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Acoustic characteristics of whistles associated with the two-voice phenomenon in two oceanic delphinids

Orientador: Prof. Dr. Artur Andriolo

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Natália Rodrigues de Souza Soares

Acoustic characteristics of whistles associated with the two-voice phenomenon in two oceanic delphinids

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“Não somos mais
Que uma gota de luz
Uma estrela que cai
Uma fagulha tão só
Na idade do céu
Não somos o que queríamos ser
Somos um breve pulsar
Em um silêncio antigo
Com a idade do céu
Calma!
Tudo está em calma”

Música: A idade do céu
Compositor: Jorge Drexler
Intérprete: Paulinho Moska

RESUMO

Os golfinhos possuem um sofisticado sistema de comunicação acústica capaz de produzir sons tonais e pulsados. A emissão simultânea de dois sinais acústicos distintos pode ocorrer devido à presença de dois pares de lábios fônicos. Embora esse fenômeno tenha sido relatado em algumas espécies de delfinídeos, sua função biológica permanece pouco compreendida. Este estudo descreve a produção simultânea de assobios e cadeias de cliques em duas espécies de delfinídeos, *Stenella attenuata* e *Steno bredanensis*, e compara os padrões de modulação e os parâmetros acústicos dos assobios emitidos de forma simultânea e não simultânea. Os dados acústicos foram coletados ao longo da margem continental brasileira utilizando uma matriz rebocada de hidrofones com 300 m de comprimento. Os padrões de modulação e os parâmetros acústicos dos assobios (frequência inicial, final, máxima, mínima, de pico, central, largura de banda e duração) foram extraídos para comparar assobios não simultâneos e simultâneos em *Stenella attenuata* (277 e 125 assobios, respectivamente) e *Steno bredanensis* (743 e 58 assobios, respectivamente). As diferenças na distribuição dos contornos dos assobios foram avaliadas por meio de testes do qui-quadrado, enquanto os parâmetros acústicos foram comparados por meio de PERMANOVA, seguida de análises de permutação. Os resultados indicaram que os padrões de modulação dos assobios não diferiram entre emissões simultâneas e não simultâneas. No entanto, foram observadas diferenças significativas nos parâmetros acústicos. Em *Stenella attenuata*, essas diferenças estiveram principalmente restritas à frequência máxima, à largura de banda e à duração dos assobios. Em *Steno bredanensis*, todos os parâmetros acústicos avaliados diferiram significativamente entre os grupos. Esses resultados demonstram que o fenômeno de duas vozes está associado a alterações nos parâmetros acústicos dos assobios, preservando, entretanto, seus padrões de modulação.

Palavras-chave: Bioacústica, odontocetos, monitoramento acústico passivo, assobios, fenômeno de duas vozes, comunicação acústica.

ABSTRACT

Dolphins have a sophisticated acoustic communication system capable of producing tonal and pulsed sounds. The simultaneous emission of two distinct acoustic signals can occur due to the presence of two pairs of phonic lips. Although the phenomenon has been reported in some species of delphinids, its biological function remains poorly understood. This study reports the simultaneous production of whistle-click trains by two delphinid species, *Stenella attenuata* and *Steno bredanensis*, and compares the modulation and acoustic parameters of whistles emitted simultaneously and non-simultaneously. Acoustic data were collected during oceanographic surveys along the Brazilian continental margin using passive acoustic monitoring (PAM). Whistle modulation patterns and acoustic parameters were compared between whistles emitted simultaneously with click trains and whistles emitted without simultaneous click trains in *Stenella attenuata* (277 and 125 whistles, respectively) and *Steno bredanensis* (743 and 58 whistles, respectively). Differences in whistle modulation and acoustic parameters were evaluated using chi-square, PERMANOVA, and permutation analyses. Whistle modulation did not differ between simultaneous and non-simultaneous emissions. In contrast, significant differences were observed in acoustic parameters. In *Stenella attenuata*, maximum frequency, bandwidth, and whistle duration differed significantly between groups. In *Steno bredanensis*, all evaluated acoustic parameters differed significantly between groups. These findings demonstrate that the two-voice phenomenon is associated with changes in whistle acoustic parameters while preserving whistle modulation patterns, providing new insights into whistle production during simultaneous whistle-click emissions in dolphins.

Keywords: Bioacoustics, odontocetes, passive acoustic monitoring, whistles, non-linear phenomenon, acoustic communication.

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LIST OF ABBREVIATIONS AND ACRONYMS

Sa - *Stenella attenuata*;

Tt - *Tursiops truncatus*;

Sf - *Stenella frontalis*;

Sl - *Stenella longirostris*;

Sb - *Steno bredanensis*;

Sc - *Stenella clymene*;

Gma - *Globicephala macrorhynchus*;

Oo - *Orcinus orca*;

Pe - *Peponocephala electra*;

Sco - *Stenella coeruleoalba;*

TV - whistles associated with the two-voice phenomenon;

N - whistles not associated with the phenomenon;

A - ascending;

D - descending;

A/D - ascending–descending;

D/A - descending–ascending;

C - constant;

M - multiple;

PCoA - Principal Coordinates Analysis (PCoA);

kHz – Kilohertez;

s – Seconds;

PMAPC - Scientific Passive Acoustic Monitoring Project;

PCMC - Cetacean Monitoring and Characterization Project;

PAM – Passive Acoustic Monitoring;

LTSA – Long Term Spectral Averaging;

FFT – Fast Fourier Transform;

dB – Decibels

PERMANOVA - Permutational Multivariate Analysis of Variance;

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1 INTRODUCTION

In cetaceans, several adaptations were required for life in the aquatic environment, among which the acoustic communication system stands out (Tyack & Clark, 2000; Janik, 2014). This system evolved in response to limitations of vision and to the physical properties of water, where sound propagates efficiently over long distances (Popper & Edds-Walton, 1997). As a result, hearing became the main channel for information acquisition and transmission in odontocetes (Tyack, 2000; Janik, 2014).

Odontocetes produce two types of acoustic signals: pulsed and tonal sounds. Pulsed sounds include echolocation clicks, used mainly for orientation, navigation, and prey detection (Au, 1993), as well as burst pulses, associated with social and agonistic contexts (Lammers et al., 2003; Oswald et al., 2007). Whistles are narrowband tonal sounds that modulate in frequency and are generally associated with social communication (Herzing, 2000; May-Collado et al., 2007).

Their acoustic structure has been related to individual identity, group cohesion, behavioral interactions, and changes in social context, making them suitable for investigating how behavioral and communicative processes influence signal structure (Herzing, 2000; May-Collado et al., 2007; La Manna et al., 2020; Papale et al., 2020; Mustun et al., 2024). Although echolocation clicks may also convey social information in some species and contexts, their main function remains associated with biosonar (Au, 1993; Dawson et al., 2010; Sorensen et al., 2018; Martin et al., 2024; (Abildtrup Nielsen et al., 2024).

This anatomical organization allows the simultaneous production of different acoustic signals and has been proposed as the mechanism underlying biphonic

production in odontocetes (Cranford et al., 1997; Madsen et al., 2013; Kriesell et al., 2014; Filatova et al., 2009). Traditionally, the term biphonation has been used to describe the simultaneous or partially overlapping production of different acoustic components in odontocetes (Jones et al., 2020; Wellard et al., 2020; Lefèvre et al., 2025). More recently, Lefèvre et al. (2025) proposed a broader definition of biphonation as the simultaneous production of two or more partially independent and non-harmonically related fundamental frequencies, regardless of whether they are generated by a single sound source or by distinct sound sources. According to these authors, this phenomenon may result from different physiological and aerodynamic processes, including asymmetries in vocal fold oscillations, sound components produced by airflow, recruitment of accessory structures, and independent action of bilateral sound sources.

Similar patterns of simultaneous sound production have also been described in birds, canids, and fish (Aubin et al., 2000; Volodin & Volodina, 2002; Rice et al., 2011). In birds, the controlled production of distinct sounds by independent sound sources located on both sides of the syrinx is commonly referred to as the two-voice phenomenon (Zollinger, 2008). The simultaneous production of signals may increase communication complexity, increase the amount of information transmitted, or reflect adaptations associated with the sound production mechanism (Lefèvre et al., 2025; Tyack et al., 2000). Comparative studies have also shown a positive relationship between social complexity and acoustic communication complexity in different groups of vertebrates (Blumstein & Armitage, 1997; Van Cise et al., 2018; Gustison et al., 2019), suggesting that the simultaneous production of different acoustic signals may represent an additional mechanism to increase the amount of information

52 transmitted during communication. However, whether the simultaneous
53 production of whistles and click trains is associated with modifications in whistle
54 acoustic structure remains largely unexplored in delphinids.

55 Despite the available records, studies describing the occurrence, function,
56 and acoustic characteristics of this form of biphonic production in delphinids
57 remain limited. Simultaneous whistle–click production has been reported in
58 *Tursiops truncatus* in captivity (Lilly et al., 1961) and in the wild (Papale et al.,
59 2015; Kriesell et al., 2014), *Stenella frontalis* in the Bahamas (Kaplan, Melillo-
60 Sweeting & Reiss, 2018), and *Stenella coeruleoalba* in the Gulf of Taranto
61 (Papale et al., 2020), as well as in more recent observations from other
62 populations. These studies described the occurrence of the phenomenon,
63 provided quantitative descriptions of the acoustic signals, and investigated
64 aspects such as signal prevalence and behavioral context. In addition,
65 quantitative analyses of other forms of biphonic vocalizations have been
66 conducted in odontocetes, particularly in *Orcinus orca*, where the relationships
67 between the two simultaneous tonal components of calls and their role in acoustic
68 recognition have been investigated (Filatova et al., 2009; Filatova, 2014).
69 However, based on the literature reviewed, no previous study has systematically
70 compared the acoustic parameters and frequency modulation patterns of whistles
71 produced with and without the simultaneous production of click trains.

