

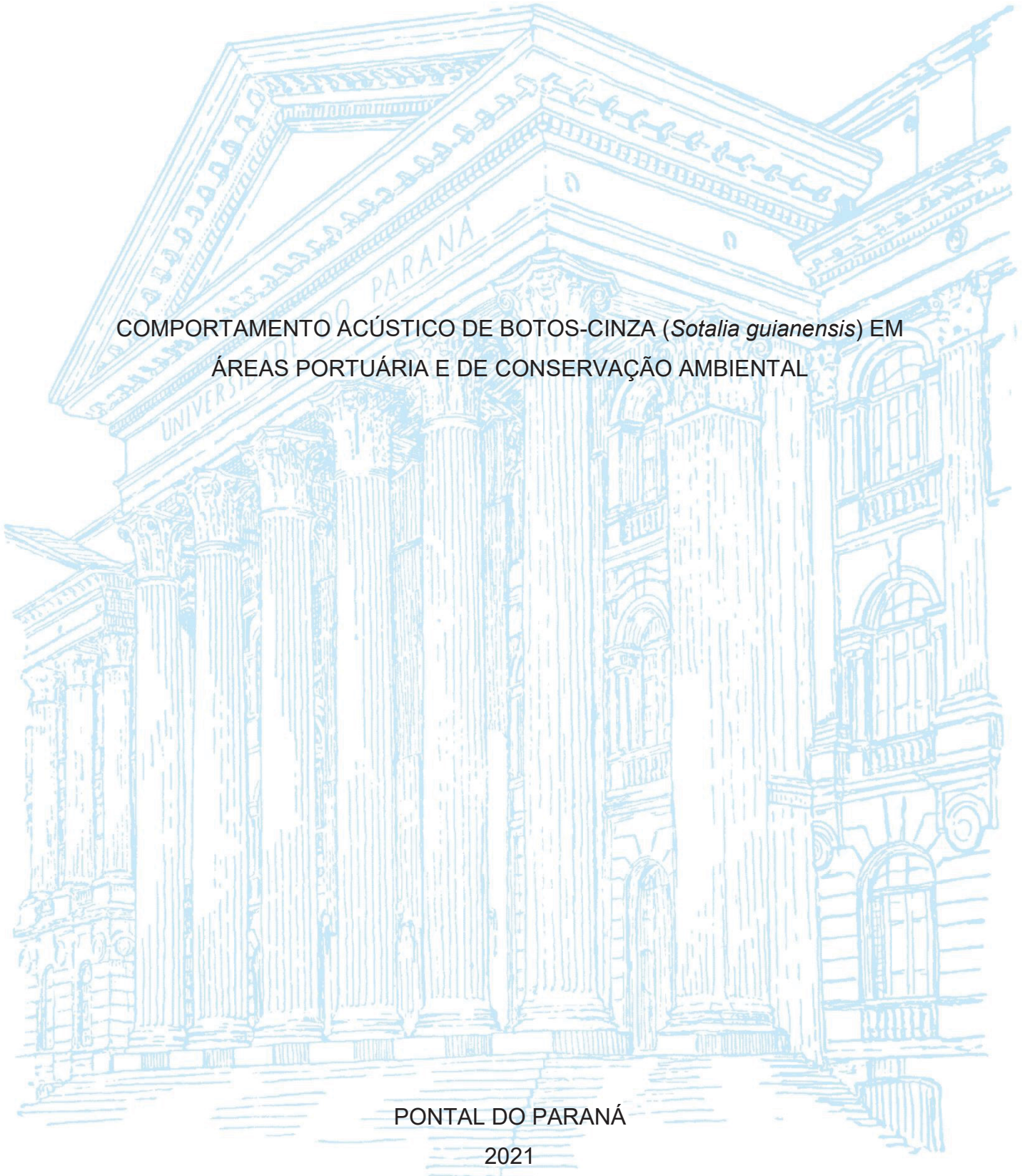
UNIVERSIDADE FEDERAL DO PARANÁ

DAIANE SANTANA MARCONDES

COMPORTAMENTO ACÚSTICO DE BOTOS-CINZA (*Sotalia guianensis*) EM  
ÁREAS PORTUÁRIA E DE CONSERVAÇÃO AMBIENTAL

PONTAL DO PARANÁ

2021



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COMPORTAMENTO ACÚSTICO DE BOTOS-CINZA (*Sotalia guianensis*) EM  
ÁREAS PORTUÁRIA E DE CONSERVAÇÃO AMBIENTAL

Dissertação apresentada ao curso de Pós-Graduação em Sistemas Costeiros Oceânicos, Setor de Ciências da Terra, Universidade Federal do Paraná, como requisito parcial à obtenção do título de Mestre em Sistemas Costeiros Oceânicos.

Orientador: Prof. Dr. Mauricio Cantor

Coorientadora: Prof<sup>a</sup>. Dra. Camila Domit

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A outorga do título de mestra está sujeita à homologação pelo colegiado, ao atendimento de todas as indicações e correções solicitadas pela banca e ao pleno atendimento das demandas regimentais do Programa de Pós-Graduação.

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*Aos botos-cinza.*

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“There are many hypotheses in science that are wrong. That's perfectly alright; it's the aperture to finding out what's right. Science is a self-correcting process. To be accepted, new ideas must survive the most rigorous standards of evidence and scrutiny”.

Carl Sagan, *“Cosmos”*

## RESUMO

O ruído antropogênico pode modificar o comportamento acústico dos animais e influenciar sua dinâmica populacional. O objetivo desta dissertação de mestrado foi caracterizar espaço-temporalmente o comportamento acústico de botos-cinza em duas de suas principais áreas de uso no Complexo Estuarino de Paranaguá (CEP). O comportamento acústico foi utilizado para inferir atividades sociais e de forrageio em áreas ambientalmente expostas a níveis distintos de interferência antrópica—região portuária e entorno de unidade de conservação. Os resultados sugerem que ruído ambiental influencia o comportamento acústico social, mas não foi possível detectar influências conclusivas no comportamento acústico de forrageio. Os parâmetros acústicos dos assobios de botos-cinza diferiram entre as áreas e contextos comportamentais; tais sinais acústicos se sobrepuseram constantemente ao ruído ambiental e antropogênico na área portuária—a mais ruidosa. A taxa de emissão de assobios correlacionou-se negativamente com os níveis relativos de ruído ambiental, sugerindo redução no uso de sons sociais em condições mais ruidosas. Esta taxa também diminuiu ao longo dos anos, possivelmente refletindo o aumento nos níveis relativos de ruído ambiental. Em especial, destaca-se a significativa redução de 75% na taxa de emissão de assobios durante o período de dragagem na região portuária, mesmo período em que os maiores níveis de ruído ambiental foram registrados. Em contrapartida, a taxa de emissão de cliques de ecolocalização foi semelhante entre as áreas, sugerindo influência mínima — se alguma — do ruído no comportamento de forrageio. Estes resultados apontam para a possibilidade de que botos-cinza diminuam a taxa de emissão de assobios em condições mais ruidosas devido à possível redução no espaço acústico de comunicação, enquanto mantêm o forrageio ativo, independentemente do ruído, como uma compensação de alimentação. Ainda, a emissão de assobios com frequências mínimas e frequências iniciais mais altas na região portuária sugere uma tentativa de evitar o mascaramento em frequências mais baixas, onde a maior intensidade de ruído se concentra. Em conjunto, estas alterações comportamentais de botos-cinza são reconhecidas como potencialmente ameaçadoras ao papel da comunicação acústica em atividades sociais, coesão de grupo e forrageio cooperativo. Estes potenciais distúrbios tendem a ser especialmente preocupantes para populações que já enfrentam outros impactos antropogênicos cumulativos, como na região do

CEP. Este estudo fornece um importante subsídio para o manejo da população de botos-cinza da região, assim como para o desenvolvimento e a implementação de medidas de mitigação para os potenciais impactos acústicos sobre a espécie, visando a sua conservação.

**Palavras-chave**

Ruído antropogênico. Áreas portuárias. Áreas de conservação ambiental. Assobios. Ecolocalização. Comportamento de forrageio.

## **ABSTRACT**

Anthropogenic noise can modify the acoustic behavior of animals and influence their population dynamics. In this dissertation, we investigate whether dolphins change their acoustic behavior relative to anthropogenic noise in two areas exposed to distinct levels of anthropogenic interference (port, conservation unit) over two sampling periods at the Paranaguá Estuarine Complex (CEP) in southern Brazil. Our findings points to noise-induced variation in social but not foraging acoustic behavior. The acoustic parameters of the dolphins' whistles differed between areas and behavioral contexts. Whistles constantly overlapped with noise levels at the noisy port area, and the whistling rate was negatively correlated with noise levels, suggesting that fewer social sounds are used in noisier conditions. By contrast, the emission of feeding buzzes, used during active foraging, was similar between areas. The whistling rate decreased over the years with the increase of the environmental noise level, with a significant 75% decrease during dredging activities at the port area. These findings suggest that Guiana dolphins decrease their whistling rate in noisier conditions due to limited space for communication while maintaining active foraging regardless of noise levels. It is possible that Guiana dolphins increase their whistles' fundamental frequencies to avoid masking in lower frequencies, where noise intensity is greater. Taken together, these findings raise concerns about the conservation of this population that already faces cumulative anthropogenic impacts, given that acoustic changes are potentially threatening to acoustic communication in social activities, group cohesion, and cooperative foraging of a population. This study provides essential information to guide conservation actions in the region, as well as baseline information for the development of mitigation measures for the potential acoustic impacts on the species.

### **Keywords**

Anthropogenic noise. Port area. Conservation area. Foraging behavior. Echolocation. Dolphins' whistles.

## RESUMO EM LINGUAGEM ACESSÍVEL

Os golfinhos se comunicam e buscam alimento por meio de sons (assobios e cliques de ecolocalização) que produzem sob a coluna d'água, no entanto, o ruído produzido por atividades subaquáticas humanas pode interferir na eficácia da comunicação e modificar o comportamento acústico dos animais. Neste sentido, o objetivo desta dissertação de mestrado foi caracterizar espaço-temporalmente o comportamento acústico de botos-cinza em duas de suas principais áreas de uso no Complexo Estuarino de Paranaguá (CEP). O comportamento acústico foi utilizado como um indicador de atividades sociais e de busca por alimento em áreas ambientalmente expostas a níveis distintos de atividades antrópicas—região portuária e entorno de unidade de conservação. Os resultados sugerem que ruído subaquático de fundo (ambiente + atividades humanas) influencia o comportamento acústico social, mas não foi possível detectar se o mesmo ocorre para o comportamento acústico de busca de alimento. As características dos assobios de botos-cinza diferiram entre as áreas e contextos comportamentais; os assobios se sobrepuseram constantemente ao ruído humano na área portuária—a mais ruidosa. O número e a taxa de assobios foram menores quando o níveis de ruído de fundo foram maiores, sugerindo redução no uso desses sons em condições mais ruidosas. Em contrapartida, o número e a taxa de cliques de ecolocalização foi semelhante entre as áreas, sugerindo menor influência do ruído no comportamento de busca por alimento. Estes resultados podem indicar que os boto-cinza conversam menos quando o ambiente está mais ruidoso, pois o ruído humano ocupa o mesmo espaço acústico que os assobios, mas ao mesmo tempo os botos mantêm a busca por alimento. A área portuária foi a mais ruidosa, devido ao intenso uso humano, e nela os botos aumentam as frequências mínimas e máximas de seus assobios, os deixando mais agudos na tentativa de evitar a sobreposição em frequências mais baixas, onde a maior intensidade de ruído se concentra. Em conjunto, estas alterações comportamentais de botos-cinza são reconhecidas como potencialmente ameaçadoras ao papel da comunicação acústica em atividades sociais, coesão de grupo e forrageio cooperativo. Estes potenciais distúrbios tendem a ser especialmente preocupantes para populações que já enfrentam outros impactos antropogênicos cumulativos, como na região do CEP. Este estudo fornece um importante subsídio para o manejo da população de botos-cinza da região, assim como para o desenvolvimento e a implementação

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## LISTA DE ABREVIATURAS OU SIGLAS

**AIC** – Akaike Information Criterion  
**BIC** – Bayesian Information Criteria  
**CA** – Conservation area  
**CEP** – Complexo Estuarino de Paranaguá  
**CV** – Coeficiente de variação  
**dB** – Decibels  
**DF** – Degrees of freedom  
**DFA** – Discriminant Function Analysis  
**FNB** – First Noise Band  
**GLM** – Generalized Linear Model  
**GMM** – Gaussian Mixture Model  
**Hz** – Hertz  
**ICI** – Inter-click-interval  
**ICL** – Integrated Completed Likelihood  
**kHz** – Kilohertz  
**ms** – Millisecond  
**PA** – Port area  
**PCA** – Principal Component Analysis  
**PC1** – First Principal Component  
**PC2** – First Principal Component  
**PC3** – First Principal Component  
**PEC** – Paranaguá Estuarine Complex  
**ppm** – Part per million  
**PTS** – Permanent Threshold Shift  
**rs** – Spearman Correlation  
**SD** – Standard Deviation  
**SNB** – Second Noise Band  
**TTS** – Temporary Threshold Shift

**V/ $\mu$ Pa** – Voltz per microPascal

**X<sup>2</sup>** – Chi-square

**MTZ**– Maximum Turbidity Zone

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## INTRODUÇÃO GERAL

A comunicação por meio de sinais acústicos é fundamental para atividades vitais de mamíferos aquáticos, em especial os cetáceos. Este sistema tornou-se evolutivamente eficiente em razão da eficiente propagação do som no ambiente aquático (TYACK, 1986; TYACK; CLARK, 2000; NOWACEK et al., 2007), possibilitando diferentes táticas de forrageio e formas de interação social (TYACK, 2000; ERBE et al., 2016), e a exploração de habitats com limitadas condições de visibilidade (TYACK, 1986; ARY et al., 2016). A eficácia da comunicação depende, porém, das condições ambientais e configurações de habitat, abrangendo desde parâmetros físico-químicos da água, como turbidez, temperatura e salinidade (DECONTO et al., 2021), até a influência das ações antrópicas no ambiente marinho, como a presença de ruídos provenientes do deslocamento de embarcações, atividades portuárias, prospecção por óleo e gás, entre outros (GOSPIĆ; PICCIULIN, 2016; LEUNISSEN; RAYMENT; DAWSON, 2019; ROBERTS et al., 2019).

