bem-estar dos animais.

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ANEXOS

ANEXO A - ATA DE QUALIFICAÇÃO

PG-ECO-INPA
PÓS-GRADUAÇÃO EM ECOLOGIA

INPA
INSTITUTO NACIONAL DE PESQUISAS DA AMAZÔNIA

GOVERNO FEDERAL
BRASIL
PAÍS RICO É PAÍS SEM POBREZA

AULA DE QUALIFICAÇÃO

PARECER

Aluno(a): ANGÉLICA CRISTINA GOUVEIA NUNES
Curso: ECOLOGIA
Nível: MESTRADO
Orientador(a): MARINA ANCIÃES
Co-orientador(es) Vera da Silva e Renata Sousa-Lima

Título:

"Turismo com botos-vermelhos (*Inia geoffrensis*) no Amazonas: alterações comportamentais e caracterização acústica de agrupamentos no Rio Negro""

BANCA JULGADORA

TITULARES:
Jansen Zuanon (INPA)
Rodrigo de Souza Amaral (INPA)
Waleska Gravena (UFAM)

SUPLENTES:
Alan Fecchio (UFAM)
Jean Paul Boubli (Univ. Salford)

	PARECER		ASSINATURA
Jansen Zuanon (INPA)	(X) Aprovado	( ) Reprovado	
Rodrigo de Souza Amaral (INPA)	(X) Aprovado	( ) Reprovado	
Waleska Gravena (UFAM)	(X) Aprovado	( ) Reprovado	
Alan Fecchio (UFAM)	( ) Aprovado	( ) Reprovado	
Jean Paul Boubli (Univ. Salford)	( ) Aprovado	( ) Reprovado	

Manaus(AM), 19 de fevereiro de 2014

OBS:

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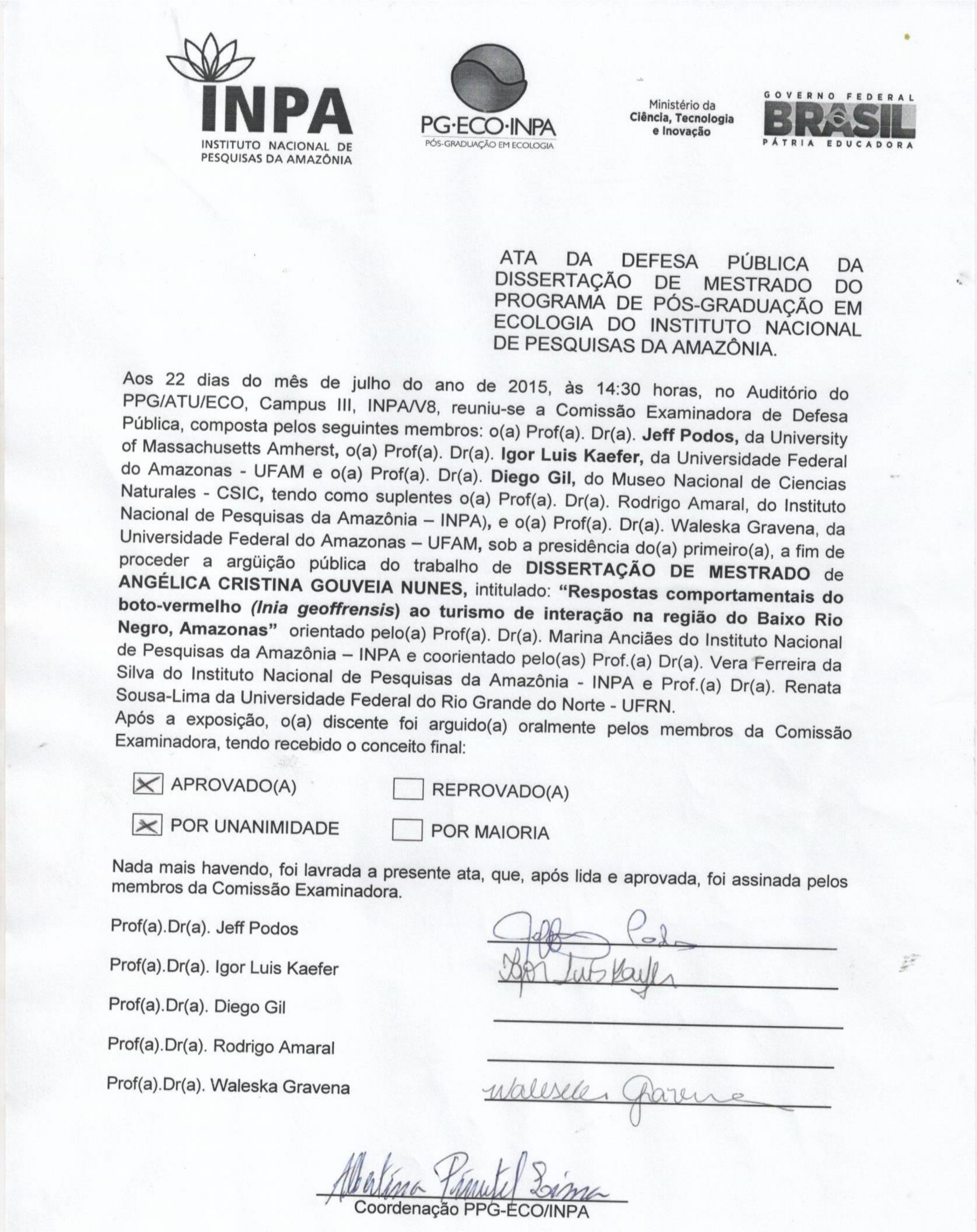
ANEXO B – ATA DA DEFESA



