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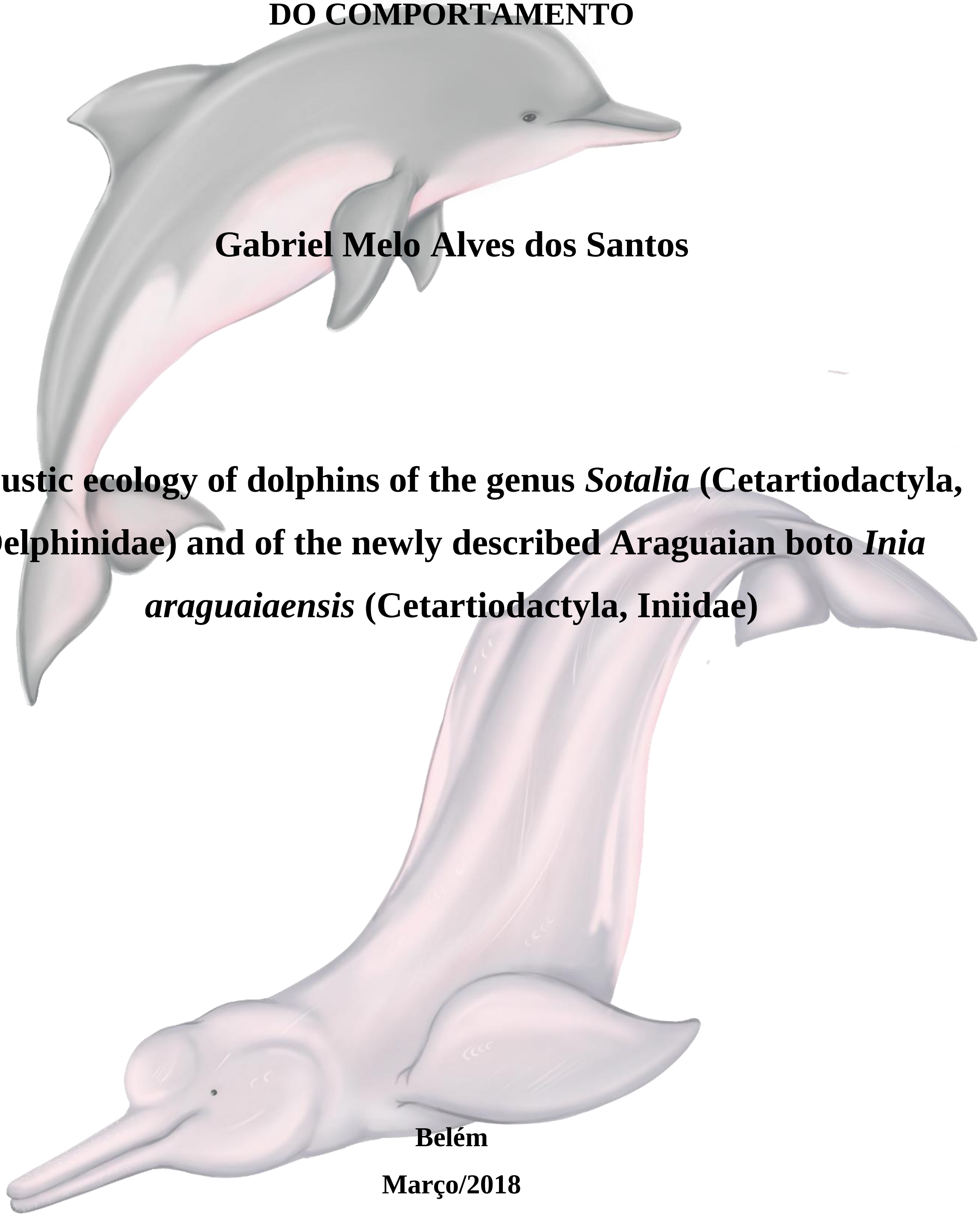

Gabriel Melo Alves dos Santos

Acoustic ecology of dolphins of the genus *Sotalia* (Cetartiodactyla, Delphinidae) and of the newly described Araguaian boto *Inia araguaiaensis* (Cetartiodactyla, Iniidae)

Belém
Março/2018

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Tese de doutorado apresentada ao Programa de Pós-graduação em Teoria e Pesquisa do Comportamento da Universidade Federal do Pará como requisito para a obtenção do grau de doutor.

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Belém
Março/2018

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Programa de Pós-Graduação em
Teoria e Pesquisa do Comportamento UFPA

Tese de Doutorado

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Aluna: Gabriel Melo Alves dos Santos.

Data da Defesa: 09 de Março de 2018.

Resultado: Aprovada.

Banca examinadora:

Maria Luiza da Silva
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Prof.ª Dr.ª Laura J. May-Collado (Co-orientadora – University of Vermont, via Skype).

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Prof.ª Dr.ª Regina Célia Souza Brito (membro 3 – UFPA).

Prof.º Dr.º Carlos David da Silva Oliveira dos Santos (membro 4 – UFPA).

Dados Internacionais de Catalogação-na-Publicação (CIP)
UFPA/Núcleo de Teoria e Pesquisa do Comportamento/Biblioteca

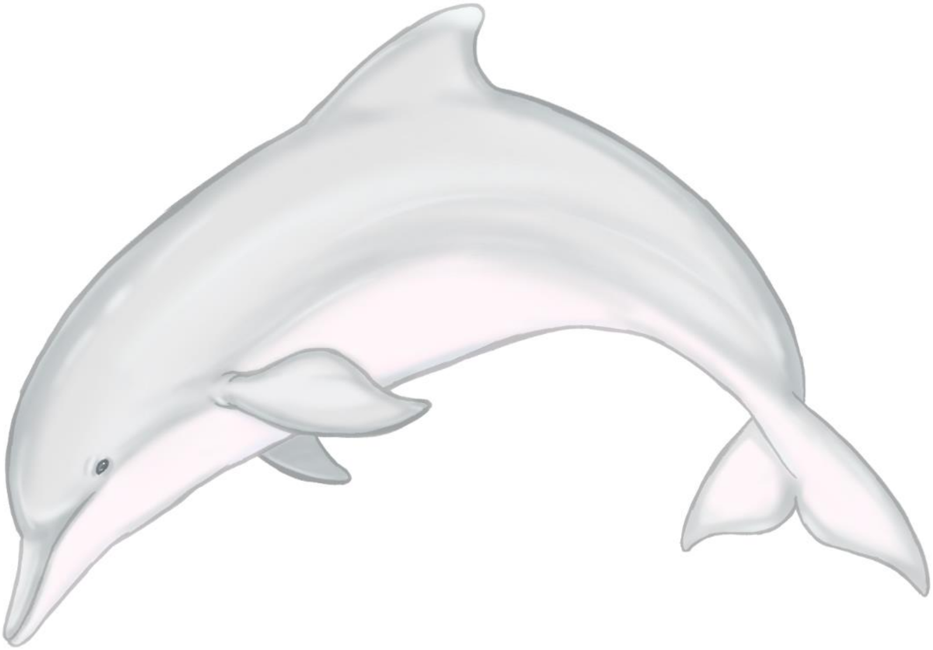
S237e Santos, Gabriel Melo Alves dos, 1990-

Acoustic ecology of dolphins of the genus *Sotalia* (Cetartiodactyla, Delphinidae) and of the newly described Araguaian boto *Inia araguaiaensis* (Cetartiodactyla, Iniidae). / Gabriel Melo Alves dos Santos. — 2018.

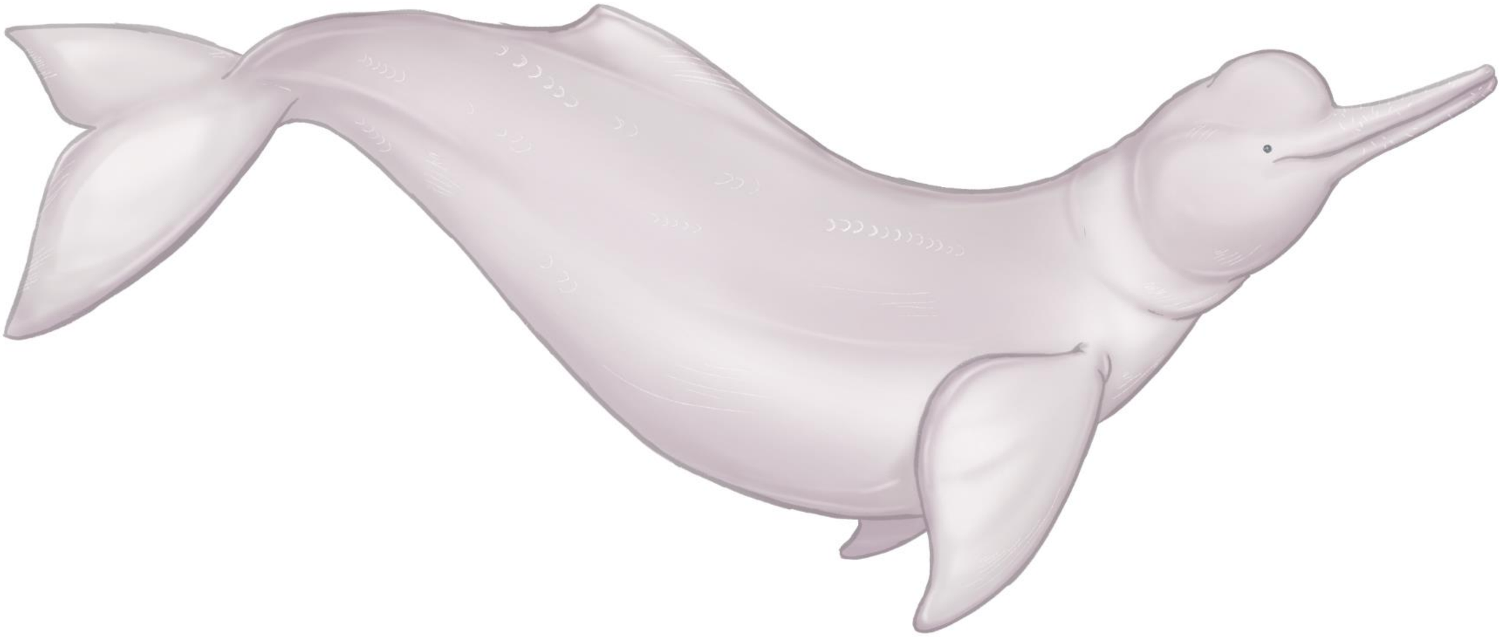
Orientador: Maria Luisa da Silva
Co-oriendor: Laura J. May-Collado
Tese (Doutorado) - Universidade Federal do Pará, Núcleo de Teoria e Pesquisa do Comportamento, Programa de Pós-Graduação em Teoria e Pesquisa do Comportamento, Belém, 2018.

1. Análise do comportamento. 2. Comportamento animal. 3. Golfinhos. 4. Ecologia Acústica - Golfinhos. I. Título.

CDD - 23. ed. 599.53



I dedicate this document to my granpa, Agenor, who left us shortly after I defended my thesis.



"I'm broke but I'm happy
I'm poor but I'm kind
I'm short but I'm healthy, yeah
I'm high but I'm grounded
I'm sane but I'm overwhelmed
I'm lost but I'm hopeful baby
What it all comes down to
Is that everything's gonna be fine fine fine
'cause I've got one hand in my pocket
And the other one is giving a high five
I feel drunk but I'm sober
I'm young and I'm underpaid
I'm tired but I'm working, yeah
I care but I'm restless
I'm here but I'm really gone
I'm wrong and I'm sorry baby
What it all comes down to
Is that everything's gonna be quite alright
'cause I've got one hand in my pocket
And the other one is flicking a cigarette
And what it all comes down to
Is that I haven't got it all figured out just yet
'cause I've got one hand in my pocket
And the other one is giving the peace sign
I'm free but I'm focused
I'm green but I'm wise
I'm hard but I'm friendly baby
I'm sad but I'm laughing
I'm brave but I'm chickenshit
I'm sick but I'm pretty baby
And what it all boils down to
Is that no one's really got it figured out just yet
'cause I've got one hand in my pocket
And the other one is playing the piano
And what it all comes down to my friends
Is that everything's just fine fine fine
'cause I've got one hand in my pocket
And the other one is hailing a taxi cab"

Alanis Morissette – Hand In My Pocket

Acknowledgments

This study was only possible due to the help and support of MANY people, and I am incredibly grateful.

First to my family, especially my parents Ney and Liliane, for always supporting me unconditionally on pursuing my objectives. Without your help I would not be able to come this far.

To Malu, my advisor, for granting me the opportunity to do this PhD, even though I would not study birds. For supporting my ideas and encouraging me to go abroad to learn more, for giving us the space and initial support when we wanted to start something new, which today is BioMA.

To my research group, BioMA, for the constant support and partnership, for the help and incredible moments in fieldwork, when writing proposals, the exchange of knowledge and last but not the least for all the moments of fun, you rock.

To Laura May-Collado, my advisor and friend, who opened so many doors for me, taught me so much and always encouraged me to pursue more! Without your constant support and eagerness this Phd would have been so different.

To Miriam Marmontel, who gave me so many opportunities, and supported me for so long, taught me so much about Amazonian aquatic mammals, and enabled me to record dolphins in the most beautiful and remote places of the Amazon, and for constantly supporting BioMA since the very beginning.

To Mark Johnson and Natacha Aguilar, for all the insights to this work, for teaching me so much, for all the opportunities you gave me, for helping me in fieldwork, for lending me a Soundtrap, believing in me and receiving me in St Andrews and in the Canaries.

To Volker Deecke, Peter Tyack and Vincent Janik for all the insights and help for this work.

To Braulio Leon-Lopez for the help with the analysis.

To all the aquatic mammals team of the Mamirauá Institute, especially my adorable friend Heloise Pavanato, for the help with the fieldwork, analyses and valuable insights and discussions. Nathali Ristau, Camila Carvalho and Mariana Paschoalini for the help in the fieldwork, insight and discussions. And of course, to all the moments of fun that allowed us to survive for long periods on board of regional boats all over the Amazon.

To my favorite aquatic mammal geneticists Sávia Moreira and Joice Farias, for all the help in the field, the conversations, that helped so much during this work.

To my friends Amanda Monte and Luis Fernando Nascimento who taught me the basics of bioacoustics when I started to record dolphins in the field and went with me to the first recording fieldwork.

To my friend-brothers Adriano Quaresma and Rafael Barata and their families, for being by my side every moment good or bad (even in the distance) for the last ten years, for the constant support, help with the analysis and on my fieldwork.

To my incredible friends Angélica Nagata and Ingrid Reale, for the constant support even living in different states, the conversations that kept me going in the darkest moments.

To my friends Rodrigo Tardin and Israel Maciel, for the support, help in the fieldwork, insights and discussions about this study, for helping me in the analysis and confectioning maps, for receiving me in Rio when we needed to do fieldwork, for all the moments of fun, you are awesome.

To my friend Julia Dombroski, even living far far way from each other has always supported this work and provided valuable insights.

To Mariana Adauto and Juliana Maciel, for receiving me in Rio, for your friendship and support.

To my friends Nathália Nascimento and Alessandra Formento, who accompanied me in field work in the Tapajós and Tocantins Rivers, respectively.

To my awesome friends from Vermont, most likely the greatest gift out of this PhD, especially Zamira Yusseff, Ricardo Burgo-Muñiz, Laura Caicedo, Kattia Palacio, Paliza and Nelish Pradhan, Vanesa Perillo, Yainna Hall, Patrick Wiencek, Silvia Justi, Muhamad Kala, Roberto Gonzalez, Raquel Lima and Jossue Gutierrez, for being my family, for making VT a cozy and warm home even with the deadly winter, for taking care of me when I got sick or hurt, for our AMAZING road trips through the East Coast of the US, for all the insights, discussions, teaching, for all the incredible fun. For supporting and caring even now that we are miles apart. I could not have done it without you!