No contexto ecológico, o comportamento acústico é reflexo das condições ambientais e fatores sociais, genéticos e antropogênicos (BITTENCOURT et al., 2016, 2017; DECONTO et al., 2021; MORON et al., 2019; ROSSI-SANTOS; PODOS, 2006). Variações intraespecíficas no comportamento acústico de odontocetos, evidenciadas principalmente por diferenças nos parâmetros físicos do som, são identificadas como habituações do repertório acústico em resposta ao ambiente, tanto físico quanto social, que podem refletir a variação geográfica das populações (BARBOSA et al., 2019; DECONTO; MONTEIRO-FILHO, 2016, 2019; MAY-COLLADO; WARTZOK, 2008; MORON et al., 2019a; ROSSI-SANTOS; PODOS, 2006)

No ecossistema marinho, atividades antrópicas geradoras de ruídos subaquáticos são apontadas como as principais fontes de poluição sonora, capazes de causar alterações acústicas, tanto nos sinais de comunicação, quanto nos de ecolocalização (HILDEBRAND, 2009; HOLT et al., 2015; WEILGART, 2007). Apesar da capacidade de habituação individual dos cetáceos às pressões ecossistêmicas por meio da adaptação de seus repertórios acústicos, impactos comuns da poluição sonora incluem efeitos diretos, como comportamentais (e.g. afugentamento e abandono de área); crônicos (e.g. estresse); de percepção (e.g. mascaramento acústico); físicos (e.g. danos

auditivos como perda de sensibilidade auditiva temporária e permanente – TTS e PTS); e efeitos indiretos (e.g. redução ou alteração na disponibilidade de presas) (CAFARO et al., 2018; ERBE et al., 2019; MARLEY; ERBE; SALGADO-KENT, 2016; NMFS, 2018). Áreas portuárias se destacam por causarem muitos destes impactos, dado o intenso tráfego de embarcações e as constantes obras e operações náuticas (LUÍS; COUCHINHO; DOS SANTOS, 2014; MCKENNA et al., 2012; VEIRS; VEIRS; WOOD, 2016). O ruído subaquático tipicamente gerado por estas atividades pode interferir na comunicação. Neste sentido, se ultrapassados os limiares de sensibilidade auditiva dos animais, o ruído pode desencadear desde respostas comportamentais temporárias até injúrias físicas graves (NMFS, 2018; LEUNISSEN; DAWSON, 2018; NACHTIGALL; PAWLOSKI; AU, 2003; NACHTIGALL; SUPIN; BREESE, 2012; PIROTTA et al., 2013) (Figura 1).

Na região sudeste e sul do Brasil, a maioria dos portos estão instalados em áreas de baías e estuários, regiões que também abrigam populações de cetáceos costeiros e alta diversidade biológica (MARCONDES et al., 2020). No eixo Leste-Oeste do Complexo Estuarino de Paranaguá (CEP), Paraná, localiza-se o segundo maior porto em cargas de grãos do Brasil (SIQUEIRA, 2016), o Complexo Portuário de Paranaguá-Antonina. A estrutura e as atividades associados a este porto são um dos potenciais causadores de impactos ao ecossistema local em decorrência do intenso e diário tráfego de embarcações, além de obras como dragagens de aprofundamento e manutenção do canal de acesso ao porto (SILVA et al., 2015). Efeitos negativos ao habitat e aos animais já foram registrados em detrimento de ações deste porto, entre estes estão as modificações das áreas manguezais (MACHADO, 2012) e bancos de gramas marinhas (GAMA et al., 2016), indicativos de colisões entre os animais marinhos e embarcações que geram lesões corporais, e a geração de altos níveis de estresse detectados em tartarugas marinhas e cetáceos, os quais por sua vez levam à depressão do sistema imunológico e aumento do risco a patologias (CABRAL; MARTINS, 2018; DOMICIANO et al., 2016; MIURA; NOERNBERG, 2020; TREVIZANI et al., 2021; VAN BRESSEM et al., 2009). Entre os impactos descritos especificamente para os cetáceos, o afastamento de áreas de uso e alterações em tamanho de grupos e interações comportamentais foram registradas durante períodos de maior intensidade de atividades geradoras de



para alguns grupos (DOMIT, 2010; MIRANDA, 2017). Apesar dos impactos gerados pelas atividades portuárias globalmente reportados, historicamente os animais permanecem utilizando áreas de intensa poluição sonora, possivelmente pela disponibilidade de alimento, proteção contra predadores, viabilidade no cuidado dos infantes e pelas interações sociais com outros indivíduos da espécie que utilizam a área (DOMIT, 2006; SANTOS et al., 2010). No entanto, a exposição diária a ambientes estressores pode levar a consequências comportamentais e fisiológicas em curto e longo prazo (AZEVEDO et al., 2017; CREMER; SIMÕES-LOPES; RODRIGUES PIRES, 2009; SCIENCES, 2017; VAN BRESSEM et al., 2009).

Em contraste à Baía de Paranaguá, a Baía de Laranjeiras, situada no eixo Norte-Sul do estuário (ANGULO, 1993), abriga a Ilha das Peças, região margeada pelas Unidades de Conservação: Parque Nacional do Superagüi e Estação Ecológica da Ilha do Mel (MMA 2017). Esta ilha é caracterizada pela presença de comunidades tradicionais e pelo moderado tráfego de embarcações de turismo e pesca artesanal (FILLA e MONTEIRO-FILHO, 2009). Os canais de desembocadura localizados nesta região constituem o principal acesso dos animais à região interna do CEP (DOMIT, 2010) e é considerada uma importante área para os grupos residentes de botos-cinza, que a utilizam para alimentação, cuidado parental e brincadeiras, principalmente devido à elevada concentração de filhotes, assim como para os grupos transientes, que são atraídos para a área principalmente pela disponibilidade de alimento (DOMIT, 2006; de MOURA et al., 2021).

A proteção de áreas como o CEP, consideradas essenciais para o desenvolvimento e sobrevivência, não só do boto-cinza (DE MOURA et al., 2021; FILLA e MONTEIRO-FILHO, 2008), mas de demais espécies que também utilizam a região, como a criticamente ameaçada de extinção, toninha (SANTOS et al., 2009) é crucial para sua conservação. Porém, o desenvolvimento de medidas efetivas de ordenamento territorial que visam à mitigação de impactos demanda o conhecimento sobre atributos ecológicos, que muitas vezes são de difícil aquisição quando considerando a dinâmica comportamental de mamíferos marinho. Neste sentido, o estudo do comportamento acústico das espécies permite inferir comportamento, fisiologia, uso de área, e potenciais ameaças aos animais, contribuindo diretamente para conhecimento de base para direcionar

esforços de conservação (CARUSO et al., 2017; MAY-COLLADO; WARTZOK, 2009; GONZÁLEZ-GARCÍA et al., 2018). Ainda, esta metodologia permite a obtenção de dados sem a necessidade de contato direto com os animais, possibilitando a redução de chances de ocorrência de possíveis injúrias e interferências em seu comportamento pela presença dos pesquisadores (BAPTISTA E GAUNT, 1997).

### ***Boto-cinza***

O boto-cinza apresenta um vasto repertório acústico, composto por sons tonais, como assobios, gargarejos, e sons de banda estreita de baixa frequência (AZEVEDO; VAN SLUYS, 2005; MONTEIRO-FILHO; MONTEIRO, 2001); e sons pulsados, como cliques e *burst-pulses* (KAMMINGA et al., 1993; MONTEIRO-FILHO; MONTEIRO, 2001; WIERSMA, 1982). Há ainda sugestões de ocorrência de assobios-assinatura no repertório acústico da espécie (LIMA; LE PENDU, 2014).

Os assobios são sinais associados às atividades sociais dos cetáceos e compõem a categoria de som mais estudada em boto-cinzas (BARBOSA et al., 2019; DE SOUSA PAIS et al., 2017; ERBER; SEMÃO, 2004; MAY-COLLADO, 2013; MORON et al., 2019a; ROSSI-SANTOS; PODOS, 2006). Em geral, estes sinais variam em frequência de 1,1 a 48,4 kHz (ANDRADE et al., 2015; MAY-COLLADO; WARTZOK, 2009) e podem ser categorizados de acordo com a modulação da frequência durante a emissão como: ascendente, descendente, ascendente-descendente, descendente-ascendente, regular ou múltiplo/outros (AZEVEDO; VAN SLUYS, 2005). Estes estudos indicam que os assobios ascendentes são mais representativos no repertório do boto-cinza.

Sinais sonoros pulsados do tipo clique podem ser categorizados de acordo com a duração de seus intervalos inter-clique (intervalo entre um clique e o subsequente – ICI) em cliques de orientação e cliques discriminantes. Cliques de orientação são utilizados para escaneamento e percepção do ambiente (MOSS; VALTER, 2004) e apresentam ICIs relativamente longos (ICI  $\leq 100$ ms) (HOLT et al., 2019; PIROTTA et al., 2013). Cliques discriminantes apresentam ICIs mais curtos e subsequentemente decrescentes (ICI  $\leq 10$ ms) são utilizados para obter informações detalhadas à medida que localizam e

tentam capturar a presa. (JOHNSON & TYACK 2004; MILLER; SOLANGI; KUCZAJ, 2010; RASMUSSEN et al., 2013).

O conhecimento sobre cliques de ecolocalização emitidos pelo boto-cinza é reduzido quando comparado aos seus assobios. Os estudos sobre cliques concentram-se na caracterização pontual de parâmetros acústicos (e.g. frequência mínima, duração, intervalo inter-clique e taxa de emissão), considerando áreas distintas, condições ambientais e possíveis influências do ruído antrópico (ANDRADE et al., 2015; DECONTO; MONTEIRO-FILHO, 2016, 2017; LEÃO; MONTEIRO-FILHO; SILVA, 2016; MONTEIRO-FILHO; MONTEIRO, 2001). A frequência dominante conhecida para os cliques de botos-cinza está em torno dos 88 kHz (SAUERLAND; DEHNHARDT, 1998, 2002). Influência antrópica conhecida sobre os cliques de botos-cinza se manifesta como mudanças nos parâmetros acústicos e diminuição em sua taxa de emissão quando na presença de embarcações (LEÃO MARTINS; ROSSI-SANTOS; LIMA SILVA, 2016). Contudo, ainda pouco se sabe sobre padrões acústicos de forrageio do boto-cinza e potenciais diferenças mediante a distintas escalas espaço-temporais—informações relevantes para o entendimento sobre a dinâmica acústico-comportamental e de uso do espaço (PIROTTA et al., 2014).

## **OBJETIVO GERAL**

Esta dissertação tem como objetivo geral a caracterização espaço-temporal do comportamento acústico da população de botos-cinza do CEP, em duas de suas principais áreas de uso. O comportamento acústico é tido como atributo de inferência para atividades sociais e de forrageio em áreas ambientalmente expostas a níveis distintos de interferência antrópica.

Ao considerar dois habitats distintos, e o caráter biológico dos cetáceos como sentinelas ambientais (DE MOURA et al., 2014; MOORE, 2008), nesta dissertação testa-se: 1) a existência de diferenças no repertório acústico dos botos-cinza entre as áreas; e 2) a existência de mudanças acústico-comportamentais, possivelmente associadas ao ruído subaquático.

## **ESTRUTURA DA DISSERTAÇÃO**

Esta dissertação contém um capítulo único, escrito em formato de artigo científico e formatado de acordo com a revista científica alvo: *Biodiversity and*

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## RESEARCH PAPER

### Spatiotemporal changes in the acoustic behavior of Guiana dolphins under increasingly noise conditions

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#### Highlights

- Relative noise levels seem to influence the social acoustic communication of Guiana dolphins;
- The whistling rate decreases with increasing relative noise levels;
- Initial and minimal whistling frequencies are higher at the noisiest area;
- The emission of echolocation clicks is maintained regardless of noise levels.

#### Abstract

Anthropogenic noise can modify the acoustic behavior of animals and influence their population dynamics. Here, we investigate whether dolphins adjust their acoustic behavior relative to anthropogenic noise in two areas exposed to distinct levels of anthropogenic interference (port and conservation unit) over two sampling periods eight

years apart. Our findings point to noise-induced changes in social behavior of Guiana dolphins in southern Brazil. The acoustic parameters of the dolphins' whistles differed between areas and behavioral contexts. Whistles highly overlapped with noise levels at the port area relative to the conservation unit. Overall, the whistling rate was negatively correlated with noise, suggesting that fewer social sounds are produced under noisier conditions. The whistling rate also decreased over the years with the increase of the environmental noise level, with a significant 75% decrease during dredging activities at the port area. By contrast, the emission rate of feeding buzzes, used during active foraging, was similar between the port and the conservation areas. These findings suggest that Guiana dolphins may decrease their whistling rate in noisier conditions due to limited space for communication while maintaining active foraging regardless of noise levels. Guiana dolphins may also increase the fundamental frequencies of their whistles to avoid masking in lower frequencies where noise is more intense. Our study raises concerns about the conservation of this population that already faces cumulative anthropogenic impacts, given that acoustic changes are potentially threatening to acoustic communication in social activities, group cohesion, and cooperative foraging of a population. This study provides essential information to guide conservation actions in the region, as well as baseline information for the development of mitigation measures for the potential acoustic impacts on the species.

