ENCALHES E ATRIBUTOS BIOECOLÓGICOS PARA A CARACTERIZAÇÃO POPULACIONAL DO BOTO-CINZA (*Sotalia guianensis*) NA COSTA NORTE DO BRASIL

GREICY FERNÁNDEZ RUENES

UNIVERSIDADE ESTADUAL DO NORTE FLUMINENSE - UENF

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Tese apresentada ao Centro de Biociências e Biotecnologia da Universidade Estadual do Norte Fluminense Darcy Ribeiro como parte das exigências para a obtenção do título de Doutor em Ecologia e Recursos Naturais.

Orientador: Prof. Dr. Salvatore Siciliano

Coorientadora: Prof.ª. Dr.ª Larissa Rosa de Oliveira

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DECLARAÇÃO

Eu, Marina Satika Suzuki, coordenadora do Programa de Pós-Graduação em Ecologia e Recursos Naturais (PPG-ERN) da Universidade Estadual do Norte Fluminense Darcy Ribeiro (UENF), seguindo a Resolução CPPG nº2 de 2021, declaro validadas as assinaturas constantes da Folha de Assinaturas da Tese intitulada "**ENCALHES E ATRIBUTOS BIOECOLÓGICOS PARA A CARACTERIZAÇÃO POPULACIONAL DO BOTO-CINZA (*Sotalia guianensis*) NA COSTA NORTE DO BRASIL"** de autoria de Greicy Fernández Ruenes, defendida no dia 27 de janeiro de 2023.

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A mi abuelita,

Es por ti que yo estoy aqui hoy

....”Somos del Sul y venimos del Sul”

.... “Temos uma gigantesca dívida social, e a necessidade de defender a Amazônia, os Mares, e nossos grandes Rios da América... (e) ... é nosso dever biológico, acima de todas as coisas, respeitar a vida e estimula-la, cuida-la, e entender que a espécie (em perigo) somos nos.”

- José (Pepe) Mujica

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APRESENTAÇÂO

O boto-cinza, *Sotalia guianensis,* é um dos cetáceos mais abundante nas costas brasileiras. Contudo, até a data não existe uma análise abrangente sobre as condições da população do boto-cinza na região Norte do Brasil, bem como a influência das pescarias e, portanto, da captura acidental como uma pressão sistemática, possivelmente afetando a taxa de mortalidade e o padrão dos encalhes da espécie na região. Além disso, aspectos relacionados à morfologia e história de vida dos indivíduos, que permitiriam a caracterização da população, também não têm sido abordados. Nesse sentido esta tese teve como objetivos descrever o padrão espacial, sazonal e de longo-prazo dos encalhes de *Sotalia guianensis* no Estuário Amazônico, ao longo de um período de 10 anos de amostragens sistemáticos. Em adição, determinar e avaliar a estrutura etária da população a partir de dados originários de eventos de encalhe. Além disso, foi proposto descrever o crescimento pós-natal de *Sotalia guianensis* do Estuário Amazônico a partir da relação da idade com o comprimento total do crânio, assim como possíveis diferenças na morfologia e no desenvolvimento do crânio com outras populações da costa Atlântica Sul-ocidental. Finalmente, objetivou-se também determinar, a partir do uso da técnica de estimação de idade e da caracterização da morfologia dentaria, a influência do comercio ilegal de partes corporais de golfinhos nos mercados tradicionais do Norte e Nordeste do Brasil, na mortalidade de *Sotalia guianensis*.

Este manuscrito conta com uma introdução geral e quatro capítulos principais, cada um dos quais foi formatado como artigo científico. Nesse sentido, o Capítulo I do documento inclui uma revisão bibliográfica sobre o processo de encalhe de mamíferos marinhos, sua caracterização, a relação com fatores naturais e as potenciais inferências ecológicas que este tipo de amostragem permite fazer. Este capítulo constitui uma síntese do exame de qualificação, e devido a sua relevância para o tema da tese foi incluído no documento final como capitulo inicial. O mesmo foi redigido em português e seguindo as normas ABNT. Os dois primeiros objetivos da tese foram sintetizados no Capítulo II do manuscrito. O mesmo foi redigido em inglês e segundo as normas da revista *Aquatic Conservation: Marine and Freshwater Ecosystems*, à qual foi submetido. O objetivo três se encontra desenvolvido no Capítulo III, o qual está redigido também em inglês e foi formatado segundo as normas da revista *Journal of the Marine Biological Association of the United Kingdom,* à qual será submetido. Finalmente, o Capítulo IV da resposta ao último objetivo, sendo redigido em inglês e formatado a partir das normas da revista *Frontiers in Marine Science,* na qual foi publicado. Todas as tabelas e figuras foram inseridas no texto para facilitar a leitura do manuscrito. Nos ANEXOS vão ser achados os materiais suplementais para cada um dos capítulos citados.

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RESUMO

Esta tese destaca a eficácia dos dados de encalhe na avaliação das populações de mamíferos marinhos e como indicadores da sua interação com as condições ambientais circundantes. Além disso, o uso da morfologia craniana e dentária dos cetáceos, especialmente o boto-cinza *Sotalia guianensis*, como fornecedora de informações sobre a variação geográfica e adaptabilidade às condições ambientais dos mamiferos marinhos, identificação de espécies, hábitos alimentares, crescimento e determinação da idade cronológica. O boto-cinza, apresenta distribuição restrita à região costeira tropical da América Central e do Sul no Oceano Atlântico. Devido ao padrão de dispersão das populações e o fluxo gênico limitado, a área de distribuição da especie tem sido dividida em 12 Unidades de Manejo (*Management Unit* – MUs), dessas, oito localizadas no Brasil, e uma delas no Estuário Amazônico. O padrão espacial, sazonal e de longo-prazo dos encalhes de S. guianensis no Estuário Amazônico foram avaliados para 12 anos de amostragem sistemático. Foi detectado um ciclo sazonal de encalhes possivelmente relacionado ao ciclo hidrológico da região e à interação com a pesca artesanal. A análise da variação geográfica do tamanho do crânio, a partir do comprimento côndilo-basal (CBL) de 361 espécimes, mostrou diferenças no crescimento e tamanho entre as diferentes MUs do Norte, Nordeste, Sudeste e Sul do Brasil, com espécimes maiores encontrados em regiões mais oligotróficas. Isso pode ser relacionado ao fato de que em regiões oligotróficas, crânios e dentes maiores possibilitariam a captura de presas de tamanhos variados, aumentando os recursos disponíveis na área. Finalmente, a partir do uso da técnica de determinação de idade e a caracterização da morfologia dentária, foram identificados 118 dentes de *S. guianensis* e *S. fluviatilis* no comercio ilegal de partes corporais de golfinhos, nos mercados tradicionais do Norte e Nordeste do Brasil. A frequência de idades desses dentes sugere uma estrutura etária parcialmente influenciada pela captura acidental. Os resultados corroboram a eficácia dos dados de encalhe para avaliar as alterações temporais nas populações costeiras de mamíferos aquáticos, especialmente no que se refere à mortalidade e estrutura populacional de Cetáceos. Alem disso, sugerem um efeito temporal e um impacto humano no Norte do Brasil e ressaltam a importância da morfometria e ontogenia para aprimorar a diferenciação entre as Unidades de Manejo estabelecidas. Conjuntamente, as analises morfométricas revelaram novas informações sobre as populações do boto-cinza do Norte e Nordeste do Brasil e destacam a adaptavilidade da espécie às diferentes condições ambientais do Atlantico Sul Ocidental.

Palavras-chaves: Conservação de Cetaceos, Morfologia craniana, crescimento, mortalidade, Estuario Amazônico

ABSTRACT

This thesis highlights the effectiveness of stranding data in assessing marine mammal populations and as indicators of their interaction with surrounding environmental conditions. Furthermore, the use of cranial and dental morphology of cetaceans, especially the Guiana dolphin *Sotalia guianensis*, as a provider of information on geographic variation and adaptability to environmental conditions of marine mammals, for species identification, feeding habits diferentiation, growth and chronological age determination. The Guiana dolphin has a distribution restricted to the tropical coastal region of Central and South America in the Atlantic Ocean. Due to the dispersal pattern of the populations and the limited gene flow, the distribution area of the species has been divided into 12 Management Units (MUs), of which eight are located in Brazil, and one in the Amazon Estuary. The spatial, seasonal and long-term patterns of strandings of *S. guianensis* in the Amazon Estuary were evaluated for 12 years of systematic sampling. A seasonal cycle of strandings was detected, possibly related to the hydrological cycle of the region and the interaction with artisanal fisheries. Analysis of geographic variation in skull size, from the condyle-basal length (CBL) of 361 specimens, showed differences in growth and size between the MUs from North, Northeast, Southeast and South of Brazil, with larger specimens found in more oligotrophic regions. This may be related to the fact that in oligotrophic regions, larger skulls and teeth would make it possible to capture prey of varying sizes, increasing the resources availability in the area. Finally, using the age determination technique and the characterization of tooth morphology, 118 teeth of *S. guianensis* and *S. fluviatilis* were identified in the illegal trade of dolphin body parts in traditional markets of the North and Northeast of Brazil. The age frequency of these teeth suggests an age structure partially influenced by incidental capture. The results corroborate the effectiveness of stranding data to assess temporal changes in coastal populations of aquatic mammals, especially regarding mortality and population structure of Cetaceans. Furthermore, they suggest a temporal effect and a human impact in Northern Brazil. The results also highlight the importance of morphometry and ontogeny to improve differentiation among the established Management Units. Moreover, the morphometric analyses reveal new information about the Guiana dolphin populations in the North and Northeast of Brazil and highlight the adaptability of the species to the different environmental conditions in the South Western Atlantic

Key words: Cetacea conservation, cranial morphology, growth, mortality, Amazon estuary

INTRODUÇÃO GERAL

Os dados originários de encalhes constituem uma ferramenta efetiva para determinar a presença de cetáceos em uma área (*e.g.,* PYENSON, 2011; TRUCHON *et al*., 2013; LÓPEZ-BERENGUER *et al*., 2020). Além disso, possibilitam a obtenção de dados sobre a biologia e ecologia das populações de mamíferos marinhos, incluindo, por exemplo, informações sobre sua história de vida e dinâmica populacional *(e.g.,* FERNANDEZ & HOHN, 1998; DI BENEDITTO *et al*., 2004; MANNOCCI *et al*., 2012), incidência de doenças e contaminantes (*e.g*., BENNET *et al.,* 2000; DE MOURA et al., 2014; FENTON *et al*., 2017), informações sobre ecologia alimentar (*e.g*., BISI *et al*., 2013; COSTA *et al*., 2020; DRAGO *et al.,* 2021) e estrutura das populações (*e.g*., CABALLERO *et al*., 2018; DOS SANTOS *et al*., 2018). Muitos destes dados de outra forma não estariam disponíveis devido as limitações ainda existentes para amostragens e no estudo deste grupo de animais no ambiente marinho.

Contudo, dados de encalhes são muitas vezes subestimados, devido à falta de controle sobre o processo de encalhe. Isto se evidencia pelo caráter principalmente oportunista das amostragens (PELTIER *et al*., 2012; PIKESLEY *et al*., 2012) e o desconhecimento da origem geográfica dos animais encalhados que é causado pela influência da oceanografia e a topografia costeira na variação periódica destes eventos (EVANS *et al*., 2005; HART *et al*., 2006; EVANS, 2012). Porém, apenas por meio da compreensão dos processos de deriva e encalhe, podem ser entendidas as relações entre esses registros e aspectos ecológicos e biológicos fundamentais relativos às populações e comunidades de mamíferos marinhos (PELTIER *et al*., 2014). Isto, possibilitando a interpretação de padrões em termos de estrutura e mortalidade das populações ao longo prazo, o que permitiria, por exemplo, a detecção de áreas ou estações com alta probabilidade de interações entre a pesca e as comunidades de cetáceos (SAAVEDRA *et al*., 2017).

No Brasil, pesquisas relativas ao padrão de encalhes de animais marinhos, incluindo aves, tartarugas e mamíferos marinhos, tem se incrementado em anos recentes, conjuntamente com o aperfeiçoamento e padronização dos métodos de amostragem e monitoramento (PRADO *et al*., 2016, 2022; MAYORGA *et al*., 2020; TAVARES; ALVARES *et al*., 2021; CREMER *et al*., 2022; RAMOS *et al*., 2022). A Rede de Encalhe e Informação de Mamíferos Aquáticos do Brasil (REMAB), coordenada pelo Ministério do Meio Ambiente por meio do Instituto Chico Mendes de Conservação da Biodiversidade (ICMBio) e do Centro Mamíferos Aquáticos (CMA), é formada pela Rede de Encalhes de Mamíferos Aquáticos do Norte (REMANOR), a Rede de Encalhes de Mamíferos Aquáticos do Nordeste

(REMANE), a Rede de Encalhes de Mamíferos Aquáticos do Sudeste (REMASE) e a Rede de Encalhes de Mamíferos Aquáticos do Sul (REMASUL). Esta associação de instituições com abrangência nacional, é um exemplo do esforço na coordenação e estandardização das metodologias de monitoramento dos encalhes. Como parte deste processo, a criação do Sistema de Monitoramento de Mamíferos Marinhos (SIMMAM), repositório integrado de dados de registros de encalhes e avistamento de mamíferos marinhos que é continuamente atualizada por pesquisadores, organizações não-governamentais (ONG), institutos de pesquisa e empresas que participam dos monitoramentos tem permitido o acesso a dados antigamente espalhados e a novas informações sobre as diferentes espécies de mamíferos registradas em águas brasileiras. A partir desses dados, estudos sobre o fenômeno do encalhe como processo físico e biológico (*e.g.*, TAVARES *et al*., 2021; PRADO *et al*., 2022), assim como pesquisas relativas aos indivíduos encalhados (*e.g.*, COSTA *et al*., 2017; CONVERSANI *et al*., 2021; CREMER *et al*., 2022), tem permitido a melhor caracterização das espécies de animais marinhos nas costas do Brasil, e principalmente das populações costeiras de cetáceos.

O boto-cinza, *Sotalia guianensis* (van Bénéden, 1864) (Cetacea, Delphinidae), é um dos cetáceos mais abundante nas costas brasileiras. Não obstante, apesar da distribuição geográfica da espécie abranger grande parte da região costeira da América Central e do Sul, no Oceano Atlântico, estendendo-se desde Santa Catarina, no Brasil, até Nicarágua, realmente pequenas populações estuarinas-costeiras tendem a estar concentradas irregularmente ao longo desse intervalo geográfico (FLORES *et al*., 2018). Além disso, o boto-cinza é caracterizado por formar grupos relativamente pequenos (dentre 3 e 13 indivíduos), de elevada filopatria, e consequentemente, com áreas de vida pequenas (AZEVEDO & VAN SLUYS, 2005; ARAÚJO *et al*., 2007; CABALLERO *et al*., 2010; BARROSO *et al*., 2014). Essas características, conjuntamente com sua preferência por áreas rasas, e o pequeno tamanho corporal dos indivíduos, limitam a sua dispersão, o que reduz notavelmente o possível intercambio gênico entre as populações (CABALLERO *et al*., 2018). Isto tem levado à categorização de 12 Unidades de Manejo (*Manegment units* – MUs) ao longo da área de distribuição da espécie, dessas oito sendo estabelecidas no Brasil (CUNHA *et al.,* 2020 *apud* DOMIT *et al*., 2021). Essa subdivisão em MUs é reforçada por resultados que confirmam diferenças na morfologia, acústica, história de vida e ecologia alimentar entre as populações da espécie residentes nas diferentes regiões (DOMIT *et al*., 2021).

Na atualidade, a captura acidental em redes de pesca artesanais permanece como a principal causa de mortalidade para o boto-cinza e gera preocupação quanto a conservação da maioria das populações. Nesse aspecto, capturas acidentais da espécie têm sido amplamente documentadas (*e.g.,* SICILIANO *et al*., 1994) DA SILVA; BEST, 1996; BENEDITTO *et al*., 1998; NERY et al., 2008; ZAPPES *et al*., 2009; REEVES *et al*., 2013; SICILIANO *et al*., 2017)*,* existindo inclusive relatos sobre caça intencional para consumo humano e para seu uso como isca na pesca de tubarão e camarão (DA SILVA & BEST, 1996). Igualmente, existem evidências sobre altos níveis de cargas de contaminantes e uma variedade de doenças emergentes (*e.g.* morbillivírus, herpesvirus, doenças de pele de etiologia desconhecida) afetando as diversas populações da espécie ao longo do litoral brasileiro (VAN BRESSEM *et al*., 2009; DE MOURA *et al*., 2014; SEADE *et al.*, 2017). Consequentemente, populações da espécie são atualmente consideradas como "vulneráveis" no Brasil, na Colômbia e na Venezuela devido aos efeitos combinados destes fatores, e a mesma tem sido avaliada pela IUCN como espécie "Quase ameaçada" (*Near Threatened* em inglês) (SECCHI *et al*., 2018). A espécie se encontra listada também no Apêndice I da Convenção sobre o Comércio Internacional de Espécies Ameaçadas de Extinção (CITES), o que proíbe sua caça e comercialização.

No Estuário Amazônico e ao longo do litoral norte do Brasil, as particularidades físico-geográficas da região costeira, delineada por séries de reentrâncias, e a presença de extensas áreas conservadas de manguezais, incluindo numerosas baías e estuários, brindam às populações de botos-cinza uma variedade de hábitats costeiros preferenciais. Isto justifica sua abundância na região (TORRES & BEASLEY, 2003; EMIN-LIMA et al., 2010). Contudo, dados sobre biologia, morfologia, genética e ecologia das populações de *S. guianensis* do litoral norte brasileiro são escassos e relativamente recentes (*e.g*., FETTUCCIA et al., 2009; LAETA *et al*., 2010; ARCOVERDE *et al*., 2014; CABALLERO *et al*., 2018; COSTA *et al*., 2020; SICILIANO et al., 2008). Os estudos mais atuais sobre estrutura genética confirmam as populações do boto-cinza da costa norte brasileira como uma unidade de manejo independente, diferenciada das populações das regiões nordeste, sudeste e sul do Brasil, e que mantém um fluxo gênico com populações vizinhas localizadas mais ao norte, como por exemplo as da Guiana Francesa (CABALLERO *et al*., 2018). Esses resultados apoiam os estudos preliminares que descrevem também diferenças morfológicas entre os indivíduos da região norte e os indivíduos das regiões nordeste, sudeste e sul (FETTUCCIA *et al*., 2009; FETTUCCIA, 2010; ARCOVERDE *et al*., 2014). A maioria desses estudos, no entanto, focaram na determinação das diferenças entre as duas espécies do gênero *Sotalia* (*S. guianensis* e *S. fluviatilis*), e abordaram de forma secundária a variação geográfica na morfologia do boto-cinza. Sendo assim, é reconhecido pelos referidos autores a necessidade de estudos mais aprofundados relacionados à biologia, morfologia e variação geográfica da espécie no litoral brasileiro.

Dentro do estuário Amazônico, *S. guianensis* parece ser a espécie de cetáceo mais abundante, mais também com o maior número de encalhes registrados (SICILIANO *et al*., 2008). A elevada produtividade desse litoral sustenta uma grande diversidade e biomassa de peixes, que faz com que a costa norte do Brasil apresente um forte desenvolvimento da atividade pesqueira (BRITO, 2012). Consequentemente, a captura acidental de *S. guianensis* nessa área, devido à interação com a atividade de pesca, tem sido amplamente registrada, indicando um elevado número de exemplares capturados, principalmente no litoral dos estados do Pará e Amapá (BELTRÁN-PEDREROS *et al*., 2006; EMIN-LIMA *et al*., 2010). Aliás, o número de mortes causadas por emalhe nas redes de pesca, bem como a agressão intencional, tem se incrementado consideravelmente ao longo dos anos na região para essa e outras espécies de mamíferos aquáticos (ALVES; ROSA, 2008; ALVES *et al*., 2012; IRIARTE & MARMONTEL, 2013). Os exemplares capturados podem ter partes retiradas para seu uso ou comercialização como amuletos tradicionais, e os restos são habitualmente descartados, pelo que frequentemente encalham na costa (SICILIANO *et al*., 2018).

Apesar da relação entre as pescarias, a captura acidental e os encalhes ser amplamente documentada no litoral brasileiro para o boto-cinza, nenhum estudo tem avaliado a variação espaço-temporal dessa mortalidade no litoral amazônico, levando em consideração aspectos biológicos da espécie, assim como possíveis mudanças nas características climáticas e oceanográficas da região. Pressões antrópicas e/ou ambientais, em um contexto mais amplo, influenciam a distribuição e aspectos de história de vida das populações de mamíferos marinhos, podendo levar, por exemplo, a seu deslocamento das áreas especificamente designadas para sua proteção, à medida que respondem a estas variações (MACLEOD, 2009; SCHUMANN *et al*., 2013; MEAGER & LIMPUS, 2014). Aliás, os poucos estudos realizados em áreas tropicais e subtropicais evidenciam a necessidade de mais investigações sobre os efeitos a longo prazo destes atributos climáticos e as pressões antrópicas sobre as populações de mamíferos marinhos nas regiões costeiras. Por outro lado, no caso de *S. guianensis* do estuário amazônico, até a data não existe uma análise abrangente sobre as condições atuais da população e a influência das pescarias e, portanto, da captura acidental como pressão sistemática sobre a espécie, possivelmente afetando a taxa de mortalidade e o padrão dos encalhes da espécie na região. Além disso, aspectos biológicos ligados a estrutura etária dos indivíduos encalhados, que pode ser afetada pela pressão da captura acidental, assim como aspectos relacionados à morfologia e história de vida dos indivíduos, que permitiriam a caracterização da população também não têm sido abordados.

Levando-se em consideração toda a informação supracitada, são propostos como objetivos desta pesquisa:

- Descrever o padrão espacial, sazonal e de longo-prazo dos encalhes de *Sotalia guianensis* no Estuário Amazônico, região norte do Brasil.
- Determinar e avaliar a estrutura etária da população de *Sotalia guianensis* do Estuário Amazônico, região norte do Brasil, a partir de dados originários de eventos de encalhe.
- Descrever o crescimento de *Sotalia guianensis* do Estuário Amazônico a partir da relação da idade com o comprimento total do crânio, assim como possíveis diferenças na morfologia e no desenvolvimento pós-natal do crânio com outras populações da costa Atlântica Sul-ocidental.
- Determinar, a partir do uso da técnica de determinação de idade e a caracterização da morfologia dentária a relação do comercio ilegal de partes corporais de golfinhos nos mercados tradicionais do Norte e Nordeste do Brasil com os encalhes de *Sotalia guianensis*.

Como hipóteses propõem-se as seguintes:

- Levando-se em consideração os aspectos ambientais que mais influenciam no padrão de encalhes dos cetáceos e as características oceanográficas e climáticas próprias da costa norte do Brasil, espera-se uma variação sazonal da frequência e distribuição dos encalhes de *S. guianensis* no Estuário Amazônico.
- Levando-se em consideração o histórico de captura acidental, espera-se que a pesca seja o principal fator influenciado na estrutura etária doa animais encalhados de *S. guianensis* na região, sendo refletido numa mortalidade maior de animais jovens e sub-adultos.
- Levando em consideração as intrínsecas variações genéticas e morfológicas da população de *S. guianensis* do Estuário Amazônico, assim como as características ambientais dessa região, espera-se que o padrão de crescimento do crânio dos indivíduos dessa população seja diferente aos das outras populações da espécie nas regiões Nordeste, Sudeste e Sul do Brasil.
- Tendo em consideração que estudos genéticos demostraram a maioria do comercio de partes corporais de “boto” para uso tradicional no Brasil é baseado em espécimes de *Sotalia guianensis*, provavelmente capturados acidentalmente, espera-se que o comércio de dentes usados como suvenires no Norte e no Nordeste do Brasil tenham a mesma origem.

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CAPÍTULO I: ENCALHES DE CETÁCEOS: CARACTERIZAÇÃO, RELAÇÃO COM FATORES NATURAIS E POTENCIAIS INFERÊNCIAS ECOLÓGICAS

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Resumo

No estudo e entendimento das populações de cetáceos, a importância da análise de dados originários de encalhes, como fonte de informação sobre a biologia, ecologia, saúde e status de conservação de espécies e populações em regiões especificas é reconhecida. O presente estudo tem como objetivos apresentar uma síntese do conhecimento atual sobre o fenômeno dos encalhes nos cetáceos, focando-se na caracterização do processo e sua complexidade (componentes físicos, biológicos e sociais). Além disso pretende-se avaliara a representatividade e limitações dos dados de encalhes, o uso de dados de encalhes como indicadores de abundancia e mortalidade natural relativas, riqueza de espécies e estrutura das comunidades de cetáceos adjacentes aos sítios de encalhes, e avaliar a relação da taxa de encalhe com fatores naturais em diferentes escalas temporais e espaciais, visando descrever possíveis padrões ecológicos que tais resultados permitem realizar. Dados de encalhes são muitas vezes subestimados, devido à falta de controle sobre o processo, o caráter oportunista das amostragens, o desconhecimento da origem geográfica dos espécimes e a complexidade na quantificação dos múltiplos componentes. Apesar dessas limitações, é bem aceito que indivíduos encalhados representam uma medida mínima da mortalidade no mar, e que apenas por meio da compreensão dos processos de deriva e encalhe, podem ser entendidas as relações entre esses registros e a dinâmica das populações dos mamíferos marinhos. Conjuntamente, o acúmulo de dados históricos de encalhes possibilitaram a análise de tendências temporais, permitindo definir a linha de base para taxas de encalhe espécie-específicas, bem como métricas biológicas e ecológicas da população encalhada e suas relações com fatores ambientais de pequena e grande escala espaço-temporal. Contudo o intervalo da maioria dos estudos ainda não é suficiente para estimar variações a escalas de macro-temporais, principalmente pela necessidade de uso de dados de coleta sistemáticas. É sugerido que estudos focados em uma espécie ou grupo de espécies de cetáceos em uma localidade geográfica específica, permitem obter definições regionais e sazonais que não são obtidos em estudos macroecológicos a partir de dados istemáticos de encalhes.

Palavras chaves: Cetacea, Mortalidade, Monitoramento de praia, Fatores ambientais, Variação espaço-temporal

1. Introdução

De forma mais ampla, o termo encalhe define qualquer mamífero marinho descoberto na linha de costa (incluindo praias, bancos de areia, ou qualquer outra área litorânea), derivado pelas correntes marinhas, vento e/ou a maré até a terra, estando vivo ou morto (GERACI & LOUNSBURY, 2005; COOMBS *et al.*, 2019). Na atualidade uma definição mais detalhada do fenômeno de encalhe nos mamíferos marinhos inclui espécimes achados mortos na linha de costa, qualquer cetáceo achado vivo, pinípedes doentes ou feridos, mamíferos marinhos fora de seu habitat originário, casos específicos de carcaças flutuantes ou reflutuantes e carcaças presas em redes de emalhe ou algum outro tipo de lixo marinho (MOORE *et al.,* 2018).

A partir dos dados de encalhes é possível obter informação sobre a presença de cetáceos em uma área (*e.g.,* PYENSON, 2011; COOMBS *et al.* 2019; ALVARADO-RYBAK *et al.,* 2020; MAYORGA *et al.,* 2020), ou sobre a saúde e *status* de conservação de espécies e populações em regiões especificas (*e.g*., FENTON *et al.,* 2017; LAVANDIER *et al.* 2019). O número de espécimes encalhados, apesar de não ser uma evidência direta da estrutura populacional, está relacionado com aspectos como o padrão de abundância e mortalidade da população, que podem variar sazonal ou anualmente (PELTIER *et al.,* 2012; PIKESLEY *et al.,* 2012). Em adição, a relação entre a distribuição espacial das populações e a ocorrência de fenômenos que possam causar essas mortalidades, seja de forma natural ou induzida pelo homem, também podem ser inferidas (TRUCHON *et al.,* 2013; MEAGER & LIMPUS *et al.,* 2014; SAAVEDRA *et al.,* 2017).

Nesse sentido, o entendimento da relação entre fatores ambientais (*e.g.,* temperatura superficial do mar, precipitações, eventos de seca ou inundações e ocorrência de furacões) e a taxa de encalhe de mamíferos marinhos, pode ser fundamental para determinar tendências temporais no comportamento das populações e comunidades, como resposta às variações do ambiente marinho, e na previsão de possíveis impactos dos eventos climáticos extremos e das mudanças climáticas em geral sobre este grupo (SCHUMANN *et al.,* 2013; MEAGER & LIMPUS, 2014). Contudo, dados de encalhes são muitas vezes criticados devido à falta de controle sobre o processo, o que se evidencia pelo caráter principalmente oportunista das amostragens e o desconhecimento da origem geográfica dos espécimes (WIESE & ELMSLIE, 2006; PELTIER *et al*., 2012; PIKESLEY *et al.,* 2012). Além disso, a complexidade na quantificação dos múltiplos componentes do processo de encalhe tem gerado incertezas em torno do valor ecológico do registro de encalhe como informação das populações no mar (HART *et al.,* 2006; PELTIER *et al.,* 2013).

Por esse motivo, para realizar inferências espaciais e/ou temporais sobre o fenômeno dos encalhes, e sua relação com variações ambientais no longo e curto prazo, a coleta sistemática de dados e amostras biológicas são imprescindíveis, o que é escasso na maioria dos processos de registro ou séries de monitoramento. Isto permitiria tanto entender o fenômeno como processo natural, influenciado por fatores bióticos e abióticos, como lograr discernir processos biológicos intrínsecos das espécies e correlações com fatores antrópicos que afetam as populações. Porem, apesar das limitações, eventos de ecalhes representam uma amostra básica do que acontece no mar, e apenas por meio da compreensão dos processos de deriva e encalhe, podem ser entendidas as relações entre esses registros e a abundância relativa, riqueza de espécies e a mortalidade dos mamíferos marinhos (PYENSON, 2011; PELTIER *et al.,* 2014).

O presente estudo tem como objetivo apresentar uma síntese do conhecimento atual sobre o fenômeno do encalhe nos cetáceos, buscando sua caracterização como processo natural, a identificação dos principais fatores relacionados, assim como inferências ecológicas possíveis de serem feitas a partir de tais dados. Nesse sentido, pretende-se realizar a) uma avaliação crítica da representatividade e limitações dos dados de encalhes, a partir da revissão de fatores físicos, biológicos e sociais envolvidos no processo de amostragem de encalhes, b) analisar o uso dos dados de encalhes como indicadores de abundância relativa, mortalidade natural relativa, riqueza de espécies e estrutura das comunidades de cetáceos adjacentes aos sítios de encalhes na literatura, e c) discutir a relação da taxa de encalhe de cetáceos com fatores naturais a diferentes escalas temporais e espaciais para delinear possíveis padrões ecológicos que tais resultados permitem realizar.

2. Materiais e Método

Uma busca bibliográfica avançada nas fontes Scopus, Periódicos Capes e Google Scholar foi realizada usando-se como palavras chaves principais "*Cetacea*", "*Mysticeti", "Odontoceti", "dolphin"* e *"whale"*, sempre combinadas com "*stranding*" e os equivalentes "*stranding rate"*, "*stranding record*" e "*natural mortality*". A essas combinações foram adicionadas as palavras "*diversity*", "*abundance*", "*temporal rate*", "*geografic distribution*", "*season*", "*environmental factors*", "*climatic*" e "*beach survey*". Somente foram selecionados na busca, artigos revisados por pares, relatórios de programas de monitoramento com séries temporais de mais de 10 anos de coleta (*e.g.*, NOAA Technical Report- NMFS, Acordo sobre Conservação de Pequenos Cetáceos do Mar Báltico, Atlântico Nordeste, Irlanda e Mar do Norte – ASCOBANS) e Dissertações e Teses de igual abrangência temporal.

A classificação dos mesmos foi realizada segundo o período de abrangência, o tópico tratado – Registro, ou Tendência, e os aspectos específicos avaliados – Diversidade, Ocorrência, Variação temporal, Variação Espacial, Sazonalidade, Fatores ambientais (Anexo Capitulo1: Tabela 1). Os artigos relacionados aos fatores ambientais também foram classificados segundo o fator discutido ou correlacionado com os encalhes, e sua distribuição espaço-temporal, em Fenômenos regionais (fatores que caracterizam as condições ambientais de cada localidade especifica), Fenômenos continentais (fenômenos climáticos de grande escala), Eventos estocásticos, Recursos locais, e Características físicas do ambiente. Isto baseado no delineamento proposto por TRUCHON *et al.* (2013) para avaliar padrões temporais nos encalhes de espécies de mamíferos marinhos residentes, migrantes e raras. Artigos que somente relataram a ocurrência de espécies ou a descrição de registros para uma área ou período determinado foram posteriormente eliminados da seleção.

3. Resultados e Discussão

Um total de 274 documentos relacionados exclusivamente a taxa de encalhe e mortalidade natural de cetáceos, publicados entre 1971 e 2022 foram encontrados. Os tópicos mais recorrentes foram o de Registro e o de Registro relacionado à Tendências temporais, contabilizando 202 documentos. Nos aspectos específicos, artigos sobre Diversidade e Distribuição espacial dos encalhes foram mais comuns, representando respectivamente 33% e 48% da amostra total. Unicamente 68 artigos (14 % da amostra total), inclueram variações espaço-temporais de eventos de encalhes diretamente viculandos a algum fator ambiental assim como analises da representatividade dos dados de encalhes como indicadores das populações e comunidade de cetáceos adjacentes (Anexo Capítulo1: Tabela 1). Desses o 63% incluiu séries temporais de registros de encalhes iguais ou superiores a 10 anos. Outras seis revisões gerais que abordavam o tema de 'encalhe' foram usadas como informação complementar.

Causas gerais da ocorrência de encalhes e sua classificação

As causas que ocasionam o encalhe de cetáceos são diversas, podendo ser classificadas como intrínsecas, quando se refere à biologia e história de vida do animal (*e.g.,* doenças, senescência, comportamento), ou extrínseca, quando relacionadas a fatores ambientais bióticos, abióticos e antrópicos, que podem atuar em diferentes escalas geográficas e temporais (PYENSON, 2011; TRUCHON, 2013). Entre as causas naturais citam-se o esgotamento físico, doenças, lesões ou ferimentos, predação, parasitismo, infanticídio, inviabilidade de neonatos e filhotes e a exposição a biotoxinas (GERACI & LOUNSBURY, 2005; GREIG *et al.,* 2005; MCFEE *et al.* 2006; DÍAZ-DELGADO *et al.,* 2018; GULLAND *et al.* 2018). Incluem-se também fatores espécie-específicos, como migrações e época de reprodução, embora a relação entre esses processos e o fenômeno do encalhe não seja diretamente causal (GERACI & LOUNSBURY*,* 2005; TRUCHON *et al.* 2013; MOORE *et al.* 2018). Fatores abióticos, como a temperatura e a variação das correntes marinhas e marés, também são incluídos. Mudanças nesses parâmetros influenciam diretamente na frequência de encalhes em regiões específicas e indiretamente guiando aspectos como a distribuição das presas e os limites de distribuição das espécies de mamíferos marinhos (HOLYOAKE *et al.,* 2013; MACLEOD *et al.,* 2014). As causas antrópicas ou de interação humana, incluem a captura incidental, emalhe nas redes de pesca, colisões com embarcações e degradação do habitat (VISHNYAKOVA & GOL'DIN, 2015; PELTIER *et al.,* 2016).

Tendo em conta o número de animais encalhados, a condição em que eles chegam às praias e as possíveis causas do evento, quatro tipos fundamentais de encalhes podem acontecer. Deles, o tipo mais comum constitui o encalhe individual, que ocorre quando o evento inclui somente um indivíduo, estando documentado para todos os táxons de mamíferos marinhos (MOORE *et al.,* 2018). O menos frequente dos tipos de eventos de encalhe são os encalhes em massa de cetáceos (do inglês: *Mass stranding events* - MSEs). Excluindo mães com filhotes, os MSEs incluem dois ou mais cetáceos encalhados relativamente no mesmo período de tempo e localidade geográfica (SERGEANT, 1982; GERACI & LOUNSBURY, 2005). Os encalhes em massa têm sido amplamente documentados para a Subordem Odontoceti (cetáceos dentados), mas não para a Subordem Mysticeti (baleias e rorquais) (SERGEANT, 1982). As espécies mais frequentemente observadas nestes eventos são: a baleia-piloto-de-peitorais-curtas, *Globicephala melas* (*e.g*., SIGURJÓNSSON *et al.,* 1993), a baleia-piloto-de-peitorais-longas, *Globicephala macrorhynchus* (*e.g*., WELLS *et al.,* 2013), a falsa-orca, *Pseudorca crassidens* (*e.g*., ANDRADE *et al.,* 2001) e o cachalote, *Physeter macrocephalus* (*e.g.,* MAZZARIOL *et al.,* 2011). Os MSEs parecem ser causados por um grande conjunto de fatores, muitos deles ainda pouco conhecidos, sendo a natureza social das espécies envolvidas o mais evidentemente relacionado, já que as mesmas são altamente gregárias e com fortes laços sociais. Entre as causas relacionadas a esse fenômeno ao longo da história, encontra-se a ocorrência de MSEs como processo regulador da densidade populacional (SERGEANT, 1982), mas com pouco suporte em estudos posteriores. Também tem sido sugerida a perda de alguns sons de baixa frequência que determinam a localização durante a procura de alimento, causando desorientação dos indivíduos em praias de areia ou lama (*e.g*., SUNDARAM *et al.,* 2006). Outras causas incluem condições ambientais incomuns (*e.g*., CLUA *et al.*, 2014; FERRARI, 2017), e mais recentes, atividades antrópicas principalmente relacionadas ao uso de sonares de média frequência durante exercícios navais (FERNÁNDEZ *et al.,* 2005; SIMONIS *et al.,* 2020).

O terceiro tipo de evento é nomeado de mortalidade em massa ou eventos pouco usuais de mortalidade (do ingês: *Unusual Mortality Events* – UMEs). Os UMEs tendem a acontecer numa escala temporal relativamente mais ampla, podendo ser causados por um único fator, como por exemplo no caso das epidemias, porém são mais comumente relacionados a vários fenômenos, sendo difícil identificar a causa mais direta (MOORE *et al.* 2018). Este tipo de evento tem sido registrado para as Subordens Odontoceti e Mysticeti, como resultado de vários encalhes individuais acontecendo num período de tempo curto, tipicamente dias ou semanas, ou de encalhes massivos onde os animais derivam mortos ou moribundos até a zona litorânea. Entre as principais causas se encontram a captura incidental em determinadas épocas do ano (LEENEY *et al.,* 2008; MEAGER & SUMPTON, 2016), o surto de doenças virais (e.g**.** *morbillivírus:* MAZZARIOL *et al.,* 2017; Van Bressem *et al.,* 2014), a exposição a biotoxinas (VAN DOLAH *et al.,* 2003; FIRE *et al.,* 2010) e a ocorrência de desastres ambientais como são os vazamentos de petróleo (LANE *et al.,* 2015, DIAS *et al.,* 2017). Mudanças em fatores ambientais e ecológicos ocasionadas pelo fenômeno El Niño, têm sido apontadas também como causantes de alguns UMEs registrados (EVANS *et al.,* 2005; HÄUSSERMANN *et al.,* 2017).

O último tipo de encalhe é descrito como indivíduos fora do habitat (do inglês: *out of habitat).* Acontece quando mamíferos marinhos são encontrados fora de seu habitat de distribuição normal ou originário. Por exemplo, golfinhos pelágicos que são avistados dentro de áreas interiores ou espécies marinhas sendo observadas no interior de rios (DE CASTRO *et al.,* 2011; CHIT *et al.,* 2012; MOORE *et al.,* 2018).

Componentes físicos no comportamento das carcaças no mar e probabilidade de encalhe

De forma geral o número de animais que encalham é muito menor que a mortalidade real que acontece no mar. PELTIER *et al.* (2012), em um estudo experimental sobre modelagem do processo de deriva de carcaças de cetáceos na costa atlântica francesa, relataram somente 8 % de recuperação das carcaças liberadas a diferentes distâncias da costa. Esses autores referem também que a maioria dos animais que derivam até o litoral tem seu ponto de origem na plataforma continental. Segundo, WELLS *et al*. (2015), a recuperação de carcaças na costa constitui unicamente um terço da mortalidade conhecida para a população particularmente estudada dos golfinho-nariz-de-garrafa *Tursiops truncatus* na baía de Sarasota, Flórida, Estados Unidos. Outros estudos relatam proporções igualmente baixas de taxa de recuperação de carcaças para diferentes localidades e espécies de cetáceos: 25% para *T. truncatus* na costa de Califórnia, Estados Unidos (CARRETA *et al.,* 2016), 0,18 para *Pontoporia blainvillei* no Brasil (Prado *et al.,* 2013), 6,2% para várias espécies de cetáceos no Golfo de México (WILLIAMS *et al.,* 2011) e < 1% para *Phocoena phocoena* no Atlântico Norte (MOORE & READ, 2008). Isto porque aspectos físico-biológicos relativos às carcaças, além dos fatores ambientais, também influenciam no fenômeno de encalhe.

A partir do momento da morte até o espécime ser documentado como encalhado, numerosos componentes vão atuar no processo aumentando ou diminuindo a probabilidade de encalhe, a localização da carcaça na praia, e posteriormente a taxa de descobrimento (PELTIER *et al.,* 2012, 2014; MOORE *et al.,* 2020) (Figura 1). Esses componentes se dividem em fatores físicos (*e.g*., vento, correntes predominantes na área, oceanografia e topografia costeira), biológicos (*e.g.,* reprodução, migração, preferências de habitat) e do comportamento humano (*e.g.* redes de monitoramento de encalhes, épocas de visitação de praias) e vão afetar diretamente as taxas registro como dado quantitativo nos monitoramentos (EVANS *et al.,* 2005; HART *et al.,* 2006; PELTIER *et al.* 2013; TEN DOESCHATE *et al.,* 2018).

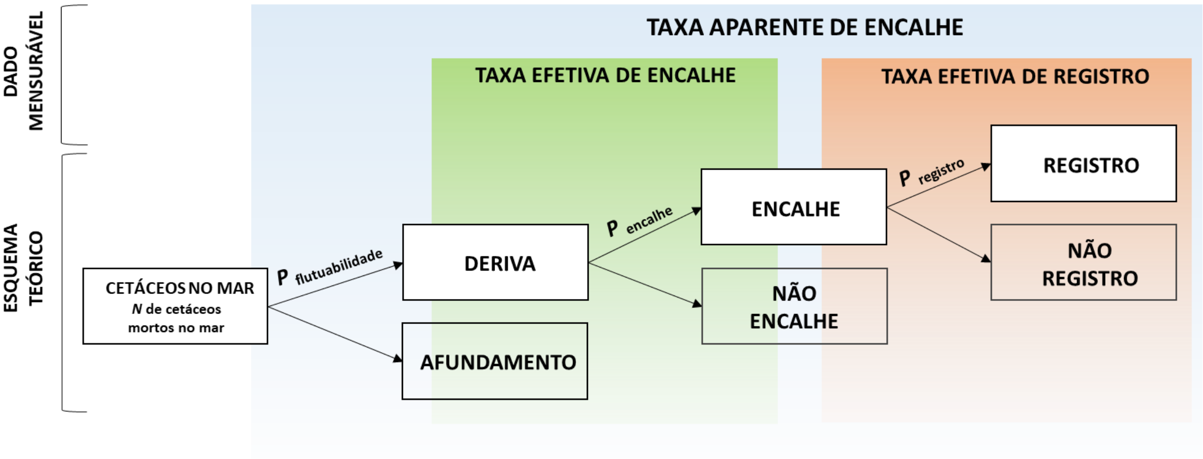

Figura 1. Esquema do processo de encalhe que descreve os diferentes fatores que podem influenciar a taxa de encalhe e registro, suas probabilidades e o dado mensurável. Modificado de: PELTIER *et al*. (2012).

Após a morte no mar, as carcaças de cetáceos podem afundar ou derivar até a linha de costa, dependendo da flutuabilidade dos restos e sua interação com os fatores ambientais, biótico e abioticos (Figura 1). A capacidade de flutuabilidade das carcaças varia entre e dentre espécies, sendo dependente da condição corporal do animal, o tamanho, o conteúdo de lipídios (principalmente nos ossos e na camada de gordura característica dos mamíferos aquáticos) e do acúmulo de ar nos pulmões e sistema digestivo. Estas características vão ser influenciadas a sua vez por variáveis físico-ambientais como a temperatura do meio e a profundidade do local da morte, além da pressão hidrostática (SMITH *et al.,* 2015; MOORE *et al.,* 2020). Carcaças com maior probabilidade de flutuar/encalhar tendem a ser de maior tamanho, apresentar flutuação positiva ou morrer ou reflutuar perto da costa, enquanto espécimes mortos, principalmente com baixa qualidade física, que afundam em águas mais profundas do que 100 m (ou fora da plataforma continental), provavelmente não vão ser descobertos nas praias (MOORE *et al.,* 2020). Isso é corroborado por resultados de modelos de deriva testados, que descrevem uma origem majoritariamente costeira dos encalhes documentados (PELTIER *et al.,* 2012, PELTIER *et al.,* 2014; HÄUSSERMANN *et al.,* 2017) e justifica a baixa taxa de recuperação de carcaças relatada para as diferentes regiões geográficas.