To my friends in St Andrews, especially, Heather Vance, Illias Foskolos, Popi Gkikopoulou and René Swift, for the warm reception and making my stay in St Andrews amazing, for the discussions, the help with freaking MATLAB, the discussions and insights into this work, and of course the fun and touring, you are great!

To Professor Regina Brito for teaching me so much about ethology.

To Dona Maria das Graças Justi, for the friendship, kindness and support.

To all the people who received me in their homes and supported me during fieldwork, Seu Jorge in the Capim Island (Abaetetuba), Dona Graça and Seu Tucão in the Sapocajuba Island

(Abaetetuba), Dona Dorita (Abaetetuba), Dona Raquel Valente (Mocajuba), Doca and Lídio (Mocajuba), to my cousin Lídia her family who hosted me in Santarém.

To all the captains who conducted me through the rivers of the Amazon and the coast of Pará, Genilson, Anderson and Seu Orlandino (Guamá River), Seu Eliseu, Gaia, Seu Flor, Jairo and Jaime (Tocantins River), Rodrigo, Momo and Odacir (Japurá and Solimões Rivers), Zé Ariranha (Juruá River), Seu Adelson (Augusto Corrêa), Seu Nazário (Quatipuru).

To Laura May-Collado, Alexandre Azevedo, Amandine Bordin, Camila Carvalho de Carvalho, Camila Domit, Dalila Teles Leão Martins, Diogo Destro Barcellos, Hector Barrios-Garrido, Israel Maciel, Kareen De Turris, Luciana Duarte Figueiredo, Luz Helena Rodriguez Vargas, Marcos César de Oliveira Santos, Marie Trone, Marta Jussara Cremer, Miriam Marmontel, Monique Pool, Rodrigo Tardin, and Sheila Marino Simão who provided me their raw recordings so this work could be possible!

To CAPES (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior) for the PhD scholarship.

To the Rufford Foundation for supporting my fieldwork and my visit to the University of St Andrews.

To Mr. William Rossiter and the Cetacean Society International team, who supported my fieldwork, trips to conferences and my second Visit to the University of Vermont, and for supporting me since the very beginning until now.

To Universidade Federal do Pará, Instituto Mamirauá de Desenvolvimento Sustentável, the University of Vermont and the University of St Andrews, who were fundamental in my formation as a scientist and to the execution of this work.

Least nut not the last, to the river dolphins, my passion, the reason of this study, the reason of so many great things that happened in my life for the last ten years, since the beginning of undergrad.

Preface

My fascination for aquatic mammals begun very early, when I was only three years old, and was greatly stimulated by my parents, who used to record all Jacques Cousteau on K7 tapes and buy loads of books. Since then, I knew I wanted to become a biologist. In 2007 I entered college and started searching for someone that could offer me the opportunity to study aquatic mammals, that was when I began my first internship in the aquatic mammals research group of Museu Paraense Emílio Goeldi. The activities there were mostly beach surveys to collect dolphins carcasses and organizing the aquatic mammals collection, though my desire was to study living animals in the wild. Short after I began my own research project, which became my undergrad dissertation and first published article, my research was focused on the behavior of river dolphins in the Guamá River, surrounding the University campus, as our fieldwork was paid with my own money and some small grants by Cetacean Society International (CSI).

Immediately after graduation I started the Masters in Zoology, harsh times were ahead of me, nine months before my deadline our research group split and with three more colleagues we started a new one BioMA, the one which I am still a part of today. With the support of Dr. Miriam Marmontel from the Mamirauá Institute, my parents, our own money, my Masters advisor, and Malu (now my PhD advisor), we were able to return to the field in the Guamá and Tocantins River so I could finish the Masters. During that time, I started recording river dolphins sounds and in the meantime my Maters advisor granted me the opportunity to record river dolphins in the middle reaches of the Tapajós River.

I finished the masters in the beginning of 2013, six months later I entered the PhD program to study what is, in my opinion, one of the most fascinating aspects of cetaceans, their communication. If the Masters was harsh in the PhD many opportunities of collaboration and new horizons appeared.

Right after entering the program we discovered the place that today is our main study site, Mocajuba, what a fascinating place! There botos come to the fish marked to be fed by locals, not only they were so close, but the water was so clear that we can, now, see them in detail. This opened up so many opportunities for our team, we were able to study aspects of these animals that would be incredibly hard or impossible to study by the traditional approaches. Our bond to the dolphins and the community there became stronger with time and we are still monitoring this group of animals.

In the beginning of 2014, two friends Nathali Ristau and Heloise Pavanato, under the supervision of Miriam, invited me to expeditions to estimate density and abundance of river dolphins in the Tocantins, Solimões and Japurá Rivers. Later that year I would return for more expeditions, to the Tapajós and in 2016, go to the Juruá Rivers, I was able to record dolphins in all those rivers. These were amazing field experiences and we were able to study these dolphins in some of the most remote and beautiful places in the Amazon.

In December 2014 came the turning point of the PhD, I went to Cartagena for the Latin American conference on aquatic mammals, there Thomas Hrbek (whom we have a partnership studying boto's genetics) introduced me to Laura May-Collado, we talked and arranged the details for my "sandwich" period of the PhD. Then in July 2015 I went to Vermont (USA) where me and Laura started working together. We reshaped the initial proposal oh this thesis for something bigger, we send an open letter for researchers of Latin America working with *Sotalia* dolphins, inviting them for a collaborative work, as far as I know, the largest in Latin America regarding dolphin acoustics. The objective of the work was to understand geographical variation on whistles of *Sotalia* dolphins and how their repertoire evolved. With the collaboration of about 30 researchers from various countries of Latin America, Europe and USA we compiled data from 16 sites along the distribution of *Sotalia*, culminating in one of the chapters of the thesis. Our second step was towards botos, more specifically the newest species *Inia araguaiaensis*, as we were studying a group of known individuals of the species in Mocajuba. Still, we did not know what these dolphins do when they left the fish market, also so little was known on *Inia* communication and social behavior, even more was in the dark for the new species. Therefore, we contacted Peter Tyack (as we knew worked with acoustic tags) whom introduced us to another great researcher Mark Johnson, both from the University of St Andrews. We had a skype meeting with Mark, when we explained to him the setting in Mocajuba and what we wanted to do, in this meeting we defined our methods, our objectives, and Mark stated that after fieldwork I would go to St Andrews for training and learning more about tags. After the meeting with Mark we wrote a basis proposal, and along with Miriam we submitted it for 10 or more funding sources.

Short after my return to Brazil in July 2016, we received an e-mail from the Rufford Foundation informing that our project would be funded. In September, we were all in the field, along with Mark came his partner Natacha Aguilar who also has vas experience with tags and acoustics. Despite of being so close to us the botos in the market did not left the tags attached for a long time, our longest deployment time was six minutes, on a male which we called Double-tooth (due to his dentition). As the tags were not as successful as we wanted to, we went for the plan b: deploying three soundtraps (autonomous sound recording systems) along the study area, one in the market, and the other two in the main river channel. Along with acoustic data we collected a variety of information including individuals photographs for photo identification, tissue and blow samples for molecular and microbiota analysis, also we promoted an environmental education activity by the end of the campaign. The project caught attention of the media and we recorded interviews in the field and in Belém with all major Brazilian TV networks, and later in 2017 we recorded a documentary for the BBC series “Wild Metropolis”, which will feature the interactions between botos and humans in Mocajuba as well as our research. We also presented part of the results of the project to the Latin American conference on aquatic mammals in Chile in 2016.

In the beginning of 2017 I went to St Andrews to visit Mark’s lab, where we defined the analytical methods for the echolocation data. I also sat on the courses of bioacoustics and biologging. In addition, I received feedback of great bioacousticians on my work, Peter Tyack, Vicent Janik and Volker Deecke provided valuable insights to the last chapter of the thesis, concerning boto’s communication signals. Moreover, Volker whom created the ARTwarp method also helped me with the analyses of the *Sotalia* chapter, improving our results.

After my visit to St Andrews, Laura and Volker encouraged me to submit the *Inia* and *Sotalia* chapters to the “4th International Symposium on Acoustic Communication by Animals” and the “22nd Biennial Conference on the Biology of Marine Mammals”, respectively. We did oral presentations for both conferences, again with valuable feedback. In October 2017 I also went to the Canary Islands to the Universidad de La Laguna, to give a talk on our research with river dolphins and to accompany Natacha’s team on fieldwork.

This PhD means so much to me, not only I was able to visit many places around the world, I met great people and made great friends from many places and cultures. I had the support of great researchers and I evolved and grew as a scientist. I am thankful for my advisors, my family and all those who supported me. I hope that this document reflects my passion for my work and the river dolphins, and that it will contribute somehow for their conservation and for Brazilian Scienc

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RESUMO

Sistemas sensoriais são vitais para que os animais obtenham informações sobre seus arredores. A informação pode ser adquirida por vias visuais, químicas, elétricas, táteis e acústicas. Estes sinais são usados em diferentes contextos ecológicos incluindo forrageamento, competição, defesa, interações sociais (e.g. corte), e também podem indicar uma condição, e estado emocional ou reprodutivo, ou a identidade do emissor do sinal. Para os mamíferos aquáticos o som é o meio de comunicação mais importante. A luz atenua rapidamente com a profundidade em ambientes aquáticos, limitando a comunicação visual. Todavia, a atenuação do som na água é baixa e ele viaja cerca de cinco vezes mais rápido na água do que no ar, o que o torna um meio de comunicação extremamente eficiente em baixo da água. Logo, o som é um aspecto fundamental da biologia dos cetáceos, já que estes animais dependem do som para comunicação, navegação e localização de presas. O gênero *Sotalia* compreende duas espécies que vivem em hábitats contratantes. O boto-cinza (*Sotalia guianensis*) que habita águas costeiras da Nicarágua ao sul do Brasil, e o tucuxi (*Sotalia fluviatilis*) restrito aos principais tributários da Bacia Amazônica. Enquanto que golfinhos do gênero *Inia* – popularmente conhecidos como botos-vermelhos – são encontrados exclusivamente nas Bacias dos rios Amazonas, Orinoco e Tocantins. Com ambos os seus estados de conservação como dados deficientes há grande demanda de informações quanto a sua biologia. Como fator-chave da biologia dos cetáceos a acústica pode nos prover uma riqueza de informações e ser usada como ferramenta para adquirir dados quanto ao uso de hábitat, números populacionais e comportamento. Entretanto, para fazê-lo, primeiro é necessário conhecer o repertório vocal das espécies em detalhe de forma que possamos diferenciá-las usando métodos acústicos. Portanto apresentamos aqui a primeira análise englobando toda a distribuição de golfinhos do gênero *Sotalia* e padrões geográficos e de diversidade de seus assobios. Além disso, apresentamos a primeira descrição do repertório vocal e de *Inia araguaiensis* focando na diversidade de repertório e em sua estrutura. O repertório de assobios de ambas as espécies de *Sotalia* é altamente estruturado, com populações da espécie fluvial apresentando um repertório menos diverso do que as populações da espécie costeira. Esta estruturação se dá provavelmente devido a áreas de vida pequenas e ao baixo fluxo gênico entre populações. As diferenças na riqueza entre o repertório destas espécies parecem estar relacionadas a uma combinação de fatores socio-ecológicos e evolutivos. Também apresentamos a primeira descrição do comportamento acústico do boto-do-Araguaia (*Inia araguaiaensis*) e mostramos que eles possuem um repertório rico composto por assobios e principalmente chamados pulsados. Assobios foram produzidos raramente, um tipo específico de chamado, chamados curtos com dois componentes, foram o tipo de sinal mais comum durante nosso estudo. Estes chamados são similares em estrutura a aqueles produzidos por orcas (*Orcinus orca*) e baleias-piloto (*Globicephala* sp.). Devido ao contexto no qual estes sinais foram produzidos, nós hipotetizamos que eles podem funcionar para a comunicação ente mãe e filhote. *Sotalia* e *Inia* podem ser distintos acusticamente baseados em seus sons sociais, já que o primeiro tem um repertório baseado em assobios e o último em chamados pulsados. A baixa emissão de assobios por *Inia* faz com que a chance de identificação errônea seja baixa. Logo, sons de ambos os gêneros podem ser usados para distingui-los durante monitoramentos acústicos passivos e podem servir como ferramentas para determinar a presença destas espécies em estudos de distribuição, uso de hábitat e abundância.

Palavras-chave: golfinhos de rio, comunicação acústica, comportamento, ecologia conservação.

Santos, G.M.A. (2017). Acoustic ecology of dolphins of the genus *Sotalia* (Cetartiodactyla, Delphinidae) and of the newly described Araguaian boto *Inia araguaiaensis* (Cetartiodactyla, Iniidae). PhD Thesis. Behavior Theory and Research Graduate Program. Belém: Universidade Federal do Pará. 93 pages.

ABSTRACT

Sensory systems are vital for animals to obtain information about their surroundings. Information can be gathered via visual, chemical, electrical, tactile and acoustic cues. These cues are used in several ecological contexts including foraging, competition, defense, social interactions (e.g. courtship behavior), and to indicate a condition, emotional or reproductive state or identity of the signal emitter. For aquatic mammals, sound is the most important mechanism of communication. Light attenuates rapidly with depth in aquatic environments limiting visual communication. In contrast, sound has low attenuation in water and it travels about five times faster in water than in air, making a very efficient way to communicate underwater. Therefore, sound is a fundamental aspect of cetacean biology, as these animals rely on acoustic signals for communication, navigation and location of prey. The genus *Sotalia* consist of two species that inhabit contrasting habitats. The Guiana dolphin (*Sotalia guianensis*) inhabits the coastal waters from Nicaragua to Southern Brazil, and the tucuxi (*Sotalia fluviatilis*) is confined to the main tributaries of the Amazon Basin. Meanwhile, river dolphins of the genus *Inia* - commonly known as botos - are found exclusively in the Amazon, Orinoco, and Tocantins River Basins. With their conservation status of both genera as data deficient there is great demand for information on their biology. As a key factor on cetacean biology acoustics can provide us with a richness of information and used as a tool to acquire data on habitat use, population numbers and behavior. However, in order to do so, first one needs to know the species vocal repertoire in detail and be able to differentiate those using acoustic methods. Thus, here we present the first distribution-wide analysis of the vocal repertoire of *Sotalia* dolphins and diversity and geographical patterns of their whistles. In addition, we present the first description of the vocal repertoire of *Inia araguaiaensis* focusing on repertoire diversity and structure. The whistle repertoire of both *Sotalia* species is highly structured, with populations of the riverine species showing a less diverse whistle repertoire than the populations of the coastal species. The highly structured repertoire is likely due to the small home ranges and low gene flow among populations. Differences in the richness of the acoustic repertoire between both species, may be due to a combination of socio-ecological and evolutionary factors. We also provide the first description of the Araguaian boto (*Inia araguaiensis*) acoustic behavior and showed that they have a rich acoustic repertoire consisting of whistles and primarily pulsed calls. While whistles were produced rarely, a specific type of call, the short two-component calls were the most common signal emitted during the study. These calls were similar in acoustic structure to those produced by orcas (*Orcinus orca*) and pilot whales (*Globicephala* sp.). Because of the context at which these signals were produced, we hypothesize that they possibly play a role in mother-calf communication. *Sotalia* and *Inia* can be acoustically distinguished based on their social sounds, as the former has a repertoire based on whistles and the latter based on pulsative calls. With the low emission rate of whistles by *Inia*, so chances of misidentification are low. Thus, sounds of both genera can be used to distinguish them from one another during passive acoustic monitoring and serve as proxies for species presence in studies of distribution, habitat use, and abundance.