**Keywords:** Anthropogenic noise. Port area. Conservation area. Foraging behavior. Echolocation. Dolphins' whistles.

### **Highlights**

- Ruídos ambiental e antropogênico influenciam a comunicação acústica social de botos-cinza;
- Taxa de emissão de assobios diminui com o aumento do nível de ruído;
- Frequências mínimas e iniciais dos assobios são mais altas em condições mais ruidosas;
- Emissão de cliques de ecolocalização se mantém independentemente do nível de ruído.

## Resumo

O ruído antropogênico pode modificar o comportamento acústico dos animais e influenciar sua dinâmica populacional. O objetivo desta dissertação de mestrado foi caracterizar espaço-temporalmente o comportamento acústico de botos-cinza em duas de suas principais áreas de uso no Complexo Estuarino de Paranaguá (CEP). O comportamento acústico foi utilizado para inferir atividades sociais e de forrageio em áreas ambientalmente expostas a níveis distintos de interferência antrópica—região portuária e entorno de unidade de conservação. Os resultados sugerem que ruído ambiental influencia o comportamento acústico social, mas não foi possível detectar influências conclusivas no comportamento acústico de forrageio. Os parâmetros acústicos dos assobios de botos-cinza diferiram entre as áreas e contextos comportamentais; tais sinais acústicos se sobrepuseram constantemente ao ruído ambiental e antropogênico na área portuária—a mais ruidosa. A taxa de emissão de assobios correlacionou-se negativamente com os níveis relativos de ruído ambiental, sugerindo redução no uso de sons sociais em condições mais ruidosas. Esta taxa também diminuiu ao longo dos anos, possivelmente refletindo o aumento nos níveis relativos de ruído ambiental. Em especial, destaca-se a significativa redução de 75% na taxa de emissão de assobios durante o período de dragagem na região portuária, mesmo período em que os maiores níveis de ruído ambiental foram registrados. Em contrapartida, a taxa de emissão de cliques de ecolocalização foi semelhante entre as áreas, sugerindo influência mínima — se alguma — do ruído no comportamento de forrageio. Estes resultados apontam para a possibilidade de que botos-cinza diminuam a taxa de emissão de assobios em condições mais ruidosas devido à possível redução no espaço acústico de comunicação, enquanto mantêm o forrageio ativo, independentemente do ruído, como uma compensação de alimentação. Ainda, a emissão de assobios com frequências mínimas e frequências iniciais mais altas na região portuária sugere uma tentativa de evitar o mascaramento em frequências mais baixas, onde a maior intensidade de ruído se concentra. Em conjunto, estas alterações comportamentais de botos-cinza são reconhecidas como potencialmente ameaçadoras ao papel da comunicação acústica em atividades sociais, coesão de grupo e forrageio cooperativo. Estes potenciais distúrbios tendem a ser especialmente preocupantes para populações que já enfrentam outros impactos antropogênicos cumulativos, como na região do CEP. Este estudo fornece um importante subsídio para o manejo da população de botos-cinza da região, assim como para o desenvolvimento e a

implementação de medidas de mitigação para os potenciais impactos acústicos sobre a espécie, visando a sua conservação.

**Palavras-chave:** Ruído antropogênico. Áreas portuárias. Áreas de conservação ambiental. Assobios. Ecolocalização. Comportamento de forrageio.

## **Introduction**

Small dolphins often inhabit coastal habitats where they find food resources, protection from predators, and mating opportunities (Jefferson et al. 2009; Miller and Wahlberg 2013). However, the rapidly growing urban development has been increasingly impacting such coastal areas. Among the many disturbances to the marine environment worldwide, man-made underwater noise is a chronic source of pollution that is particularly threatening to dolphins, since they rely on sounds for spatial orientation, social communication, and foraging (Richardson et al. 1995; Erbe et al. 2018; Vieira et al. 2021). Dolphins can acoustically habituate to these environmental pressures, such as by changing their acoustic repertoires to noise (Bejder et al. 2009). However, noise exposure commonly inflict multiple negative effects on dolphins—behavioral (e.g., avoidance and displacement), perceptual (e.g., auditory masking and Lombard effect), physical (e.g., hearing damage), chronic (e.g., stress), and indirect disturbances (e.g., altering prey availability) (Marley et al. 2016; Erbe et al. 2018). These effects are particularly concerning for resident dolphins populations (Forney et al. 2017) inhabiting coastal waters under high anthropogenic interference levels, such as port areas (Bittencourt et al. 2014; Merchant et al. 2014; Pine et al. 2016b).

The Guiana dolphin (*Sotalia guianensis*), a small coastal dolphin endemic to the southwestern Atlantic (Filla and Monteiro-Filho 2009; Bonin et al. 2017), is considered near-threatened by IUCN. In southern Brazil, the Paranaguá Estuarine Complex (PEC) is an essential habitat for a population estimated to be comprised of about 2,000 resident and transient individuals (Miranda 2017). The conservation area around the Peças Island is a crucial habitat for Guiana dolphins' foraging, parental care, and infant social learning (Domit, 2006; Fila & Monteiro-Filho, 2009; de Moura et al., 2021). The PEC also comprises the second-largest bulk port of Latin America, and this area is also frequently used by foraging Guiana dolphins. The port area implies in intense and frequent activities such as dredging, pile-driving constructions, and daily traffic of large vessels (Santos et al. 2007; Miura and Noemberg, 2020; Marcondes et al. 2020).

Despite the presence of ports and marine infrastructure throughout the Guiana dolphin distribution (Marcondes et al. 2020), few studies evaluated the influence of underwater noise pollution on their acoustic behavior (Reis 2013; Bittencourt et al. 2017; de Sousa Pais et al. 2017), and little is known about this relationship in the PEC (Domit 2010; Oliveira 2011). Here we provide a spatiotemporal acoustic characterization of Guiana dolphins during social and foraging activities in two areas of the PEC exposed to distinct levels of anthropogenic interference. Over two sampling periods (2012-2013 and 2018-2020), we evaluated (i) the relative environmental noise levels, (ii) the acoustic parameters of whistles used in different behavior contexts, and (iii) the echolocation inter-click intervals used in foraging contexts.

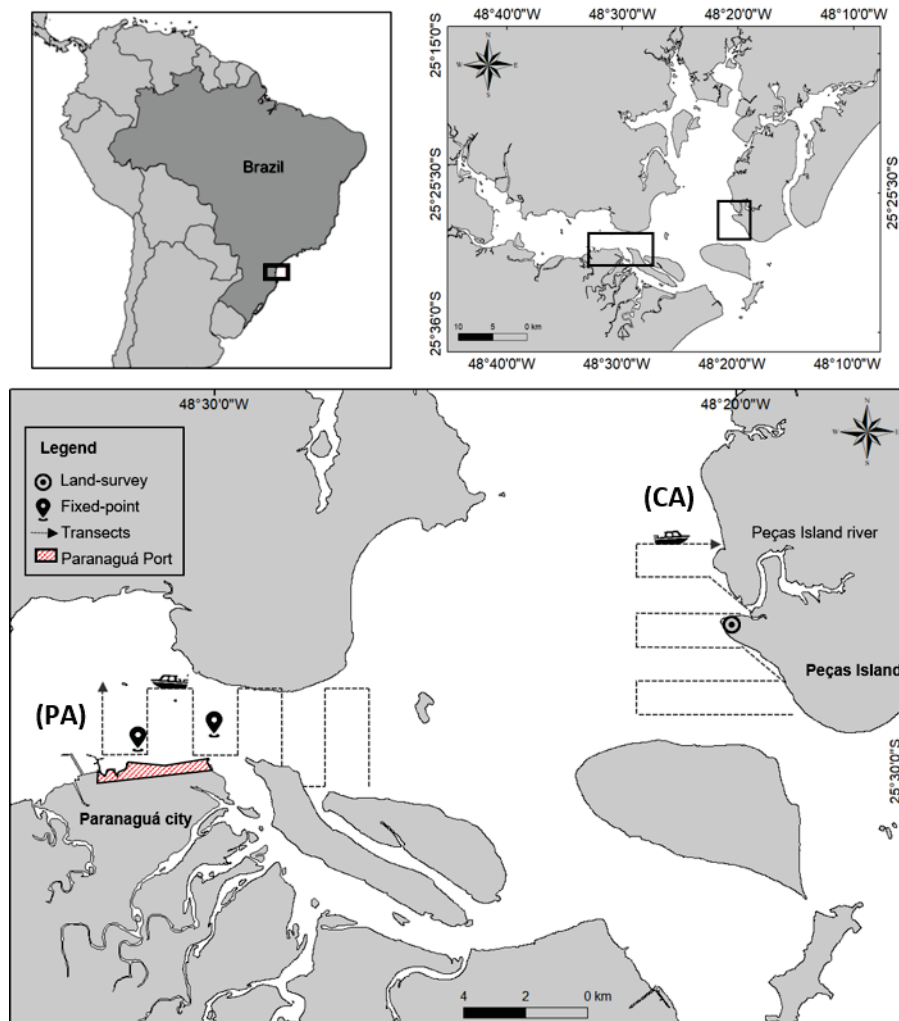
## **Material and Methods**

### *1. Study Areas*

The study was carried out in two areas with distinct levels of anthropogenic interference in the Paranaguá Estuarine Complex (PEC), southern Brazil (Figure 1). The first area comprises the Laranjeiras Bay (25°27'49" S, 48°18'11" W) at the Peças Island, which is surrounded by two Conservation Units (Superagüi National Park and Ilha do Mel Ecological Station). As it constitutes one of the world's largest remnants of the Atlantic Forest biome, UNESCO has designated the area as the "Atlantic Forest Biosphere Reserve" (MMA, Ministério do Meio Ambiente 2000; UNESCO 2012). This area constitutes the estuarine mouth at the N-S axis (Lana et al. 2001), where depth can reach up to 30 meters. It is also a rocky bottom region with high-speed currents that greatly influence the saline wedge, with an average salinity of ~ 30 ppm (Lana et al. 2001, Noernberg et al. 2006, 2007). The presence of traditional human communities and the moderate traffic of tourism and artisanal fishing vessels are the main anthropogenic activities in this area (Fila and Monteiro-Filho, 2009; Miura and Noernberg, 2020).

The second area is located at the Paranaguá Bay (25°29'39" S, 48°30'33" W), located approximately 20 km from the first area, along the E-W axis of the estuary. Water depth can reach 15 meters at the Paranaguá Port; however, Islands and submerged rock outcrops are located between Cobras and Gererês Island, where there is a zone of maximum turbidity (ZMT). An increase in primary productivity occurs at this zone due to the trapping of fine sediments from the drainage basins of Serra do Mar. Salinity varies in a seaward horizontal gradient among the seasons (Marone et al. 2005). Driven by tidal

currents, the region's heterogeneous bottom influences the hydrodynamic and sedimentary processes (Angulo et al. 2006; Cattani and Lamour 2016).



**Fig.1** Estuarine Complex of Paranaguá, southern Brazil, indicating study locations and the methods used to collect acoustic data. Both sampled areas are equivalent to approximately 16km<sup>2</sup>. CA= Conservation area and PA= Port area

## 2. Acoustic sampling

To evaluate temporal and spatial patterns, we analyzed acoustic recordings obtained during land- and boat-based surveys in both sampled areas (Figure 1) from 2012-2013; and October 2019 until February 2020. The port area was also sampled between 2018 - 2019, period that dredging activities were occurring in the area. Land-surveys were carried out three times a month at the same locations. The acoustic recordings started every time at least one dolphin was noticed and ended when they were no longer seen or heard for than 10 minutes. Boat-based surveys were carried out two or three times a month

(as weather allowed), following predefined transects to cover both areas. Upon encountering at least one dolphin, the boat approached the group (no closer than 20 meters), and the engine was turned off to minimize environmental noise. Samples were recorded for a total of 10 minutes split into five two-minute files to allow for posterior subsampling at random (see below).

Acoustic data were recorded with an omnidirectional C55 Hydrophone (-165 dB re: 1V/ $\mu$ Pa, frequency response: 1 kHz to 96 kHz) and a Fostex digital recorder (192 kHz sampling rate, 24 bits, signal-to-noise ratio: -165 dB), placed at 1.5–3m deep. To describe the dolphins' behavioral context during the acoustic sampling, we simultaneously recorded the surface behavioral state (foraging and traveling), group size, and composition of all dolphins in sight within a radius of 300 meters. Thus, we considered all dolphins engaged in the same behavioral state in the area as a group. In addition, to investigate possible anthropogenic influences on acoustic emissions, we registered the number, type, and activity of vessels (e.g., ships, dredgers, and fishing) in the area during the acoustic recordings within a radius of 500 meters.

### *3. Acoustic analysis*

To ensure the same sampling effort between study areas and across study periods, we randomly selected 60 2-min recordings for each area in each sampled period (using native functions in R 3.2.4; R Core Team, 2020), except for the Conservation area in 2018-2019 for which there was no data available. Thus, the total sample size was 300 2-min recordings. To enable spatial comparisons, we composed the period of 2018-2020 at the port area of 30 random samples from 2018-2019 and 30 from 2019-2020 (Table 1). All acoustic analyses were performed using Raven Pro 1.5 software (Cornell Laboratory of Ornithology, New York) with a 512-point Hamming window and 50% overlap, according to the default settings of the analysis window (brightness:50; contrast: 50).