72 Given this gap in knowledge, the present study aimed to identify species
73 that exhibit simultaneous whistle–click production in Brazilian oceanic waters and
74 to evaluate whether the occurrence of this phenomenon is associated with
75 changes in whistle acoustic parameters and frequency modulation patterns. We
76 hypothesized that whistles produced simultaneously with click trains would differ

in their acoustic parameters from whistles produced in isolation while maintaining their overall frequency modulation pattern.

2 OBJECTIVES

2.1 General Objective

To identify and document the dolphin groups that use the two-voice phenomenon in their acoustic repertoire.

2.2 Specific Objectives

Chapter 1: To describe the acoustic parameters of signals produced individually and simultaneously, and to compare the acoustic parameters among the different species that exhibit the two-voice phenomenon.

3 MATERIAL AND METHODS

3.1 Study area and data collection

The data used in this study were obtained from surveys conducted by Instituto Aqualie as part of different cetacean monitoring programs along the Brazilian waters. The study area encompassed the Equatorial Atlantic (Northern and Northeastern Brazil, including the Brazilian Equatorial Margin) and the Southwester. The dataset comprised 13 oceanographic surveys conducted between 2017 and 2026 as part of two cetacean monitoring programs: the Scientific Passive Acoustic Monitoring Project (PMAPC; 2017–2022, six surveys) and the Cetacean Monitoring and Characterization Project (PCMC; 2024–2026, seven surveys). Although the monitoring programs differed in their primary objectives, both employed comparable acoustic acquisition protocols, allowing the datasets to be integrated for the purposes of this study.

Sampling strategies varied according to the objectives of each monitoring program. PMAPC consisted of opportunistic Passive Acoustic Monitoring (PAM) conducted from support vessels during seismic operations, with continuous recordings except during operational maneuvers. PCMC was primarily based on systematic line-transect surveys following distance sampling method (Buckland et al., 2001), integrating continuous passive acoustic monitoring and standardized visual effort. Part of the surveys also included targeted searches along the continental slope to locate focal species for satellite tagging, combining continuous visual monitoring with opportunistic acoustic recordings.

Acoustic data were collected using towed linear hydrophone arrays operated in real time with PAMGuard software. Different acquisition systems were employed throughout the surveys, including AUSET Technology

hydrophone arrays and autonomous SoundTrap ST600HF recorders. Detailed information on equipment specifications, acquisition settings, survey duration, and sampling effort is provided in Table 1.

Visual monitoring was conducted continuously during daylight hours and was suspended only under poor sighting conditions, such as sea state ≥ 6 on the Beaufort scale and/or heavy rainfall. Observations were carried out from the highest observation platform available on each vessel using the naked eye or reticle binoculars (Fujinon™ Mariner). For each sighting, information on group location, behavior, size, and composition was recorded. Species identification was subsequently confirmed by experienced observers based on photographic documentation whenever possible.

Table 1. Summary of the acoustic monitoring surveys used in this study across the Equatorial and Southwestern Atlantic sectors of the Brazilian continental margin.

Project	Cruise	Sampling year	Acquisition system	Equipment and array length (m)	Number of channels	Sampling rate (kHz)	Sampling duration (days)	Recording effort (hours)
PMAPC	1	2017	Roland Octa-Capture	AUSET (300m)	4	192	35	354.6
PMAPC	2	2019	SailDaq	AUSET (300m)	4	500	28	299.3
PMAPC	3	2019–2020	SailDaq	AUSET (300m)	4	500	20	413.2
PMAPC	4	2020	SailDaq	AUSET (300m)	4	500	31	645.6
PMAPC	5	2021	SailDaq	AUSET (400m)	4	500	40	611.1
PMAPC	6	2022	SailDaq	AUSET (400m)	4	500	58	650.4
PCMC	1	2024	SailDaq	AUSET (300m)	4	500	38	677.1
PCMC	2	2024	SoundTrap	ST600 HF (250m)	2	384	21	349.1
PCMC	3	2025	SailDaq	AUSET (250m)	4	500	30	475.5
PCMC	4	2025	SailDaq	AUSET (250m)	4	500	8	123.8
PCMC	5	2025	SailDaq	AUSET (250m)	4	500	20	257
PCMC	6	2025	SailDaq	AUSET (250m)	4	500	29	613.5
PCMC	7	2026	SailDaq	AUSET (250m)	4	500	30	430

3.2 Acoustic processing and detection definition

Acoustic processing began with the generation of Long-Term Spectral Averages (LTSAs) using Triton software (Scripps Institution of Oceanography) implemented in MATLAB to facilitate the screening of recordings and the identification of potential acoustic detections. Acoustic detections were defined

as continuous sequences of vocalizations separated by no more than 10 min of silence; a criterion adopted to delimit independent acoustic events.

Candidate acoustic detections were subsequently validated by manual inspection in Raven Pro 1.6 (Cornell Laboratory of Ornithology), using spectrograms generated with a Hann window, a 2048-point FFT, and 50% overlap. Detection times were then matched with simultaneous visual records, allowing acoustic detections to be assigned to visually identified species whenever possible.

Species identification was primarily based on the association between acoustic detections and simultaneous visual records. Exceptionally, one detection without visual confirmation was included based on a previous acoustic identification as *Steno bredanensis* from the Instituto Aqualie reference acoustic database. For the present study, this identification was independently reassessed using a Random Forest classifier trained with reference recordings from the same database, and the classification confirmed its assignment to *Steno bredanensis*. Details of the classification procedure and its validation are provided in Supplementary Material S1.

3.3 Identification of the two-voice phenomenon

The two-voice phenomenon was identified based on the temporal co-occurrence of whistles and click trains within the same acoustic detection (Figure 1), through visual inspection of spectrograms in Raven Pro 1.6, considering only detections that had been previously validated. A detection was classified as exhibiting the two-voice phenomenon when a whistle and a click train showed a clear period of temporal overlap, with the click-train structure clearly distinguishable. Whistles associated with the two-voice phenomenon were

classified as TV, whereas whistles not associated with the phenomenon were classified as N. Temporal overlap did not require the whistle and click train to start or end simultaneously. Because individual sound sources could not be distinguished, classification relied on the repeated occurrence of the same whistle–click association within a single acoustic detection (Figure 1). To reduce the likelihood of misclassifying coincident vocalizations from different individuals as the two-voice phenomenon, this association had to be observed at least twice within the same detection. Detections in which temporal overlap could not be unequivocally confirmed, as well as those with excessive noise preventing reliable identification of both acoustic components, were excluded from the analyses.

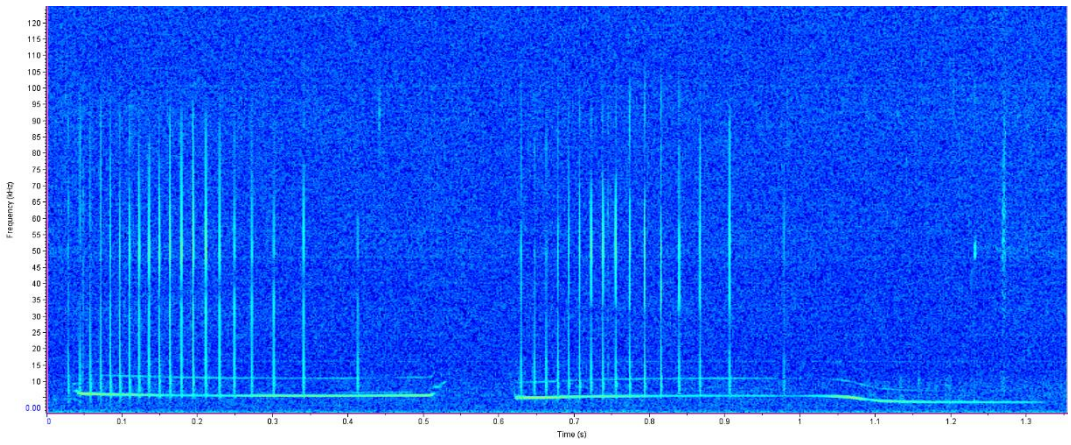


Figure 1. Representative spectrogram of a *Steno bredanensis* acoustic detection analyzed in the present study. The figure illustrates the criteria used to identify the two-voice phenomenon, including the temporal overlap between whistles and click trains and the repeated occurrence of the same whistle–click association within a single acoustic detection. Temporal overlap did not require both signals to start or end simultaneously.

3.4 Statistical analyses

Intraspecific analyses were restricted to species in which the two-voice phenomenon was recorded and that were represented by sufficient numbers of both TV and N whistles to support reliable statistical comparisons and adequately

represent within-species acoustic variation (Weaver et al., 2020). Species without records of the phenomenon or with insufficient numbers of TV whistles were excluded from inferential analyses.

3.4.1 Descriptive analyses

Descriptive statistics were calculated separately for TV and N whistles, including the mean, standard deviation, median, and percentile ranges for each acoustic parameter.

3.4.2 Whistle modulation patterns

Selected whistles were classified according to their frequency modulation pattern into six categories: ascending (A), descending (D), ascending–descending (A/D), descending–ascending (D/A), constant (C), and multiple (M), following the criteria described by Azevedo et al. (2007) and Ansmann et al. (2007).