A intensidade e direção dos ventos constituem um dos fatores fundamentais no processo de deriva e encalhe, determinando onde, quando e se as carcaças chegarão à linha costeira (NORMAN *et al.,* 2004; EVANS *et al.,* 2005; HART *et al.* 2006, MOURA *et al.,* 2016; HÄUSSERMANN *et al.,* 2017). A ação do vento vai deslocar as carcaças influenciando o movimento superficial das águas a partir da formação da deriva de Ekman (ARDHUIN *et al.,* 2009) e atuando sobre a área exposta das mesmas por cima da superfície (BIBBY & LLOYD, 1977; NORMAN *et al.,* 2004). Estudos experimentais sobre a influência do vento na taxa de recuperação de objetos flutuantes na linha costeira demonstraram que mudanças na direção do vento pode afetar a taxa de encalhe, fazendo a mesma variar de 0,7 % a 61 % para a mesma localidade geográfica (FLINT & FOWLER, 1997; PELTIER *et al.,* 2012). Porém, para áreas de praia mais extensas a influência dos ventos locais parece afetar mais a posição específica onde os encalhes vão acontecer. Isto, porque apesar dos ventos guiarem o padrão geral de encalhe das carcaças, a interação dos mesmos com as correntes marinhas locais é o aspecto que vai definir o ponto final de deposição (HLADY & BURGER, 1993). Assim, nas épocas do ano onde correntes marinhas predominarem no interior da plataforma continental, será esperado o aumento dos encalhes (HART *et al.,* 2006). Por exemplo, para algumas regiões costeiras do Oceano Atlântico, a orientação ocidental do litoral, assim como sua localização no hemisfério norte possibilitaram que ventos constantes do norte e noroeste desloquem as águas superficiais longe da costa. Este evento, com predominância nos meses de verão, reduz a probabilidade de encalhes, ao deslocar as carcaças para áreas mais profundas (SAAVEDRA *et al.,* 2017; FOORD *et al.,* 2019). Outros estudos têm confirmado essa forte influência do vento no padrão temporal e espacial dos encalhes para outras regiões geográficas (*e.g.*, Costa Sul da Austrália: EVANS *et al.,* 2005; Costa do Brasil: MOURA *et al.,* 2016; Península da Florida, Estados Unidos: WALKER *et al.,* 2005).

Finalmente, a geografia da linha costeira conjuntamente com o tipo de praia ao qual as carcaças derivam, vão afetar a preservação das mesmas, proporcionando que as taxas de registro sejam sítio-específicas (GOL'DING *et al.,* 2003; LIEBIG *et al.*, 2003; FAERBER & BAIRD 2010; HAMILTON, 2018). Saliências da linha costeira e a formação de pequenos redemoinhos pelas correntes marinhas parecem favorecer pontos geográficos de deposição de carcaças, criando sítios de acumulação (BRABYN & MCLEAN, 1992; DIGIACOMO & HOLT, 2001; DANIL *et al.,* 2010; PYENSON *et al.* 2014; HAMILTON, 2018). Para algumas regiões mais frias, como é o caso do Mar Negro, a preservação das carcaças é mais influenciada pela intensidade do processo de abrasão e acumulação de sedimento do que pela posição geográfica, orientação, ventos ou a direção das correntes (GOL'DING *et al.,* 2013). A ação e intensidade do processo de abrasão vai depender dos fatores que controlam o movimento das partículas do sedimento, como o tamanho da partícula e a energia da onda, a geometria da superfície rochosa e a granulometria do sedimento acumulado (FEAL-PEREZ & BLANCO-CHAO, 2012). A atividade sazonal das ondas favorece o lavado e enterramento das carcaças nos meses de outono e inverno. Já condições climáticas mais áridas podem criar ambientes mais favoráveis para a mumificação e a preservação (GOL'DING *et al.,* 2013). Outro aspecto geográfico que parece ter uma relação importante com a distribuição dos encalhes ao longo do litoral constitui a largura da plataforma continental. Em áreas onde a plataforma é mais extensa, espécies costeiras ou carcaças de menor tamanho terão uma maior probabilidade de chegar nas praias. Contrariamente, em regiões onde a plataforma é mais estreita um maior número de espécies oceânicas vão encalhar (NORMAN *et al.,* 2004; PRADO *et al.,* 2016; COSTA *et al.,* 2017). A dinâmica de macro-marés diárias existentes em algumas zonas geográficas também pode interferir no número e na frequência dos encalhes (COSTA *et al.,* 2017; HAMILTON, 2018).

Componentes antrópicos e biológicos ifluenciando no registro de encalhes

Registros históricos e importância das redes de monitoramento de encalhe

A análise de dados de encalhes de cetáceos é um processo evidentemente complexo. Segundo AUTHIER *et al*. (2014), para confirmar de forma robusta a relação de causalidade entre os encalhes e os fatores que o influenciam, é necessário a construção de modelos interpretáveis com boa capacidade de predição e que considerem possíveis fatores de confusão, como a taxa de detecção e/ou de registro. Porém, dados sistemáticos padronizados e de ampla escala temporal, necessários para a construção de tais modelos só foram obtidos nas últimas décadas e para algumas regiões geográficas.

A documentação sistemática de encalhes em determinadas regiões, como parte de planos de monitoramento a longo prazo, tem permitido a compilação padronizada de séries temporais (*e.g*. EVANS *et al.,* 2005; PELTIER *et al.,* 2012; COOMBS *et al.,* 2019; RUSSELL *et al*., 2022). A mais longa compilação sistemática de encalhes tem sido realizada no Reino Unido, onde registros são publicados anualmente desde 1913 (COOMBS *et al.,* 2019) e existem programas de monitoramento contínuo. Outras redes de encalhes foram estabelecidas posteriormente, como são a Rede Francesa Nacional de Encalhes, instituída nos anos 1980s (PELTIER *et al.,* 2019), a Rede de Encalhes de Mamíferos Marinhos dos Estados Unidos, com coletas sistematizadas desde 1991 (HEYNING, 1991) e o Banco de Dados de Encalhes de Cetáceos da Austrália Ocidental – NMMD, criado em 1982 (GROOM

& COUGHRAN, 2012). Em áreas tropicais, embora muito recentemente, a Rede Caribenha de Encalhes (MIGNUCCI-GIANNONI, 1996; NIÑO-TORRES *et al.,* 2015), a Base de Dados WIS - *Whale Stranding Indonesia* da Indonésia (CHAN *et al.,* 2017) e a Rede de Encalhe e Informação de Mamíferos Aquáticos do Brasil – REMAB conjuntamente com o Programa de Monitoramento de Praias – PMP da Bacia de Santos, litoral Sudeste e Sul do pais, constituem alguns exemplos desses esforços (*e.g*. DÍAZ-DELGADO *et al.,* 2019, GROCH *et al.,* 2020).

Devido ao custo econômico que exige a pesquisa com mamíferos marinhos, cada vez mais esses planos de monitoramento sistemático de encalhes constituem uma alternativa eficiente, sendo reconhecida sua relevância ecológica, ótimo custo-benefício e sua crescente credibilidade estatística quando o processo é realizado de forma padronizada (CAUGHLAN & OAKLEY, 2001). Contudo, o desenvolvimento de indicadores baseados em dados de encalhes (*e.g*. riqueza e composição de espécies, abundância relativa, taxa de mortalidade), precisa levar em consideração todos os processos que vinculam o encalhe com as populações de cetáceos no mar, a partir de comparações com outras técnicas de monitoramento, possibilitando o aperfeiçoamento de seu significado ecológico (PELTIER *et al*., 2014). A confirmação da fidelidade desses dados vai então permitir traduzir o evento de encalhe em diferentes parâmetros ecológicos relativos às comunidades e populações de cetáceos.

Esforço amostral e possíveis padrões de variação natural nos encalhes de cetáceos

A técnica de amostragem mais comumente usada nos estudos de ecologia de cetáceos vivos em ambiente natural, é a de amostragem por transecto (HAMMOND, 2010). Devido ao fato que essa técnica depende fortemente do esforço amostral, métricas que levam em consideração tal efeito tem sido desenvolvidas (BUCKLAND *et al*., 2001). No caso dos encalhes de cetáceos, análises similares são escassos na literatura, principalmente relacionados ao processo de deriva (PELTIER *et al.,* 2012, PELTIER *et al.,* 2014) e em menor quantidade à taxa de chegada das carcaças (EVANS *et al.,* 2005; PELTIER *et al.,* 2012; CUNHA-RAMOS *et al*., 2022). Vieses temporais no número de encalhes para determinadas regiões têm sido relacionados com: mudanças em longo prazo no esforço observacional; resultado do incremento do interesse do público nos mamíferos marinhos; a expansão habitacional; o incremento das atividades costeiras e a criação de políticas ambientais governamentais, dentre delas a criação e implementação das redes de encalhes (EVANS *et al.* 2005, LEENEY *et al.,* 2008; MOURA *et al.,* 2016; GÓMEZ-HERNÁNDEZ *et al.,* 2020). Por exemplo, estudos realizados no sul da Austrália e no Pacífico sul do Canadá, demostraram uma variação espaço-temporal na taxa de descobrimento das carcaças de mamíferos marinhos depositadas na praia, sendo esta maior durante dias ensolarados ou finais de semana, e em praias arenosas que em litorais rochosos (HLADY & BURGER, 1993; EVANS *et al.,* 2005).

Na análise de dados oportunistas ou registros históricos de encalhes, um dos *proxies* usados como alternativa de esforço amostral é o crescimento da população humana costeira, já que se espera que, com o incremento da ocupação da linha de costa exista um aumento nos registros de encalhes (EVANS *et al.,* 2005; PYENSON, 2011; COOMBS *et al*., 2019; LIU *et al*., 2022). Porém esta relação parece ser também local-específica e precisa ser avaliada de forma individual para cada pesquisa e área geográfica (*e.g*., MCLEOD *et al.,* 2005; MALDINI *et al.,* 2005; PYENSON, 2011). Novamente, o uso de amostragens sistemáticas ao longo prazo parece ser o procedimento ideal na avaliação temporal dos encalhes de cetáceos, já que garante um esforço equitativo das amostragens. Quando o processo de monitoramento é realizado de forma sistemática, novos registros serão de carcaças já documentadas, o que indica uma maioria das carcaças na linha de costa sendo descobertas (AUTHIER *et al*., 2014). Contariamente, na ausência de amostragem sistematizada, até 69% das carcaças que chegam à costa podem não ser documentadas (HUGGINS *et al*., 2015).

Apessar desses fatores de confusão, os encalhes parecem demostrar variações relacionadas à ecologia e historia de vida das populações e comunidades de cetáceos presentes em uma região. Desse modo, a suposição mais básica quando se avaliam dados de encalhe é que os predadores marinhos morrem em geral onde vivem, o que parece ser supostamente verdadeiro na escala populacional (PELTIER, 2014). Portanto, mudanças na distribuição espacial das populações de cetáceos podem causar aumento nos registros de encalhes de diversas formas. Se as populações de cetáceos se movimentam para perto da costa, a probabilidade de que uma grande parte dos indivíduos mortos derive até o litoral será maior, aumentando também o número de registros no monitoramento. Se as populações mudarem para áreas de elevada atividade pesqueira, o número de indivíduos capturados também aumentará, o que será refletido num número maior de encalhes (LEENEY *et al.,* 2008). Dados de encalhes também podem mostrar tendências sazonais, dependendo dos padrões de migração e movimento das espécies (DANIL *et al*., 2010). Assim, um aumento na frequência de encalhe de neonatos e filhotes pode estar indicando épocas de reprodução, o que tem sido confirmado para espécies como a baleia-jubarte, *Megaptera novaeangliae* e a baleia-minke-antártica, *Balaenoptera bonaerensis* (KEMPER & LING, 1991; KASAMATSU *et al*., 1995). Outra suposição, confirmada para várias regiões geográficas, é que comumente as espécies raras serão menos representadas (TRUCHON *et al*., 2013).

Contudo, devido ao fato que os encalhes são fortemente dependentes dos fatores físicos- oceanográficos, os mesmos podem simplesmente refletir uma região geral de ocorrência e não estar relacionado com preferências específicas de habitat das espécies (NORMAN *et al.,* 2004). Concluindo, os dados de encalhes precisam ser usados com cautela, deixando claro as premissas assumidas para as análises, devido principalmente a quantidade de variáveis vinculadas ao processo, e tendo em conta que as possíveis deduções ecológicas dependeram da sistematicidade, do período e a área de amostragem, e do conhecimento existente sobre os grupos-alvo para as regiões específicas de estudo.

Encalhes como indicadores da riqueza, abundancia e composição de espécies

A representatividade dos dados de encalhes na análise da riqueza e composição de espécies tem sido comprovada no nível espacial e temporal a partir de comparações com registros de populações e comunidades vivas de cetáceos. Nesse sentido, os registros de encalhes podem ser interpretados como as assembleias-mortas nos estudos tafonómicos (LIEBIG *et al*., 2003; PYENSON, 2010), já que constituem uma amostra representativa da comunidade de cetáceos habitando as águas adjacentes. Sendo assim, para análises de fidelidade, as linhas costeiras poderiam ser consideradas como armadilhas naturais, análogas a cavernas, poços de alcatrão, inclusões de âmbar ou pântanos. As mesmas constituem áreas de captação restritas lateralmente que fornecem instantâneos de diversidade com algum grau de variação temporal, mas que não necessariamente refletem o habitat original da comunidade (PYENSON, 2010; KIDWELL & TOMASOVYCH, 2013).

Por outro lado, os resultados obtidos de monitoramentos sistemáticos de encalhes que avaliam estas assembleias de espécies, tendem a refletir a riqueza e composição de espécies raras e comuns de cetáceos que habitam uma região e seu entorno (PYENSON, 2010; 2011). Isto acontece principalmente em grandes escalas espaciais, quando as linhas costeiras monitoradas são superiores a 1000 km de extensão (PYENSON, 2010; 2011). Contudo, para extenções menores de 200 km, também tem sido confirmada essa representatividade, inclusive apresentando uma melhor acuracia quando comparados com dados de amostragens de animais vivos, principalmente usando amostragens padronizados por períodos superiories aos 15 anos de monitoramento (MALDINI *et al.,* 2005; DANIL *et al.,* 2012; PIKESLEY *et al.,* 2012; LIU *et al*., 2022). Curvas de rarefação obtidas por PYENSON (2010) e (2011), usando dados padronizados de assembleias e populações de cetáceos vivos, demonstraram que linhas costeiras longas e categorias taxonômicas superiores acumulam táxons mais rapidamente que zonas costeiras pequenas e classificações de maior resolução. PYENSON (2011) também sugere que, nas áreas que incluem um amplo transecto latitudinal, o incremento da amostragem espacial otimiza a amostragem taxonômica. Contrariamente, áreas restritas latitudinalmente falham na representação adequada da riqueza de espécies de cetáceos, apesar de terem amostragens robustas temporariamente. Num nível espaço-temporal, em linhas costeiras maiores que 1000 km, a saturação da curva de descubrimento será relativamente rápida. Já, em linhas costeiras com comprimentos entre 10 e 1000 km será necessário um maior tempo de amostragem, dentre 10 e 15 anos, para níveis de gênero e espécie ter a representatividade adequada. Em nível de família, amostragens com menos de 10 anos parecem ser suficientes (*e.g.*, MACLEOD *et al.,* 2005; PYENSON, 2010; MAYORGA *et al.,* 2020; LIU *et al*., 2022).

A partir da boa representatividade na riqueza de espécies pelos dados de encalhes é possível realizar inferências de importância ecológica, relacionadas a mudanças na composição das comunidades de cetáceos, inclusive sua correlação com variações ambientais a diferentes escalas temporais e espaciais (EVANS *et al.,* 2005; MACLEOD *et al.,* 2005; TRUCHON *et al.,* 2013). Diferenças entre as curvas de acumulação de espécies esperadas e observadas, por exemplo, podem indicar mudanças na composição da comunidade causadas pela aparição de novas espécies ao longo das séries temporais, devido a mudanças climáticas, oceanográficas, ou ao impacto das pescarias (MACLEOD *et al.,* 2005).

A abundância, é outro dos descritivos de grande importância no estudo da ecologia das populações e comunidades de cetáceos. A partir dos dados de encalhes a abundância absoluta não pode ser estimada diretamente. Isso porque na maioria dos casos se desconhecem aspectos da população, como a taxa de mortalidade, a proporção de indivíduos mortos que atinge a costa e/ou a proporção dos encalhes que são efetivamente registrados nos monitoramentos e os que não são (PELTIER *et al.*, 2012; SAAVEDRA *et al.,* 2017). Contudo, nas análises de tendências temporais, os dados de encalhes podem ser usados como índices de abundância relativa, indicando variações no estado de conservação das populações de cetáceos ao longo do tempo e a estimação de padrões de variação sazonais, ou de maior escala temporal (MACLEOD *et al.,* 2005; MCGOVEN *et al.,* 2016; COOMBS *et al.,* 2019; RUSSELL et al., 2022). Nesse sentido, a variação espaço-temporal na abundância relativa é um dos indicadores primários das mudanças populacionais de cetáceos, permitindo detectar mudanças antes que sejam observáveis a partir de dados de monitoramento de animais vivos (MACLEOD *et al.,* 2005).

Adicionalmente, os dados de encalhes parecem refletir o *ranking* de abundância das espécies dentro da comunidade, assim como dos gêneros e das famílias (PYENSON, 2010; LIU *et al.,* 2022). Contudo, essa caracterização parece ter também restrições espaciais e temporais dependendo da amostragem realizada. Para linhas costeiras entre 1000 e 1500 km de extensão as assembleias de espécies encalhadas parecem refletir a estrutura de abundância da comunidade de cetáceos vivos. Porém, em linhas costeiras com uma extensão superior a 6000 km a correspondência entre as abundâncias das espécies que compõem as assembleias e as da comunidade de animais vivos é perdida devido ao efeito de escala (PYENSON, 2011). Isto provavelmente acontece porque variações de aspectos ambientais regionais específicos, como é o caso das correntes oceânicas, variações climáticas locais e a geomorfologia costeira, vão influenciar nos encalhes (*e.g*., DANIL *et al.,* 2012; SAAVEDRA *et al.,* 2017; COOMBS *et al.,* 2019). Especificamente, amostragens de linhas costeiras que incluem múltiplos sistemas de correntes oceânicas parecem amostrar diferentes comunidades e fusionar os *rankings* de abundância, sugerindo que a estrutura das comunidades de cetáceos obtidas a partir de dados de encalhes sejam mais estáveis e precisas a escalas regionais ou locais que a escalas continentais (PYENSON, 2011). Outro aspecto a ter em conta é, que pelo menos em nível de família, espécies costeiras ou relacionadas a plataforma continental apresentaram sempre uma maior representatividade (PYENSON, 2010), o que é esperado também nos dados de avistagens.

Taxa de encalhe de cetáceos e sua relação com variações climáticas

Os eventos de encalhes, além de serem usados como indicadores indiretos de abundância, presença e distribuição dos mamíferos marinhos, têm confirmado seu uso como bioindicadores em tempo real devido a sua forte relação com as variações climáticas (EVANS *et al.,* 2005, PIKESLEY *et al.,* 2012, TRUCHON *et al.,* 2013; MEAGER & LIMPUS, 2014; SAAVEDRA *et al.,* 2017; COOMBS *et al.,* 2019). Não obstante, as respostas ecológicas dos mamíferos marinhos às mudanças ambientais são extremamente variáveis e complexas. Em sua análise, é preciso considerar parâmetros ambientais de dimensões continentais (fenômenos de grande escala) e regionais (fenômenos de escala local), criando uma avaliação hierarquizada que permitirá o claro entendimento dos possíveis processos que afetam as espécies (TRUCHON *et al.,* 2013). Conjuntamente, tais análises precisam também levar em consideração os diferentes níveis de distribuição geográfica das espécies, a disponibilidade regional de recursos e a ocorrência de eventos estocásticos (PIERCE *et al.,* 2007; TRUCHON *et al.,* 2013; MEAGER & LIMPUS, 2014; COOMBS *et al.,* 2019; WARLICK *et al*., 2022) (Figura 2).

A relação hierarquizada de todos esses componentes determinará as características ambientais locais, e suas variações em grande escala temporal, influenciando os padrões de distribuição das populações de cetáceos, e afetando de forma diferente sua sobrevivência em dependência da estratégia de dispersão das espécies (*e.g*., residentes ou migrantes) e o padrão de uso do habitat (*e.g*. reprodução ou alimentação) (TRUCHON *et al.,* 2013) (Figura 2). Assim, por exemplo, espécies residentes, raras e migrantes terão respostas diferentes às variações ambientais específicas de uma localidade, mas poderão ser afetadas da mesma forma por mudanças oceânicas que ocasionam, de forma direta ou indireta, distúrbios nas populações de predadores marinhos topos (DELABIANCA *et al.,* 2012; TRUCHON *et al.,* 2013; SAAVEDRA *et al.*, 2017). Porém, essas respostas não são sempre claras e os padrões de causa e efeito podem ser difíceis de detectar (HEMERY *et al.,* 2008; TRUCHON *et al.,* 2013).

Nesse padrão hierarquizado, os fenômenos de escala continental (Figura 2), são considerados condutores da variabilidade ecológica, por poderem atuar sobre a hidrologia e meteorologia local, provocando respostas específicas para cada área. Os mesmos incluem todos os índices climáticos de grande escala assim como suas teleconexões atmosféricas ((HEMERY *et al*., 2008). A influência destes fenômenos sobre as populações de cetáceos, atua de forma indireta ao ocasionar mudanças no ambiente que afetam principalmente a disponibilidade local de recursos (EVANS *et al.,* 2005, BERINI 2009; TRUCHON *et al.,* 2013; SEYBOTH *et al.,* 2016; HAMILTON, 2018). Dentre essas mudanças locais encontram-se as variações na temperatura superficial do mar e o conteúdo de calor do oceano, variações das correntes marinhas no transporte do calor e alterações da camada de gelo nas regiões árticas e subárticas (STENSETH *et al.,* 2002; STENSETH *et al.,* 2003). As respostas dos cetáceos e outros mamíferos marinhos às mudanças ocasionadas pelos fenômenos continentais vão desde alterações no comportamento migratório, em áreas de distribuição das espécies, até afetar a condição corporal e sobrevivência dos indivíduos (JOHNSTON *et al.,* 2012; TRUCHON *et al.,* 2013; SAAVEDRA *et al.,* 2017; GROSE *et al.,* 2020).

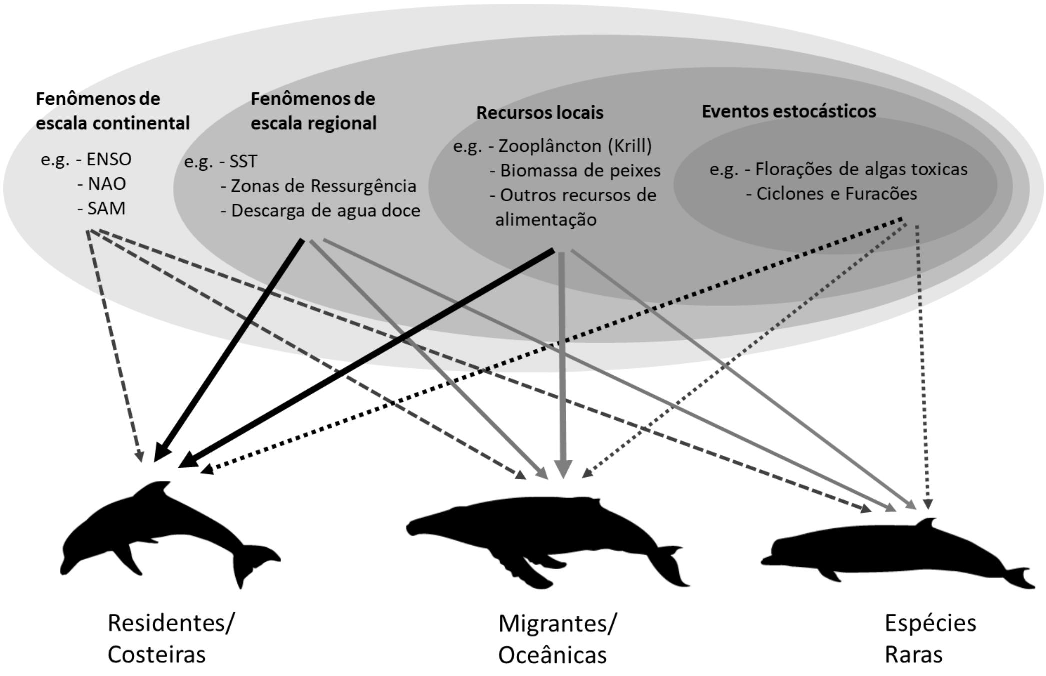

Figura 2. Influência hierarquizada dos fatores ambientais sobre a frequência de encalhes dos diferentes grupos de cetáceos. Fenômenos de escala continental afetam indiretamente a frequência de encalhe das espécies residentes/costeiras, migrantes/oceânicas e das espécies raras. Fenômenos de escala regional e sua influência nos recursos locais afetam de forma diversa e em diferentes intensidades. Eventos estocásticos podem afetar direta ou indiretamente os três grupos, mas seu efeito mais duradouro será sobre as espécies residentes/costeiras. Modificado de TRUCHON *et al.* (2013). ENSO: EL Niño: Oscilação do Sul; NAO: Oscilação do Atlântico Norte; SAM: Modo Anular Sul.

Fenômenos continentais

A influência dos fenômenos continentais sobre a frequência e distribuição dos encalhes de cetáceos tem sido avaliada através da análise de índices específicos climatológicos como El Niño-Oscilação Sul (do inglês *El Niño-Southern Oscillation* - ENSO) (TREMBERTH, 1997) e seu índice correlacionado SOI, a Oscilação do Atlântico Norte (do inglês *North Atlantic Oscillation* - NAO) (Hurrell, 1995), o Modo Anular do Hemisfério Sul (do inglês *Southern Annular Mode* – SAM) (MARSHALL, 2003), e os índices multivariados Índice Multivariado-ENSO (do inglês *Multivariate ENSO Index* - MEI) (HEMERY *et al.* 2008) e Índice Multivariado Oceano-Climático (do inglês *Multivariate Ocean-Climatic Index* - MOCI) (HEMERY *et al.* 2008). Os mesmos afetam tanto espécies costeiras como oceânicas, e tanto grupos residentes como migrantes, o que é refletido em variações temporais da taxa de encalhe. Além disso, alguns dos fatores físicos ambientais influenciados por eles parecem ter uma ação imediata sobre a ocorrência e mortalidade de espécies de cetáceos, enquanto outros vão afetar fatores biológicos a longo prazo, refletindo-se principalmente através da teia trófica (EVANS *et al.,* 2005, BERINI, 2009; TRUCHON *et al.,* 2013).

A modelagem de dados temporais dos índices do ENSO, SOI e MEI confirmam essa relação direta e a longo prazo com a taxa de encalhe de cetáceos para regiões específicas. Na costa da Austrália, variações temporais nos encalhes de baleia-jubarte, *Megaptera novaeangliae*, espécie migratória com distribuição tropical-polar, parecem estar fortemente correlacionados com o aumento do índice SOI, mas com uma defasagem de 4 anos, o que indica um efeito indireto a longo prazo (MEYNECKE & MEAGER, 2016). Mudanças nas temperaturas ocasionadas pelo evento afetam a localização e concentração do krill, o que tem um efeito direto na sincronização das migrações da baleia-jubarte, na produção das reservas energéticas necessárias e no seu sucesso reprodutivo (SEYBOTH *et al.,* 2006; MEYNECKE & MEAGER, 2016). O efeito defasado do SOI também foi demonstrado sobre a mortalidade de alguns golfinhos costeiros como no caso de *Tursiops aduncus, Sousa chinensis* e *Orcaella heinsohni* em áreas tropicais e subtropicais da Austrália (MEAGER & LIMPUS, 2014). Índices

SOI positivos tem um efeito direto na temperatura do ar e consequentemente no esfriamento das águas superficiais do mar, o que pode afetar diretamente a saúde das espécies tropicais de cetáceos (WILSON *et al.,* 1999), assim como a disponibilidade de presas (MUNDAY *et al.,* 2008; MEAGER & LIMPUS, 2014). Uma correlação positiva entre o número de encalhes mensais e anuais de *Kogia breviceps*, espécie de cetáceo oceânica com distribuição em áreas tropicais e temperadas, com o índice MEI também foi estimado para a costa atlântica dos Estados Unidos usando dados coletados entre os anos 1992 e 2006. A influência direta na frequência de encalhes desta espécie de cetáceo foi mais evidente nos anos 1997 e 1998, quando os valores do índice foram os mais elevados e o número de encalhes duplicou. Contudo, não foram obtidas correlações com valores defasados do índice de anos anteriores (BERINI, 1999).

No hemisfério Norte, os índices NAO e NAO-invernal são os mais usados como preditores climáticos de grande escala devido a sua forte correlação com o Modo Anular de variabilidade climática do Hemisfério Norte (NAM), também conhecido como Oscilação Ártica (HURRELL, 1995). No noroeste da Espanha, é suposto que o comportamento do golfinho-comum *Delphinus delphis* e seu padrão migratório é marcado pelo deslocamento da espécie para águas mais calmas, longe da área costeira durante NAO negativos. Isso diminui sua abundância nas áreas litorâneas, o que se vê refletido no menor número de encalhes. Essa influência é mais evidente para o NAO-invernal, onde o maior número de encalhes tem sido relacionado à fase positiva do fenômeno e não à negativa (SAAVEDRA *et al.,* 2017). No Oceano Atlântico Norte Ocidental foi estimada uma correlação positiva do índice NAO-invernal do ano anterior com a maior frequência de eventos de encalhe no próximo ano (defasagem de 1 ano), para as espécies de cetáceos residentes (beluga, *Delphinapterus leucas*), e do NAO do ano em curso para espécies migratórias mais abundantes (*Lagenorhynchus acutus* e *Balaenoptera acutorostrata*) e as raras (*e.g*. baleia-piloto-de-peitorais-longas *Globicephala melas*, baleia-bicuda-de-cabeça-plana-do-norte *Hyperoodon ampullatus*) (TRUCHON *et al.,* 2013). NAO positivos ocasionam uma diminuição da área marinha coberta pelo gelo devido às temperaturas mais elevadas, o que permite a ocupação de novas áreas de forrageio pelas espécies migratórias de cetáceos e aumenta sua abundância em localidades da plataforma continental. A exposição da linha costeira pelo não congelamento das águas permitirá por sua vez que um maior número de carcaças chegue à costa. Conjuntamente, a temperatura relativamente mais elevada ocasiona que espécies com uma distribuição temperada quente-subpolar ou tropical-polar ocorram na região. Contrariamente, para espécies de cetáceos residentes e algumas migratórias que usam a camada intermediária fria de água para forrageio, o aumento do número de encalhes tem sido relacionado à diminuição da abundância de krill e a consequente diminuição da biomassa de peixes demersais (TRUCHON *et al.*, 2013; LAIR *et al.,* 2016).

Fenômenos locais

O segundo grupo de fenômenos a incluir na análise hierarquizada são os parâmetros ambientais locais, como a temperatura da superfície do mar (TSM), a concentração de clorofila-a (EVANS *et al.,* 2005; MOURA *et al.* 2016; WARLICK *et al*., 2022), a disponibilidade local de recursos (TRUCHON *et al.,* 2013) e a descarga dos rios nas áreas costeiras (MEAGER & LIMPUS, 2014). Os mesmos são também influenciados pelos fenômenos climáticos continentais (MO & WHITE, 1985), e podem afetar direta e/ou indiretamente os cetáceos, criando respostas diferentes na frequência dos encalhes entre espécies residentes, migrantes e raras (Figura 2).

A TSM constitui um dos principais fatores ambientais regionais que vai regular a dispersão e ocorrência das espécies cetáceos, e consequentemente a frequência e/ou distribuição dos encalhes (LAMBERT *et al.,* 2011; SEGURA *et al.* 2008; MCLEOD *et al.,* 2005; TRUCHON *et al.* 2013; WARLICK *et al*., 2022). Organismos distintos requerem condições diferentes de temperatura e salinidade. Sendo assim, a TSM constitui um indicador da abundância e frequência com que as espécies habitam áreas específicas ao longo do tempo, e de seus possíveis predadores e presas (BRUSIUS *et al.,* 2020). Em áreas tropicais e subtropicais a diminuição atípica ou sustentada da TSM pode ocasionar estresse térmico nas espécies residentes de pequenos cetáceos e afetar a disponibilidade de presas para as espécies migrantes e costeiras. Isto é refletido numa baixa condição nutricional dos indivíduos, aumento na mortalidade e mudanças na distribuição espacial das populações (MILLER, 1992; YEATES & HOUSER, 2008). Nas regiões polares o efeito contrário de variação de temperatura será o esperado, sendo que a mortalidade de espécies de mamíferos marinhos residentes, será superior com o aumento deste parâmetro (TRUCHON *et al.,* 2013).

Já no caso de espécies raras, a relação dos encalhes com a TSM pode indicar preferências de habitat ou mudança nas áreas de distribuição (LAMBERT *et al.,* 2011; GROOM & COUGHRAN, 2012; TRUCHON *et al.,* 2013). No Atlântico Sul Ocidental, a maior frequência na distribuição dos encalhes de espécies raras ou oceânicas, como *Kogia breviceps* ao Sul do Brasil, parece estar fortemente correlacionada com uma TSM menor, que reflete a preferência desta espécie por águas temperadas e subtropicais mais frias e ricas em nutrientes. A outra espécie do gênero, *K. sima,* apresenta uma forte correlação na distribuição dos encalhes com TSMs mais elevadas, evidenciando sua preferência por águas tropicais oligotróficas (MOURA *et al.,* 2016). Por outro lado, a variação sazonal na frequência de encalhes da baleia-bicuda-de-Gray *Mesoplodon grayi* na região sul-oeste da Austrália, tem sido relacionada com mudanças nas temperaturas ocasionadas por correntes oceânicas do Sul que chegam com mais força no verão, esfriando as áreas costeiras até temperaturas comparáveis com as águas subantárticas temperadas comumente usadas pela espécie (GROOM & COUGHRAN, 2012).

No marco das mudanças climáticas, os aumentos contínuos das TSMs anuais estão também correlacionados com mudanças na frequência e distribuição espaço-temporal dos encalhes das espécies de cetáceos. Isto ganha mais importância tendo em conta que mudanças nas comunidades de mamíferos marinhos parecem ser mais rapidamente detectadas a partir de dados de encalhes que a partir das frequências relativas obtidas no monitoramento de animais vivos (MCLEOD *et al.,* 2005; PYENSON, 2010). No noroeste da Escócia, mudanças na frequência relativa de encalhes e avistamento do golfinho-comum *Delphinus delphis* e do golfinho-de-bico-branco *Lagenorhynchus albirostris,* confirmam mudanças na comunidade de cetáceos local causadas pelo aumento da temperatura ao longo dos anos, com a ocorrência na região de espécies anteriormente restritas a águas cálidas e a diminuição do habitat disponível para espécies de águas frias. Nesse sentido, *D. delphis,* e em menor grau, *Stenella coeruleoalba,* espécies de golfinhos pouco frequentes na região antes dos anos 80's, constituem as espécies com maior recorrência nos encalhes em anos recentes. Por outro lado, espécies restritas a águas temperadas-frias, como é o caso de *L. albirostris, Hyperoodon ampullatus* e *Mesoplodon bidens,* diminuíram significativamente a taxa relativa de encalhes nas datas mais atuais (Mcleod *et al.* 2005). No Mar do Norte, a variação multi-decadal da frequência de encalhes de *D. delphis* está fortemente correlacionada com mudanças temporais na TSM, confirmando resultados modelados a partir de dados de avistamento, e predizendo mudanças na área de distribuição da espécie com o aquecimento contínuo das águas (LAMBERT *et al.,* 2011).

Um outro fenômeno de escala regional, apesar de pouco estudada sua relação com os encalhes de cetáceos, são os sistemas hidrológicos e a sua influência nos ecossistemas costeiros. O nível de descarga de água doce para as regiões costeiras e sua variação sazonal, têm um forte efeito na estrutura e funcionamento do ecossistema marinho local, ao afetar aspectos como a salinidade, concentração de nutrientes e partículas, além da temperatura (CARMICHAEL *et al.,* 2012; MEAGER & LIMPUS 2014; LEÃO-MOURA *et al.,* 2016). O impacto da descarga de água doce nos ecossistemas costeiros não é limitado aos ciclos de inundação, já que estudos demonstram que períodos sustentados de baixo ou alto fluxo podem afetar fortemente os pastos marinhos e as assembleias de peixes, comprometendo a disponibilidade de recursos alimentares (GIBSON, 2002). Além disso, a descarga acumulativa de água doce parece também afetar a saúde dos mamíferos marinhos ao aumentar a exposição a patógenos e contaminantes (ROWE *et al.,* 2010; COLBERT *et al.,* 1999). Sendo assim, o impacto da descarga de água doce sobre zonas costeiras baixas, áreas interiores e áreas relacionadas a estuários, vai ser mais pronunciado que em zonas abertas ou mais profundas, afetando principalmente as espécies de cetáceos estritamente costeiras ou estuarinas, como é o caso de *T. truncatus* na região norte do Golfo do México (CARMICHAEL *et al.,* 2012) e *T. aduncus* no Nordeste da Austrália (MEAGER & LIMPUS, 2014). Contrariamente, espécies de cetáceos que não se alimentam nas áreas costeiras ou com baixa permanência nessas zonas, como são as espécies de cetáceos oceânicas, transeuntes e migratórias, não vao ser notavelmente afetadas por esse fator (MEAGER & LIMPUS, 2014).

Finalmente, estudos recentes indicam de forma indireta, a influência de sistemas hidrológicos de grande escala nos ecossistemas marinhos adjacentes e sua consequente repercussão nos mamíferos marinhos, e sugerem que nestas regiões as espécies de cetáceos que encalham em sua maioria fazem uso da área. Por exemplo, a composição de espécies de cetáceos encalhados na costa norte/nordeste do Brasil, assim como suas relações tróficas (SICILIANO *et al.,* 2008, COSTA *et al.,* 2017; COSTA *et al.,* 2020), sugerem que os ciclos naturais de descarga de água doce no Estuário Amazônico influenciam à produção primária e a estrutura da comunidade de peixes da região costeira relacionada, o que a sua vez interfere na presença de determinados predadores de topo de cadeia alimentar. Um estudo similar para o estuário do Rio de La Plata, indica o uso da região costeira estuarina para alimentação pela maioria das espécies de cetáceos, tantos costeiras como de águas mais profundas da plataforma continental, que são registradas nos encalhes (DRAGO *et al.,* 2020).

Outros dos fatores regionais que têm uma relação importante com a frequência e distribuição dos encalhes é a produtividade primária e sua correlação com as zonas de ressurgência. O processo de ressurgência é geralmente guiado pelos ventos predominantes em áreas específicas e sua interação com as correntes marinhas, e acontece em três formas fundamentais: a ressurgência costeira, a ressurgência equatorial e a ressurgência *ice-edge* (KAEMPF & CHAPMAN, 2016). As variáveis ambientais e biológicas que tem sido utilizadas como proxies do fenômeno de ressurgência, assim como do consequente incremento de produtividade primária, são a intensidade dos ventos favoráveis ao processo (WALKER *et al.,* 2005), a diminuição do nível e a temperatura do mar na zona costeira (GREMILLET *et al.* 2008,; WALKER *et al.* 2005), o aumento na concentração de clorofila-a (GREMILLET *et al.,* 2008; COOMBS *et al.* 2019, MOURA *et al.* 2016; WARLICK *et al*., 2022) e o incremento na densidade de consumidores primários (TRUCHON *et al.* 2013). A partir destas variáveis, vários estudos relacionam a presença e dinâmicas destas áreas de produtividade com a distribuição espacial e temporal dos encalhes, indicando mudanças na comunidade de cetáceos para áreas geográficas específicas, assim como variações na abundância relativa e mortalidade de algumas das espécies.

Por exemplo, no Sul da Austrália, o processo de ressurgência costeira nas zonas do Golfo de *Spencer* e *Bonney Coast*, ocasionam a movimentação de espécies oceânicas, como *Globicephala* spp. e *P. macrocephalus*, para áreas interiores da plataforma continental com uma frequência maior que o usual. Isto ligado às características geográficas da região, que apresenta uma reduzida plataforma continental, fazem que a probabilidade de encalhe seja maior criando pontos de *hotspot* de encalhes ao longo do litoral (SEGAWA & KEMPER, 2015; FOORD *et al.,* 2019). Nesse sentido, uma correlação entre a alta diversidade de cetáceos e as áreas/ou épocas de ressurgência, devido aos altos níveis de associação de presas nestes pontos, tem sido sugerido, sendo isto refletido nos dados de encalhes (FORCADA, 2009; FOORD *et al.* 2019). As mudanças na diversidade de espécies de cetáceos encalhados ao longo do Atlântico Sul Ocidental, pode ser também relacionada com a existência ou não de áreas de elevada produtividade, constituindo um exemplo claro desta relação espacial e temporal. A estrutura da comunidade de cetáceos do Estuario Amazónico, região tropical, é representada por uma assembleia de 15 espécies registradas em 12 anos de monitoramento, tendo uma maior similaridade com as comunidades do Caribe que com comunidades descritas para a região Nordeste do Brasil (COSTA *et al.*, 2017). Na região Sudeste do Brasil, além de existir uma zona de ressurgência de relativa importância, a plataforma continental é muito mais estreita que nas regiões Norte e Sul, com um maior número de espécies oceânicas registradas nos encalhes (ALVES-JUNIOR *et al.,* 1996, RAMOS *et al.,* 2001, MEIRELLES *et al.,* 2009, MOURA *et al.,* 2016). Já para a região do Atlantico Subtropical, a variação sazonal na frequência de encalhes tem sido relacionada com o incremento da produtividade primária, com o consequente aumento da biomassa de espécies de peixes demersais e pelágicos durante o inverno e a primavera. Esta região parece apresentar uma alta diversidade de espécies de cetáceos, sendo 37 as espécies representadas nos encalhes num máximo de 35 anos de monitoramento (PRADO *et al.* 2016; TAVARES et al., 2021; PRADO et al., 2022).

Eventos estocásticos

Finalmente, eventos estocásticos locais, que não podem ser preditos de forma espacial, nem para extensas escalas temporais, influenciam também nos encalhes de cetáceos, e vão ser o último componente da análise hierarquizada sugerida por TRUCHON *et al*. (2013). Eles afetam igualmente a mortalidade de espécies residentes/costeiras como migrantes/oceânicas presentes, em dependência da magnitude e duração do evento. Dois exemplos clássicos deste tipo de evento são as florações de algas tóxicas e a formação de ciclones ou furacões em localidades específicas. Na maioria dos casos estes fatores causam eventos pouco usuais de mortalidade massiva ou encalhes em massa das espécies afetadas, sejam estas residentes migrantes ou raras (MOORE *et al*, 2018; TRUCHON *et al.,* 2013). Eventos de floração de algas tóxicas nas zonas de alimentação aumentam de forma direta a probabilidade de intoxicação e mortalidade ao entrar em contato com as biotoxinas (principalmente neurotoxinas), e indiretamente, devido a sua transferência ao longo da teia trófica marinha (LANDSBERG, 2002; TWINER *et al*., 2011; TRUCHON *et al.,* 2013). Várias espécies de cetáceos são afetadas por florações de algas tóxicas devido a sua sobreposição com áreas importantes de alimentação, entre elas espécies residentes/costeiras como *T. truncatus, P. phocoena* e *D. leucas,* e espécies migratórias como *Balaenoptera acutorostrata, Balaenoptera borealis* e *Lagenorhynchus acutus* (TRUCHON *et al.* 2013; STARR *et al.,* 2017; HÄUSSERMANN *et al.* 2017). Estes eventos de mortalidade podem durar vários meses sempre afetando um número importante de indivíduos ao longo de todas as faixas etárias (LITZ *et al.* 2014).

A influência de furacões e ciclones sobre os padrões de encalhes de cetáceos tem sido muito menos estudado. Estes fenômenos atmosféricos apresentam áreas geográficas específicas de maior incidência, podendo causar importantes impactos em longo prazo nos habitats marinhos (GREENING *et al.* 2006). Os mesmos podem ocasionar variações nos padrões de encalhes e consequentemente na mortalidade natural das espécies de cetáceos, mas também podem induzir encalhes em massa e deslocamento de populações (ELLISER *et al.* 2011; MEAGER & LIMPUS, 2014; SAAVEDRA *et al.,* 2017; MOORE *et al.*, 2018), afetando com maior probabilidade espécies costeiras e residentes de pequenos cetáceos, do que as oceânicas (LAWLER *et al.,* 2007; Schumann *et al*., 2013; MEAGER & LIMPUS, 2014). Não obstante, carcaças de espécies oceânicas podem ser transportadas de forma atípica até a linha costeira devido aos fortes ventos característicos destes fenômenos (MACLEOD *et al.,* 2004). Contudo, a maior relação dos ciclones e furacões com os encalhes de cetáceos aparece de forma indireta. Entre os impactos registrados estão o aumento excessivo das concentrações de nutrientes com as consequentes florações de algas tóxicas, a diminuição das populações locais de peixes e invertebrados, a intrusão de grandes quantidades de detritos e matéria orgânica causado principalmente por inundações costeiras e grandes escala e a diminuição da salinidade e de oxigênio no ecossistema costeiro (GREENING *et al.,* 2006). Isto aumentará a mortalidade das espécies de cetáceos afetadas, via diminuição dos recursos alimentares, alteração do hábitat e pela possível aparição de doenças e novos patógenos (MEAGER & LIMPUS, 2014).

4. Conclusão

A distribuição espaço-temporal das populações e espécies de cetáceos está fortemente determinada por características físicas como profundidade, temperatura e salinidade, e pela disponibilidade dos recursos alimentares como principal fator biótico. As variações nestes fatores vão determinar onde os cetáceos habitam ao longo da vida, mas também onde morrem. Sendo assim, a mortalidade natural destas espécies, expressada na frequência dos encalhes, pode certamente refletir respostas ecológicas das espécies e populações às condições do meio. Contudo o intervalo temporal da maioria dos estudos ainda não é suficiente para estimar variações a escalas de tempo muito superiores, principalmente pela necessidade de coletas sistemáticas de dados. Um único estudo multi-regional tentou avaliar a influência de fatores continentais (índice NAO), regionais (TSM local), e estocásticos (número de tempestades), sobre os eventos de encalhes de cetáceos ao longo de toda a costa do Reino Unido e Irlanda, não achando resultados conclusivos. É sugerido, portanto que, no caso da análise de dados de encalhe, estudos focados em uma espécie ou grupo de espécies de cetáceos em uma localidade geográfica específica, permitem obter definições regionais e sazonais que não são obtidos em estudos macroecológicos (COOMBS *et al*, 2019). Isto porque fatores como o NAO constituem fenômenos de grande escala, mas que ocasionam respostas locais específicas dos ecossistemas e das comunidades de cetáceos que os compõem.

