Variation in the diversity of *Sotalia* dolphin (Cetacea: Delphinidae) whistle repertoires at a continental scale

Gabriel Melo-Santos[1, 2,3,4,5,6,7]*, Sam F. Walmsley[1,8], Volker B. Deecke[9], Heloíse Pavanato[10], Braulio Leon-Lopez[11], Alexandre F. Azevedo[12], Diogo Destro Barcellos[13], Hector Barrios-Garrido[14,15,16], Amandine Bordin[17], Camila Carvalho de Carvalho[6, 18], Marta J. Cremer[19,20,21], Kareen De Turris-Morales[14, 22], Maria Claudia Diazgranados[23], Camila Domit[24], Nínive Espinoza-Rodríguez[14,25], José Lailson-Brito Jr[12]., Miriam Marmontel[6], Dalila Teles Leão Martins[26], Angélica Lúcia Figueiredo Rodrigues[2], Israel Maciel[3,4,5], Marcos César de Oliveira Santos[13], Natacha Aguilar de Soto[27], Rodrigo Hipólito Tardin[5], Marie Trone[28], Luz Helena Rodriguez-Vargas[29], Maria Alice dos Santos Alves[4], Maria Luisa da Silva[7,30], Laura J. May-Collado[31,32]*, Vincent M. Janik[1]*

[1]Sea Mammal Research Unit, Scottish Oceans Institute, University of St Andrews, Fife KY16 8LB, UK

[2]Biologia e Conservação de Mamíferos Aquáticos da Amazônia (BioMA), Universidade Federal Rural da Amazônia (UFRA), Belém, Pará, Brazil

[3] Programa de Pós-Graduação em Ecologia e Evolução, Universidade do Estado do Rio de Janeiro, Rio de Janeiro, CEP 20550-011, Brasil.

[4]Departamento de Ecologia, Universidade do Estado do Rio de Janeiro, Rio de Janeiro, CEP 20550-011, Brasil.

[5]Laboratório Ecologia e Conservação Marinha (ECoMAR), Departamento de Ecologia, Universidade Federal do Rio de Janeiro (UFRJ), Rio de Janeiro, Brazil.

[6]Grupo de Pesquisa em Mamíferos Aquáticos Amazônicos (GPMAA), Instituto de Desenvolvimento Sustentável Mamirauá (IDSM), Amazonas, Brazil

[7]Programa de Pós-Graduação em Teoria e Pesquisa do Comportamento, Núcleo de Teoria e Pesquisa do Comportamento, Universidade Federal do Pará (UFPA), Belém, Pará, Brazil

[8]Department of Biology, Dalhousie University, 1355 Oxford Street, Halifax, Canada, NS B3H 4J1, Canada.

[9]Institute of Science and Environment, University of Cumbria, Ambleside LA22 9HA, UK

[10] Proteus Research and Consulting Limited, Outram, New Zealand

[11]Museo de la Ballena y Ciencias del Mar, Laboratorio de Osteología, La Paz, B.C.S, Mexico

[12]Laboratório de Mamíferos Aquáticos e Bioindicadores "Profa. Izabel Gurgel", Faculdade de Oceanografia, Universidade do Estado do Rio de Janeiro (MAQUA/UERJ)

[13]Laboratório de Biologia da Conservação de Mamíferos Aquáticos, Instituto Oceanográfico, Universidade de São Paulo, Brazil.

[14]Laboratorio de Ecología General, Centro de Modelado Científico (CMC), Departamento de Biología; Facultad Experimental de Ciencias. La Universidad del Zulia. Maracaibo-Venezuela. 04002.

[15]TropWATER - Centre for Tropical Water and Aquatic Ecosystem Research. James Cook University. Australia.

[16]Beacon Development Comfpany (BDC). King Abdullah University of Science and Technology. Thuwal. Saudi Arabia.

[17] Group d`Etude et de Protection des Oiseaux en Guyane, Rémire-Montjoly, French Guiana, France

[18]Aquasis - Associação de Pesquisa e Preservação de Ecossistemas Aquáticos, Ceará, Brazil

[19]Laboratório de Ecologia e Conservação de Tetrápodes Marinhos e Costeiros, Universidade da Região de Joinville, São Francisco do Sul, Brazil.

[20]Programa de Pós-Graduação em Ecologia, Universidade Federal de Santa Catarina, Florianópolis, Brazil

[21]Programa de Pós- Graduação em Saúde e Meio Ambiente, Universidade da Região de Joinville, Joinville, Brazil.

[22]Fundación Fauna Caribe Colombiana (FFCC), Barranquilla, Colombia.

[23]Conservation International, Brussels, Belgium.

[24]Laboratório de Ecologia e Conservação, Centro de Estudos do Mar, Universidade Federal do Paraná, Brazil.

[25]Centro de Rescate de Especies Marinas Amenazadas (CREMA). San José, Costa Rica.

[26]Instituto Federal Catarinense, Campus Abelardo Luz, Santa Catarina, Brazil.

[27]University of La Laguna (ULL), Tenerife, Canary Islands, Spain

[28]Valencia College, Kissimmee, Florida, USA.

[29]Plymouth Marine Laboratory, Plymouth, England, United Kingdom

[30]Laboratório de Ornitologia e Bioacústica, Instituto de Ciências Biológicas, Universidade Federal do Pará (UFPA), Belém, Pará, Brazil.

[31]Department of Biology, University of Vermont, 109 Carrigan Drive, Burlington, VT 05405, USA

[32]Smithsonian Research Tropical Institute, Luis Clement Avenue, Bldg. 401, Tupper Balboa Ancon Panama, Republic of Panama.

*Corresponding authors: botogabriel@gmail.com, vj@st-andrews.ac.uk, lmaycoll@uvm.edu

Conflict of interest: no conflict of interest

Authors Contributions: Gabriel Melos-Santos, Laura J May-Collado, and Vincent M. Janik conceived the ideas and designed the methodological approaches for the study. All authors collected acoustic data for this study. Gabriel Melos Santos processed all acoustic data. Gabriel Melos-Santos and Sam F Walmsley performed data analysis in ARTwarp and iNext. Gabriel Melos-Santos, Laura J May-Collado, Vincent M. Janik and Sam F Walmsley lead the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

Statement of Inclusion: This study brings together scientists from most countries throughout the distribution of Sotalia dolphins in Central America and South America. All authors were regularly updated on the use of their recordings and were engaged in all aspects of the manuscript development.

Data availability statement: This repository contains the data and analysis scripts necessary to reproduce the results: https://github.com/swalmsley/Intraspecific-Sotalia/tree/main

Acknowledgements

We thank Anne Chao for guidance in using iNEXT software. We also thank Elizabeth Zwamborn for helpful comments on the manuscript. We thank Helena Katherine Gaffney, Ben Robert Davis, Nina McDonne, Emmin J. Cabral, Caitlin Durkin for helpin on the initial data processing. G. Melo-Santos is under a postdoctoral fellowship by Fundação Carlos Chagas Filho de Amparo à Pesquisa do Estado do Rio de Janeiro (FAPERJ) (PDR10, process E-26/204.304/2021). G. Melo-Santos recieved a scholarship through the Sandwich PhD Program (2015-2016) from the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES, Brazilian Ministry of Education) (process: PDSE 99999.003276/2015-05), and a PhD scholarship through 2013-2018. Funding was provided by the Swarosvki Foundation and World Wide Fund for Nature Brazil (Programa de Estimação de Abundância de Golfinhos de Rio da América do Sul). Fundação Carlos Chagas Filho de Amparo à Pesquisa do Estado do Rio de Janeiro (FAPERJ) currently supports the research of R.Tardin (JCNE process E-6/200.238/2023). The National Council for Scientific and Technological Development – CNPq and Fundação Carlos Chagas Filho de Amparo à Pesquisa do Estado do Rio de Janeiro - FAPERJ, process SEI 151239/2023-1 supported I. Maciel.

Abstract

1. While cetaceans are known to produce large and complex acoustic repertoires, the challenges of exhaustively sampling sounds at sea and counting relevant signals has precluded an understanding of their true repertoire diversity.
2. Here we quantify and compare the whistle repertoires of 16 populations in the genus *Sotalia*, belonging to two sister species, the Guiana dolphin (*Sotalia guianensis*) and the tucuxi (*Sotalia fluviatilis*), both endemic to Latin America. We used an adaptive resonance theory neural network combined with dynamic time-warping (ARTwarp) to categorize whistles into types. Applying recent methods from community ecology, we then determined the size of each population's repertoire and estimated β-diversity between sites while accounting for differences in sampling effort.
3. Our analysis included a total of 1,817 whistles from 16 populations along the Atlantic coast of Latin America and six in the Amazon Basin, distributed throughout the range of the genus *Sotalia*. Contrary to previous studies comparing acoustic parameters alone, this whistle contour type based analysis reveals significant differences in the composition of each population's repertoire, with 21% of types being unique to a single population. We also identified surprisingly large variation in repertoire size, estimating some populations to have up to 20-times more whistle types than others.
4. Our findings reveal substantial intraspecific variation in the whistle repertoires of two sister delphinid species. This suggests that repertoire diversity and size are strongly influenced by population-specific processes, rather than being constant within species. Further application of

these methods for comparison across unevenly sampled populations will open the door for new insights into the evolutionary and ecological drivers of odontocete repertoires, and can also be applied to other animal groups.

Keywords: intraspecific variation, acoustic behaviour, vocal repertoire, cetacean, Guiana dolphin, tucuxi

1 Introduction

Animals emit a diversity of sounds to communicate across a range of situations. The whole of these sounds is called acoustic repertoire. Only a tiny portion of vocal repertoires have been characterized, songbirds (Leighton & Birmingham 2021) and non-human primates among the best studied (McComb & Semple 2005). Acoustic repertoire size has been linked to mating success (Reid et al., 2004), territory size (Aweida, 1995), brain size (Sewall et al., 2013), cognition (Creanza et al., 2016), population dynamics (Hill & Pawley 2019), and urbanization (Deoniziak & Osiejuk 2019). A growing number of phylogenetic comparative studies show support for an evolutionary increase in vocal repertoire size with social ´complexity´ (non-human primates: McComb & Semple 2005, Bouchet et al., 2013; birds: Leighton & Birmingham 2021). Therefore, understanding the makeup of vocal repertoires can reveal how animals organize and respond to changes in their environment (Gagne et al., 2022), and the overall evolutionary basis of their communication (May-Collado et al., 2007a-b). In conservation, acoustic repertoires can be a useful tool because many species produce species-specific acoustic signals, that can be used to study temporal and spatial changes in distribution, habitat use and population density (Marques et al., 2013; Deoniziak & Osiejuk, 2019). In some cases, analysis of vocal repertoires can provide demographic information, as some signals within the vocal repertoire are individual-specific and can reveal cues on age, sex and body size (Charlton et al., 2007, 2012).