Keywords: river dolphins, acoustic communication, behavior, ecology, conservation.

Acoustic ecology of dolphins of the genus *Sotalia* (Cetartiodactyla, Delphinidae) and of the newly described Araguaian boto *Inia araguaiaensis* (Cetartiodactyla, Iniidae)

Chapter 1 - General Introduction

Sensory systems are vital for animals to obtain information about their surroundings. Information can be gathered via visual, chemical, electrical, tactile and acoustic cues (Bradbury & Vehrencamp, 1998). These cues are used in several ecological contexts including foraging, competition, defense, social interactions (e.g. courtship behavior), and to indicate a condition, emotional or reproductive state, or identity of the signal emitter (Fitch *et al.*, 2002; Gannon *et al.*, 2005; Janik *et al.*, 2006; King & Janik, 2013; Pagnucco *et al.*, 2008; Smith, 1991; Watts & Holekamp, 2007; Woods *et al.*, 2007 Zuk *et al.*, 2006).

1.1. Sound and aquatic mammals

For aquatic mammals, sound is the most important mechanism of communication. Light attenuates rapidly with depth in aquatic environments limiting visual communication. In contrast, sound has low attenuation in water and travels about five times faster in water than in air, making a very efficient way to communicate underwater (Berta *et al.*, 2006; Tyack, 2000).

Among aquatic mammals pinnipeds (seals, sea lions and fur seals) and cetaceans (whales and dolphins) are known to modify their vocal repertoire according to their soundscape and can increase repertoire size and/or modify complexity of their vocal repertoire according to the sonorous stimuli they perceive along their lives (Berta *et al.*, 2006; Filatova *et al.* 2012; King & Janik, 2013; Morisaka, 2012; Reiss & McCowan, 1993; Tyack, 1986, 2000, 2008; Vergara &

Barrett-Lennard, 2008). For example, several species of dolphins can modify the acoustical characteristics of their sounds in response to stress (Esch *et al.*, 2009), sound pollution (Au *et al.*, 1985; Lesage *et al.*, 1999; Morisaka, 2012; Wright *et al.* 2007), and during intra and interspecific interactions and (Tyack, 1986, 2000; Janik, 2009, May-Collado, 2010). Their plasticity is so impressive they are known to imitate each other and even human speech (Abramson *et al.*, 2018; Ridgway *et al.*, 2012). Interestingly, unlike other groups of vertebrates that rely on sound for communication, the frequency of cetacean's communicative signal frequencies is no significantly impacted by morphological constraints (*i.e.*, body size) (May-Collado, *et al.* 2007a; Martin *et al.*, 2017).

1.2. Cetaceans

There are about 80 species of cetaceans present in all oceans of the planet and in large river systems of Asia and South America (Berta *et al.*, 2006; Jefferson *et al.*, 2008). The living species are divided into two major groups: Odontoceti and Mysticeti. The odontocetes or toothed whales, are the cetaceans that possess teeth. This is the most diverse group of cetaceans with approximately 70 species including dolphins, river dolphins, porpoises, sperm whales beaked whales, beluga and narwhal. Meanwhile, there are about 14 species of mysticetes also known as baleen whales. These animals lack teeth after the embryonic phase and so use structures called baleen to filtrate their pray after engulfing huge amounts of water (Berta *et al.* 2006; Jefferson *et al.*, 2008).

Life in groups is common among cetaceans (Gygax 2002; Mann *et al.* 2000). Group formation in baleen whales is related mostly to the distribution of resources and their strong annual migration cycle, during which the costs of grouping vary considerably along the year (Clapham 2000; Connor 2000). On the other hand, toothed whales are, mostly, social animals, nevertheless there is huge variation on the social structure of these species, from highly structured societies of resident orcas (*Orcinus orca*) and sperm whales (*Physeter microcephalus*) (Bigg *et al.*, 1990; Baird, 2000; Whitehead & Weilgart, 2000) to loose fusion-fission societies as found in bottlenose dolphins (*Tursiops truncates*) and Guiana dolphins (*Sotalia guianensis*) (Connor *et al.* 2000; Santos & Rosso, 2008).

1.2.1. Sound emission and reception in toothed whales

The structure responsible for sound production in toothed whales is located inside the blowhole in the nasal sacs region. This apparatus consists in a structural anatomical complex associated to the superior nasal passages and it is known as monkey lips/dorsal bursa complex (MLDB complex) (Figure 1). The name monkey lips derived from the appearance of this structure in sperm whales. All toothed whale species have the MLDB complex bilaterally disposed, with the exception of sperm whales. The monkey lips or phonic lips are located under the ventral portion of the vestibular air sac. The MLDB complexes are composed by a pair of anterior and posterior dorsal bursae to which a pair of muscular phonic lips are connected. In addition, there are a bursal cartilage and a blowhole ligament. All the structure is found suspended in a complex of muscles and air spaces (Berta *et al*.2006; Cranford *et al.* 1996, 1997).

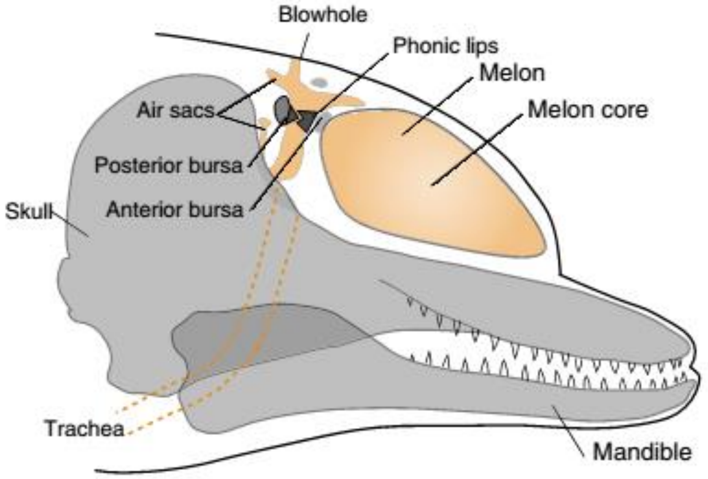

Figure 6. Schematics of the sound production apparatus in odontocetes, taken from Berta *et al.* (2006).

A sound propagation structure, the melon, is associated to the sound production system of odontocetes (except for the sperm whales, physiterids), located above the skull and anterior to the MLDB complex. The melon is composed by low-density lipids that function as an acoustic lens, in other words, this structure focus and directs the emitted signals into the water (Berta *et al*.2006; Cranford *et al.* 1996, 1997). In sperm whales the spermaceti organ is homologous to the melon and in those species the MLDB complex is anterior to the spermaceti organ on the tip of the head (Berta *et al*.2006; Cranford *et al.* 1996, 1997).

As for sound reception, behavioral studies conducted both in the wild and in captivity indicate that odontocetes have sensibility to a broad spectrum of frequencies (Au, 1993; Berta *et al*., 2006). All cetaceans lack pinna (auricle), nevertheless the external auditory canal is similar to those of terrestrial mammals and connects the external to the middle ear. This canal is extremely narrow in odontocete, thus playing a small role in sound reception, on the other hand the lower jaw six times more sensible than the external auditory canal (Berta *et al*. 2006; Brill *et al*., 1998). The posterior part of toothed whale's lower jaws are known as pan bones, which are thin and translucent, filled with fat bodies. Like the melon, these fat bodies are made of low-density lipids and function as sound conductors. The pan bones are directly connected to the lateral wall of the tympanic bulla, this is the main path for sound reception in toothed whales (Figure 2). Moreover, there is an area on each side of the melon as sensitive to sound reception as the pan bones (Berta *et al.*2006; Cranford *et al.* 1996, 1997, 2010).

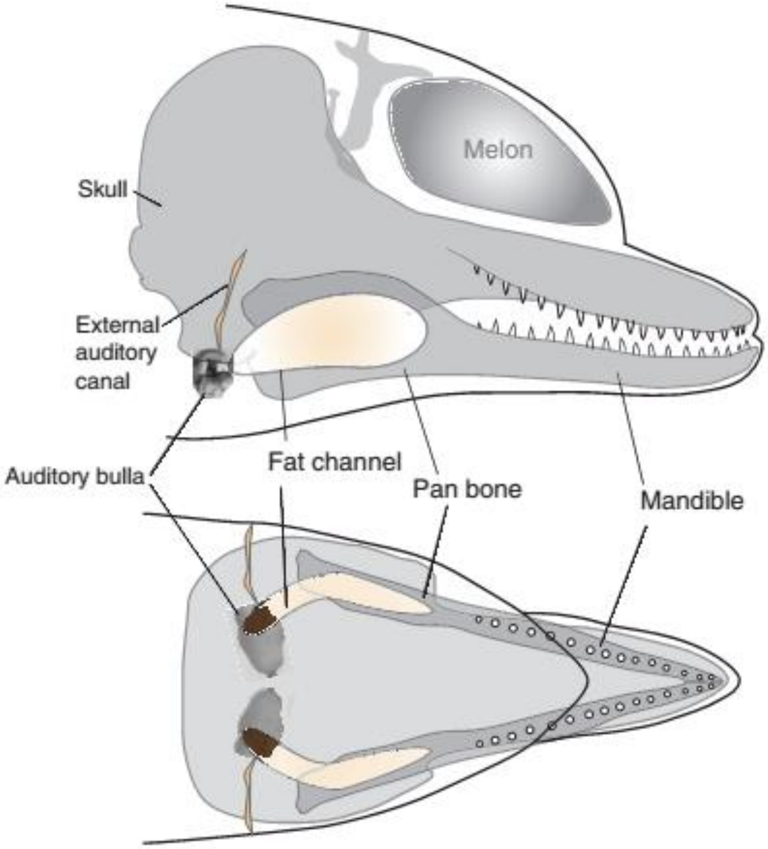

Figure 7. Schematics of sound reception structures in odontocete, taken from Berta *et al.* (2006).

The odontocete ear has two components the tympanic and the periotic, bones that form the tympanic-periotic complex, which are extremely dense of pachyostotic (Berta *et al.*, 2006; Oelschlager, 1986a, 1986b). In cetaceans these bones have a different shape, construction and cranial associations than those found in other mammals. In the odontocetes the tympanic bulla is separate from all neighboring bones by peribullar sinuses, which in turn are filled with an emulsion of mucus, oil and air. This complex is suspended by a thin network of connective tissue (Figure 3). Thus, the tympanic-periotic complexes function as independent sound receptors with better capability to locate sound sources and reflected echoes (Berta *et al.*, 2006; Oelschlager, 1986a, 1986b).

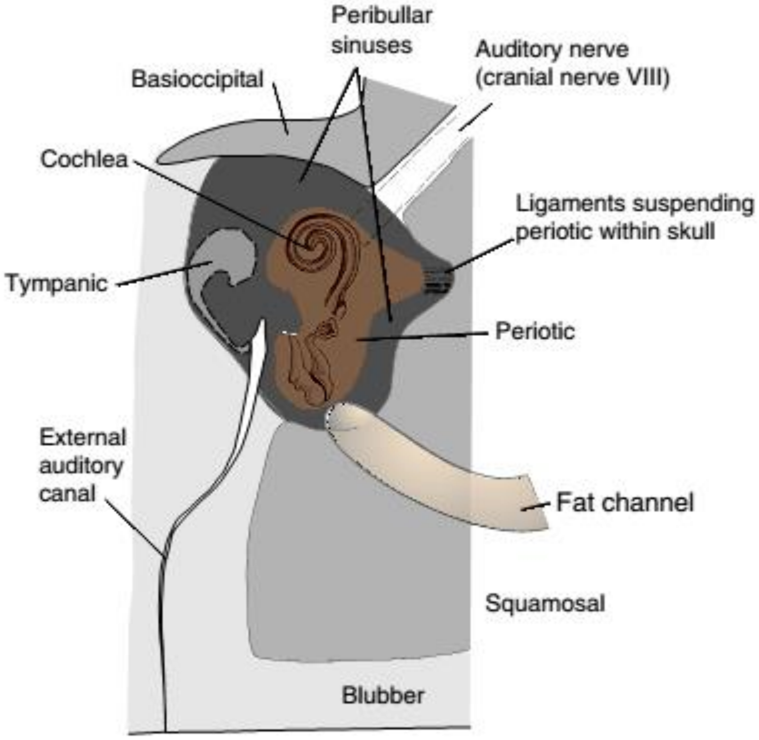

Figure 8. Schematics of the ventral view of the ear of an odontocete (without the tympanic), taken from Berta *et al.* (2006).

Sound is transmitted to the inner ear through the tympanic bone (more specifically by the tympanic plate) (Berta *et al.*, 2006; Cranford, *et al*. 2010; Hemila *et al.*, 1999, 2001). The malleus is connected to the tympanic plate by a *processus gracilis*, and the sound vibrations are transmitted to the oval window and then to the fluid of the inner ear by the ossicular chain (Berta *et al*., 2006; Cranford, *et al*. 2010; Hemila *et al*., 1999, 2001).

The inner ear and the cochlea of odontocetes work similar to the humans auditory system. The main differences are in the density of flagella per cell in hair cells (2:1 in humans and 5:1 in bottlenose dolphins *Tursiops* sp., making the cells of the latter more sensible to sound vibrations), in the number of turns in the cochlea, and the distribution of membrane support structures. Moreover, the basilar membrane is thicker and stiffer in odontocetes, which supposedly enhance sensitivity to higher frequencies (Berta *et al*., 2002; Ketten *et al*., 1992; Spoor *et al*., 2002) (Figure 3).

Odontocetes produce a variety of sounds that fall into two major groups tonals (whistles) and pulsed sounds (echolocation clicks and pulsative calls). Echolocation clicks are used to obtain information of the surrounding environment, in other words, these sounds are used to navigation and prey location. By emitting high frequency clicks forward into the water and receiving reflected echoes, odontocetes can obtain information on the volume, density, shape and distance of the objects which the echoes reflected from (Au 1993; Berta *et* al., 2006; Ketten 1992; Madsen *et al.* 2005; Morisaka *et al.* 2012; Tyack, 2000; Surlykke *et al.* 2014). So far there are no evidences that baleen whales use echolocation clicks, as they do not possess melon, the MLDB complex or any similar structure (Berta *et al.*, 2006; Tyack, 2000). However, there is evidence that these animals do use low frequency sounds to communicate through long distances (Clark, 1994, 1995; Tyack, 2000). Baleen whales might also use the echoes of these low frequency sounds to navigate through the oceans (Clark, 1994, 1995; Tyack, 2000).

On the other hand, pulsative calls and whistles are used for social communication. Whistles are tonal sounds commonly used by most delphinid species. While in delphinids these sounds are thought to play key role in maintaining group cohesion, and in the case of *Tursiops* sp. issuing individual identity, the function of whistles in other odontocete families is not yet fully comprehended (Berta *et al.*, 2006; Cremer *et al.* 2017; Filatova *et al.* 2012; Janik *et al.*, 2006; King & Janik, 2013; May-Collado *et al.*, 2007b; May-Collado, 2013; Morisaka, 2012; Tyack, 2000; Wright *et al.* 2007). Pulsative calls are also widespread through the odontocete phylogenetic tree but have been mostly studied in orcas (*Orcinus orca*) and pilot whales (*Globicephala* sp.). In these species such calls are very stereotyped and may convey individual's social affiliation (Deecke *et al.* 2010; Ford 1989; Pérez *et al.* 2017; Zwamborn & Whitehead 2017; Yurk *et al.* 2002).