Segundo BRADSHAW *et al*. (2006), apesar destas correlações não oferecerem resolução dos mecanismos de causa e efeito dentro do processo, permitem formular hipóteses mais sólidas com relação aos encalhes de cetáceos. Por exemplo, se águas ricas em nutrientes determinam a presença de cetáceos em zonas costeiras, é esperado que a probabilidade de encalhe nessas regiões específicas seja maior. Conjuntamente, mudanças na distribuição e disponibilidade dos recursos de alimentação em função de variações climáticas e oceanográficas de grande escala parecem determinar padrões de migração, sobrevivência, distribuição e comportamento de forrageio dos cetáceos, e podem ser avaliados a partir da coleta sistematizada de registros de encalhes, pelo menos em escala regional (PYENSON, 2010, COOMBS *et al.* 2019; WARLICK *et al*., 2022).

Os resultados obtidos até a data na análise de encalhes, embora reduzidos, parecem ratificar estudos realizados com populações vivas de localidades específicas, permitindo entender as respostas das populações e comunidades de cetáceos às variações ambientais. Nesse sentido, apesar das limitações inerentes associadas aos dados de encalhes, a análise hierarquizada sugerida por TRUCHON *et al*. (2013), e presente em outras pesquisas (*e.g.*, MEAGER & LIMPUS 2014), possibilita compreender como fatores globais, regionais e estocásticos, assim como suas interconexões, afetam de forma direta e indireta o padrão de encalhe das espécies de cetáceos. Por sua vez, permite criar inferências ecológicas sobre os padrões populacionais dos cetáceos (abundância relativa, mortalidade natural, reprodução) e a estrutura das comunidades (migrações e uso do habitat) nos ambientes onde habitam, o que corrobora a importância dos dados de encalhe como indicadores de respostas a longo prazo e fonte de informação biológica fundamental.

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CAPÍTULO II: UPS AND DOWNS: AN INSIGHT ON THE STRANDING PATTERN OF GUIANA DOLPHINS, *Sotalia guianensis,* IN THE AMAZON ESTUARY, NORTHERN BRAZIL

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Abstract

1. Long-term systematic records of cetacean strandings have been proved as good indicators of temporal responses of specific cetacean populations and communities to natural or anthropogenic pressures, as well as an important key to guide management objectives and conservation actions.
2. Generalized additive models (GAMs) were used aiming to describe long-term and seasonal patterns of Guiana dolphin (*Sotalia guianensis*) strandings in the Amazon Estuary region, North Brazilian coast, through the analysis of 12 years (2004 – 2016) of systematic and opportunistic surveys. Spatial distribution of strandings were assessed via Kernel density maps and 120 individuals were aged in order to describe the age-at-dead structure of this Management Unit (MU).
3. Model results indicated a marked seasonality in the stranding and therefore, in the mortality of Guiana dolphin on the Amazon Estuary, with inverse timing between inner estuarine regions and more coastal areas. Thus, reflecting the seasonality characteristic of this environment. Long-temporal decline of strandings in the Amazon Estuary inner region and high unusual mortality of young sexually mature individuals for this area were also detected, possibly related to the interaction of the species with fishing gillnets.
4. Conservation efforts must have into consideration the detected seasonality of strandings in the management of the Northern Brazilian MU of the species. Our study corroborate the effectiveness of stranding data as a source of biological and ecological information, and their use as a tool for gathering information about human actions affecting specific groups of coastal populations of aquatic mammals. The novel results obtained highlighted the necessity of urgent studies to clarify the influence of bycatch on the observed mortality, since these hypotheses may be the basis for effective conservation actions related to the local preservation of the Guiana dolphin, and for the characterization of these poorly-known MU.

Keywords

Amazon, bycatch, Cetacea, coastal dolphin, mortality, seasonality, spatial distribution, strandings

1. Introduction

Conservation monitoring plans, as a means to detect the impact of environmental changes – natural or human-induced, on wildlife populations, are expected to be ecologically relevant, cost-effective, and have statistical credibility (Hinds, 1984; Caughlan & Oakley, 2001). In this scenario, the use of indicators and sentinel species has proven to be a useful tool to optimize the monitoring of wildlife and environments conditions (Wells et al., 2004; IJsseldijk et al., 2018), as well as an important key to guide management objectives and conservation actions (Carignan & Villard, 2002; Bal et al., 2018; IJsseldijk et al., 2018). Since marine mammals are long-lived species, in many cases, with, long-term resident populations, and constitute high-level feeding predators, their usefulness as models to assess ecosystem changes has been renowned (Bossart, 2011; Evans & Waggitt, 2020). However, marine mammal populations are still relatively difficult and expensive to access in their natural habitat. Therefore, strandings monitoring programs offers an opportunity to obtain key biological information that otherwise, would be unavailable due to logistical and financial limitations of survey these species at sea (Peltier et al., 2014; Geraci & Lounsbury, 2005; Saavedra et al., 2017).

Systematic documentation of cetacean strandings, as part of long-term monitoring plans, has endorsed the standardized compilation of stranding time series for several regions (e.g., Australia - Evans et al., 2005, France - Peltier et al., 2012, UK - Coombs et al., 2019). This increase in accuracy of data collection has enabled the correlation of strandings with other biological, and environmental variables, allowing the generation and testing of more robust and complex hypotheses about the phenomenon of stranding (Pyenson, 2010; Pyenson, 2011; Authier et al., 2014). Thus, ecological studies based on systematic long-term stranding datasets have allowed a better understanding of the strandings, being possible its validation as indicators of temporal responses of specific cetacean populations to the action of natural and/or anthropogenic pressures (e.g., Meager & Limpus, 2014; Peltier et al., 2016; IJsseldijk et al., 2021).

Sampling limitations of stranding data records, related to factors affecting the provability of stranding and discovery rates, have been also widely discussed (Peltier et al., 2012). Despite that, it is recognized that stranded individuals represent a minimum measure of mortality at sea (Peltier et al., 2014), allowing the detection of temporal tendencies such as variations in nearly population's relative abundance, annual mortality patterns, and temporal changes in the structure of the local marine mammal community (PELTIER et al., 2012, 2014; PIKESLEY et al., 2012; MEAGER; SUMPTON, 2016). Furthermore, the use of long-term stranding series collected by systematic survey programs seems to do available the definition of baselines for species-specific stranding rates, as well as biological and ecological metrics for the stranded populations (TEN DOESCHATE et al., 2018).

The Guiana dolphin, *Sotalia guianensis,* is a small size dolphin, hat occurs strictly in Central and South America, inhabiting bays, estuaries, and shallow coastal waters along the Western South Atlantic Ocean and Caribbean Sea (FLORES; DA SILVA; FETTUCCIA, 2018b). On the Brazilian coast, current studies on genetic population structure confirm the existence of eight independent management units (MUs) of the species, one of these placed in the Amazon Estuary, in the tropical North region of the country (Caballero et al., 2018; Domit et al., 2021). Despite stranding records of Guiana dolphins have been frequently reported throughout the Brazilian coast (e.g., Santos et al., 2010; Batista et al., 2012; Vianna et al., 2016; Mayorga et al., 2020), studies on stranding patterns of this species have been more frequent in the Northeastern, Southeastern, and Southern regions, being focused mainly on fishery-related mortality (Monteiro-Neto et al., 2000; Netto & Barbosa, 2003; Moura et al., 2009), and, in many times, with temporally restricted data analysis (Meirelles et al., 2010; Santos et al., 2010). However, in the Amazon coastal region, only two studies had assessed the structure of the stranded local cetacean community, including the Guiana dolphin as a part of the records, but with no long-temporal or spatial evaluation (Siciliano et al., 2008; Costa et al., 2017).

The physical-geographical peculiarities of the Amazon Estuary, outlined by a series of inlets, channels, creeks and large mangroves areas compounding several bays and estuaries named in Portuguese as *reentrâncias amazônicas* (Souza-Filho et al., 2005), provide an optimal preferential habitat for Guiana dolphin's populations from Northern Brazil coast. Moreover, the Amazon Estuary is heavily influenced by the Amazon and adjacent river discharges (e.g. Pará and Araguaia-Tocantins Rivers), and a marked rainfall season that creates characteristic annual cycles of changes in the environmental conditions. Thus, many coastal regions varied from marine/or salty to freshwater prevalence throughout the year (Rosário et al., 2016), leading to cyclical modifications on different ecological levels (Barthem & Schwassmann, 1994; Camargo & Isaac, 2001; Barletta & Lima, 2019), and probably influencing changes in populations of marine top predators such as the case of Guiana dolphins. This species is also one of the most affected aquatic mammals by anthropic pressures, being classified as vulnerable in the Brazilian Red List of Threatened Species (Directive MMA 148/2022), and the most frequently observed cetacean stranding at the Amazon Estuary and Northern Brazilian region (Siciliano, 1994; Costa et al., 2017). The main factor suggested as causing these high stranding rates are the incidental capture in fishing nets due to the intense fishing activity in the region (Emin-Lima et al. 2008), with the overlapping of favorable areas for gillnetting with optimal areas of the species (Siciliano, 1994;

Siciliano et al., 2008; Filgueira et al., 2021). For instance, is supposed that approximately 90% of the ~600 Guiana dolphins specimens deposited in the marine mammal collection of the Museu Paraense Emílio Goeldi – located at Pará, one of the States comprising the Amazon Estuary – were incidentally caught in fishing gillnets in the region (Siciliano et al., 2018).

Since 2005, beach monitoring programs have recorded stranding events of Guiana dolphins in the Amazon Estuary, creating an important long-term database for the Northern Brazilian management unit of the species, which lives in a complex and unique coastal environment. The investigation of this dataset could generate a baseline pattern of Guiana dolphin stranding rates as a part of the initial steps for improving conservation efforts of the species in this region. This is the first approach to the Guiana dolphin stranding events in the Amazon estuarine region focused on a temporal tendencies analysis. The aims of the present study were to describe: i) the seasonal and long-term variations of strandings through the analysis of 12 years of continuous systematic and opportunistic samplings ii) the spatial distribution of the stranding events in the region, and iii) estimate the age composition of stranded specimens to understand the age-at-dead structure of this Guiana dolphin Management Unit.

2. Methods

Sampling Area

The Amazon Estuary (Figure 1A) is based merely on the mixing of river water and seawater, characterized by a humid tropical monsoon type of clime (Alvares et al., 2013), and mainly influenced by the interaction of the Amazon and Pará Rivers with the Atlantic Ocean (Souza-Filho et al., 2005; Smoak, Krest & Swarzenski, 2006) which forms the region named as Marajó Bay. It comprises an area of 3,850,253 ha, including 700 km of continuous mangroves stretch and the larger fluvial-maritime archipelago of the world (Abell et al., 2008). According to the physical-geographical attributes – i.e. geological characteristics from Amazon mouth and shelf, the freshwater discharge and the action of the Equatorial Current, this region is divided into three segments from South to North: 1) *reentrâncias amazônicas* from Northeast portion of the state of Pará, 2) Marajó Bay, Marajó Island, and the Amazon River mouth region and 3) Northern Amazon Estuary Coast (Isaac & Barthem, 1995). The discharge of fresh water into the marine ecosystems differs along the coast, being strongly influenced in the North by the Amazon River and further East by the Pará River and Araguaia-Tocantins basin, creating a semi-diurnal tidal regime with mean variations of 4 m (Menezes et al., 2013). In addition, the region is strongly influenced by a well-marked rainfall regime, with a rainy season (from December to May), characterized by high precipitation, low salinity, and high-water turbidity, and a dry season (from June to November), characterized by relatively low precipitation, high salinity, and low water turbidity (Moraes et al., 2005). Consequently, many rivers in the Amazon and Araguaia-Tocantins basins go through annual cycles of floods and ebb tides that result in dramatic seasonal changes in the Amazon estuarine environment. The local winds regime has also a seasonal variation, driven by shifts in the position of the Intertropical Convergence Zone over the year. Thus, winds are more intense between January and April, with direction predominantly from the northeast, and less intense between June and October when they mainly blow to the west/northwest (Abreu et al., 2020).

Data collection

Guiana dolphin stranding data were collected from 2005 to 2016, in the two southeastern segments of the Amazon Estuary, including the Marajó Bay, Marajó Island, and a section of the systems of *reentracias* from North Pará coast named Salgado Paraense (Figure 1A). The strandings were recorded for three shoreline sections of this segments encompassing approximately 150 km of extension (Figure 1B): 1) ~50 km coastline section inside the Marajó Bay (MB), between the coordinates 00°56’S/48°34’W and 00°26’S/48°26W; 2) ~40 km coastline segment in the Northeastern Atlantic coast of Marajó Island (NMI), extended from 00°13’S/48°46’W to 00°20’S/48°22’W and; 3) ~60 km of Salgado Paraense coast (SP), in the North Atlantic coast of Pará, between the coordinates 00°33’S/47°55’W and 00°37’S/47°31’W. Since 2005, the GEMAM (*Grupo de Estudos de Mamíferos Aquáticos da Amazônia*) has recorded stranding events in this location every two weeks by a regular beach monitoring program and opportunistic observations. For each record it was collected the date, geographical coordinates, biological data – i.e., number of stranding specimens, sex, body length, biological samples, decomposition states – (1) alive; (2) freshly dead; (3) moderate decomposition; (4) advanced decomposition; (5) mummified or skeletal remains, based in Geraci & Lounsbury (2005), and additional information of interest – i.e., evidence of interaction with fishing, *post-mortem* damage. All specimens of Guiana dolphins collected as part of the GEMAM's beach monitoring program were deposited in the mammal collection of the Museu Paraense Emílio Goeldi (MPEG), located in Belém, Pará State, Brazil.

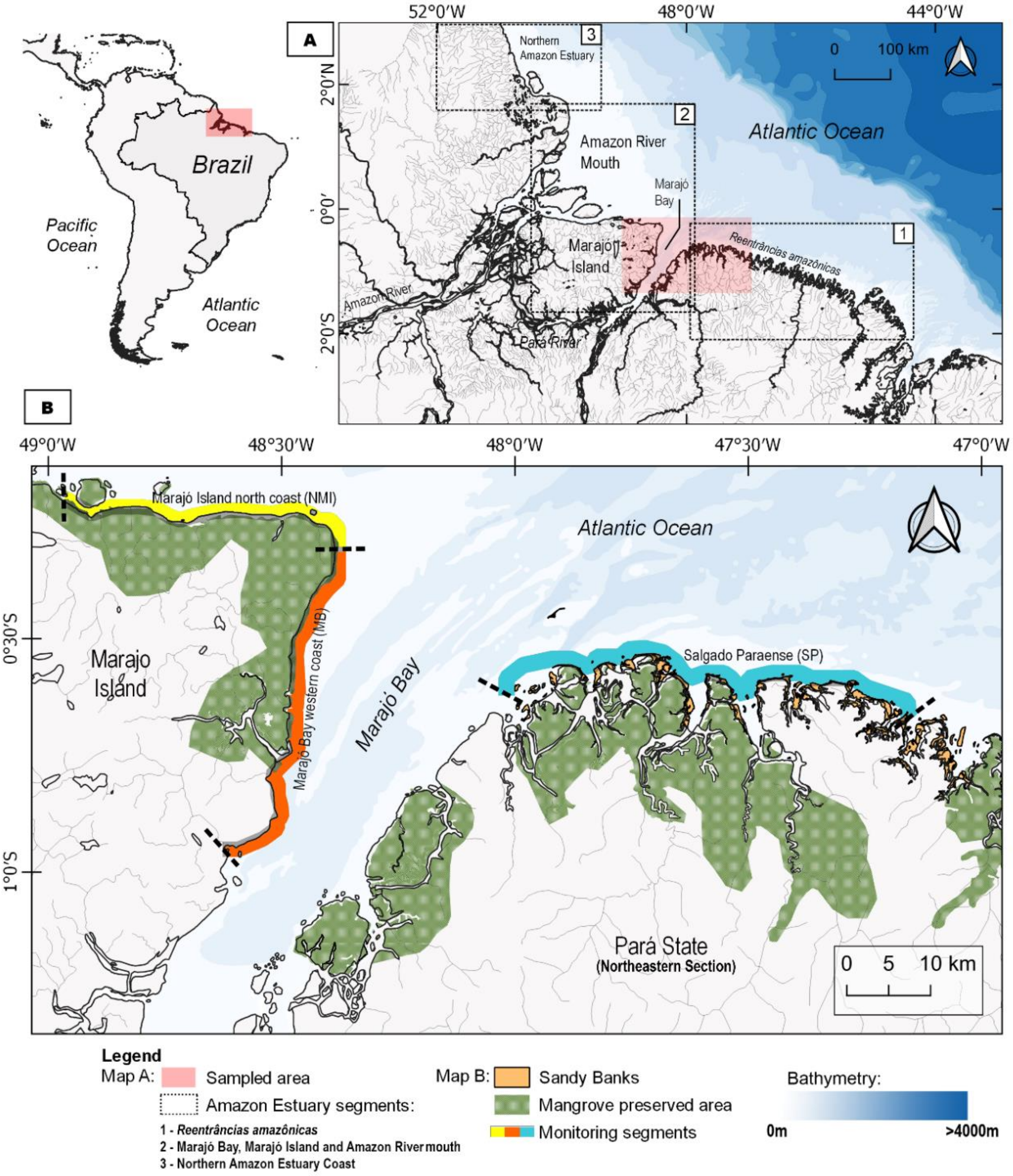

Figure 1. Study area in the Southern Amazon Estuary, Northern Brazilian coast. A: Amazon Estuary sections and sampled area including part of sections 1 and 2. B: Sampling area of the stranding monitoring program (2004 and 2016), including three coastal sections: Marajó Island north coast (NMI – in yellow), Marajó Bay western coast (MB – in orange), and Salgado Paraense (SP – in blue).

Spatial and temporal analysis

The geographic coordinates of stranding events of Guiana dolphins in the sampled area of Southern Amazon Estuary were plotted and used to create a Kernel density map including all the records collected from 2005 to 2016. Stranding events were categorized by region using the three principal sampled areas (NMI, MB, and SP) and by seasons. Seasonality was based on the local rainy (December - May) and dry (June - November) seasons related to the hydrodynamic cycle of the Amazon coastal region. Temporal trends in the stranding pattern were assessed through Generalized Additive Models (GAMs). Due to the lack of sampling for the years 2015 and 2016 on the North Atlantic Pará coast (SP region), the time interval used in the temporal analysis only included the years between 2005 and 2014. Records from the Northeastern Atlantic coast of Marajó Island (NMI) were also not included because of the limited sampling period and the segmentation of this dataset, mostly opportunistic.

The number of specimens stranded by month over a time series of 10 years was used as the response variable in the GAMs construction. The modeling method by GAMs is the most recommended in time-series analyses, where the relationship between predictors and the response variable is more complex and assumed to be non-linear (Wood, 2017). The relationship between the mean of the response variable and a “smoothed” function of the predictor variables is described by a specific link function (Guisan, Edwards & Hastie, 2002). The effective degrees of freedom (edf) will describe the degree of non-linearity of this relationship (Woods, 2017). As predictors variables were used Years (Date term to detect long-term temporal trends), Month (a specific term for seasonal trends related to the months), and Place. The latter variable was included in the model candidates as a factor representing the sampling subareas Marajó Bay western coast (MB) and Salgado Paraense coast (SP) because both regions embody different ecological characteristics. A binomial-negative link function was used to control for the over-dispersion of the models tested. A cubic spline was used to model the smoothness of the seasonal term, allowing continuity between the months of December and January. Due to differences in the number of beach monitoring over the period and subareas, an offset term was added, referring to sampling effort, counted as the number of field days *per* month *per* sampling year for each region. Four GAMs candidate models were tested and compared with a null model (M5), relative to a random temporal variation in the stranding pattern of the Guiana dolphin (Table 1). A model where strandings were not split between areas were also included as candidate (M2, Table 1). The best fit model was selected according to the Akaike Information Criterion corrected for small sample sizes (AICc) as well as the adjusted coefficient of determination (R^2) and validated using the diagnostic plots (Q-Q plot, residuals *vs*. linear predictors, histogram of residuals, and response *vs*. fitted values).

Table 1. Candidates of Generalized Additive Models (GAMs) tested to describe the temporal pattern of stranding of Guiana dolphins in the Southern Amazon Estuary. M5 described the null model.

No.	GAMs candidates
M1	*f* (Years, Place) + *f* (Month, Place) + *offset (log*(*effort*))
M2	*f* (Years) + *f* (Month) + *offset(log(effort))*
M3	*f* (Years, Place) + *offset(log(effort))*
M4	*f* (Month, Place) + *offset(log(effort))*
M5	1 + *offset(log(effort))*

Age-at-death frequency distribution

Specimens of Guiana dolphin with collected teeth were used to assess the age-at-death distribution of the stranded population by counting the Growth Layer Groups (GLGs) deposited in the teeth's dentine, considering the described by Ramos, Di Bemeditto & Lima (2000). A tooth of each specimen was subsequently worn, decalcified with RDO®, cut with a manual freezing microtome in sections of 20-30 µm of thick, stained with Mayer's hematoxylin, and finally, blued in Ammonia 2% (Ramos, Di Bemeditto & Lima, 2000). The GLGs present in the dentin were counted three different times by specialists to determine the chronological age of the specimens and having into consideration the correspondence of 1 GLG and 1 year, previously described for Guiana dolphin (Ramos, Di Bemeditto & Lima, 2000; Di Beneditto & Ramos, 2004). Age frequency were described by area and seasons.

Data exploration, statistical analyses, and graphics were developed in the free software RStudio v.2022.02.1 (RStudio Team, 2020). The packages used were 'mgcv' (Woods, 2017), 'AICcmodavg' v.2.3-1 (Mazerolle, 2020), 'itsadug' v.2.4 (van Rij et al., 2020) and 'countreg' v.0.2-1 (& Zeileis & Kleiber, 2020). Plots and data handling were due using the packages 'ggplot2' (Wickham, 2016) and 'tidyverse' (Wickham et al., 2019). The Kernel density heat maps were created using the Free and Open Source software QGis v. 3.10.13 (QGIS.org, 2022).

3. Results

Between the years 2005 and 2016, 609 stranded specimens (Median = 41strandings *per* year, Media = 47 strandings *per* year ± 27 SD, Modes= 49, 64) of Guiana dolphins were recovered in 284 field days along the Southern Amazon Estuary coast, being 71 males, 70 females, and 468 specimens whose sex could not be identified. The sex ratio of the sexed sampled was 1.01. The years 2006, 2007, and 2013 recorded the higher number of strandings, with 15 to 24 dolphin carcasses found in the months of higher frequency. The lower number of strandings was recorded for the years 2005 and 2016, with frequencies between 1 and 4, and between 1 and 7 specimens stranded *per* month, respectively (Figure 2A and Supplementary Figure S1). A rapid increase in strandings was observed between the years 2007 and 2009 and between the years 2011 and 2015 (Figure 2A).

The stranding pattern of Guiana dolphins was highly different between the sampled regions (NMI, MB, and SP). In general, a higher number of strandings was reported for the MB region, with 368 records cumulated in 178 field days along the 12 years of sampling (Figure 2A and Supplementary Figure S1), ranging between 0 and 24 specimens stranded by month (Median = 6, Mode = 4). In the SP region, 204 stranded specimens were recorded 93 field days for same the sampling period (Figure 2A and Supplementary Figure S1), with a tendency to increase in the later years, and being founded 16 specimens as maximum in a month (Median = 4, Mode = 2, 3). Monthly cumulative records indicated a high frequency of strandings records between August and February inside the MB, and between March and June for the SP region (Figure 2B). Strandings from the NMI region were only opportunistically reported in four years of the sampling period (2007 and 2012-2014), being recorded 41 specimens in 13 field days for this area, and with similar cumulative records for the months of June, October, and, December (Figure 2B). These data were only used for the heat map shown in Figure 3.

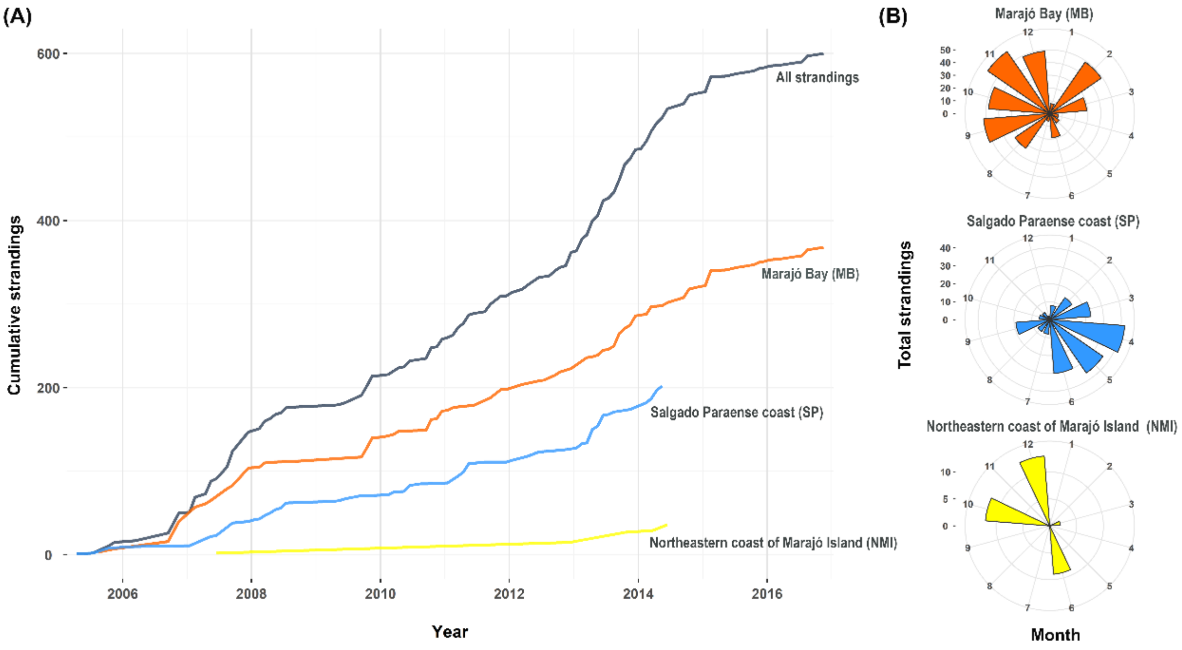

Figure 2. Temporal variation of Guiana dolphin strandings between 2005 and 2016 in the Southern Amazon Estuary. (A) Cumulative strandings by sampled segments in the study area and total cumulative strandings for the period 2005 – 2016. (B) Total strandings by months for the three sampled segments in the study area.

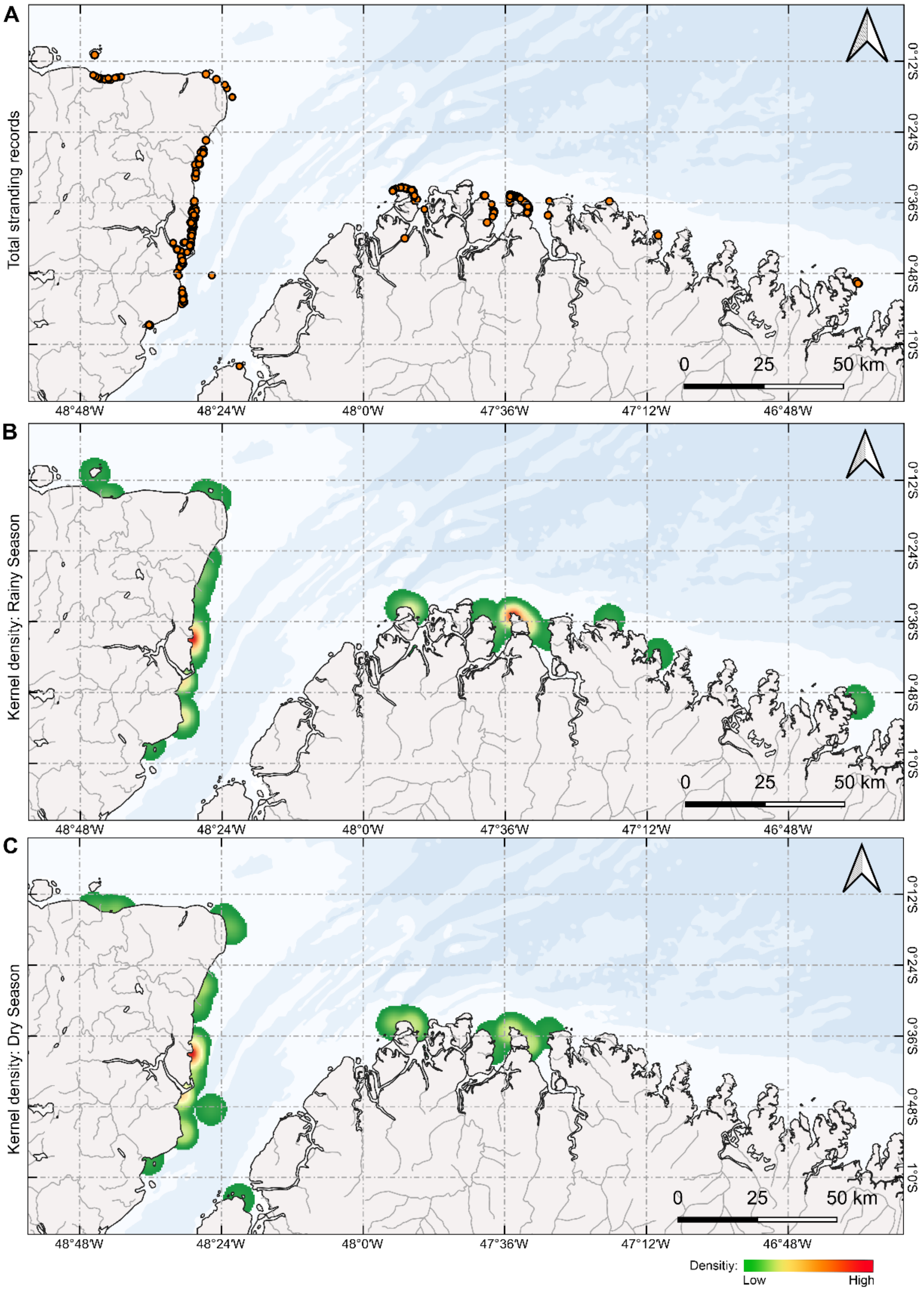

Figure 3. Geographic distribution of stranding events of Guiana dolphin on the Southern Amazon Estuary. A. Stranding records of Guiana dolphins from 2005 to 2016 (orange points); B): Kernel density map for the cumulative stranding records in the rainy season (December-May); C): Kernel density map for the cumulative stranding record in the dry season (June-November).

GAMs results indicated that the best fit generalized additive model was the model including Years and Month as predictors related to the Place (M1: AICc= 495.84, Total df=8,34 and R^2adj=0.55). Different from the other models, the M1 explained the 28% of the temporal variation of Guiana dolphin strandings in the Southern Amazon Estuary between the years 2005 and 2014 (Table 2).

Table 2. Generalized additive models (GAMs) candidates evaluated for the temporal analysis of Guiana dolphin strandings in the Southern Amazon Estuary ranked by the deviance explained and AICc (Akaike Information Criterion corrected for small samples). ΔAICc: Differences in AICc between models. Φ: Parameter of overdispersion. Φp: Probability of Φ ≈1 by Chi^2 test using $p < 0.05$ as significance level. R2 adj: Adjusted coefficient of determination.

No.	**Models**	**Deviance explained**	**AICc**	**ΔAICc**	**Φ**	**Φp**	**R^2adj**
M1	*f* (Years, Place) + *f* (Month, Place) + *offset(log(effort))*	28%	495.84	0.00	1.05	0.321	0.55
M4	*f* (Month, Place) + *offset(log(effort))*	13.5%	500.51	4.67	1.07	0.287	0.42
M3	*f* (Years, Place) + *offset(log(effort))*	14%	503.40	7.56	1.15	0.142	0.47
M2	*f* (Years) + *f*(Month) + *offset(log(effort))*	2.5%	506.86	11.02	1.17	0.114	0.41
M5	1 + *offset(log(effort))*	9.13e-09%	507.29	11.45	1.16	0.115	0.36

The GAM analysis indicated a significant long-term temporal variation in the stranding rate for the MB, showing a linear negative tendency over time (Table 3, Figure 4A). For the SP region, the results suggest a slight decrease in the number of Guiana dolphins strandings for the initial years (2006 – 2010), followed by a posterior increase, but with no statistically significant oscillations (Table 3 and Figure 4B).

Table 3. Parameters of the selected Generalized Additive Model (GAM) for the temporal analysis of Guiana dolphin strandings in the southern Amazon Estuary. SE: Standard Error; CI: 95% Confidence Intervals; IRR: Incidence Rate Ratios. Edf: Effective degrees of freedom; Ref. edf: Reference degrees of freedom; p: provability value with statistical significance settled at $p < 0.05$).

	Predictors (M1)	**Coefficient**	**ES**	**IRR**	**Z-Valor**	**p**
	Intercept	0.67	0.07	1.95 (CI:1.72 – 2.22)	10.35	<2e-16
		Edf	**Ref. edf**	**IRR**	**Chi^2**	***p***
Smooth Terms	s(Years): MB	1.00	1.001	2.72	9.984	0.002
	s(Years): SP	2.47	3.073	11.77	6.767	0.082
	s(Month): MB	2.05	8	7.80	8.807	0.005
	s(Month): SP	1.82	8	6.14	6.014	0.021

A significant seasonal pattern was detected in the stranding rate for both regions, MB and SP, but with an opposite tendency (Table 3, Figures 4: C and D). Thus, beginning in December, and over the initial five months of the years, from January to May, strandings showed a gradual decrease in the MB and a gradual increase in the SP region. From June to November, a tendency was an increase of stranded animals inside the MB and a decrease in the SP region. Positive peaks in the trends were observed in November in the MB region and in June for the SP region, while negative peaks were detected between May and June, and between December and January in the respective localities (Figure 4: C and D).

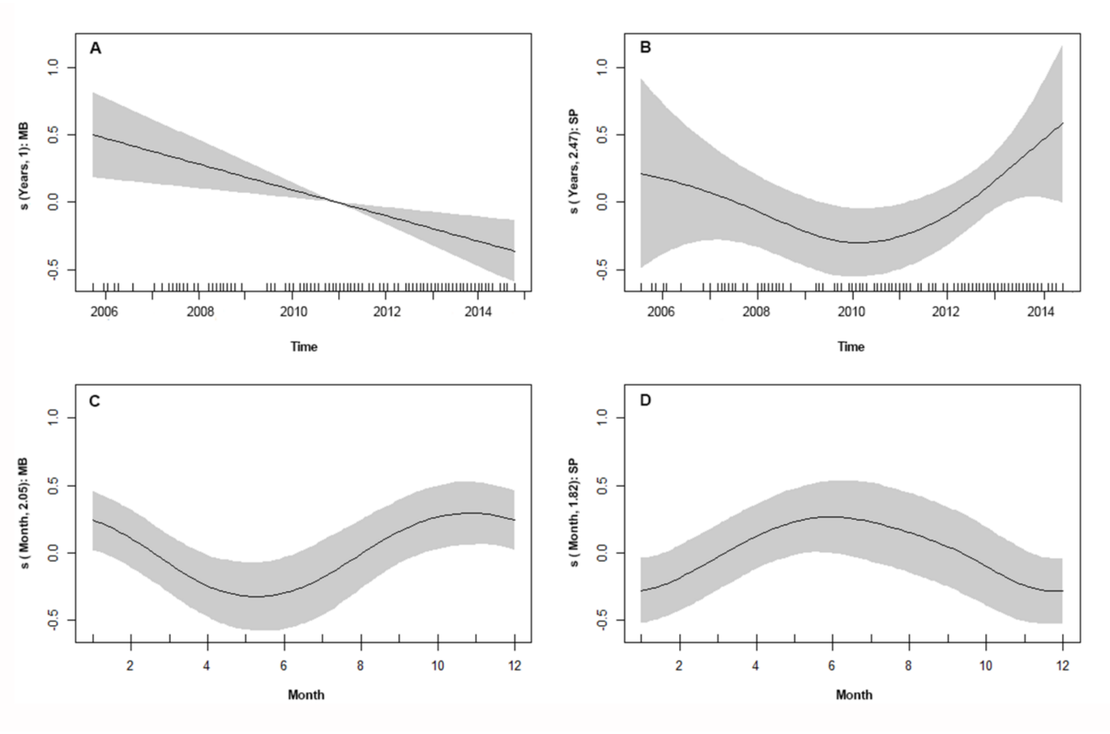

Figure 4. Temporal trend in stranding rate of Guiana dolphin in the southern Amazon Estuary. The solid line represents the estimated Smooth function, while shadow areas represent 95% confidence intervals. A and C: GAM models fitted for Years and Month, respectively, as predictors of strandings in Marajó Bay (MB). B and D: GAM models fitted for Years and Month, respectively, as predictors of strandings in the Salgado Paraense region (SP).

The spatial distribution of strandings occurred between 2005 and 2016, described via Kernel density map (Figure 3 and Supplementary Figure S2), revealed seasonal density changes, and specific geographic points of strandings accumulations inside the MB and in the SP area. Density changes between rainy and dry seasons were more evident for the SP area, on the Atlantic coast.

Additionally, the age-at-death frequency distribution of 120 of the stranded specimens was estimated, being 70 from MB, 45 from SP, and 7 from NMI, ranging between 0 and 33 years, with a Median of 10 years. The intervals between 0 and 3 years, and the age class of 10 years, were the most frequently ages observed, totalizing 34% of the sample (Figure 5A, Supplementary Table S1). Age-at-death distribution was different between seasons (Figure 5B). For the MB area, among the 46 aged specimens stranded in the dry season, 39% ranged between the ages of 0-6 years, other 39% ranged between 7-20 years and 22% included animals older than 20 years. In the rainy season, these proportions changed to 28% corresponding to animals between the ages of 0-6 years, 68% corresponding to ages 7-20 years, and 20% older than 20 years. For the SP region, only 8 animals stranded in the dry season were aged, being 50% represented by the ages between 0-6 years and the other 50% by the ages between 7-20 years. No animals were detected with ages older than 20 years for this area in the dry season. Distinctly, in the rainy season, the 31 animals aged for SP were represented in 37% by the ages between 0-6 years, 47% by the ages between 7-20 years, and only 5% of the animals were older than 20 years. Two mortality peaks were detected for MB in the rainy season, with the ages between 7 and 10 years, and between 17 and 20 years being the more frequently observed (Figure 5B, Supplementary Table S1). In the SP, observed frequency were always higher for the ages < 4 years.

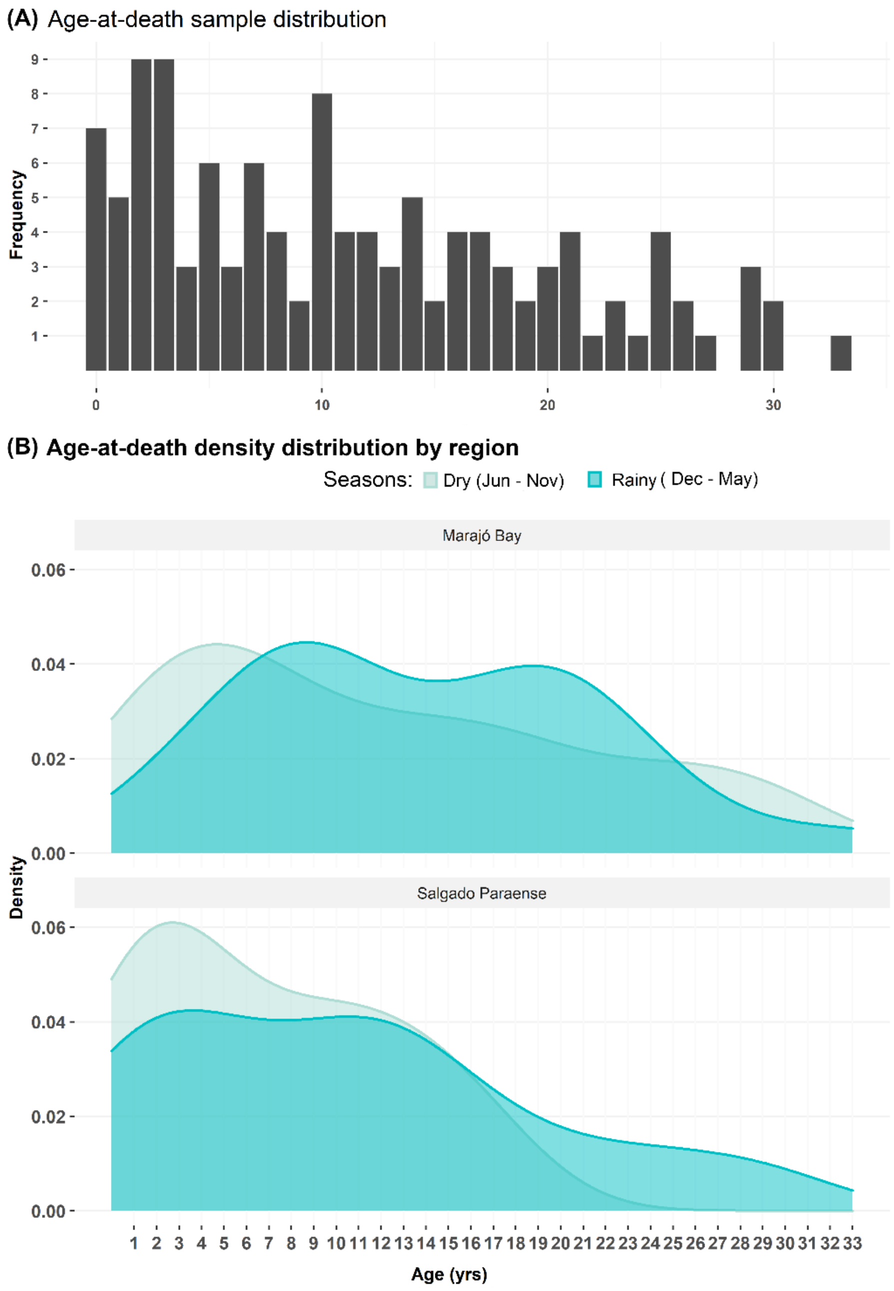

Figure 5. Age distribution of the sample of 120 specimens of Guiana dolphin stranded on the southern coast of the Amazon Estuary. (A) Total age-at-death distribution including the three

sampled sections on the north coast of Marajó Island – NMI, Marajó Bay – MB, and Salgado Paraense – SP; (B) Age-at-death density distribution by seasons for the regions Marajó Bay and Salgado Paraense.

4. Discussion

Results indicated a high frequency of stranding events for Guiana dolphins in the Amazon Estuary. Additionally, was detected a seasonal pattern between the years 2005 and 2014, with strandings gradually decreasing during the rainy season in Marajó Bay (MB) inner region – December to May, and posteriorly increasing throughout the dry season – June to November. An opposite pattern was identified for Salgado Paraense (SP) coastal region. The long-term analysis also indicated a significant decrease in stranding events over the years for the MB and no significant long-temporal change in the SP coast. Likewise, age-at-death distribution showed different patterns of mortality between these regions, with a predominance of young-adult classes stranding in the rainy season specifically for the MB. The spatial distribution of the strandings indicated also different processes of stranding spatial accumulation between the areas and confirm the detected seasonality. Thus, at a regional scale, the strandings pattern of Guiana dolphins differed seasonally and on a larger temporal scale between coastal and more inner regions of the Amazon Estuary, suggesting different pressures acting on the mortality of the Guiana dolphin community at the different areas of this ecosystem.

Strandings and sampling bias

The 609 specimens of Guiana dolphins stranded on the Southern coast of the Amazon Estuary between the years 2005 and 2016 were high when compared to stranding records reported for this species in other regions of Brazil, in a similar or longer period (Northeast: 160 events in 14 years – Meirelles et al., 2010; Southeast: 344 events in 40 years – Mayorga et al., 2020; South: 97 events in 32 years – Vianna et al., 2016). Although a similar number of Guiana dolphin stranded was described recently for the Southeastern Brazilian region – 621 events in 5 years period, based on a more intense beach survey program in a larger latitudinal range (Prado et al., 2022), the current results indicated higher mortality for Guiana dolphin in the Brazilian Northern region, in contrast to the other Management Units in the South Atlantic Ocean. This high mortality rate was suggested by Emin-Lima et al. (2010) in a preliminary study on the Amazon Estuary and seems to persist over the years. Thus, presumably, indicating a high abundance of the species in this environment and, probably, a constant exposure to anthropic and local environmental pressures – temporal climatic changes (e.g. da Costa et al., 2022), fishing activity (e.g. Freire et al., 2021), increase of shipping traffic and tourism (e.g. Pereira, 2018), and coastal environmental degradation (e.g. Barletta & Lima, 2019; Lima et al., 2021).