Compared to their terrestrial relatives, the breadth of the acoustic repertoires of most cetaceans remains poorly understood. However, because acoustic behaviour is a key part of their lives it can reveal information on their cognitive and social capacities (Martino et al., 2007). Several studies on odontocetes have shown that they are cognitively adept and socially complex with highly advanced acoustic production abilities (Janik, 2009, 2013). However, few studies have quantified the diversity of their signals in the context of vocal repertoires. This is a particularly true for the toothed whales. Their vocal learning abilities can result in repertoire variation between groups and populations (Janik & Slater, 2003).

Such patterns have been described for example in killer whales, *Orcinus orca* (Filatova et al., 2015), botos, *Inia araguaiensis* (Melo-Santos et al., 2020), pantropical spotted dolphins, *Stenella attenuate* (Rege-Colt et al., 2023), common dolphins *Delphinus* spp. (Oswald et al., 2021) and bottlenose dolphins *Tursiops truncatus* (May-Collado & Wartzok, 2008).

Many odontocetes produce narrowband, and frequency modulated tonal sounds referred as whistles for communication (Tyack, 2000). In bioacoustics studies, researchers typically extract frequency and temporal information from whistles often classified based on general patterns of their contours (i.e., constant, upsweep, downsweep, concave, concave, sine) (Bazúa-Durán & Au, 2004; Azevedo & Van Sluys, 2005). However, such simplified categorizations do not fully capture the diversity of the signals in their repertoire, and makes it challenging to compare repertoires across populations and species. Furthermore, because many toothed whales have broad distributions, studies are limited in their sampling efforts to a few, often-neighboring populations (Bazúa-Durán & Au, 2004; Ansmann et al., 2007). Such limitations make it very difficult to disentangle group-specific variation from population differences and to estimate the vocal repertoire representative of the species.

Here we investigate the whistle repertoire of two sister species, the Guiana dolphin (*Sotalia guianensis*) and the tucuxi (*S. fluviatilis*), both endemic to Latin America with relatively small distributions and contrasting habitats. The Guiana dolphin is found in coastal waters, from Nicaragua to Santa Catarina State, in Southern Brazil (Simões-Lopes, 1988; Edwards & Schnell, 2001), while the tucuxi is confined to the main tributaries of the Amazon Basin (Da Silva & Best, 1994, 1996). Phylogeographic and movement data suggest that these species are separated into multiple populations across riverine and marine habitats in South and Central America (Caballero et al., 2018)~~.~~ Studies indicate that Guiana dolphins produce whistles with simple contour shapes and a wide frequency range (1.38 to 48.4 kHz) (Azevedo & Van Sluys, 2005; May-Collado & Wartzok, 2009).

In this study, we present the first description of the acoustic repertoire of Guiana and tucuxi dolphins covering most of their distribution. First, we extract their whistle contours and second, we use an adaptive resonance theory neural network approach combined with dynamic time-warping (ARTwarp) (Deecke & Janik, 2006), to categorize whistle contours into types. We then apply statistical methods from community ecology to measure the compositional similarity and size of the whistle type repertoires of both dolphin species populations, accounting for differences in sampling effort. Finally, we explore whether differences in the composition or size of vocal repertoires are linked to geographical distance, species, habitat type, and group size.

2 Methods

Fieldwork

Recordings were collected between 1998 to 2017 at 16 sites across the distribution of both Guiana and tucuxi dolphins (Figure 1). Recordings were made with various recorders and frequency ranges (See electronic supplement, Table S1). Groups were defined according to (Quick & Janik, 2008), that is each individual was at least within 10m of another member of the group.

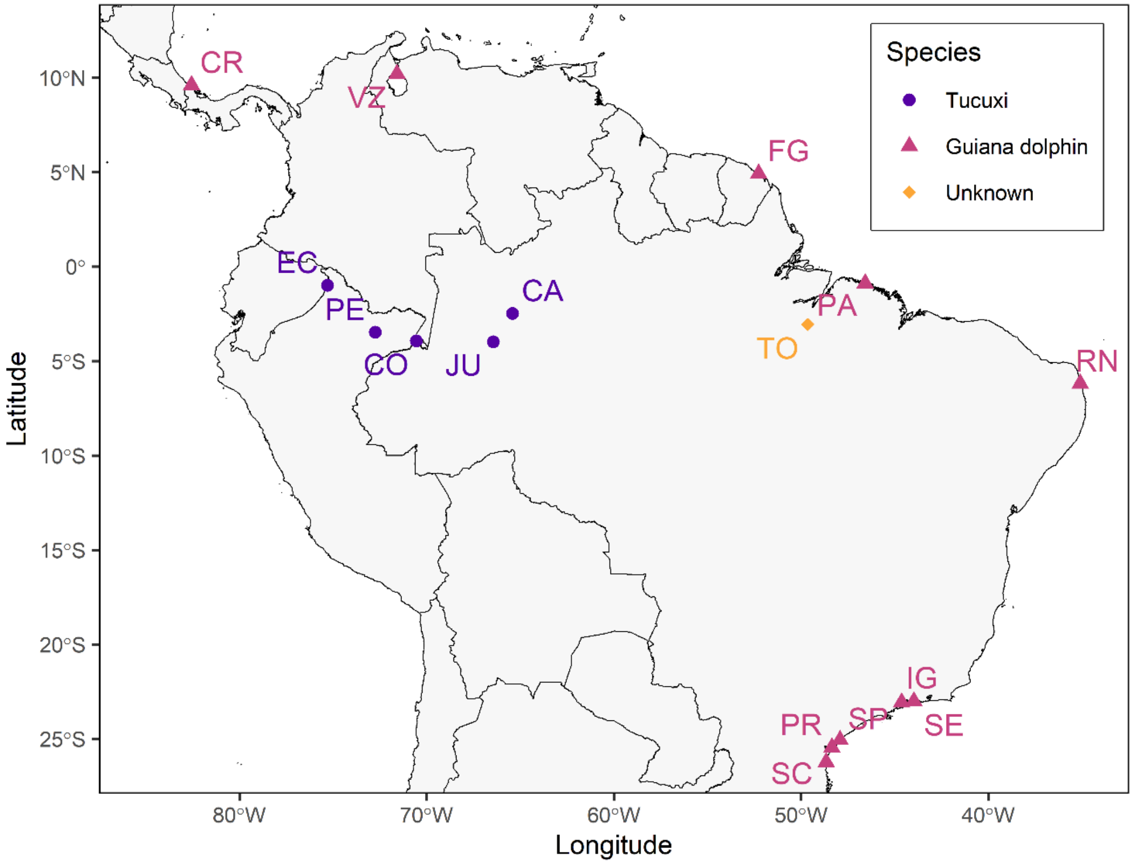

Figure 1 – Locations of study populations showing all detected, population-specific whistle types. Types are represented as time-warped reference contours from ARTwarp (varying durations) with frequency range 0-24 kHz for visualization. Only population-specific types representing 2 or more whistles are included.

While both Guiana and tucuxi dolphins are sympatric with other toothed whales along their distribution (Da Silva & Best,1994, 1996; May-Collado, 2010), obtaining single species recording was possible for most of our sites for this study. In addition, we applied statistical tools to avoid the inclusion of other species sounds in our study (see below).

Acoustic analysis

Spectrograms were constructed and examined in Raven Pro 1.5 (Cornell Laboratory of Ornithology, NY, USA), using a Hamming window, 1024 FFT, and 90% overlap. We selected the whistles with signal-to-noise ratio ≥ 6dB available from each population for comparison, up to an upper limit of 200 whistles (Table S2). We considered whistles to be tonal narrow-band signals of at least 100ms in duration. Whistles less than 200ms apart were considered as one whistle (Bazúa-Durán & Au, 2004). Whistles were selected across as large a range of different recording encounters as possible to reduce oversampling specific individuals or contexts. We used a Matlab routine, Beluga, to manually extract the frequency contour of each whistle (Deecke & Janik, 2006).

Automated whistle categorization

We used an adaptive resonance theory neural network combined with dynamic time-warping (ARTwarp) to group whistles into types, as described by Deecke & Janik (2006). This method categorizes frequency contours based on the vigilance, a critical similarity value. Because Guiana and tucuxi dolphin's whistles have relatively simple contours (Azevedo & Van Sluys, 2005) we used a vigilance of 96% to account for fine-scale differences in our classifications. While little is known about vocal signal perception in *Sotalia* dolphins, the 96% vigilance threshold is proven to optimally identify signature whistles of bottlenose dolphins (Deecke & Janik, 2006).

Rather than comparing single acoustic parameters like frequency or duration, ARTwarp groups whistles according to contour shape (i.e., pattern of frequency change over time), allowing for time-warping contours up to a factor of three, ensuring maximum overlap in the frequency domain (Deecke & Janik, 2006). This increases the chances of categorizing whistles into biologically significant categories. First, we applied ARTwarp to each population separately to quantify local repertoires, resampling whistle contours at a 10 ms resolution. For each whistle category identified, ARTwarp generates a reference contour that represents a typical whistle of that category. We then conducted a continent-wide analysis using the reference contours generated from each of the local analyses to identify whistle types similarities among populations. Contours were not re-sampled for the continent-wide analysis.

Acoustic signals cannot be reliably measured at a frequency greater than half the sampling rate of the recording (i.e., the Nyquist frequency) (Shannon, 1949). Thus, the ability to detect higher-frequency whistles from populations recorded at higher sampling rates could bias estimates of similarity and total repertoire size. To provide unbiased comparisons across populations, we applied our analyses to two data subsets: (1) using all recordings but excluding any whistles with frequency values greater than 22 kHz (hereafter "low-frequency dataset"), and (2) using only recordings with a sampling rate of at least 96 kHz, and excluding any whistles with frequency values greater than 48 kHz (hereafter "high-frequency dataset").

Patterns of whistle repertoires across populations

With incomplete sampling, populations with identical vocal repertoires will appear to have some unique signals by chance alone. We used a permutation test to determine if the measured proportion of population-specific whistle types was greater than expected by sampling processes alone. For this test, we limited our analysis to signal categories representing two or more recorded whistles. Over 1,000 permutations, we randomly shuffled whistle types across each population in the original dataset, calculating a two-tailed p-value as the proportion of permutations with a more extreme proportion of population-specific whistles than the observed value.

Compositional similarity between populations

Diversity measures in community ecology are typically weighted by the relative abundance of each species in an assemblage, beyond the simplest measure of species richness (Jost et al., 2011). Following recent methods in community ecology, we estimate diversity across a continuous parameter q, which indicates the measurement's sensitivity to the abundance of each species/type (Chao & Jost, 2015).

Considering diversity across non-0 orders of q achieves a similar effect as excluding singleton whistle types (categories represented by single whistle) and provides two additional benefits for our study. First, focusing on more common whistle types can be a way to safeguard against under-sampling: these types should almost always be well-represented, even in small samples. Second, rare types may include some interrupted whistles or sounds from other species. Beyond the general intuition that abundance matters when measuring compositional similarity, de-emphasizing the importance of these rare signals should help to identify the most representative sounds from each *Sotalia* population.