#### *3.1 Relative background noise levels*

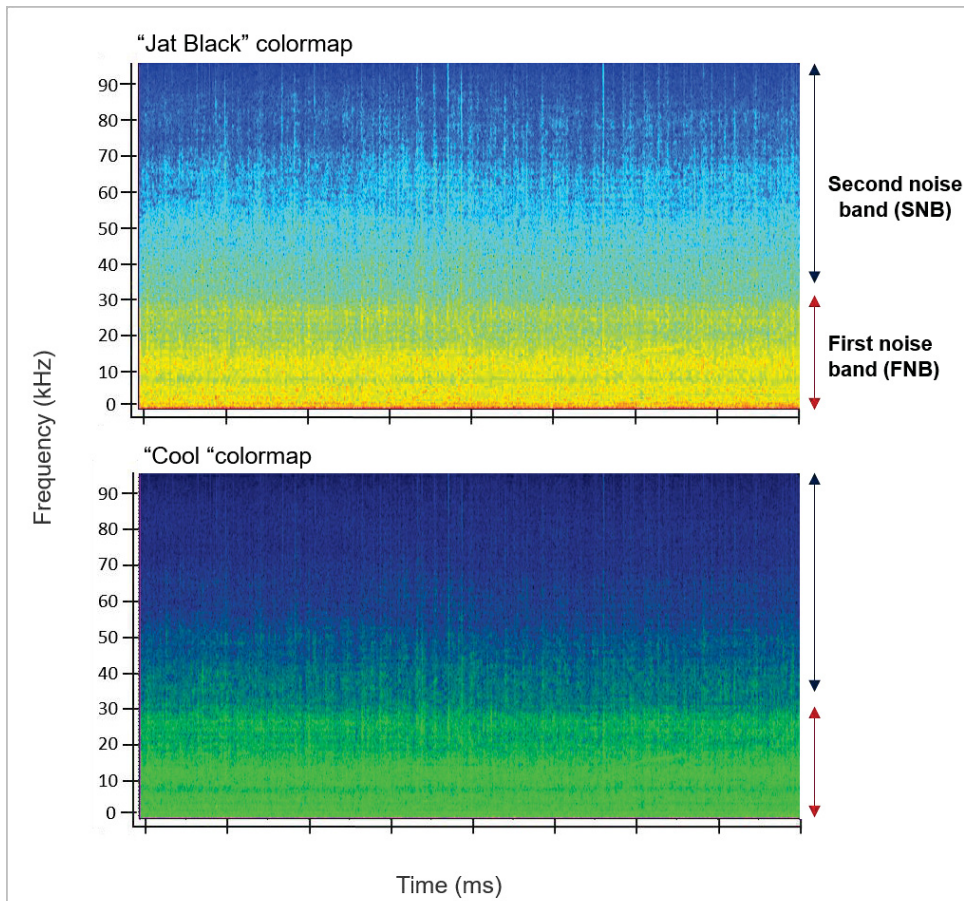
To investigate and characterize the relative levels of background noise to the sampled areas and recognize possible changes between years, we visually measured the frequency range (kHz) and the relative energy (dB) of background noise based on the “Cool” and “Jet Black” color maps available in Raven Pro 1.5. In these maps, the color shown in each pixel of the spectrogram represents the logarithm of the relative power at the corresponding spectrum frequency. Hence, the color maps allow distinguishing

relative spectral power density (dB) based on color intensity, where darker spectrogram pixels represent higher power spectral density values.

We created two frequency range categories based on color intensity (Figure 2). The first noise band (FNB) was the darkest one, reflecting higher power spectral density levels that can reach 20 - 30 kHz. The second noise band (SNB) was the extension of FNB, but with a lighter color range and lower power spectral density values, reaching frequencies between 60 and 95 kHz. Next, we identified the maximum frequency for each noise band and obtained their relative spectral energy selecting the entire duration of the recording, which was calculated as:

$$\left( \sum_{t=t1}^{t2} \sum_{f=f1}^{f2} (W_0 \cdot 10^{(S_{t,f}/10)}) \right) \Delta f$$

where  $f1$  and  $f2$  are the lower and upper-frequency limits of the selection,  $t1$  and  $t2$  are the beginning and ending frame numbers of the selection,  $W_0$  is the power dB reference value,  $S_{t,f}$  is the spectrogram power spectral density in frame  $t$  at frequency  $f$  (in dB), and  $\Delta f$  is the frequency bin. In Raven, the reference power  $W_0$  is always equal to unity. In total, we analyzed 40 minutes of recordings from both conservation (CA) and port areas (PA) in each sampled period, totaling 200 minutes.



**Fig.2** Raven Pro’s “Cool” and “Jet Black” spectrograms color maps with the two frequency range categories used to measure the relative background noise

### 3.2 Whistle analysis

To avoid interference from our boat in the acoustic samples, we discarded the first minute of all recordings. We visually and aurally identified all whistle signals during the remaining 60s but analyzed only those of high quality (i.e., with an entire spectral contour visible). We manually measured the following acoustic parameters: duration (s); initial, final, minimum, and maximum frequencies (kHz); delta frequency (kHz); relative energy (dB), and number of harmonics (Rossi-Santos and Podos 2006; Deconto and Monteiro-Filho 2017; Moron et al. 2019). Then we classified whistles into six types of frequency modulation (ascending, descending, ascending-descending, descending-ascending, multiple, and flat) (Azevedo and Van Sluys 2005; May-Collado and Wartzok 2008). We emphasize that it was impossible to localize vocalizing dolphins with our recording system to calculate energy source levels, and Raven Pro 1.5 calculates energy based on power spectral density. Thus we carefully interpreted the energy parameters (e.g.,

whistles' and background energy) as the relative values from spectral power within the spectrogram selection.

We first investigated if whistles parameters differed across areas, sampled periods, and surface behavior using Kruskal–Wallis test (as in May-Collado 2013), followed by a post hoc Dunn test (significant level of  $\alpha = 0.5$ ) with Bonferroni correction due to the multiple comparisons. Then we used Principal Component Analysis (PCA) to reduce the multidimensionality of quantitative acoustic parameters and to visually explore if whistles tended to differentiate between areas of greater (PA) and lesser anthropogenic impact (CA). Next, we built three discriminant functions analysis (DFA) models to search for a combination of the acoustic parameters (independent variables) that described any differences between the study areas, sampled periods, and behavior (response variables). The saturated DFA model was simplified by stepwise leave-on-out procedure, and the most parsimonious model was selected using the classification accuracy. Since whistles were recorded mostly when dolphins were foraging, we used the same number of recordings for behavior-related analysis.

We fitted Generalized Linear Models (GLMs) with a negative binomial error structure to investigate whether the whistling rate differed within the environmental context. We build models with the number of whistles per minute as a response variable in function of background noise levels, dolphin group size, presence of calves, and the number of boats in the area. Before the GLM, we performed data exploration to check for outliers and collinearity. Then, the full model was reduced step-by-step (Zuur et al. 2005), followed by graphical diagnosis, and the best model was selected using Akaike Information Criteria (AIC). All statistical analyses were performed on the R environment, version 4.1.1 (R Core Team, 2020).

#### *4. Foraging activities*

We used the temporal patterning of echolocation clicks to distinguish between different foraging stages, which are generally classified through the duration of their inter-click intervals (ICIs; see Pirotta et al. 2014b; Bergès et al. 2020). At least the three following different echolocation processes can be identified from ICIs, which potentially reflect different stages of the foraging behavior. First, the buzzes, characterized by trains of clicks with very short ICIs (e.g., 0.007-0.05s; Jones et al. 2019), are associated with active foraging, including attempted prey capture (Miller et al. 2004; Carlström 2005; Todd et al. 2009). Second, the regular clicks, characterized by longer, regularly-spaced

ICIs, are typically associated with navigation and scanning (Jones et al. 2019). Finally, inter-train clicks have long and more variable ICIs and may represent pauses between different click trains (Au 1994; Carlström 2005; Madsen et al. 2005).

#### *4.1 Foraging activities classification*

To identify these different echolocation processes in our data, we use a total of 88 2-min recordings (not the exact same for whistles analysis) equally divided into 44 recordings of 2-subsequent minutes for each area from the most recent sampled periods (2019-2020 for the conservation area and 2018-2020 for the port area).

First, we developed a customized algorithm (in Matlab; The MathWorks, Inc.) to count all the echolocation clicks per second and calculate their ICIs. The click detector considered a threshold of 2.5ms interval between detections, roughly corresponding to the duration of a dolphin click plus its echoes (Deconto and Monteiro-Filho 2016, 2017; Leão Martins et al. 2016a; Leão et al. 2016; Andrade et al. 2017). To minimize interference from background noise, we defined a cut-off frequency for a high-pass filter of 15k Hz and a detection threshold of 17 dB re noise for the recordings from the CA (Peças Island), and a threshold of 20 dB for the recording from the (noisier) PA.

Second, to the temporal series of detected ICIs in each location, we fit finite Gaussian mixture models using the “Mclust” package in R with k-components varying from one to seven to specified the component distributions that represent the different echolocation processes detected, similarly to previous studies (Pirodda et al. 2014b, 2015; Garrod et al. 2018; Bailey et al. 2019). All clicks were then assigned to one of the three echolocation processes (buzz, regular or inter-train click) as per the mean ICI of the GMM component they were classified into. The most parsimonious model – with an unequal variance – was selected and validated based on both Bayesian Information Criteria (BIC) and Integrated Completed Likelihood (ICL) (Scrucca et al. 2016). Finally, we used feeding buzzes as a proxy to describe the potential active foraging between areas.

## **Results**

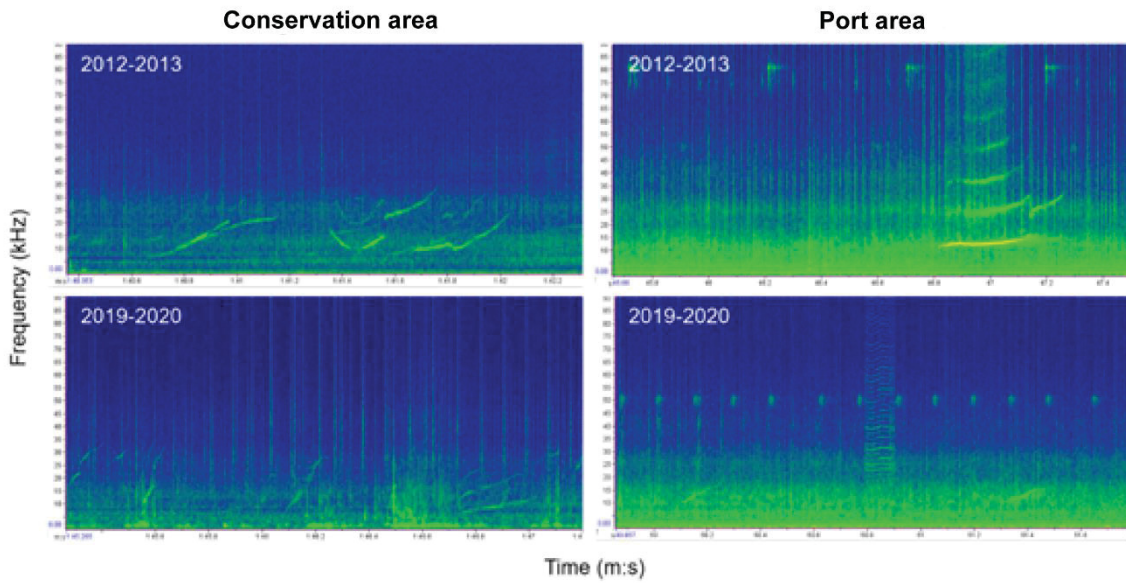
### *Relative background noise levels*

The port area was noisier than the conservation area around Peças Island. Between years, the total noise frequency range (FNB plus SNB) ranged from 0.39 kHz to 75 kHz at CA and from 2.38 up to 95 kHz at the PA. At CA, the total relative spectral energy ranged across years from 100 dB up to 193 dB, while at the PA, it ranged from 105 up to 204.4 (Table 1 and Figure 3).