It is important to acknowledge that some sampling bias could influence the actual results. Initially, the aggregation of strandings at specific geographic points inside the MB and SP were probably related to the physical-geographic characteristics of the Amazon Estuary – e.g. seasonal flooding, tidal gradient, and extended mangrove belts, which made continuous monitoring of the area impossible and may have caused differences in sampling effort between the monitored segments. Additionally, the interval between beach surveys, which were carried out every two weeks (~10-15 days), may have influenced the recovery of carcasses due to the exposition time, underestimating the observed stranding rate. Recovery rates of carcasses in periodic beach surveys could be affected by the carcass decomposition process, the action of scavengers, or the carcass removal by locals (e.g. Peltier et al., 2012; Tavares et al., 2021), and the probability of recovery decrease with the increase of the sampling interval. For instance, adult cetacean carcasses decompose rapidly to stage 4 in warmer conditions, and the process is also affected by the body size (Gol'din, Vishnyakova & Gladilina, 2013; Moore et al., 2020). Likewise, monitoring of cetacean strandings commonly underestimated neonate mortality, due to the rapid decomposition occurring in this age class regardless of species or body size (Gol'din, Vishnyakova & Gladilina, 2013). Thereby, the high mean annual temperatures characteristics of the tropical monsoon climate of the Amazon coastal region (Köppen classification, in Alvares et al., 2013) certainly could cause a faster decomposition of carcasses resulting in a low discovery rate of some ages classes, such as newborns – only 7 specimens <1 year old recorded in 12 years of sampling. Despite this bias, the results indicated that factors other than sampling are influencing the frequency of strandings of Guiana dolphins in this region. Nevertheless, it is reasonable to assume that the mortality of Guiana dolphins in the Amazon Estuary must be higher than observed.

Seasonal changes in environmental conditions, such as tidal changes, precipitations, rivers' discharge, sea currents variation, and wind strength and direction, may have also contributed to the observed spatial distribution and temporal variation of strandings. It is acknowledged that some of these ecological stressors could influence the temporal pattern of cetacean strandings, such as in the case of the coastal species *Tursiops aduncus, Sousa chinensis,* and *Orcaella heinsohni* (Meager & Limpus, 2014)*,* the highly migratory species *Megaptera novaeangliae* (Meynecke & Meager, 2016) and the oceanic species *Kogia* spp*.* (Moura et al., 2016). Furthermore, local environment conditions could affect the drift of cetacean carcasses, one of the aspects that have been seated as directly influencing the strandings rates (Peltier et al., 2012; Peltier et al., 2013; Moore et al., 2020; Tavares, 2021). Environmental factors were not considered in this study since the goal was to characterize the pattern of stranding and mortality of Guiana dolphins in the Amazon Estuary, as first step for the construction of a baseline of indicators for this population. However, more specific research is needed to characterize the environmental predictors conditioning the stranding process of Guiana dolphin in this ecosystem.

Seasonality of strandings

Based on the results it is possible to assume the existence of a marked seasonality in the stranding frequency and therefore, in the mortality of Guiana dolphin in the Amazon Estuary, with inverse timing between inner estuarine regions and more coastal areas. In this respect, seasonal environmental changes in the Amazon Estuary, related to variations in the quality and conditions of this ecosystem, may not only be able to shape the physical pattern of strandings, but also could influence the distribution pattern of the live populations of Guiana dolphin in the area, and thus being reflected in the strandings. The annual hydrological cycle in interaction with a marked tidal variation governs all the ecological processes in the Amazon Estuary (Barthem & Schwassmann, 1994; Menezes et al., 2013; Carneiro, Prestes & Rollnic, 2020). In the rainy season (December- May), the coastal salty waters of MB are replaced by freshwater due to the high levels of water discharge from the Pará River, Amazon River and Araguaia-Tocantins basin to the estuary (Q mean > 30000 m^3s^{-1}, Prestes et al., 2020). Consequently, there is an increase in the abundance and diversity of riverine fish species in the area. In the dry season (June-November), this discharge decreases (Q mean < 16000 m^3s^{-1}, Prestes et al., 2020), and, with the incoming of coastal water inside the MB, the salinity gradually increases, and the fish species composition changes, adding marine species to the area (Barthem, 1985; Oliveira & Fredoú, 2011; Mourão, Ferreira, & Lucena-Frédou, 2014; Mourão, Frédou, & Lucena-Frédou, 2018). Thus, if the food ability/quality decrease inside the MB during the rainy season, some groups of dolphins may change their distribution to more coastal areas during this period following these processes of floods and droughts. Consequently, the number of strandings may decrease inside the MB – inner riverine region of the estuary and increase in the SP – marine coastal region, and the opposite will be observed in the dry season.

Habitat use and changes in preferential areas driven by shifts in the abundance of major prey, seasonal salinity gradient, and foraging strategies have been previously indicated for populations of Guiana dolphins from Eastern and Southern Brazil (Wedekin, Daura-Jorge & Simões-Lopes, 2010; Rodrigues et al., 2020; Ferro de Godoy, Mendonça & Andriolo, 2020). The species *Trichiurus lepturus*, *Bagre bagre*, *Chathrops spixii*, *Mugil curema*, and *Anchoa spinifer* have been described as preferential prey for Guiana dolphins inhabiting the Amazon coast (Beltrán-Pedreros & Pantoja, 2006). Four of these species are more abundant inside the

MB between the months of transition and high salinity, May to December (Barthem, 1985). Stomach contents of Guiana dolphins stranded in MB and SP included also the demersal fishes with estuarine cycle *Macrodon ancylodon*, *Stellifer rastrifer*, and *Gobioides broussonnetii*, and the estuarine benthic species *Lycengraulis grossidens* as principal preys (Vieira, 2014). Although these species transit between freshwater, brackish, and coastal environments, distributions of adults and juveniles have been related to the seasonal increase in salinity occurring in the dry season in inner regions of the estuary (Barthem, 1985; Camargo & Isaac, 2005; Mai & Vieira, 2013). The abundance of these preys, predominantly in MB, coincides with the months of higher frequency of Guiana dolphin strandings inside the bay, may be reflecting seasonal changes in the spatial distribution of the Guiana dolphin populations on the Amazon coast.

In addition, part of the observed seasonal increase in Guiana dolphin strandings could be also explained by the species' interaction with the fishing local activity. The fishing effort in the Brazilian North Region has been described as intense throughout the year, representing 29% of the annual fishing production of Brazil (Ministério da Pesca e Aquicultura, 2011). The hydrological cycle, the biological cycle of the target species – mainly with migratory habits, and the regional climate influence the fishing operations (Isaac, Milstein & Ruffino, 1996), with the action in the rainy season of both, local artisanal float, and industrial fishing float in the more coastal areas, such as SP. In the dry season, the capture is usually concentrated by the artisanal fishing float, since small vessels are more capable of getting into the internal estuarine areas and channels (Oliveira et al., 2007). The principal fishing method is gillnet, being increasingly used over the dry season (Oliveira et al., 2007; Isaac & Ferrari, 2017). Thus, fishers in the region seasonally explore ocean, coastal water, estuarine environments and the inner riverine waters based on the temporal abundance of the target species (Barthem, 1995; Oliveira et al., 2007).

Due to the exclusively coastal habits of Guiana dolphins, fishing activities are the main threat to populations of this species along the Brazilian coast, resulting in a high number of specimens incidentally caught *per* year (Siciliano, 1994; Di Beneditto et al., 1998; Netto & Di Beneditto, 2008; Meirelles et al., 2010). In the MB and SP coast fisheries operations are conducted principally by small vessels in deeps between 3m and 50m (Barthem & Fabré, 2004; Espírito-Santo & Isaac, 2012). Incidental catches of Guiana dolphins in gillnets have been previously reported for the Amazon Estuary (Siciliano, 1994; Emin-Lima et al., 2008), with at least one animal estimated to be caught in each fishing operation (Emin-Lima et al., 2008; Martins, 2015). Gillnets are positioned mainly within Guiana dolphin's preferential areas (Zappes et al., 2009), which justifies the intensity of this interaction, reinforcing the hypothesis of a high mortality related to incidental capture, i.e. bycatch. Ethno-ecological research also indicated that most of the Guiana dolphin specimens accidentally captured are discarded (Lima-Martins, 2015; Filgueira et al., 2021), which may explain the high number of stranded dolphins observed over the years at the coast of the estuary. Moreover, the use of carcasses by local populations as bait and for the traditional trade of anatomical parts, such as teeth, eyes, and skin, is well documented (Gravena et al., 2008; Siciliano et al., 2018; Ruenes et al., 2022) and may indicate increased pressure over this Guiana dolphin's Management Unit.

The overall age-at-death distribution showed a representation of the selected sample for most ages among 0 and 33 years. When this distribution was divided between regions and seasons, the age density seems to differ, with the age distribution being more stable for animals stranded on the coast of SP. Thus, showing the expected larger number of younger specimens, followed by juveniles, adults, and a small number of older age classes (Mannocci et al., 2012). The same pattern was also detected for the MB in the dry season. However, in the rainy season, this pattern changes in the MB with an evident increase in mortality for the ages between 6 and 10 years and the ages between 18 and 20 years. This predominance of younger adults in the sample (especially between 6 and 10 years) and the bimodal distribution of the ages, may be caused by a selective effect of bycatch on these ages. The effect of fishing activity on the mortality of younger sexually mature adults due to their inexperience has been reported previously for other species of dolphins, such as *Cephalorhynchus hectori* and *Delphinus delphis* (Slooten & Lad, 1991; Mannocci et al., 2012). For Guiana dolphins, the effect of bycatch on young mature individuals has been reported in Northeastern and Southeastern coastal regions of Brazil (Santos, Rosso & Ramos, 2003; Di Beneditto & Ramos, 2004; Meirelles et al., 2010), and suggested by Ruenes et al. (2022) for Northern region.

Long-temporal trends

The long-temporal significant decrease of strandings over the years inside MB could led by some hypothetical scenarios, also related to the local fishing activity. First, a time trend of decreasing fishing effort in this specific inner area. This, with a consequent reduction in incidental catch, but only if incidental catch was the only aspect affecting the observed stranding frequency of Guiana dolphins, which seems to be unlikely. Despite no formal regional data of annual fishing production/effort was available for the sampling period (2005-2016), between the years 1999 and 2003, fishing landings increase from 41200t to 50000t in the ports located in the estuarine area of MB (Oliveira & Frédou, 2011). Reconstruction of the marine commercial landing of artisanal fisheries indicates also an increase for all the Brazilian Northern region starting in 2000 and extending until 2015 (Freire et al., 2021). Thus, fishing activity seems to increase in the South Amazon Estuary throughout the years, not decrease. Another, and more plausible hypothesis is the decrease of the population of Guiana dolphins inside Marajó Bay between the years 2005 and 2014. The increase of the fishing effort in the area could be led to both, a diminution of prey availability and, as consequence, the habitat abandonment for part of the population, or an increase of Guiana dolphin's bycatch with the same result of population declining.

No long-temporal change was detected in strandings on the SP coast. This may indicate an insufficient sampling interval to detect large-scale temporal patterns of strandings in this area, or other factors different from those supposed for MB, acting on stranding's pattern of Guiana dolphins at this coast. Furthermore, a lower intensity of anthropogenic effects on Guiana dolphin populations in this region could lead to similar results. Previous study suggested a high abundance of Guiana dolphins in the SP, which principally occupy interior bays and channel systems for feeding, and mainly interact with the traditional fishing gear named as *curral* in this area (see Torres & Beasley, 2003). This fishing gear does not appear to be a threat to Guiana dolphins and this interaction has been reported in other regions of Brazil (Monteiro-Filho, 1995; Ferro de Godoy et al., 2020). However, population information for more open coastal areas is missing in the Amazon Atlantic coast. Thus, others studies are needed to confirms this supposition.

Implications for conservation

Guiana dolphin strandings in the Southern Amazon Estuary reflected the seasonality characteristic of this environment. The fishing, principal human activity in this region, also seasonally structured, could be related to the detected changes in the strandings and the high number of records, since historical dolphin bycatches are well documented for the Brazilian North coast. Tavares et al. (2021) suggested that strandings of resident marine species such as Guiana (*S. guianensis*) and Franciscana (*Pontoporia blainvillei*) dolphins, due to their predominance and the constant interaction of these species with fisheries, could be classified as predictable resources in the South and Southeastern coasts of Brazil. This predictability could be also assumed for the Northern Brazilian coast, based on the seasonality detected in the strandings frequency of Guiana dolphins. In this scenario, conservation efforts must have into consideration this seasonality in the management of the Northern Brazilian Management Unit of the species. In addition, systematic monitoring of strandings in this region could be a way to preserve other adjacent ecosystems on the Amazon Estuary, such as mangroves, sandy beaches, and flooded forests, which probably are supplied for this seasonal income. Furthermore, urgent studies to clarify the influence of bycatch on the observed mortality rises as principal future step. This, because the necessity to understand if the observed mortality rates reflected a population depreciation caused by anthropic pressures, as could be the case of MB, or, if it is a result of the interaction of a high populations densities with the intense local fishing activity. The clarification of these hypotheses may be the basis for effective conservation actions related to the preservation of the species in the Northern Brazilian region.

On the other hand, the validation of strandings as population indicators needs for comparison with other kinds of population data (Peltier et al., 2014; Carretta et al., 2016; IJsseldijk et al., 2021). Therefore, further studies are needed including an assessment of the living population of Guiana dolphin – e.g. population size, and life history traits, to support the observed patterns of the strandings. More than that, these studies are essential for the characterization of this poorly-known Management Unit. Our study is an initial evaluation of temporal fluctuations in the population mortality of Guiana dolphin from the Amazon Estuary, using effectively, strandings as a source of biological and ecological information. The results confirmed the potentiality of stranding data to assess temporal changes on coastal populations of aquatic mammal species and as indicators of surrounding environmental conditions, and suggest a temporal effect and a human impact in this region.

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CAPÍTULO III: FROM NORTH TO SOUTH: GEOGRAPHIC VARIATION IN SKULL SIZE AND GROWTH OF GUIANA DOLPHINS IN BRAZILIAN WATERS AND THE INFLUENCE OF LOCAL ENVIRONMENT

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Abstract

Skull morphology have been useful tools to assess geographic variation and adaptation of cetaceans to environmental conditions through their evolution and current distribution. In this context, the condylobasal length (CBL), teeth length (TL), and age of 352 Guiana dolphins (*Sotalia guianensis*) were used to assess differences in skull size and growth of populations from north (n= 96), northeast (n= 31), southeast (n= 101), and southern (n= 39) Brazilian management units (MUs). In addition, the relation between CBL and environmental variables related to the species distribution was tested, including temperature, latitude, primary productivity, continental shelf extension, and estuarine environment dimensions of each region. As a result, Gompertz models described well the skull growth for the different MUs. The asymptotic skull' size (CBL_{∞}), the CBL of adults, and the TL showed similar patterns, with sizes increasing from North to Northeast, and decreasing from Northeast to Southeast and South populations. The slowdown/retardation in the skull growth seems to have different consequences, resulting in large or small forms, probably related to local environmental conditions and genetic differentiation between populations. A negative influence of sea surface concentration of chlorophyll-a (Chl-a) on the skull's size was detected, with the presence of larger animals in the oligotrophic coastal regions of Brazil. Although the shelf extension and estuaries' dimensions had a similar negative influence, explained fewer of the data variation. Thus, the geographical variation in the skull size of Guiana dolphins from the Brazilian coast may be related to different feeding habits, since lager skulls and teeth could allow the access to larger prey or a variety of sizes of prey, which increases the availability of feeding resources in oligotrophic regions. Finally, this could be advantageous for the incursion in open coastal environments subjected to competition or predation.

Keywords

Sotalia, Asymptotic size, Primary productivity, South Atlantic Ocean, Brazilian Management Units, Growth Layers Groups, Estuarine dolphin, cranial morphology

1. Introduction

Geographic variation in morphology could be a result of distinct selective pressures acting in different environmental conditions (Gould & Johnston, 1972). For marine mammals, most geographic variation in morphology and the underlying genetic basis is more presumably due to differential selection, i.e., ecological divergence, than to genetic drift (Perrin, 2009). Aspects such as temperature, primary productivity, feeding habits, and population density have been found to lead to intraspecific variations in morphological structures, resulting in differentiations among populations (Galatius & Gol'din, 2011; Perrin *et al*., 2011; Sepúlveda et al., 2013; Gol'din & Vishnyakova, 2016). For instance, in some cetacean species, such as *Tursiops truncatus*, and *Globicephala* spp., populations from colder areas seem to attain larger body sizes than those from warmer regions, which could be accompanied by the latitudinal gradient (Adamczak *et al.*, 2019; van Aswegen et al., 2019). In addition, high productivity areas appear to be associated with the attaining of larger bodies and skulls in both, cetaceans (e.g., Galatius & Gol'din, 2011) and pinnipeds (e.g., Sepúlveda *et al*., 2013), suggesting a link with some ecogeographic rules – e.g., Bergmann's rule (Torres-Romero *et al.*, 2016), Allen's (Adamczak *et al.*, 2019), and Rosenzweig's hypothesis (Sepúlveda *et al.*, 2013).

Bergmann's rule indicates that specimens from colder areas attain larger body sizes in comparison with those from warmer regions, as a response to avoid heat loss (Blackburn *et al*., 1999). In addition, changes in primary productivity could lead to the same pattern of increase of size (Rosenzweig hypothesis – (Rosenzweig, 1968), sometimes directly related to Bergmann's rules, but sometimes suggested as having an inverse relationship – i.e., in opposition to this rule. Thus, it has been proposed that warmer environmental conditions may increase food availability and save the energy required for thermoregulation, having a positive effect on the increase of body size for some marine mammals, such as in *Enhydra lutris* and *Phoca vitulina* (Meiri *et al.*, 2007; Naya *et al.*, 2017). Finally, Allen's rule indicates a decrease of appendix size with the increase of latitude, alos related to change in temperature (Allen, 1877), having a minor attention in the case of marine mammals (Adamczak *et al.*, 2019).

Another way to comprehend such geographic variations is through the analysis of the somatic growth of individual and how this growth influences the final size of the morphological structures – *i.e.*, asymptotic size. The increase in growth rates or the delay of sexual maturity could lead to larger adult sizes, while small adult sizes could be the result of premature sexual maturation or reduced intensity of growth (McNamara, 2012). Life history traits such as growth rate, and size in general are linked to reproduction, sexual maturity, and ontogeny, and could be affected by extrinsic environmental pressures (Koons *et al*., 2008). On cetaceans' populations, the habitat characteristics (e.g., Gol'din & Vishnyakova, 2016), food availability (*e.g.*, Kerem *et al*., 2013), temperature (*e.g.*, van Aswegen *et al*., 2019), and anthropic pressures such as bycatch and comertial hunting (e.g., Read & Gaskin, 1990; Lockyer, 2007), are factors which have been also associated to changes in these growth components – e.g., growth rate, asymptotic size, age of maturity, being detected variations at temporal and spatial scales (Murphy *et al*., 2020).

For cetacean species, the majority of the data available on morphology came from skulls and skeletons deposited in museum collections, which have been acquired through stranding monitoring programs (e.g., Coombs *et al*., 2019). In this scenario, whereby data quality depends on carcass conditions, and external morphometry is not available in many times, the use of skeletal morphology comes as the first source of information. As well as body length, skull morphology – based on shape and size, have been useful tool to assess geographic variation, life habits, and adaptation of cetaceans to environmental conditions through their evolution and current distribution (*e.g*., Monteiro-Filho *et al*., 2002; Barreto, 2016; Galatius *et al*., 2020; Laeta *et al.*, 2021). Moreover, since dolphins, in general, are a homodont group, tooth morphology has been assessed in intend to understand changes in physiology related to environmental conditions (Wittmann *et al*., 2016), feeding habits (Bianucci *et al*., 2022), and geographic variation in shape and size (Akin, 1988), as well as tools for the determination of specimens' chronological age (Read *et al*., 2018).

The Guiana dolphin *Sotalia guianensis* is a small marine cetacean with a distribution restricted exclusively to coastal shallow waters of Central and South America, and significantly associated with estuarine environments and mangroves (Flores *et al*., 2018). The distribution of the species is limited by temperature and depth, not exceeding the latitude of 28°S and the 50 m isobaths (Secchi *et al*., 2018). Males can reach 230 cm in body length, and sexual maturity is reached at the ages of 6 - 7 years with sizes of 170-180 cm, while females reached its maturity at the ages 5-7 years, with sizes of 160-169 cm (Rosas & Monteiro-Filho, 2002; Ramos *et al*., 2010). Across Guiana dolphin distribution range, discrete populations of the species tend to occur, driven by a reduced individuals' home range and the species small body size, which has led to 12 genetically different management units (MUs) (Cunha *et al*., 2020 *apud* Domit *et al.*, 2021). The population with the highest genetic diversity in South America is the Northern Brazilian population, placed at the Amazon Estuary region. However, this diversity tends to decrease towards the Eastern and Southern Brazil MUs (Caballero *et al*., 2018).

Despite the many studies related to the skull morphology of Guiana dolphins, the majority of them was focused on the delimitation of this species from the riverine form of the genera, *Sotalia fluviatilis* (*e.g*., Borobia, 1989; Monteiro-Filho *et al*., 2002; Fettuccia, 2006;

Fettuccia *et al.,* 2012). Other studies had assess body size, skull morphology, and body growth but only focused on specific populations (Ramos *et al.,* 2000; Santos *et al*., 2003; Sydney *et al*., 2012; Lima *et al.,* 2016). Moreover, most of the morphometrics and biological information related to the geographic variation of the species have being focused on body length, and in much less proportion on skull length, being limited to the Southeastern and Southern Brazilian MUs (*e.g*., Ramos *et al.,* 2000; Rosas & Monteiro-Filho, 2002; Santos *et al.*, 2003; Di Beneditto & Ramos, 2004; Ramos *et al.,* 2010; Lima *et al*., 2016; Conversani *et al*., 2021). Meanwhile, Guiana dolphin populations from the Northeast and North have been poorly investigated (*e.g*., Laeta et al., 2010; Arcoverde et al., 2014; Novais *et al*., 2020). Therefore, most of the life-history traits described for the species (*e.g*., growth, sexual maturity, asymptotic size, and size-at-birth) have been reported from the southern range of its geographic distribution range.

Due to this lack of information about geographic variation on the morphology and life-history traits of Guiana dolphin on the South Atlantic coast, the goals of our study were a) to describe the skull size and growth for Guiana dolphins from five management units (MUs) placed at north, northeast, southeast, and southern Brazilian coast, and compare the obtained growth parameters between them; b) to analyze the relation between the skull size and environments factors of along the MUs in Brazilian coast; and c) to analyze the relation between teeth size and skull size, as well as the influence of sex and locations (MUs) in this relation.

2. Material and Methods

Brazilian coast characterization

The Brazilian coastline extends for 7,400 km of the South Atlantic Ocean, between Cape Orange (latitude 4°N) and Chuí (latitude 34°S) (Muehe & Neves, 1995). The geographical and hydrological characteristics of the coastal zone indicate five principal regions: the North, Northeast, East, Southeast, and South (Knoppers *et al.*, 1999). The North zone includes the Amazon Estuary and the extensive mangrove complex named *Reentrâncias Parenses* (Pará State – PA) and *Reentrâncias Maranhenses* (Maranhão State – MA), which are considered the second largest mangrove corridor in the world. This region is characterized by a marked macro-tidal regimen, an intense rainy season, influenced by the Brazil North Current, and the high run-off of the Amazon River and tributaries and Parnaíba River and tributaries. The Northeast coastal zone is characterized by a narrow open shelf in the east section, directly influenced by the oligotrophic South Equatorial Current (SEC) and with limited run-off from the continent, creating a tropical low-productivity area (Knoppers *et al.*, 1999). The run-off gradually increases through Southeast and South regions by the influence of the

Brazilian Current (BC) in interaction with the enlargement of the continental shelf and Malvinas Current (MC), promoting the increase of diversity and productivity (Knoppers *et al.*, 1999; Kämpf & Chapman, 2016). Between Northeast-Southeast limit and Southern Brazil, three primary upwelling zones are placed: the South Bahia upwelling zone (15°S–19°S) in the Northeast, the Cabo Frio upwelling zone (27°S–32°S) in the Southeast, and the Santa Marta upwelling zone (27°S–32°S), in the South ((Kämpf & Chapman, 2016). The Brazilian littoral includes more than 41 estuaries complex larger than 40 km^2, encompassing areas with different topography, climate, tidal ranges, and coastal drift (Lessa *et al.*, 2018). The climate is mainly tropical/humid in the North (Am Köppen type), varies between tropical dry/semiarid (Bsh) and tropical humid (Aw) in the Northeast, still tropical/humid (Af/Aw) in the East, and it changes from Tropical humid (Af) to subtropical humid (Cfb) at Southeast and South (Cfa/Cfb) (Alvares *et al.*, 2013).

Sampling data

In this study we segmented the data using the genetic population structure proposed for Guiana dolphins, *Sotalia guianensis*, which established 12 Management Units (MUs) throughout the species distribution range, being 8 located on the Brazilian coast (Cunha *et al.,* 2020 *apud* (Domit *et al.*, 2021). The MUs sampled were Brazilian Northern MU (BRNO), Brazilian Northeastern MUs (BRNE1 and BRNE3), Brazilian Southeastern MUs (BRSE1 and BRSE2), and Brazilian Southern MU (BRSE/S) (Figure 1).

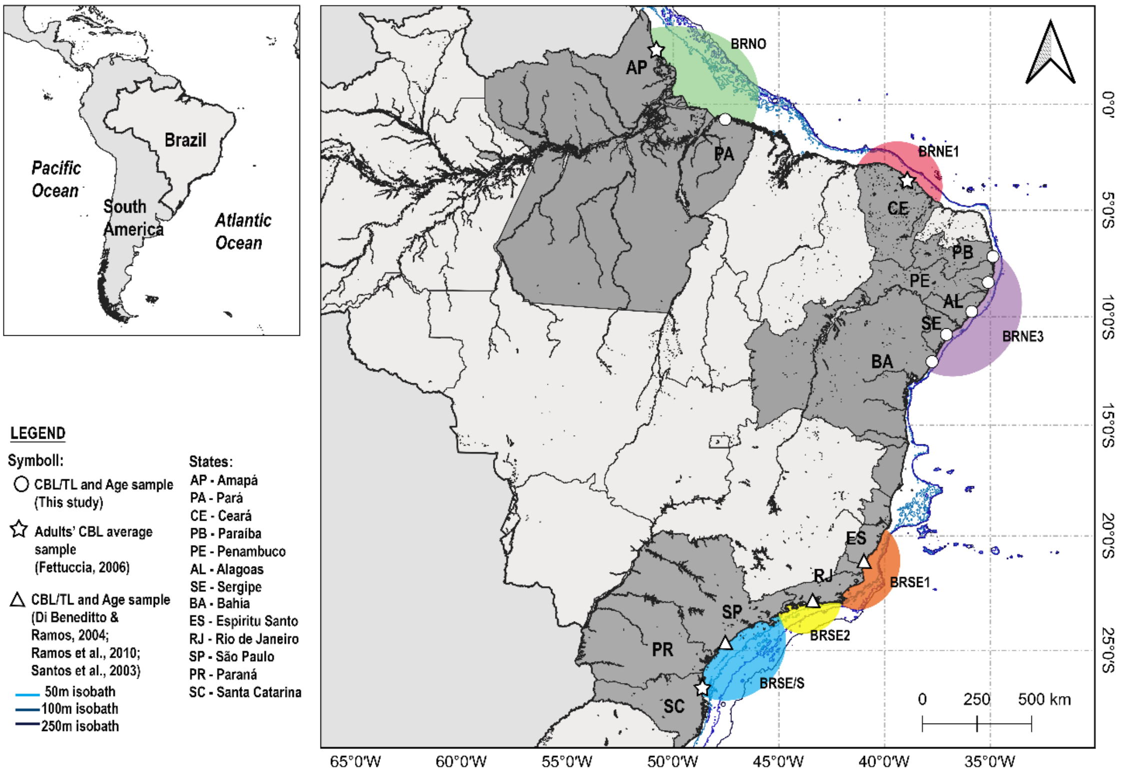

Figure 1. Sampling locations on the management units (MUs) of Guiana dolphins from the Brazilian coast: Brazilian North Region MU (BRNO), Brazilian Northeastern MU-3 (BRNE3), Brazilian Southeastern MU-1 (BRSE1), Brazilian Southeastern MU-2 (BRSE2), and Brazilian South MU (BRSE/S). CBL: Condylobasal length, TL: tooth length.

North and Northeast Brazilian regions

We analyzed data from 96 Guiana dolphins stranded on the Southern Amazon Estuary, that represented the Brazilian North coast MU (BRNO) (26 females, 35 males, and 35 individuals where sex was not possible to identify), between the years 2005 and 2016. This sample was collected throughout the beach monitoring program of the *Grupo de Estudos de Mamíferos Aquáticos da Amazônia* (GEMAM) and is part of the marine mammal collection of the *Museu Paraense Emilio Goeldi* (MPEG), placed in Belém, Pará State, Brazil. Further details on the monitoring program conducted along the coast of Pará can be found in Siciliano *et al.,* (2008) and Costa *et al.*, (2017, 2020). Based on the stranding location and previous genetic studies (Cunha *et al*., 2010; Caballero, 2018) we assumed all of these individuals as bellowing to the Brazilian Northern MU (BRNO) of the species.

Were added to the dataset 31 Guiana dolphins (13 females, 13 males, and 5 without sex identification) stranded in the Northeastern region of Brazil, between the coordinates 6°50’S/34°54’W and 11°49’S/37°33’W, including the states of Paraíba (PB), Pernambuco (PE), Alagoas (AL), Sergipe (SE) and the north sector of Bahia (BA). This data was collected as part of the stranding monitoring programs led by the *Centro Mamíferos Aquáticos* (CMA/ICMBio) and the *Fundação Mamíferos Aquáticos* (FMA) in this region. Based on the stranding locations we assume these individuals as bellowing to the Brazilian Northeastern MU-3 of the species (BRNE3).

Measurements

To characterize the growth of the Guiana dolphin populations from Brazilian coast, the variables used were sex, Condylobasal length (CBL), tooth length (TL), and the chronological age of each individual. The CBL was used as a proxy of body length, since this measurement is not always available in samples collected from strandings. CBL was measured as described by (Perrin, 1975), from the tip of the rostrum to the hindmost margin of occipital condyles of the skull. The TL was measured in each tooth used for the age determination as described by Ramos *et al.*, 2000, in a straight line from the tip of the crown to the end of the rood. Measurements were taken with a caliper or a measuring tape, to the nearest 0.05 mm or 0.50

mm, respectively for teeth and skulls. The chronological age was obtained as described in the *Age determination* section (Table 1).

Data source for other populations

Previously published data were used to assess the age and CBL measurement of Guiana dolphins stranded or accidentally captured on the Southeastern and South Brazilian coast (Santos *et al.*, 2003; Di Beneditto & Ramos, 2004; Ramos *et al.*, 2010), including the South of Espírito Santo state (ES) and Rio de Janeiro Northern Region (RJN) as the Brazilian Southeastern MU-1 (BRSE1) and Rio de Janeiro Southern Region (RJS) as the Brazilian Southeastern MU-2 (BRSE2). The sample from these MUs included, respectively, 128 (47 females, 63 males, and 18 individuals without sex identification), and 39 Guiana dolphins (17 females, 12 males, and 10 individuals without sex identification). Data from 67 dolphins (17 females, 10 males, and 40 individuals without sex identification) stranded in São Paulo (SP) and North Paraná regions (PR), representing the South MU (BRSE/S) of the species were also added. For the referenced dataset, TL was only available for the specimens from BRSE1, deposited in the collection of marine mammals from the *Museu Nacional, Universidade Federal do Rio de Janeiro* (MN/UFRJ), Rio de Janeiro, Brazil. In this case, the measurement was taken by the first author selecting always the straightest, longest, and less worn of the teeth in the sample (Table 1). In addition, the average CBL for adults from the localities of Amapá – AP (BRNO), Ceará – CE (Brazilian Northeastern MU-1 – BRNE1), and Santa Catarina – SC (BRSE/S), available in Fettuccia (2006), were used on the geographic variation analysis (see *Statistical Analysis* section).

Table 1. Sample of Guiana dolphin (*Sotalia guianensis*) from different regions along the Brazilian coast. See Supplementary Table S1 for data from all variables.

Total Sample	**N adults**	**Teeth sample**	**Region**	**Mus**	**References**
–	6	-	Amapá (AP)	BRNO	Fettuccia (2006)
96	60	82	Pará (PA)	BRNO	This study
–	34	-	Ceará (CE)	BRNE1	Fettuccia (2006)
3	2	3	Paraíba (PA)	BRNE3	This study
6	3	4	Pernambuco (PE)	BRNE3	This study
6	4	5	Alagoas (AL)	BRNE3	This study
14	13	14	Sergipe (SE)	BRNE3	This study
2	2	2	Bahia-North (BA)	BRNE3	This study
17	10	-	Espírito Santo-South (ES)	BRSE1	Ramos *et al*. (2010)
111	28	65	Rio de Janeiro-North (RJN)	BRSE1	Di Beneditto & Ramos (2004); Ramos *et al*. (2010),

					This study (Teeth sample)
39	26	-	Rio de Janeiro-South (RJS)	BRSE2	Ramos *et al*. (2010)
67	42	-	São Paulo (SP) / Paraná- North (PR)	BRS/SE	Santos *et al.* (2003); Ramos *et al*. (2010)
–	26	-	Santa Catarina (SC)	BRS/SE	Fettuccia (2006)

Age Determination

The teeth of the stranded specimens from BRNO and BRNE3 were used for age determination, always choosing the largest and straightest tooth of the available sample. This selection allows us to obtain the most accurate age for each individual, since straight and bigger teeth must reflect a more realistic deposition of dentine through the years (Hohn, 1980). A longitudinal grinding was initially done on both sides of the longitudinal plane of each tooth, to facilitate subsequent decalcification. The central sections of 2-3 mm thickness resulting were posteriorly decalcified with RDO® (commercial rapid decalcification agent) for a period between one and 12 hours, depending on the size and dentin density of each tooth. Once decalcified, the teeth were cut longitudinally into 20-30 µm thick sagittal sections with the aid of a freezing microtome (model RHM-THIACRON®). The obtained sections, belonging to the central axis of the tooth, were selected to stain with Mayer's hematoxylin and blued with Ammonia 2%. As result, it was obtained a tooth section differentially stained according to the structure of the dentin which allowed the counting of the deposited layers of dentine (Rosas *et al.*, 2003).

The chronological age was determined by counting the Growth Layer Groups (GLGs) deposited on the teeth's dentine, as described by Hohn (1980) and Rosas *et al.* (2003). For the readings, the equivalence between one GLG and one year of life for the species was assumed for the species following Rosas *et al.* (2003). The best sections of each tooth were photographed using a Leica S9i Stereomicroscope with an attached digital camera and processed using Leica Application Software v4.12 (Leica Microsystems, 2018), with a maximum magnification of 55x. The reading was conducted in the digital images on three different occasions by two experts, and without prior knowledge of the sex, and total length information from specimens. The final age was determined using the coincidence of at least two of the three initial readings. In case of a discrepancy of < 2 years between readings, the average of the three readings was used as the final age. When the difference between readings was greater, a final reading was performed by the experts together taking into account the body length of the individual, if available.

For descriptive purposes and data analysis, specimens were classified based on the ages into three different categories: juveniles (age ≤ 6), young adults (6 < age ≤ 12), and adults (13 ≥ ages), taking into account: a) 7 years as the age of sexual maturity for the species (Rosas & Monteiro-Filho, 2002; Rosas *et al.*, 2003) and b) 7 – 12 years as the range of ages when the complete fusion of the sutures of the occipital complex occurs, concurring with the age of sexual maturity (Fettuccia, 2010; Novais *et al.*, 2020).

Data analysis

Growth models

The CBL was used to perform non-linear regressions adjusted to the ages obtained, in order to determine the parameters and the equation that best describe the growth of the skull for the Guiana dolphins MUs with available data: BRNO, BRNE2/BRNE3, BRSE1, BRSE2, and BRS/SE. The Gompertz model, two forms of the von Bertalanffy equation, and the Richard and Logistic growth models were tested since they are the models most widely used in the evaluation of the growth in cetaceans, and particularly in the Guiana dolphins (Di Beneditto & Ramos, 2004b; Ramos *et al.*, 2010; Barreto, 2016; Conversani *et al.*, 2021).

The equations from the models were used as:

Gompertz (GOM): $L_{(t)} = L_{\infty} * e^{-ae^{-kt}}$ (Ricker, 1979)

Logistic (LG): $L_{(t)} = \frac{L_{\infty}}{1+e^{\left(-g_i*(t-t_i)\right)}}$ (Ricker 1979)

Richards (RD): $L_{(t)} = L_{\infty} * \left(1 - a * e^{(-k*t)}\right)^{b}$

Tipica von Bertalanffy (TVB): $L_{(t)} = L_{\infty} * (1 - e^{(-k(t-t_0))})$ (Beverton & Holt, 1993)

Original von Bertalanffy (OVB): $L_{(t)} = L_{\infty} - (L_{\infty} - L_0) * e^{(-kt)}$

Where: $L_{(t)}$ is the size at time t, L_0 is the size at birth; L_{∞} is the asymptotic value of size; g_i or g represents the instantaneous growth rate at the inflection point (when $t = t_0$); k is the growth constant (years^{-1}) or Brody growth rate, and represents how quickly the L_{∞} is reached; and a is the constant of integration (Fitzhugh Jr, 1976; Zullinger *et al.*, 1984). Time t is expressed in years, with a time equal to zero at birth (Laird, 1966), t_0 being the theoretical age when the length is zero, and t_i representing the inflection point.

The equations of the models were fitted without taking into account the sex of the specimens since previous morphological research found no sexual dimorphism in *Sotalia* *guianensis* (Ramos *et al.*, 2000; Monteiro-Filho *et al.*, 2002; Rosas *et al.*, 2003). Candidate models were fitted by a nonlinear iterative method using the packages ‘FSA’ v. 0.8.32 (Ogle, 2017) and ‘nlstools’ v.1.0-2 (Baty *et al.*, 2015) from RStudio v.1.3.959 (RStudio Team, 2020). Confidence Intervals (CI) and medians of model parameters were obtained by bootstrap resampling with 1000 iterations. The growth models were ranked using the package ‘AICcmodavg’ v.2.3-1 (Mazerolle, 2020) based on the corrected Akaike’s Information Criteria for small samples (AICc) and AICc weight (ωi). Models with differences in AICc (*i.e.,* Δi) of less than 2 units were considered to be comparable (Burnham & Anderson, 2004).

The age of attaining the skull’s maturation was used according to Barreto (2016) based on the growth curve parameters, being the point where after one year of age, growth will be less than 1% of the asymptotic size. Geographic differences in the skull growth were assessed through the comparison of nested subsets of the final growth model selected (Table 2), using the grouping factor “MU” as a dummy variable (Ogle, 2016; Ogle & Isermann, 2017). Models were tested initially comparing the more complex model (1) and the less complex model (8). If statistical differences exist, model 1 was compared with the nested subsets models (models 2 to 7). Log-Likelihood ratio tests (Kimura, 1980), extra sum-of-squares tests (Ritz & Streibig, 2008), and AICc were used to the comparisons. Comparisson were made with the R packages ‘FSA’ v. 0.8.32 (Ogle, 2017).

Table 2. Example of nesting models for Gompertz equation, tested for differentiation in skull’s growth between the Guiana dolphin management units (MUs). L_∞*:* asymptotic value of the body measurement; *k*: growth constant (years-1); *t*: individual’s age: and groups represent MUs: BRNO, BRNE3, BRSE1, BRSE2, and BRSE/S.

ID	Model description	Model Equations
1	L_∞, *a, k* differs between groups	$L \sim L_\infty[groups] * e^\wedge((-a[groups] * e^\wedge(-k[groups] * t)))$
2	L_∞, *a,* differs between groups	$L \sim L_\infty[groups] * e^\wedge((-a[groups] * e^\wedge(-k * t)))$
3	L_∞, *k* differs between group	$L \sim L_\infty[groups] * e^\wedge((-a * e^\wedge(-k[groups] * t)))$
4	*a, k* differs between groups	$L \sim L_\infty * e^\wedge((-a[groups] * e^\wedge(-k[groups] * t)))$
5	L_∞ differs between groups	$L \sim L_\infty[groups] * e^\wedge((-a * e^\wedge(-k * t)))$
6	*a* differ between groups	$L \sim L_\infty * e^\wedge((-a[groups] * e^\wedge(-k * t)))$

7	*k* differ between groups	$L \sim L_{\infty} * e\wedge((-a * e\wedge(-k[groups] * t)))$
8	All parameters are equals between groups	$L \sim L_{\infty} * e\wedge((-a * e\wedge(-k * t)))$

Size and environmental factors

Two different approaches were followed to describe the geographical variation in skull size among populationsof the Guiana dolphin across Brazilian Atlantic coast and its relation with local environmental conditions. First, we evaluated which environmental variables could influence the skull size along the latitudinal and climatic gradient. Afterwards, we analyze the potential relationship between teeth size and skull size, and how this relationship might change in the different regions.

The average CBL of adult specimens (>6 years old) was used as a measurement of size. Samples were available for 13 specific locations, comprising the MUs of BRNO, BRNE1, BRNE3, BRSE1, BRSE2, and BRS/SE (Table 1, Figure 1). We split the data by specific sampling locations – *i.e.,* States, instead of MUs to account for a major latitudinal gradient of variation. For all statistical procedures were used the R packages 'stats' v.3.6.2, 'AICcmodavg' v.2.3-1 (Mazerolle, 2020), 'MASS v.7.3-58.1' (Venables & Ripley, 2002) and 'MuMIn' v.1.43.17 (Barton, 2009).

To explore the changes in skull size of Guiana dolphins related to geographic variation , taking into account the characteristics of their habitat throughout the Brazilian coastline, 11environmental factors were considered: the annual average of sea surface temperature – SST in °C, the SST minimal average – SST_{min}, calculated using the winter months average for the Southern hemisphere (June, July, and August), the SST maximal average – SST_{max}, calculated using the summer months average for the Southern hemisphere (December, January, and February), and the SST range – SST_{r}, representing the differences between SST_{min} and SST_{max} for each location. These values were taken from http://www.seatemperature.org, which uses historic data and daily SST updates from the National Oceanic and Atmospheric Administration (NOAA), USA. The latitude was also included as a variable since latitude and SST could influence the size in cetaceans being related to Bergmann's rule ((McNab, 2010; van Aswegen *et al.*, 2019). The mean average of sea-surface chlorophyll-a concentration (Chl-a in mgm^{-3}) was used as a proxy for primary productivity because primary production, and it is also recognized as an important environmental factor affecting the size and growth of marine mammals (Rosenzweig productivity hypothesis – (Amano & Miyazaki, 1992; Sepúlveda *et al.*, 2013). The Chl-a was extracted from https://giovanni.gsfc.nasa.gov/giovanni/, using monthly data generated by the NASA Ocean Biogeochemical Model (NOBM) based on data assimilation of remotely-sensed chlorophyll-a.

As a proxies to characterize the physical and geographic characteristics of the different environments from the Brazilian coastal line, the distance to the 50m, 100m, and 250m depth of the continental shelf ($DS_{50,}$ $DS_{100,}$ and DS_{250}, km) for each sampled location were used. This distance was measured using QGIS v.3.10.13 software, on the middle point of each location, at an angle of 90° from the continental border to the bathymetric lines of 50m, 100m, and 250m depth of the continental shelf. The measurement was made using the ESRI Ocean base-map (https://www.esri.com/) and the GEBCO ocean bathymetric card (https://www.gebco.net/). The bathymetric lines were used as a proxy of habitat structure/characteristic, taking into consideration the depths' limit for Guiana dolphins – 50m (Secchi *et al.,* 2018), and the habitat on the continental shelf on the main fish species detected as preferential preys (Supplementary Table S2). Finally, since the species had a strong relationship with the estuary environment, the summarization of the area (ESA - km^2) and length (ESE - km) of estuaries larger than 40 km^2 of each location were also included as variables. This data was selected from Lessa *et al.* (2018), see these authors for the methodology of geographic data extraction.

Generalized linear models (GLMs) were performed to understand the relationship between the skull size and the mentioned environmental variables. The models were adjusted with Gamma distribution error and identity link function, since data were lambda transformed for counter overdispersion and heteroscedasticity issues. Due to the high collinearity between predictors, models were performed individually for each environmental variable. Models were validated using the diagnostic plots (Q-Q plot, residuals vs. linear predictors, histogram of residuals, and response vs. fitted values). Model selection was made by their ranking, using corrected Akaike Information Criteria for small sample sizes (AICc), the model weight (w), and the adjusted coefficient of determination R^2.

Finally, an analysis of covariance (two-way ANCOVA) was used to explore the relationship between skull size and tooth size, and how it might change geographically. Since previous studies have indicated that in some Guiana dolphin populations, females and males could have different prey preferences or feeding strategies (Beltrán-Pedreros & Araújo-Pantoja, 2006; Pansard *et al.*, 2011) and this might be reflected in the shape of skull components, the sex of specimens was also added to this model. Thus, the ANCOVA included the independent continuous variable CBL (mm), as factors sex and location, and as the dependent variable the TL (mm). Interaction between predicting variables was also tested. The model was validated using the diagnostic plots Q-Q plot, residuals vs. linear predictors, histogram of residuals, and response vs. fitted values, and continuum variables were lambda-transformed to attain the analysis' premises.

All statistical analyses were performed in RStudio v.1.3.959 (RStudio Team, 2020) free software. Graphics were generated using the R packages 'ggplot2' v.3.4.0 (Wickham *et al.*, 2016).