When applied to whistle repertoires, measures of between-population (*beta*) diversity, an estimate at $q = 0$ considers overlap in all whistle types, $q = 1$ emphasizes overlap in types weighted proportionally by their relative abundance, and $q = 2$ emphasizes overlap in very common or dominant types (weighted by squared relative abundances). For measures of within-population (*alpha*) diversity, orders of $q = 0$, 1, and 2 estimate the number of any, common, and very common types, respectively.

First, we estimated overall compositional similarity between populations using multiple-community extensions of the Horn ($q = 1$, similarity in common whistle types), and Morisita-Horn indices ($q = 2$, similarity in very common types) (Chao et al., 2012). These estimators are sensitive to undetected species/types, meaning that they are robust to under-sampling (Chao et al., 2008). Multiple-community measures consider the non-independence of pairwise differences, and thus are preferable to simply averaging pairwise comparisons (Chao et al., 2008; Diserud & Ødegaard, 2006). Standard errors and 95% confidence intervals were estimated by bootstrapping with 200 replications (Chao et al., 2008). We applied non-metric dimensional scaling (NMDS) analysis to distances estimated using the Horn and Morisita-Horn method to visualize pairwise similarity between populations. In addition, we used a scree plot of stress vs. number of dimensions to identify the optimal number of dimensions for each visualization. Similarities were estimated in SpadeR (Chao et al., 2016), converted into distance matrices, and passed to the metaMDS function in *vegan* for fitting (Oksanen et al., 2019). Hull polygons were overlaid for both species, allowing to qualitatively assess the importance of phylogeny on repertoire similarity. Using the same distance matrices (estimated Horn and Morisita-Horn), we then used Mantel tests with non-parametric (Spearman) correlation to assess if geographic distance predicts differentiation in repertoire composition. Geographic distance was calculated with the Haversine method.

Estimating and comparing repertoire sizes using iNEXT

To estimate and compare repertoire size across populations, we followed steps adapted from Chao *et al.* (2020) for estimating the diversity of species in assemblages with incomplete data. For each population, we 1) assessed sample completeness, 2) estimated total repertoire size, and 3) estimated restricted whistle repertoire size at a matched level of sample coverage. These analyses were carried out using iNEXT (Hsieh et al., 2016). As before, we focused on whistle repertoire size estimates using orders of $q = 1$ and $q = 2$, de-emphasizing importance of rare sounds.

The asymptotic estimators provided by Chao and Jost (2015) generate accurate measures of diversity, i.e., the number of whistle types a population of Guiana and tucuxi dolphins produces, that are robust to incomplete data with undetected signals. With severe under-sampling though (more often occurring for low orders of q), the estimate should be interpreted as a lower bound of true whistle type diversity (Chao & Jost, 2012). Sufficient sampling can be verified empirically by examining whether sample-size based rarefaction and interpolation curves begin to flatten when extended to twice the sample size (Magurran, 2004). We calculated asymptotic diversity estimates as a function of whistle type diversity order q, presenting the special cases of richness ($q = 0$), Shannon diversity ($q = 1$), and Simpson diversity ($q = 2$). When populations differ in whistle repertoire size, equal sample sizes will capture different *proportions* of each repertoire. Therefore, it is preferable to standardize by sample coverage (Chao & Jost, 2012). Sample coverage is defined as the proportion of acoustic signals a population produces that are detected in a given sample (Chao et al., 2020). Though this method does not provide an estimate of total repertoire size, it allows for statistically robust comparisons at c_{max}, the smallest coverage achieved in any single population when each is extrapolated to twice its original sample size.

Drivers of repertoire size across populations

We used simple linear regressions to explore relationships between repertoire size and several covariates. First, we estimated the relationship between whistle repertoire size and the number of encounters analysed for each population. Although our repertoire size estimates accounted for general differences in sampling effort, populations with fewer encounters may still be under-sampled if usage of specific whistle types varies according to behavioural state or group composition. Thus, a positive link between recording encounters and vocal diversity would be diagnostic of down-biased estimates for populations recorded fewer times. Next, to explore differences related to phylogenetic history, we fitted regressions of repertoire size on species. We also tested the relationship between repertoire size and habitat (riverine or marine), given the division between species across habitats, this was nearly identical to the species model, but included the Tocantins River population (where dolphin species identity is unknown). Lastly, we regressed repertoire size on median group size estimated for recordings for each population. For each predictor of interest, separate models were fitted using Shannon diversity and Simpson diversity as the response variable respectively, each estimated using the coverage-based rarefaction-extrapolation method at c_{max} (for q=1: 64.8% coverage, q=2: 70.0% coverage). We incorporated standard errors for each diversity estimate as measurement error in each model. This allowed us to propagate uncertainty in measures of vocal diversity through the analysis. All analyses were conducted in R (version 4.3.1) and organized into a reproducible pipeline using the *targets* package. Regressions were fit using the *brms* package using flat priors (Landau, 2021).

Results

Overview of recorded signals

We analysed 2,467 whistles from 16 populations distributed throughout the range of *Sotalia* dolphins (Fig. 1), 10 Guiana dolphin populations, five tucuxi dolphin populations, and one population in the Tocantins River (Brazil), where dolphin species is unknown. The number of whistles extracted, and encounters analysed varied by population (Table S2). Excluding whistles with maximum frequencies above 22 kHz resulted in a low-frequency dataset of 1,817 whistles from the 16 study populations. These 1,817 whistles were grouped into 281 whistle categories by the ARTwarp analysis. When excluding 155 categories represented by only a single whistle, we identified 126 whistle types across all populations, each type containing between two and 95 individual whistles (median 5 whistles). Of the 126 whistle types, 26 were exclusively produced by one population, and the remaining types were shared among 2-11s populations (median three populations). The proportion of whistle types that were unique to a single population (21%) was much greater than expected by sampling processes alone ($\text{mean}_{\text{permuted}}$= 1.9%, p = 0.001).

We were able to include 1,921 whistles in the high-frequency subset (sampling rate of 96 kHz or above and whistles up to 48 kHz). However, this required the complete exclusion of recordings from the Colombian Amazon (CO) and Lake Maracaibo, Venezuela (VZ) populations, which had a sampling rate of 44kHz and thus were not directly comparable. Given our interest in assessing large-scale geographic patterns, we focus primarily on the results of the low-frequency subset, with diversity estimates for the higher-frequency subset available in Table S4.

Compositional similarity of whistle repertoires between species and populations

Across all populations, we estimated Horn (q = 1) and Morisita-Horn (q = 2) similarities to be 0.51 ± 0.02 (s.e.) and 0.15 ± 0.01, respectively. We detected higher levels of differentiation among tucuxi populations (Horn: 0.36 ± 0.04, Morisita-Horn: 0.09 ± 0.02), while the Guiana populations in our study tended to have more similar vocal repertoires (Horn: 0.51 ± 0.03, Morisita-Horn: 0.17 ± 0.01). The finding of lower similarity values at higher orders of q suggests increased differentiation in the more commonly used whistle types. The NMDS based on common Horn and Morisita-Horn similarities converged with stress values of 0.20 and 0.18. Polygons overlaid onto each species overlapped substantially, suggesting limited separation of vocal repertoire composition between Guiana and tucuxi dolphins (Figure 2). Tocantins River population (TO), whose species membership is disputed, appeared to be most closely grouped with Guiana dolphin populations.

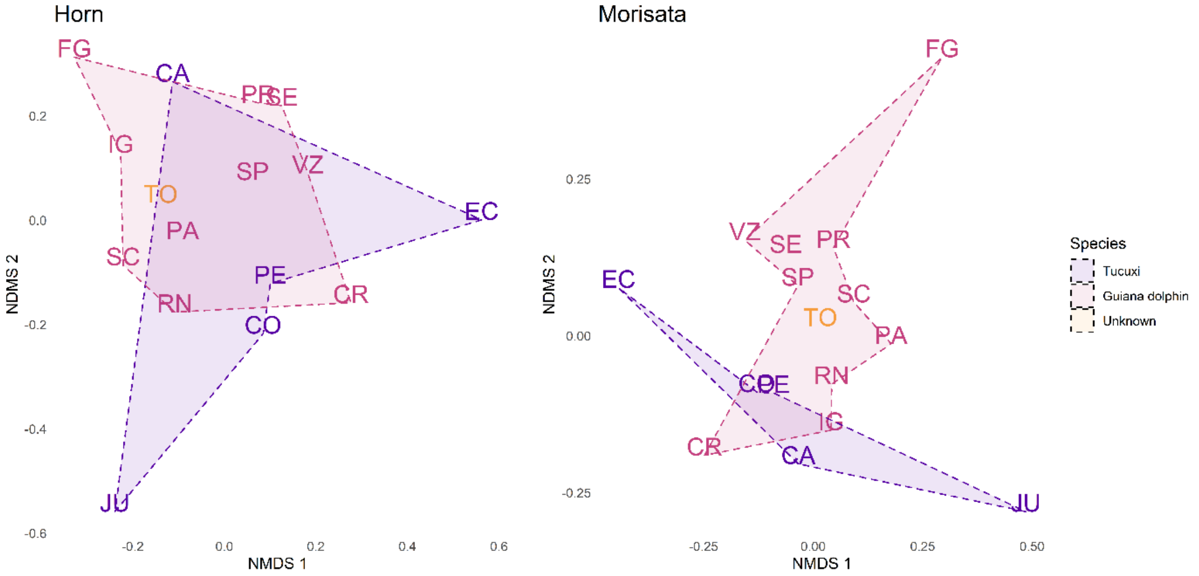

Figure 2 – Non-metric dimensional scaling of whistle repertoires recorded from *Sotalia* dolphin populations. Pairwise distances estimated with the Horn and Morisita-Horn methods in SpadeR (53), taking into account the relative abundance of whistle types. Polygons represent species-specific hulls.

When compositional similarity was weighted by whistle abundance ($q = 1$), we found a significant correlation between acoustic and geographic distances (Mantel test; $R = 0.21$, $p = 0.02$). A positive, but non-significant relationship was detected when focusing on abundant whistle types only ($q = 2$; $R = 0.11$, $p = 0.11$).

Variation in repertoire size between species and populations

As expected, estimates of repertoire diversity were more likely to plateau with higher orders of q (Figure 3). This supports the idea that comparisons of whistle repertoire composition and size would be most accurate for measures at $q = 2$ (Figure 3). Both the asymptotic and coverage-based estimates of diversity revealed significant differences in repertoire size among populations. While we were unable to completely account for possible individual- or context-specific whistle types having an influence of estimated vocal diversity, we found no strong relationship between the number of encounters analysed and repertoire size (Table S4), suggesting that context-specificity was not a major source of bias in these analyses. More generally, we found stark differences in repertoire size across populations, even when levels of sampling were similar. For example, the estimated repertoire of common whistle types of the Guiana dolphin population from Costa Rica (200 analysed whistles across nine encounters) exceeded that of the population from the Paranaguá Estuary (199 analysed whistles across eight encounters) by 40 whistle types (Figure 3). Regarding other potential drivers of estimated repertoire size, we found no clear relationships between habitat type or species and acoustic diversity (Figure 4B, 4C). Larger group sizes were associated with larger total repertoire size estimates, though this effect was quite uncertain (Figure 4D) and should only be interpreted as preliminary/weak evidence for a link between group size and vocal diversity.