Echolocation clicks are produced by the pneumatic pressurization into the intranarial spaces. The air sacs force the air through the phonic lips, so as the MLDB complexes vibrate. The periodic opening and closing of the phonic lips interrupt the air flow and generate the click repetition rate (Berta *et al*., 2006; Cranford *et al*., 1996). Meanwhile, whistles are likely produced when the nasal plugs and their nodes, the blowhole ligament and other membranes regulate the air flux in the passages dorsal to the nares (Berta *et al*., 2006; Cranford *et al*., 1996).

1.3. Cetaceans in riverine environments

Among the nearly 80 species of cetaceans only seven genera (*Inia*, *Sotalia*, *Pontoporia*, *Lipotes*, *Platanista*, *Orcaella* and *Neophocaena*) have adapted to freshwater environments in South America and Asia (Best & Da Silva, 1989, 1993; Brownell & Herald, 1972; Crespo *et al*., 1998; Da Silva & Best, 1994, 1996; Di Beneditto *et al*., 2001; Jefferson & Hung, 2004; Leatherwood & Reeves, 1994; Smith *et al*., 2007; Smith & Jefferson, 2002; Stacey & Arnold, 1999). Four of those genera (*Inia*, *Lipotes*, *Platanista* and *Pontoporia*) are known as “river dolphins”, although one of these species, the Franciscana (*Pontoporia blainvillei*), lives in estuarine waters instead of rivers. The three remaining taxa have adapted to riverine environments through millions of years. The tucuxi (*Sotalia fluviatilis)* and the Irrawaddy dolphin (*Orcaella* spp.) are both into the family Delphinidae and along with the finless porpoise (*Neophocaena phocaenoides)*, family Phocoenidae, represent a more recent incursion into riverine systems (Da Silva & Best, 1994, 1996; Caballero *et al.*, 2007; Jefferson & Hung, 2004; Stacey & Arnold, 1999).

Despite the differences in their evolutionary history, those seven genera share some behavioral characteristics: most of the cetacean species inhabiting freshwater habitats are not gregarious, their strongest association is between mother and calves, while larger aggregations are formed to feed on large fish schools and/or mating, and they are not very conspicuous in the surface. Notwithstanding, little is known on the societies of river cetaceans (Best & Da Silva, 1989, 1993; Brownell & Herald, 1972; Crespo *et al.*, 1998; Da Silva & Best, 1994, 1996; Da Silva, 2009; Di Beneditto *et al.*, 2001; Jefferson & Hung, 2004; Leatherwood & Reeves, 1994; Martin & Da Silva, 2004; Reeves & Martin, 2009; Smith *et al.*, 1997, 2007; Smith & Jefferson, 2002; Stacey & Arnold, 1999; Zhou *et al.*, 1994, Zhou, 2009). In addition, those species are among the most threatened cetaceans, as their habitat and resources are also used by human populations and because of great water development projects, as dams (Leatherwood & Reeves, 1994; Reeves & Martin, 2009; Reeves et al., 2000, 2003; Smith et al., 2007; Smith & Jefferson, 2002). The baiji (*L. vexillifer*) is the first small cetacean to be reported extinct by human action, representing the loss of a whole cetacean linage (Tuvey *et al.* 2007).

1.3.1. The genus Sotalia

The genus *Sotalia* comprises two species: the tucuxi (*S. fluviatilis*) and the Guiana dolphin (*S. guianensis*). (Caballero *et al*.2007; Cunha *et al*., 2005, Da Silva & Best, 1994, 1996; Flores & Da Silva 2009) (Figure 4). The tucuxi is the only exclusively riverine delphinid and is found in the main tributaries of the Amazon River Basin. Its dorsum is gray and the belly is pinkish, the dorsal fin is triangular and the flippers are slightly larger than those of other coastal delphinids, though the fluke is similar in shape. The melon is round, and the rostrum is slender, with 26 to 36 teeth, both on the lower and upper jaw, maximum length recorded is 1.52m (Caballero *et al*.2007; Cunha *et al*., 2005, Da Silva & Best, 1994, 1996; Flores & Da Silva 2009).

Figure 9. Dolphins of the genus Sotalia: A – tucuxi (*Sotalia fluviatilis*), photo by Pedro Fruet; B – Guiana dolphin (*Sotalia guianensis*), photo by Rodrigo Tardin.

Sotalia fluviatilis have a variety of aerial behavior including vertical and lateral jumps, summersaults and “surfing” on waves made by boats (Da Silva & Best, 1994, 1996). Group size is typically two to six individuals, though groups of up to 30-60 animals can be seen where the river is deeper and wider (Da Silva & Best, 1994, 1996; Da Silva et al., 2010, Faustino & Da Silva, 2006; Herrando-Pérez et al., 2006; Martin & Da Silva, 2004; Martin et al., 2004; Santos et al., 2012; Vidal et al., 1997). These dolphins do not enter the flooded forests as far as botos (*Inia* sp.) and are more restricted to the river main channels (Da Silva *et al*., 2010; Faustino & Da Silva, 2006; Martin *et al.*, 2004). Tucuxi movement patterns are dictated by the seasonal flooding cycle in Central Amazon, expanding the area of use during flood season and narrowing it down to main channels during the dry season (Faustino & Da Silva, 2006). These dolphins are usually associated to low current habitat that serve as shelter to prey (Gomez-Salazar *et al.*, 2012a, 2012b; Martin *et al.*, 2004).

The Guiana dolphin is characterized by marine and estuarine habits usually associated to fresh water bodies. This species occurs from the coast of Nicaragua (Central America) to the northern coast of Santa Catarina (South Brazil) (Edwards & Schnell, 2001; Simões-Lopes, 1988). *Sotalia guianensis* is very similar in morphology and color patterns to its sister species da Silva e Best, 1994, 1996; Flores & da Silva, 2009), however they are larger and have more teeth (Flores e da Silva, 2009). These dolphins can reach up to 1.9cm, but the larger recorded animal had 2.20m (Flores & da Silva, 2009).

Mean group size varies along the geographical distribution of the species (Da Silva *et* al., 2010; Emin-Lima *et al.*, 2010; Flores & Da Silva, 2009; Santos & Rosso, 2007). The larges aggregations of this species are found in the coast of Rio de Janeiro State, southeastern Brazil in

Ilha Grande Bay, where they form groups of up to 450 animals (Lodi & Hetzel, 1998; personal observation).

Studies conducted using the photo identification technique (individual identification by natural marks) along the distribution of Guiana dolphins showed that these dolphins have site fidelity and do not perform long movements (Azevedo *et al.* 2004; Flores & Bazzalo, 2004; Oshima *et al.*, 2010; Rossi-Santos *et al.*, 2007; Santos *et al.*, 2010). Both *Sotalia* species are classified as data deficient by IUCN (2008).

1.3.2. The genus Inia

Botos (*Inia sp.*) are the largest of river dolphins. They are present in the Amazon, Tocantins and Orinoco River Basins (Araújo & Wang, 2012; Best & Da Silva, 1989, 1993; Hrbek et al., 2014; Santos et al., 2012, 2014) (Figure 5). This genus was thought to be monospecific, however a study by Hrbek and colleagues (2014) showed that the genus actually comprises three species, namely: *Inia geoffrensis* (Amazon River Basin), *Inia boliviensis* (Madeira River Sub-basin) and *Inia araguaiaensis* (Tocantins River Basin).

Figure 10. Dolphins of the genus *Inia* (botos): A - Amazonian river dolphin (*Inia geoffrensis*), photo by Gabriel Melo-Santos; B – Araguaian boto (*Inia araguaiaensis*), photo by Gabriel Melo-Santos; C – Bolivian river dolphin (*Inia boliviensis*), photo by W. Gravena.

Inia are evolutionary unique dolphins, with a set of characteristics that distinguish them from marine dolphins. Their ancestors are thought to inhabit the Amazonian Basin for about 15 million years (Hamilton *et al.* 2001; May-Collado & Agnarsson, 2011; Pyenson *et al.*, 2015). Females measure up to 2.30m and males 2.70m. They are robust animals though very flexible, as their first two vertebrae (*atlas* and *axis*) are not fused. The backbone is also more flexible than in marine dolphins allowing them to bend and twist more than their marine counterparts. The flukes are big and triangular, instead of a dorsal fin there is a keel extending from the mid-dorsum to the caudal peduncle. The flippers are broad and big, paddle-like, and capable of independent circular movements, enabling botos to swim backwards. All these characteristics allow botos to explore a variety of habitats and go far into flooded forests. The rostrum is long and slender, and the melon is small and round, and the eyes are small. Coloration varies from dark grey to bright pink. Calves are born grey and tend to get clearer with age (Best & Da Silva, 1989, 1993).

These animals are slow swimmers and usually do not jump or breach. The strongest associations are between mother and calf, though larger groups may be formed for mating and feeding on large fish schools (Best & Da Silva, 1989, 1993; Martin *et al.*, 2008). They are curious animals and may approach boats and swimmers but can also be cryptic (Best & Da Silva, 1989, 1993). Martin & Da Silva (2004) reported a large spectrum of movement patterns for botos in the Mamirauá Reserve of Sustainable Development in the Central Amazon Brazil, where they observed both resident and transient animals. This species is also associated with slow current habitats with high density prey (Gomez-Salazar *et al.* 2012a, 2012b; Martin *et al.*, 2004).

According to Martin *et al.* (2008), botos are the only species of aquatic mammals to use objects for social-sexual display, and one of the few species of mammals to do so, this behavior was only reported for chimpanzees (*Pan* sp.) and humans (Ingmanson 1996; Martin *et al.* 2008). This display normally occurs in large aggregations with many males, where they carry objects out on the surface, carried objects can be stones or vegetation (Martin *et al.*, 2008). *Inia* dolphins are classified as data deficient by IUCN (2008).

1.3.3. Acoustics of Sotalia and Inia

Sound production has been studied for both *Sotalia* and *Inia*. Although the whistle repertoire of *Sotalia* have been studied in several areas along their distribution these studies are often punctual and focus only on isolated populations (Azevedo and Van Sluys 2005; Ding *et al.* 2001; May-Collado 2010a, 2010b; May-Collado &Wartzok 2009, 2010; Pivari & Rosso 2005; Rossi-Santos & Podos, 2006). And even though, some studies (Azevedo and Van Sluys 2005; Rossi-Santos & Podos, 2006) evaluated geographical differences in whistles produced by *Sotalia* dolphins, little is known on the diversity of the repertoire of this genus is and how different the repertoire is between populations. On the other hand, the vocal behavior of *Inia* has been greatly underestimated, and the species has been reported to have low vocal activity and limited repertoire (Podos *et al.*, 2002). However, other studies of captive and free-ranging animals reported several sounds including burst-pulsed sounds, pulsative calls, whistles and echolocation clicks (Ding *et al.* 2001; May-Collado and Wartzok 2007; Diazgranados & Trujillo 2002). All these studies are restricted to the western portion of the Amazon and so far, no studies were conducted for the most recently described species *Inia araguaiaensis*.

With their conservation status of both genera as data deficient there is great demand for information on their biology. As a key factor on cetacean biology, the study of acoustics can provide us with a richness of information and used as tools to acquire information on habitat use, population numbers and behavior (Au 1993; Berta *et* al., 2006; Cremer *et al.* 2017; Deecke *et al.* 2010; Filatova *et al.* 2012; Ford 1989; Janik *et al.*, 2005; Ketten 1992; King & Janik, 2013; Madsen *et al.* 2005; May-Collado, 2010, 2013; May-Collado *et al.*, 2007; Collado & Wartzok 2007, 2008; ; Morisaka, 2012; Pérez *et al.* 2017; Surlykke *et al.* 2014 Tyack, 2000; Yurk *et al.* 2002; Zwamborn & Whitehead 2017). However, in order to do so, first one needs to know the species vocal repertoire in detail and be able to differentiate them using acoustic methods. Thus, here we present the first distribution-wide analysis of the vocal repertoire of *Sotalia* dolphins, and the diversity and geographical patterns of their whistles (Chapter 2, formatted according to the guidelines of **Nature Communications**). Im addition, we present the first description of the vocal repertoire of *Inia araguaiaensis* focusing on repertoire diversity and structure (Chapter 3 formatted according to the guidelines of **Scientific Reports**).

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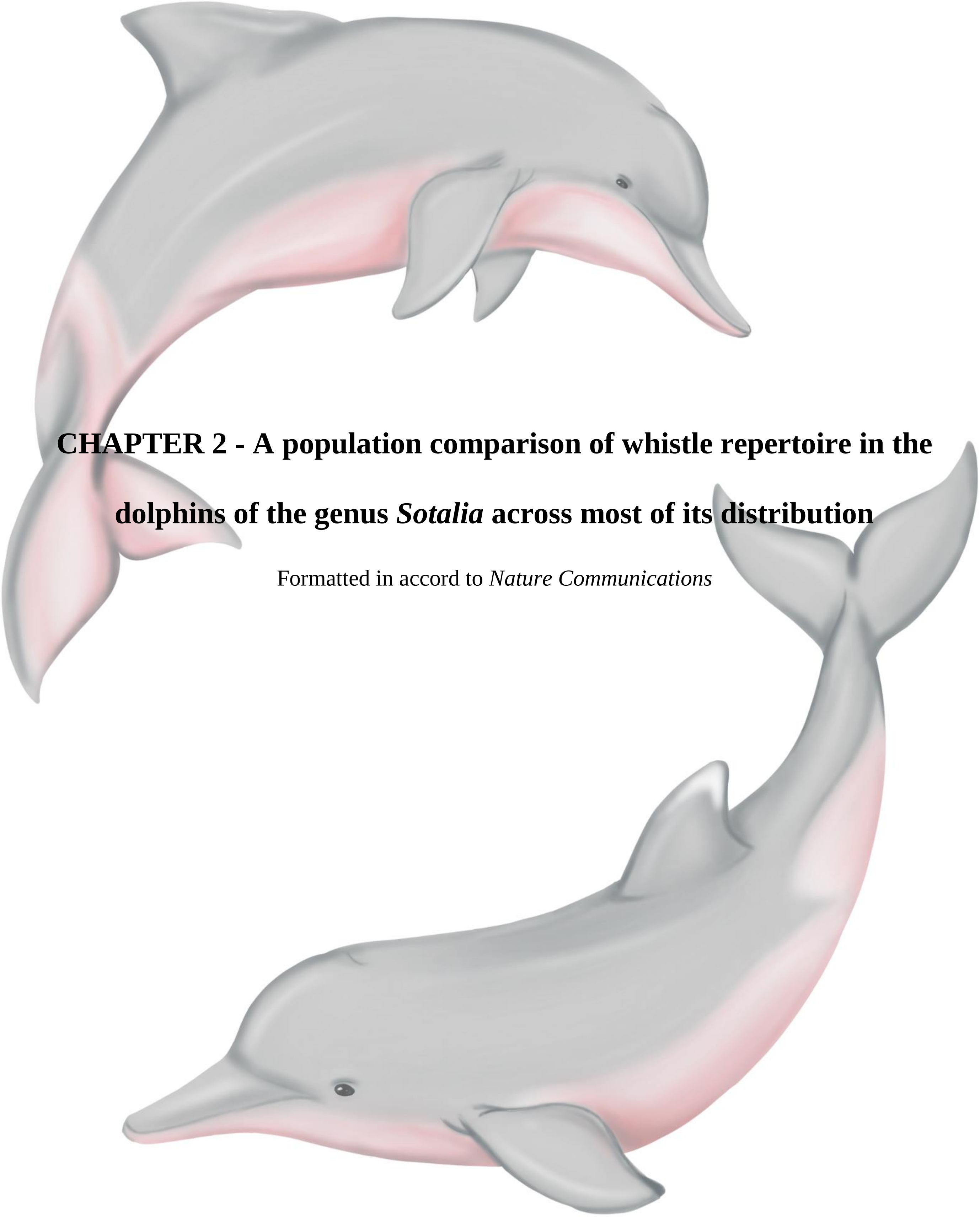

CHAPTER 2 - A population comparison of whistle repertoire in the dolphins of the genus *Sotalia* across most of its distribution

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A population comparison of whistle repertoire in the dolphins of the genus *Sotalia* across most of its distribution

The genus *Sotalia* consist of two species that inhabit contrasting habitats. The Guiana dolphin (*Sotalia guianensis*) inhabits the coastal waters from Nicaragua to Southern Brazil, and the tucuxi (*Sotalia fluviatilis*) is confined to the main tributaries of the Amazon Basin. The whistle acoustic structure of these species has been exhaustively studied primarily at the local level. Here we present the first large scale analysis of *Sotalia* whistles contours using recordings from 10 coastal populations ranging from Costa Rica to Southern Brazil, and six riverine populations. For each population we extracted a maximum of 200 whistle frequency contours and used an adaptive resonance theory neural network combined with dynamic time-warping (ARTwarp) to classify each contour. We showed that *Sotalia* whistle repertoires are highly structured with a few widespread whistle contours along its distribution. The coastal species had a more diverse repertoire than the riverine species. Of the 16 populations included in this analysis those from Costa Rica, Venezuela, Ilha Grande, Ecuador, Juruá and Tocantins River had the most distinct whistle repertoires. These results indicate that the diversity and structure of acoustic repertoire of *Sotalia* dolphins seems to be correlated to a series of factors including behavior, social organization, movement patterns, habitat, genetic structure and diversity.