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PÓS-GRADUAÇÃO EM ECOLOGIA

Ministério da Ciência, Tecnologia e Inovação

GOVERNO FEDERAL
BRASIL
PÁTRIA EDUCADORA

ATA DA DEFESA PÚBLICA DA DISSERTAÇÃO DE MESTRADO DO PROGRAMA DE PÓS-GRADUAÇÃO EM ECOLOGIA DO INSTITUTO NACIONAL DE PESQUISAS DA AMAZÔNIA.

Aos 22 dias do mês de julho do ano de 2015, às 14:30 horas, no Auditório do PPG/ATU/ECO, Campus III, INPA/V8, reuniu-se a Comissão Examinadora de Defesa Pública, composta pelos seguintes membros: o(a) Prof(a). Dr(a). **Jeff Podos,** da University of Massachusetts Amherst, o(a) Prof(a). Dr(a). **Igor Luis Kaefer,** da Universidade Federal do Amazonas - UFAM e o(a) Prof(a). Dr(a). **Diego Gil,** do Museo Nacional de Ciencias Naturales - CSIC, tendo como suplentes o(a) Prof(a). Dr(a). Rodrigo Amaral, do Instituto Nacional de Pesquisas da Amazônia – INPA), e o(a) Prof(a). Dr(a). Waleska Gravena, da Universidade Federal do Amazonas – UFAM, sob a presidência do(a) primeiro(a), a fim de proceder a argüição pública do trabalho de **DISSERTAÇÃO DE MESTRADO** de **ANGÉLICA CRISTINA GOUVEIA NUNES,** intitulado: **"Respostas comportamentais do boto-vermelho *(Inia geoffrensis)* ao turismo de interação na região do Baixo Rio Negro, Amazonas"** orientado pelo(a) Prof(a). Dr(a). Marina Anciães do Instituto Nacional de Pesquisas da Amazônia – INPA e coorientado pelo(as) Prof.(a) Dr(a). Vera Ferreira da Silva do Instituto Nacional de Pesquisas da Amazônia - INPA e Prof.(a) Dr(a). Renata Sousa-Lima da Universidade Federal do Rio Grande do Norte - UFRN.

Após a exposição, o(a) discente foi arguido(a) oralmente pelos membros da Comissão Examinadora, tendo recebido o conceito final:

☒ APROVADO(A) ☐ REPROVADO(A)

☒ POR UNANIMIDADE ☐ POR MAIORIA

Nada mais havendo, foi lavrada a presente ata, que, após lida e aprovada, foi assinada pelos membros da Comissão Examinadora.

Prof(a).Dr(a). Jeff Podos ____________________
Prof(a).Dr(a). Igor Luis Kaefer ____________________
Prof(a).Dr(a). Diego Gil ____________________
Prof(a).Dr(a). Rodrigo Amaral ____________________
Prof(a).Dr(a). Waleska Gravena Waleska Gravena

Albertina Pimentel Lima
Coordenação PPG-ECO/INPA