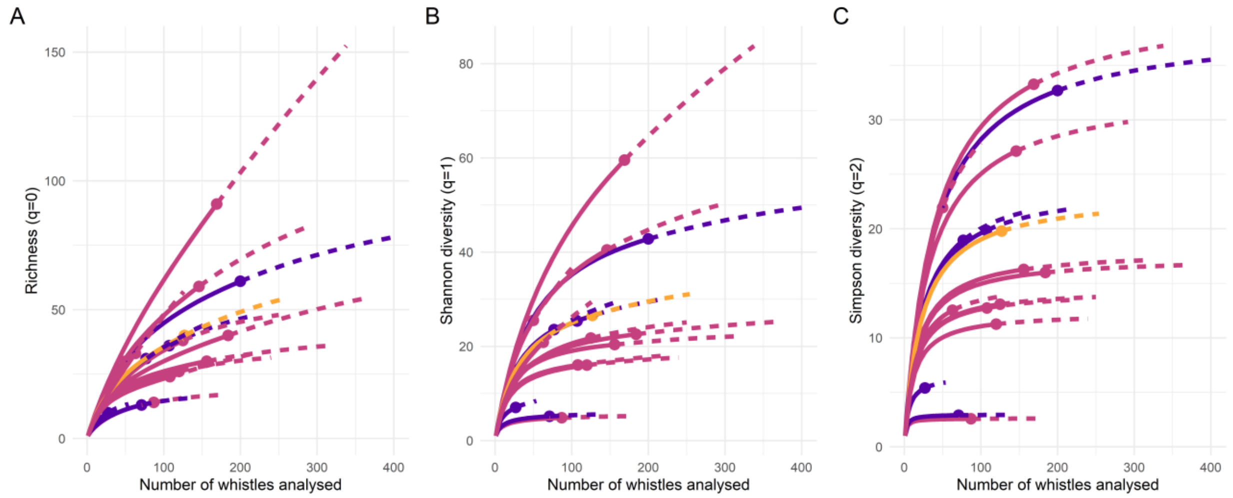

Figure 3 – Sample size-based rarefaction and extrapolation curves showing diversity estimates for each recording site organized by parameter q. Curves are extrapolated up to twice each population's original sample size. The levelling-off of curves for q = 2 (*right*) suggest that the estimates of the total number of dominant whistle types is accurate. Estimates for lower orders of q (*left*, *middle*), derived by extending curves to their asymptotes, may be down-biased, and are best interpreted as lower bounds of true diversity.

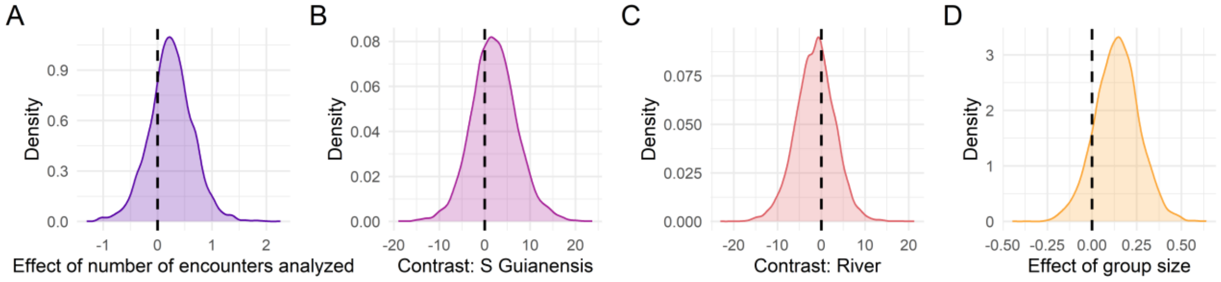

Figure 4 – Posterior distributions of effects of various predictors on whistle diversity, including the number of encounters analyzed (A), species (B), habitat (C), and group size (D). Diversity was calculated for common whistle types (q=2), and effects were estimated from models 2, 4, 6, and 8, respectively (Full details in supplement).

Discussion

Our analyses reveal substantial geographic variation in the diversity of tucuxi and Guiana dolphin whistle repertoires. Interpopulation similarity was partially explained by geographic distance among populations, along with some evidence of greater similarity within each species. Most striking was the magnitude of geographic differences in repertoire size, with some populations estimated to have up to 20-times more whistle types than others. This contrasts with previous studies, which have compared *Sotalia* whistles among populations using broad shape categories (e.g., upsweep, convex) or acoustic parameters (e.g., maximum frequency, duration) and found few differences. For example, Azevedo & Van Sluys (2005) were unable to distinguish between adjacent populations of *Sotalia* because of the way the range of parameters overlapped (Azevedo & Van Sluys, 2005; Rossi-Santos & Podos, 2006; May-Collado & Wartzok, 2009; Moron et al., 2019). Building on these efforts, we tested for differences when using a routine designed for identifying biological signal types in bottlenose dolphins (ARTwarp). The discovery of previously masked variation suggests that the combined application of ARTwarp and statistical tools from community ecology is a powerful approach for further investigation of whistle repertoires in cetaceans.

We identified substantial intraspecific variation in the composition of *Sotalia* whistle repertoires, with geographically closer populations having more similar repertoires. These differences were balanced by similarities among non-adjacent populations, possibly the result of their shared evolutionary history (Caballero et al., 2018), or convergence on similar signal types. Nevertheless, for cetacean species repertoire differences often do not correlate with geographic distance between sampled groups (Camargo et al., 2006; May-Collado & Wartzok, 2008, Filatova et al., 2012). Despite the overlap in the repertoires of the tucuxi and Guiana dolphins, non-metric dimensional scaling analyses revealed some clustering of populations from each species together. The two species diverged recently (Caballero et al., 2018), so that some similarities in their acoustic signals can be expected. Intra-species variation appeared to be especially high among the tucuxi populations. Caballero *et* al. (2018) proposed that there is greater genetic differentiation between tucuxi from the center of their distribution (parallel to the course of the Amazon River) to the smaller tributaries. This might explain, in part, the dissimilarity found in repertoires of riverine populations, as isolation might have driven genetic and acoustic differentiation along different tributaries. The most central locations in our dataset are CA and CO while others are smaller tributaries. However, little is known on the movements and site fidelity of *S. fluviatilis* other than they are more restricted to main river channels and have their habitats expanded during high water season (Martin et al., 2004; Faustino & Da Silva, 2004; Da Silva et al., 2010). Still there is no physical barrier among these rivers, with inter-population isolation being driven by differences in space-use and home ranges.

Compared to the tucuxi, the Guiana dolphins showed greater similarity in their repertoires. This was partially driven by the fact that five of the ten populations in our study were relatively close geographically (SE-SC), all located in the southern portion of the Brazilian Coast; these also presented lower values of acoustic diversity and repertoire size, except for the population from Ilha Grande Bay (IG) (Figure 1). According to Caballero et al. (2018), Brazilian Coast populations originated by a historic founder event with subsequent population expansion. As these are the populations at the extreme of the species distribution, they are probably the last to have diverged and thus have a more similar and less diverse whistle repertoire, this might be an effect of founder population dynamics, similar to the findings of Hill & Pawley (2019). These southern populations are also the ones under the greatest anthropogenic pressure (great harbours, traffic of large vessels, large urban centres). Fouda et al. (2018) reported the reduction in the complexity of bottlenose dolphins (*Tursiops truncatus*) whistles when noise levels are higher due to vessel traffic. While these authors did not correlate the decrease of complexity with reduction of diversity/repertoire size this may be the case of Guiana dolphin populations on the Southern Brazilian Coast. The relationship between noise levels and acoustic repertoires demands further investigation, especially for coastal species like the Guiana dolphins that are more vulnerable and closer to human activities.

We found some evidence that *Sotalia* dolphin populations which form larger groups also produce more whistle types. However, this must be interpreted with caution as the posterior distribution of the effect of group size contained non-trivial density below 0 (Figure D). The presence of substantial variation in repertoire size *not* explained by group size suggests that other factors may be more important drivers of acoustic diversity. For example, the population from Ilha Grande, Brazil, had a smaller repertoire size than the Costa Rica population, despite Ilha Grande having the largest group sizes in our study (Da Silva et al., 2010; Lodi & Hetzel, 1998). One such factor is the effect of interspecies interactions on a given vocal repertoire. Members of the Costa Rican population regularly interact with bottlenose dolphins (*Tursiops truncatus*), and May-Collado (2010) reported that Costa Rican Guiana dolphins produce whistles with intermediate characteristics when they are in mixed groups. Some of the population-specific types from Costa Rican Guiana dolphins show some qualities present in bottlenose dolphin whistles. Since cetaceans are known to be capable of vocal learning (Janik 2009, 2013), the Costa Rican population of Guiana dolphins could be incorporating bottlenose dolphin features in their repertoire through vocal learning. However, we are unaware of any tests of vocal learning in *Sotalia* dolphins. Similarly, to the

Costa Rican coast, Ilha Grande Bay is used by other whistling species including the Atlantic Spotted dolphin (*Stenella frontalis*) (Azevedo et al., 2010), rough-toothed dolphin (*Steno bredanensis*) (Lodi & Hetzel, 1999), and bottlenose dolphin (Lodi et al., 2008). However, these species are often observed in the outer waters of Ilha Grande Bay, away from the Guiana dolphins core area (Tardin et al., 2020), the only exception being the franciscana dolphin (*Pontoporia blainvillei*) (Neves et al., 2023). Thus, while mixed groups between Guiana dolphins and these other species have not been observed, acoustic contact may occur and, therefore, may contribute to the diversity of Ilha Grande Bay's population repertoire.

Multivariate analyses placed the Tocantins River population (TO) among the Guiana dolphins, showing that despite living in a riverine environment, their whistle repertoires were more like those of their marine relatives. This region of the Tocantins River is part of the Amazon Estuary, an area of possible sympatry between the two *Sotalia* species (Caballero et al., 2007). Guiana dolphins have tolerance to living in freshwater environments, given they are present in the Orinoco River (Caballero et al., 2017). Because the Tocantins River is part of the possible sympatry area between *Sotalia* species and given that *Sotalia guianensis* tolerates freshwater environments, the genetic identity of Tocantins dolphins is still unknown. Hybrid populations are also a possibility. Dos Santos et al. (2018) did not find any hybrids between the two species, but only analyzed samples from outer waters of the Amazonian Coast. Furthermore, the Amazon Estuary is a known hybrid zone for Amazonian and West Indian manatees (*Trichechus inunguis* and *Trichechus manatus*, respectively) (Lima et al., 2019). To the extent that variation in signal types can reveal population structure in other cetaceans, such as blue whales (*Balaenoptera musculus*) (Balcazar et al., 2015), we hypothesize that the Tocantins River population likely has more recent ties (genetic or otherwise) to coastal *S. guianensis*. Nonetheless, further investigation of the taxonomic identity of the Tocantins dolphins is necessary.