The majority of dolphin species use narrow-banded frequency modulated signals called whistles to communicate [1]. Dolphin whistles play a key function in the maintenance of social groups cohesion, communicating with partners and keeping the contact between mother and offspring [2,3]. For some species (e.g. bottlenose dolphins, *Tursiops* sp.) whistles also serve to issue individual identity [4,5] and possibly rely species-specific information [6]. Therefore, these sounds are an essential part of a dolphin biology.

Dolphins of the genus *Sotalia* are small social delphinids, two sister species are recognized in this genus: the Guiana dolphin (*Sotalia guianensis*) and the tucuxi (*Sotalia fluviatilis*) [7-9]. Guiana dolphins are found in coastal estuarine waters from Nicaragua (Central America) to Santa Catarina (Southern Brazil) [10,11]. Normally found in groups of 2 to 15 animals, although mean group size varies along the geographical distribution of the species [12-17]. The largest aggregations of this species are found in the coast of Rio de Janeiro State, southeastern Brazil in

Ilha Grande Bay, where they form groups of up to 450 animals [18, personal observation]. These dolphins live in a fusion-fission society where animals have small home-ranges [19-23].

Meanwhile, the tucuxi occurs in the main tributaries of the Amazon River Basin and is the only exclusively riverine delphinid [24,25]. Groups are typically from 2 to 6 animals, also varying along its distribution, however groups of up to 60 animals can be observed where the river is wide enough [14,24-30]. Their movements are linked to the flooding regime in the Amazon. Their habitat is expanded during the high-water season and then tucuxis are confined to the main river channels when water levels are low [24,25,27].

The two species of *Sotalia* are thought to have diverged about 1 mya [9]. Caballero et al. [31] verified that populations of *Sotalia guianensis* are highly structured with higher genetic diversity on the Caribbean Sea populations and lower genetic diversity in Brazilian Coast populations. On the other hand, populations of *Sotalia fluviatilis* are less genetically structured, with increasing genetic differentiation in tributaries that are more isolated [31].

Although the whistles of *Sotalia* dolphins have been extensively studied these studies were primarily focus on whistle emission and structure at a small geographical scale [6,32-38]. These studies revealed that *Sotalia* dolphin’s whistles are normally simple (with few inflection points) and with a relatively wide frequency range. In several of the studied populations the Sotalia’s whistle frequency was over 24kHz, with coastal populations having the highest frequency span from 1.38 to 48.4kHz [33,36,37]. Unfortunately, attempts for comparisons among populations have proven to be such difficult or unsuitable, partly due to differences in recordings systems and classification methods. To this day, there is no information about the repertoire diversity for each of these species, nor an adequate analysis that allows for comparison at various geographical scales.

Here, we present the first large scale analysis of the whistle repertoire of *Sotalia* Specifically, we quantify and compare the diversity of whistle repertoire among 16 populations throughout the entire distribution of the genus using an adaptive resonance theory neural network combined with dynamic time-warping (ARTwarp) to classify whistle contours.

Results

We analyzed a total of 2469 whistles of 16 populations (Figure 1) of *Sotalia* which were classified into 426 categories (Figure 2) from which 53% (n=227) were found exclusive for *S. guianensis* and 11% (n=48) only for *S. fluviatilis*, 4% (n=16) whistle types were found exclusive for Tocantins River *Sotalia* dolphins, but as the taxonomic identity of these dolphins remain uncertain we did not include them in the former two groups. In addition, only 2% (n=10) whistles types were found in 8-12 populations, showing a trend towards highly structured repertoires for the genus.

The populations with larger repertoire were Ilha Grande Bay with 114 whistle types (from which 64 were unique to that site), followed by Costa Rican dolphins with 111 whistle types (from which 57 were unique). The Ecuadorian dolphins had the smallest repertoire with only 12 whistle types (from which two were unique). Overall *Sotalia guiaenensis* has a more diverse repertoire than *Sotalia fluviatilis*. Figure 1 summarizes the results for repertoire size for all populations, while Table 1 shows the repertoire size and the relative number of unique whistles for each population relative to their respective repertoire size.

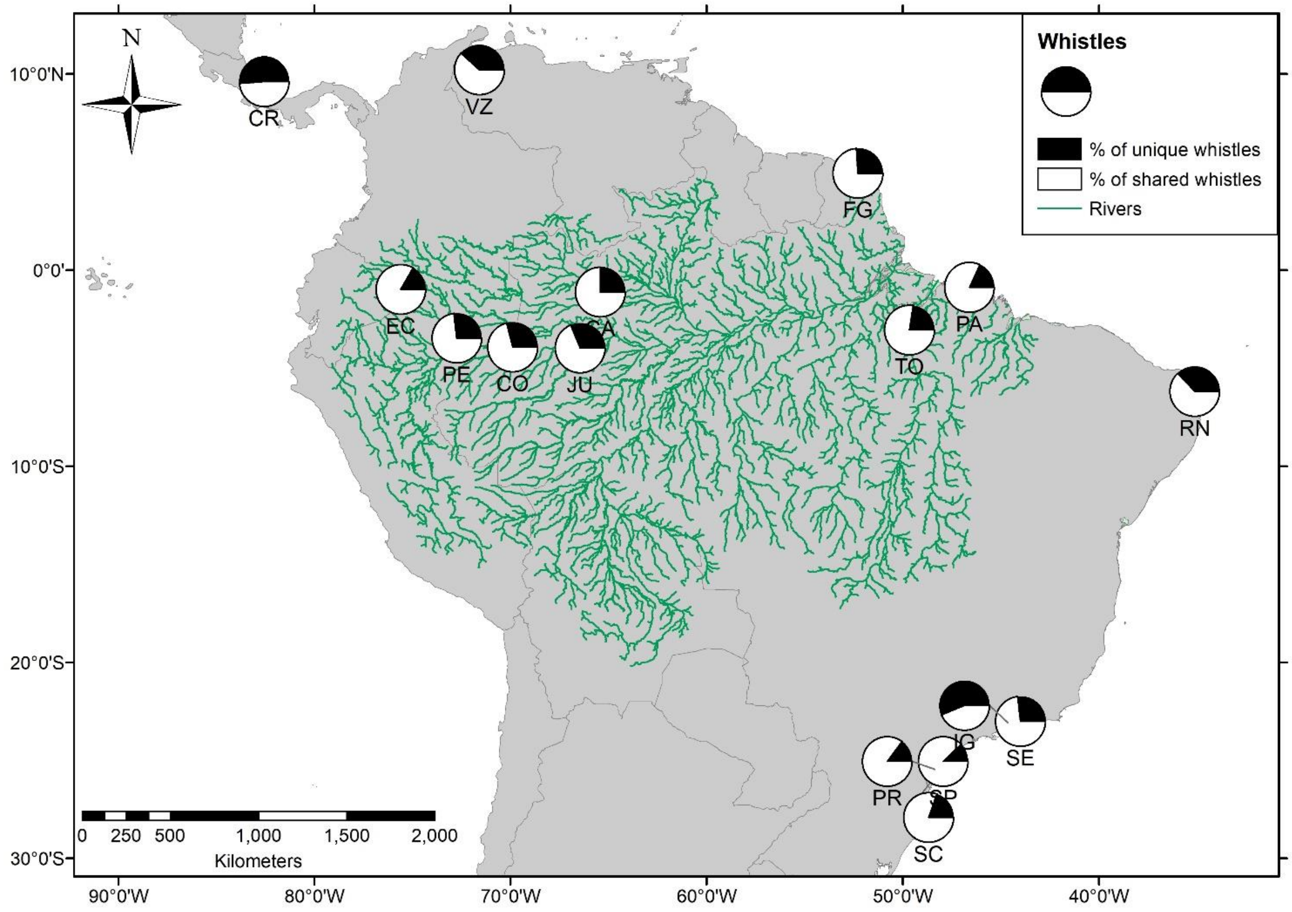

Figure 1. Location of the recording sites of *Sotalia* dolphins, namely: Costa Rica coast (CR), Lake Maracaibo and Gulf of Venezuela (Venezuela Coast) (VZ), French Guiana coast(FG), the coast of Pará State (Northern Brazil) (PA), the Tocantins River (Pará State, Northern Brazil) (TO), Japurá and Solimões Rivers (Central Amazon, Amazonas State, Northern Brazil) (CA), Juruá River (Amazonas State, Northern Brazil) (JU), Colombian Amazon (CO), Peruvian Amazon (PE), Napo River (Ecuadorian Amazon) (EC), the coast of Rio Grande do Norte State (Northeastern Brazil) (RN), Sepetiba Bay (coast of Rio de Janeiro State, Southeastern Brazil) (SE), Ilha Grande Bay (coast of Rio de Janeiro State, Southeastern Brazil) (IG), Cananeia Estuary (São Paulo State, Southeastern Brazil) (SP), Paranaguá Estuary (Paraná State, Southern Brazil) (PR) and Babitonga Bay (Santa Catarina, Southern Brasil) (SC). The circles display the amount of whistle types unique to each population (in black) and shared whistle types (in white).

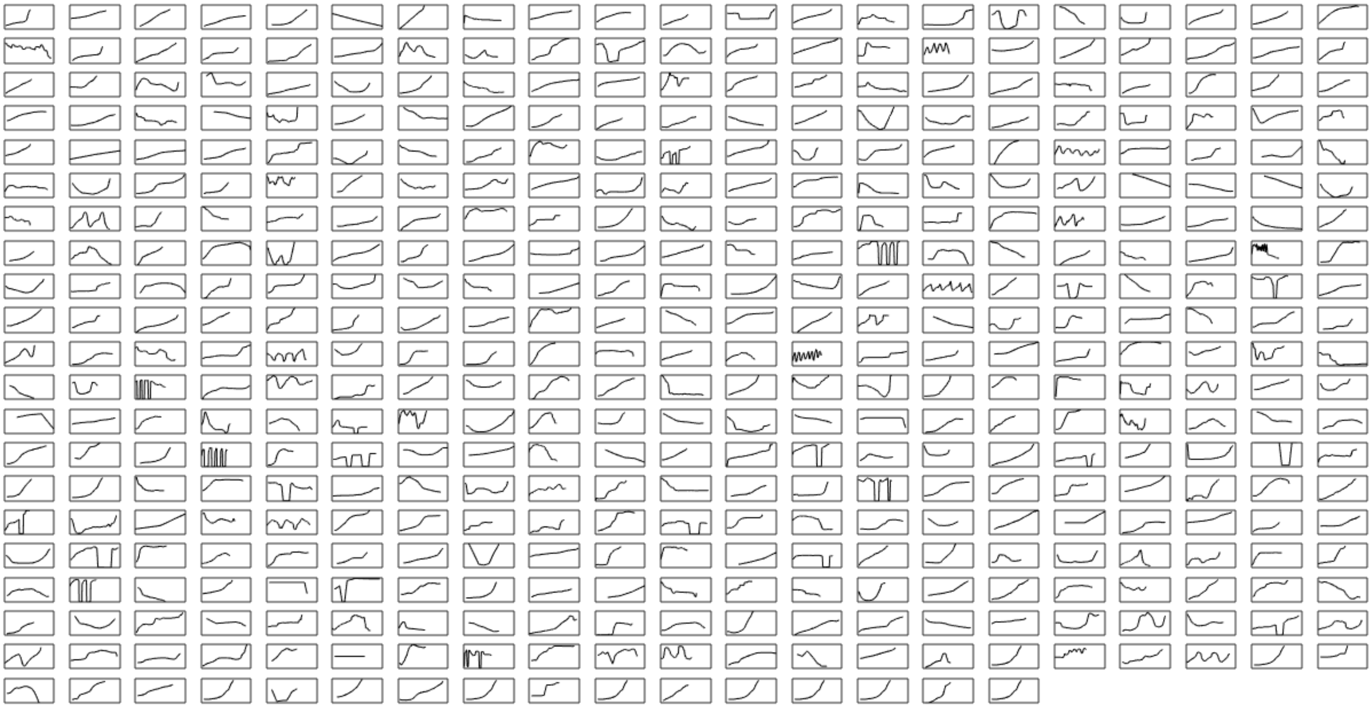

Figure 2. Graphic representation the whole *Sotalia* dolphins' repertoire. Each window contains a reference contour (whistle type) found by the ART-warp analysis.

Table 1. Summary of whistle repertoire sizes of *Sotalia* dolphins, showing the number of whistles used in the local analysis, the number of whistle types and number of whistle types unique to each population, along with relative uniqueness level considering each population repertoire size. Highlighted numbers indicate populations with more unique repertoire.

Species	Population	Number of whistles analyzed	Number of whistle types	Number of unique whistles	Relative number of unique whistles (%)

S. fluviatilis	**Central Amazon (CA)**	112	40	10	25.00
S. fluviatilis	**Colombian Amazon (CO)**	200	69	20	28.99
S. guianensis	**Costa Rica (CR)**	200	111	57	**51.35**
S. fluviatilis	**Napo River, Ecuador (EC)**	30	12	2	16.67
S. guianensis	**French Guiana (FG)**	114	27	7	25.93
S. guianensis	**Ilha Grande Bay (IG)**	200	114	64	**56.14**
S. fluviatilis	**Juruá River (JU)**	83	16	5	31.25
S. guianensis	**Pará Coast (PA)**	199	72	13	18.06
S. fluviatilis	**Peruvian Amazon (PE)**	91	41	11	26.83
S. guianensis	**Paranaguá Estuary, Paraná (PR)**	199	61	9	14.75
S. guianensis	**Rio Grande do Norte Coast (RN)**	185	86	32	37.21
S. guianensis	**Babitonga Bay, Santa Catarina (SC)**	200	56	11	19.64
S. guianensis	**Sepetiba Bay (SE)**	199	56	15	26.79
S. guianensis	**Cananéia Estuary, São Paulo (SP)**	199	49	6	12.24
***Sotalia* sp.**	**Tocantins River (TO)**	195	71	16	22.54
S. guianensis	**Lake Maracaibo, Venezuela (VZ)**	63	34	13	38.24

The highest amount of whistle types shared by pair of *Sotalia* populations is 14 and the lowest is zero (Table 2). The non-metric multidimensional scaling (NMDS) analysis shows that dolphins from Costa Rica, Venezuela, Ilha Grande Bay, Tocantins, Napo and Juruá Rivers have the most distinct repertoire. Sepetiba Bay is closer to its neighbor population, Ilha Grande, than to the remaining groups. In addition, the southernmost populations of *Sotalia guianensis* (São Paulo, Paraná and Santa Catarina) form a tight cluster indicating their similar repertoire. The remaining populations do not seem to form any clear clustering (Figure 3).