To investigate potential differences in the distribution of modulation patterns between TV and N whistles, contingency tables were constructed separately for each species that exhibited the two-voice phenomenon.

Differences in the frequency of occurrence of modulation categories between the two emission types were assessed using Pearson's chi-squared test of independence. Because some categories contained low expected frequencies, significance values were estimated using Monte Carlo simulations with 10,000 permutations. Standardized residuals were examined to identify categories that contributed most strongly to deviations from the expected distribution.

3.4.3 Acoustic parameter comparisons

Acoustic parameters of TV and N whistles were compared separately for each species using permutation-based independence tests with 999 random resamples. Because multiple acoustic parameters were tested simultaneously, p-values were adjusted using the Benjamini–Hochberg procedure to control the false discovery rate.

3.4.4 Multivariate analyses

Differences in the multivariate acoustic structure of whistles were evaluated using Permutational Multivariate Analysis of Variance (PERMANOVA) based on Euclidean distances calculated from standardized acoustic parameters (z-scores). For each species, a PERMANOVA was performed with 999 permutations, using whistle type (N vs. TV) as the explanatory factor.

The homogeneity of multivariate dispersions was assessed using betadisper, followed by permutation tests with 999 resamples. This analysis was used to determine whether differences detected by PERMANOVA reflected shifts in group position within the multivariate space or differences in within-group dispersion.

To visualize multivariate patterns, a Principal Coordinates Analysis (PCoA) was performed using the same Euclidean distance matrices employed in the PERMANOVA. The first two axes were used to represent whistle distribution in multivariate acoustic spaces. Additionally, Random Forest classification models were used to assess group discrimination. Models were trained using 60% of the data and validated with the remaining 40%, employing 1,000 decision trees. Further methodological details can be found in Machado et al. (2024). All

statistical analyses were conducted in R (R Core Team, 2025) using the packages vegan, coin, dplyr, tidyr, and ggpubr.

4. RESULTS

4.1 Sampling Effort and Occurrence of the Two-Voice Phenomenon

A total of 99 acoustic detections associated with visually identified odontocetes were analyzed. The taxonomic composition of the records was dominated by *Stenella attenuata* and *Tursiops truncatus*, which accounted for the majority of detections (45 and 28, respectively). The remaining species were recorded less frequently and included *Stenella frontalis*, *Stenella longirostris*, *Steno bredanensis*, *Stenella clymene*, *Peponocephala electra*, *Globicephala macrorhynchus*, *Orcinus orca*, and *Stenella coeruleoalba*. Mixed-species groups involving different combinations of odontocetes were also recorded during the study (Figure 2).

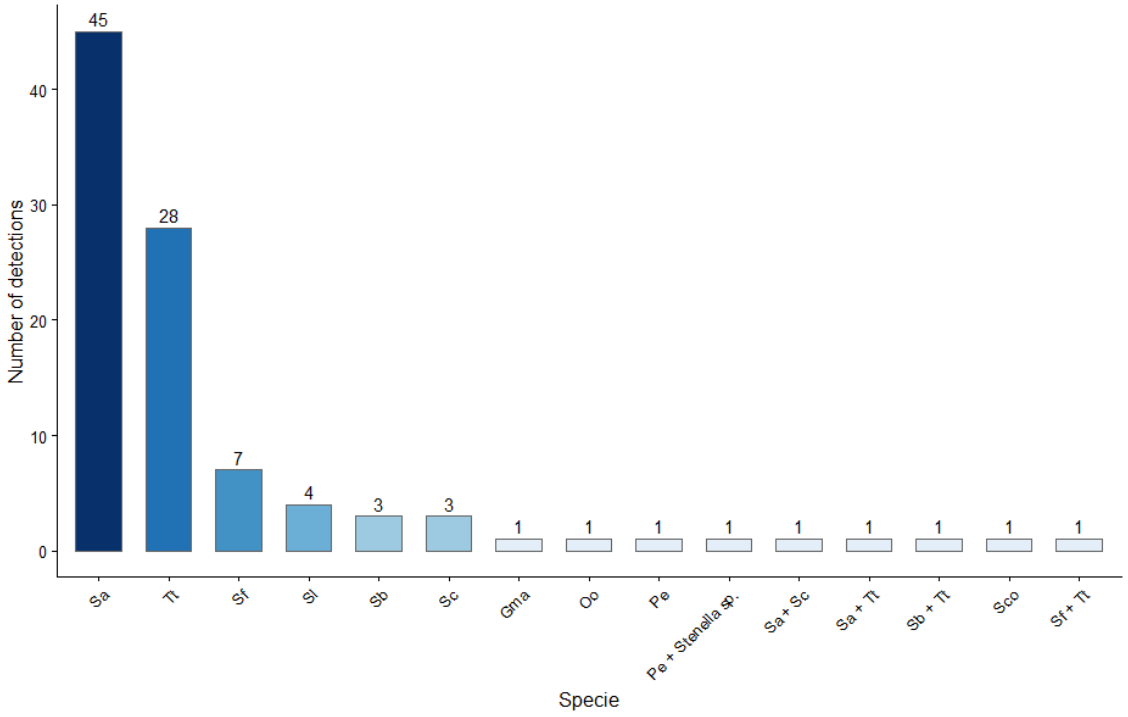


Figure 2. Number of acoustic detections associated with visually identified odontocetes. Bars represent single-species and mixed-species groups recorded during the monitoring period. Values above the bars indicate the number of detections. Abbreviations: Sa = *Stenella attenuata*; Tt = *Tursiops truncatus*; Sf = *Stenella frontalis*; Sl = *Stenella longirostris*; Sb = *Steno bredanensis*; Sc = *Stenella clymene*; Gma = *Globicephala macrorhynchus*; Oo = *Orcinus orca*; Pe = *Peponocephala electra*; Sco = *Stenella coeruleoalba*.

Among the detections attributed to *Steno bredanensis*, one lacked visual confirmation and was assigned to the species based on acoustic classification validated using a Random Forest classifier (Supplementary Material S1).

The two-voice phenomenon was recorded in 24 acoustic detections. Most detections were attributed to *Stenella attenuata* (n = 20), whereas two were attributed to *Steno bredanensis*. The geographic distribution of detections exhibiting the two-voice phenomenon is shown in Figure 3. The phenomenon was also observed in two mixed-species detections involving associations between *S. attenuata* and *T. truncatus* (n = 1) and between *S. attenuata* and *P. electra* (n = 1). However, these records were excluded from subsequent analyses because

of the limited number of whistles available and the inability to determine which species produced the simultaneous whistle-click emissions.

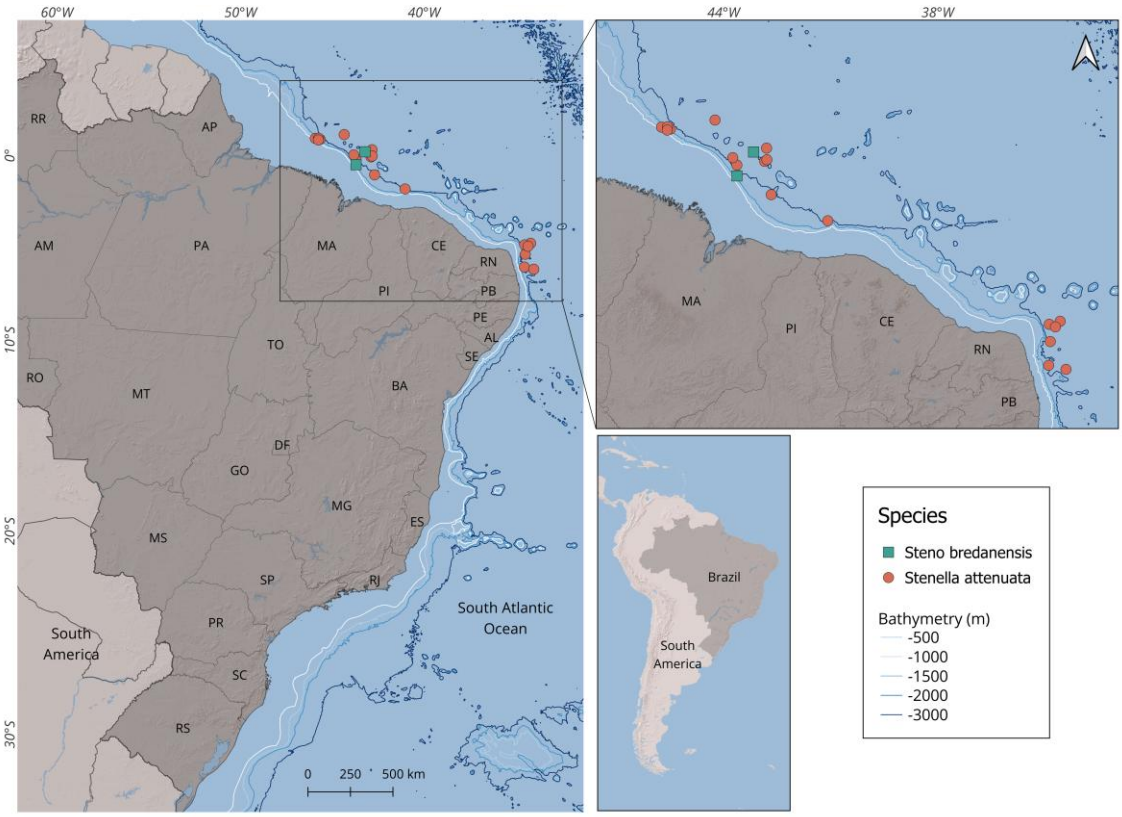


Figure 3. Geographic distribution of acoustic detections of the two-voice phenomenon recorded in *Stenella attenuata* and *Steno bredanensis*. Mixed-species detections were not included.