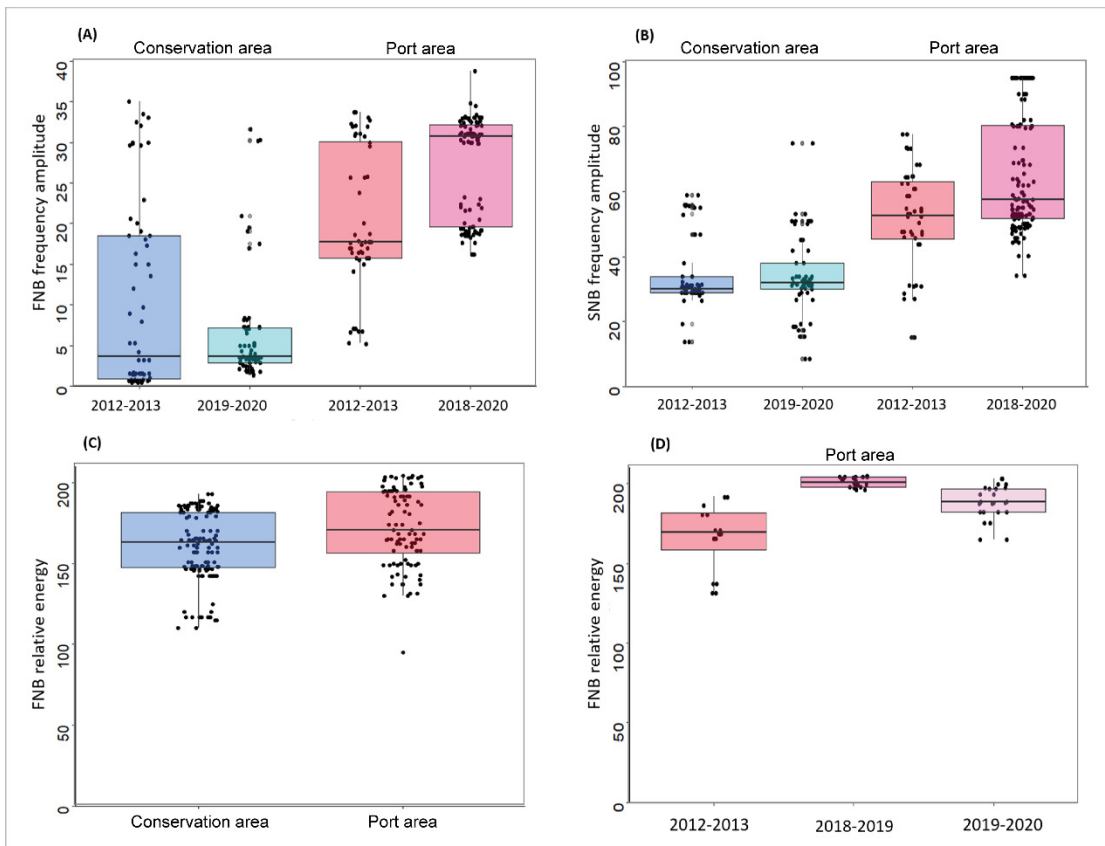
The *FNB frequency range* was higher across both study periods at the port area ( $X^2 = 52.1$ ,  $df = 3$ ,  $p = 2.86 \cdot 10^{-11}$ ), such as the *FNB's relative spectral energy* ( $X^2 = 47.3$ ,  $df = 3$ ,  $p = 3.04 \cdot 10^{-10}$ ) and *SNB frequency range* for 2018-2020 ( $X^2 = 34.1$ ,  $df = 3$ ,  $p = 1.9 \cdot 10^{-7}$ ). During the dredging period (2018-2019) the mean *FNB spectral energy* was higher than those from 2012-2013 and 2019-2020 ( $X^2 = 14.651$ ,  $df = 2$ ,  $p < 0.0007$ ), which had no significant difference between them at this area. Post hoc multiple comparisons are present in the supplementary material (Table S1) and distribution boxplots in Figure 4.

**Table 1.** Descriptive statistics for two frequency range categories of relative background noise levels recorded at Conservation area and Port area - Southern Brazil, according to the sampled period. (Frequency in kHz; Spectral energy in dB; standard deviation; CV = coefficient of variation).

Location	Statistic	First noise band	FNB relative energy	Second noise band	SNB relative energy
Conservation area 2012-2013	<b>Mean ± SD</b>	<b>12.48 ±9.8</b>	<b>152.5 ±14.1</b>	<b>34.76 ±13.1</b>	<b>144.0 ±13.01</b>
	CV	0.78	0.09	0.37	0.09
	Range	0.39-32.0	116.9-170.3	13.72-60.5	119.3-160.5
Conservation area 2019-2020	<b>Mean ± SD</b>	<b>7.19 ±8.7</b>	<b>166.6 ±26.5</b>	<b>30.19 ±13.6</b>	<b>160.5 ±24.4</b>
	CV	1	0.15	0.45	0.15
	Range	1.43-20.63	110.0-193.1	8.39-75.01	100.0-189.1
Port area 2012-2012	<b>Mean ± SD</b>	<b>19.52 ±9.4</b>	<b>160.9 ±16.0</b>	<b>49.34 ±15.3</b>	<b>143.9 ±15.6</b>
	CV	0.48	0.09	0.31	0.11
	Range	5.23-33.73	131.3-191.7	15.01-77.68	117.9-165.5
Port area 2018-2019	<b>Mean ± SD</b>	<b>22.16 ±5.6</b>	<b>198.9 ±4.8</b>	<b>62.76 ±18.1</b>	<b>172.0 ±16.6</b>
	CV	0.25	0.02	0.29	0.09
	Range	15.71-32.96	189.2-204.4	33.27-95.00	120.0-185.1
Port area 2019-2020	<b>Mean ± SD</b>	<b>20.09 ±7.7</b>	<b>177.6 ±26.5</b>	<b>44.96 ±16.1</b>	<b>159.8 ±24.8</b>
	CV	0.38	0.15	0.36	0.15
	Range	2.38-34.75	111.2-203.1	29.85-80.0	105.0-186.2
*Port area 2018-2020	<b>Mean ± SD</b>	<b>21.99 ±9.8</b>	<b>186.0 ±33.4</b>	<b>55.85 ±20.2</b>	<b>165.0 ±31.6</b>
	CV	0.41	0.18	0.36	0.19
	Range	2.38-34.5	112.2-204.4	29.85-95.0	105.0-186.2



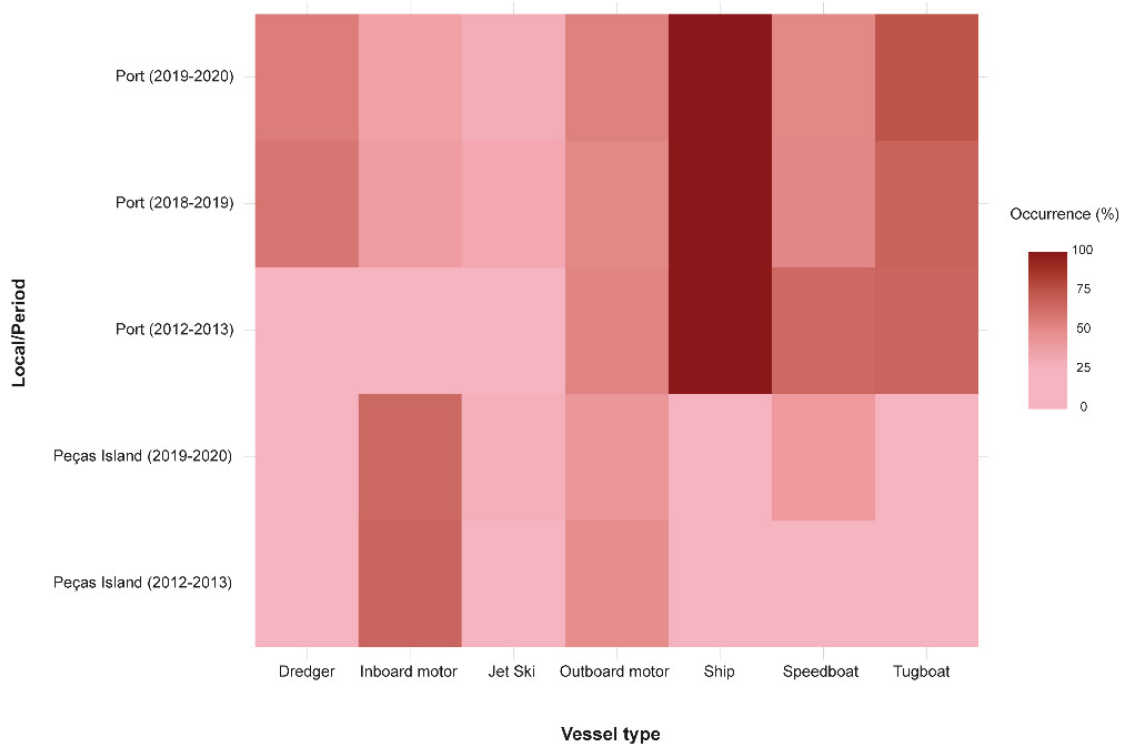
**Fig. 3** Spectrograms examples of Conservation area and Port area background noise (continuous green stripe) according to the sample periods ( $N = 300$ ). The relative noise levels vary with the green color intensity



**Fig. 4** Acoustic distribution of the (A) First noise band (FNB) and (B) second noise bands (SNB) frequency range. (C) FNB relative spectral energy at Conservation area and Port area according to the study periods. (D) the acoustic distribution of FNB relative spectral energy recorded at Port area per year

*Presence of vessels*

As two distinct areas of anthropogenic activities, the number, and type of vessels registered during sampling differed between them. At the port area, the average number of vessels present at the area per recording was 20.4 (SD = 5.97, range = 6 – 27, mode = 21), of which container and bulk ships, tug boats, dredger vessel, speedboats, and outboard motor fishing vessels were the majority type. In contrast, pilot crafts, inboard motors, and Jet Ski were typical but less seen (Figure 5). Meanwhile, the average number of vessels at the conservation area was 3.95 (SD = 7.37, range = 0 – 24, mode = 0), of which in- and outboard motor fishing vessels and speedboats were the most common ones, while Jet Skis were rare.



**Fig. 5** Frequency of presence of each vessel category per recording by area and study period. The y-axis represents the accumulated number of recordings where vessels were present (number of recordings per area and period = 60; total = 300)

### *Group Composition and behavior context*

Dolphins were predominantly foraging during our surveys: at CA, dolphins were seen foraging around 75% of the time, while at the PA were foraging 82% of the time (Table 2). The mean group sizes and percentage of calf presence were similar among areas and periods (group sizes:  $X^2 = 8.86$ ,  $df = 4$ ,  $p$ -value = 0.06 | calves %:  $X^2 = 0.03$ ,  $df = 4$ ,  $p = 0.9$ ). Since whistles were recorded mostly when dolphins were foraging, we used the same number of recordings for behavior-related analysis (N =200 in each behavioral context).

**Table 2.** Group composition and main surface behavior frequency of all groups sighted during the recordings at Conservation and Port areas by sampled periods (CA= Conservation area, and PA = Port area)

Local	Period	Total number of recordings	Sighted groups	Group size			Calves	Behavior	
				Mean±SD	Min.	Max.	Presence	Foraging	Travel
CA	2012-2013	60	25	6.2	1	27	71.4%	80%	10%
	2019-2020	60	24	4.5	1	25	62.5%	70%	30%
PA	2012-2013	60	20	4.8	1	19	76.1%	90%	10%
	2018-2019	60	10	4.0	1	8	60.0%	90%	10%
	2019-2020	60	20	6.1	2	30	75.0%	66.6%	33.4%

### *Guiana dolphin whistles repertoire*

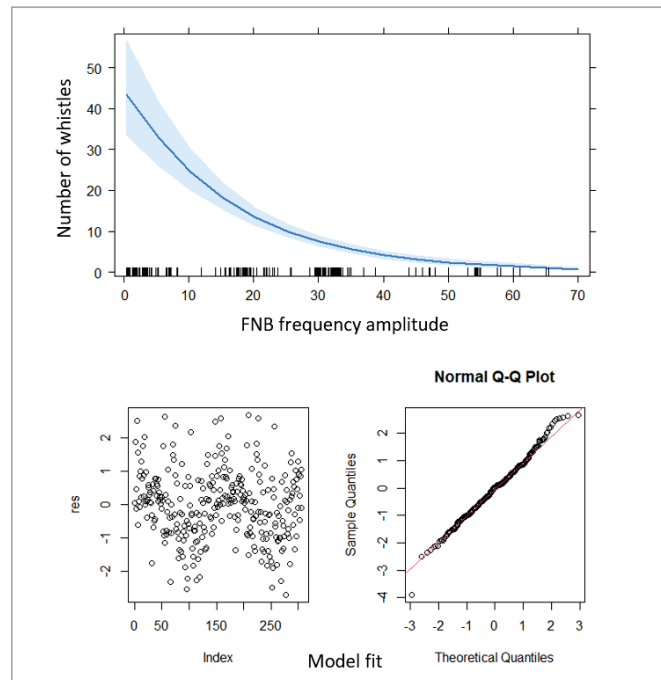
To the total of 300 minutes of recordings, we identified 5,610 Guiana dolphin whistles and analyzed 1,749. The total number of whistles by area and sampling periods and their emission rates are in table 3.

**Table 3.** According to the sampled period, the total number of Guiana dolphins' whistles and average whistling rates per dolphin per minute for two areas of the Estuarine Complex of Paranaguá.