3. Results

Ninety-six Guiana dolphins *Sotalia guianensis* stranded on the Pará coast, and bellowing to the BRNO of the species (BRNO) were aged. The mean age of these specimens was 11 ± 8 SD. The most frequent age classes were 2 and 10 years, with females ranging between 0 and 36 years – the maximum age observed for this species, and males ranging between 0 and 29 years. Twenty-nine percent of the sample had ages less than 7 years, being considered as immatures, 25 % had ages ranging between 7 and 12 years, being considered as young adult and the other 28 % represented completely mature adults. The ages of 31 specimens from the BRNE3 region ranged between 0 and 27 years. The older identified female and male were 25 and 23 years old, respectively. The mean age was 13 ± 7 SD and the most common age observed was 15 years. Age categories were represented in 16 % by immatures, 25 % by young adults, and 45 % by mature specimens. In the data available for the Southeastern and Southern Brazilian coast (Table 1), the 129 specimens from the Brazilian Southeastern MU-1 (BRSE1) the ages ranged between 1 and 30 years old, with a mean of 7 ± 5 SD, and modes of 4 and 5 years. Seventy percent of this sample was compounded by immature animals, 18 % by young adults, and 11 % by older specimens. The BRSE2 region included 39 specimens with ages ranging from 0 to 30 years, mean of 11 ± 9 SD, and mode of 8 years. In this case, younger and young adult consisted equally 33 % of the sample, and older specimens were 34 %. Finally, the sample of 67 specimens from the Southern MU (BRSE/S) ranged in age from 0.5 to 29 years, with a mean of 11 ± 9 SD and as most frequent age 3 and 6 years. Younger immature specimens consisted of 36 %, while young adults were 25 % and older specimens represented 39 % of the sample.

Concerning to skull's dimension, the CBL of Guiana dolphins from BRNO ranged from 240 mm to 283 mm (N = 96, Median = 354mm, Mean = 346.4 mm ± 25.86 SD), while for the BRNE3 dimensions were between 235 mm and 404 mm (N = 31, Median = 389 mm, Mean = 380 mm ± 32.57 SD). Ralated to compiled data (Table 1), management units from for BRSE1 had CBLs ranging between 292 and 410 mm (N = 118, Median = 370 mm, Mean = 368.8 mm ± 25.86 SD) and for BRSE2 between 225 and 395 mm and (N = 39, Median = 375 mm, Mean = 369 mm ± 28.84 SD). The most Southern MU with data available, the BRSE/S, showed CBL dimensions between 236 mm and 396 mm (N = 69, Median = 366 mm, Mean = 346.4 mm ± 23.60). Adult means were 359.5 mm for BRNO, 388.4 mm for BRNE3, 381 mm for BRSE1, 379.5 for BRSE2, and 371.1 mm for BRSE/S (Supplementary Figure S1).

Teeth size varied from North to Southeast of Brazil with specimens from BRNO showing teeth lengths between 8.5 and 18.8 mm (N = 82, Median = 17 mm, Mean = 16.7 mm ± 1.39 SD). For the BRNE3, teeth length ranged from 15.3 to 20.3 mm (N = 31, Median = 18.7 mm, Mean = 18.4 mm ± 1.13 SD). Finally, for the other MU with data available, BRSE1, teeth sizes ranged between 13.76 and 20.78 mm (N = 65, Median = 17.9 mm, Mean = 17.9 mm ± 1.22 SD).

Growth

All growth models fitted similarly for all management units, except for the Richards model, which showed a good adjustment only for the BRNO. Differences in the predicted asymptotic skull size across all models within each MU were always less than 0.75, with models' parameters and CI values being also equivalents (Table 3). Thus, ∆AICc values for Gompertz, Logistic, von Bertalanffy typical, and von Bertalanffy original growth models were less than two units, being accepted as valid models to describe the growth of the skull of Guiana dolphins from the Brazilian coast (Table 3, Supplementary Figure S2). Since the Gompertz model has been used before to describe the age-growth relationship in Guiana dolphins populations (*e.g.,* Ramos et al., 2000; Di Beneditto & Ramos, 2004; Ramos *et al*., 2010), and showed a relatively good adjustment for data from all MUs analyzed, it was chosen for further comparisons between regions (Table 3, Figure 2).

In this regard, comparisons between the nested Gompertz models indicated statistical differences between the growth curves of the sampled MUs of Guiana dolphins (Supplementary Table S3). The model selected (Supplementary Table S3, Model 1), explained differences between all MUs in asymptotic condylobasal length (CBL_{∞}) growth constant (*k)* and the integration constant (*a*) of the equation (Model 1: AICc = 2858.75; LL = -1412.56; RSS = 64324; w = 0.61), with only marginal differences in the Likelihood ratio, extra sums-of-squares and AICc with the next less complex model selected (LL p = 0.04717; RSS p = 0.05527, ΔAICc = 0.92) (Supplementary Table S3, Model 2). However, since Model 1 had a superior AICc weigth (ω) in the ajustment to the dataset, was selected to describe differences in growth and asymptotic size of the skull between the MUs from Brazilian waters.

Table 3. Growth parameters related to total condylobasal length (CBL – mm) for Guiana dolphins, *Sotalia guianensis,* from the Brazilian North Region Management Unit (BRNO), Brazilian Northeastern Management Unit-3 (BRNE3), Brazilian Southeastern Management Unit-1 (BRSE1), Brazilian Southeastern Management Unit-2 (BRSE2), and Brazilian South Management Unit (BRSE/S). GOM: Gompertz model; LGT: Logistic; RCD: Richards, VBO: von Bertalanffy Original; VBT: von Bertalanffy Typical; L_∞: asymptotic size for CBL; L_0: size at birth; g_i/g: instantaneous growth rate at the inflection point; k: growth constant (years^{-1}); t_0: the age when the length is zero; t_i: age at the inflection point; CI: 95 % confidence intervals; AICc: corrected Akaike's information criteria for small samples; and ω: Akaike model weights, Cum. ω: Cumulative weight and LL: Likelihood. In bold the Gompertz model, selected for further comparisons.

MUs	**Models**	**L_∞ (mm) (95% CI)**	**L_0 (mm) (95% CI)**	**k; g_i (95% CI)**	**t_0; t_i (95% CI)**	**AICc**	**ΔAICc**	**ω**	**Cum. ω**	**LL**
BRNO	**RCD**	360.60 (357.76 - 364.61)	-	0.41 (0.31 - 0.53)	-3.28 (-4.64 - -2.37)	777.65	0.00	0.25	0.25	-383.49
N=96	**VBT**	360.63 (356.72 -364.61)	-	-	-3.78 (-5.33 - -2.80)	777.88	0.23	0.22	0.48	-384.72
	VBO	360.91 (357.15 - 364.68)	277.81 (266.46 - 287.85)	0.38 (0.29 - 0.49)	-	777.88	0.23	0.22	0.70	-384.72
	GOM	**360.48 (356.71 - 363.88)**	**-**	**0.41 (0.32 - 0.53)**	**-**	**778.43**	**0.78**	**0.17**	**0.87**	**-385.00**
	LGT	360.57 (356.95 - 364.40)	-	0.43 (0.33 - 0.56)	-2.84 (-4.01 - -2.00)	778.99	1.34	0.13	1.00	-385.27
BRNE3	**LGT**	390.06 (384.13 - 395.34)	-	0.52 (0.38 - 0.74)	-0.73 (-1.43 - 0.05)	241.69	0.00	0.35	0.35	-116.01
N=31	**GOM**	**389.99 (383.87 - 396.10)**	**-**	**0.48 (0.34 - 0.69)**	**-**	**242.39**	**0.70**	**0.25**	**0.59**	**-116.36**

	VBT	390.40 (383.96 - 396.41)	-	0.42 (0.30 - 0.65)	-2.13 (-3.21 - -1.25)	243.06	1.37	0.18	0.77	-116.70
	VBO	390.32 (383.86 - 396.09)	233.32 (188.13 - 257.47)	0.43 (0.31 - 0.63)	-	243.06	1.37	0.18	0.94	-116.70
	RCD	390.01 (384.01 - 395.97)	-	0.47 (0.35 - 0.69)	-1.39 (-2.23 - -0.65)	245.33	3.64	0.06	1.00	-116.36
BRSE1	**LGT**	380.79 (376.28 - 385.95)	-	0.53 (0.36 - 0.78)	-2.48 (-4.52 - -1.21)	995.00	0.00	0.24	0.24	-493.33
N=129	**GOM**	**380.84 (376.25 - 386.04)**	**-**	**0.51 (0.35 - 0.74)**	**-**	**995.05**	**0.05**	**0.23**	**0.47**	**-493.35**
	VBT	380.77 (375.93 - 387.00)	-	0.50 (0.34 - 0.76)	-2.92 (-5.34 -1.54)	995.12	0.11	0.22	0.69	-493.39
	VBO	380.77 (376.16 - 386.33)	293.85 (255.09 - 321.02)	0.50 (0.39 - 0.75)	-	995.12	0.11	0.22	0.92	-493.39
	RCD	380.64 (375.56 - 385.62)	-	0.63 (0.37 - 29.90)	-1.32 (-4.46 - 5.00)	997.07	2.07	0.08	1.00	-493.27
BRSE2	**LGT**	379.36 (374.79 - 384.13)	-	0.69 (0.56 - 0.87)	-0.59 (-0.95 - -0.12)	309.90	0.00	0.24	0.24	-150.35
N=39	**GOM**	**379.81 (374.76 - 384.42)**	**-**	**0.62 (0.50 - 0.78)**	**-**	**309.94**	**0.03**	**0.24**	**0.48**	**-150.36**
	VBT	380.04 (374.67 - 384.85)	226.54 (195.97 - 241.77)	0.55 (0.45 - 0.72)	-	309.98	0.07	0.23	0.71	-150.38
	VBO	380.06 (374.59 - 385.02)	-	0.56 (0.45 - 0.72)	-1.61 (-2.16 - -1.11)	309.98	0.07	0.23	0.94	-150.38

	RCD	379.65 (374.48 - 384.73)	-	0.62 (0.30 - 0.98)	-1.08 (-5.99 - 0.46)	312.60	2.70	0.06	1.00	-150.36
BRSE/S	**VBT**	371.28 (367.26 - 375.07)	-	0.71 (0.52 - 0.95)	-1.21 (-2.11 - -0.65)	543.01	0.00	0.30	0.30	-267.18
N=67	**VBO**	371.27 (367.63 - 375.07)	215.73 (169.41 - 253.47)	0.71 (0.52 - 0.95)	-	543.01	0.00	0.30	0.61	-267.18
	GOM	**371.23 (367.34 - 375.01)**	**-**	**0.74 (0.56 - 1.00)**	**-**	**543.89**	**0.88**	**0.20**	**0.81**	**-267.62**
	LGT	371.30 (367.33 - 375.34)	-	0.79 (0.59 - 1.07)	-0.69 (-1.41 - -0.18)	544.67	1.66	0.13	0.94	-268.01
	RCD	371.14 (367.27 - 375.11)	-	0.75 (0.55 - 1.00)	-0.94 (-3.00 - 3.01)	546.23	3.22	0.06	1.00	-267.62

The nested models selected by pairwise comparison between the MUs are shown in Table 4. The selected models and the curves parameters indicated the smaller asymptotic size for the BRNO population, with asymptotic condylobasal length (CBL_{∞}**)** defined in 360.48 mm (CI: 56.71 mm - 363.88 mm), being significantly different from the MUs of Northeast (BRNE3), Southeast (BRSE1 and BRSE2) and South (BRSE/S) of Brazil (Table 4, Figure 2). The CBL_{∞} of dolphins from BENE3 was defined at 390.06 mm (CI: 384.13 mm - 395.34 mm), being significantly larger than $CBL_{\infty s}$ from BRNO, BRSE1, BRSE2, and BRSE/S. The MUs from Southeastern coast, BRSE1 and BRSE2, show no statistical differences between them in the asymptotic skull size, with CBL_{∞} of 380.84 mm (376.25 mm - 386.04 mm) and 379.88 mm (374.76 mm - 384.42 mm), respectively. Guiana dolphins from BRSE/S were statically different in CBL_{∞} from the other regions, with CBL_{∞} defined at 371.23 mm (CI: 367.34 mm - 375.01mm) (Table 2, Figure 2). Growth rates increased from North to South coast of Brazil (Table 2), but with no statistical differences between BRNO and BRNE3, between BRNE3 and BRSE1, and between both Southeastern MUs (BRSE1, BRSE2) and BRSE/S (Table 4, Figure 2).

Table 4. Nested Gompertz model pairwise comparisons between the management units (MUs) of Guiana dolphins Sotalia guianensis from the Northern (BRNO), Northeastern (BRNE3), Southeastern (BRSE1, BRSE2), and South (BRSE/S) coast of Brazil. The model column indicates the selected model compared to the more complex model M1, in which all curve parameters between MUs were different; df: degree of freedom; LL: Log-Liklyhood; LL diff: differences in LL between models; p: Probability values with significance for p>0.05. Significant values of p indicates that the more complex model (M1) were selected.

MUs	**Models**	**df**	**LL**	**df (M1)**	**LL (M1)**	**df**	**LL Diff**	**Chisq**	**p**
	M1: L_{∞} *a k* differs								
BRNO *vs.* BRNE3	M2: L_{∞} *a* differs	119	-497.24	118	-497.090	1	-0.154	0.309	0.578
BRNO *vs.* BRSE1	M3: L_{∞} *k* differs	212	-878.62	211	-878.590	1	-0.035	0.071	0.790

BRNO *vs.* BRSE2	M2: L_{∞} *a* differs	130	-541.439	129	-538.932	1	-2.5065	5.013	0.025
BRNO *vs.* BRSE/S	M2: L_{∞} *a* differs	158	-656.335	157	-652.628	1	-3.707	7.414	0.007
BRNE3 *vs.* BRSE1	M2: L_{∞} *a* differs	144	-605.675	143	-605.567	1	-0.108	0.217	0.642
BRNE3 *vs.* BRSE2	M3: L_{∞} *k* differs	62	-265.965	61	-265.968	1	-0.001	0.000	0.990
BRNE3 *vs.* BRSE/S	M3: L_{∞} *k* differs	90	-379.762	89	-379.715	1	-0.047	0.095	0.758
BRSE1 *vs.* BRSE2	M4: *a k* differs	155	-647.722	154	-647.672	1	-0.050	0.099	0.753
BRSE1 *vs.* BRSE/S	M2: L_{∞} *a* differs	183	-762.435	182	-761.269	1	-1.167	2.333	0.126
BRSE2 *vs.* BRSE/S	M3: L_{∞} *a* differs	101	-421.930	100	-421.501	1	-0.429	0.857	0.355

Growth curve results also indicated the attainnmet of skull's asymptotic size at the age of 6 years for the populations of BRSE1, BRSE2, and BRSE/S. For the BRNO, asymptotic size was reached between 6 and 7 years, and for the BENE3 the process occurs between 7 and 8 years. The skull's size-at-age zero, predicted by the Gompertz equation, was defined at 278.68 mm for dolphins from BRNO, 232.1 mm for dolphins from BRNE3, 297.97 mm and 225.23 mm for those from BRSE1 and BRSE2 respectably, and 227.18 mm for specimens from the BRSE/S. However, some of these values were maybe overestimated because the small number of specimens with one year or less in the sample.

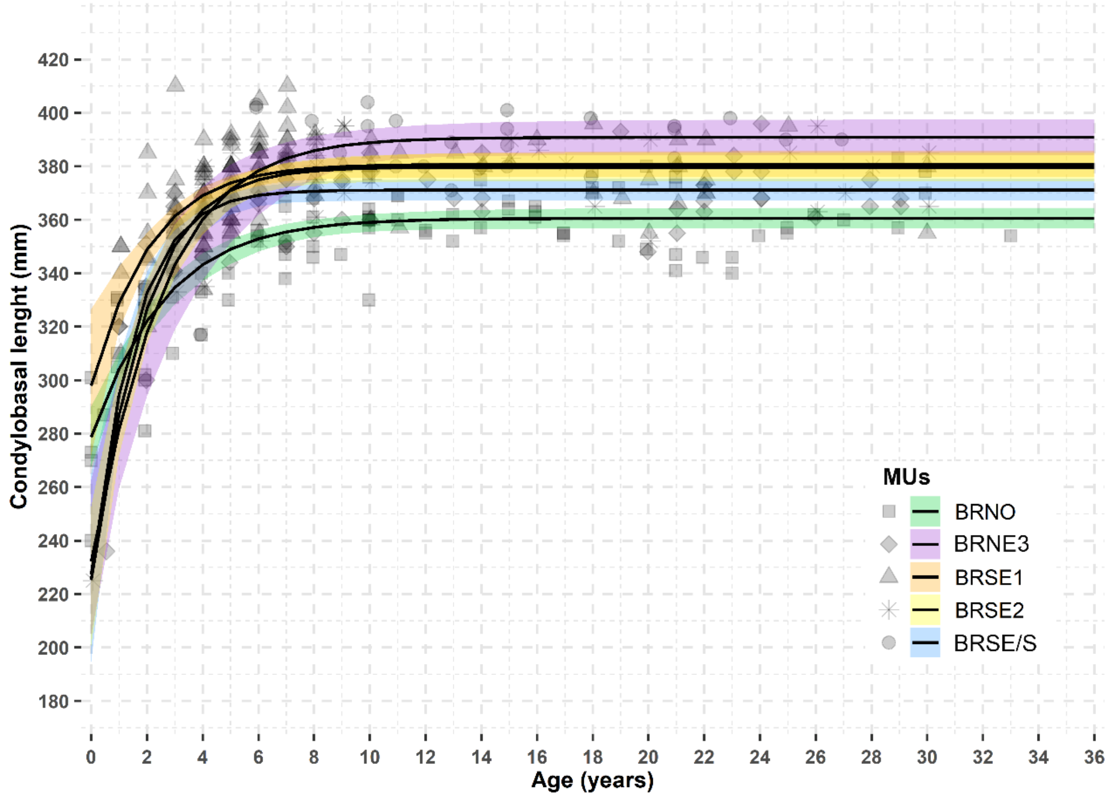

Figure 2. Gompertz growth model for age and condylobasal length (CBL) for Guiana dolphins, *Sotalia guianensis,* from the Brazilian coast, with the predicted confidence interval in a light color: Brazilian Northern Management Unit (green/square): BRNO, Brazilian Northeastern Management Units-3 (purple/diamond): BRNE3, Brazilian Southeastern Management Unit–1 (orange/tringle): BRSE1, Brazilian Southeastern Management Unit–2 (yellow/ asterisk): BRSE2 and Brazilian South Management Unit (blue/circle): BRSE/S.

Environmental factors

GLM model results are shown in Table 5. Sea surface temperature (SST$_{a}$), minimal SST (SST$_{min}$), maximal SST (SST$_{max}$), SST range (SST$_{r}$), and latitude (Lat) were not statistically significant factors influencing the average skull size of adult Guiana dolphins inhabiting the Brazilian coast. The models including only the predictors chlorophyll-a concentration – Chl-a (Model AICc = 96.8; ω = 0.685; df =12; LL = -44.084), continental shelf length until 50 m depth– DS$_{50}$ (Model AICc = 99.2; ω = 0.21; df = 12; LL = -45.261), continental shelf length until 100 m depth– DS$_{100}$ (Model AICc = 101.1; ω = 0.082; df = 12; LL = -46.203) and continental shelf length until 200 m depth – DS$_{200}$ ((Model AICc = 104.5; ω = 0.019; df = 12; LL = -47.692) showed a significant inverse relationship of this factors with CBL (Table 5; Supplementary Figure S3-S8). The other two models, relates to estuarine region length (ESE) and area (ESA) showed a similar negative, but weak relation with skull average size. The ESE, ESA, DS$_{50}$, DS$_{100}$, and DS$_{200}$ models exhibited ΔAICc > 2. Therefore, the model relating to Chl-a was considered to have the best performance, explaining 75 % of data variation (Table 5, Figure 3).

Table 5. Results from the different Generalized Linear models tested using as predictor variables sea surface temperature (SST$_a$), minimal SST (SST$_{min}$), maximal S|ST (SST$_{max}$), sst range (SST$_r$), latitude (Lat), chlorophyll-a concentration (Chl-a), the continental shelf length until 100 m (DS$_{100}$), and continental shelf length until 200 m (DS$_{200}$) of depth. CI: 95 % confidence interval; p: Probability values with significance for p>0.05. In bold the model with ΔAICc < 2.

Models	**Predictors**	**Estimates**	**CI**	**p(>|t|)**	**R^2**	**AICc**	**ω**
~Chl-a	**(Intercept)**	**391.44**	**387.39 – 395.54**	**< 2e-16**	**0.75**	**96.8**	**0.685**
	Chl-a	**-9.28**	**-11.36 – -7.15**	**3.32e-06**			
~DS$_{50m}$	(Intercept)	395.73	390.28 – 401.23	< 2e-16	0.70	99.2	0.211
	DSH50m	-0.27	-0.34 – -0.20	1.46e-05			
~DS$_{100m}$	(Intercept)	396.27	390.49 – 402.13	< 2e-16	0.67	101.1	0.082
	DSH100m	-0.19	-0.24 – -0.14	2.2e-05			
~DS$_{200m}$	(Intercept)	395.42	387.45 – 403.56	< 2e-16	0.57	104.1	0.019
	DSH200m	-0.14	-0.20 – -0.08	0.0007			
~ESE	(Intercept)	392.07	384.42 – 399.87	< 2e-16	0.34	104.5	0.001
	ESE	0.08	-0.12 – -0.04	0.00159			
~ESA	(Intercept)	389.22	379.97 – 398.72	<2e-16	0.22	111.6	0.00
	ESA	-0.01	-0.02 – -0.00	0.026			
~Lat	(Intercept)	375.25	361.47 – 389.56	1.26e-14	0.084	113.8	0.00
	Lat	-0.47	-1.38 – 0.44	0.324			
~SST$_{max}$	(Intercept)	447.304	273.84 – 623.57	0.0003	0.047	114.3	0.00
	SSTmax	-2.412	-8.82 – 3.92	0.466			
~SST$_a$	(Intercept)	503.90	308.20 – 1068.09	0.101	0.032	114.05	0.00
	SSTa	-52.85	-295.72 – 181.55	0.659			
~SST$_{min}$	(Intercept)	394.22	314.32 – 477.27	1.19e-06	0.022	114.7	0.00
	SSTmin	-0.53	-3.82 – 2.69	0.756			
~SST$_r$	(Intercept)	381.92	365.06 – 398.78	9.23e-1	0.017	114.7	0.00
	SSTr	-0.28	-6.15 – 5.60	0.929			

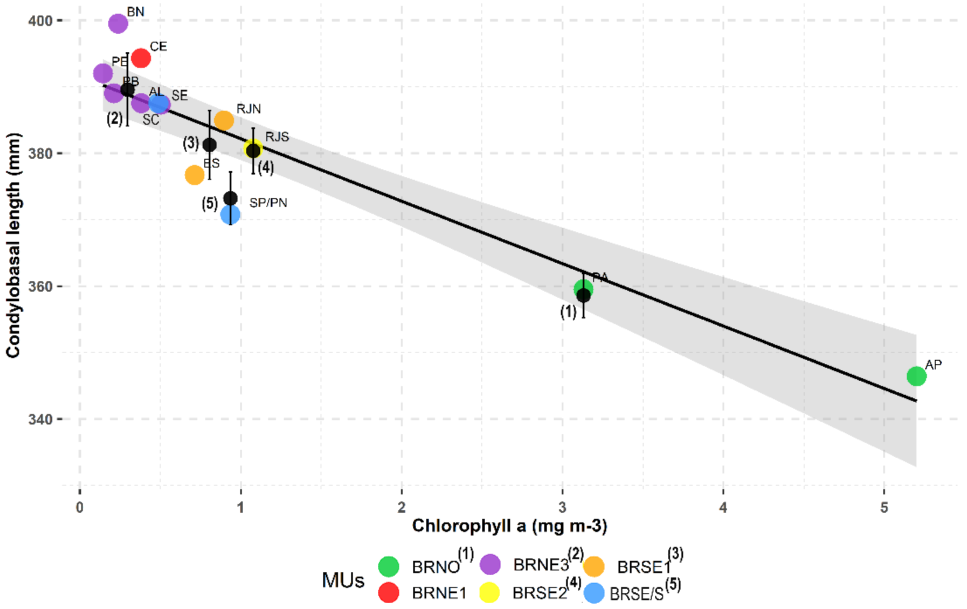

Figure 3. Relation between condylobasal length average of adults Guiana dolphins, *Sotalia guianensis,* from the Brazilian coast and the Chlorophyll-a concentration (Chl-a) of the different regions sampled. Brazilian Northern management unit – BRNO (green), Brazilian Northeastern management units 1 – BRNE1 (red), 2 –and 3 – BRNE3 (purple), Brazilian Southeastern management units 1 – BRSE1 (orange) and 2 – BRSE2 (yellow), Brazilian Southern management unit – BRSE/S (blue). Black dots with straight line indicated asymptotic Condylobasal length and 95% confidence intervals for the management units (1) BRNO, (2) BRNE3, (3) BRSE1, (4) BRSE2, and (5) BRSE/S.

The ANCOVA results, related to the influence of CBL, sex, and region on teeth dimension for Guiana dolphins indicated a statistically significant influence of skull size and location on teeth size (Figure 4). This model explained 36% of the data variation, with teeth size increasing with the skull size (CBL estimate = 0.017905; SD = 0.003215; p = 1.47e-07). Specimens from BRNO had significantly smaller teeth in comparison with specimens from BRNE3 and BRSE1 (BRNO estimate = -0.7952; SD = 0.258353; p = 0.0026). In addition, specimens from BRSE1 had also smaller teeth than those from BRNE3, but with no statistical differences between them (BRSE1 estimate = -0.1470; -0.241182; p = 0.3327). No influence of sex in teeth size was detected, although males show slightly larger dimensions than females for some MUs (Figure 5).

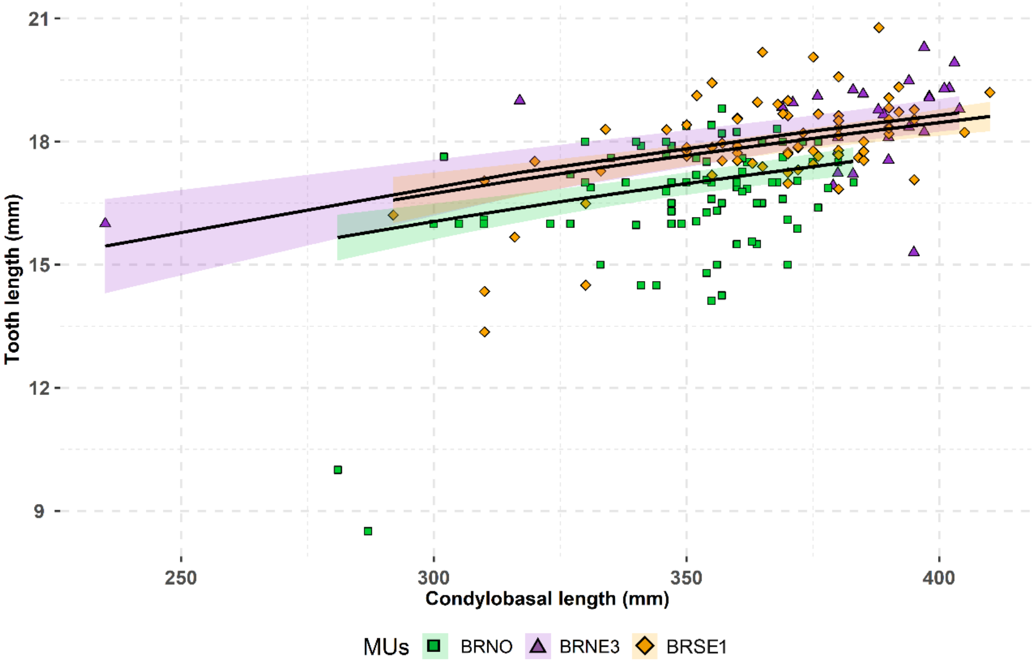

Figure 4. Relation between tooth length and condylobasal length for Guiana dolphin *Sotalia guianensis* from the Brazilian Northern (BRNO), Brazilian Northeastern (BRNE3) and Brazilian Southeastern (BRSE1) management units.

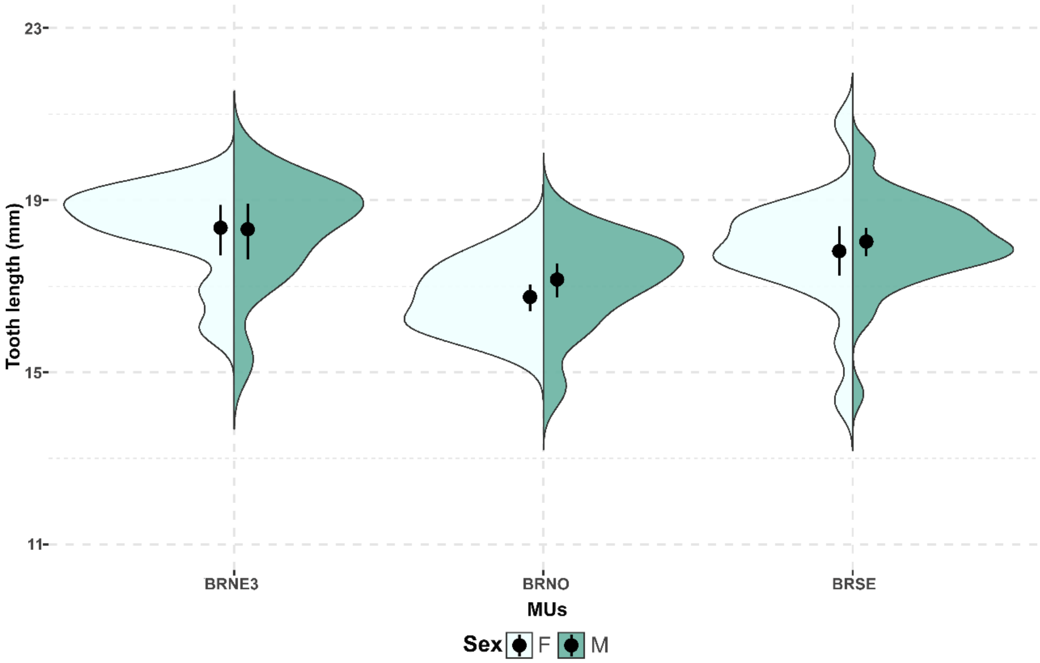

Figure 5. Violin plot showing the distribution of the sample of teeth length by sex and management units of Guiana dolphins: Brazilian Northern (BRNO), Brazilian Northeastern (BRNE3), and Brazilian Southeastern (BRSE1). Black dots with straight line indicate the sample mean and 95% confidence interval.

4. Discussion

The growth and size of the Guiana dolphin *Sotalia guianensis* changed markedly from North to South on the Brazilian coast. The asymptotic skull' size (CBL_{∞}), the average length of skulls from adult specimens (CBL_a), and teeth' length (TL) showed similar patterns, with sizes increasing from North (BRNO) to Northeast (BRNE3), and decreasing from Northeast to Southeast (BRSE1 and BRSE2) and South (BRSE/S). Growth rate (related to *k*), seems to change geographically in a different way, slightly increasing from North to South, and with the Southeastern and Southern populations reaching before the asymptote, at 6 years old in comparison to the remaining populations. The BRNE3 population seems to grow for a longer time until the age of 8 years. This span of growth time justified the largest asymptotic skull size detected for this location. Age distribution from BRNO indicated also 36 years as the oldest age found for the species. In addition, no influence of sea superficial temperature (SST_a, SST_{min}, SST_{max}, SST_r) or latitude (Lat) on the skull's size was detected. However, the relation of the skull with the other environmental factors indicated a negative influence of sea surface chlorophyll-a concentration (Chl-a) on the skull's size, with the presence of larger animals in the oligotrophic coastal regions of Brazil. This inverse association was also detected for the width of the continental shelf – principally until 50m of depth (DS_{50}), and, in weaker intensity, for the length and area of the estuarine region (ESE, ESA). However, the models with these predicted variables performed worse than the "Chl-a" model.

Growth

Growth curves related to skull dimensions has being investigated before only by Ramos *et al.* (2010) and Sydney *et al.* (2012), for the Southeastern and Southern Brazilian MUs of Guiana Dolphin. For the North and Northeastern region of Brazil, cranial morphology has been approached through linear morphometry (Fettuccia, 2006; Arcoverde *et al.*, 2014), geometric morphometrics (Monteiro-Filho et al. 2002) and non-metric characters (Fettuccia, 2010; Novais *et al.*, 2020). Some of these morphometric studies suggested a size increase from North to Northeast, with the skull's size changing from 351.3 mm in adult specimens from Amapá (AP) and Pará (PA) coasts – Northern Brazil, to 394.3 mm in adult specimens from Ceará (CE), at the Northeast continental ends (Fettuccia, 2006). Length of the periotic-tympanic complex from skulls seems to change similarly, with the small structures detected in specimens from PA, sizes gradually increasing for specimens from Maranhão (MA) – middle-Northeast region, and for those from CE, and with these latest locations having larger sizes than Rio de Janeiro Northern region (RJN) – at the Southeast coast (Arcoverde *et al.*, 2014). Asymptotic size obtained in the present study seems to follow the same pattern North–Northeast, with specimens from BRNO attaining the lowest CBL_{∞} and those from BRNE3 attaining the largest.

Related to BRNE3, no information was found available on morphology or life history traits for this Northeastern MU. The average body length of 22 adult specimens from the adjacent management unit, BRNE4, placed at Southern Bahia (BA) – Northeast/Southeast regional limit of the Brazilian coast, reached 186.9 cm of size (Rodrigues *et al.*, 2020). Thus, similar to specimens from Southern Rio de Janeiro (RJS) and slightly smaller than those from RJN. Since no other information existed, new studies are necessary for both MUs, BRNE3 and BRNE4, to improve the current results and support the largest asymptotic skull' size obtained for Northeastern Brazil, in comparison to all the other MUs of the species in the South Atlantic coast.

Skull asymptotic length from the Southeastern and South regions of Brazil were smaller than those from BRNE3 and very similar to those described by Ramos et al. (2010). Thus, CBL_{∞} decreased from the Northeast to Southeast (RJN – 380.8 mm; RJS – 379.8 mm) and Southern region (BRSE/S – 371.3 mm). Ramos et al. (2010) indicated CBL_{∞} of 374.73 mm for the Espírito Santo (ES) population, 382.77 mm for the RJN population, 378.09 mm for RJS, and 370.58 mm for specimens from São Paulo. These similarities between results were expected since we use partially the same dataset as the authors, although including the ES and RJN data as from the same BRSE1 management unit. In addition, the observed pattern of decreasing in skull size supports similar changes in asymptotic body length previously reported for these regions (Santos *et al.*, 2003; Di Beneditto & Ramos, 2004b; Ramos *et al.*, 2010; Conversani *et al.*, 2021). Thus, our results confirm at large-scale a geographical gradient for the size of Guiana dolphins in the Brazilian coast, with skulls increasing in size North-to-Northeast and decreasing Northeast-to-South.

The slight variation in the growth constant between MUs implies that specimens from the Northeastern regions reach largest skull dimensions and grow longest, while specimens from the Southeastern and Southern regions grow slightly faster but stop growing earlier. In the North region, growth seems to be slower than in the South, Southeast and sortheast, and individuals reach smallest sizes despite they grow for one year more. However, in general, the asymptotic size of skulls is reached between 6 and 8 years, relatively near to the age of sexual maturity described for the species, between 5 and 8 years (Rosas & Monteiro-Filho, 2002; Di Beneditto & Ramos, 2004a). Comparatively, the riverine species of the genus, *Sotalia fluviatilis* seems to grow more slowly and in much less proportion, attaining smaller body asymptotic sizes than Giana dolphins, and sexual maturity at the ages between 11 and 13 years (Fettuccia, 2010). Thus, in the dispersion of the species of the genus *Sotalia*, the slowdown/speedup in the growth seems to have different consequences, resulting in large or small forms most probably related to local environmental conditions and genetic differentiation between populations (Ramos *et al.*, 2000; Rosas & Monteiro-Filho, 2002; Cunha *et al.*, 2010; Sydney *et al.*, 2012). Furthermore, since the growth constant increased slightly from North to Northeast, and subsequently to the Southeast, it is plausible to assume that aspects such as temperature and latitudinal gradients may be influencing this growth aspect, at least partially.

Nerveless, the differences detected between sampled units on CBL_{∞} reinforced geographical variations between the MUs suggested for the delimitation of Guiana dolphin populations across its distribution (Cunha, *et al.,* 2020 *apud* Domit *et al.*, 2021). Genetic studies have indicated a fine-scale population structure for Guiana dolphin related to the species' dispersal behavior, reduced home range, and small body size (Cunha *et al.*, 2005; Caballero *et al.*, 2010; Hollatz *et al.*, 2011; Caballero *et al.*, 2018). These subdivisions have been also supported by some findings on morphology (*e.g*., Fettuccia, 2006; Arcoverde *et al.*, 2014), acoustic (*e.g*., Azevedo & Van Sluys, 2005; Leão *et al.*, 2016), diet preferences (*e.g*., Bisi *et al.*, 2013; Rodrigues *et al.*, 2020) and reproduction and life history traits (*e.g*., Ramos et al., 2010; Conversani et al., 2020).

Environmental factors

Unexpectedly, no relationship was found between temperature or the latitudinal gradient and skull size of Guiana dolphins in our study along the Brazilian coast. Thermal gradient has been related to differences in the body' and skull' sizes between populations of cetaceans worldwide (Bergmann's rule – Bergmann, 1847), being the case of *Tursiops truncatus* (Cheney et al., 2018), *T. aduncus* (van Aswegen *et al.*, 2019), *Phocoena phocoena* (Hohn *et al.*, 1996), *Stenella* spp. (Perrin, 1975; Perrin *et al.*, 1999) and *Delphinus delphis* (Murphy & Rogan, 2006). The two other predominant coastal species of small cetacean inhabiting the Western South Atlantic coast – the common bottlenose dolphin *T. truncatus* and the Franciscana dolphin *Pontoporia blainvillei*, seem to show also a latitudinal/thermal relationship with size, at least partially. The size of *T. truncatus* seems to increase throughout higher latitudes, since specimens from Southeaster/Southern Brazil, belonging to the *"T. truncatus*" morphotype, appear to be smaller than their counterparts inhabiting from Southern Brazil to Argentina coasts and described as the "*T. gephyreus*" morphotype (Costa *et al.*, 2016; Hohl *et al.*, 2020). In the case of *P. blainvillei*, only the male's size seems to increase with the increase in latitude and the related decrease in temperature (Danilewicz *et al.*, 2022). Interestingly, these two species had a geographic distribution that included tropical and subtropical waters, until 43°S and 45°S latitudinal degrees (Crespo, 2018; Wells & Scott, 2018). Guiana dolphins appear to be a more restricted species, confined to strictly tropical waters, reaching only 28°S of latitude (Flores *et al.*, 2018). This restricted thermal gradient of residence may not make evident the effect of temperature on skull or body sizes. In this scenario, where the decreasing temperature is not a pressure for the development of larger bodies, other environmental components may be more influential or evident in driving the size of individuals.

In our study, the inverse relationship between skull sizes of Guiana dolphin with the sea superficial Chl-a concentration, used as a proxy of primary productivity, was identified as a better-adjusted model to the data. Geographically, this result implied that specimens from the Northeast, inhabiting a more oligotrophic region, had larger skulls than those from the Southeast and South, where the concentration of Chl-a is relatively higher, while specimens with smallest skulls inhabited the more productive areas placed in Northern Brazil. Although we don't have a direct justification for this relation of larger skull sizes with less productive areas, in other small odontocete species, such as *Phocoena phocoena* and *Cephalorhynchus eutropia*, large sizes have been associated with inhabiting areas of high primary productivity but with intense fluctuations of food availability (Galatius & Gol'din, 2011; Dawson, 2018). In addition, the notable small size characteristic of the species of harbour porpoises *P. phocoena* and the Comerson' dolphin (*C. commersonii*), because their constant necessity of restitute the metabolic energy storage, seems to imply inhabiting highly and predictively productive environments (Galatius, 2010). This could be the case of the high productive area from Northern Brazil (BRNO), where Guiana dolphins attain smaller sizes than in other Brazilian MUs. However, it does not explained the large sizes of Guiana dolphins inhabiting the tropical oligotrophic coast of Brazilian Northeast.

The Northern and Southeastern-South of Brazil had a wider continental shelf, large river discharges to vast estuarine areas, and/or the influence of upwelling zones (Knoppers *et al.*, 1999). Differently, the Northeastern Brazilian coast is characterized by the narrowness of the continental shelf, the income of oceanic currents more onshore, the strong action of winds, and a minor river discharge (Knoppers *et al.*, 1999; Maia *et al.*, 2018; Pimenta *et al.*, 2019). The primary productivity in all these regions seems to be more directly a consequence of these geographical features (Knoppers *et al.*, 1999; Neto *et al.*, 2015); being seasonally variable, but major differences are related to variation in Chl-a total concentration between the regions, and not to local fluctuations (Neto *et al.*, 2015). However, primary production and coastal characteristics may shape fish's communities and morphology in these regions (Pinheiro *et al.*, 2018). For instance, an opposite correlation of primary production with fish biomass has been suggested for Brazilian reefs, including those from the Northeast (Morais *et al.*, 2017), although comparative studies from estuarine habitats have not been found in the literature. Guiana dolphin is classified as a generalist species whose principal preferential preys are mainly small or juvenile fishes from estuarine environments or related to them, with demersal habits and few are pelagic (Ramos *et al.*, 2010; Pansard *et al.*, 2011; Lopes *et al.*, 2012). Stable isotopes also indicated that in the Brazilian Northern estuarine region the species feed at a low trophic level (Costa *et al.*, 2020) whereas in the Southeastern and Southern coasts seem to feed at a similar level when compared to the other marine odontocetes from the respective communities (Bisi *et al.*, 2013; Hardt *et al.*, 2013; Drago *et al.*, 2021). At the Northeast, in Abrolhos Bank region, preferential preys seem to be piscivorous fishes, and a positive correlation between Guiana dolphin body size and prey size has been recently found (Rodrigues *et al.*, 2020). In CE, also Northeast, the species seem to prefer sandstone reefs and estuarine areas for foraging (Gurjão *et al.*, 2003).

Therefore, we propose that the large skull size of Guiana dolphins in the Northeast and RJN – Southeast of Brazil, could be related to feeding habits. In biting odontocetes, such as the Guiana dolphin, it is suggested that skull size and mandible shape are related to maximum prey size (McCurry *et al.*, 2017; Drago *et al.*, 2021). This matches with the premise that larger predators capture larger prey, and are still able to feed on smaller ones (McCurry *et al.*, 2017), as an advantageous foraging strategy. Furthermore, as found in the present study, a positive relationship between skulls and longer teeth could be important for catching preys of a wider range of sizes, reinforcing the hypothesis of a relationship skull size – prey size. The skull size – tooth size correlation has been associated with feeding adaptations in other small cetaceans such as *T. truncatus* and *Stenella longirostris* from the North Atlantic and Pacific oceans (Akin, 1988; Perrin *et al.*, 1999, 2011). Furthermore, as in these species, larger teeth imply fewer teeth, and this was confirmed for Guiana dolphins from Northeast throughout the comparison of mandibular dental series with specimens from North and South of Brazil (Fettuccia, 2006). Small odontocetes experiences high heat loss and has a limited capacity for energy storing, and so they must eat regularly, therefore depends highly on predictable resource (Galatius, 2010). Thus, if the low primary productivity in the Northeast region implies a less number of juvenile fishes related to other age classes of fishes with larger lengths, in comparison with the Northern and the Southern regions, a lagers skull and teeth could allow access to a larger variety of preys, increasing the food availability for Guiana dolphins. In addition, it could permit the incursion on more open coastal environments, increasing the disposable food resources in oligotrophic regions or in areas such as RJN, where resurgence could implies larger fiches availables.

Another supposition is that a larger body size would be also advantageous for animals living in an environment subjected to competition or predation (Borobia, 1989). The narrowness off the continental shelf in the Northeast region of Brazil, and the species-limited distribution – estuaries, bays, and coastal areas <50 m depth, might limit the habitat availability for the Guiana dolphin in this region. In addition, this geographic characteristic increases the presence of oceanic, or larger-range coastal species of marine mammals closer to the shore (Alves Júnior *et al.*, 1996; Meirelles *et al.*, 2009), potentially increasing the competition for feeding resources, restricting Guiana dolphins to some specific locations, and increasing the action of predators. For instance, in the Babitonga Bay and Baía Norte, South Brazil, the distribution areas of Guiana dolphins and *T. truncatus* populations seem to be mostly mutually exclusive, with Guiana dolphins restricted to interior zones < 3 m depth and some antagonistic encounters have been described between the species (Flores & Fontoura, 2006; Vianna *et al.*, 2016). In addition, shark attacks are a mentioned threat for Guiana dolphins on the Northeastern and Southeastern coast of Brazil (Bornatowski *et al.*, 2012; Flores *et al.*, 2018) that transit open shore areas to move between closely spaced estuaries (Araújo *et al.*, 2007). Thus, larger skull size could allow better performance in aggressive interactions, and in the competition for feeding resources, while large body size – taking skull size as a proxy, could improve the propulsion and locomotion in the open coastal areas from the Northeast Brazilian coast, as a way to avoiding predation.

The relationship between size and environment is difficult to evaluate because of the interconnection between factors, the impossibility of directly testing all the environmental components, and the action of species' intrinsic factors, such as sex and ontogeny (Peters & Peters, 1986). In the case of Guiana dolphins, we have tried to understand some of these environmental components, but it is important to raise some observations. First, we included in the environment analysis 13 different population means of adult skull sizes, although in some cases the mean was based on small sample sizes. For three MUs only the CBL mean and standard deviation was reported in the literature and we are aware that possible measurement errors could occur. In this regard, we intend to use only data from morphology studies, with the adult's sampled number and location reported, as a means to reduce the possible vies. In addition, we added to the graphic representation of the model results the asymptotic skull size obtained for five of the MUs, as a way to detect whether the CBL_∞ followed the same pattern as described in the models. The superposition of both measurements occurred in all cases. Finally, we understand that a direct relationship between some of the tested environmental factors with the skull size of the dolphins could be ambiguous, as they might represent proxies for many other environmental components difficult to delineate. So, even though geographic variation in the pattern of skull growth appears to be accurate and reinforced by previous studies on the morphology of this species, new studies are needed to improve the sample size and parameters accuracy, and confirm our findings related to the influence of environmental traits on skull size. Furthermore, here we bring novel information about the North and Northeast MUs of Guiana dolphin related to life-history traits and stand the importance of morphology and ontogeny research to improve populations' differentiations.