An important qualification in our findings is that tucuxi and Guiana share their habitat with other dolphin species that also produce whistles. Tucuxi overlap with botos (*Inia* spp.) throughout much of their range. Botos (*Inia* spp.) also produce whistles (May-Collado & Wartzok, 2007; Melo-Santos et al., 2019) although not frequently. Melo-Santos *et al*. (2020) also reported that *Inia araguaiaensis* produces bouts of downsweep whistles, but these too are uncommon. Guiana dolphins interact with marine species that tend to produce tonal signals (e.g., bottlenose dolphins). However, compared to botos which can be quite cryptic, it was possible to be more certain that Guiana dolphin recordings were collected from single-species groups. Nevertheless, given the extent of *Sotalia*'s overlap and interaction with sometimes difficult-to-spot dolphin species, we cannot exclude the possibility that some tonal sounds from other species were included in our analysis. However, excluding whistle categories represented by a single whistle and weighing measures of compositional similarity and repertoire size by the relative abundance of each signal type, the influence of any (presumably rare) whistles from other species should be minimal. Thus, we do not expect that this represents a significant source of bias in our findings.

Another factor that can lead to differences in dolphin whistle repertoires is the presence of signature whistles in many delphinid species (Janik & Sayigh, 2013; van Parjis & Corkeron, 2001; Gridley et al., 2014; Sayigh et al., 2013; Fearey et al., 2019). Signature whistles are individually distinctive whistle types that are mostly used by the signature owner or its close associates. Since it has been suggested that *Sotalia* dolphins have signature whistles (Figueiredo & Simão, 2009; Lima & Pendu, 2014), some of the patterns found could be caused by differences in signature whistles. Particularly, the significant relationship between group size and repertoire diversity is to be expected if signature whistles are used. However, only 26 of 126 whistle types were found in only one population. These could have been signature whistles but the remaining 79% of whistle types were found in more than one population which make them less likely to signal individual identities. Future studies are needed to investigate the occurrence of signature whistles in *Sotalia* dolphins.

Together, our results point to a striking amount of unexplained variation in repertoire sizes for this genus, which seems unrelated to simple covariates such as species or habitat. Beyond the weak association with group size, we expect that finer-grain variation in environmental heterogeneity, life history, social structure, demography, foraging strategies, and interspecies interactions are factors that have been reported to drive intraspecific variation in other traits of animal ecology and may underlie these intraspecific differences in *Sotalia* vocal repertoires (Isvaran, 2007; Herczeg & Välimäki, 2011; Schradin , 2013; Schradin et al., 2018; Jaeggi et al., 2021). While molecular data points to differences in home-ranges (movements) between *Sotalia* species (Caballero et al., 2018) and that Guiana dolphins present different foraging strategies along their distribution (Santos, 2010; Tardin et al., 2011; Tannure et al. 2020), much of these factors are poorly studied for most *Sotalia* populations. These findings reinforce the need to consider intraspecific diversity when using comparative approaches to understand variation in behaviour across species, as well as between and within populations (Herczeg & Välimäki, 2011; Jaeggi et al., 2021). Unravelling the causes of this impressive vocal diversity will require fundamental inquiries into the biology of these understudied species.

References

Ansmann, I. C., Goold, J. C., Evans, P. G. H, Simmonds, M. & Keith, S. G. (2007). Variation in the whistle characteristics of short-beaked common dolphins, *Delphinus delphis*, at two locations around the British Isles. *Journal of the Marine Biological Association of the United Kingdom*, 87, 19–26. https://doi:10.1017/S0025315407054963

Aweida, M. K. (1995). Repertoires, territory size and mate attraction in Western meadowlarks. *Condor*, 97, 1080–1083. https://doi.org/10.2307/1369552

Azevedo, A. F. & Van Sluys, M. (2005). Whistles of tucuxi dolphins (*Sotalia fluviatilis*) in Brazil: Comparisons among populations. *The Journal of the Acoustical Society of America*, 117, 1456–1664. https://doi:10.1121/1.1859232

Azevedo, A.F., Flach, L., Bisi, T.L., Andrade, L.G., Dorneles, P.R. & Lailson-Brito, J. (2010). Whistles emitted by Atlantic spotted dolphins (*Stenella frontalis*) in southeastern Brazil. *The Journal of the Acoustical Society of America*, 127, 2646-2651. https://doi.org/10.1121/1.3308469

Balcazar, N.E., Tripovich, J.S., Klinck, H., Nieukirk, S.L., Mellinger, D.K., Dziak, R.P. & Rogers, T.L. (2015). Calls reveal population structure of blue whales across the southeast Indian Ocean and the southwest Pacific Ocean. *Journal of Mammalogy*, 96, 1184-1193. https://doi.org/10.1093/jmammal/gyv126

Bazúa-Durán, C. & Au, W.W.L. (2004). Geographic variations in the whistles of spinner dolphins (*Stenella longirostris*) of the Main Hawaiian Islands. *The Journal of the Acoustical Society of America*, 116**,** 3757-3769. https://doi.org/10.1121/1.1785672

Bolgan, M., Pereira, B.P., Crucianelli, A., Mylonas, C.C., Pousão-Ferreira, P., Parmentier, E., Fonseca, P.J. & Amorim, M.C.P. (2020). Vocal repertoire and consistency of call features in the meagre *Argyrosomous regius* (Asso, 1801). *Plos one*,15, p.e0241792. https://doi.org/10.1371/journal.pone.0294105

Bouchet, H., Blois-Heulin, C., & Lemasson, A. (2013). Social complexity parallels vocal complexity: a comparison of three non-human primate species. *Frontiers in Psychology*, 4, 390. https://doi.org/10.3389/fpsyg.2013.00390

Boughey, M. J. & Thompson, N. S. (2019). Song variety in the brown thrasher (*Toxostoma rufum*). *Ethology*, 56, 47–58. https://doi.org/10.1111/j.1439-0310.1981.tb01283.x

Bradbury, J. W. & Vehrencamp, S. L. (2011). Principles of Animal Communication. (2nd ed.). Sinauer Associates.

Caballero, S., Trujillo, F., Vianna, J.A., Barrios-Garrido, H., Montiel, M.G., Beltrán-Pedreros, S., Marmontel, M., Santos, M.C., Rossi-Santos, M., Santos, F.R. & Baker, C.S. (2007). Taxonomic status of the genus *Sotalia*: species level ranking for “tucuxi”(*Sotalia fluviatilis*) and “costero”(*Sotalia guianensis*) dolphins. *Marine Mammal Science*, 23, 358-386. https://doi.org/10.1111/j.1748-7692.2007.00110.x

Caballero, S., Hollatz, C., Rodríguez, S., Trujillo, F. & Baker, C.S. (2018). Population structure of riverine and coastal dolphins *Sotalia fluviatilis* and *Sotalia guianensis*: patterns of nuclear and mitochondrial diversity and implications for conservation. *Journal of Heredity*, 109, 757-770. https://doi:10.1093/jhered/esy049

Caballero, S., Trujillo, F., del Risco, A., Herrera, O. & Ferrer, A. (2017). Genetic identity of *Sotalia* dolphins from the Orinoco River. *Marine Mammal Science*, 33, 1214-1223. https://doi.org/10.1111/mms.12422

Camargo, F. S., Rollo Jr, M. M., Giampaoli, V. & Bellini, C. (2006). Whistle variability in South

Atlantic spinner dolphins from the Fernando de Noronha Archipelago off Brazil. *The Journal of the Acoustical Society of America*, 120, 4071-4079. https://doi:10.1121/1.2359704

Chao. A., Jost, L., Chiang, S. C., Jiang, Y. H. & Chazdon, R. L. (2008) A two-stage probabilistic approach to multiple-community similarity indices. *Biometrics*, 64, 1178–1186. https://doi:10.1111/j.1541-0420.2008.01010.x

Chao A., Chiu, C. H., Hsieh, T. C. & Inouye, B. D. (2012a). Proposing a resolution to debates on diversity partitioning. *Ecology*, 93, 2037–2051. https://doi:10.1890/11-1817.1

Chao, A. & Jost, L. (2012b). Coverage-based rarefaction and extrapolation: Standardizing samples by completeness rather than size. *Ecology*, 93, 2533–2547. https://doi:10.1890/11-1952.1

Chao, A. & Jost, L. (2015). Estimating diversity and entropy profiles via discovery rates of new species. *Methods in Ecology and Evolution*, 6, 873–882. https://doi:10.1111/2041-210X.12349

Chao, A., Ma, K., Hsieh, T. & Chiu, C. (2016). SpadeR (Species-richness prediction and diversity estimation in R).

Chao, A., Kubota, Y., Zelený, D., Chiu, C.H., Li, C.F., Kusumoto, B., Yasuhara, M., Thorn, S., Wei, C.L., Costello, M.J. & Colwell, R.K. (2020). Quantifying sample completeness and comparing diversities among assemblages. *Ecological Research*, 35, 292-314. https://doi:10.1111/1440-1703.12102

Charlton, B. D., Reby, D. & McComb, K. (2007). Female perception of size-related formant shifts in red deer, *Cervus elaphus*. *Animal Behaviour*, 74, 707–714. https://doi:10.1016/j.anbehav.2006.09.021

Charlton, B. D., Reby, D., Ellis, W. A. H., Brumm J. & Fitch, W. T. (2012). Estimating the active space of male koala bellows: propagation of cues to size and identity in a eucalyptus forest. *Plos*

One, 7, 1–9. https://doi:10.1371/journal.pone.0045420

Creanza, N., Fogarty, L. & Feldman, M. W. (2016). Cultural niche construction of repertoire size and learning strategies in songbirds. *Evolutionary Ecology* 30, 285–305. https://doi:10.1007/s10682-015-9796-1

Da Silva, V. M. F. & Best, R. C. Tucuxi. (1994). *Sotalia fluviatilis* (Gervais, 1853) In SH Ridgway, R Harrison (Eds.) *Handbook of Marine Mammals, Volume 5, The first book of dolphins* (pp 43-69) London Academic Press

Da Silva, V. M. F. & Best, R. C. (1996). *Sotalia fluviatilis*. *Mammalian Species* 527, 1–7.