Table 2. Pairwise comparison displaying the number of whistle types shared by *Sotalia* dolphin's populations along their geographical range. Bold numbers represent the total number of whistle types for each population.

Population	***CA***	***CO***	***CR***	***EC***	***FG***	***IG***	***JU***	***PA***	***PE***	***PR***	***RN***	***SC***	***SE***	***SP***	***TO***	***VZ***
CA	**40**															
CO	4	**69**														
CR	1	3	**111**													
EC	2	3	4	**12**												
FG	2	5	2	1	**27**											
IG	3	7	7	2	3	**114**										
JU	4	4	2	2	3	2	**16**									
PA	6	11	11	3	9	9	3	**72**								
PE	3	8	3	4	3	6	4	9	**41**							
PR	4	9	10	5	6	13	2	13	8	**61**						
RN	4	9	5	3	6	7	7	14	4	12	**86**					
SC	3	8	4	3	5	10	2	12	6	14	4	**56**				
SE	3	7	10	3	2	10	0	7	5	11	8	8	**56**			
SP	5	9	8	5	6	10	4	12	8	14	9	5	13	**49**		
TO	7	8	8	3	5	10	4	10	8	9	13	14	7	13	**71**	
VZ	5	5	6	1	4	6	1	11	4	7	6	1	7	4	6	**34**

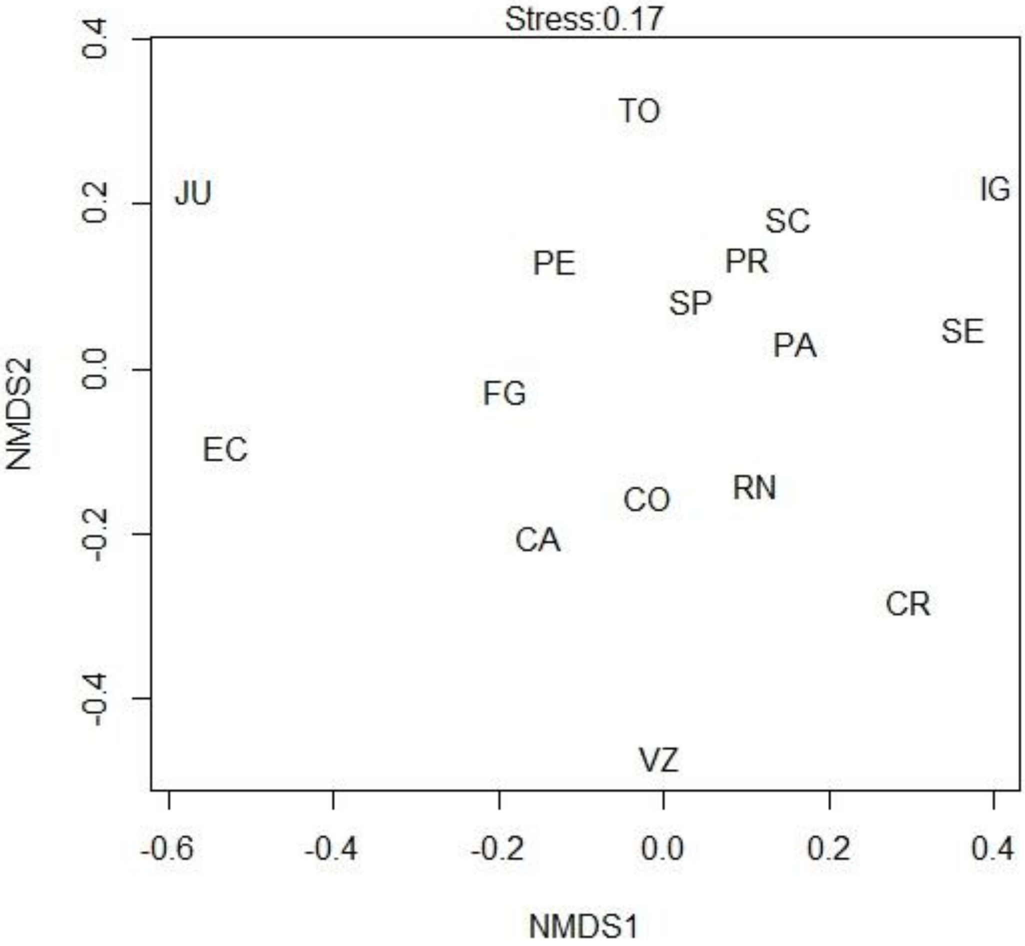

Figure 3. Non-metric multidimensional scaling, using presence and absence data of whistle types along the distribution of the genus. The graph displays the relationship of *Sotalia* dolphins' repertoire along the distribution of the genus.

Discussion

Our results demonstrate that the vocal repertoire of both *Sotalia* species is highly structured with only a few of whistle contours shared among the studied populations. This pattern may result from low dispersal capabilities and small home-ranges [19-23]. We also found that the whistle repertoire of the coastal species (S. *guianensis*) is more diverse repertoire than that of the riverine species (*S. fluviatilis*). This might be related to the more recent evolutionary history of *S. fluviatilis* and they are less genetically structured, this indicates some connectivity between populations [31]. Combined with a more dynamic habitat, water level may vary 15m along the year, drastically impacting tucuxis habitat use [27, 28, 39]. Furthermore, smaller group sizes (typically 2-6 animals) these factors might have contributed to a less diverse repertoire [14, 24- 27, 30]. In contrast, *S. guianensis* has older evolutionary history and populations are more genetically structured [9,31], which translates into lower connectivity between populations. In addition, their habitat is relatively more stable, site fidelity is higher and group sizes are larger [13-25]. Altogether, these factors might relate to the more diverse repertoire of *S. guianensis*.

Costa Rica and Ilha Grande populations stand out not only by repertoire size but also due to their distinctiveness. Guiana dolphins from Costa Rica are not only in an area of high genetic diversity [31], but they have constant encounters with bottlenose dolphins (*Tursiops truncatus*) [6]. The frequent interaction with bottlenose dolphins might influence on Costa Rican Guiana dolphin's acoustic repertoire. May-Collado [6] reported that when both species are together they produce whistles that are an intermediate between both dolphins. Thus, *S. guianensis* may be incorporating part of *Tursiops* whistle features into their own. This can be observed in some highly modulated whistles found in Costa Rican repertoire (Figure 4). While Ilha Grande Bay is located in an area with low genetic diversity [31], it is the area where largest groups of *Sotalia* dolphins are found in all distribution of the genus, groups up to 450 individuals [14, 18]. These large aggregations often engage in coordinate feeding tactics to prey on schooling fish, moreover coordinate feeding groups often had calves present [40]. These behavioral traits might have caused selective pressure for a more complex acoustic communicative repertoire.

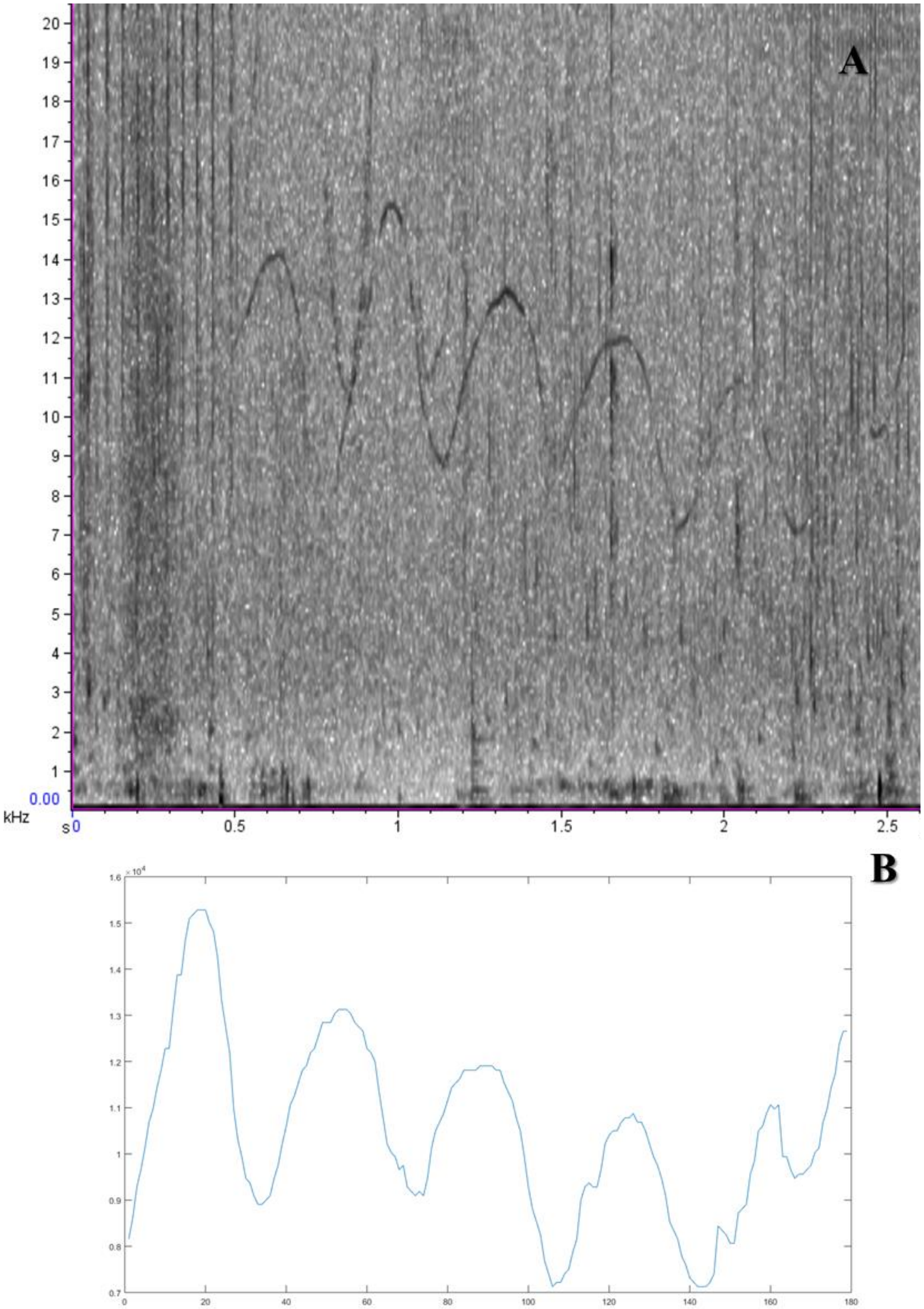

Figure 4. A – Whistle emitted by a Guiana dolphin *Sotalia guianensis* from Costa Rica, in B- is the frequency contour extracted from the whistle.

The population of Guiana dolphins from the Gulf of Venezuela and Lake Maracaibo, Venezuela also have a distinct vocal repertoire, this agrees with genetic and morphological data, which indicates that this population is differentiated from the rest of the Caribbean Sea Guiana dolphins [31,41,42]. Indeed, Caballero et al. [31] states that Venezuelan dolphins originated from a founder event from Caribbean dolphins. Moreover, these authors also report that the Brazilian Coast populations originated by a historic founder event with subsequent population expansion, probably 12000 ya. This recent expansion event along with lower genetic diversity might explain the low differentiation of repertoires from French Guiana to the coast of Rio Grande do Norte (Figure 3). Similarly, the three southernmost populations (São Paulo, Paraná and Santa Catarina) have more similar repertoire than other populations of *S. guianensis* along the Brazilian Coast. As these are the population the extreme of the species distribution they are probably the last to have diverged and thus have more similar whistle repertoire.

Riverine populations of *Sotalia* have a lower degree of genetic structure, with growing genetic differentiation to more isolated tributaries, suggesting that there is some connectivity between populations [31]. This is also shown by our results, populations more central to the distribution of *Sotalia fluviatilis* (Central, Colombian and Peruvian Amazon) have a less distinct repertoire and do not cluster (Figure 3). On the other hand, more peripherical tributaries of the Amazon River (Tocantins, Napo and Juruá) have more differentiated repertoire, even though Juruá tucuxis did not present an elevated relative uniqueness of whistle types the number of whistle types shared to each pair was low. Similarly, even though our sample from the Napo River was low, the amount of whistle types shared with each paired population was low. Furthermore, the Tocantins River does not discharge directly into the Amazon River Basin for about 1.8mya likely due to the detachment of the Marajó Island from the main land [43], before the divergency of the two *Sotalia* lineages. Indeed, this event is thought to be related to the divergence of the lineages of *Inia,* and the origin of *Inia araguaiaensis* [44]. Therefore, even though the identity of *Sotalia* dolphins from the Tocantins remains unknown it is clear that populations of this river are isolated from the main Amazon River Basin. It is also unlikely that there is movement of individuals from the Coast of Pará into the Tocantins River and vice-versa. All these events may explain the distinctiveness of the acoustic repertoire of Tocantins River *Sotalia* dolphins.

In summary, the diversity and structure of acoustic repertoire of *Sotalia* dolphins seems to be correlated with to a series of factors including behavior, social organization, movement patterns, habitat, genetic structure and diversity [1-6,45-51]. Furthermore, our results provide a catalogue of the acoustic repertoire of *Sotalia* that can be used for future studies (mainly passive acoustic monitoring) to differentiate *Sotalia* whistles from those of other odontocete species. In addition, we recommend for acoustic distinctiveness to be taken into account for future conservation action plans.

Methods

Data collection

Our database comprised recordings collected between 1998 to 2017 in 16 different sites along the distribution of both *Sotalia* species, namely: Costa Rica coast (CR), Lake Maracaibo and Gulf of Venezuela (Venezuela Coast) (VZ), French Guiana coast(FG), the coast of Pará State (Northern Brazil) (PA), the Tocantins River (Pará State, Northern Brazil) (TO), Japurá and Solimões Rivers (Central Amazon, Amazonas State, Northern Brazil) (CA), Juruá River (Amazonas State, Northern Brazil) (JU), Colombian Amazon (CO), Peruvian Amazon (PE), Napo River (Ecuadorian Amazon) (EC), the coast of Rio Grande do Norte State (Northeastern Brazil)

(RN), Sepetiba Bay (coast of Rio de Janeiro State, Southeastern Brazil) (SE), Ilha Grande Bay (coast of Rio de Janeiro State, Southeastern Brazil) (IG), Cananeia Estuary (São Paulo State, Southeastern Brazil) (SP), Paranaguá Estuary (Paraná State, Southern Brazil) (PR) and Babitonga Bay (Santa Catarina, Southern Brasil) (SC).

Acoustic and Statistical analysis

The recordings were visually inspected using Raven Pro 1.5 (Cornell Laboratory of Ornithology). We selected at maximum 200 whistles with good signal-to-noise ratio from each population (Table 1). We considered whistles tonal narrow-band signals of 100ms or longer. Sound emissions less than 200ms apart were considered the same signal (whistle). We opted to select whistles further away from each other as possible, given our sample size for each population, to increase the probability of englobing as many different individuals as possible. After the whistles were selected we used a Matlab-based routine (Beluga) to extract their frequency contours.