Location	Period	Total minutes analyzed	Total number of whistles	Number of analyzed whistles	Whistling rate per dolphin/minute
Conservation area	2012-2013	60	2,264	732	7.54
Conservation area	2019-2020	60	1,732	533	8.85
Port area	2012-2013	60	930	313	4,06
Port area	2018-2019	60	230	50	1.03
Port area	2019-2020	60	454	121	1.45
Total		300	5,610	1,749	-

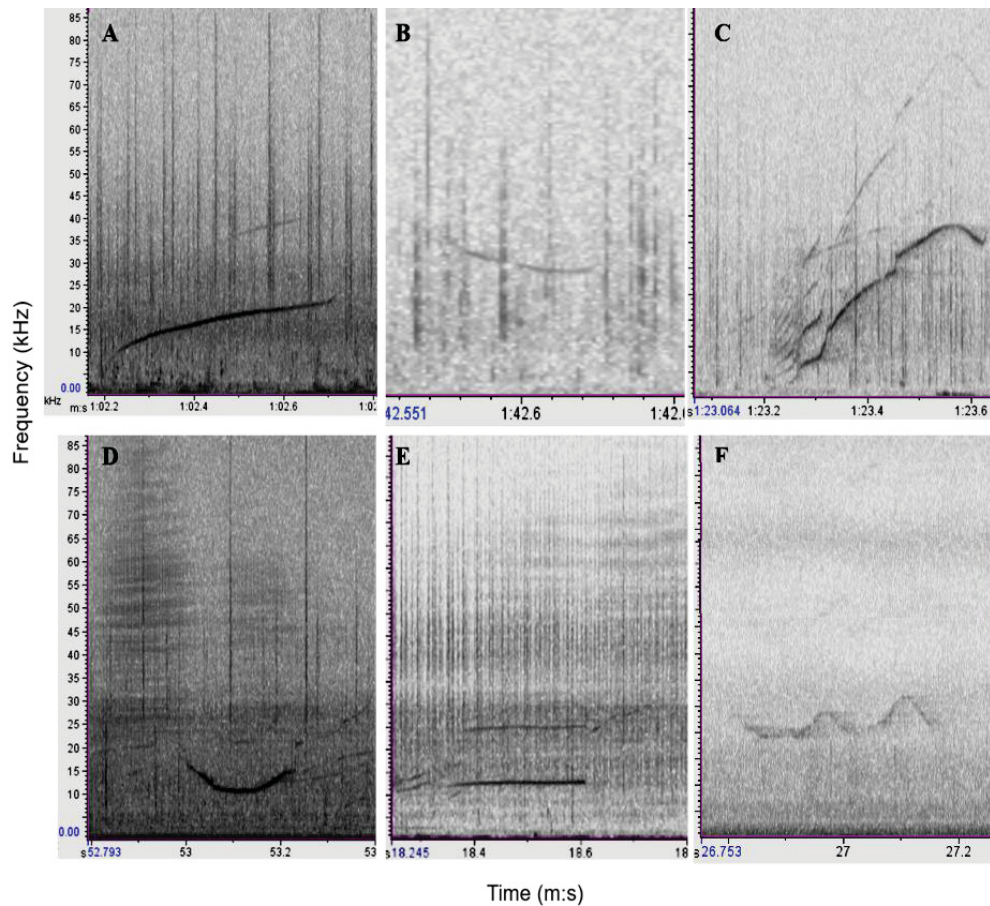
The total number of whistles and whistling rate per minute differed spatial- and temporally ( $X^2 = 43.877$ ,  $df = 4$ ,  $p = 6.804 \cdot 10^{-9}$ ) and were negatively correlated with the background noise range ( $r_s = 0.70$ ,  $p < 2.2 \cdot 10^{-16}$ ). Although the most parsimonious negative-binomial GLM model explained only 25.2% of the data variation, it had a satisfactory fit (Figure 6). The model retained as a predictor only the background noise variable: *FNB frequency range*, which had a negative relationship with the number of whistles ( $-0.0592 \pm 0.005$ ,  $z = -11.53$ ,  $p < 2.0 \cdot 10^{-16}$ ).

Overall, the whistling rate was higher at CA and when dolphins were foraging ( $X^2 = 12.507$ ,  $df = 2$ ,  $p = 0.001923$ ; Table 3). Across years, the whistling rate decreased around 64.3 % at the PA, with a major decrease of about 75% between the dredging period (2018-2019) and the previous one (2012-2013). Meanwhile, the conservation area presented an increase of around 14.8% throughout the years.



**Fig. 6** Negative Binomial GLM: Upper subplot shows the predicted curve for the variable FNB frequency (95% confidence intervals), which was the most important predictor variable for explaining the differences in the number of whistles emitted by Guiana dolphins; The bottom subplot shows the model fit quality based on randomized quantile residues

Regarding whistles characterization, we identified whistles from all six frequency modulation categories (Figure 7). The ascending type was the most frequent (87.9%,  $n=4,931$ ), representing more than 70% of the whistles identified in both study areas and periods (CA: 2012-2013= 2,014; 2019-2020 = 1,506; PA: 2012-2013= 790; 2018-2019= 184; 2019-2020= 331). The other five types accounted for less than 5% of the total whistles analyzed.



**Fig. 7** Examples of each type of whistle frequency modulation emitted by the Guiana dolphin in the areas of Conservation and Port areas - southern Brazil. A = Ascending, B= Descending, C= Ascending-descending, D= Descending-Ascending, E= Regular, D = Multiple

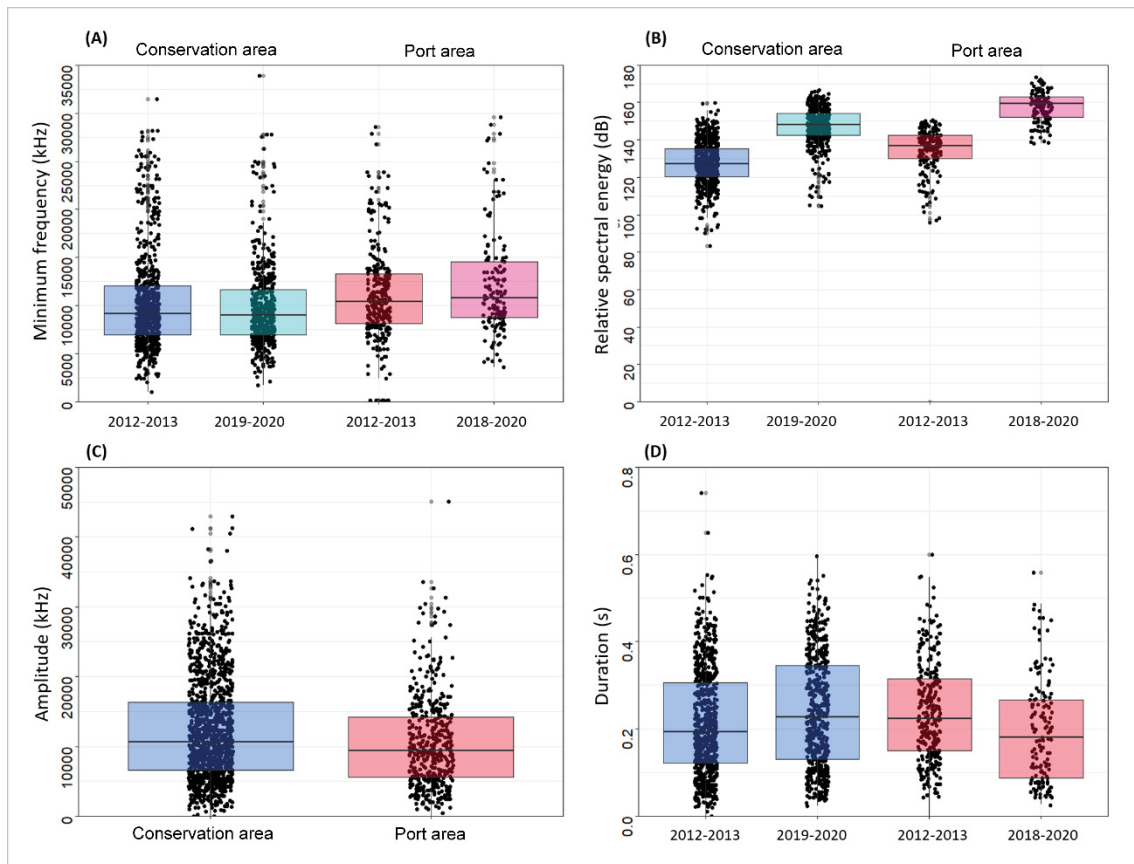
The whistles of the Guiana dolphin population from PEC ranged in frequency from 1.04 to 55.96 kHz and duration from 0.02 up to 0.74 seconds. Only 21.7% (=379) of all analyzed whistles (1,749) contained harmonics, of which 37% (=140) had sufficient signal-to-noise ratio for measurements. Whistles with up to 9 harmonics were found, but those with one or two corresponded to 78.9% of the total (n=299). Overall, harmonic frequencies ranged from 2.23 up to 90 kHz, with a duration varying from 0.042 to 0.436 seconds.

Three whistle parameters differed across behavior contexts. Whistles were longer ( $X^2 = 9.7976$ ,  $df = 1$ ,  $p = 0.005$ ), and had higher maximum ( $X^2 = 26.027$ ,  $df = 1$ ,  $p = 0.002$ ) and delta frequency ( $19.992$ ,  $df = 1$ ,  $p = 0.042$ ) when dolphins were traveling. Overall, across study periods, the initial frequency ( $X^2 = 47.995$ ,  $df = 3$ ,  $p = 2.13 \cdot 10^{-10}$ ), minimum frequency ( $X^2 = 43.624$ ,  $df = 3$ ,  $p = 1.81 \cdot 10^{-9}$ ), and relative spectral energy of whistles

( $X^2 = 511.34$ ,  $df = 3$ ,  $p < 2.2 \cdot 10^{-16}$ ) were higher in the noisier area (PA). Meanwhile, whistles' delta frequency was smaller ( $X^2 = 17.562$ ,  $df = 1$ ,  $p = 2.78 \cdot 10^{-5}$ ). Over the years, whistles' relative spectral energy has increased in both areas, but whistles' duration varied differently. While at the CA, the whistles' duration increased by an average of 30 milliseconds, it decreased by 40 milliseconds at PA, (Table 4; Figure 8). This pattern was observed only for foraging whistles. Post hoc multiple comparisons are present in the supplementary material (Table S2).

**Table 4.** Descriptive statistics of the acoustic parameters of Guiana dolphins whistles in Conservation and Port areas, southern Brazil, in each study period. Frequency in kHz; relative Spectral Energy in dB; Duration in seconds; SD = standard deviation; CV = coefficient of variation.

Location/ Period	Statistic	Initial frequency	Final frequency	Low frequency	High frequency	Frequency- range	Spectral energy	Duration
Conservation area 2012-2013	<b>Mean ± SD</b>	<b>10.64 ± 5.8</b>	<b>22.03 ± 7.4</b>	<b>10.33 ± 5.5</b>	<b>22.30 ± 7.3</b>	<b>12.72 ± 7.7</b>	<b>125.4 ± 11.7</b>	<b>0.21 ± 0.1</b>
	CV	0.54	0.33	0.53	0.32	0.6	0.09	0.53
	Range	1.04 – 54.74	7.79 – 55.96	1.04 – 54.74	8.17 – 55.96	7.07 – 42.89	83.3 - 159.5	0.02 - 0.74
Conservation area 2019-2020	<b>Mean ± SD</b>	<b>10.02 ± 4.7</b>	<b>20.83 ± 6.8</b>	<b>9.86 ± 4.5</b>	<b>21.08 ± 6.7</b>	<b>11.65 ± 7.3</b>	<b>145.2 ± 10.4</b>	<b>0.24 ± 0.1</b>
	CV	0.46	0.32	0.45	0.31	0.62	0.07	0.55
	Range	1.73 – 40.64	1.89 - 41.87	1.73 - 39.83	4.03 – 42.68	6.13 – 36.50	104.8 – 166	0.02 - 0.55
Port area 2012-2013	<b>Mean ± SD</b>	<b>12.49 ± 6.2</b>	<b>21.69 ± 6.8</b>	<b>12.39 ± 6.0</b>	<b>21.83 ± 6.9</b>	<b>10.66 ± 7.1</b>	<b>132.5 ± 11.9</b>	<b>0.23 ± 0.1</b>
	CV	0.49	0.31	0.48	0.31	0.67	0.09	0.47
	Range	2.43 – 34.52	7.27 – 45.14	2.43 – 34.52	7.27 – 45.14	21.27 – 45.02	95.90 – 150	0.04 - 0.60
Port area 2018-2019	<b>Mean ± SD</b>	<b>11.98 ± 3.7</b>	<b>20.95 ± 5.2</b>	<b>10.86 ± 2.7</b>	<b>21.42 ± 4.8</b>	<b>9.67 ± 5.6</b>	<b>158.4 ± 6.0</b>	<b>0.22 ± 0.12</b>
	CV	0.31	0.24	0.25	0.22	0.57	0.04	0.56
	Range	7.48 - 20.98	13.77 - 31.77	7.48 - 17.22	13.89 - 31.77	1.84 – 22.70	144.8 - 169.4	0.05 - 0.56
Port area 2019-2020	<b>Mean ± SD</b>	<b>13.21 ± 5.8</b>	<b>21.22 ± 6.3</b>	<b>13.36 ± 5.7</b>	<b>21.33 ± 6.3</b>	<b>9.84 ± 6.0</b>	<b>155.1 ± 8.6</b>	<b>0.18 ± 0.1</b>
	CV	0.44	0.3	0.42	0.29	0.61	0.05	0.61
	Range	1.79 – 28.80	10.82 – 38.74	4.11 – 28.80	10.82 – 38.74	1.04 – 29.82	137.9 - 171.5	0.02 - 0.47
* Port area 2012-2020	<b>Mean ± SD</b>	<b>12.37 ± 5.6</b>	<b>21.74 ± 6.5</b>	<b>12.16 ± 5.4</b>	<b>21.95 ± 6.4</b>	<b>9.79 ± 5.9</b>	<b>157.62 ± 8.2</b>	<b>0.19 ± 0.11</b>
	CV	0.46	0.3	0.45	0.29	0.59	0.05	0.61



**Fig. 8** Distribution of whistles (A) minimum frequency and (B) relative spectral energy emitted by *Sotalia guianensis* at the Conservation and Port areas – Southern Brazil, according to study periods

### *Principal Component Analysis*

The two first principal components of the PCA explained 86.02% of total data variation (PC1 = 50.31% and PC2 = 35.71%; Figure 9). Initial and minimum frequency were highly correlated ( $r_s = 0.95$ ) and represented the most important variables for PC1. The same effect was apparent for final and maximum frequency ( $r_s = 0.98$ ), which were more important for PC2. The duration parameter was not related to any frequency parameter analyzed and was more important for PC3. Due to the high correlation between initial and minimum frequency and final and maximum frequency, we chose to use minimum and maximum frequency to proceed with the discriminant analysis since they

were the most important variables for each component. Duration was also considered due to its low correlation with frequency parameters.