Da Silva, V.M.F., Fettuccia, D., Rodrigues, E.D.S., Edwards, H., Moreno, I.B., Moura, J.F., Wedekin, L.L., Bazzalo, M., Emin-Lima, N.R., Carmo, N.A.S., Siciliano, S. & Utreras V.B., (2010). Report of the working group on distribution, habitat characteristics and preferences, and group size. *Latin American Journal of Aquatic Mammals*, 31-38. https://doi.org/10.5597/lajam00151

Deecke, V. B. & Janik, V. M. (2006). Automated categorization of bioacoustic signals: Avoiding perceptual pitfalls. *The Journal of the Acoustical Society of America*, 117, 2470–2470. https://doi.org/10.1121/1.2139067

Deoniziak, K. & Osiejuk, T. S. (2019). Habitat-related differences in song structure and complexity in a songbird with a large repertoire. *BMC Ecology*, 19, 1–11. https://doi:10.1186/s12898-019-0255-7

Diserud O. H. & Ødegaard F. (2006). A multiple-site similarity measure, *Biology Letters*, 3, 20–22. https://doi.org/10.1098/rsbl.2006.0553

Dorph, A. & McDonald, P.G. (2017). The acoustic repertoire and behavioural context of the vocalisations of a nocturnal dasyurid, the eastern quoll (*Dasyurus viverrinus*). *PloS one*, 12, p.e0179337. https://doi.org/10.1371/journal.pone.0179337

Dos Santos, T.E., Da Silva, V.M., Do Carmo, N.A., Lazoski, C. & Cunha, H.A. (2018). *Sotalia* dolphins in their potential sympatry zone: searching for hybrids in the Amazonian estuary.*Journal of the Marine Biological Association of the United Kingdom*, 98, 1211-1215. https://doi.org/10.1017/S0025315418000401

Edwards, H. H. & Schnell, G. D. (2001). Status and ecology of *Sotalia fluviatilis* in the Cayos Miskito Reserve, Nicaragua. *Marine Mammal Science*, 17, 445–472. https://doi.org/10.1111/j.1748-7692.2001.tb00998.x

Faustino, C. & Da Silva, V. M. F. (2006). Seasonal use of Amazon floodplains by the tucuxi *Sotalia fluviatilis* (Gervais 1853), in the Central Amazon, Brazil. *Latin American Journal of Aquatic Mammals*, 5, 95-104. https://doi.org/10.5597/lajam00100

Fearey, J., Elwen, S. H., James, B. S., & Gridley, T. (2019). Identification of potential signature whistles from free-ranging common dolphins (*Delphinus delphis*) in South Africa. *Animal Cognition*, 22, 777-789. https://doi.org/10.1007/s10071-019-01274-1

Figueiredo, L.D., & Simão, S. M. (2009). Possible occurrence of signature whistles in a population of *Sotalia guianensis* (Cetacea, Delphinidae) living in Sepetiba Bay, Brazil. *The Journal of the Acoustical Society of America*, 126, 1563-1569. https://doi.org/10.1121/1.3158822

Filatova, O.A., Deecke, V.B., Ford, J.K., Matkin, C.O., Barrett-Lennard, L.G., Guzeev, M.A., Burdin, A.M. & Hoyt, E. (2012). Call diversity in the North Pacific killer whale populations: implications for dialect evolution and population history. *Animal behaviour*, 83, 595-603. https://doi:10.1016/j.anbehav.2011.12.013

Filatova, O.A., Samarra, F.I., Deecke, V.B., Ford, J.K., Miller, P.J. & Yurk, H. (2015). Cultural evolution of killer whale calls: background, mechanisms and consequences. *Behaviour*,152, 2001-2038. https://doi:10.1163/1568539X-00003317

Foote, A.D., Vijay, N., Ávila-Arcos, M.C., Baird, R.W., Durban, J.W., Fumagalli, M., Gibbs, R.A., Hanson, M.B., Korneliussen, T.S., Martin, M.D. & Robertson, K.M. (2016). Genome-culture coevolution promotes rapid divergence of killer whale ecotypes. *Nature communications*, 7, p.11693. https://doi.org/10.1038/ncomms11693

Fouda, L., Wingfield, J. E., Fandel, A. D., Garrod, A., Hodge, K. B., Rice, A. N. & Bailey, H. (2018). Dolphins simplify their vocal calls in response to increased ambient noise. *Biology letters*, 14, p.20180484. https://doi.org/10.1098/rsbl.2018.0484

Freeberg, T. M., Dunbar, R. I. M. & Ord, T. J. (2012). Social complexity as a proximate and ultimate factor in communicative complexity. *Philosophical Transactions of the Royal Society B*, 367, 1785–1801. https://doi:10.1098/rstb.2011.0213

Gagne, E., Perez-Ortega, B., Hendry, A. P., Melo-Santos, G., Walmsley, S. F., Rege-Colt, M., Austin, M & May-Collado, L. J. (2022). Dolphin communication during widespread systematic noise reduction-a natural experiment amid COVID-19 lockdowns. *Frontiers in Remote Sensing*, 3, 934608. https://doi.org/10.3389/frsen.2022.934608

Gridley, T., Cockcroft, V. G., Hawkins, E. R., Blewitt, M. L., Morisaka, T., & Janik, V. M. (2014). Signature whistles in free-ranging populations of Indo-Pacific bottlenose dolphins, *Tursiops aduncus*. *Marine Mammal Science*, 30, 512-527. https://doi.org/10.1111/mms.12054

Herczeg, G., & Välimäki, K. (2011). Intraspecific variation in behaviour: effects of evolutionary history, ontogenetic experience and sex. *Journal of Evolutionary Biology*, 24, 2434-2444. https://doi.org/10.1016/j.ecolind.2023.110711

Hsieh, T. C., Ma, K. H. & Chao, A. (2016). iNEXT: an R package for rarefaction and extrapolation of species diversity (Hill numbers). *Methods in Ecology and Evolution*, 7, 1451–1456. https://doi:10.1111/2041-210X.12613

Hill, S. D. & Pawley, M. D. M. (2019). Reduced song complexity in founder populations of a widely distributed songbird. *Ibis* ,161, 435–440. https://doi:10.1111/ibi.12692

Isvaran, K. (2007). Intraspecific variation in group size in the blackbuck antelope: the roles of habitat structure and forage at different spatial scales. *Oecologia*, 154, 435-444. https://doi.org/10.1007/s00442-007-0840-x

Jaeggi, A. V., Miles, M. I., Festa-Bianchet, M., Schradin, C., & Hayes, L. D. (2020). Variable social organization is ubiquitous in Artiodactyla and probably evolved from pair-living ancestors. *Proceedings of the Royal Society B*, 287, 20200035. https://doi.org/10.1098/rspb.2020.0035

Janik, V. M. & Slater, P. J. B. (2003). Tradition in mammalian and avian vocal communication In D.M. Fragaszy & S. Perry (Eds.), *The biology of traditions: models and evidence*. Cambridge University Press Cambridge, United Kingdom.

Janik, V.M. (2009). Acoustic communication in delphinids. *Advances in the Study of Behavior*, 40,123-157. https://doi.org/10.1016/S0065-3454(09)40004-4

Janik, V.M. (2013). Cognitive skills in bottlenose dolphin communication. *Trends in cognitive sciences*, 17, 157-159. https://doi:10.1016/j.tics.2013.02.005

Janik, V.M. & Sayigh, L.S., (2013). Communication in bottlenose dolphins: 50 years of signature whistle research. *Journal of Comparative Physiology A*, 199, 479-489. https://doi.org/10.1007/s00359-013-0817-7

Jost, L., Chao, A. & Chazdon, R. L. (2011). Compositional similarity and β (beta) diversity In

A.E. Magurran & B.J. McGill (Eds.), *Biological Diversity: Frontiers in Measurement and Assessment*, (pp.66–84). Oxford University Press, New York, NY.

King, S. L., Sayigh, L. S., Wells, R. S., Fellner,W. & Janik, V. M. (2013). Vocal copying of individually distinctive signature whistles in bottlenose dolphins. Proceedings of the Royal Society B: Biological Sciences, 280, 20130053–20130053, (2013). https://doi:10.1098/rspb.2013.0053

Landau W.M. (2021). The targets R package: a dynamic Make-like function-oriented pipeline toolkit for reproducibility and high-performance computing. *Journal of Open Source Software*, 6, 2959. https://doi.org/10.21105/joss.02959.

Leighton, G. M., & Birmingham, T. (2021). Multiple factors affect the evolution of repertoire size across birds. *Behavioral Ecology*, 32, 380-385. https://doi.org/10.1093/beheco/araa139

Lima, A., & Le Pendu, Y. (2014). Evidence for signature whistles in Guiana dolphins (*Sotalia guianensis*) in Ilhéus, northeastern Brazil. *The Journal of the Acoustical Society of America*, 136, 3178-3185. https://doi.org/10.1121/1.4900829

Lima, C.S., Magalhaes, R.F., Marmontel, M., Meirelles, A.C., Carvalho, V.L., Lavergne, A., Thoisy, B.D. & Santos, F.R. (2019). A hybrid swarm of manatees along the Guianas coastline, a peculiar environment under the influence of the Amazon River plume. *Anais da Academia Brasileira de Ciências*, 91. https://doi.org/10.1590/0001-3765201920190325

Lodi, L. & Hetzel, B. (1998) Grandes agregações do boto-cinza (Sotalia fluviatilis) na Baía da Ilha Grande, Rio de Janeiro. *Bioikos*, 12, 26-30.

Lodi L. & Hetzel B. (1999). Rough-toothed dolphin, *Steno bredanensis*, feeding behaviors in Ilha Grande Bay. *Biociências*, 7, 29-42.

Lodi, L., Wedekin, L. L., Rossi-Santos, M. R. & Marcondes, M. C. (2008). Movements of the bottlenose dolphin (*Tursiops truncatus*) in the Rio de Janeiro State, southeastern Brazil. *Biota Neotropica*, 8, 0-0. https://doi.org/10.1590/S1676-06032008000400020

Magurran, A.E. (2004) *Measuring biological diversity*.

Marino, L., Connor, R. C., Fordyce, R. E., Herman, L. M., Hof, P. R., Lefebvre, L., Lusseau, D., McCowan, B., Nimchinsky, E.A., Pack, A.A., Rendell, L., Reidenberg, J.S., Reiss, D., Uhen, M.D, Van der Gucht, E. & Whitehead, H. (2007). Cetaceans have complex brains for complex cognition. *PLoS biology*, 5, e139. https://doi.org/10.1371/journal.pbio.0050139

Marques, T.A., Thomas, L., Martin, S.W., Mellinger, D.K., Ward, J.A., Moretti, D.J., Harris, D. & Tyack, P.L. (2013). Estimating animal population density using passive acoustics. *Biological reviews*, 88, 287-309. https://doi:10.1111/brv.12001

Martin, A. R., Da Silva, V. M. F. & Salmon, D. L. (2004) Riverine habitat preferences of botos (*Inia geoffrensis*) and tucuxis (*Sotalia fluviatilis*) in the central Amazon. *Marine Mammal Science*, 20, 189-200. https://doi.org/10.1111/j.1748-7692.2004.tb01150.x

May-Collado, L. J., Agnarsson, I., & Wartzok, D. (2007a). Phylogenetic review of tonal sound production in whales in relation to sociality. *BMC Evolutionary Biology*, 7, 1-20. https://doi.org/10.1186/1471-2148-7-136

May-Collado, L. J., Agnarsson, I., & Wartzok, D. (2007b). Reexamining the relationship between body size and tonal signals frequency in whales: a comparative approach using a novel phylogeny. *Marine Mammal Science*, 23, 524-552. https://doi.org/10.1111/j.1748-7692.2007.02250.x

May-Collado, L. J. & Wartzok, D. (2007). The freshwater dolphin *Inia geoffrensis geoffrensis* produces high frequency whistles. *The Journal of the Acoustical Society of America*, 121, 1203-1212. https://doi.org/10.1121/1.2404918

May-Collado, L. J. & Wartzok, D. (2008). A Comparison of bottlenose dolphin whistles in the Atlantic Ocean: factors promoting whistle variation. *Journal of Mammalogy*. 89, 1229–1240. https://doi:10.1644/07-mamm-a-310.1

May-Collado, L. J. & Wartzok, D. (2009). A characterization of Guiana dolphin (*Sotalia guianensis*) whistles from Costa Rica: The importance of broadband recording systems. *The Journal of the Acoustical Society of America*, 125, 1202–1213. https://doi:10.1121/1.3058631

May-Collado, L. J. (2010). Changes in whistle structure of two dolphin species during interspecific associations. *Ethology*, 116, 1065-1074. https://doi.org/10.1111/j.1439-0310.2010.01828.x

McComb, K., & Semple, S. (2005). Coevolution of vocal communication and sociality in primates. *Biology letters*, 1, 381-385. https://doi.org/10.1098/rsbl.2005.0366

McElreath, R. (2020). *Statistical Rethinking2: A Bayesian Course with Examples in R and Stan* (2nd ed.).