We then used an adaptive resonance theory neural network combined with dynamic time-warping (ARTwarp) to classify the contours (as described by Deecke & Janik, [52]). This method categorizes frequency contours based on the vigilance, a critical similarity value. Because *Sotalia* whistles have relatively simple contours [33] we used a vigilance of 96% to account for minimal differences in our classifications. Unlike the methods previously used to compare *Sotalia* whistles, which normally use a series of separately measured variables; ARTwarp takes the whole whistle contour into consideration when running classifications, it also allows stretching and shrinking the contours up to a factor of three, ensuring maximum overlap in the frequency domain, usually more relevant for animal perception of acoustic signals [52]. This increases the chances of classifying whistles into biologically significant categories. To reduce effect of ambient noise and technical artifacts from the different recording systems used to collect the data we re-sampled every frequency contour at 10ms. We first analyzed each population separately to quantify local repertoires. We then conducted a distribution-wide analysis using the reference contours (neurons) generated from each the local analyses to identify whistle types that are shared ad unique among populations. Input contours of the distribution-wide analysis were not re-sampled, as they were already re-sampled on the local analyses. Those analyses were performed using a Matlab-based routine called ARTwarp.

To evaluate the relationships among populations, we conducted a non-metric multidimensional scaling ordination analysis, using presence and absence of whistle types along the distribution of *Sotalia* dolphins. We used Jaccard's index as a measure of distance. The analysis was conducted on R using the package vegan.

Acknowledgments

The first author was granted a scholarship from the CAPES foundation (Brazilian Ministry of Education).

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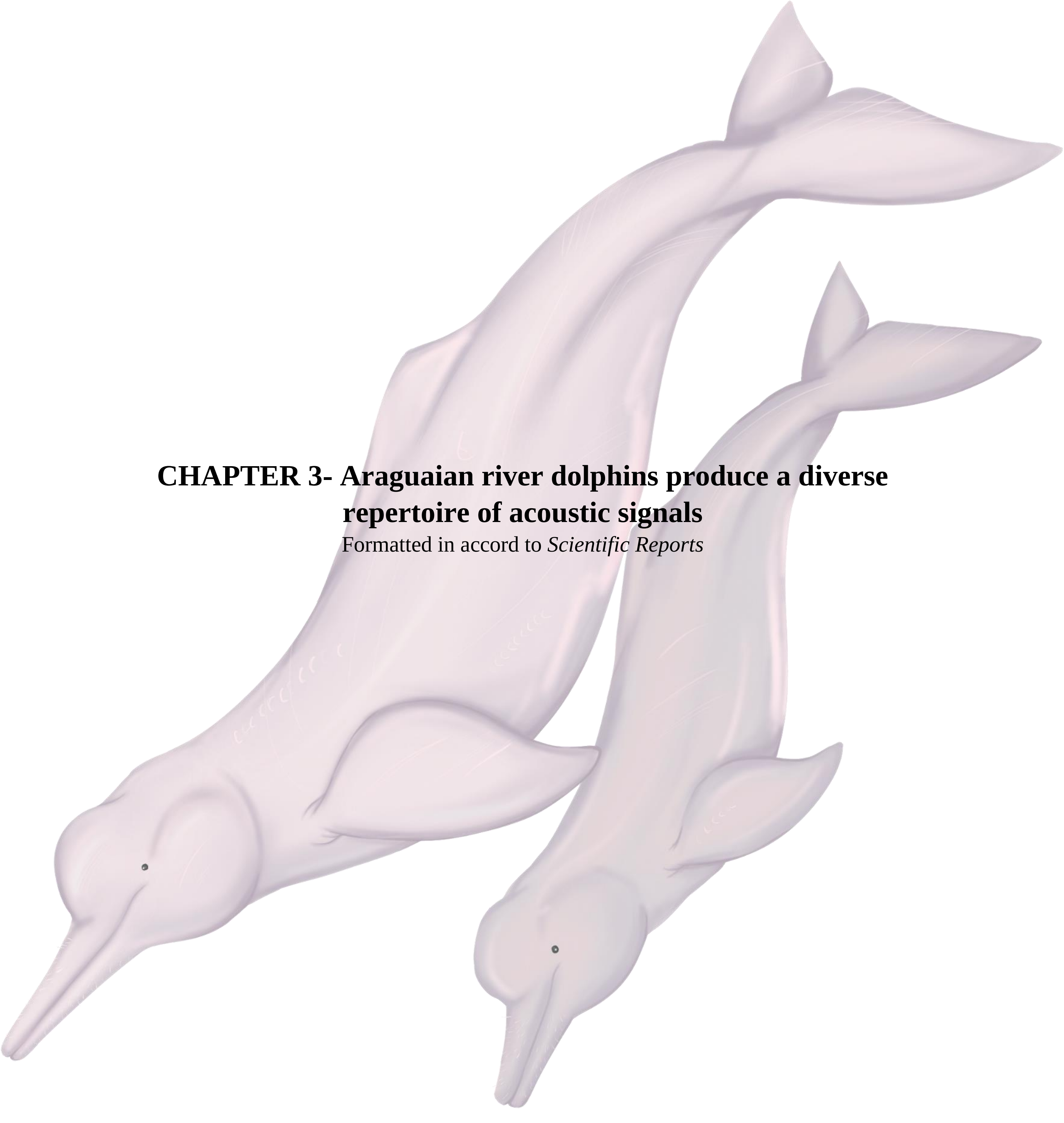

CHAPTER 3- Araguaian river dolphins produce a diverse repertoire of acoustic signals

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Araguaian river dolphins 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 [1-7]. 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 [1, 2, 8-16]. Botos have a preference for habitats with slow currents and high prey concentration such as bays, confluences, small streams, and channels and island margins [17-21]. However, residency patterns vary within locations from long-term residency to occasional visitors [17]. 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 [1, 2, 22].

The acoustic repertoire of botos has traditionally been thought to be limited to a few sounds [23], 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 [23-31]. Ding et al. [28] also described the emission of low-frequency whistles (up to 5 kHz) for Peruvian botos. However, this discovery was disputed [23] due to the presence of sympatric tucuxi dolphins (*Sotalia fluviatilis*) known to emit whistles. Later, May-Collado and Wartzok [30] 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 [28-30] and likely play a different social role as the one described for delphinids [30]. Podos et al. [23] 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.* [31] 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 [1,2,8-16]. Here we studied the acoustic repertoire of free-ranging Arauguaian botos that regularly visit a fish market in Mocajuba in Northern Brazil [7]. This semi-controlled setting gave us the unique opportunity to combine acoustic technology with underwater behavioural observations.

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.1). 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).

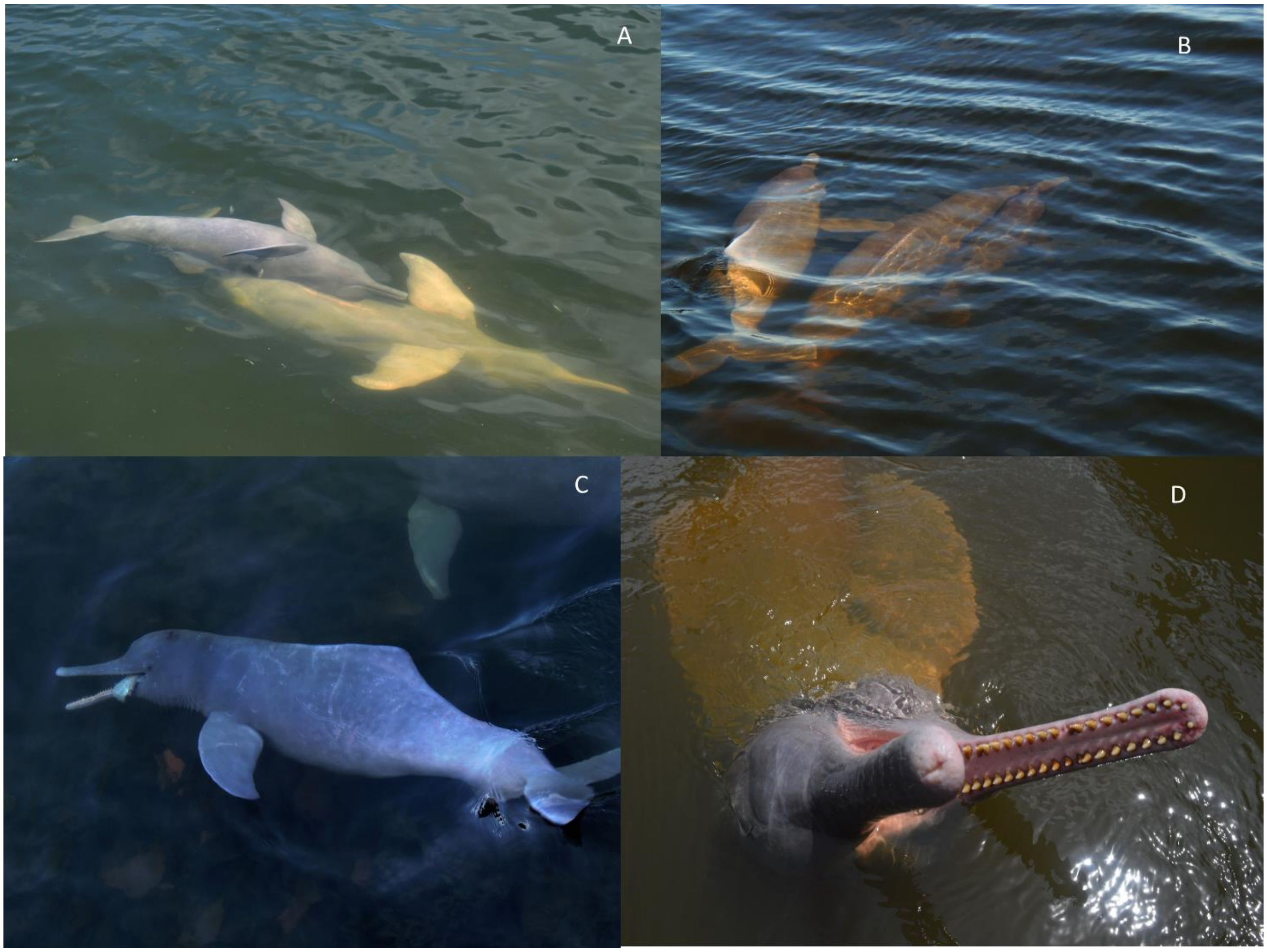

Figure 1. The two behaviours observed during this study were socialization and feeding. (A) and (B): botos engaged in social activity, slow-swimming and physical contact, (C): boto feeding on a fish and (D): waiting to be fed. Photos (A), (B) and (C) by Gabriel Melo-Santos and (D) by Luiza Pereira.

Based on signal quality we selected at 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. 2, 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.3).

However, botos do seem to produce some signals more abundantly than others (Fig. 4). 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.5).

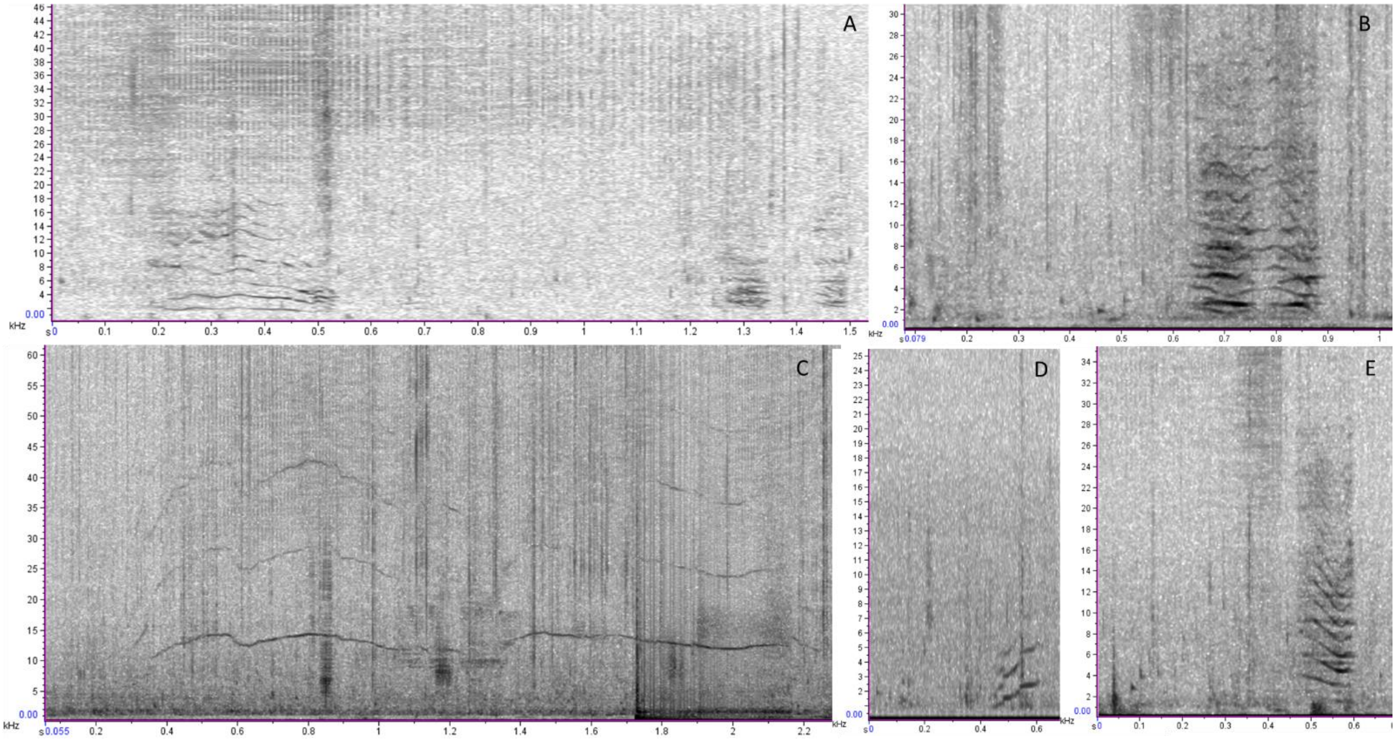

Figure 2. Examples of acoustic signals recorded during our study:(A)- Long call with subharmonics followed by two short two-component calls; (B) -Long two-component call; (C) - Narrow-banded frequency modulated whistle and the longest sound registered in this study; (D) – Short call with no non-linear 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.