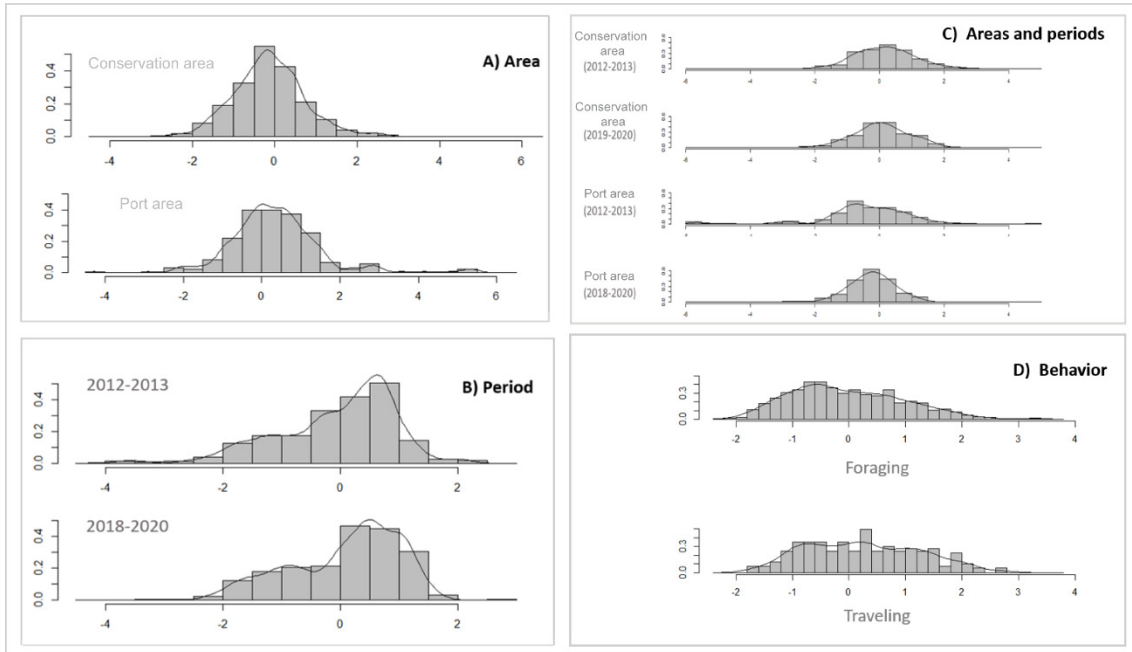


**Fig. 9** Principal component analyses (PCA) plot for Guiana dolphin whistles recorded at Conservation and Port areas, southern Brazil. PC1: First principal component, PC2: Second principal component, with corresponding explained variation.

#### *Discriminant Function Analysis*

In agreement with PCA results, DFA further suggested that whistle samples could not be separated by areas nor by years (Figure 10). The most accurate DFA model attempting to distinguish whistles samples by areas had a relatively low ability to separate groups (52.9%). The final DFA model retained two acoustic parameters ( $Study\ area \sim 2.16 \cdot 10^{-4} \text{ minimum frequency} - 7.02 \cdot 10^{-5} \text{ maximum frequency} + 4.49 \text{ duration}$ ). Similarly, the most accurate DFA model ( $Year \sim 1.83 \cdot 10^{-5} \text{ minimum frequency} - 1.47 \cdot 10^{-4} \text{ maximum frequency}$ ), aiming to distinguish whistles samples over time (2012-2013 and 2018-2020), also had low ability to separate the study periods (11.5%). Finally, a DFA model combining areas and periods ( $Mix \sim 2.04 \cdot 10^{-4} \text{ minimum frequency} + 8.51 \cdot 10^{-5} \text{ maximum frequency} - 6.47 \text{ duration}$ ) also had low accuracy (20.9%). Although the model attempting to distinguish whistles by behavior had a relatively high ability to separate groups (72.49%) ( $Behavior \sim 6.51 \text{ duration} - 5.4 \cdot 10^{-5} \text{ duration} + 1.12 \cdot 10^{-5} \text{ maximum frequency}$ ) the whistle samples overlapped along the first linear discriminant axis (Figure 10). Overall, these results suggest that dolphins' whistles emitted at Conservation area could not be discriminated from those emitted at the Port area, nor

those from when dolphins were foraging or traveling. However, some whistles parameters differed among areas, periods, and behavior.



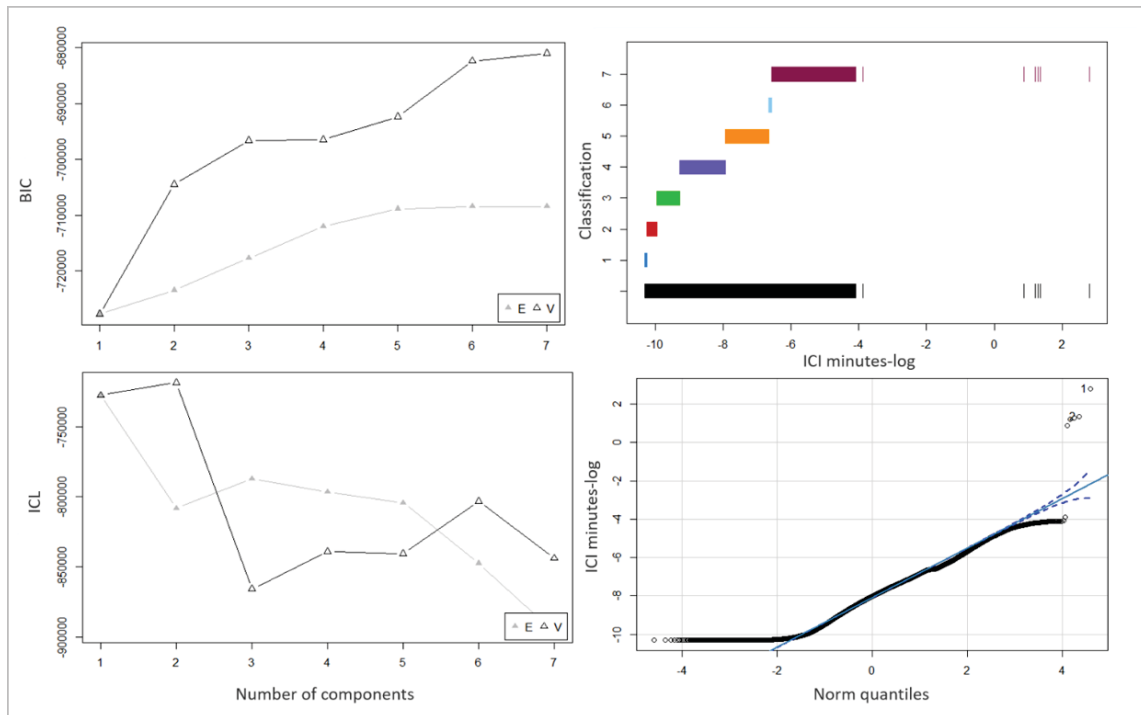
**Fig. 10** Distribution of whistles' acoustic parameters along the first linear discriminant axes of the three discriminant function analysis (DFA) models that attempted to separate whistle samples by (a) Areas (b) Sampled periods (c) mixing both (d) behavior.

*Foraging activity (echolocation clicks analysis)*

The GMM with seven components was the most parsimonious model at specified identifying different echolocation processes. This classification into components corroborated with the three expected echolocation processes (Figure 11). As the natural logarithm of each ICI value in minutes was classified into one of the seven components, we assigned, based on the mean ICI and previous studies, the three first components,  $k = 1, 2,$  and  $3$  to feeding buzzes,  $k = 5$  and  $6$  to regular clicks, and  $k = 7$  to inter-train clicks (Table 5). We designated the fourth component as “undefined” due to the unclear separation between the expected echolocation processes. The two first components could also be assigned to the Guiana dolphin’s burst-pulse categories 1 and 2, recently described by Andrade et al. (2021).

Active foraging was similar in both areas. From 225,181 ICIs classified (CA: 133,802; PA: 91,379), the proportion of feeding buzzes was 22.7% at the conservation and 14.5% at the port area ( $n =$  Island: 30,336; Port: 13,230;  $X^2 = 0.016842,$   $df = 1,$   $p =$

0.8967). Overall feeding buzzes ICIs ranged from 2.0 to 5.5 milliseconds and mean  $\pm$  SD of inter-click-interval were similar (CA: 3.2ms  $\pm$  1.08; PA: 3.3ms  $\pm$  1.04).



**Fig.11** BIC, AIC, ICIs (log minutes) Q-Q plot, and components classification by ICIs-minutes log for the GMM model that better specified the component distributions representing different echolocation processes (number of components  $k = 7$ )

**Table 5.** Gaussian mixture model components characterization for each assigned echolocation process (ICI in milliseconds)

Class	Mean - ICI	SD	CV	Min. - ICI	Max. - ICI	Echolocation process
<b>K= 1</b>	2.04	0.04	0.02	2.01	2.11	Feeding buzz
<b>K= 2</b>	2.43	0.2	0.08	2.12	2.83	Feeding buzz
<b>K= 3</b>	4.08	0.7	0.17	2.83	5.56	Feeding buzz
<b>K= 4</b>	12.58	4.5	0.36	5.57	21.36	Undefined
<b>K= 5</b>	40.49	4.8	0.12	21.36	77.49	Regular click
<b>K= 6</b>	80.56	1.5	0.02	77.49	83.78	Regular click
<b>K= 7</b>	185.75	141.3	0.76	83.79	998.05	Inter-train click
Log-likelihood	n	df	BIC	ICL		
	-340367.2	225187	20	-680980.9	-843317.6	

## Discussion

Our study points to spatiotemporal differences in Guiana dolphin acoustic behavior reflecting the intensity of anthropogenic noise. The whistling rate was lower at the noisiest port area relative to the conservation area, and it decreased over the years in both areas with the increase of the background noise level. Whistle acoustic parameters also differed between areas, mirroring the spatiotemporal background noise tendency. On the other hand, the emission of foraging clicks was similar between areas. In what follows, we discuss how these findings suggest that Guiana dolphins from the Paranaguá Estuarine Complex vary their acoustic behavior under distinct levels of anthropogenic interference and the possible biological implications for their social and foraging activities.

We first found that the whistling rate was lower, and that whistles had higher initial and minimal frequencies, at the port area relative to the conservation area around Peças Island. Temporally, whistles' relative spectral energy increased in both areas, following the background noise levels. Nonetheless, the whistling rate had the opposite pattern: a significant 75% decrease during the period of maximum noise. This decrease could be related to the dredging activities that occurred at the port area (Paranaguá Bay), since we found significantly higher levels of background noise during its occurrence. Similar acoustic responses in other studies show how dolphins under elevated noise conditions shift their whistles frequencies to a frequency range with lower noise interference (Rako Gospić and Picciulin 2016; van Ginkela et al. 2018).

Dolphins' response to noise can change depending on their behavior state (May-Collado and Quiñones-Lebrón 2014; Gospić and Picciulin 2016). In our study, whistle duration changed only when dolphins were foraging and such changes were different between areas. Between study periods, whistle duration increased at the conservation area but decreased at the port area, despite a similar overall average duration. Both increased (Reis 2013) and decreased whistle duration (Bittencourt et al. 2017) have been documented for Guiana dolphins in response to noise. Such difference may be expected in a population living under different background conditions directly reflecting acoustic habituation (May-Collado and Quiñones-Lebrón 2014). Moreover, since the Guiana dolphin presents different population stocks throughout its distribution, such differences could also be related to the genetic variability and behavioral capacity (Cunha et al. 2010; Domit et al. 2021).

Anthropogenic noise can also affect dolphins' foraging activity, reducing prey capture as recently found for resident killer whales at the Salish Sea (Holt et al. 2021).

However, we did not find significant differences in active foraging for Guiana dolphins: there were similar proportions of feeding buzzes in both the port and the conservation areas. This result can indicate that social communication is more susceptible to elevated noise levels than foraging activity, as least in this population. Thus, the availability and movement of prey across the CEP may influence the active foraging of Guiana dolphins than noise levels alone. Similarly, noise level alone and the mere presence of boats seem not to affect the buzzing activity of Yangtze finless porpoise in busy port areas (Wang et al. 2014), neither bottlenose dolphins off eastern Scotland (Pirotta et al. 2014); instead, dolphins appear to temporarily interrupt their foraging when vessels pass by, staying in the area and quickly resuming foraging activities as vessels moved away. Nevertheless, repeated disruptions of foraging activity by vessel traffic can potentially reflect reduced energy intake (New et al. 2013; Noren et al. 2017).

This potential consequence is particularly concerning for animals using port areas since they tend to be exposed to many anthropogenic activities (de Sousa Pais et al. 2017; Marcondes et al. 2020) that vary in the distribution, type, and persistence of the noise produced (Hildebrand 2009; Miksis-Olds and Nichols 2016; Fredianelli et al. 2021). In our study, a combination of anchored ships, vessels traffic, and recurrent dredging-related activities resulted in a moderate positive correlation between noise levels and the number of vessels. Further, the noise emitted by these anthropogenic activities contributes to increasing background noise as they accumulate at low-, mid and high frequencies. (Bittencourt et al. 2014, 2020; Li et al. 2015; Veirs et al. 2016; Deconto et al. 2021; Vieira et al. 2021). Consequently, we found that the background noise at the port area constantly overlaps with the range of whistles' fundamental frequencies, up to the whistles' harmonics and echolocation signals. Such condition may influence the visual detection of these signals in the spectrograms and may also explain the reduced whistling rates found in the area. In contrast, the overall noise magnitude at the conservation area was lower, and noise overlapping occurred during specific occasions and for shorter periods, such as when vessels were moving near the dolphins or the hydrophone.