5. References

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CAPÍTULO IV: WHO IS IN HANDICRAFTS: TOOTH MORPHOLOGY AND AGE DETERMINATION AS TOOLS TO ASSESS VULNERABLE DOLPHINS *SOTALIA* SPP. SUPPLYING THE TRADE OF ARTISANAL CHARMS IN BRAZIL

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Abstract

In Brazil, dolphins' body parts are commonly used as traditional amulets, named as love-charms, while dolphins' teeth are commercialized in handcrafted, necklaces and bracelets. Recent studies on forensic genetics confirmed the Guiana dolphin, *Sotalia guianensis*, as the principal target of the love-charms trading, with specimens incidentally captured in gillnets suggested as the primary source. As additional support for these investigations, we characterized tooth morphotypes and ages of dolphins used in the construction of traditional crafts, with the main objective of detecting the dolphin species and population groups most affected by this trade. Teeth collected from necklaces, bracelets, and earrings, sold in public markets from three major cities of Northern and Northeastern Brazil (Belém = 99, São Luís = 4, and Fortaleza =15), were analyzed using four morphological measurements: tooth total length (TL), root diameter (RD), crown length (CL), and cingulum diameter (CID). An unweighted pair group method using arithmetic means (UPGMA), with Euclidean distances as a measure of dissimilarity, multivariate analysis of variance (MANOVA), and principal component analysis (PCA) were used to detect tooth morphotypes in the samples. Ages were obtained by counting the growth layer groups (GLGs) deposited in dentine. Two different groups of teeth were detected presumably belonging to the species *Sotalia guianensis* and *S. fluviatilis*. Handicrafts showed a diverse age composition, ranging from 2 to 30 years, with a multimodal distribution (Mean =11 ± 7 SD, Median = 10, Mode = 4, 8, 19), similar to dolphin populations affected by fishery interaction. Results suggested that juveniles individuals and adults younger than 20 years, from both *Sotalia* species, probably incidentally captured, are the principal source of handicrafts, and raise concern about the greater vulnerability of young age categories presented in this trade.

Keywords: Age, Sotalia, Guiana dolphin, Tucuxi, Love-charms, Teeth, Northern Brazil, Amazon

1. Introduction

The regular trade of cetacean products is long-standing in Brazil, still today. Folkloric beliefs from the Amazon region have the riverine dolphins (*Sotalia* spp. and *Inia* sp.) as central characters, fomenting a ritualistic usage of dolphin body parts with believed magical powers, renowned as “love charms” (Siciliano et al., 2018). These charms are commonly used and sold as traditional amulets, principally in Amazonian city markets, but have also been reported in coastal regions of the North, Northeast, and Southeast of the country (Siciliano, 1994; Sholl et al., 2008; Siciliano et al., 2018).

In Northern Brazil, the demand for boto (*Inia* sp.) derived amulets is high (Smith, 1996) and includes genital organs, eyes, skin, and teeth as principal products (Gravena et al., 2008; Siciliano et al., 2008). Since local traditional legends talk about boto dolphins changing to human form and seducing young women, it is believed that possessing one of these body parts brings luck in love and good sexual performance to the carrier (Cravalho, 1999; Vidal et al., 2019). However, recent research using genetic material from love charms collected in traditional markets of eight main cities from Northern to Southeastern Brazil indicated the estuarine dolphin *Sotalia guianensis* as the only cetacean species supporting this dolphin-derived charms trade (Siciliano et al., 2018).

Dolphins of the genus *Sotalia* are small-size odontocetes with geographical distribution restricted to Central and South America. The genus includes two species, *Sotalia guianensis*, a marine species limited to shallow coastal and estuarine habitats from Nicaragua to southern Brazil, and *Sotalia fluviatilis*, a relatively smaller riverine species, exclusively found in the Amazon basin, including southern Peru, eastern Ecuador, southeastern Colombia, and northern Brazil (Flores et al., 2018) (Figure 1). Both species are widely affected by incidental catch caused by the interaction with the intense fishing activity existing throughout their geographic range (Siciliano, 1994; Zappes et al., 2009; Meirelles et al., 2010, Iriarte and Marmontel, 2013a). Despite being fully protected by federal laws, intentional killing has been also documented for some regions of Brazil, related to their use as bait, in traditional medicine, and for human consumption (Siciliano et al., 2008; Alves and Rosa, 2008; Iriarte and Marmontel, 2013b; Barbosa-Filho et al., 2018, Dos Santos-Filgueira, 2021). In addition, *S. guianensis* and *S. fluviatilis* are intensely threatened by habitat degradation and pollution related to anthropic action, such as dam constructions and mining in the Amazon, besides city growth, boat traffic, and the destruction of mangroves and salt marshes in coastal areas (Flores et al., 2018; Secchi et al., 2018).

Genetic forensic researches, such as Siciliano et al. (2018), are significantly important to assess the species more vulnerable or affected by illegal human trade, mainly when this trading uses body parts with no diagnostic characteristics or when the commercialized species are more difficult to identify. This achieves greater importance for the conservation effort of species classified as endangered, which is the case of *Sotalia* genus in Brazil (Flores et al., 2018; Secchi et al., 2018; Da Silva et al., 2020). As additional support to these genetic investigations, morphological characterization of specimens, as well as age determination could be a valuable tool, making it possible to obtain specific information about demographic groups and populations of species that have been more exposed to this human activity. As part of the market of dolphin-derived charms, dolphin teeth are another widely traded item in the Northern Brazil region, mainly as ornaments for handmade necklaces, earrings, and bracelets, and often sold in streets and traditional markets. The morphology of teeth from aquatic mammals has been used before in the identification of populations stock (*Stenella longirostris* - Akin, 1988), for identification of species (Brunner, 2004), and sexual morphometric differentiation in pinnipeds (*Otaria byronia* - Tarnawski et al., 2014), and for ecotype characterizations based in dental alterations related to use, as in the case of *Orcinus orca* (Ford et al., 2011). Furthermore, the dentine and cement from aquatic mammals' teeth are classified as recording structures, being shaped by growth layers with continuous deposition which allows accessing the exact age of individuals (Scheffer and Myrick, 1980; Hohn et al., 1980; Klevezal, 1996). These layers, named growth layer groups (GLGs), are cyclically deposited in parallel to the tissue formation's surface, creating a pattern that can be counted. The validation of 1 year – 1 GLG is needed in order to confirm this chronological correspondence, and has been widely demonstrated for several species (Hohn et al., 1989; Da Silva, 1994; Ramos et al., 2000; Read et al., 2018). This research aimed to identify teeth from *Sotalia* spp. found in handicrafts sold at traditional markets of Northern and Northeastern Brazil, and assess the more frequent age classes used in their production, and therefore, affected by the trading of dolphin's body parts.

2. Materials and Methods

Sampling

Handicrafts, purchased by authors REL, AFC, and SS for this study, at five traditional or public markets from major cities of Northern and Northeastern Brazil were analyzed in addition to teeth obtained from stranded dolphins, used for comparison purposes only (Figure 1B). From these handicrafts, 118 teeth, being 99 acquired in Belém (Pará State), 4 in São Luís (Maranhão State), and 15 in Fortaleza (Ceará State), were used to assess the *Sotalia* spp. dolphins and the frequent ages commercialized in the trade of dolphin-derived artisanal objects (Table 1).

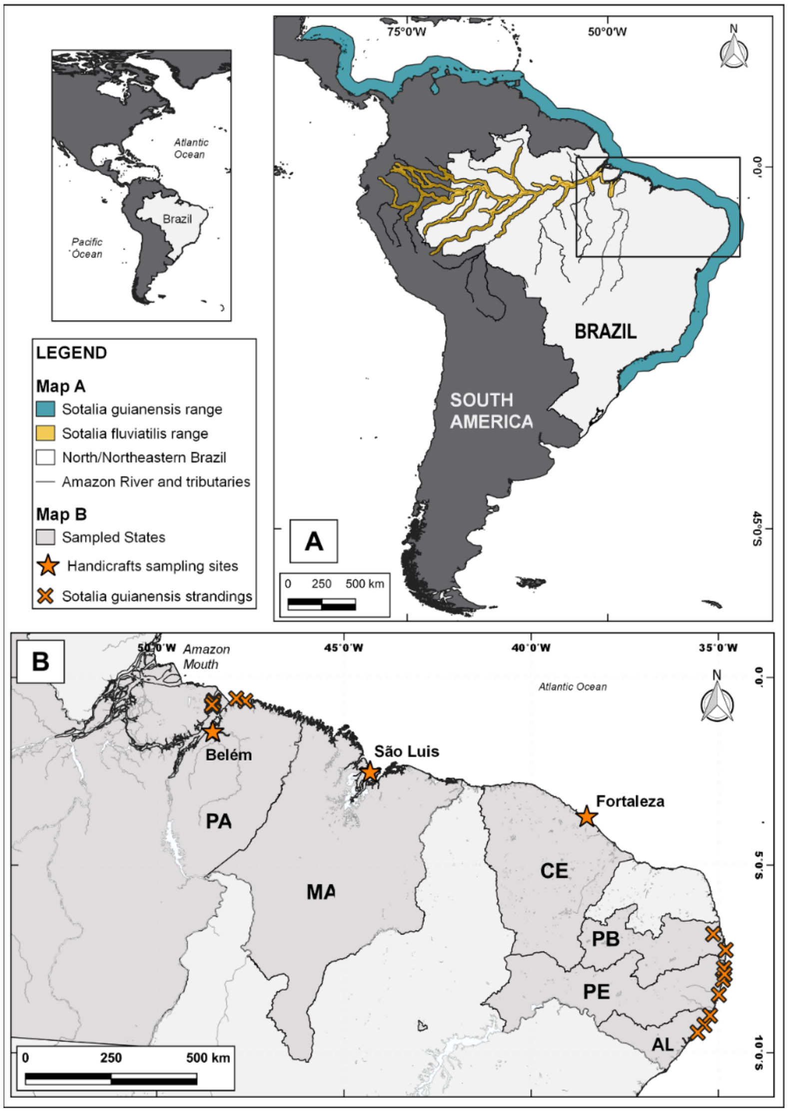

Figure 1. Distribution range of the species from the genus *Sotalia* in South America (Map A) and sampling points in the Northern (Map B: PA - Pará State) and Northeastern (Map B: MA - Maranhão State, and CE - Ceará State, PB - Paraíba State, PE - Pernambuco State and AL - Alagoas State) regions of Brazil were handicrafts and stranded animals were collected.

Table1. Handicrafts collected in three major cities from the Brazil Northern and Northeastern regions.

Object	**Place**	**City**	**State**	**Number of Teeth**
Earrings 1	Mercado Central	São Luís	Maranhão	2
Earrings 2	Mercado Central	São Luís	Maranhão	2
Bracelet 1	Loja Artesanato da Amazônia	Belém	Pará	10
Bracelet 2	Loja Artesanato da Amazônia	Belém	Pará	10
Bracelet 3	Loja Artesanato da Amazônia	Belém	Pará	10
Bracelet 4	Loja Artesanato da Amazônia	Belém	Pará	11
Bracelet 5	Feira da Praça da República	Belém	Pará	9
Necklet 1	Loja Artesanato da Amazônia	Belém	Pará	26
Necklet 2	Mercado Ver-o-Peso	Belém	Pará	23
Necklet 3	Loja Praia de Iracema	Fortaleza	Ceará	15

Data classification

External characteristics of the teeth adorning necklaces, bracelets, and earrings (Figure 2A-C), were used to confirm the genus/species to which they belonged. Each tooth was visually compared with teeth from small cetaceans preserved in the mammal collection at the Museu Paraense Emílio Goeldi, in Pará, Northern Brazil. This collection includes ~600 specimens of Delphinidae recovered in Pará, same geographical region where teeth are commercialized. Based on visual inspection all teeth used in this study were similar to *Sotalia* sp. specimens from the referred collection. Furthermore, since two different sizes of teeth from adult specimens were detected in the sample (Figure 2D and 2E), four external measurements were used to assess possible differences in teeth morphology that indicated different species of *Sotalia* genus being used in the handicrafts (Ramos et al., 2000): tooth total length (TL), measured from the apical extremity of the crow to the end of the root, root diameter (RD) measured as the maximum width of the root, crown length (CL), measured from the apical extremity of the crown to the root cingulum, and cingulum diameter (CID), measured as a diameter of the tooth at the intersection area of the crown with the root. In addition, teeth from 10 *S. guianensis* specimens stranded at the coast of Pará State (PA) and 10 *S. guianensis* specimens stranded in the Northeastern region of Brazil (NE) (Figure 1) were measured and used for further comparisons as two different control groups (see statistical analyses section). Measurements were taken with a caliper to the nearest 0.05 mm and only teeth relatively straight and with no notable wear of the crow were analyzed.

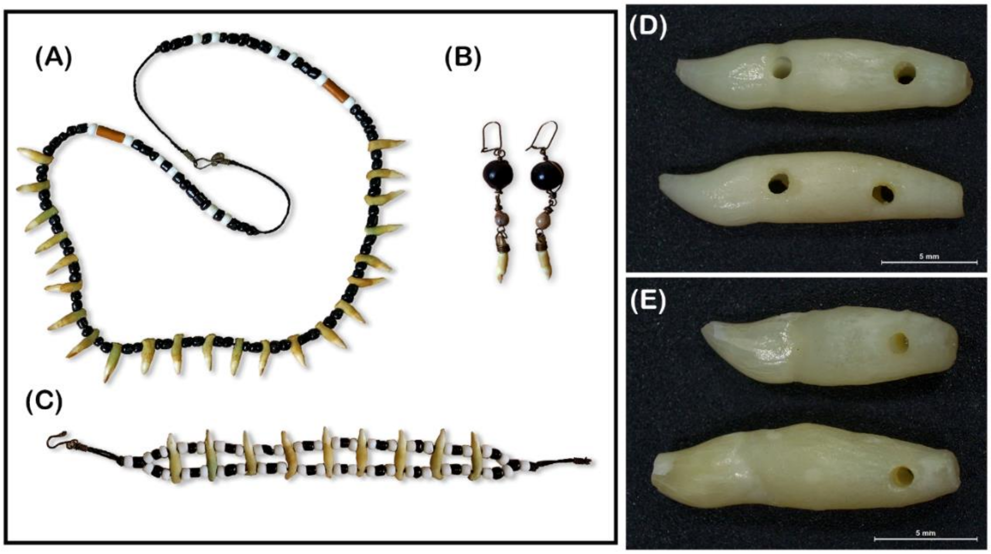

Figure 2. Sample of handicrafts manufactured with teeth of *Sotalia* dolphins and commercialized in the trade of artisanal objects in the North-Northeastern of Brazil: A) necklace, B) earrings, C) bracelet, D) *Sotalia guianensis* teeth from a bracelet, E) Two different types of *Sotalia* sp. teeth detected in the necklaces.

Age determination

The age frequency of the handicrafts was assessed by counting the growth layer groups (GLGs) deposited in the teeth' dentine (Figure 3), and considering the described by Rosas et al. (2003). Each tooth was subsequently wearing, decalcified with RDO®, and cut with a manual freezing microtome in thin sections of 20-30 µm. Posteriorly, the thin sections were stained with Mayer's hematoxylin, blued in Ammonia 2%, and finally, mounted in glycerin 100% (Rosas et al., 2003; Conversani et al., 2020). After being mounted, thin sections were photographed using a Leica S9i Stereomicroscope with an attached digital camera, and the images were processed by Leica Application Software v4.12 (Leica Microsystems, 2018) with a maximum magnification of 55x at Mammal Ecology Laboratory, Universidade do Vale do Rio dos Sinos, Brazil. Readings were made in the images three different times by two researchers separately to determine the chronological age of each tooth. The GLGs were counted in the dentine, having in consideration a correspondence of 1 GLG and 1 year of life described previously for the genus *Sotalia* (Da Silva, 1994; Rosas et al., 2003; Di Beneditto and Ramos, 2004). In some cases, the final age was classified as minimum age because the perforations made for the craft construction coincided with the center of the pulp cavity (Figure 2 and Figure 3), making it impossible to read the most recently deposited GLGs. The best age estimate was defined as the coincidental readings of at least two from the three times. In the case of a discrepancy ≤ 2 GLGs between readings, final age was considered as the mean of the three different ages. If a discrepancy between readers occurs, final ages were obtained by a consensus reading involving the two researchers. Readings with differences ≥ 3 GLGs were not considered in the analysis.

Finally, based on the age readings, teeth were classified as belonging to three different age categories: juveniles (age ≤ 6), young adults (6 < age ≤ 12), and adults (13 ≥ ages), taking into account that a) the age of 7 years as the age of sexual maturity for *Sotalia guianensis* (Rosas and Monteiro-Filho, 2002; Rosas et al., 2003) and b) the ages between 7 and 12 years as the range when occurs the complete fusion of the sutures of the occipital complex, concurring with the age of sexual maturity in *S. guianensis* and *S. fluviatilis* (Fettuccia et al., 2009; Novais et al., 2020).

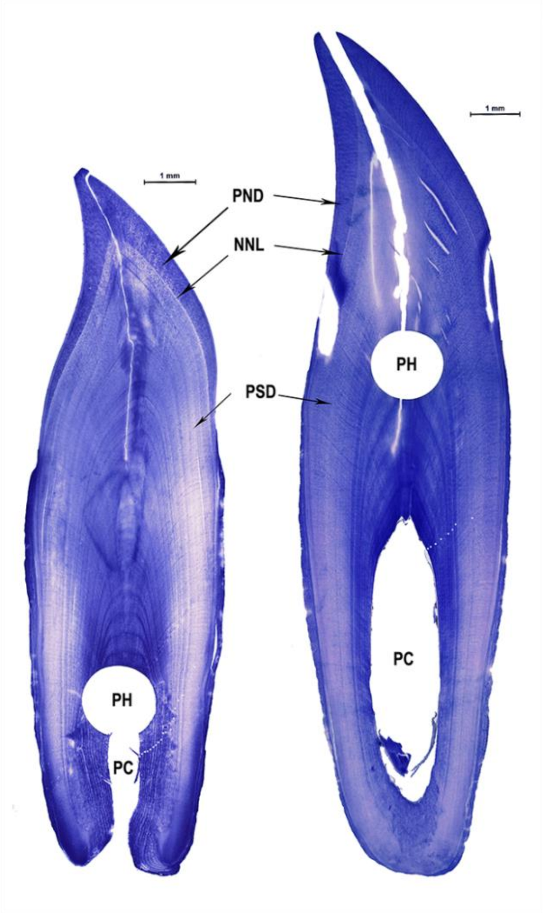

Figure 3. Thin sections from two *Sotalia* spp. teeth collected from handicrafts (left: tooth N1-10 with TL= 13.16 mm; right: tooth B2-5 with TL=17.4 mm). PND: Prenatal dentine, NNL: Neonatal line, PSD: Postnatal dentine, PC: Pulp cavity, PH: Holes made for craft construction. White dots indicate the GLGs counts in the dentine corresponding to the age.

Statistical analyses

Since two species from the genus *Sotalia* are described in the literature (*S. guianensis* and *S. fluviatilis*), we intend to answer three questions through the statistical analysis. First, if two different groups of *Sotalia* sp. teeth were present in the collected handicrafts. Secondly, if some of these groups were more similar to teeth from *S. guianensis* specimens stranded in two different coastal localities of the sampling area (Figure 1B), and if these differences were influenced by age or if they were exclusively morphological differences. To this end, we included data from stranded specimens as two control groups (PA and NE), assuming that they adequately represented the morphological variation of the species *S. guianensis*, since individuals from populations with the smallest body size (PA region, Novais et al., 2020) and populations with the largest body size (NE region, Monteiro-Filho et al., 2002) described for the species were added. Finally, as a third question, we wanted to know what the most frequent ages in these products were for the detected groups.

Teeth from handicrafts were initially grouped through a cluster analysis using the four external measurements (TL, RD, RC, and CID) in order to find potential groups related to the tooth external characteristics of the two species of *Sotalia*. The dendrogram was constructed based on the unweighted pair group method using arithmetic means, UPGMA (Hale and Dougherty, 1988), and Euclidean distances (Podani, 2000) as a measure of dissimilarity. To evaluate the stability of the cluster, indicated by the representativeness of the structure of the data, a bootstrap resampling with 1000 iterations was performed and obtained the Jaccard coefficient of similarity between sets (Henning, 2007). Cluster results were visualized including also the sampling localities to check if the geographical distribution of teeth was also related to the grouping.

Resulting groups from cluster analysis and teeth groups of stranded specimens (Tooth Type factor), the age category of teeth (Age class factor), and as dependent variables the four morphological measurements, were combined in a two-way multivariate analysis of variance (MANOVA). Also, the interaction between factors was evaluated in this analysis. Box-Cox transformation was used for two dependent variables (RC, and CID), to fulfill the MANOVA assumptions of homogeneity of the variance-covariance matrices and multivariate normality. Since multivariate significant differences were detected, we performed analyses of variance (ANOVAs) for each dependent variable. We used an *a posteriori* Tukey test to find out where the significant differences were in the ANOVA results. Likewise, to determine which morphological variables most influenced the composition of the teeth groups, a principal component analysis (PCA) was performed using also the four external measurements from teeth. Critical value for coefficients of correlation between variables and components, *i.e.* loading, was set to 0.5. In the graphical visualization of the PCA results, data were identified using the classification from the clusters, the two control groups, and the growth categories of teeth as a way to visualize the MANOVA results. Thus, to see if the distribution of the scores had some relation with the age or were only influenced by the morphological variables indicating similarities in teeth characteristics in the groups, and therefore, a high probability to belong to the same species.

We used $p < 0.05$ as the critical p-value of statistically significant. Descriptive statistics such as Range, Mean, Median, Standard deviation (SD), and 95% confidence interval (CI) were also acquired. Finally, the age distribution of the sample was obtained using the age estimates, and differences in the age distribution between age classes and groups of teeth from handicrafts were described.

All statistical analyses and graphics were conducted in the free software RStudio v.1.4.1717 (RStudio Team, 2021). The packages used were *stat*, from software R v.4.0.1 (R Core Team, 2020), *car* v.3.0-12 (Fox and Weisberg, 2019), *ggdendro* v. 0.1.22 (De Vries and Ripley, 2020), *fpc* v. 2.2-9 (Hennig, 2020), and *factorextra* v. 1.0.7 (Kassambara and Mund, 2020). Graphics were created using the packages *ggplot2* v.3.3.5 (Wickham, 2016) and *gridExtra* v.2.3 (Auguie, 2017).

3. Results

Two clusters were generated by the analyses (Euclidean distance for separation = 3.843), indicating two different morphotypes of teeth from dolphins of the genus *Sotalia* in the sample (Figure 4). The Jaccard coefficients of the two clusters, 0.94 and 0.90, indicated good representability of the data structure by the dendrogram. The morphotype named as Type 1 included 67 teeth from handicrafts, while the morphotype named as Type 2 included 42 teeth. Related to geographical distribution, Type 1 morphotype was found in all sampled markets of Northern and Northeastern Brazil, while Type 2 was found only in two markets of Belém, the capital city of Pará State, placed at the southeastern Amazon Estuary (Figure 4).

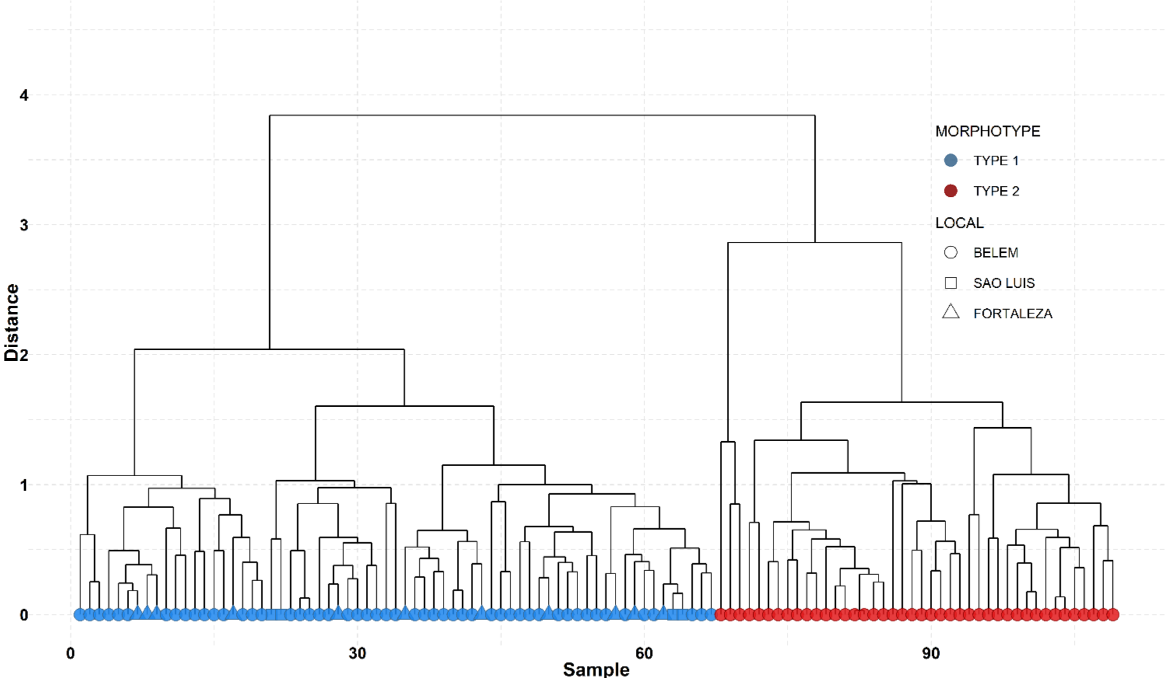

Figure 4. Dendrogram from the clustering analysis showing the two principal clusters separating tooth types. Colors indicate the teeth morphotype groups and symbols indicate the sample's locality in the North and Northeast regions of Brazil.

Type 1 morphotype of teeth shows more similar dimensions to PA and NE control groups than Type 2 morphotype (Figure 5). The two-way MANOVA results indicated that measurements differed between the four groups of teeth (Type 1, Type 2, NE and PA) (df = 3, Pillai Trace = 1.080, approximate F = 15.900, p < 2.2e-16), age classes (juveniles, young adults, and adults) (df = 2, Pillai Trace = 0.283, approximate F = 4.600, p = 3.178e-05) and also between teeth morphotypes in relation to the ages classes (df = 6, Pillai Trace = 0.3698, approximate F = 1.900, p = 0.005). Individual ANOVAs detected statistical differences between groups of teeth for the measurements total length (TL), root diameter (RD), crown length (CL), and cingulum diameter (CID), while differences between the age classes were only related to root diameter (RD) (Supplementary Table S1). Type 1 morphotype were similar to PA control group in TL (Mean differences = -0.80, p = 0.152) and CID (Mean differences = 0.28, p = 0.971), and similar to NE control group in CID (Mean differences = 1.417, p = 0.109), and CL (Mean differences = -1.92, p = 0.052). Type 2 shows the smallest dimensions for the four measurements, being statistically different from the others groups of teeth in TL, CID and CL (Figure 5 and Table S2). Type 2 morphotype were also different from Type 1 and NE control group in RD, but similar to PA teeth

(Figure 5 and Supplementary Table S2). Related to the ages classes, juveniles (Mean differences = -0.35, p = 0.0001) and young adults (Mean differences = - 0.27, p = 0.0032) show smaller RD dimensions, being statistically different from adults (Supplementary Table S2).

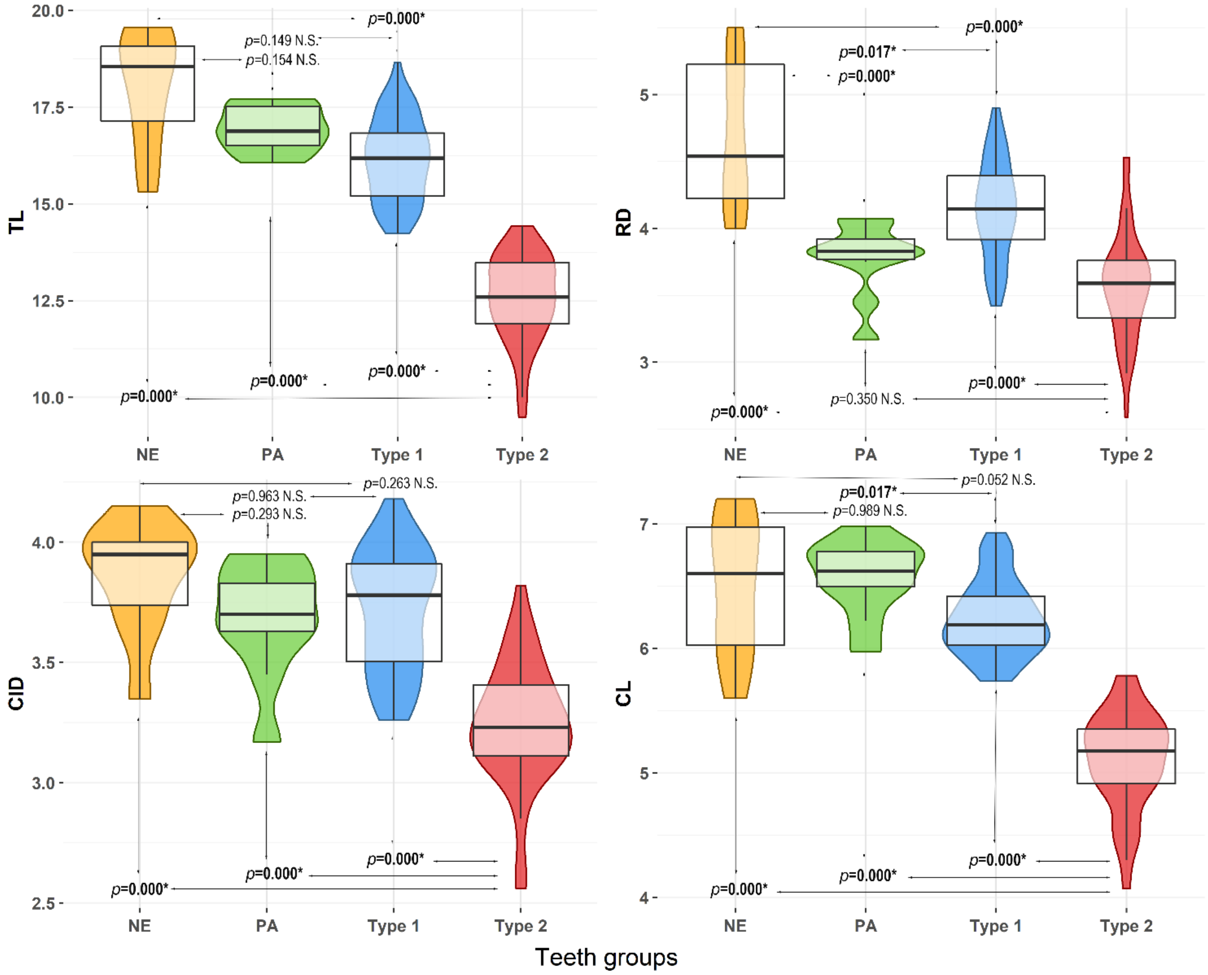

Figure 5. Violin data representation and pairwise comparison between groups of teeth for the measurements of total length (TL), root diameter (RD), cingulum diameter (CID), and crown length (CL). Type 1 and Type 2 comprised groups of teeth resulting from cluster analysis and the localities of Pará coast (PA) and Northeast Brazil (NE) comprised only stranded specimens of *Sotalia guianensis* used as control groups. Box represents values between the first and third quartile, the horizontal dark line represents the median, and vertical lines inside the violin shapes represent the lowers and upper values.

Interaction between factors influenced the CID, with some age categories being different between the types of teeth, but always related with Type 2 morphotype having smaller dimensions (Supplementary Table S2). Thus, juveniles, young adults and adults teeth from Type 1 morphotype had, respectively, significant higher dimensions than juveniles (Mean differences = 2.14, p=0.00), young adults (Mean differences = 1.68, p=2.862E-03) and adults (Mean differences = 1.68, p=1.040E-05) from Type 2 morphotype. Juveniles teeth from Type 2 morphotype were also smaller than juveniles from PA (Mean differences = -2.42, p=8.267E-04) and NE (Mean differences = -2.46, p=1.210E-05) control groups, and smaller than adults from Type 1 morphotype (Mean differences =-2.19, p=0.00), PA (Mean differences= -1.90, p= 0.0162), and NE (Mean differences = -2.55, p=0.00) control groups. Young adult teeth from Type 2 were smaller than juveniles (Mean differences = -1.74, p= 0.0001) and adults (Mean differences = -1.81, p= 2.870E-05) from Type 1 morphotye, and juveniles (Mean differences = -2.13, p= 0.0038) and adults (Mean differences = -2.23, p= 1.660E-05) from NE control group. Finally, adults from Type 2 were smaller than juveniles (Mean differences = -1.61, p=5.600E-05) and young adults (Mean differences = -1.55, p=0.004) from Type 1 morphotype (Mean differences = -2.55, p=0.00), and juveniles (Mean differences = -2.02 p=0.008) from NE control group.

The PCA grouping shows a similar data structure to the clustering analysis with the first two dimensions explaining 87.1% of the data ordination (Figure 6, Supplementary Table S3). The first principal component (PC1) explained 72.9% ± 0.22 SD of the total variability. The measurement with higher loading for this PC was the TL (Loading = 0.6405), describing the size difference between the groups, followed by the CL but with a smaller correlation (Loading = 0.4818). The PC2 explained only 13.8% ± 0.09 SD of the variance, being more related to the DR (Loading = 0.8172), indicating an increase in diameter through the age categories (Figure 6) and supporting the differences between adults and the others ages classes founded in the ANOVA analysis.

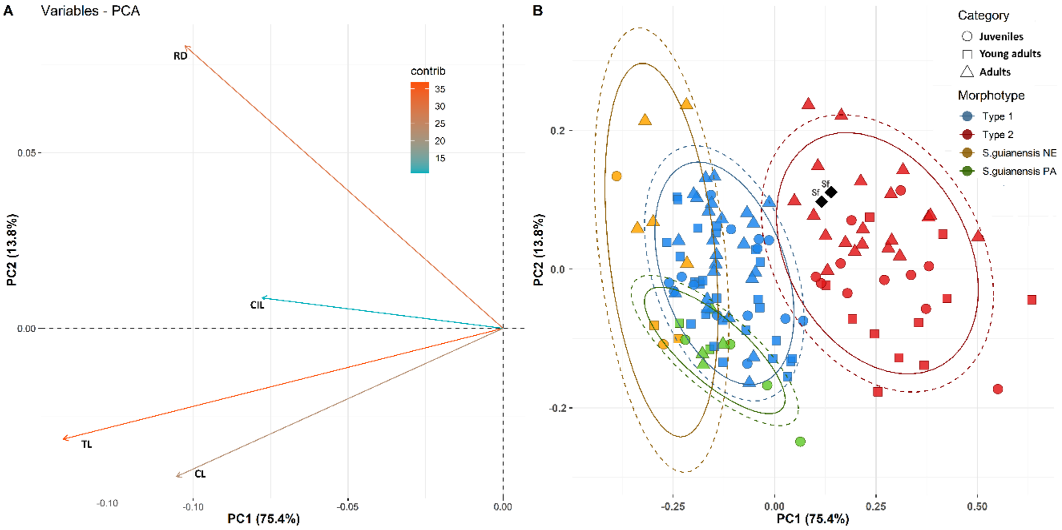

Figure 6. Principal component analysis for teeth measurements obtained from samples of *Sotalia* dolphins from North and Northeastern Brazil. A: Contribution of the variables total length (TL), root diameter (RD), crown length (CL), and cingulum diameter (CID) to the principal component dimensions (PCs). B: Scores distribution in the two dimensions of the PCs. The category indicates juveniles, young adults, and adults in the sample, and colors indicate the teeth morphotypes or species. The localities of Pará coast (PA) and Northeast Brazil (NE) included only stranded specimens of *Sotalia guianensis*. The black diamonds represent the size of the teeth of an adult specimen of *Sotalia fliuviatilis* preserved at the Museu Nacional in Rio de Janeiro, Brazil.

Ages of 116 teeth adorning the handicrafts were possible to obtain, with 64 teeth bellowing to Type 1, 42 bellowing to Type 2, and 10 teeth with no classification. The total sample showed a diverse age composition, ranging between 2 and 30 years (Mean =11 ± 7 SD, Median = 10, Mode = 3, 4, 8, and 19 years). Some teeth from the ages 25, 28, and 29 years were aged as minimal age because of the loss of part of the pulp cavity in the construction of handicraft (see Figure 2), thus probably being older than was detected. Juveniles dolphins (ages ≤ 6) were 34% of the teeth sample, while young adults (6 < ages ≤ 12) corresponded for 27% and adults (ages ≥ 13) for 41%. Where the sample was split by teeth morphotype, Type 1 was composed of 40% juveniles, 22% by young adults, and 38% by adults. The type 2 sample was divided into 24% of juveniles, 25% of young adults, and 49% of adults. Age composition for the teeth classified as Type 1 ranged between 2 and 30 years (Mean =10 ± 7 SD), with the age class of 3 years being the most frequent in this group (17% of the sample), followed by the ages of 4 and 13 years which represented in both cases 11% of the sample. The ages of teeth classified as Type 2 ranged between 4 and 28 years (Mean=14 ± 6 SD), with the ages of 5 (12%) and 16 years (10%) being the most frequently observed. Adults, between the ages of 7 and 20 years, represent, respectively, 52% and 63% of Type 1 and Type 2 morphotypes. The distribution of ages for both morphotypes of teeth showed a multimodal frequency distribution (Figure 7).

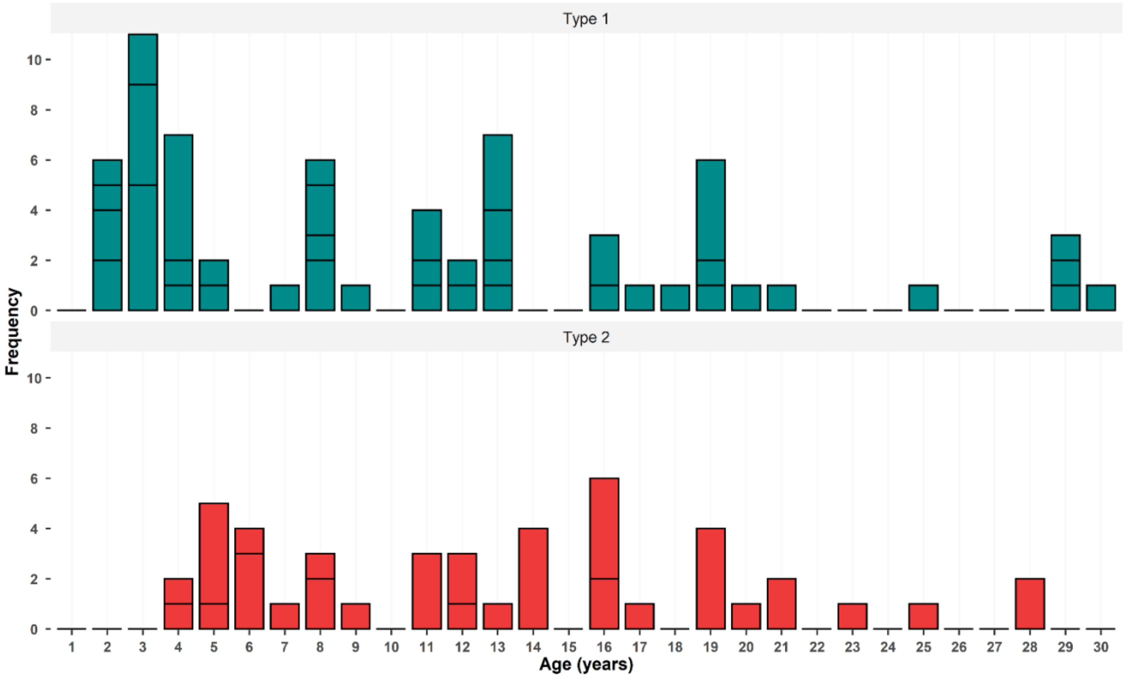

Figure 7. Age distributions for the two morphotypes of *Sotalia* spp. dolphin teeth detected in the trade of handcrafted objects in the North and Northeast regions of Brazil. Branches indicated the frequency of each age class in the handicraft objects.

4. Discussion

Two morphotypes of teeth, presumably belonging to both species from the genus *Sotalia*, *S. guianensis,* and *S. fluviatilis* were found on handmade necklaces, bracelets, and earrings sold in public markets of three main cities of the North and Northeast coast of Brazil. Age distribution of teeth indicated that juveniles and young adult and adult dolphins, between the age of 2 and 8 years, and between the age of 11 and 20 years, are more used in these trades since were the most frequent ages observed in the different handicraft objects.

Some aspects of geographical variation in morphology of the genus *Sotalia* have been more frequently investigated, mainly related to general body size and osteology (Borobia, 1989; Monteiro-Filho et al., 2002; Fettuccia et al., 2009; Ramos et al., 2010; Fettuccia, 2010; Fettuccia et al., 2012; Arcoverde et al., 2013). For these results, it is confirmed a variation in body length and cranial size for *S. guianensis* on the Brazilian coast, with the smallest individuals habiting the Amazon estuarine region, and a gradual increase in size for populations inhabiting coastal areas from more Northeastern, Southeastern and Southern regions (Fettuccia, 2010; Emin-Lima, 2012; Arcoverde et al., 2013). Additionally, *S. fluviatilis*, the riverine species of the genus, is described as significantly smaller in body dimensions compared to *S. guianensis*, with slower body development and with the preservation of some pedomorphic characters (Fettuccia et al., 2009; Fettuccia, 2010). In our study, it was possible to detect this geographic variation in size in the teeth collected from the handicrafts and stranded animals. Thus, teeth of stranded specimens from the Northeastern Brazilian region (NE), had the highest dimensions, while teeth from specimens stranded in the coastline of Pará state (PA), North region, were relatively smaller in size, but with no statistical difference between the two regions for the majority of measurements. Overlapping with these two groups, the teeth classified as Type 1 showed a similar interval of size, although being statistically different from those collected in Northeastern Brazil in total length and root diameter. On the other hand, the Type 2 morphotype, detected in two of the handicrafts, showed evident smaller dimensions being separated from the other three groups in the ordination analysis and with a statistically significant difference in almost all measurement comparisons. Furthermore, in the only variable where the interaction of age classes and tooth type was identified (CID), the results indicated that all age classes of the Type 2 morphotype were mostly smaller than the age classes of other tooth groups, and no differences were found within any group. Thus, suggesting that the differences found are related to the morphological characteristics of the groups and not to possible modifications throughout growth. In addition, the Type 2 morphotype was found only in the Southeastern Amazon Estuary region, while Type 1 was found in all North-Northeastern localities. Based on these results it is suggested that the Type 1 teeth morphotype belongs to the species *S. guianensis*, while the Type 2 morphotype of teeth belongs to the riverine species *S. fluviatilis*.

The use of body parts of *Sotalia* dolphins has been widely reported in South America. In Brazil and Venezuela, estuarine dolphins *S. guianensis* accidentally captured in gillnet are commonly used for human consumption and bait in longline-fishing or shark-related-fishing

(Siciliano et al., 2008; Briceño et al., 2021). In addition, some practices related to Amazon traditional culture from northern Brazil, *i.e*., the use of dolphin parts as love's charms (Siciliano et al., 2018), have been linked to the increased trade of genitals, eyes, dorsal fin, skin, and teeth of Amazon dolphin species in traditional markets (Siciliano, 1994; Da Silva and Best, 1996; Siciliano et al. 2008; Sholl et al., 2008). More recently, forensic genetics results have indicated that *S. guianensis* is the most used cetacean in the majority of dolphin's love charms sold in traditional Brazilian markets, including the markets in the cities of Belém, São Luís and Fortaleza sampled in our research (Gravena et al., 2008; Sholl et al., 2008; Siciliano et al., 2018). Thus, our results are consistent with the geographical distribution of this trade in Northern and Northeastern Brazil and reveal specific segments of *S. guianensis* populations being more frequently used.

For the riverine species *S. fluviatilis*, its use as bait principally for the piracatinga (*Calophysus macropterus*) fishery, has been documented in the Amazonian regions of Peru and Brazil (Brum et al., 2015; Campbell et al., 2020), and medicinal and magical religious use has been also reported in Brazilian, Peruvian and Bolivian Amazon region (Alves et al., 2013). Based on the analysis of genetic markers on the tissue samples from love's charms acquired in Brazilian traditional markets, Siciliano et al. (2018) did not detect the use of *S. fluviatilis* individuals in this kind of trade. However, handicraftsmen who were carrying a notable quantity of *Sotalia* teeth, interviewed near Belém, Pará, reported to the authors that they had acquired the teeth from several fishing communities in their trip through the Amazon. Therefore, it is possible the presence of *S. fluviatilis* teeth brought from riverine regions in handicrafts sold at public markets of Belém or another coastal region of the southeastern Amazon. Siciliano et al. (2018) also described those necklaces and bracelets made with *Sotalia* teeth were found in traditional open-air market squares from Belém and were the only charm found in street markets from Fortaleza, Ceará State. In this context, it could be possible that the trading of teeth and handicrafts made with them, would take a different path to these sites, more related to these travelers selling handicrafts at squares and street markets, and not with the traditional trading of other dolphin body parts. This fact could also explain the absence of *S. fluviatilis* in the love's charm samples from the study conducted by Siciliano et al. (2018).