A summary of the occurrence of the two-voice phenomenon in *S. attenuata* and *S. bredanensis*, including the number of detections, total whistles analyzed, sampling duration, and whistle emission rates, is presented in Table 2.

Table 2. Occurrence of the two-voice phenomenon in *S. attenuata* and *S. bredanensis*. The table summarizes the number of detections, number of detections containing the phenomenon, sampling duration, total whistles analyzed, number of whistles exhibitin the two-voice phenomenon, and the proportion of two-voice whistles.

Species	Detections (n)	Two-voice detections (n)	Sampling duration (min)	Total whistles (n)	Whistles with TV n	TV whistle occurrence rate (%)
Stenella attenuata	45	20	430	402	125	31
Steno bredanensis	3	2	58	801	58	6.9

4.2 Acoustic Characteristics of Whistles

A total of 402 whistles from *Stenella attenuata* (125 TV and 277 N) and 801 whistles from *Steno bredanensis* (58 TV N and 743 N) were analyzed. Descriptive statistics for TV and N whistles are presented in Tables 3 and 4, together with published values available for each species. In both species, the acoustic parameters measured in the present study generally fell within the range of values previously reported, although some parameters exhibited differences among studies. In *Stenella attenuata*, both TV and N whistles were generally comparable to previously published values, whereas in *Steno bredanensis*, N whistles closely matched the published ranges, and TV whistles showed a tendency toward lower frequency values. These apparent differences were formally evaluated in the subsequent statistical analyses.

Table 3. Comparison of acoustic parameters of TV and N whistles in *Stenella attenuata* with values previously reported for the species. Frequency values are expressed in kHz and duration in seconds. Abbreviations: No. whistles = number of whistles; MinF = minimum frequency; MaxF = maximum frequency; IniF = initial frequency; FinF = final frequency; DeltaF = frequency range (maximum frequency − minimum frequency); CenterF = center frequency; PeakF = peak frequency; IP = inflection points; ST = steps; Harmonics = number of harmonics. [a] Values are presented as mean ± standard deviation (SD), with values in parentheses indicating the median, followed by the 10th (P10) and 90th (P90) percentiles. [c] Values are presented as mean ± standard deviation (SD), with

values in parentheses indicating the minimum and maximum values. – Data not available.

Acoustic parameter	North Pacific (Ding et al., 1995a) Mean ± SD (min–max) c	Eastern tropical Pacific (Oswald et al., 2003) Mean ± SD [a]	Eastern tropical Pacific (Oswald et al., 2004) Mean ± SD [a]	North/eastern tropical Pacific (Oswald et al., 2007) Mean ± SD [a]	North Pacific (Silva et al., 2016) Mean ± SD [a]	Western South Atlantic (Pires et al., 2015) Mean ± SD (min–max) c	Western South Atlantic (This study - N) Mean ± SD (median; P10–P90) b	Western South Atlantic (This study - TV) Mean ± SD (median; P10–P90) b
N°. whistles	144	97	100	399	746	155	277	125
Recording sessions	-	7	5	26	2	8	20	20
MinF (kHz)	8.73 ± 2.04 (3.13-14.70)	8.2 ± 1.7	9.0 ± 3.9	8.41 ± 2.39	9.7 ± 2.8	8.6 ± 2 (4.2-14.5)	7.74 ± 1.95 (7.32; 5.80-10.19)	7.47 ± 1.67 (7.04; 5.77-9.97)
MaxF (kHz)	15.72 ± 2.12 (7.73-21.4)	18.7 ± 3.0	19.4 ± 5.2	17.99 ± 4.69	19.8 ± 4.2	19.8 ± 3.5 (9.5-31.1)	21.21 ± 3.96 (21.08; 15.85-26.36)	23.02 ± 3.57 (22.47; 19.56-27.81)
IniF (kHz)	11.04 ± 3.72 (3.13-18.47)	9.5 ± 2.9	10.4 ± 4.5	9.92 ± 3.94	11.2 ± 3.9	10.2 ± 3.3 (4.7-22.0)	8.97 ± 3.45 (8.08; 6.00-12.68)	9.14 ± 4.23 (7.63; 5.99-13.28)
FinF (kHz)	14.66 ± 3.21 (4.3-21.4)	15.3 ± 5.2	16.8 ± 6.4	14.92 ± 5.66	16.8 ± 5.3	14.9 ± 5.7 (5.3-30.0)	14.65 ± 7.12 (12.68; 6.90-24.76)	15.38 ± 7.72 (13.18; 6.72-26.17)
DeltaF (kHz)	-	-	10.6 ± 3.3	-	10.1 ± 4.9	11.3 ± 3.6 (1.4-22.7)	13.45 ± 4.33 (13.13; 8.24-19.14)	15.41 ± 3.58 (15.29; 10.79-19.65)
CenterF (kHz)	-	-	-	-	-	-	12.71 ± 2.86 (12.70; 8.69-16.36)	12.96 ± 3.13 (12.85; 9.45-17.09)
PeakF (kHz)	-	-	-	-	-	-	12.48 ± 4.02 (12.45; 7.81-17.58)	12.01 ± 4.34 (10.99; 7.35-18.54)
Duration (s)	0.53 ± 0.27 (0.09-1.95)	0.9 ± 0.4	0.63 ± 0.40	0.75 ± 0.38	0.7 ± 0.5	0.9 ± 0.4 (0.1-1.7)	0.98 ± 0.93 (0.94; 0.62-1.28)	1.02 ± 0.21 (1.01; 0.75-1.27)
IP	0.7 ± 0.92 (0-4)	1.9 ± 1.8	1.2 ± 1.8	1.29 ± 1.45	1.2 ± 1.2	1.3 ± 1.5 (0-8)	-	-
ST	-	-	2.8 ± 3.4	-	-	2.3 ± 3.2 (0-20)	-	-
Harmonics	-	-	-	-	-	0.6 ± 0.7 (0-3)	-	-

Table 4. Comparison of acoustic parameters of whistles produced with (TV) and without (N) the two-voice phenomenon in *Steno bredanensis* with values previously reported for the species. Frequency values are expressed in kHz and duration in seconds. Abbreviations: No. whistles = number of whistles; MinF = minimum frequency; MaxF = maximum frequency; IniF = initial frequency; FinF = final frequency; DeltaF = frequency range (maximum frequency − minimum frequency); CenterF = center frequency; PeakF = peak frequency; IP = inflection points; ST = steps; Harmonics = number of harmonics. [a] Values are presented as mean ± standard deviation (SD), with values in parentheses indicating the median, followed by the 10th (P10) and 90th (P90) percentiles. [b] Values are presented as mean ± standard deviation (SD), with values in parentheses indicating the minimum and maximum values. c Parameter not reported. d Parameter not evaluated in the present study. – Data not available.

Acoustic parameter	**Western South Atlantic – Southeastern Brazil (Lima et al., 2012)** Mean ± SD [a]	**Eastern Tropical Pacific (Oswald et al., 2003)** Mean ± SD [a]	**Eastern and Central Pacific (Oswald et al., 2007b)** Mean ± SD [a]	**Western South Atlantic (This study - N)** Mean ± SD (median; P10–P90) [b]	**Western South Atlantic (This study - TV)** Mean ± SD (median; P10–P90) [b]
N°. whistles	340	68	192	743	58
Recording sessions	4	5	14	2	2
MinF (kHz)	6.08 ± 1.46	6.3 ± 2.5	6.46 ± 2.33	6.19 ± 1.49 (5.95; 4.56-8.91)	4.29 ± 0.87 (4.57; 3.21-5.25)
MaxF (kHz)	7.96 ± 1.57	9.1 ± 3.00	9.53 ± 2.97	8.11 ± 1.47 (7.8; 6.20-10.20)	7.24 ± 1.42 (6.91; 5.79-9.55)
IniF (kHz)	6.56 ± 1.7	6.8 ± 2.9	7.41 ± 3.15	7.05 ± 1.61 (6.74; 5.16-9.57)	5.86 ± 1.18 (6.01; 4.21-7.25)
FinF (kHz)	7.4 ± 1.72	8.5 ± 3.1	8.33 ± 2.95	7.25 ± 1.81 (6.99; 5.14-9.79)	6.09 ± 2.22 (6.07; 3.45-9.46)
DeltaF (kHz)	1.89 ± 1.42	2.8 ± 2.1	-	1.91 ± 1.05 (1.66; 0.80-3.33)	2.94 ± 1.44 (2.58; 1.35-5.31)
CenterF (kHz)	-	-	-	7.10 ± 1.34 (6.84; 5.49-9.40)	5.67 ± 0.65 (5.86; 4.88-6.84)
PeakF (kHz)	-	-	-	7.07 ± 1.36 (6.84; 5.49-9.40)	5.85 ± 0.61 (5.86; 4.88-6.84)
Duration (s)	0.347 ± 0.236	0.6 ± 0.4	0.64 ± 0.36	0.45 ± 0.18 (0.46; 0.21-0.68)	0.55 ± 0.18 (0.52; 0.31-0.80)
IP	0.36 ± 0.73	1.3 ± 2.8	2.56 ± 3.00	-	-
ST	-	1.3 ± 1.6	1.51 ± 1.84	-	-
Harmonics	-	-	-	-	-

Whistles were classified into six frequency modulation categories. The distribution of modulation patterns was similar between TV and N whistles in both species (Figure 4). In *Stenella attenuata*, ascending–descending (A/D) whistles were the predominant modulation category in both whistle types, followed by ascending (A) whistles. In *Steno bredanensis*, ascending (A) and descending (D) whistles were the predominant modulation categories in both whistle types.