Vocalization type	**Number of sub-categories**	**Number of sounds produced**
Long two-component calls - pulsed calls longer than 0.200s, with biphonation as the most marking characteristic, may exhibit subharmonics	9	18
Long calls with subharmonics- calls longer than 0.200s, may exhibit biphonation	5	11
Short two-component calls - pulsed calls shorter than 0.200s, with biphonation as the most marking characteristic, may exhibit subharmonics	29	246
Short calls - signals shorter than 0.200s with no non-linear phenomena	7	29
Short calls with subharmonics - calls shorter than 0.200s, may exhibit biphonation	15	74
Whistles - tonal narrow-banded signals longer than 0.100s, may exhibit harmonics	13	15
Total	**78**	**393**

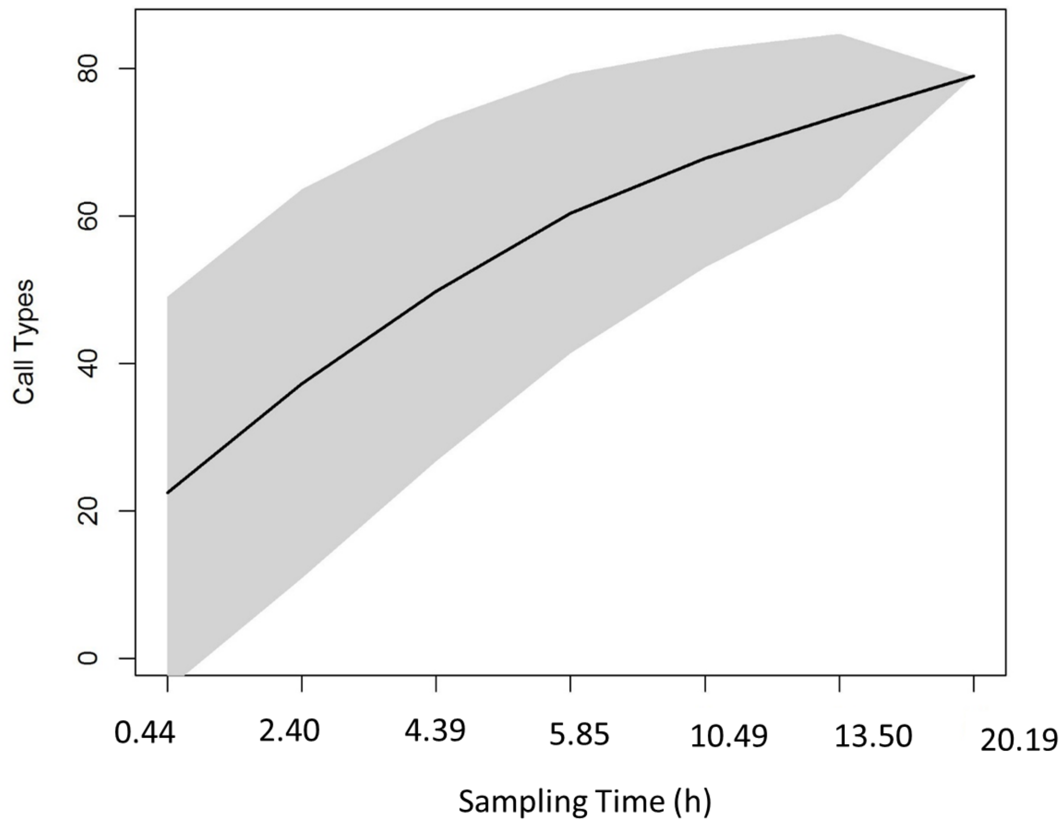

Figure 3. Rarefaction curve showing the cumulative number of call types detected with increasing sampling time (hours of recordings analysed). The curve suggests that 20 hours of acoustic sampling is not enough to capture most of the acoustic repertoire of the Araguaian river dolphin.

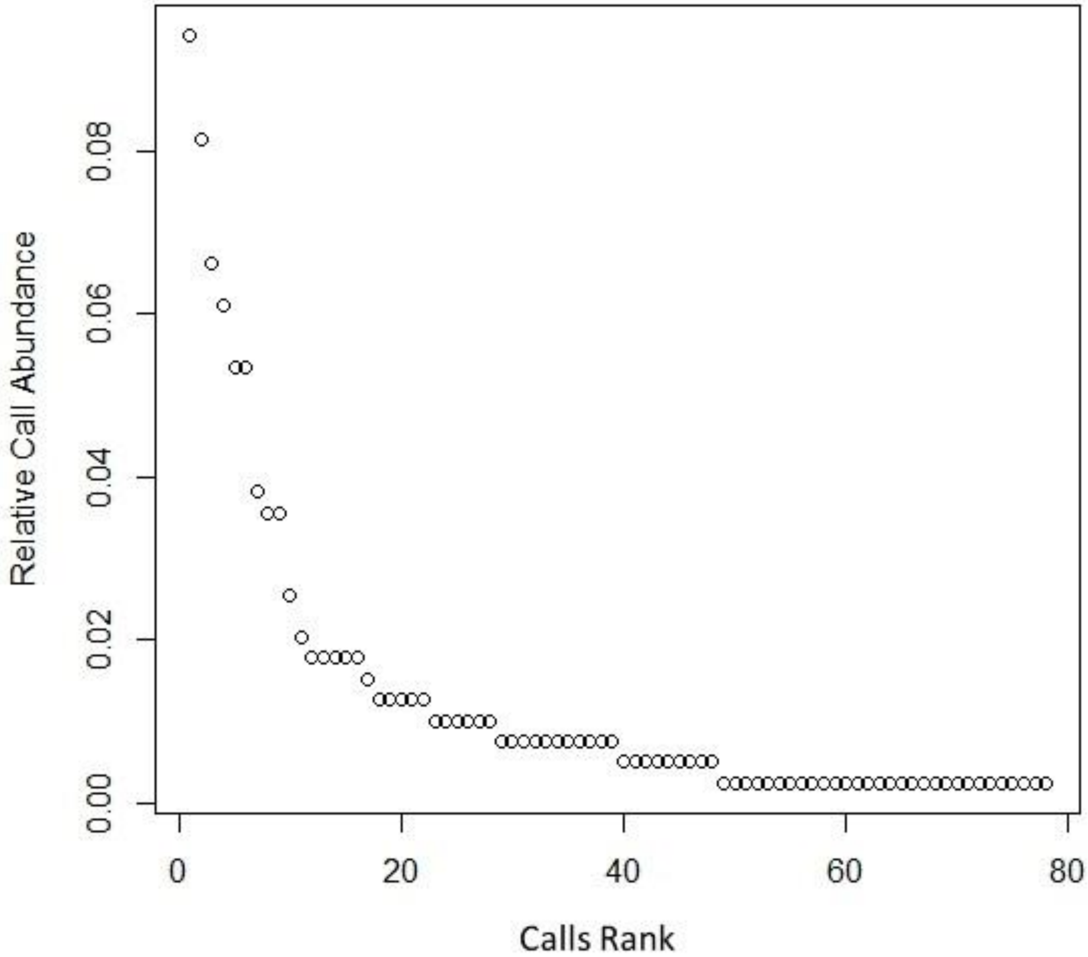

Figure 4. Whittaker diagram displaying the occurrence of calls emitted by Araguaian botos with most calls produced rarely.

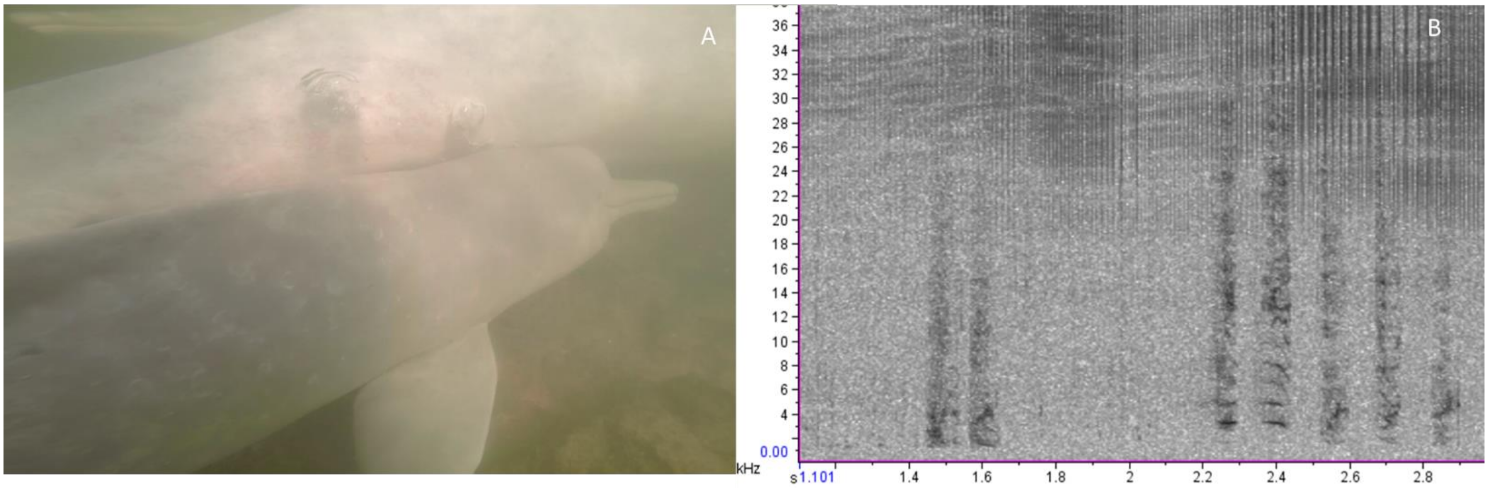

Figure 5. (A): Vocalizing calf as indicated by the bubbles blowing from its blowhole and associated short-two-component call, taken from video footage by Paulo Castro. (B) Spectrogram of calls produced by a calf of Araguaian boto, followed by a bubble stream.

Discussion

Our results show that the Araguaian river dolphin has a more diverse acoustic repertoire than previously documented for the genus *Inia* [23-31]. Early studies described members of the genus *Inia* as silent animals [23] or with a limited acoustic repertoire [23-31]. 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 [28] and Ecuadorian botos [30] and other river dolphins like the Franciscana and the baiji [32-34]. In these studies, the function of emitted whistles was unclear. May-Collado and Wartzok [37] 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 [35-37] 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 [38]. Given the strength of mother-calf associations in botos [1,2] 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 [32].

Several species of toothed whales emit calls of similar acoustic nature as the ones described here for botos [39-54]. 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 [44,46,53,54]. Similarly, Marcoux et al. [50] 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 [43,52]. 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 79 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.

Methods

Study area

This study took place along the Tocantins River in the town of Mocajuba in Pará State, Brazil (Fig. 6). 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 [55]. 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 [56]. There is also a daily cycle of tide pulses [56,57]. 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. 7).

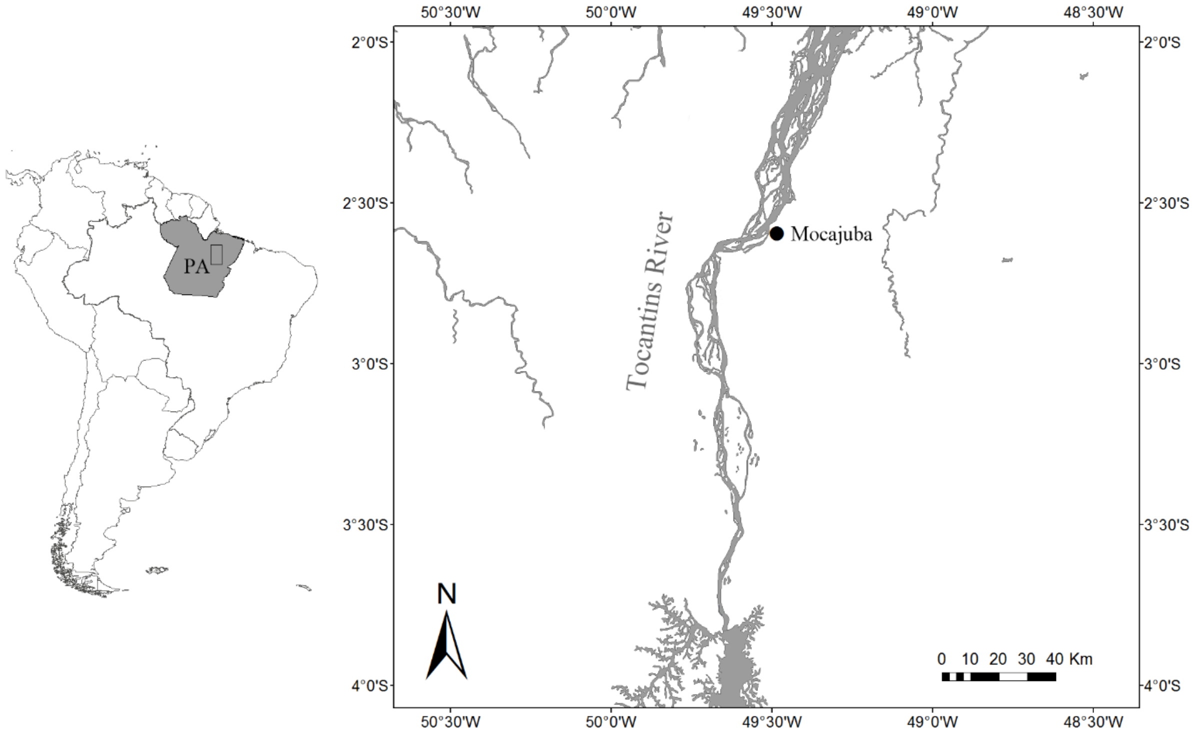

Figure 6. Location of the Mocajuba fish market at the margins of the Tocantins River

Figure 7. Mocajuba’s fish market, clear and shallow waters allow for detailed observation of Araguaian botos’ behaviour. (A): Taken from drone footage by the team of the Laboratório de Geologia de Ambientes Aquáticos/Universidade Federal Rural da Amazônia (LGAA/UFRA). (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 [7]. Therefore our observations took place in the morning. Behavioural observations were collected in a continuous all-events sampling [58]. 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 [7]. 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.

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 Zeland, 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 a *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 1/3 of the fundamental frequency- and (b) biphonation - signals with the presence of two independent fundamental frequencies [59,60]. Pulsed calls produced in a succession where considered a single signal if they were separated by less than 200 ms. Using a rarefaction curve [61], we evaluated how much of the acoustic repertoire was registered during our sampling period. Using a Whittaker diagram [61], we assessed the occurrence of the signals recorded as part of these animal’s repertoire. Analyses were conducted in R.

Acknowledgments

We are thankful to the Rufford Foundation and Cetacean Society International for financing our field work. The first author was granted a scholarship from the CAPES foundation (Brazilian Ministry of Education). We are also thankful to the community of Mocajuba for the support to our work in the market. We are also thankful to Volker Deecke by the contributions to this manuscript.

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4. Final Considerations and Future steps

While we showed that the dolphin whistle repertoire of both *Sotalia* species is highly structured, populations of the riverine species showed a less diverse whistle repertoire than the populations of the coastal species. The high structured repertoire is likely related to the small home ranges and low gene flow among populations. Differences in the richness of the acoustic repertoire between both species, may be due to a combination of socio-ecological and evolutionary factors. An interesting finding in this study, is that the NMDS analysis find the he Tocantins River dolphin population to be a distinct in their acoustic repertoire from other riverine populations of *Sotalia*. Previous studies indicate that the Tocantins River is isolated from the main Amazonian Basin for about 1.8 million years. While molecular and morphological tools are necessary to shed light on the taxonomic identity of these dolphins, the differences in their acoustic repertoire and the geological events suggest that this population might be distinct from other *Sotalia* populations. Thus, we recommend that more efforts are directed on the research of this population of *Sotalia* dolphins.

We also provide the first description of the Araguaian boto (*Inia araguaiensis*) acoustic behavior and showed that they have a rich acoustic repertoire consisting of whistles and primarily pulsed calls. While whistles were produced rarely, a specific type of call, the short two-component calls were the most common signal emitted during the study. These calls were similar in acoustic structure to those produced by orcas (*Orcinus orca*) and pilot whales (*Globicephala* sp.). Because of the context at which these signals were produced, we hypothesize that they possibly play a role in mother-calf communication. To confirm that this acoustic diversity is not influenced by an environment with significant human presence, we are analyzing data from dolphin groups recorded along the course of the Tocantins River to determine if the same level of acoustic diversity is present away from the Mocajuba market.

Sotalia and *Inia* can be acoustically distinguished based on their social sounds as the former has a repertoire based on whistles and the latter based on pulsative calls. With the low emission rate of whistles by *Inia*, so chances of misidentification are low. Thus, sounds of both genera can be used to distinguish them from one another during passive acoustic monitoring and serve as proxies for species presence in studies of distribution, habitat use, and abundance. Furthermore, I am analyzing echolocation from *Sotalia* and *Inia* of the Tocantins River, to improve acoustic detection and classification of these dolphins by using a combination of acoustic signals. Preliminary results indicate that indeed these both riverine species do differ in their echolocation clicks spectral profiles.

Furthermore, we plan to use acoustics for monitoring dolphins along Amazonian Rivers, mainly the Tocantins River. This river is under constant impact by human activities and more development projects are planned for the region. Thus, there is urgent for research and conservation research, given that the river is inhabited by such distinctive lineages of river dolphins.