The age distribution obtained in our study indicated that teeth from juvenile individuals, between the ages of 2 and 6 years, and young adults and adults, between 8 and 19 years, are more frequently used in handicrafts. Both *Sotalia* species show this pattern of age distribution, being more evident in *S. guianensis*. Although some of the teeth may be from the same individuals, we believe that the repetition of ages on the different objects collected indicates a recurrence of animals of these age classes as a source of teeth for handicrafts. In addition, no teeth of specimens younger than two years old were found, maybe because crafts with smaller or finer teeth can be more difficult to manufacture and/or because teeth providing this trade could be collected from specimens accidentally caught in fishing gillnets, being newborns and yearlings probably discarded or less affected by this activity. This last hypothesis needs further confirmation; however, some inferences are possible to be made. In marine mammal populations under a stable age distribution, natural mortality rates are expected with the greatest frequency of yearlings (animals < 1year), followed by juveniles and adults with much less frequency (Reilly and Barlow, 1986; Barlow and Boveng, 1991; Mannocci et al., 2012). When mortality is affected by the action of bycatch (where animals are accidentally caught in gillnets during fisheries), the expected age distribution changes because not all ages are equally affected in the process (Moore and Read, 2008; Mannocci et al., 2012). Thus, an increase in juveniles' and young adults' mortalities relative to the other age classes is observed. This had been detected in different dolphin populations, such as striped dolphin (*Stenella coeruleoalba*) and common dolphin (*Delphinus delphis*) in the Northeast Atlantic Ocean (Mannocci et al., 2012; Brown and Rogan, 2014), and common bottlenose dolphins (*Tursiops truncatus*) in North Carolina coast (Byrd and Hohn, 2017).

On the North Pará coast, *S. guianensis* is the most frequent marine mammal found stranded, as a result of its interactions with local fishing activity (Siciliano, 1994; Siciliano et al., 2008; Costa et al., 2017). The use of dolphin carcasses, stranded and/or incidentally captured in fisheries as a source of products found in regional markets of Northern Brazil has been previously suggested (Siciliano, 1994; Da Silva and Best, 1996; Sholl et al., 2018; Siciliano et al., 2018), as well as its use as bait or for human consumption (Siciliano, 1994; Tosi *et al*., 2009; Zappes et al., 2009; Dos Santos-Filgueira et al., 2021). Moreover, studies on *S. guianensis* bycatch for populations of Northeastern, Southeastern, and Southern Brazil indicated that juveniles and adult individuals, with body length superior to 160 cm (> 6 years old), have more vulnerability to by caught in fishing nets representing until 80% of the captures (Rosas et al., 2003; Di Beneditto and Ramos, 2004; De Moura et al., 2009; Meirelles et al., 2010; Lima et al., 2016). For *S. fluviatilis,* despite interactions with fishing activities are extremely difficult to record, punctual data of bycatch and intentional catches in Western Brazilian Amazon indicated a high incidence of juveniles (of ~138 cm of body length) and adults (reproductively matures animals) captured in tambaqui (*Colossoma* *macropomum*) and pirapitinga (*Piaractus brachypomus*) fishing gillnets (Best and Da Silva, 1996; Iriarte and Marmontel, 2013). Furthermore, Iriarte and Marmontel (2013), raised concern about the use of Amazonian River dolphin carcasses as a productive cost-effective means of obtaining bait for fishermen. The cost, viability, and effectiveness as drivers for the use of dolphins as bait by fishermen have been recently confirmed on the Peruvian coast, where similar human-related pressures increased the mortality of coastal small cetaceans (Campbell *et al*., 2020).

Whether the origin of our sample is from stranded or accidental capture, remains unknown, however, based on our results, and having into consideration the most frequent age categories observed in our sample, juveniles and adults younger than 20 years, it is possible to suggest that handicrafts commercialized as part of dolphin charm trade in the North and Northeast of Brazil and *Sotalia* dolphins' bycatch seems to affect the same population segment, and they are probably related in this region. Public policies and conservation actions have been encouraged in Brazil to reduce dolphin bycatch. However, the real impact of this activity on populations of *Sotalia* spp. dolphins from Northern-Northeastern Brazil are still unknown. Ethnoecological studies with fishermen's communities indicated a high incidence of this anthropic action in the region, with animals being mostly discarded and finally stranding at the coast (Siciliano, 1994; Brito, 2012; Dos Santos-Figueira, 2021). The overlapping in the occurrence of the dolphin population's groups with preferential areas for gillnet placements has been suggested as one of the principal causes of the high incidence of bycatch (Martins et al., 2004; Beltrán-Pedreros and Petrere, 2010). In this scenario, the sale of body parts could be both, indicative of the high mortality of these species in the region, and a pressure that can influence this mortality, increasing the vulnerability of the local dolphin populations.

In summary, our study attempted to detect different cetaceans teeth used in the confection of handicrafts traded as artisanal dolphin-derived products in North and Northeastern Brazil using linear morphometry as a tool. We detected two different morphotypes of teeth from the genus *Sotalia* in the handicrafts, presumably belonging to the species *S guianensis* and *S. fluviatilis* however further studies are needed to confirm this inference. In addition, we see the necessity of future researchers related to cetaceans' tooth morphology, that confirm the use of teeth as an effective tool for distinguishing species where other data is unknown. We also attempted to access the segments within the *Sotalia* spp. populations more used in this trade by determining the age of the teeth. We found that juveniles and adults younger than 20 years are more frequently used in these handicrafts ornamented with teeth, coinciding with the age categories that have the highest incidence of cetaceans mortality related to bycatch in this region. Thus, the technic of age determination showed its applicability as a tool in gathering information about human actions, directly and indirectly, affecting specific groups of coastal populations of *Sotalia* dolphins in Northern and Northeastern Brazil. In addition, this result raises concerns about the increased vulnerability of the two species, but especially related to the threatened species *S. fluviatilis*, as scarce data have been reported on their life-history traits and only one age-related research was found available. Finally, we believe that this improved data could allow for more realistic management policies to help in the conservation of the two *Sotalia* species in the Amazon region, guiding enforcement actions, and improving environmental education with traders and local fishermen. However, it is imperative to assess the real impact of fisheries and bycatch on the *S. guianensis* population of the Northern Brazilian coast, as a way to understand how the population has been affected over the years and how this trade is supplied.

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Considerações Finais

Este manuscrito aponta novas informações relacionadas à população de *Sotalia guianensis* do Estuário Amazônico, pertencente à Unidade de Manejo da espécie do Norte do Brasil (BRNO). Nesse sentido, a primeira hipótese proposta, referente ao padrão sazonal dos encalhes da espécie na região, baseada nas características ambientais locais, foi corroborada. Além disso, foi detectada uma diminuição de encalhes ao longo dos anos para a Baía de Marajó o que poderia ser consequência da intensa atividade pesqueira nessa área do estuário. A atividade pesqueira parece estar influenciando também na distribuição de idades observada nos exemplares encalhados, sendo detectado uma maior mortalidade de espécimes jovens. Nesse sentido, dados sistemáticos de encalhes confirmam-se como ferramenta útil para detectar variações a curto e longo-prazo na mortalidade de espécies costeiras de cetáceos. Além disso, os resultados indicam mudanças temporais e espaciais na distribuição de *S. guianensis* no Estuário Amazônico que podem ser importantes para o manejo das populações da espécie na região. Porém, novos estudos são recomendados para avaliar diretamente a influência da atividade de pesca na mortalidade da espécie nesta região geográfica como principal ameaça para sua conservação.

Por outro lado, a análise da variação do tamanho do crânio ao longo da distribuição da espécie na costa brasileira corroborou a hipótese proposta sobre uma diferenciação morfológica dos indivíduos da região Norte com respeito a espécimes do Nordeste, Sudeste e Sul do país. Além disso, uma influência negativa da Clorofila-a no tamanho do crânio foi detectada, justificando-se pela presencia de animais maiores em regiões de baixa produtividade primaria, e animais menores em regiões mais produtivas. Estes resultados confirmam o tamanho e crescimento do crânio como caracteres importantes na delimitação das unidades de manejo da espécie, e demostram um gradiente de crescimento para a especie Norte-Nordeste, e um gradiente de diminuição Nordeste-Sul. Nesse sentido, se propõe como hipótese para a especie uma plasticidade no tamanho do corpo relacionada a regiões de baixa produtividade primaria, onde o maior tamanho do crãinio permitiria a exploração de uma maior variedade de recursos de alimentação, sendo o contrario observado em regiões de alta produtividade. É sugerido novas pesquisas sobre a morfologia de *S. guianensis,* focados na variação geográfica da espécie e com ênfases na região Nordeste, sendo uma das regiões com menos informação disponível sobre esta espécie de cetáceo costeiro.

Finalmente, a partir da técnica de determinação de idade e o uso das medidas morfométricas dos dentes, foi possível determinar diferenças na morfologia dentaria da espécie ao longo da sua distribuição, principalmente entre o Norte do Brasil, e outras regiões geográficas. Ainda, usar essas diferenças para detectar o uso de dentes de *S. guianensis*, e sua contraparte ribeirinha, *Sotalia fluviatilis,* no comercio ilegal de amuletos “mágicos” tradicionais e suvenires no Norte e Nordeste brasileiros. Sendo assim, a morfometria e morfologia dentaria podem ser ferramentas importantes, junto com a genética forense, na detecção de espécies de Cetáceos usadas tradicionalmente ou sendo comercializadas de forma ilegal em determinadas regiões. Assim, contribuir a planos de manejo e monitoramento de mamíferos marinhos mais acertados.

ANEXOS

MATERIAL SUPLEMENTAR (CAPÍTULO I)

Tabela1. Referencias documentadas com 10 ou mais anos de intervalo de estudo, incluindo Artigos, Teses/Dissertações e Relatórios. Revisões sem datas especificas também foram incluídas e sinalizadas com (?). O grupo especifico de estudo foi classificado por Ordem, Subordem, Família, Gênero ou Espécie segundo a abrangência da pesquisa. Os fatores ambientais discutidos ou diretamente avaliados, sinalizados com (*), foram classificados segundo a análise hierarquizada discutida na revisão. UME: Indica evento de mortalidade em massa; MSE: Indica evento de encalhe em massa

Autores	**Ano de publicação**	**Intervalo**	**Grupo**	**Tipo**	**Tópico**	**Aspectos específicos**	**Fator Ambiental**	**Revista**	**DOI**
Sheldrick, M.C.	1976	1913 - 1972	Cetacea	Artigo	Registro/ Tendência	Diversidade/ Variação temporal	Recursos locais	Mammal Review	10.1111/j.1365-2907.1976.tb00197.x
Berrow, S. & Rogan, E.	1994	1901 -1995	Cetacea	Artigo	Registro/ Tendência	Diversidade/ Variação temporal/ Sazonalidade/Fatores ambientais	Fenômenos regionais	Mammal Review	0.1111/j.1365-2907.1997.tb00372.x
Brabyn M. & Frew R.V.C.	1994	?	Cetacea	Artigo	Registro	Diversidade/Distribuição espacial	Características físicas do ambiente	Marine Mammal Science	10.1111/j.1748-7692.1994.tb00261.x
Evans et al.	2002	1911 - 1998	Odontoceti/ *P. macrocephalus*	Artigo	Registro	UME/Ocorrência/ Fatores ambientais	Fenômenos continentais	Marine Mammal Science	
Santos et al.	2002	1990 - 1999	Cetacea	Artigo	Registro/ Tendência	Diversidade/ Variação temporal/ Sazonalidade	Fenômenos regionais/ Recursos locais* (Encalhe & avistamento)	Journal Of The Marine Biological Association Of The United Kingdom	10.1111/j.1748-7692.2002.tb01021.x
Macleod et al.	2003	1979 - 2001	Odontoceti/ Ziphiidae	Artigo	Revisão/Registro	Distribuição espacial	Recursos locais	Journal Marine Biological Association Of The United Kingdom	10.1017/S0025315407054380

Norman, et al.	2004	1930 - 2002	Cetacea	Artigo	Registro/ Tendência	Distribuição espacial/ Variação temporal/ Sazonalidade/ Fatores ambientais /Esforço	Fenômenos regionais	Journal Of Cetacean Research And Management	10.3354/meps300229
Palacios et al.	2004	1923 - 2003	Cetacea	Artigo	Registro/ Tendência	Diversidade/ Distribuição espacial/ Variação temporal/ Esforço	Fenômenos regionais/ Recursos locais*	Latin American Journal Of Aquatic Mammals	IWC
Evans et al.	2005	1920 - 2003	Odontoceti	Artigo	Tendência	Variação temporal/ Sazonalidade/ Fatores ambientais	Fenômenos regionais*	Biology Letters	10.5597/lajam00058
Macleod et al.	2005	1948 - 2003	Cetacea	Artigo	Registro/ Tendência	Diversidade/ Variação temporal/ Fatores ambientais	Fenômenos regionais*	Biological Conservation	10.1098/rsbl.2005.0313
Maldini et al.	2005	1937 - 2002	Odontoceti	Artigo	Registro/ Tendência	Diversidade/Distribuição espacial/ Variação temporal	(Encalhe & avistamento)	Pacific Science	10.1016/j.biocon.2005.02.004
Vanselow, K.H. & Ricklefs, K.	2005	1712 - 2003	Odontoceti/ *P. macrocephalus*	Artigo	Tendência	Variação temporal/ Sazonalidade/ Fatores ambientais	Eventos estocásticos*	Journal Of Sea Research	10.1578/016754203101023924
Walker et al.	2005	1977 - 2001	Cetacea	Artigo	Tendência	Variação temporal/ Sazonalidade/UME/ Fatores ambientais	Fenômenos regionais/ Recursos locais*	Marine Mammal Science	10.1016/j.seares.2004.07.006
Branch et al.	2007	1825 - 2005	Mysticeti	Artigo	Registro	Diversidade/ Distribuição espacial/ Fatores ambientais	Recursos locais	Mammal Review	10.1016/j.seares.2006.01.003
Pierce et al.	2007	1563 - 2004	Odontoceti/ *P. macrocephalus*	Artigo	Tendência	Distribuição espacial/ Variação temporal/	Fenômenos regionais*/ Recursos locais*	Fisheries Research	

						Fatores ambientais			
O'Brien, N.M.	2008	1977 - 2005	Odontoceti/ Kogia	Tese	Registro/ Tendência	Distribuição espacial/ Variação temporal/ Sazonalidade/ Fatores ambientais	Fenômenos regionais*	Nova Southeastern University	
Berini, C.	2009	1992 - 2006	Odontoceti/ *K. breviceps*	Tese	Registro/ Tendência	Distribuição espacial/ Sazonalidade/ Fatores ambientais	Fenômenos regionais*	College Of Charleston	10.1017/S0025315408000155
MacLeod, C. D.	2009	?	Cetacea	Artigo	Revisão	Distribuição espacial/ Variação temporal/ Fatores ambientais	Mudanças climáticas	Endangered Species Research	10.1017/S0025315409000307
Meirelles et al.	2009	1992 -2005	Cetacea	Artigo	Registro/ Revisão/ Tendência	Diversidade/ Distribuição espacial/ Sazonalidade	Fenômenos regionais/ Recursos locais	Journal Of The Marine Biological Association Of The United Kingdom	10.3354/esr00197
Simmonds, M.P. & Eliott, W. J.	2009	?	Cetacea	Artigo	Revisão	Ocorrência/ Fatores ambientais	Fenômenos continentais/ Fenômenos regionais	Journal Of The Marine Biological Association Of The United Kingdom	
Vanselow et al.	2009	1712 - 2003	Odontoceti/ *P. macrocephalus*	Artigo	Tendência	Fatores ambientais	Eventos estocásticos*	The Open Marine Biology Journal	10.1017/S0025315408003196
Taguchi et al.	2010	1966 - 2009	Odontoceti/ *P.phocoena*	Artigo	Tendência	Distribuição espacial/ Sazonalidade	Fenômenos regionais	Mammal Study	10.1590/s1679-87592010000200004
Bearzi et al.	2011	1555 - 2009	Odontoceti/ *P. macrocephalus*	Artigo	Registro/ Tendência	Ocorrência/ Distribuição espacial/ Fatores ambientais/ MSE/UME	Características físicas do ambiente	Mammal Review	

Lambert et al.	2011	1930 - 2003	Odontoceti/ *D.delphis*	Artigo	Tendência	Variação espacial/ Variação temporal/ Fatores ambientais	Fenômenos continentais*/ Fenômenos regionais*	Endangered Species Research	
Fruet et al.	2012	1993 - 2006	Odontoceti/ *T. truncatus*	Artigo	Tendência	Variação temporal	Outras causas	Journal Of The Marine Biological Association Of The United Kingdom	10.5597/lajam00175
Groom, C.J. & Coughran, D.K.	2012	1981 - 2010	Cetacea	Artigo	Registro/ Tendência	Diversidade/ Distribuição espacial/ Variação temporal/ Fatores ambientais	Fenômenos regionais/ Características físicas do ambiente	Journal Of The Royal Society Of Western Australia	
Kemper et al.	2013	1884 - 2007	Odontoceti/ *C. marginata*	Artigo	Registro/ Tendência	Distribuição espacial/ Variação temporal/ Sazonalidade/ Fatores ambientais	Fenômenos continentais* / Recursos locais*	New Zealand Journal Of Zoology	
Peltier et al.	2013	1990 - 2009	Odontoceti/ *P.phocoena*	Artigo	Tendência	Distribuição espacial/ Deriva de carcaças/ Variação temporal/ Sazonalidade/ Fatores ambientais	Fenômenos regionais/ Características físicas do ambiente	Plos ONE	10.2478/s11756-013-0237-8
Rubio-Guerri et al.	2013	1990 -2011	Mysticeti/ *S. coeruleoalba*	Artigo	Tendência	Variação temporal/ Fatores ambientais	Eventos estocásticos	Bmc Veterinary Research	10.1371/journal.pone.0062180
Schumann et al.	2013	?	Cetacea	Artigo	Revisão	Fatores ambientais	Fenômenos continentais/Fenômenos regionais/Recursos locais	Australian Journal Of Zoology	10.1186/1746-6148-9-106
Thompson et al.	2013	1991 -2011	Odontoeti	Artigo	Registro/ Tendência	Distribuição espacial/ Variação temporal/ Sazonalidade/	Recursos locais	Biological Conservation	10.1071/ZO12131

						Fatores ambientais			
Truchon et al.	2013	1994 - 2008	Cetacea	Artigo	Tendência	Distribuição espacial/ Variação temporal/ Sazonalidade/ Fatores ambientais	Fenômenos continentais*/ Fenômenos regionais*/ Recursos locais*/ Eventos estocásticos*	Plos ONE	10.1016/j.biocon.2012.07.017
Smultea, M. & Jefferson, T.	2014	1950 - 2012	Cetacea	Artigo	Registro/ Tendência	Ocorrência/ Variação temporal	(Encalhe & avistamento)	Aquatic Mammals	10.1017/S0025315413000337
Byrd et al.	2014	1987 - 2008	Cetacea	Artigo	Registro/ Tendência	Distribuição espacial/ Variação temporal/ Sazonalidade	(Encalhe & avistamento)	Fishery Bulletin	10.1007/s10531-014-0741-3
Casalone et al.	2014	1987 - 2013	Cetacea	Artigo	Registro/ Tendência	Ocorrência/ UME Distribuição espacial/ Fatores ambientais	Mudanças climáticas/ Eventos estocásticos	Diseases Of Aquatic Organisms	10.7755/FB.112.1.1
Hamilton L.J. & Lindsay K.	2014	1871 - 2010	Odontoceti	Artigo	Registro/ Tendência	Distribuição espacial/ Variação temporal/ Fatores ambientais	Fenômenos regionais	Journal Of Cetacean Research And Management	IWC
Hamilton L.J. & Lindsay K.	2014	1868 - 2010	Odontoceti	Artigo	Registro/ Tendência	Distribuição espacial/ Fatores ambientais	Características físicas do ambiente	Journal Of Cetacean Research And Management	IWC

Harlan, A.J.	2014	1992 - 2013	Odontoceti/ Kogia	Tese	Tendência	Distribuição espacial/ Deriva de carcaças/ Variação Temporal/ Fatores ambientais	Fenômenos regionais	College Of Charleston	IWC
Litz et al.	2014	1990 - 2009	Cetacea	Artigo	Registro/ Tendência	Distribuição espacial/ Variação temporal/ Fatores ambientais/ UME	Eventos estocásticos	Diseases Of Aquatic Organisms	
MacLeod et al.	2014	1992 - 2004	Odontoceti/ *G. griseus*	Artigo	Tendência	Distribuição espacial/ Fatores ambientais	Recursos locais (Encalhe & avistamento)	Hydrobiologia	10.3354/dao02807
Meager, J. & Limpus, C.	2014	1996 - 2012	Odontoceti	Artigo	Tendência	Variação temporal/ Fatores ambientais	Fenômenos continentais*/ Fenômenos regionais*/ Recursos locais*/ Eventos estocásticos*	Plos ONE	10.1007/s10750-013-1555-0
Pyenson et al.	2014	Registro fosil	Cetacea	Artigo	Tendência	Distribuição espacial/ Fatores ambientais/ UME	Fenômenos regionais/ Recursos locais/ Eventos estocásticos	Proceedings Of The Royal Society B: Biological Sciences	10.1016/j.ecolind.2013.12.018
Vishnyakova, K. & Gol'din, P.	2014	1999 - 2014	Odontoceti/ *P.phocoena*	Artigo	Tendência	Variação temporal/ Sazonalidade/ Fatores ambientais	Fenômenos regionais/ Recursos locais*	Marine Biology	10.1098/rspb.2013.3316
Berini et al.	2015	1992 - 2006	Odontoceti/ *K.breviceps*	Relatório	Tendência	Variação temporal/ Sazonalidade/ Fatores ambientais	Fenômenos continentais*/ Fenômenos regionais*	Noaa Technical Memorandum Nos Nccos	10.3366/anh.2014.0252

Harry, C.T.	2015	1990 - 2014	Odontoceti	Tese	Tendência	Variação temporal/ Sazonalidade/ Fatores ambientais	Fenômenos continentais*	University Of Rhode Island	10.1111/mms.12264
Segawa, T. & Kemper, C.	2015	1881 - 2008	Cetacea	Artigo	Tendência	Distribuição espacial/ Variação temporal/ Sazonalidade/ Fatores ambientais/Esforço	Fenômenos regionais/ Recursos locais/ Características físicas do ambiente	Australian Mammalogy	10.1016/j.envsci.2015.07.013
Toledo et al.	2015	1960 - 2010	Odontoceti/ *G. griseus*	Artigo	Registro/ Revisão	Ocorrência		Marine Biodiversity Records	10.1071/AM14029
Ferrari, T.E.	2016	1998 - 2012	Cetacea	Artigo	Registro/ Tendência	Variação temporal/ Fatores ambientais	Eventos estocásticos*	International Journal Of Astrobiology	10.1186/s41200-016-0050-5
McGeady et al.	2016	2003 - 2015	Odontoceti	Artigo	Tendência	Distribuição espacial/ Variação temporal/ Fatores ambientais	Fenômenos regionais*/ Características físicas do ambiente*	Proceedings Of Meetings On Acoustics	10.1016/j.chemosphere.2016.01.026
Meynecke, J.O. & Meager, J.J.	2016	1989 - 2014	Mysticeti/ *M.noveangliae*	Artigo	Tendência	Distribuição espacial/ Variação temporal/ Fatores ambientais	Fenômenos continentais*/ Fenômenos regionais*	Journal Of Coastal Research	10.5597/lajam00227
Moura et al.	2016	1965 - 2014	Odontoceti/ Kogia	Artigo	Tendência	Distribuição espacial/ Sazonalidade/ Fatores ambientais	Fenômenos regionais*/ Recursos locais*	Plos ONE	10.2112/SI75-180.1
Prado et al.	2016	1976 - 2013	Cetacea	Artigo	Registro/ Tendência	Diversidade/ Distribuição espacial/ Variação temporal/ Sazonalidade/ Fatores ambientais	Fenômenos regionais/ Recursos locais/ Características físicas do ambiente	Plos ONE	10.1016/bs.amb.2016.07.007

Bengtson et al.	2017	1965 - 2017	Cetacea	Artigo	Tendência	Variação temporal/ Sazonalidade//UME/ Fatores ambientais	Recursos locais*/ Eventos estocásticos*	Bulletin Of Environmental Contamination And Toxicology	
Figueiredo et al.	2017	2000 - 2015	Mysticeti/ *E.australis*	Artigo	Registro/ Revisão	Distribuição espacial/ Variação temporal/ Sazonalidade	(Encalhe & avistamento)	Aquatic Mammals	10.3354/dao03080
Saavedra et al.	2017	2000 - 2013	Odontoceti	Artigo	Registro/ Tendência	Distribuição espacial/ Variação temporal/ Sazonalidade/ Fatores ambientais	Fenômenos continentais*/ Fenômenos regionais*/ Recursos locais*	Marine Biology	10.1578/AM.43.1.2017.52
Westdal et al.	2017	1840 - 2013	Odontoceti/ *O.orca*	Artigo	Registro/ Revisão	Distribuição espacial/ Fatores ambientais	Características físicas do ambiente	Polar Biology	10.1007/s00227-017-3200-3
Augé et al.	2018	1880 - 2015	Cetacea	Artigo	Registro/ Tendência	Diversidade/ Distribuição espacial/ Variação temporal/ Sazonalidade/ Fatores ambientais	Características físicas do ambiente	Journal Of Cetacean Research And Management	https://scholar.oxy.edu/scas/vol117/iss1/2
Grant et al.	2018	1999 - 2010	Cetacea	Artigo	Registro/ Tendência	Diversidade/ Distribuição espacial/ Variação temporal/ Fatores ambientais	Eventos estocásticos*	Animals	doi.org/10.3390/ani8020018
Hamilton, L.J.	2018	1963 - 2018	Odontoceti	Artigo	Revisão/ Tendência	Distribuição espacial/ Fatores ambientais/ MSE/UME	Fenômenos regionais/ Recursos locais/ Características físicas do ambiente	Journal Of Cetacean Research And Management	10.3390/ani8020018
Nielen, J.H.	2018	1949 - 2016	Odontoceti/ *P. macrocephalus*	Tese	Registro/ Tendência/UME	Distribuição espacial/ Variação temporal/ Fatores ambientais	Fenômenos regionais*/ Eventos estocásticos*	Utrecht University	10.1016/b978-0-12-804327-1.00249-1

Williams, O.	2018	1988 - 2006	Cetacea	Tese	Registro/ Tendência	Distribuição espacial/ Variação temporal/ Sazonalidade/ Fatores ambientais	Fenômenos regionais/ Características físicas do ambiente	Tese	10.1016/j.ocecoaman.2018.07.006
Coombs et al.	2019	1913 - 2015	Cetacea	Artigo	Tendência	Distribuição espacial/ Variação temporal/ Sazonalidade/ Fatores ambientais	Fenômenos continentais*/ Fenômenos regionais*/ Recursos locais*/ Eventos estocásticos*	Marine Mammal Science	doi.org/10.1111/mms.12610
Betty et al.	2020	1978 - 2017	Odontoceti/ *G. melas*	Artigo	Registro/Tendência/	Distribuição espacial/ Variação temporal/ Sazonalidade/ Fatores ambientais/ UME	Fenômenos continentais/ Fenômenos regionais/ Recursos locais	Biodiversity And Conservation	10.1038/s41598-020-66484-x
Mayorga et al.	2020	1975 - 2005	Cetacea	Artigo	Registro/ Tendência	Diversidade/ Distribuição espacial/ Variação temporal/ Sazonalidade	Recursos Locais	Zookeys	10.47536/JCRM.V21I1.180
Sanderson C.E. & Alexander K.A.	2020	1955–2018	Cetacea	Artigo	Tendência/UME	Distribuição espacial/ Variação temporal/ Fatores ambientais	Fenômenos continentais*/ Fenômenos regionais*/ Eventos estocásticos*	Global Change Biology	10.3389/fmars.2020.00333
Zellar et al.	2021	1999 - 2017	Ctacea	Artigo	Tendência/UME	Distribuição espacial/ Variação temporal/MSE	Fenômenos continentais*/ Fenômenos regionais*/	Geophysical Research Letter	doi.org/10.1029/2021GL093697
WARLICK et al.	2022	2000 - 2019	Cetacea	Artigo	Tendência/UME	Diversidade/ Distribuição espacial/ Variação temporal/ Sazonalidade	Fenômenos continentais*/ Fenômenos regionais*/ Eventos estocásticos*	Frontiers in Marine Science	10.3389/fmars.2022.758812

MATERIAL SUPLEMENTAR (CAPÍTULO II)

UPS AND DOWNS: AN INSIGHT ON THE STRANDING PATTERN OF GUIANA DOLPHINS, Sotalia guianensis, IN THE AMAZON ESTUARY, NORTHERN BRAZIL

Table S1. Number of stranded specimens of Guiana dolphins in the South of the Amazon estuary related to age, season, and sampled region. MB: Marajó Bay, SP: Salgado Paraense, NMI: Northern Atlantic coast of Marajó Island.

Region	Season	Age	No. Strandings
MB	dry	0	1
MB	dry	1	2
MB	dry	2	4
MB	dry	3	2
MB	dry	4	3
MB	dry	5	4
MB	dry	6	2
MB	dry	7	2
MB	dry	8	1
MB	dry	10	2
MB	dry	11	2
MB	dry	12	1
MB	dry	13	1
MB	dry	14	1
MB	dry	15	1
MB	dry	16	2
MB	dry	17	3
MB	dry	18	-
MB	dry	19	2
MB	dry	20	-
MB	dry	21	1
MB	dry	22	1
MB	dry	23	-
MB	dry	24	1
MB	dry	25	1
MB	dry	26	1
MB	dry	27	2
MB	dry	28	-
MB	dry	29	1
MB	dry	30	2
MB	dry	31	-
MB	dry	32	-
MB	dry	33	-
MB	dry	33	-
MB	rainy	0	-
MB	rainy	1	-
MB	rainy	2	2

MB	rainy	3	-
MB	rainy	4	-
MB	rainy	5	1
MB	rainy	6	-
MB	rainy	7	3
MB	rainy	8	1
MB	rainy	9	2
MB	rainy	10	3
MB	rainy	11	-
MB	rainy	12	-
MB	rainy	13	-
MB	rainy	14	1
MB	rainy	15	1
MB	rainy	16	1
MB	rainy	17	-
MB	rainy	18	2
MB	rainy	19	-
MB	rainy	20	3
MB	rainy	21	2
MB	rainy	23	1
MB	rainy	24	-
MB	rainy	25	-
MB	rainy	26	1
MB	rainy	27	-
MB	rainy	28	-
MB	rainy	29	-
MB	rainy	30	-
MB	rainy	31	-
MB	rainy	32	-
MB	rainy	33	1
MB	rainy	33	-
SP	dry	0	-
SP	dry	1	2
SP	dry	2	-
SP	dry	3	2
SP	dry	5	-
SP	dry	6	-
SP	dry	7	-
SP	dry	8	1
SP	dry	9	-
SP	dry	10	1
SP	dry	11	-
SP	dry	12	-
SP	dry	13	1
SP	dry	14	-
SP	dry	15	-
SP	dry	16	1
SP	dry	17	-
SP	dry	19	-
SP	dry	20	-
SP	dry	21	-
SP	dry	22	-
SP	dry	23	-
SP	dry	25	-
SP	dry	26	-

SP	dry	27	-
SP	dry	28	-
SP	dry	29	-
SP	dry	30	-
SP	dry	31	-
SP	dry	32	-
SP	rainy	0	3
SP	rainy	1	1
SP	rainy	2	3
SP	rainy	3	3
SP	rainy	5	1
SP	rainy	6	1
SP	rainy	7	1
SP	rainy	8	1
SP	rainy	9	-
SP	rainy	10	3
SP	rainy	11	2
SP	rainy	12	2
SP	rainy	13	1
SP	rainy	14	3
SP	rainy	15	-
SP	rainy	16	-
SP	rainy	17	1
SP	rainy	18	-
SP	rainy	18	1
SP	rainy	19	-
SP	rainy	20	-
SP	rainy	21	1
SP	rainy	22	-
SP	rainy	23	1
SP	rainy	25	1
SP	rainy	26	-
SP	rainy	27	-
SP	rainy	28	-
SP	rainy	29	2
SP	rainy	30	-
SP	rainy	31	-
SP	rainy	32	-
NMI	dry	0	1
NMI	dry	3	1
NMI	dry	25	1
NMI	NA	0	1
NMI	NA	4	1
NMI	NA	12	1
NMI	rainy	3	-
NA	NA	0	1
NA	NA	4	1

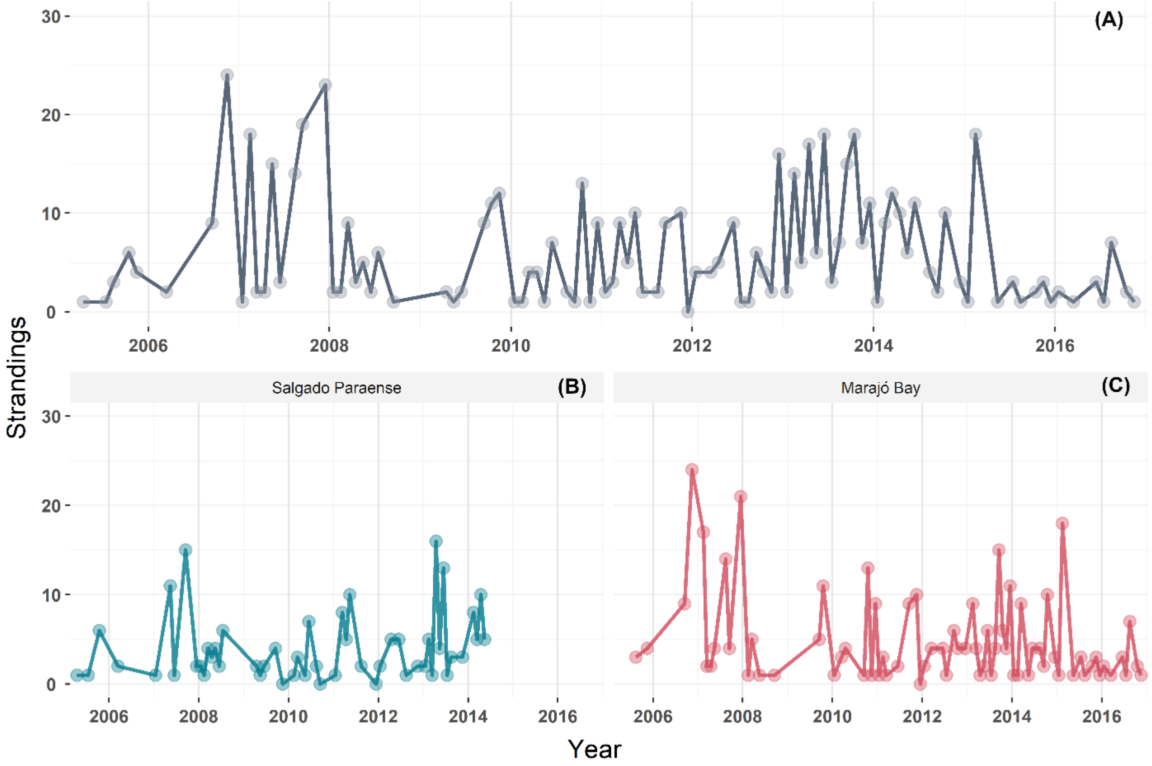

Figure S1. Temporal series of Guiana dolphin strandings in the South of the Amazon Estuary. A: Total strandings detected between the years 2005 and 2016. B: Strandings detected for the coastal region of Salgado Paraense. C: Strandings detected in the inner region of Marajó Bay.

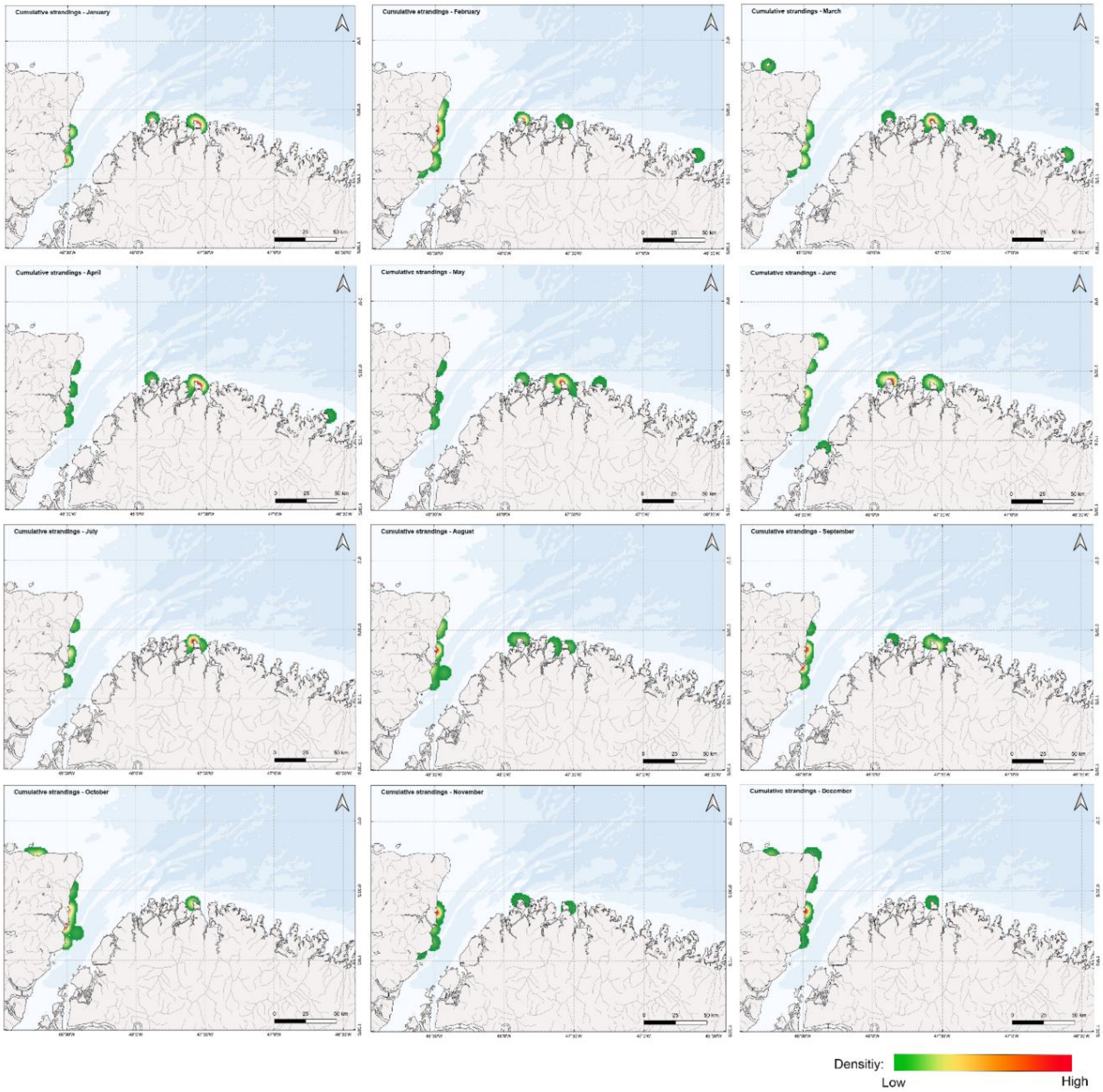

Figure S2. Kernel density map of monthly cumulative strandings of Guiana dolphins in the South region of Amazon Estuary.

MATERIAL SUPLEMENTAR (CAPÍTULO III)

CAPITULO III: FROM NORTH TO SOUTH: GEOGRAPHIC VARIATION IN SKULL SIZE AND GROWTH OF GUIANA DOLPHIN FROM THE BRAZILIAN COAST AND THE INFLUENCE OF LOCAL ENVIRONMENT

Table S1. Sample of Guiana dolphin from the different regions of Brazilian coast used in the analysis of geographic variation in the skull size. CBL_a: Condylobasal length average for adults obtained in this study or compiled in the literature (see References column); SST_a: Sea Surface Temperature average; SST_{min}: Minimal SST; SST_{max}: Maximal SST; SST_r: SST range; Lat: Latitude; Chl-a: Chlorophyll-a concentration; DS_{100}: Continental shelf length until 100 m depth; DS_{200}: Continental shelf length until 250 m of depth; Region: Specific region or State were animals stranded. MUs: Management Units.