Chi-square tests with Monte Carlo simulations revealed no significant association between whistle type and modulation category in either *Stenella attenuata* ($\chi^2 = 3.39$, $p = 0.513$) or *Steno bredanensis* ($\chi^2 = 8.72$, $p = 0.116$). These results indicate that whistle modulation patterns did not differ significantly between TV and N whistles in either species.

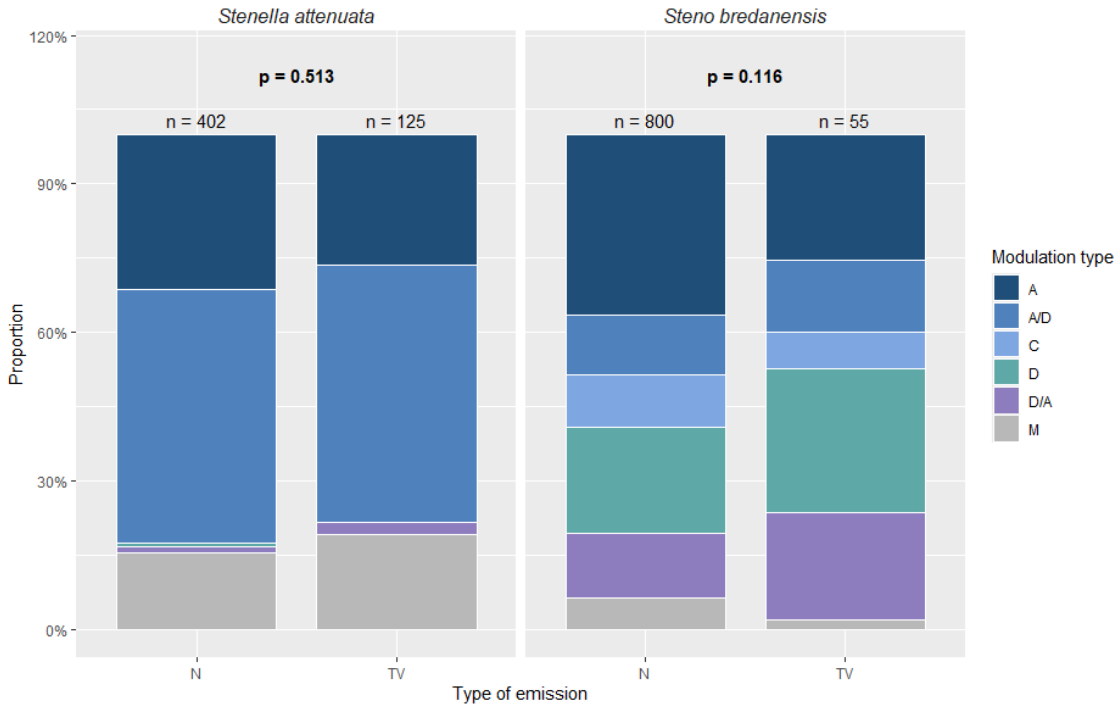


Figure 4. Distribution of whistle modulation patterns in *S. attenuata* and *S. bredanensis* according to whistle type. TV = whistles associated with the two-voice phenomenon; N = whistles not associated with the phenomenon. Modulation categories: A = ascending, D = descending, A/D = ascending–descending, D/A = descending–ascending, C = constant, and M = multiple.

4.3 Acoustic differences associated with the two-voice phenomenon

PERMANOVA revealed significant differences in the multivariate acoustic structure of TV and N whistles in both species. In *Stenella attenuata*, whistle type (TV vs. N) accounted for 2.0% of the total acoustic variation ($F = 7.56$, $R^2 = 0.020$, $p = 0.001$). In *Steno bredanensis*, differentiation between whistle types was more pronounced, with whistle type explaining 4.4% of the total acoustic variation ($F = 34.92$, $R^2 = 0.044$, $p = 0.001$).

Multivariate dispersion analysis detected no significant differences in within-group dispersion for either species (*Stenella attenuata*: $F = 0.010$, $p = 0.921$; *Steno bredanensis*: $F = 0.974$, $p = 0.332$), indicating that the PERMANOVA results reflected differences in group centroids rather than differences in dispersion.

To identify which acoustic parameters contributed to these multivariate differences, permutation tests were performed for each acoustic parameter individually. In *Stenella attenuata*, maximum frequency, frequency range, and duration remained significantly different after Benjamini–Hochberg correction (Figure 5). TV whistles exhibited higher

maximum frequencies, broader frequency ranges, and longer durations than N whistles.

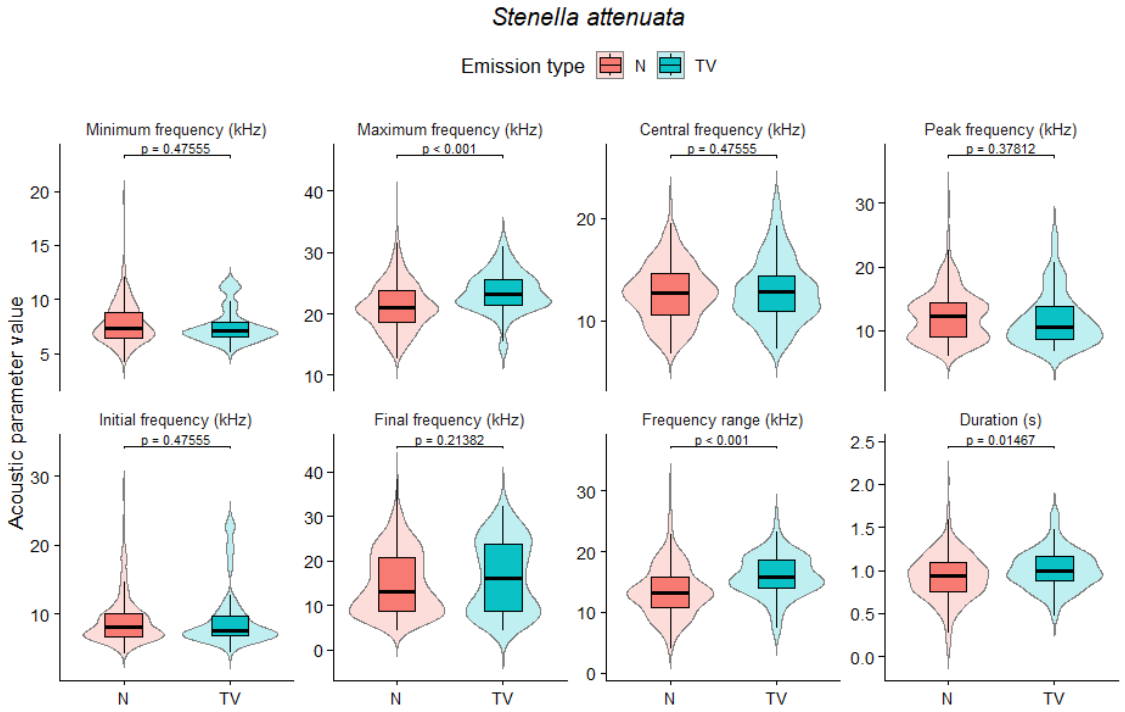


Figure 5. Distribution of acoustic parameters of whistles with (TV) and without (N) the two-voice phenomenon in *S. attenuata*. P-values above each panel indicate the significance of differences between whistle types.

In *Steno bredanensis*, all acoustic parameters evaluated differed significantly between TV and N whistles after Benjamini–Hochberg correction (Figure 6). Overall, TV whistles exhibited lower minimum, maximum, center, initial, final, and peak frequencies, together with

broader frequency ranges and longer durations than N whistles.

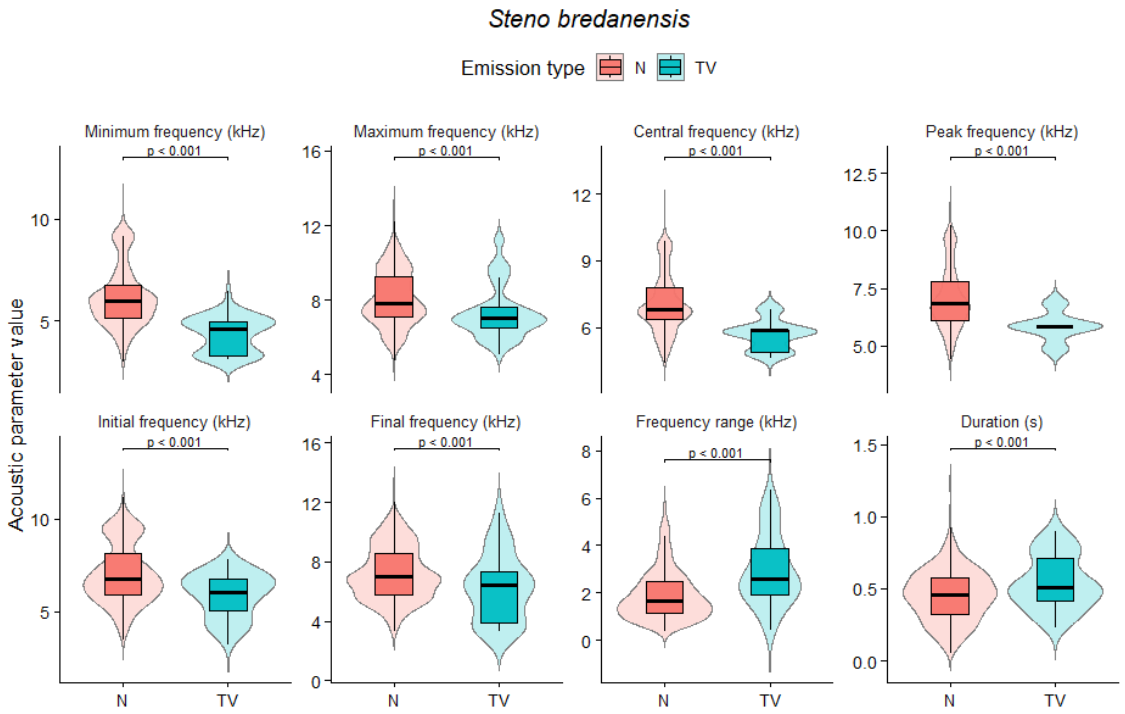


Figure 6. Distribution of acoustic parameters of whistles with (TV) and without (N) the two-voice phenomenon in *S. bredanensis*. P-values above each panel indicate differences between whistle types.