Bandwidth overlap between noise and the dolphins' signals raises the possibility of perceptual effects on dolphins' social behavior, such as auditory masking and cutting down communication space (Erbe et al. 2016; Putland et al. 2018). Several species, including Guiana dolphins, can compensate for these effects by increasing vocalizing rates (Buckstaff 2004; Nowacek et al. 2007; Leão Martins et al. 2016b; Bittencourt et al. 2017) and changing the frequencies and duration of acoustic signals (Rako Gospić and

Picciulin 2016; van Ginkela et al. 2018; La Manna et al. 2020). However, since shifts in acoustic efforts can cause energetic and metabolic costs (Noren et al. 2013; Holt et al. 2015), these animals may need to trade off acoustic changes in noisy environments (Pirota et al. 2014a) avoiding short and long-term health consequences. We, therefore, hypothesize that Guiana dolphins from PEC decrease their whistling rate in noisier conditions due to limited space for communication while maintaining active foraging as a food intake trade-off. They may also increase their whistles' fundamental frequencies to avoid masking in lower frequencies of background noise, in which greater sound intensity is concentrated. Moreover, cetaceans and other vertebrates can increase signals' amplitude under rising noise levels environments—a phenomenon known as Lombard Effect (Lombard, 1991; Holt et al. 2009; Hotchkin and Parks 2013; Kragh et al. 2019). Our results show that whistles' relative spectral energy increased at both port and conservation areas, according to the local noise levels. Since we could not access whistles' source levels with our recording system, whether Guiana dolphins increase whistles' amplitude in response to noise—particularly when simultaneously exposed to multiple noise sources—remains an open question for next studies.

Another biological effects of noise on cetaceans' behavior are avoidance of the noise source and displacement of the impacted area (Castellote et al. 2012; Rako et al. 2013; La Manna et al. 2014; Small et al. 2017). Noise from marine constructions, pile-driven operations, dredging, and other common activities around port areas can change the use of foraging areas, displace cetaceans from essential habitats, and induce the risk of permanent threshold shift (PTS) (Pirota et al. 2013, 2014a; Leunissen and Dawson 2018). For example, a critical case of almost eight-year displacement of a Guiana dolphin population with high site fidelity was associated with dredging, and pile-drivers activities in Babitonga Bay, southern Brazil (Cremer et al. 2009). Although the precise consequences of such avoidance behaviors remain to be fully understood, they likely include increased stress and reduced reproduction (Forney et al. 2017).

Guiana dolphins using the port area seem to tolerate acoustic disturbance rather than avoid or displace from the site as a possible trade-off given the high local availability of crucial resources, such as food (Wang et al. 2015; Possatto et al. 2017; Lin et al. 2020). However, persistent exposure to increasing anthropogenic noise levels raises concerns beyond short-term behavioral responses, such as physical or physiological hearing damage (e.g., Temporary and permanent thresholds shifts - TTS and PTS (Kastelein et al. 2015; NMFS 2018). Given that the Paranaguá Estuarine Complex is a coastal area

exposed to several other anthropogenic pressures (Cabral and Martins 2018; Miura and Noernberg 2020; Trevizani et al. 2021), the synergetic effect of these stressors can result in cumulative impacts on the local fauna (Sciences 2017; Marcondes et al. 2020; Lesage 2021). Some of these cumulative effects may reflect in poorer health conditions. Guiana dolphins from CEP have been showing changes in health conditions since 2007, such as skin lesions and pathological disease-related to immunosuppression. (Van Bressen et al 2008; Domiciano et al., 2016). Other species that inhabit the PEC, such as the critically endangered Franciscana dolphin (*Pontoporia blainvillei*) (Santos et al. 2009), should also be evaluated in terms of acoustic thresholds for behavior and physical/physiological disturbance in this habitat (NMFS 2018). To predict precise risk levels for hearing disruption for such species, future studies need to evaluate sound exposure levels considering noise pollution in coastal areas under the perspective of cumulative impacts, thus allowing the development of efficient mitigation actions.

#### *Implications for Conservation*

These results contribute to in both national and international levels by i) providing data that corroborate the achievement of goal 7 (Reducing and Mitigating Noise Pollution) of the National Plan for the Conservation of Endangered Cetaceans (ICMBIO, 2019); and ii) corroborating with essential data on current knowledge about Guiana dolphins' threats under the International Whaling Commission's framework of cumulative impact assessment (IWC/67b/SM/WP/12). We hope these findings can guide decision-making processes regarding conservation in areas already established as priorities for the species, thus strengthening new research into the context of the Decade of Ocean Science (2021–2030).

#### *Conclusion*

Our study on the acoustic changes of Guiana dolphins reflects eight years of exposure to an increasingly noisy environment. These findings suggest that the Guiana dolphin population from PEC uses different acoustic strategies to cope with noise under different background conditions and behavioral contexts. However, for a resident species living in fission-fusion social dynamics, short-term noise exposure can disrupt the role of acoustic communication in social activities, group cohesion, and cooperative foraging. Moreover, long-term exposure to degraded habitat conditions can induce fine-scale changes (e.g. slow progressive degeneration of auditory cells), and other health condition losses.



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## IMPLICAÇÕES PARA A CONSERVAÇÃO

Considerando que *Sotalia guianensis* é uma espécie classificada como “vulnerável” na lista vermelha da fauna ameaçada do Brasil e como “quase ameaçada” na lista internacional da IUCN (2018), os resultados desta dissertação de mestrado tem relevância nacional e internacional para a sua conservação. Em âmbito nacional, este estudo segue as ações prioritárias do Plano Nacional para Conservação de Cetáceos Marinhos Ameaçados de Extinção (ICMBIO, 2019), fornecendo dados que corroboram para o alcance do objetivo 7 (Diminuição e mitigação dos sons antropogênicos identificados como distúrbios sonoros sobre os cetáceos marinhos), por meio das metas específicas 7.1, 7.2 e 7.3. Internacionalmente, a *International Whaling Commission* (IWC/SC67b) elencou *S. guianensis* como uma das espécies prioritárias para a avaliação de impactos cumulativos. Neste contexto, os resultados desta dissertação contribuem diretamente para o atual conhecimento sobre o panorama de ameaças à espécie, bem como fornecem informações essenciais a serem considerados no desenvolvimento de Planos de Conservação de Espécies Ameaçadas de Extinção no âmbito da IWC.

Considerando a importância do Complexo Estuarino de Paranaguá – em especial as áreas portuária e da Ilha das Peças – como área de uso, desenvolvimento e sobrevivência, não só do boto-cinza (FILLA e MONTEIRO-FILHO, 2008; DOMIT et al., 2009; de MOURA et al., 2021), mas também de espécies criticamente ameaçadas de extinção, como a toninha, esta dissertação pode ainda:

- 1) servir de referência para futuras avaliações e monitoramento dos possíveis efeitos causados por impactos acústicos aos cetáceos na região;

- 2) contribuir positivamente para o planejamento, manejo e ordenamento territorial da região; e

- 3) fortalecer políticas públicas e tomadas de decisões relacionadas ao delineamento e efetivação de medidas mitigatórias para os atuais impactos, impulsionando assim novas pesquisas em contexto de sustentabilidade e resiliência para a Década do Oceano (2021-2031), como proclamado pela UNESCO.

## CONCLUSÃO GERAL

O comportamento acústico da população de botos-cinza do Complexo Estuarino de Paranaguá diferiu espaço-temporalmente, de acordo com a intensidade de interferência antrópica. Os níveis de ruído de fundo foram negativamente correlacionados com taxas de emissão de assobio, as quais foram significativamente menores no ambiente portuário (mais ruidoso), principalmente durante o período em que ocorreram atividades de dragagem na Baía de Paranaguá, para o qual os maiores níveis de ruído ambiental foram registrados.

As principais diferenças encontradas nos parâmetros acústicos dos assobios (frequências inicial e mínima, delta frequência e duração) refletem as diferentes respostas acústicas utilizadas pelos botos-cinza em resposta aos diferentes níveis de ruído de fundo entre as áreas, e seu aumento ao longo dos anos. No entanto, mudanças acústico-comportamentais induzidas por ruído ameaçam o papel da comunicação acústica em atividades sociais, coesão de grupo, forrageio cooperativo e outras demandas vitais associadas ao uso do som. A constante sobreposição de sinais biológicos por ruídos antropogênicos pode culminar ainda na redução de espaço acústico para a comunicação entre os animais, expondo-os a ameaças. Exposições em longo prazo a crescentes níveis de ruído antrópico podem induzir impactos permanentes, como perda auditiva.

Ambas as áreas de estudo são consideradas habitats-chave para *S. guianensis*, e neste sentido são prioritárias para a conservação da espécie. Apesar de expostas a níveis distintos de interferência antrópica, ao longo de oito anos, ambas sofreram aumento nos níveis de ruído ambiental em detrimento das atividades humanas. Não obstante, ambas as áreas estão expostas a múltiplos estressores ecossistêmicos (e.g. resíduos sólidos, contaminação química, poluentes emergentes, pesca incidental, turismo), que somados geram impactos cumulativos à fauna. Neste cenário, a integração entre ciência, governança, empreendedores e sociedade é essencial para o planejamento, desenvolvimento e aplicação de soluções efetivas de mitigação de impactos individuais e cumulativos, visando a conservação da espécie e do ambiente marinho.

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## ANEXO S - Supplementary data Appendix

**Table S1.** Kruskal-Wallis post hoc multiple comparisons of relative environmental noise levels acoustic parameters obtained through Dunn Test with Bonferroni correction. (CA = Conservation area and PA = Port area).

Parameter	Group 1	Group 2	n1	n2	Statistic	p-value	p-value adjusted
<i>FNB</i> frequency amplitude	CA (2012-2013)	PA (2012-2013)	40	40	4.11	$4.01 \cdot 10^{-5}$	$2.40 \cdot 10^{-4}$
	CA (2019-2020)	PA (2018-2020)	40	40	5.11	$1.80 \cdot 10^{-7}$	$1.08 \cdot 10^{-6}$
FNB relative energy between areas (2018-2020)	CA(2012-20213)	PA (2018-2020)	40	40	3.66	$2.57 \cdot 10^{-5}$	$1.54 \cdot 10^{-3}$
<i>FNB</i> relative energy among years	PA (2012-2013)	PA (2018-2020)	40	40	5.69	$1.29 \cdot 10^{-8}$	$1.29 \cdot 10^{-7}$
	PA (2019-2020)	PA (2018-2020)	40	40	-3.40	$6.84 \cdot 10^{-4}$	$6.84 \cdot 10^{-3}$
	PA (2012-2013)	PA (2019-2020)	40	40	2.60	0.009	0.09
<i>SNB</i> frequency amplitude	CA (2019-2020)	PA (2018-2020)	40	40	5.7	$1.37 \cdot 10^{-7}$	$8.25 \cdot 10^{-7}$

**Table S2.** Kruskal-Wallis post hoc multiple comparisons of whistles acoustic parameters obtained through Dunn Test with Bonferroni correction. (CA= Conservation area and PA= Port area).

Parameter	Group 1	Group 2	n1	n2	Statistic	p-value	p-value adjusted
Inicial frequency	CA (2012-2013)	PA (2012-2013)	1000	336	4.05	$5.10 \cdot 10^{-5}$	$3.06 \cdot 10^{-4}$
	CA (2019-2020)	PA (2018-2020)	867	200	5.10	$3.45 \cdot 10^{-7}$	$2.07 \cdot 10^{-6}$
Maximum frequency	CA (2012-2013)	PA (2012-2013)	1000	336	3.24	$1.18 \cdot 10^{-3}$	$7.06 \cdot 10^{-3}$
	CA (2019-2020)	PA (2018-2020)	867	200	5.73	$1.0 \cdot 10^{-8}$	$6.0 \cdot 10^{-8}$
Whistles spectral energy	CA (2012-2013)	CA (2019-2020)	1000	867	18.3	$1.56 \cdot 10^{-4}$	$9.38 \cdot 10^{-4}$
	PA (2012-2013)	PA (2018-2020)	336	200	11.7	$7.64 \cdot 10^{-32}$	$6.0 \cdot 10^{-3}$
	CA (2012-2013)	PA (2012-2013)	1000	336	4.57	$6.64 \cdot 10^{-6}$	$3.98 \cdot 10^{-5}$
	CA (2019-2020)	PA (2018-2020)	867	200	5.50	$3.57 \cdot 10^{-8}$	$2.20 \cdot 10^{-7}$
Whistles duration	CA (2012-2013)	CA (2019-2020)	1000	867	3.33	$8.76 \cdot 10^{-4}$	$5.26 \cdot 10^{-3}$
	CA (2012-2013)	PA (2012-2013)	336	200	3.20	$1.35 \cdot 10^{-3}$	$8.11 \cdot 10^{-3}$
	CA (2019-2020)	PA (2018-2020)	1000	336	-3.73	$1.94 \cdot 10^{-4}$	$1.16 \cdot 10^{-3}$
	PA (2012-2013)	PA (2018-2020)	867	200	-3.74	$1.81 \cdot 10^{-4}$	$1.10 \cdot 10^{-3}$