Total Sample	**N adult**	**CBL_a (mm)**	**Chl-a (mg.m^{-3})**	**SST_a (C°)**	**SST_{min} (C°)**	**SST_{max} (C°)**	**SST_r (C°)**	**Lat (°)**	**DS_{50} (Km)**	**DS_{100} (Km)**	**DS_{250} (Km)**	**ESA (Km^2)**	**ESE (Km)**	**Region**	**MUs**	**References**
–	6	346.4	5.202	28.1	27.3	29.0	1.7	2	163.38	221.99	254.37	2357	422.7	Amapá	BRNO	Fettuccia (2006)
96	60	359.5	3.131	28.4	27.6	29.2	1.6	-0.6	162.00	218.81	223.37	2357	422.7	Pará	BRNO	This study
–	34	394.3	0.381	27.3	26.6	28.0	1.4	-3.5	42.43	46.22	49.35	192.6	56.8	Ceará	BRNE1	Fettuccia (2006)
3	2	389.0	0.212	27.7	26.9	28.4	1.5	-7	27.92	28.70	30.14	203.7	71.9	Paraíba	BRNE2	This study
6	3	392.0	0.144	27.5	26.7	28.3	1.5	-8	28.66	29.535	30.48	89.2	13.4	Pernambuco	BRNE2	This study
6	4	387.5	0.382	27.4	26.7	28.2	1.5	-10	34.46	36.11	37.72	95.0	28.4	Alagoas	BRNE3	This study
14	13	387.3	0.503	27.2	26.5	28	1.5	-11	26.59	27.78	28.82	252.3	73.3	Sergipe	BRNE3	This study
2	2	399.5	0.239	27.1	26.2	27.9	1.8	-12.6	14.48	18.31	19.43	203.6	44	Bahia	BRNE3	This study
17	10	376.7	0.714	25.2	24.0	26.5	2.4	-19.6	44.49	46.13	48.22	44.6	24.3	Espirito Santo	BRSE1	Ramos et al. (2010)
111	28	384.9	0.897	23.9	22.4	25.3	2.9	-21.6	31.78	72.68	84.17	223.0	58.8	Rio de Janeiro- North	BRSE1	Di Beneditto & Ramos (2004); Ramos et al. (2010)
39	28	380.7	1.078	23.7	21.7	25.4	3.7	-23	21.34	46.86	117.59	996.5	96.2	Rio de Janeiro- South	BRSE2	Ramos et al. (2010)
67	42	370.75	0.9354	23.8	21.7	26.2	4.5	-25	62.07	134.78	217.21	1152	180.8	São Paulo/North Paraná	BRS/SE	Santos et al. (2003); Ramos et al. (2010)
–	26	387.5	0.4874	20.4	19.4	25.6	6.2	-26.5	43.571	110.53	199.52	257.6	55	Santa Catarina	BRS/SE	Fettuccia (2006)

Table S2. Preferential fish species for Guiana dolphins in Brazilian coast. Asterisk indicate more important prey for specific location

Family	Species	Enviroment	Habit	MUs	Local	Reference
Ariidae	*Aspistor luniscutis*	Fluvial/Estuarine/Marine Depth: 1m - 4m	Benthopelagic	Southeast: (BRSE1/BRSE2)	South RJ*	Araujo, 2012
Ariidae	*Bagre bagre*	Estuarine/Marine /river mouths Depth: 0 - 50m	Demersal	North (BRNO)	Pará	Vieira, 2014
Ariidae	*Bagre sp.*	-	-	Northeast (BRNE2/BRNE3/BRN4)	Abrolhos*	Rodrigues et al., 2019
Ariidae	*Bagre sp.*	-	-	Southeast (BRSE1/BRSE2)	North RJ	Di Beneditto & Ramos, 2004
Carangidae	*Chloroscombrus chrysurus*	Estuarine/Marine/soft bottoms of the continental shelf	Pelagic	North (BRNO)	Parnaíba*	Vieira, 2014
Carangidae	*Chloroscombrus chrysurus*	Estuarine/Marine/soft bottoms of the continental shelf	Pelagic	Southeast (BRSE1/BRSE2)	South RJ*	Araujo, 2012
Centropomidae	*Centropomus sp.*	Bays, estuaries, mangrove and riverine area	-	Southeast (BRSE1/BRSE2)	South RJ	Araujo, 2012
Centropomidae	*Centropomus sp.*	Bays, estuaries, mangrove and riverine areas		South (BRSE/S)	Paraná*	Ougo, 2012
Clupeidae	*Pellona harroweri*	Estuarine/Marine/inshore, along beaches and down to at least 16 m	Pelagic	North (BRNO)	Pará	Vieira, 2014
Clupeidae	*Pellona harroweri*	Estuarine/Marine/inshore, along beaches and down to at least 16 m	Pelagic	Southeast (BRSE1/BRSE2)	South RJ	Araujo, 2012
Clupeidae	*Pellona harroweri*	Estuarine/Marine/inshore, along beaches and down to at least 16 m	Pelagic	South (BRSE/S)	Paraná*	Ougo, 2012
Clupeidae	*Sardinella brasiliensis*	Found in coastal waters, often forming compact schools. Depth: 0 - 82m	Demersal	Northeast (BRNE2/BRNE3/BRN4)	Abrolhos*	Rodrigues et al., 2019
Clupeidae	*Sardinella brasiliensis*	Found in coastal waters, often forming compact schools. Depth: 0 - 82m	Demersal	Southeast (BRSE1/BRSE2)	South RJ	Araujo, 2012
Congridae	*Ariosoma opistophthalmus*	Marine (range depth 110m - 600m)	Demersal	Southeast (BRSE1/BRSE2)	North RJ*	Di Beneditto & Ramos, 2004
Engraulidae	*Anchoa filifera*	Estuarine/Marine/ coastal Depth: 1 -10m	Pelagic	Southeast (BRSE1/BRSE2)	North RJ*	Di Beneditto & Ramos, 2004

Engraulidae	*Anchoa sp.*	-	-	Southeast (BRSE1/BRSE2)	South RJ	Araujo, 2012
Engraulidae	*Anchoa spinifer*	Estuarine/Marine Depth: 1 -55m	Pelagic	North (BRNO)	Pará	Vieira, 2014
Engraulidae	*Cetengraulis edentulous*	Estuarine/Marine (inshore areas)	Pelagic	Southeast (BRSE1/BRSE2)	South RJ*	Araujo, 2012
Gobiidae	*Gobioides broussonnetii*	Fluvial/Estuarine/Marine Depth: 1 -10m	Demersal	North (BRNO)	Pará*	Vieira, 2014
Haemulidae	*Conodon nobilis*	Found along sandy shores and over shallow muddy bottoms	Demersal	Southeast (BRSE1/BRSE2)	North RJ*	Di Beneditto & Siciliano, 2007
Haemulidae	*Haemulon sp.*	Marine Depth:2-30 m	Demersal	North (BRNO)	Parnaíba*	Vieira, 2014
Haemulidae	*Haemulon sp.*	Marine Depth:2-30 m	Demersal	Northeast (BRNE2/BRNE3/BRN4)	Rio Grande do Norte	Pansard et al 2010
Gerreidae	*Diapterus rhombeus*	Fluvial/Estuarine/Marine Depth: 0 -70m	Demersal	Northeast (BRNE2/BRNE3/BRN4)	Rio Grande do Norte	Pansard et al 2010
Gerreidae	*Diapterus rhombeus*	Fluvial/Estuarine/Marine Depth: 0 -70m	Demersal	Southeast (BRSE1/BRSE2)	South RJ	Araujo, 2012
Gerreidae	*Diapterus rhombeus*	Fluvial/Estuarine/Marine Depth: 0 -70m	Demersal	South (BRSE/S)	Paraná	Ougo, 2012
Lutijanidae	*Lutjanus synagris*	Marine/coastal sandy botton/reef Depth:1-400m	Demersal	North (BRNO)	Parnaíba*	Vieira, 2014
Lutijanidae	*Lutjanus synagris*	Marine/coastal sandy botton/reef Depth:1-400m	Demersal	Northeast (BRNE2/BRNE3/BRN4)	Rio Grande do Norte	Pansard et al 2010
Mugilidae	*Mugil curema*	Estuarine/Marine/sandy coasts Genera depth: 0 - 125m	Demersal	North (BRNO)	Pará	Vieira, 2014
Mugilidae	*Mugil curema*	Estuarine/Marine/sandy coasts Genera depth: 0 - 125m	Demersal	Northeast (BRNE2/BRNE3/BRN4)	Rio Grande do Norte	Pansard et al 2010
Mugilidae	*Mugil sp.*	-	-	Northeast (BRNE2/BRNE3/BRN4)	Abrolhos*	Rodrigues et al., 2019
Mugilidae	*Mugil sp.*	-	-	Southeast (BRSE1/BRSE2)	North RJ	Di Beneditto & Ramos, 2004
Mugilidae	*Mugil sp.*	-	-	Southeast (BRSE1/BRSE2)	South RJ*	Araujo, 2012
Mugilidae	*Mugil sp.*	-	-	South (BRSE/S)	SP	Santos et al., 2002
Penaeidae	*Lycengraulis grossidens*	Estuarine/Marine Depth: 1 -40m	Pelagic	North (BRNO)	Pará*	Vieira, 2014
Batrachoididae	*Porichthys porosissimus*	Marine Depth: 30 - 200 m	Demersal	Northeast (BRNE2/BRNE3/BRN4)	Abrolhos	Rodrigues et al., 2019

Batrachoididae	*Porichthys porosissimus*	Marine Depth: 30 - 200 m	Demersal	Southeast (BRSE1/BRSE2)	North RJ*	Di Beneditto & Ramos, 2004
Batrachoididae	*Porichthys porosissimus*	Marine Depth: 30 - 200 m	Demersal	Southeast (BRSE1/BRSE2)	South RJ	Araujo, 2012
Batrachoididae	*Porichthys porosissimus*	Marine Depth: 30 - 200 m	Demersal	South (BRSE/S)	Paraná	Ougo, 2012
Sciaenidae	*Cynoscion guatucupa*	Marine Depth: 5 - 100 m	Demersal	Southeast (BRSE1/BRSE2)	North RJ*	Di Beneditto & Ramos, 2004
Sciaenidae	*Cynoscion guatucupa*	Marine Depth: 5 - 100 m depth	Demersal	Southeast (BRSE1/BRSE2)	South RJ	Araujo, 2012
Sciaenidae	*Cynoscion jamaicensis*	Estuarine/Marine/Sand or mud Depth: 5 - 100 m depth	Demersal	North (BRNO)	Pará	Vieira, 2014
Sciaenidae	*Cynoscion jamaicensis*	Estuarine/Marine/Sand or mud Depth: 5 - 100 m depth	Demersal	Southeast (BRSE1/BRSE2)	South RJ*: Baia de Guanabara	Melo, 2010
Sciaenidae	*Cynoscion jamaicensis*	Estuarine/Marine(Sand or mud Estuarine/Marine/Sand or mud Depth: 5 - 100 m depth	Demersal	Southeast (BRSE1/BRSE2)	South RJ*	Araujo, 2012
Sciaenidae	*Isopisthus parvipinnis*	Estuarine/Marine /Shallow coastal Depth: 0 - 45m	Demersal	North (BRNO)	Pará	Vieira, 2014
Sciaenidae	*Isopisthus parvipinnis*	Estuarine/Marine /Shallow coastal Depth: 0 - 45m	Demersal	Northeast (BRNE2/BRNE3/BRN4)	Rio Grande do Norte	Pansard et al 2010
Sciaenidae	*Isopisthus parvipinnis*	Estuarine/Marine /Shallow coastal Depth: 0 - 45m	Demersal	Northeast (BRNE2/BRNE3/BRN4)	Abrolhos*	Rodrigues et al., 2019
Sciaenidae	*Isopisthus parvipinnis*	Estuarine/Marine /Shallow coastal Depth: 0 - 45m	Demersal	Southeast (BRSE1/BRSE2)	North RJ	Di Beneditto & Ramos, 2004
Sciaenidae	*Isopisthus parvipinnis*	Estuarine/Marine /Shallow coastal Depth: 0 - 45m	Demersal	Southeast (BRSE1/BRSE2)	South RJ	Araujo, 2012
Sciaenidae	*Isopisthus parvipinnis*	Estuarine/Marine /Shallow coastal Depth: 0 - 45m	Demersal	South (BRSE/S)	SP	Santos et al., 2002
Sciaenidae	*Larimus breviceps*	Estuarine/Marine Depth: 1 - 60m	Demersal	North (BRNO)	Parnaíba*	Vieira, 2014
Sciaenidae	*Larimus breviceps*	Estuarine/Marine Depth: 1 - 60m	Demersal	Northeast (BRNE2/BRNE3/BRN4)	Rio Grande do Norte	Pansard et al 2010
Sciaenidae	*Larimus breviceps*	Estuarine/Marine Depth: 1 - 60m	Demersal	South (BRSE/S)	SP	Santos et al., 2002
Sciaenidae	*Larimus breviceps*	Estuarine/Marine Depth: 1 - 60m	Demersal	South (BRSE/S)	Paraná	Ougo, 2012

Sciaenidae	*Larimus breviceps*	Estuarine/Marine Depth: 1 - 60m	Demersal	Southeast (BRSE1/BRSE2)	South RJ	Araujo, 2012
Sciaenidae	*Macrodon ancylodon*	Estuarine/Marine/ coastal waters Depth: <60 m	Demersal	North (BRNO)	Pará	Vieira, 2014
Sciaenidae	*Macrodon ancylodon*	Estuarine/Marine/ coastal waters Depth: <60 m	Demersal	North (BRNO)	Parnaíba*	Vieira, 2014
Sciaenidae	*Macrodon ancylodon*	Estuarine/Marine/ coastal waters Depth: <60 m	-	Southeast (BRSE1/BRSE2)	North RJ	Di Beneditto & Ramos, 2004
Sciaenidae	*Macrodon ancylodon*	Estuarine/Marine/ coastal waters Depth: <60 m	Demersal	South (BRSE/S)	SP	Santos et al., 2002
Sciaenidae	*Macrodon sp.*	-	-	Northeast (BRNE2/BRNE3/BRN4)	Abrolhos*	Rodrigues et al., 2019
Sciaenidae	*Micropogonias furnieri*	Estuarine/Marine/coastal waters Depth 0 -80m	Demersal	North (BRNO)	Parnaíba	Vieira, 2014
Sciaenidae	*Micropogonias furnieri*	Estuarine/Marine/coastal waters Depth 0 -80m	Demersal	Southeast (BRSE1/BRSE2)	North RJ	Di Beneditto & Ramos, 2004
Sciaenidae	*Micropogonias furnieri*	Estuarine/Marine/coastal waters Depth 0 -80m	Demersal	Southeast (BRSE1/BRSE2)	South RJ*: Baia de Guanabara	Melo, 2010
Sciaenidae	*Micropogonias furnieri*	Estuarine/Marine/coastal waters Depth 0 -80m	Demersal	Southeast (BRSE1/BRSE2)	South RJ*	Araujo, 2012
Sciaenidae	*Micropogonias furnieri*	Estuarine/Marine/coastal waters Depth 0 -80m	Demersal	South (BRSE/S)	SP*	Santos et al., 2002
Sciaenidae	*Micropogonias furnieri*	Estuarine/Marine/coastal waters Depth 0 -80m	Demersal	South (BRSE/S)	Paraná*	Ougo, 2012
Sciaenidae	*Paralonchurus brasiliensis*	Estuarine/Marine/Muddy bottoms Depth 1-50m	Demersal	South (BRSE/S)	Paraná	Ougo, 2012
Sciaenidae	*Paralonchurus brasiliensis*	Estuarine/Marine/Muddy bottoms Depth 1-50m	Demersal	South (BRSE/S)	SP*	Santos et al., 2002
Sciaenidae	*Paralonchurus brasiliensis*	Estuarine/Marine/Muddy bottoms Depth 1-50m	Demersal	Southeast (BRSE1/BRSE2)	North RJ*	Di Beneditto & Ramos, 2004
Sciaenidae	*Plagioscion squamosissimus*	Fluvial/Estuarine Depth ~10m	Benthopelagic	North (BRNO)	Pará	Vieira, 2014
Sciaenidae	*Stellifer brasiliensis*	Marine/Mangroove/interior continental shelf Depth 1- 30m	Demersal	North (BRNO)	Pará	Vieira, 2014
Sciaenidae	*Stellifer brasiliensis*	Marine/Mangroove/interior continental shelf Depth 1- 30m	Demersal	South (BRSE/S)	SP	Santos et al., 2002

Sciaenidae	*Stellifer sp.*	Occurs in coastal waters/ soft, muddy bottoms and brackish estuarine regions	-	Southeast (BRSE1/BRSE2)	North RJ	Di Beneditto & Ramos, 2004
Sciaenidae	*Stellifer sp.*	Occurs in coastal waters/soft, muddy bottoms and brackish estuarine regions	-	Southeast (BRSE1/BRSE2)	South RJ*	Araujo, 2012
Sciaenidae	*Stellifer sp.*	Occurs in coastal waters/soft, muddy bottoms and brackish estuarine regions	-	South (BRSE/S)	Paraná	Ougo, 2012
Sciaenidae	*Stellifer rastrifer*	Estuarine/Marine Depth 1- 40m	Demersal	North (BRNO)	Pará*	Vieira, 2014
Sciaenidae	*Stellifer rastrifer*	Estuarine/Marine Depth 1- 40m	Demersal	Northeast (BRNE2/BRNE3/BRN4)	Abrolhos*	Rodrigues et al., 2019
Sciaenidae	*Stellifer rastrifer*	Estuarine/Marine Depth 1- 40m	Demersal	South (BRSE/S)	SP*	Santos et al., 2002
Trichiuridae	*Trichiurus lepturus*	Estuarine/Marine/ coastal waters Depth: 0 - 589m	Benthopelagic	North (BRNO)	Pará	Vieira, 2014
Trichiuridae	*Trichiurus lepturus*	Estuarine/Marine/ coastal waters Depth: 0 - 589m	Benthopelagic	North (BRNO)	Parnaíba	Vieira, 2014
Trichiuridae	*Trichiurus lepturus*	Estuarine/Marine/ coastal waters Depth: 0 - 589m	Benthopelagic	Northeast (BRNE2/BRNE3/BRN4)	Rio Grande do Norte	Pansard et al 2010
Trichiuridae	*Trichiurus lepturus*	Estuarine/Marine/ coastal waters Depth: 0 - 589m	Benthopelagic	Northeast (BRNE2/BRNE3/BRN4)	Abrolhos*	Rodrigues et al., 2019
Trichiuridae	*Trichiurus lepturus*	Estuarine/Marine/ coastal waters Depth: 0 - 589m	Benthopelagic	Southeast (BRSE1/BRSE2)	North RJ*	Di Beneditto & Ramos, 2004
Trichiuridae	*Trichiurus lepturus*	Estuarine/Marine/ coastal waters Depth: 0 - 589m	Benthopelagic	Southeast (BRSE1/BRSE2)	South RJ	Araujo, 2012
Trichiuridae	*Trichiurus lepturus*	Estuarine/Marine/ coastal waters Depth: 0 - 589m	Benthopelagic	South (BRSE/S)	SP	Santos et al., 2002
Trichiuridae	*Trichiurus lepturus*	Estuarine/Marine/ coastal waters Depth: 0 - 589m	Benthopelagic	South (BRSE/S)	Paraná	Ougo, 2012

Table S3. Nested Gompertz model comparisons between the management units of Guiana dolphins *Sotalia guianensis* from the Northern (BRNO), Northeastern (BRNE3), Southeastern (BRSE1, BRSE2), and South (BRSE/S) coast of Brazil. Probability values with significance for $p>0.05$. In bold the models with ΔAICc< 2

						Models vs. 1 (LL)			**Models vs. 1 (RSS)**	
Models	**K**	**AICc**	**ΔAICc**	**ω_i**	**LL**	**Chisq**	**p(>Chi)**	**RSS**	**F**	**p(>F)**
1	**16**	**2858.75**	**0.00**	**0.61**	**-1412.56**			**64324**		
2	**12**	**2859.67**	**0.92**	**0.39**	**-1417.38**	**9.6285**	**0.04717**	**66113**	**2.3361**	**0.05527**
5	8	2890.29	31.54	0.00	-1436.93	48.7472	7.11E-05	73908	6.2575	1.48E-04
3	12	2893.42	34.67	0.00	-1434.25	43.3717	8.66E-06	72784	11.0481	1.98E-05
4	12	2923.39	64.64	0.00	-1449.24	73.3487	4.45E-12	79274	19.5224	1.85E-11
7	8	2948.57	89.82	0.00	-1466.07	107.0245	< 2.2e-16	87256	14.9733	< 2.2e-16
6	8	2974.38	115.63	0.00	-1478.98	132.8398	< 2.2e-16	93916	19.3215	< 2.2e-16
8	4	3001.36	142.60	0.00	-1496.62	168.1175	< 2.2e-16	103845	17.2034	< 2.2e-16

Table S4. Sample of specimens measured for the analysis of geographic variation in growth and size of skull and teeth from Guiana dolphins in Brazilian waters. Regions: PA: Pará, PB: Paraíba, PE: Penambuco, AL: Alagoas, SE: Sergipe, BA: Bahía, NRJ: North region from Rio de Janeiro State. MUs: Manegment Units of the species

Specimen cod.	Region	State	MUs	Collection
GEMAM 001	North	PA	BRNO	Museu Paraense Emilio Goeldi - MPEG
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01c1412/118	Northeast	PE	BRNE3	Centro Mamíferos Aquâticos - CMA
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01c1411/165	Northeast	PE	BRNE3	Centro Mamíferos Aquâticos - CMA
01c1410/145	Northeast	AL	BRNE3	Centro Mamíferos Aquâticos - CMA
01c1410/152	Northeast	AL	BRNE3	Centro Mamíferos Aquâticos - CMA
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FMAC 1421/76	Northeast	SE	BRNE3	Fundação Mamíferos Aquâticos - FMA
FMAC 1421/90	Northeast	SE	BRNE3	Fundação Mamíferos Aquâticos - FMA
FMAC 1421/160	Northeast	SE	BRNE3	Fundação Mamíferos Aquâticos - FMA
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FMAC 1421/19	Northeast	SE	BRNE3	Fundação Mamíferos Aquâticos - FMA
FMAC 1422/86	Northeast	SE	BRNE3	Fundação Mamíferos Aquâticos - FMA
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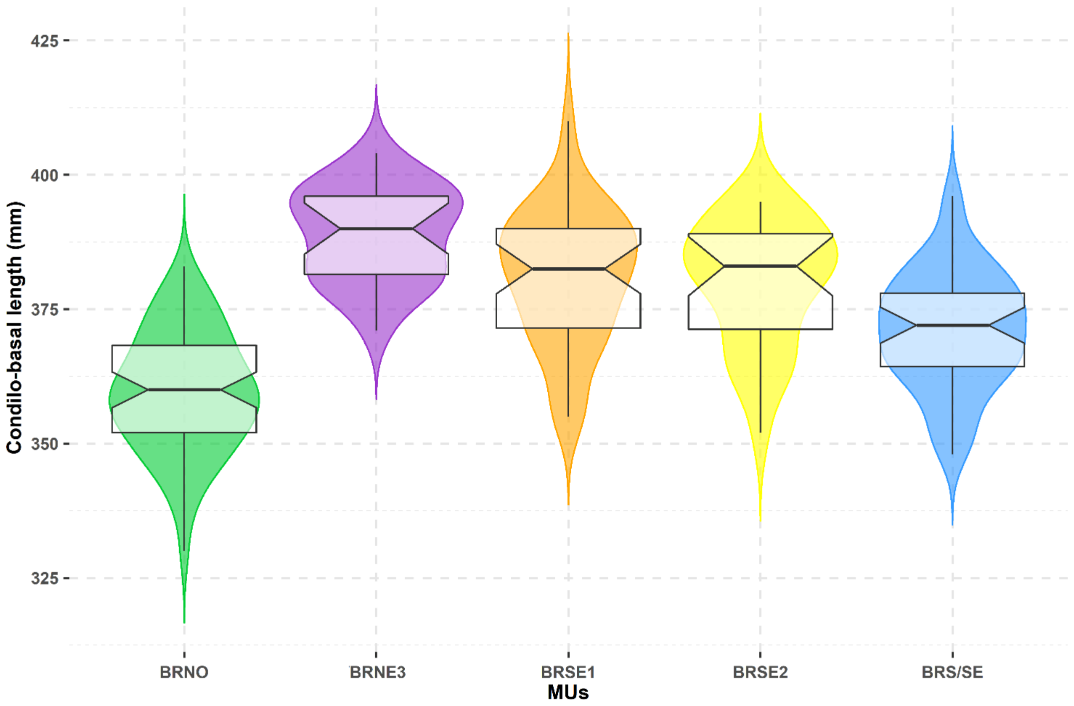

Figure S1. Condylobasal length for adults specimens of Guiana dolphin frm Brazilian coast. Regions: Brazilian Northern Management Unit – BRNO (green), Brazilian Northeastern Management Units 3 – BRNE3 (purple), Brazilian Southeastern Management Units1 – BRSE1 (orange) and 2 – BRSE2 (yellow), Brazilian Southeastern/South Management Unit – BRSE/S (blue).

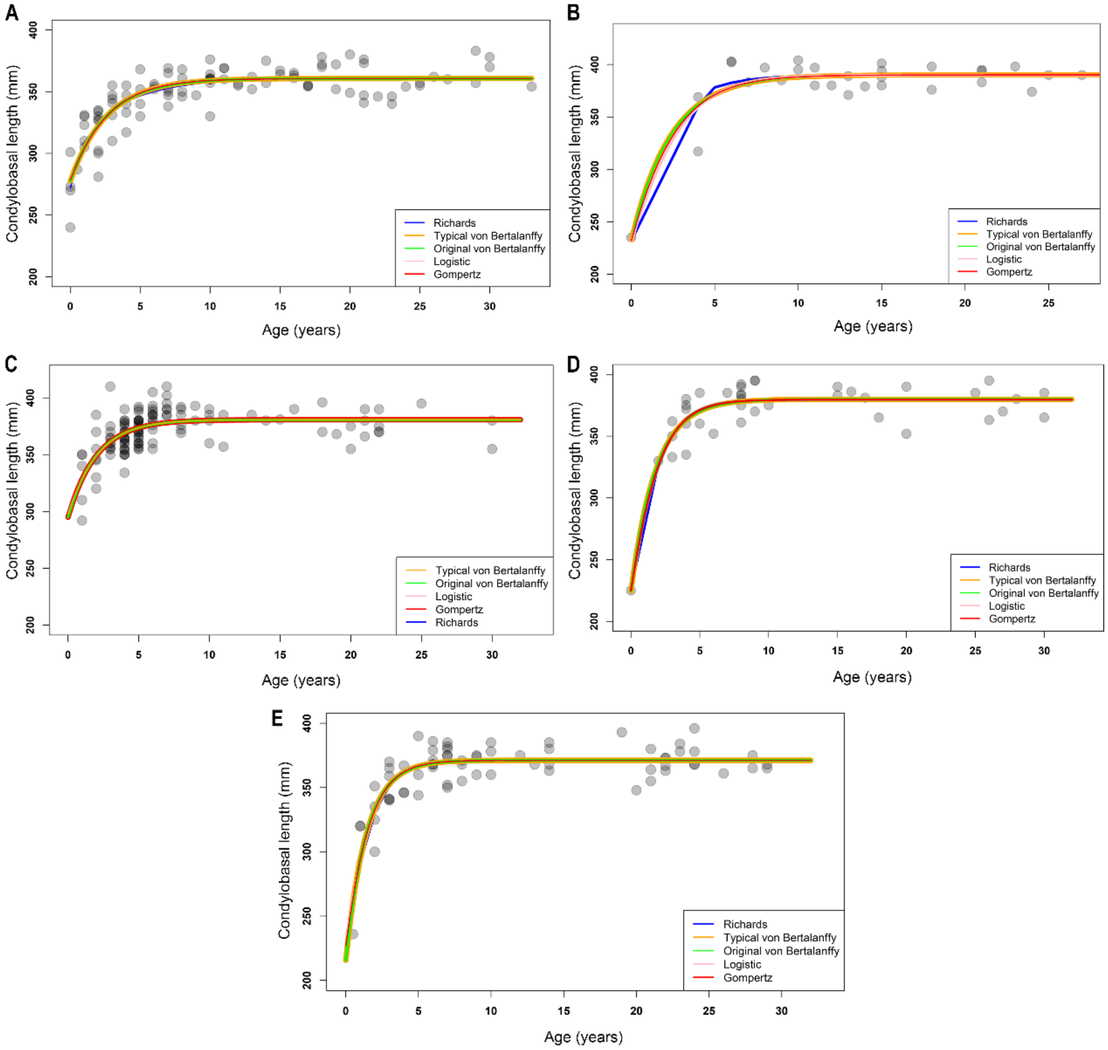

Figure S2. Growth models for CBL tested for the management units: a) Brazilian Northern Management Unit – BRNO, b) Brazilian Northeastern Management Units 3 – BRNE3, c) Brazilian Southeastern Management Units1 – BRSE1 c) Brazilian Southeastern Management Units 2 – BRSE2, and d) Brazilian Southeastern/South Management Unit – BRSE/S

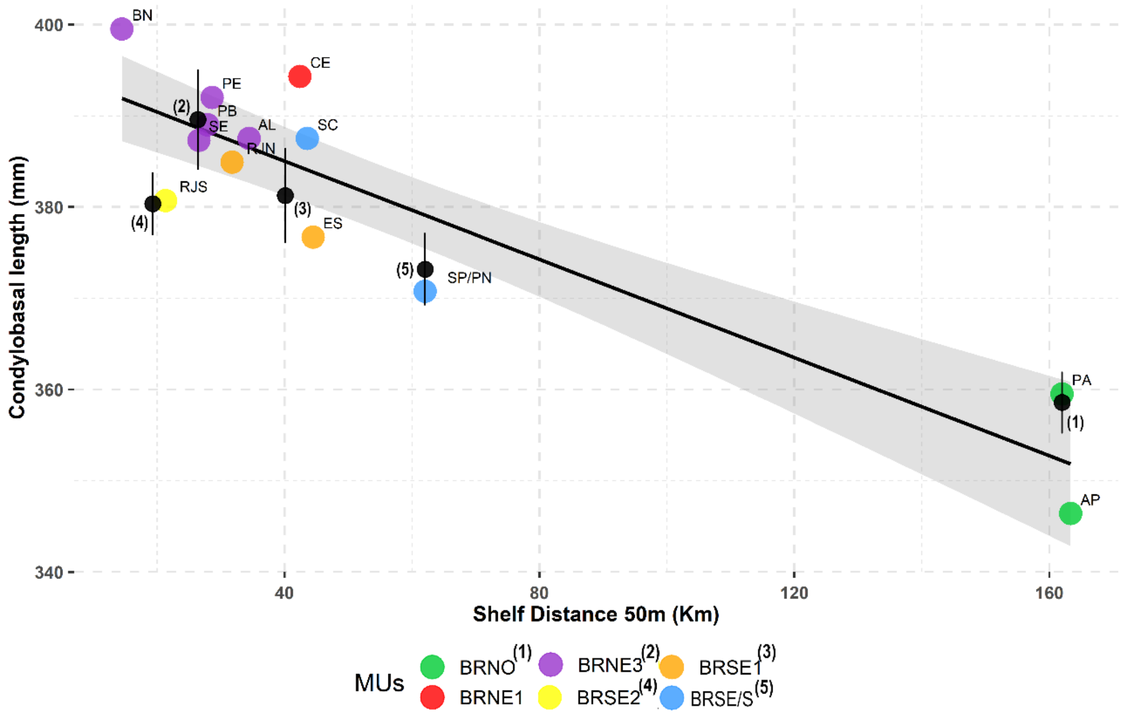

Figure S3. Relation between Condylobasal length average and the environmental factor Continental shelf length until 50 m depth, for Guiana dolphins *Sotalia guianensis* from the Brazilian coast. Regions: Brazilian Northern Management Unit – BRNO (green), Brazilian Northeastern Management Units 1 – BRNE1 (red) and 3 – BRNE3 (purple), Brazilian Southeastern Management Units 1 – BRSE1 (orange) and 2 – BRSE2 (yellow), Brazilian Southeastern/South Management Unit – BRSE/S (blue). Black dots with straight line indicated asymptotic Condilobasal length and 95% confidence intervals for the management units (1) BRNO, (2) BRNE2/ BRNE3, (3) BRSE1, (4) BRSE2, and (5) BRSE/S.

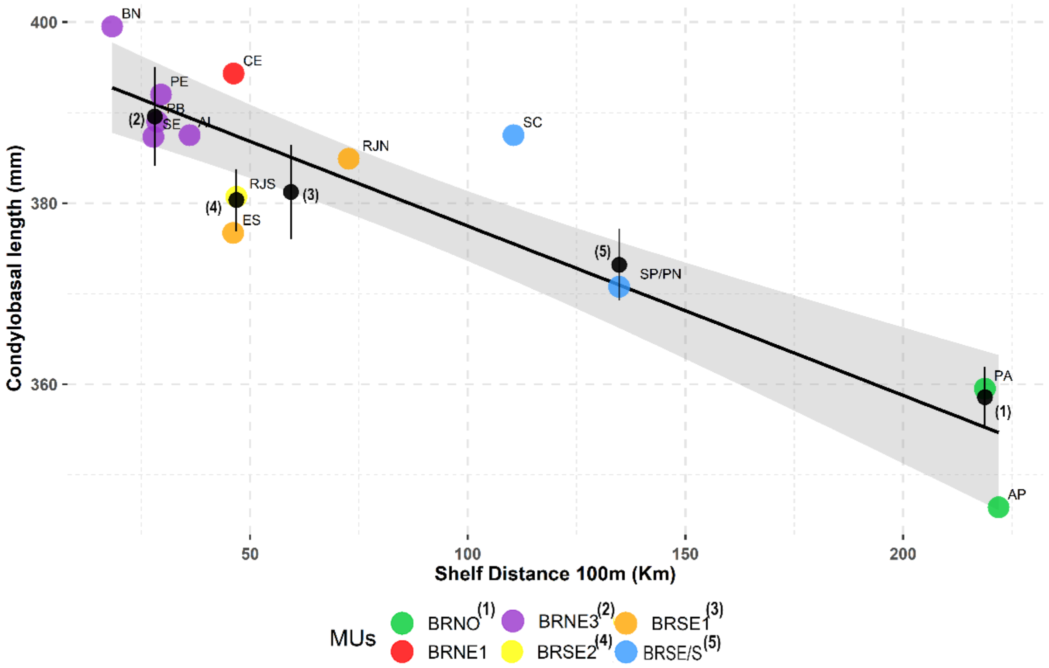

Figure S4. Relation between Condilobasal length average and the environmental factors Continental shelf length until 100 m depth for Guiana dolphins Sotalia guianensis from the Brazilian coast. Regions: Brazilian Northern Management Unit – BRNO (green), Brazilian Northeastern Management Units 1 – BRNE1 (red) and 3 – BRNE3 (purple), Brazilian Southeastern Management Units 1 – BRSE1 (orange) and 2 – BRSE2 (yellow), Brazilian Southeastern/South Management Unit – BRSE/S (blue). Black dots with straight line indicated asymptotic Condilobasal length and 95% confidence intervals for the management units (1) BRNO, (2) BRNE2/ BRNE3, (3) BRSE1, (4) BRSE2, and (5) BRSE/S.

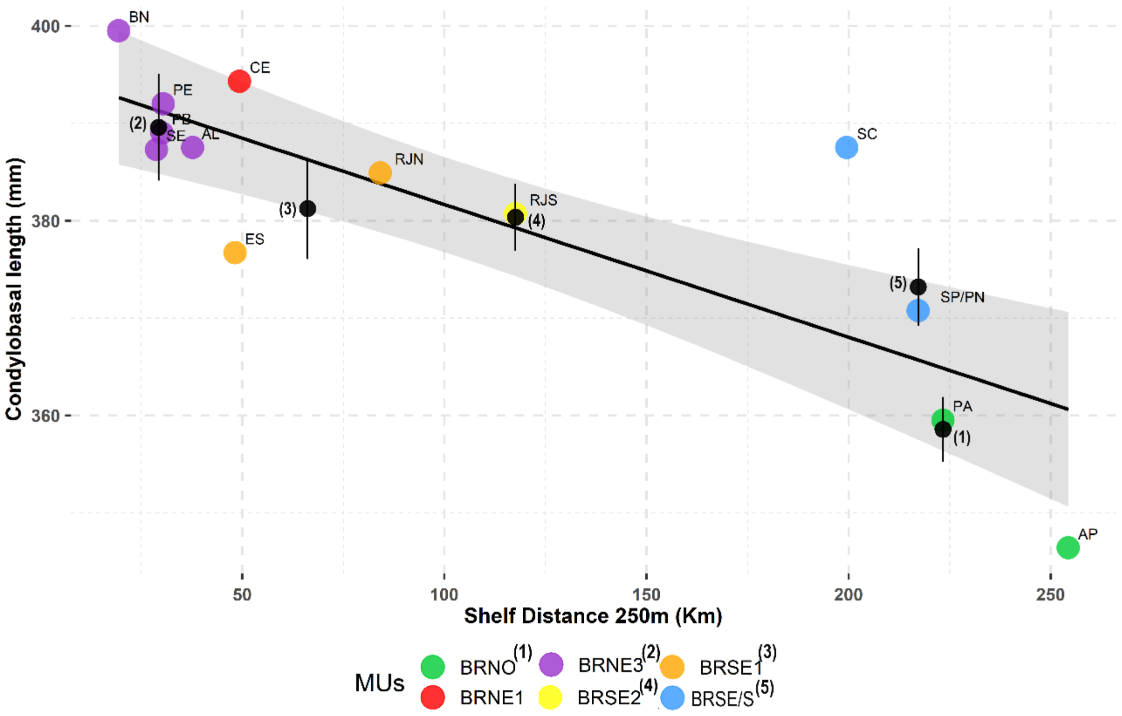

Figure S5. Relation between Condilobasal length average and the environmental factors Continental shelf length until 250 m depth for Guiana dolphins *Sotalia guianensis* from the Brazilian coast. Regions: Brazilian Northern Management Unit – BRNO (green), Brazilian Northeastern Management Units 1 – BRNE1 (red) and 3 – BRNE3 (purple), Brazilian Southeastern Management Units 1 – BRSE1 (orange) and 2 – BRSE2 (yellow), Brazilian Southeastern/South Management Unit – BRSE/S (blue). Black dots with straight line indicated asymptotic Condilobasal length and 95% confidence intervals for the management units (1) BRNO, (2) BRNE2/ BRNE3, (3) BRSE1, (4) BRSE2, and (5) BRSE/S

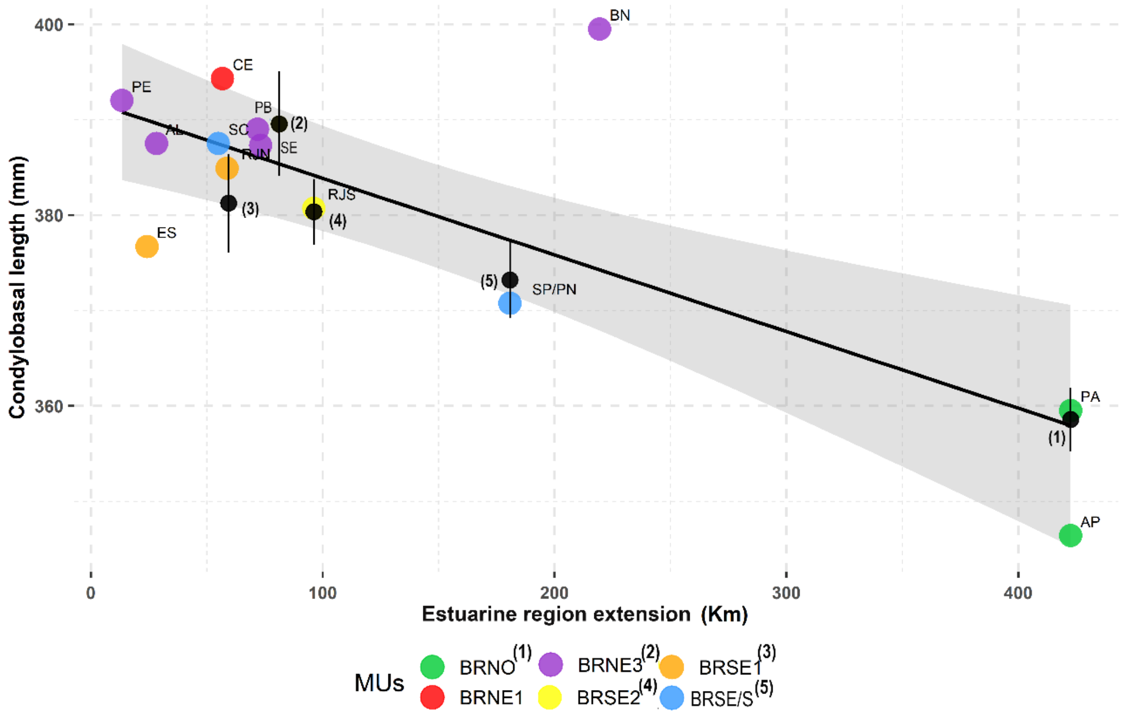

Figure S6. Relation between Condilobasal length average and the environmental factor estuarine region extension (ELE) for Guiana dolphins *Sotalia guianensis* from the Brazilian coast. Regions: Brazilian Northern Management Unit – BRNO (green), Brazilian Northeastern Management Units 1 – BRNE1 (red) and 3 – BRNE3 (purple), Brazilian Southeastern Management Units 1 – BRSE1 (orange) and 2 – BRSE2 (yellow), Brazilian Southeastern/South Management Unit – BRSE/S (blue). Black dots with straight line indicated asymptotic Condilobasal length and 95% confidence intervals for the management units (1) BRNO, (2) BRNE2/ BRNE3, (3) BRSE1, (4) BRSE2, and (5) BRSE/S.

SUPPLEMENTARY MATERIAL (CAPITULO IV)

CAPITULO IV: WHO IS IN HANDICRAFTS: TOOTH MORPHOLOGY AND AGE DETERMINATION AS TOOLS TO ASSESS VULNERABLE DOLPHINS SOTALIA SPP. SUPPLYING THE TRADE OF ARTISANAL CHARMS IN BRAZIL

Table S1. Two-way ANOVA models results for the factors Type of teeth and Age class.

Response	**Factor**	**Df**	**Sum Sq**	**Mean Sq**	**F value**	**Pr(>F)**
TL	Type	3	448.90	149.632	125.76	**<2.2e-16**
	Age class	2	4.6	2.3	1.9332	0.1492
	Type: Age class	6	5.52	0.921	0.7648	0.5991
	Residuals	114	137.25	1.204		
RD	Type	3	14.9653	4.9884	38.827	**<2.2e-16**
	Age class	2	3.284	1.642	12.782	**283e-06**
	Type: Age class	6	1.463	0.244	1.9912	0.0725
	Residuals	114	13.954	0.122		
CID	Type	3	336.87	112.289	37.0778	**<2.2e-16**
	Age class	2	14.93	7.466	2.4652	0.08928
	Type: Age class	6	40.26	6.711	2.3673	**0.03422**
	Residuals	114	323.15	2.835		
CL	Type	3	5475.9	1825.30	103.1475	**<2.2e-16**
	Age class	2	22.3	11.17	0.6312	0.5337
	Type: Age class	6	148.5	24.76	1.429	0.2096
	Residuals	114	1975	17.32		

Table S2. Factor pairwise comparisons from ANOVA models using the dependent variables total length (TL), root diameter (RD), cingulum diameter (CID), and crown length (CL). n: sample size, Range: Intervals for each measurements, SD: Standard deviation, CI: 95% Confidence interval. Diff: Means' differences types of teeth compassions, age class comparisons and factors interaction, Lwr: lower value, Upr: Upper value, *p* adj: p-value of statistically significant using critical value of $p < 0.05$.

	Factor: Type								
Measurements	**Type 1**	**Type 2**	**NE**	**PA**	**Type Comparisons**	**Diff**	**Lwr**	**Upr**	***p* adj**
TL (mm)									
n	67	64	10	10	Type 1 - NE	-1.965	-2.944	-0.987	0.000
Range	14.24 - 18.65	9.49 - 14.42	15.3 - 19.6	16.06 - 17.9	Type 1 - PA	-0.796	-1.775	0.182	0.152
Mean	16.15	12.54	18	16.90	Type 2 - NE	-5.553	-6.565	-4.540	0.000
Median	16.2	12.2	18.6	16.9	Type 2 - PA	-4.384	-5.396	-3.371	0.000
SD	1.08	1.13	1.48	0.58	Type 2 - Type 1	-3.587	-4.158	-3.016	0.000
CI	15.89 - 16.42	12.20 - 12.89	17.08 - 18.92	16.72 - 17.08	PA - NE	-1.169	-2.456	0.118	0.089
RD (mm)									
n	67	64	10	10	Type 1 - NE	-0.571	-0.888	-0.253	0.000
Range	3.42 - 4.9	2.59 - 4.53	4.0 - 5.5	3.17 - 4.07	Type 1 - PA	0.366	0.049	0.684	0.017
Mean	4.14	3.57	4.69	3.78	Type 2 - NE	-1.151	-1.480	-0.822	0.000
Median	4.14	3.59	4.54	3.83	Type 2 - PA	-0.214	-0.543	0.115	0.235
SD	0.37	0.40	0.56	0.28	Type 2 - Type 1	-0.580	-0.766	-0.395	0.000
CI	4.05 - 4.23	3.44 - 3.69	4.35 - 5.03	3.50 - 4.06	PA - NE	-0.937	-1.355	-0.519	0.000
CID (mm)									
n	67	64	10	10	Type 1 - NE	-1.024	-1.595	0.665	0.263
Range	3.26 - 3.89	2.56 - 3.94	3.17 - 4.09	3.35 - 4.30	Type 1 - PA	0.525	-1.103	1.330	0.963
Mean	3.72	3.27	3.72	3.91	Type 2 - NE	-2.083	-2.425	-1.671	0.000
Median	3.78	3.23	3.95	3.70	Type 2 - PA	-1.736	-2.135	-1.212	0.000

SD	0.25	0.27	0.26	0.27	Type 2 - Type 1	-1.813	-2.039	-1.554	0.000
CI	3.66 - 3.78	3.19 - 3.35	3.64 - 3.80	3.82 - 3.99	PA - NE	-1.149	-1.814	0.798	0.297
CL (mm)									
n	67	64	10	10	Type 1 - NE	-1.501	-2.507	1.315	0.052
Range	5.74 - 6.05	4.07 - 5.78	3.17 - 3.95	5.0 - 7.20	Type 1 - PA	-2.068	-2.880	-0.527	0.017
Mean	6.25	5.10	3.68	6.29	Type 2 - NE	-1.509	-1.924	-1.094	0.000
Median	6.19	5.18	6.60	6.62	Type 2 - PA	-1.710	-2.125	-1.295	0.000
SD	0.30	0.39	0.23	0.77	Type 2 - Type 1	-1.282	-1.516	-1.047	0.000
CI	6.17 - 6.32	4.98 - 5.22	3.61 - 3.76	6.05 - 6.52	PA - NE	1.425	-1.805	2.699	0.989
	Factor: Age Class								
	Juvenile	**Young adult**	**Adult**		**Age Class Comparison**	**Diff**	**Lwr**	**Upr**	***p* adj**
RD (mm)									
n	42	31	53		Juvenile - Adult	-0.356	-0.532	-0.180	0.000
Range	2.92 - 4.84	2.59 - 5.5	3.24 - 5.4		Juvenile - Young adult	-0.085	-0.287	0.117	0.578
Mean	3.84	3.84	4.14		Young adult - Adult	-0.271	-0.464	-0.079	0.003
Median	3.54	3.45	3.63						
SD	0.503	0.532	0.479						
CI	3.69 - 3.99	3.65 - 40.3	4.01 - 4.27						
	Type:Age Class interaction								
	Juvenile (J)	**Young adult (YA)**	**Adult (A)**		**Significant Comparisons**	**Diff**	**Lwr**	**Upr**	***p* adj**
Type 1									
n	26	14	24		J -Type 2 J	2,142	2,57	1,602	0,000
Range	3.26 - 4.12	3.26 - 3.99	3.42 - 4.18		YA -Type 2 J	2,099	2,583	1,464	0,000
Mean	3.69	3.75	3.35		A - Type 2 J	2,198	2,622	1,669	0,000
Median	3.78	3.78	3.74		A -Type 2 YA	1,811	2,308	1,112	0,000
SD	0.285	0.276	0.197		J - Type 2 YA	1,743	1,009	2,249	0,0001

					YA - Type 2 YA	1,690	2,263	0,770	0.0029
					A - Type 2 A	1,687	2,132	1,069	0,000
					J - Type 2 A	1,613	0,965	2,067	0,0001
					YA - Type 2 A	1,556	0,680	2,092	0.0039
Type 2									
	n	11	11	20					
	Range	2.56 - 3.49	3.05 - 3.61	3.04 - 3.82					
	Mean	3.03	3.28	3.35					
	Median	3.08	3.27	3.34					
	SD	0.273	0.172	0.213					
PA									
	n	2	4	4	J - Type 2 J	2,424	3,192974	1,247882	0.0008
	Range	3.83 - 3.95	3.17 - 3.95	3.63 - 3.83	YA - Type 2 J	1,909	2,631542	0,603388	0.016
	Mean	3.89	3.58	3.68	A - Type 2J	2,080	2,758118	1,022877	0.0014
	Median	3.89	3.6	3.64	J - Type 2 J	2,424	3,192974	1,247882	0.0009
	SD	0.0849	0.342	0.0971					
NE									
	n	3	2	5	J - Type 2J	2,467	3,121558	1,557272	0.000
	Range	3.85 - 4.00	3.35 - 3.65	3.7 - 4.15	A - Type 2J	2,554	3,090843	1,868844	0,000
	Mean	3.92	3.5	3.97	J - Type 2YA	2,1296	0,935743	2,862629	0.004
	Median	3.9	3.5	4.00	A - Type 2YA	2,230	2,829104	1,39395	0.000
	SD	0.0764	0.212	0.164	A - Type 2A	2,130	2,710423	1,314513	0.000
					J - Type 2A	2,024	0,787549	2,752715	0.008

Table S3. Principal component analysis results. Loadings indicate coefficients of correlation between variables total length (TL), root diameter (RD), cingulum diameter (CID), and crown length (CL) and the components (PC).

		Components			
	Variables	**PC1**	**PC2**	**PC3**	**PC4**
Loadings	**TL**	-0.6404	-0.4110	0.5856	-0.2788
	RD	-0.4719	0.8171	0.1873	0.2727
	CID	-0.3670	0.1559	-0.6155	-0.6797
	CL	-0.4819	-0.3726	-0.4929	0.62114
Standard deviation		0.2196	0.0939	0.0693	0.0460
Proportion of Variance		0.7540	0.1379	0.0751	0.0330
Cumulative Proportion		0.7540	0.8919	0.9670	100.000