The PCoA ordination (Figure 7A) showed that the first two axes explained 64.7% and 16.4% of the total variation, respectively. The first axis primarily separated *Stenella attenuata* and *Steno bredanensis*, whereas TV and N whistles showed substantial overlap within each species. Nevertheless, a slight displacement between the two groups was evident, particularly in *Steno bredanensis*. The Random Forest confusion matrix (Figure 7B) supported this pattern, showing that classification errors occurred primarily between TV and N whistles within the same species, whereas confusion between species was minimal. Together, these multivariate analyses indicate that species identity accounted for most of the acoustic variation, whereas the two-voice phenomenon was associated with more subtle within-species differences.

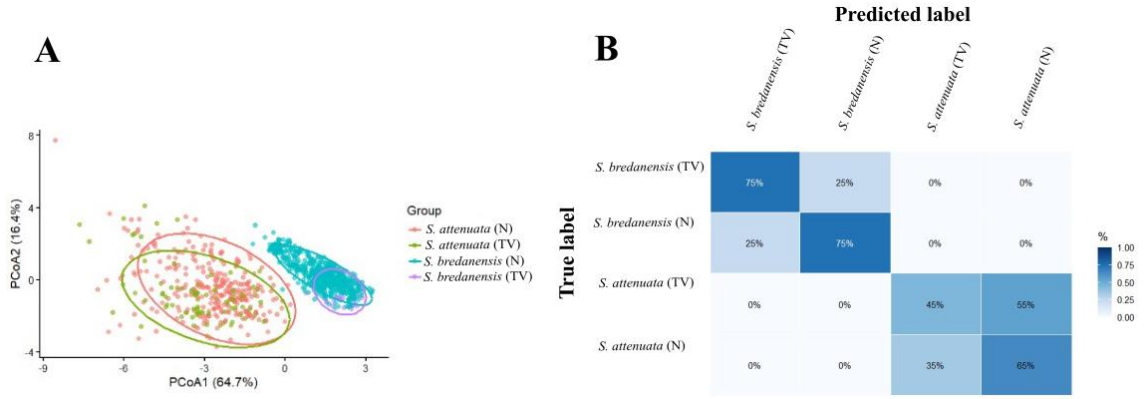


Figure 6. Complementary analyses of whistle acoustic differentiation in *Stenella attenuata* and *Steno bredanensis*. (A) Principal Coordinates Analysis (PCoA) based on Euclidean distances calculated from standardized acoustic parameters. Ellipses represent 95% confidence intervals around group centroids. (B) Random Forest confusion matrix based on standardized acoustic parameters.

5. DISCUSSION

The present study described and compared acoustic signals produced simultaneously by S. attenuata and S. bredanensis. The simultaneous production of acoustic signals has been described using different terms in the cetacean bioacoustics literature, including two voice (Miller et al., 2007), combined signals (Gallo et al., 2023), and biphonation (Kaplan et al., 2018; Papale et al., 2020; Jones et al., 2020). Zollinger et al. (2008) distinguished biphonation from the two-voice phenomenon based on whether the acoustic components originate from a single sound source or from independent sound sources. More recently, Lefèvre et al. (2025) proposed a broader definition of biphonation that does not depend on the number of sound sources, acknowledging that the underlying production mechanism is often difficult to determine in free-ranging animals. Although this broader conceptual framework facilitates comparisons across studies, here we use the term two-voice phenomenon to specifically describe the simultaneous production of whistles and click trains.

Biphonic signals have been documented in several odontocete species, including *Tursiops truncatus* (Lilly & Miller, 1961; Kriesell et al., 2014; Papale et al., 2015; Sportelli et al., 2022), *Orcinus orca* (Tyson et al., 2007; Filatova et al., 2016), *Stenella frontalis* (Kaplan

et al., 2018), *Stenella coeruleoalba* (Papale et al., 2020), and *Grampus griseus* (Corkeron et al., 2000). However, previous studies have focused primarily on describing these signals, characterizing their acoustic components, quantifying their occurrence, and investigating the behavioral contexts in which they occur (Lilly et al., 1961; Miller et al., 2007; Kriesell et al., 2014; Papale et al., 2015, 2020; Kaplan et al., 2018; Jones et al., 2020). Although some studies have quantitatively described the acoustic characteristics of biphonic signals, we found no systematic comparisons of acoustic parameters and whistle frequency-modulation patterns between TV and N whistles. Thus, the present study addresses this gap and documents the two-voice phenomenon in *Stenella attenuata* and *Steno bredanensis* for the first time. Our results further show that TV and N whistles differed in specific acoustic parameters, whereas their frequency-modulation patterns remained similar.

Although the two-voice phenomenon has previously been reported in *Tursiops truncatus* (Lilly & Miller, 1961; Kriesell et al., 2014; Papale et al., 2015; Sportelli et al., 2022), it was not detected in the present study despite the large number of detections analyzed for this species. Previous reports of simultaneous signal production in *T. truncatus* have been limited to a small number of individuals or isolated events (Papale et al., 2015), suggesting that this type of vocal behavior may occur only occasionally in this species. This may partly explain why the phenomenon was not detected in the present dataset.

In the two species in which the two-voice phenomenon was detected, *S. attenuata* and *S. bredanensis*, whistle acoustic parameters were generally consistent with the variability previously described for both species. In *S. attenuata*, the values obtained were similar to those reported for both Brazilian and Pacific Ocean populations (Ding et al., 1995a; Oswald et al., 2003, 2004, 2007; Silva et al., 2016; Pires et al., 2021). Likewise, the acoustic parameters of *S. bredanensis* whistles were generally consistent with those described for populations from southeastern Brazil and the Pacific Ocean (Oswald et al., 2003, 2007b;

Seabra de Lima et al., 2012). However, TV and N whistles differed in frequency- and duration-related parameters, with distinct patterns between the two species.

Inferential analyses conducted separately for each species revealed significant differences between TV and N whistles, although these differences were not consistent across species. In *S. attenuata*, differences were detected only in maximum frequency, frequency range, and duration, whereas in *S. bredanensis*, all acoustic parameters evaluated differed significantly. Whistle frequency- and duration-related parameters are known to exhibit considerable plasticity and may vary among species and populations, as well as according to behavioral context, motivational state, geographic distribution, ecotype, and ambient acoustic conditions (Caldwell et al., 1990; Janik et al., 1994; Oswald et al., 2004; Papale et al., 2013; Silva et al., 2016; La Manna et al., 2020; Pires et al., 2021; Rege-Colt et al., 2023; Pace et al., 2024; Ovsyanikova et al., 2025). In *S. attenuata*, for example, differences in whistle duration and frequency parameters have been reported among ecotypes, highlighting considerable intraspecific variation in these acoustic characteristics (Rege-Colt et al., 2023). In this context, the present results indicate that the two-voice phenomenon is associated with changes in whistle acoustic characteristics in both species, although these changes remain within their known ranges of acoustic variation. However, the available literature does not explain why these differences encompassed a broader range of parameters in *S. bredanensis* than in *S. attenuata*. Further comparative studies are needed to determine whether this contrast reflects interspecific differences in acoustic plasticity or other ecological, behavioral, or physiological factors.

The distribution of whistle modulation categories, however, did not differ significantly between TV and N whistles in either species. Modulation patterns constitute an important component of whistle structure and have been widely used to characterize and compare odontocete vocal repertoires (Bazúa-Durán & Au, 2002; Oswald et al., 2003; Silva et al.,

2016; Pires et al., 2021). Thus, although specific acoustic parameters differed between TV and N whistles, the two-voice phenomenon was not associated with a detectable change in the distribution of modulation categories. This result suggests that changes associated with the phenomenon occur in quantitative whistle characteristics without necessarily altering their overall organization into modulation categories.

Principal Coordinates Analysis showed substantial overlap in acoustic space between TV and N whistles within each species, despite the significant multivariate differences detected by PERMANOVA. However, this distinction explained only a small proportion of the variation in both *S. attenuata* ($R^2 = 0.020$) and *S. bredanensis* ($R^2 = 0.044$). The absence of significant differences in multivariate dispersion further suggests that this pattern was not associated with detectable differences in within-group heterogeneity. Similarly, Random Forest classification showed greater confusion between TV and N whistles within the same species than between species, indicating that the two whistle types were not clearly discriminated based on the acoustic parameters evaluated. Previous studies have shown that whistle acoustic characteristics can differ among odontocete species and provide useful information for species discrimination despite intraspecific variation associated with individuals, populations, or behavioral contexts (Oswald et al., 2000; Lin et al., 2015; Amorim et al., 2019, 2022; Machado et al., 2024). The pattern observed here is consistent with this broader interspecific differentiation: although differences in acoustic parameters were detected between TV and N whistles, these differences did not result in clearly distinct acoustic groups within species. Taken together, these results suggest that the acoustic variation associated with the two-voice phenomenon occurs largely within the broader acoustic variation of each species.