Melo-Santos, G., Rodrigues, A.L.F., Tardin, R.H., de Sá Maciel, I., Marmontel, M., Da Silva, M.L. & May-Collado, L.J. (2019). The newly described Araguaian river dolphins, *Inia araguaiaensis* (Cetartiodactyla, Iniidae), produce a diverse repertoire of acoustic signals. *PeerJ*, 7, p.e6670. https://doi.org/10.7717/peerj.6670

Melo-Santos, G., Walmsley, S. F., Marmontel, M., Oliveira-Da-Costa, M. & Janik, V. M. (2020) Repeated downsweep vocalizations of the Araguaian river dolphin, *Inia araguaiaensis*. *The Journal of the Acoustical Society of America*, 147, 748-756. https://doi:10.1121/10.0000624

Moron, J.R., Lopes, N.P., Reis, S.S., Mamede, N., Reis, S.S., Toledo, G., Corso, G., Sousa-lima, R.S. & Andriolo, A. (2019). Whistle variability of Guiana dolphins in South America: Latitudinal variation or acoustic adaptation?.*Marine Mammal Science*,35, 843-874.

https://doi:10.1111/mms.12572

Neves, M. C., Vannuci-Silva, M., Montanini, G., Azevedo, A. F., Lailson-Brito, J., & Bisi, T. L. (2023). From narrow and overlapped to wide and segregated: The isotopic niche of a tropical cetacean community. *Ecological Indicators*, 154, 110711. https://doi.org/10.1016/j.ecolind.2023.110711

Oksanen, J., Blanchet, F.G., Friendly, M., Kindt, R., Legendre, P., McGlinn, D., Minchin, P.R., O'hara, R.B., Simpson, G.L., Solymos, P. & Stevens, M.H.H. (2019). Package 'vegan'.*Community ecology package, version*,2

Oswald, J.N., Walmsley, S.F., Casey, C., Fregosi, S., Southall, B. & Janik, V.M. (2021). Species information in whistle frequency modulation patterns of common dolphins. *Philosophical Transactions of the Royal Society B*, 376, p.20210046. https://doi.org/10.1098/rstb.2021.0046

Quick, N. J. & Janik, V. M. (2008). Whistle rates of wild bottlenose dolphins (*Tursiops truncatus*): Influences of group size and behavior. *Journal of Comparative Psychology*, 122, 305–311. https://doi.org/10.1037/0735-7036.122.3.305

R Core Development Team. (2021). *R: A language and environment for statistical computing*. R Foundation for Statistical Computing.

Reid, J.M., Arcese, P., Cassidy, A.L., Hiebert, S.M., Smith, J.N., Stoddard, P.K., Marr, A.B. & Keller, L.F. (2004). Song repertoire size predicts initial mating success in male song sparrows, *Melospiza melodia*. *Animal behaviour*, 68, pp.1055-1063. https://doi:10.1016/j.anbehav.2004.07.003

Riesch R., Barrett-lennard, L. G., Ellis, G. M., Ford, J. K. B. & Deecke, V. B. (2012). Cultural traditions and the evolution of reproductive isolation: ecological speciation in killer whales? *Biological Journal of the Linnean Society*, 106, 1–17. https://doi.org/10.1111/j.1095-

8312.2012.01872.x

Rossi-Santos M. R. & Podos J. (2006). Latitudinal variation in whistle structure of the estuarine dolphin *Sotalia guianensis*, *Behaviour*, 143, 347-364. https://doi:10.1163/156853906775897905

Santos, M. C. O. (2010). Guiana dolphins (*Sotalia guianensis*) displaying beach hunting behavior in the Cananéia estuary, Brazil: social context and conservation issues. *Brazilian Journal of Oceanography*, 58, 143-152.

Schradin, C. (2013). Intraspecific variation in social organization by genetic variation, developmental plasticity, social flexibility or entirely extrinsic factors. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 368, 20120346. https://doi.org/10.1098/rstb.2012.0346

Schradin, C., Hayes, L. D., Pillay, N., & Bertelsmeier, C. (2018). The evolution of intraspecific variation in social organization. *Ethology*, 124, 527-536. https://doi.org/10.1111/eth.12752

Sewall, K. B., Soha, J. A., Peters, S. & Nowicki, S. (2013). Potential trade-off between vocal ornamentation and spatial ability in a songbird. *Biology Letters* 9, 16–18. https://doi:10.1098/rsbl.2013.0344

Shannon, C.E. (1949). Communication in the presence of noise. *Proceedings of the IRE*, 37, 10-21. https://doi.org/10.1109/JRPROC.1949.232969

Tannure, N.C., Barbosa, F.S., Barcellos, D.D., Mattiuzzo, B., Martinelli, A., Campos, L.B., Conversani, V.R. & Marcos, C.D.O. (2020). Acoustic description of beach-hunting Guiana dolphins (*Sotalia guianensis*) in the Cananéia Estuary, Southeastern Brazil. *Aquatic Mammals*, 46, 11-20. https://doi.org/10.1578/AM.46.1.2020.11

Tardin, R. H., Especie, M. A., Nery, M. F., D'Azeredo, F. T. & Simão, S. M. (2011). Coordinated feeding tactics of the Guiana dolphin, *Sotalia guianensis* (Cetacea: Delphinidae), in Ilha Grande Bay, Rio de Janeiro, Brazil. *Zoologia (Curitiba)*, 28, 291-296. https://doi.org/10.1590/S1984-46702011000300002

Tardin, R. H., Maciel, I. S., Espécie, M. A., Melo-Santos, G., Simao, S. M., & Alves, M. A. S. (2020). Modelling habitat use by the Guiana dolphin, *Sotalia guianensis*, in south-eastern Brazil: Effects of environmental and anthropogenic variables, and the adequacy of current management measures. *Aquatic Conservation: Marine and Freshwater Ecosystems*, 30, 775-786. https://doi.org/10.1002/aqc.3290

Tyack, P. L. (2000). Functional aspects of cetacean communication. In J. Mann, R.C. Connor, P.L. Tyack, H. Whitehead H (Eds.) *Cetacean societies: field studies of dolphins and whales*. (pp 270-307) The University of Chicago Press.

van Parijs, S. M., & Corkeron, P. J. (2001). Evidence for signature whistle production by a Pacific humpback dolphin, *Sousa chinensis*. *Marine Mammal Science*, 17, 944-949. http://dx.doi.org/10.1111/j.1748-7692.2001.tb01308.x

Electronic supplement

Variation in the diversity of *Sotalia* dolphin (Cetacea: Delphinidae) whistle repertoires at a continental scale.

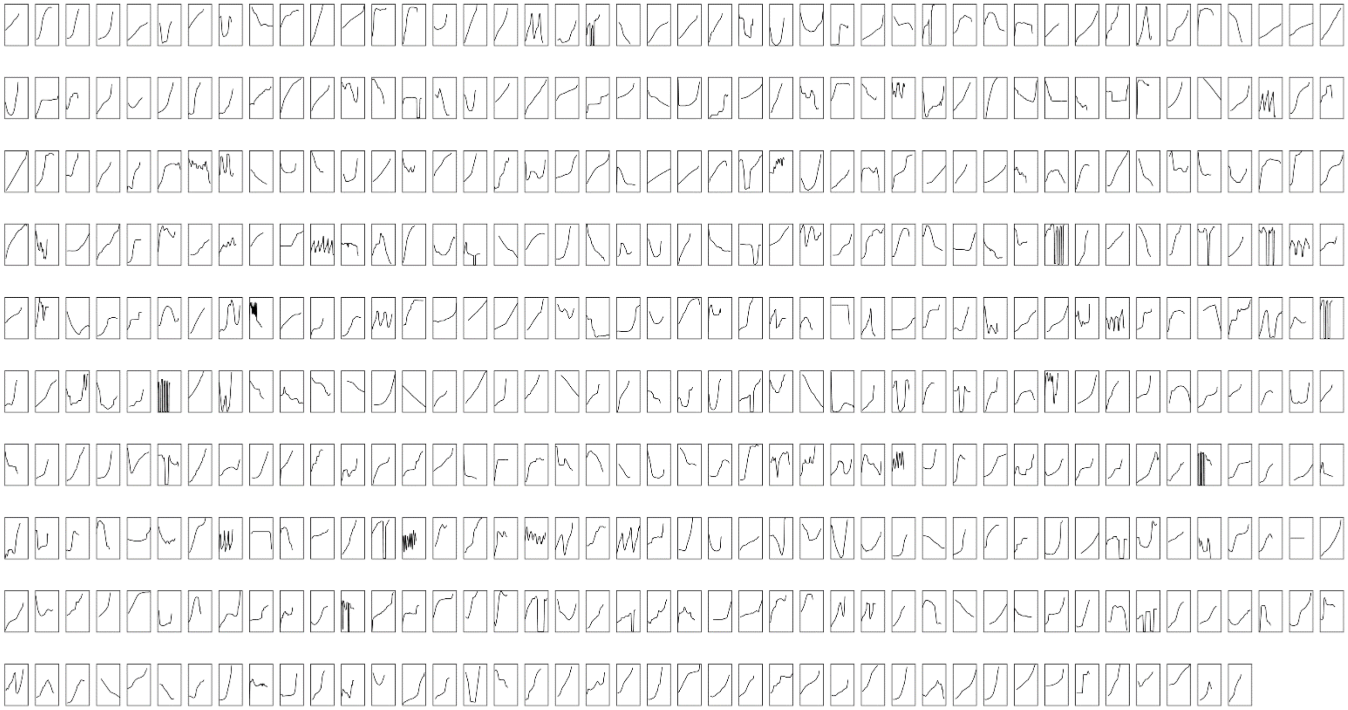

Figure S1 – Reference contours resulted from the continent-wide analysis of *Sotalia* dolphins whistle repertoire.