One possible explanation for the acoustic differences observed between TV and N whistles relates to the demands associated with the simultaneous production of two

acoustically distinct signal types. Such production may involve adjustments in whistle acoustic characteristics. This interpretation is biologically plausible given the organization of the odontocete sound-production system: the two pairs of phonic lips can operate with some degree of independence, allowing the simultaneous production of different acoustic signals (Cranford et al., 2011; Ridgway et al., 2015), while communication and echolocation signals involve distinct operating regimes of the nasal sound source (Madsen et al., 2023). Thus, the acoustic differences observed here may reflect biomechanical adjustments associated with the simultaneous production of whistles and clicks. However, because sound-production mechanisms were not directly evaluated, this interpretation remains hypothetical.

Across several vertebrate groups, the simultaneous production of multiple acoustic components has been proposed as a mechanism through which vocalizations may convey complementary types of information, including cues related to individual identity, emitter orientation, and social recognition (Aubin et al., 2000; Volodina et al., 2006; Filatova et al., 2009; Kriesell et al., 2020). Whether this also applies to the simultaneous production of whistles and click trains in odontocetes remains unknown. Whistles are primarily associated with social communication, whereas click trains are more commonly used for echolocation. Because echolocation clicks are highly directional and their received characteristics vary with emitter orientation (Hamilton et al., 2021; Wang et al., 2022; Koschinski et al., 2023), nearby conspecifics could potentially have access to spatial cues associated with click production while simultaneously receiving the social signal conveyed by the whistle. However, whether odontocetes attend to both components in this manner remains unknown. The simultaneous production of whistles and clicks may therefore provide a mechanism through which different sources of acoustic information are available within the same event, a hypothesis that requires direct behavioral investigation.

In *Stenella coeruleoalba*, simultaneous signal production has been recorded primarily during socializing and traveling behavior (Papale et al., 2020), indicating that this type of emission may occur across different behavioral contexts, including social interactions. This possibility also warrants investigation in *Steno bredanensis*, a species characterized by cohesive social associations and highly coordinated swimming behavior within a fission–fusion social system (Götz et al., 2006; Kuczaj II & Yeater, 2007). Repeated whistles have also been proposed to contribute to communication and cohesion among individuals of this species (Redaelli et al., 2026). These characteristics make it relevant to investigate whether the occurrence of the two-voice phenomenon in *S. bredanensis* is associated with social contexts or coordinated group activities. However, because behavioral context was not systematically assessed in the present study, no direct association between the two-voice phenomenon and these behaviors can be established.

Records of the two-voice phenomenon from mixed-species groups were excluded from the comparisons because of the small number of occurrences and the inability to determine with confidence which of the species present produced the signal. This caution is also warranted because differences in whistle acoustic characteristics have been observed between monospecific groups and interspecific odontocete associations (May-Collado, 2010; Viana et al., 2022; Austin et al., 2025), indicating that the interspecific context itself may represent an additional source of acoustic variation.

The mechanisms underlying the two-voice phenomenon remain unresolved, and the hypotheses proposed here are not mutually exclusive. The simultaneous production of whistles and clicks may involve biomechanical adjustments associated with producing two acoustically distinct signals while also potentially making different sources of acoustic information available to nearby receivers. Future studies integrating standardized behavioral observations, analyses of both components—whistles and clicks—and recordings from

identified individuals will be necessary to investigate the mechanisms and potential functions underlying this phenomenon.

6. CONCLUSION

Overall, the present study expands the current knowledge of simultaneous sound production in odontocetes by documenting the two-voice phenomenon for the first time in *S. attenuata* and *S. bredanensis*. In contrast, despite the analysis of numerous *Tursiops truncatus* detections, the phenomenon was not recorded in the present dataset, supporting previous observations that it may represent an uncommon vocal behavior in this species. The results further demonstrate that the two-voice phenomenon is associated with measurable changes in whistle acoustic parameters, while the distribution of modulation categories remained similar between whistles produced with and without the phenomenon. The extent of these changes differed between *S. attenuata* and *S. bredanensis*, indicating that the acoustic differences associated with the two-voice phenomenon were not uniform across species. Together, these findings provide a foundation for future studies investigating the mechanisms underlying the two-voice phenomenon and its potential communicative significance.

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8. SUPPLEMENTARY MATERIAL

8.1 Acoustic Classification Analysis

As análises de classificação envolveram a criação de modelos com 1.000 árvores utilizando o algoritmo *Random Forest* (RF) (pacote *randomForest*) no software estatístico R. O RF tem se mostrado uma abordagem eficaz para estudos bioacústicos, pois não é afetado por dados não paramétricos e consegue acomodar inúmeras variáveis correlacionadas. Essa análise, conforme proposta por Breiman (2001), configura uma coleção de árvores de decisão que crescem por meio do particionamento binário dos dados. Cada partição binária é baseada na variável que resulta nos nós mais homogêneos. A importância das variáveis foi medida utilizando o decréscimo médio da acurácia por meio do pacote randomForest no R. Ao combinar milhares de árvores, o RF explora as diferenças entre as espécies e maximiza o poder preditivo.

O encontro exclusivamente acústico (EAE), foco do presente estudo, foi incorporado separadamente a uma biblioteca de dados construída com base nos parâmetros acústicos de encontros com correspondência acústica e visual (AVM) no Atlântico Sudoeste Ocidental, conforme descrito por Machado et al. (2024). Esse conjunto de dados encontra-se depositado no Laboratório de Ecologia Comportamental e Bioacústica (LABEC) da Universidade Federal de Juiz de Fora (UFJF). Para lidar com as diferenças no número de sinais entre as espécies, foram aplicados pesos às classes para compensar o desbalanceamento entre elas, atribuindo prioridades iguais a todas as classes, conforme descrito por Amorim et al. (2022).

A biblioteca de assobios é composta pelas seguintes espécies: *Orcinus orca* (*O. orca*), *Globicephala melas* (*G. melas*), *Delphinus delphis* (*D. delphis*), *Peponocephala electra* (*P. electra*), *Grampus griseus* (*G. griseus*), *Stenella attenuata* (*S. attenuata*), *Steno bredanensis* (*S. bredanensis*), *Stenella clymene* (*S. clymene*), *Stenella frontalis* (*S. frontalis*), *Sotalia guianensis*

(*S. guianensis*), *Tursiops truncatus* (*T. truncatus*) e *Stenella longirostris* (*S. longirostris*).

As árvores construídas utilizaram 60% dos dados para treinamento, e o erro **OOB** (*Out-of-Bag*) foi calculado para o modelo final de classificação. Os resultados da classificação por Random Forest foram apresentados por meio de uma matriz de confusão, que representa a porcentagem de predições corretas e incorretas em comparação com as classificações reais do conjunto de dados. A análise da curva ROC e das respectivas áreas sob a curva (AUC) foi utilizada para avaliar o desempenho e o poder preditivo do modelo. Adicionalmente, foi realizada uma análise de dispersão para visualizar o posicionamento multivariado dos dados, fornecendo informações sobre a proximidade multidimensional entre o encontro exclusivamente acústico (EAE) e as espécies de delfinídeos que compuseram o modelo de classificação.

8.2. Results

The classification analyses involved building models with 1,000 trees using the Random Forest (RF) algorithm (randomForest package) in the R statistical software environment. RF has proven to be an effective approach for bioacoustic studies because it does not require parametric assumptions and can accommodate numerous correlated variables. As proposed by Breiman (2001), RF consists of an ensemble of decision trees grown through binary partitioning of the data. Each binary split is based on the variable that produces the most homogeneous nodes. Variable importance was assessed using the mean decrease in accuracy implemented in the randomForest package in R. By combining thousands of decision trees, RF captures differences among species and maximizes predictive performance.

The exclusively acoustic encounter (EAE), which was the focus of the present study, was incorporated separately into a reference dataset constructed from the acoustic parameters of encounters with matched acoustic and visual records (AVM) in the western South Atlantic, as described by Machado et al. (2024). This dataset is archived at the Laboratory of Behavioral Ecology and Bioacoustics (LABEC) of the Federal University of Juiz de Fora (UFJF). To account for differences in the number of signals among species, class weights were applied to compensate for class imbalance, assigning equal priority to all classes, as described by Amorim et al. (2022).

The whistle reference library comprised the following species: *Orcinus orca* (*O. orca*), *Globicephala melas* (*G. melas*), *Delphinus delphis* (*D. delphis*), *Peponocephala electra* (*P. electra*), *Grampus griseus* (*G. griseus*), *Stenella attenuata* (*S. attenuata*), *Steno bredanensis* (*S.*

bredanensis), *Stenella clymene* (*S. clymene*), *Stenella frontalis* (*S. frontalis*), *Sotalia guianensis* (*S. guianensis*), *Tursiops truncatus* (*T. truncatus*), and *Stenella longirostris* (*S. longirostris*).

The trees were built using 60% of the data for training, and the out-of-bag (OOB) error was calculated for the final classification model. Random Forest classification results were summarized using a confusion matrix, which represents the percentages of correct and incorrect predictions relative to the true classifications in the dataset. Receiver operating characteristic (ROC) curve analysis and the corresponding areas under the curve (AUCs) were used to evaluate model performance and predictive ability. Additionally, a multidimensional scaling analysis was performed to visualize the multivariate positioning of the data, providing information on the multidimensional proximity between the exclusively acoustic encounter (EAE) and the delphinid species included in the classification model.