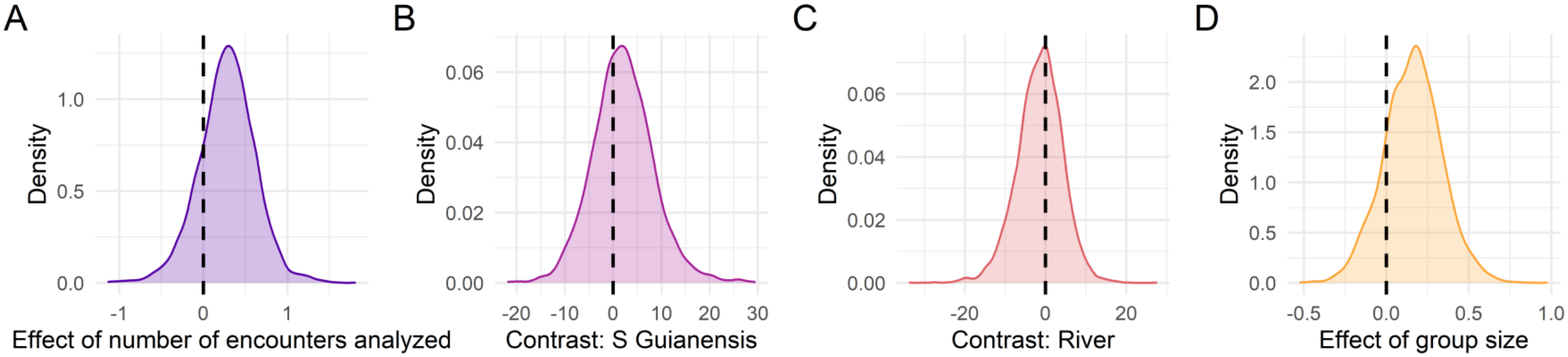

Figure S2 – Reference contours. Posterior distributions of effects of various predictors on whistle diversity, including the number of encounters analyzed (A), species (B), habitat (C), and group size (D). Diversity was calculated for common whistle types (q=1), and effects were estimated from models 1, 3, 4, and 7, respectively.

Table S1 – Summary of recording locations across 16 sites and data sets analysed in this study, regarding Guiana (*Sotalia guianensis*) and tucuxi (*S. fluviatilis*) dolphins.

Recording Locality	**Recording system**	**Sampling rate (kHz)**
Gandoca-Manzanillo, Costa Rica	RESON hydrophone + AVISOFT Recorder + Ultra Sound Gate 116	400-500
French Guiana	Cetacean Research Technology C75 + TASCAM DR 680 Recorder	192
Lake Maracaibo, Venezuela	Cetacean Research Technology CR1 + M-Audio Micro Tack II Recorder	44.1
Pará Coast, Brazil	Cetacean Research Technology CR1 + Tascam DR-44WL Recorder	96
Tocantins River, Brazil	Cetacean Research Technology CR1 + Tascam DR-44WL Recorder, Sound Trap ST300 HF autonomous recorder	96, 576
Central Amazon, Brazil	Cetacean Research Technology SQ26-H1 + Zoom Recorder	96
Juruá River, Brazil	Cetacean Research Technology CR1 + Tascam DR-44WL Recorder	96
Colombian Amazon, Colombia	Offshore Acoustics + Maranrz CP430 Recorder	44.1
Peruvian Amazon, Peru	Cetacean Research Technology CR3 + I/O Tech A-to-D converter	500
Napo River, Ecuador	RESON hydrophone + AVISOFT Recorder + Ultra Sound Gate 116	400-500
Curral Bay/Pipa, Rio Grande do Norte, Brazil	Cetacean Research Technology C55 + M-Audio Microtrack II Recorder	96
Sepetiba Bay, Rio de Janeiro, Brazil	Celesco LC-10 + Sony WM-D3, Cetacean Research Technology C54 hydrophone + M-Audio Microtrack II	44.1, 96
Ilha Grande Bay, Rio de Janeiro, Brazil	Cetacean Research Technology + Marantz PMD 671 Recorcer, Sound Trap ST300 HF autonomous recorder	576

Cananéia Estuary, São Paulo, Brazil	DSG-ST HF Loggerhead Instruments + Sony Linear PCM Recorder PCM-M10, Reason -208 + Sony Linear PCM Recorder PCM-M10	288
Paranagua Estuarine Complex, Paraná, Brazil	Cetacean Research Technology C55S + Fostex FR2 Recorder	192
Babitonga Bay, Santa Catarina	Reson TC 4032 + Fostex FR2 Recorder	192

Table S2. Summary of tucuxi and Guiana dolphin populations analyzed, organized by species. NR indicates "not recorded".

Population	Abbreviation	Species	Encounters analysed	Median group size	Whistles extracted	Min. Nyquist 22 kHz	Min. Nyquist 48 kHz	Sampling rates (kHz)
Central Amazon, Brazil	CA	*S. fluviatilis*	9	4	112	107	112	96
Colombian Amazon	CO	*S. fluviatilis*	NR	NR	200	200	0	44
Napo River, Ecuador	EC	*S. fluviatilis*	2	5	30	27	30	500
Juruá River, Brazil	JU	*S. fluviatilis*	2	8	83	71	83	96
Peruvian Amazon	PE	*S. fluviatilis*	NR	NR	90	77	90	96

Costa Rica	CR	*S. guianensis*	9	7	200	169	200	300, 384, 375
French Guiana	FG	*S. guianensis*	3	10	114	87	114	96, 192
Ilha Grande Bay, Rio de Janeiro, Brazil	IG	*S. guianensis*	6	80	200	50	174	96, 576
Pará Coast, Brazil	PA	*S. guianensis*	5	30	199	125	199	96
Paranaguá Estuary, Paraná, Brazil	PR	*S. guianensis*	8	3	198	119	198	192
Rio Grande do Norte Coast, Brazil	RN	*S. guianensis*	2	3	185	146	185	96
Babitonga Bay, Santa Catarina, Brazil	SC	*S. guianensis*	23	9	200	108	198	176, 192
Sepetiba Bay, Rio de Janeiro, Brazil	SE	*S. guianensis*	7	20	199	184	22	44, 96

Cananéia Estuary, São Paulo, Brazil	SP	*S. guianensis*	3	2	198	156	120	48, 96
Lake Maracaibo, Venezuela	VZ	*S. guianensis*	7	2	63	63	0	44
Tocantins River, Brazil	TO	*Sotalia* sp.	12	6	195	127	195	576

Table S4 – Estimates of key diversity measures for Sotalia whistle repertoires, organized by increasing sensitivity to rare signal types with parameter q. Only whistles below the standardized Nyquist frequency of 22 kHz are included here.

Population	Repertoire size (asymptotic estimate)			Repertoire size at 64.8% coverage		
	q = 0	q = 1	q = 2	q = 0	q = 1	q = 2
Tucuxi						
Central Amazon, Brazil	56	34	24	19	16	14
Colombian Amazon	92	55	39	32	27	23
Napo River, Ecuador	16	10	6	5	5	4
Juruá River, Brazil	17	6	3	3	2	2
Peruvian Amazon	73	37	25	20	17	14
Guiana dolphin						
Costa Rica	419	145	41	132	76	36
French Guiana	18	5	3	2	2	2
Ilha Grande Bay, Rio de Janeiro, Brazil	128	64	38	41	33	26
Pará Coast, Brazil	52	28	14	18	14	10
Paranaguá Estuary, Paraná, Brazil	34	19	12	11	9	8
Rio Grande do Norte Coast, Brazil	110	60	33	37	30	22
Babitonga Bay, Santa Catarina, Brazil	69	21	14	11	9	8
Sepetiba Bay, Rio de Janeiro, Brazil	76	28	17	15	13	10

Cananéia Estuary, São Paulo, Brazil	40	23	18	14	12	10
Lake Maracaibo, Venezuela	199	56	15	57	29	14
Unknown sp.						
Tocantins River, Brazil	70	35	23	20	17	14

Table S5 – Higher-frequency dataset: Estimates of key diversity measures for Sotalia whistle repertoires, organized by increasing sensitivity to rare signal types with parameter q. Only whistles below the standardized Nyquist frequency of 48 kHz are included here.

Population	Repertoire size (asymptotic estimate)			Repertoire size at 70.0 % coverage		
	$q = 0$	$q = 1$	$q = 2$	$q = 0$	$q = 1$	$q = 2$
Tucuxi						
Central Amazon, Brazil	72	38	25	24	20	16
Napo River, Ecuador	20	10	6	7	6	4
Juruá River, Brazil	24	7	3	5	4	3
Peruvian Amazon	98	45	27	29	23	18
Guiana dolphin						
Costa Rica	410	159	49	176	98	43
French Guiana	35	11	4	10	6	4
Ilha Grande Bay, Rio de Janeiro, Brazil	242	115	54	96	65	43
Pará Coast, Brazil	107	51	28	35	27	20
Paranaguá Estuary, Paraná, Brazil	69	35	21	23	18	15

Rio Grande do Norte Coast, Brazil	165	83	44	59	45	32
Babitonga Bay, Santa Catarina, Brazil	193	45	28	25	21	18
Sepetiba Bay, Rio de Janeiro, Brazil	20	16	11	12	10	8
Cananéia Estuary, São Paulo, Brazil	57	40	30	27	23	19
Unknown sp.						
Tocantins River, Brazil	106	52	33	34	27	22

Model 1 results

Term	Estimate	*SE*	CI-Lower	CI-Upper
Intercept	9.02	3.07	3.41	15.59
number.encounters.analyzed	0.28	0.34	-0.41	0.93

Model 2 results

Term	Estimate	*SE*	CI-Lower	CI-Upper
Intercept	8.93	3.57	2.04	16.34
number.encounters.analyzed	0.25	0.40	-0.55	1.03

Model 3 results

Term	Estimate	*SE*	CI-Lower	CI-Upper
Intercept	13.33	4.90	3.34	23.11
SpeciesSotaliaguianensis	2.07	6.38	-10.08	15.54

Model 4 results

Term	Estimate	*SE*	CI-Lower	CI-Upper
Intercept	10.98	3.92	3.07	18.57
SpeciesSotaliaguianensis	2.10	5.03	-7.38	12.57

Model 5 results

Term	Estimate	*SE*	CI-Lower	CI-Upper
Intercept	15.41	3.62	8.76	23.04
HabitatRiverine	-1.45	5.68	-13.09	9.15

Model 6 results

Term	Estimate	*SE*	CI-Lower	CI-Upper
Intercept	13.22	2.70	8.16	18.73
HabitatRiverine	-1.42	4.51	-10.44	7.22

Model 7 results

Term	Estimate	*SE*	CI-Lower	CI-Upper
Intercept	11.30	3.26	5.06	18.01
as.numericmedian_group	0.16	0.18	-0.19	0.51

Model 8 results

Term	Estimate	*SE*	CI-Lower	CI-Upper
Intercept	9.76	2.55	4.82	14.95
as.numericmedian_group	0.14	0.12	-0.11	0.38