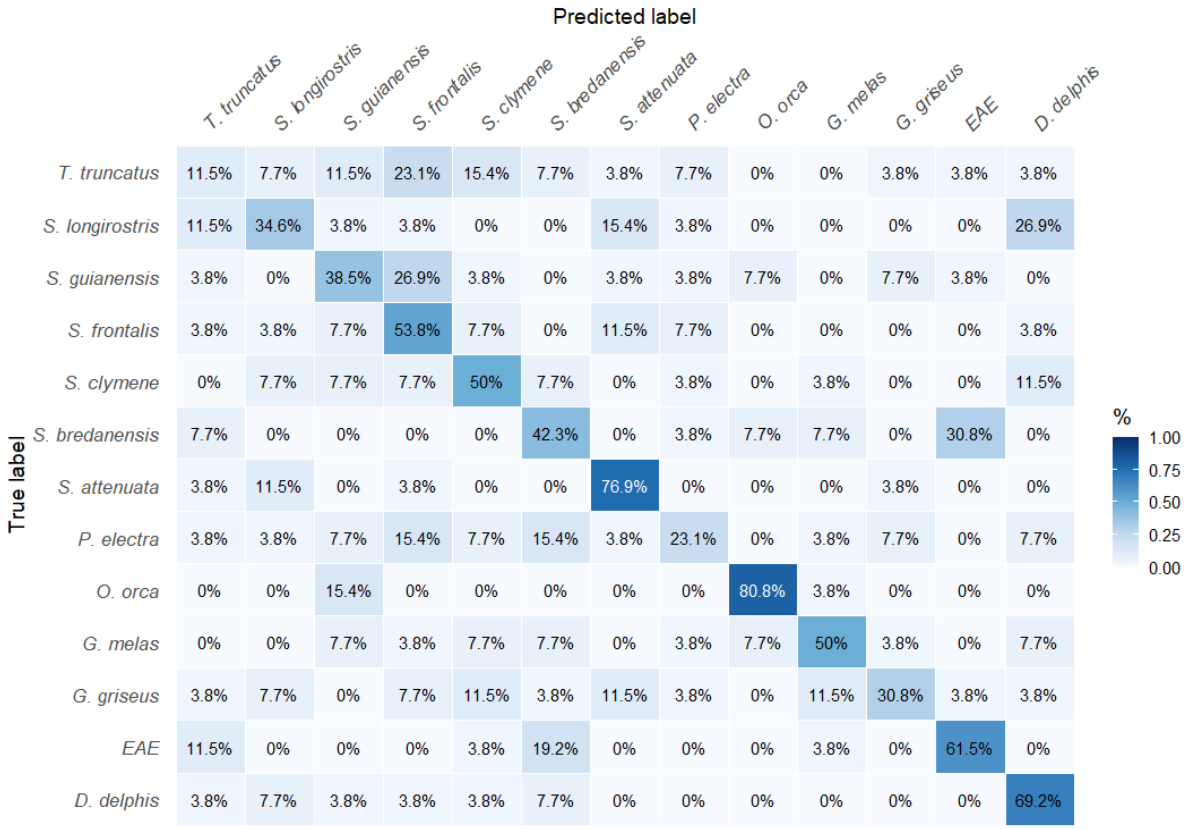


Figure 1. Confusion matrix generated by the first Random Forest classification model, including the delphinid species and the exclusively acoustic encounter (EAE).

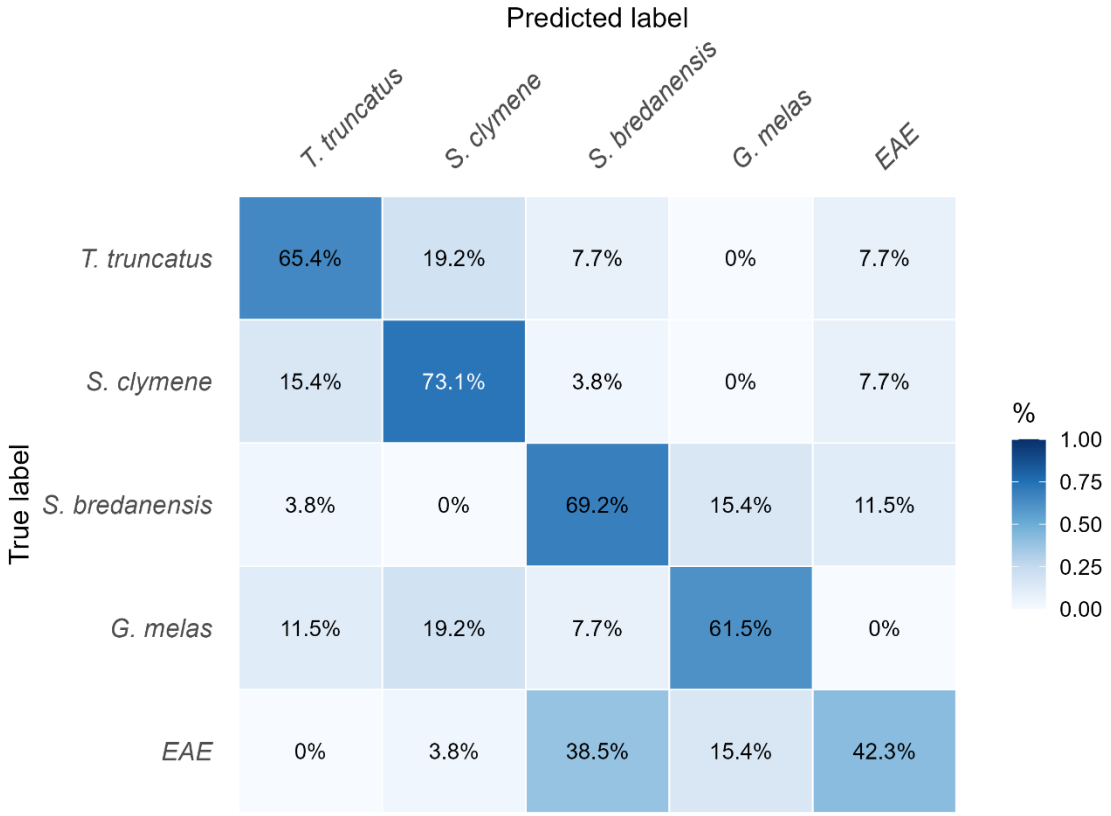


Figure 2. Confusion matrix generated by the final Random Forest model, including the exclusively acoustic encounter (EAE) and the species most acoustic similar to EAE. Abbreviations: Pe = *Peponocephala electra*; Sb = *Steno bredanensis*; EAE = exclusively acoustic encounter.

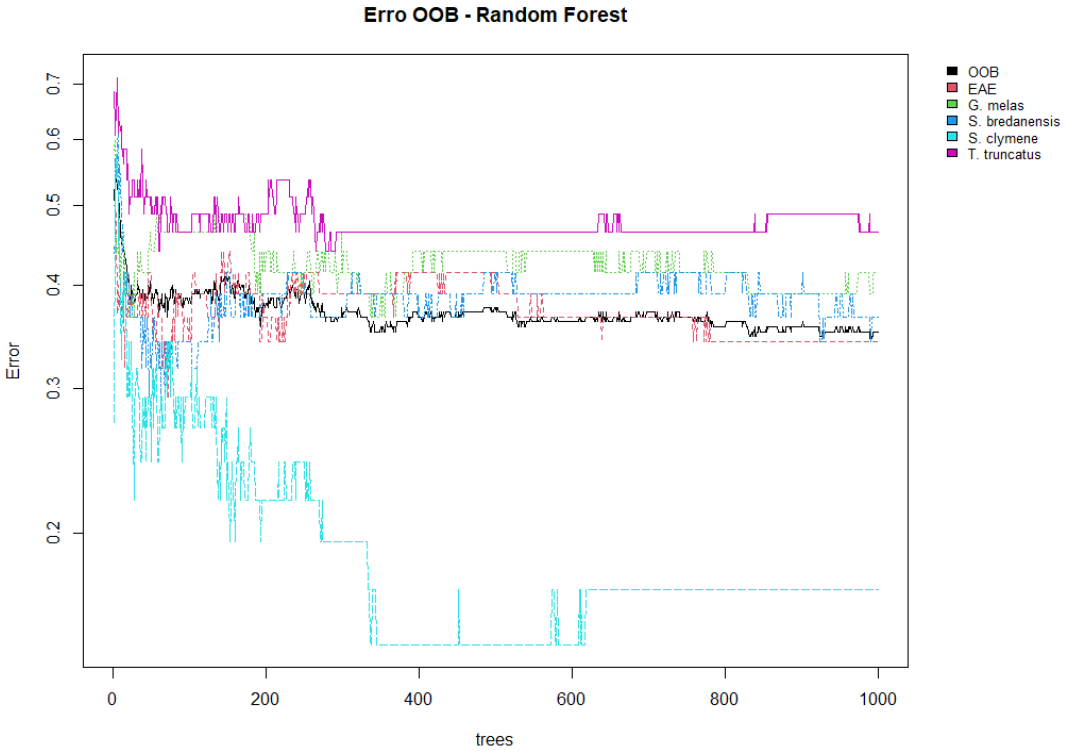


Figure 3. Out-of-bag (OOB) error rates for the final Random Forest classification model. Abbreviation: OOB = *out-of-bag*..

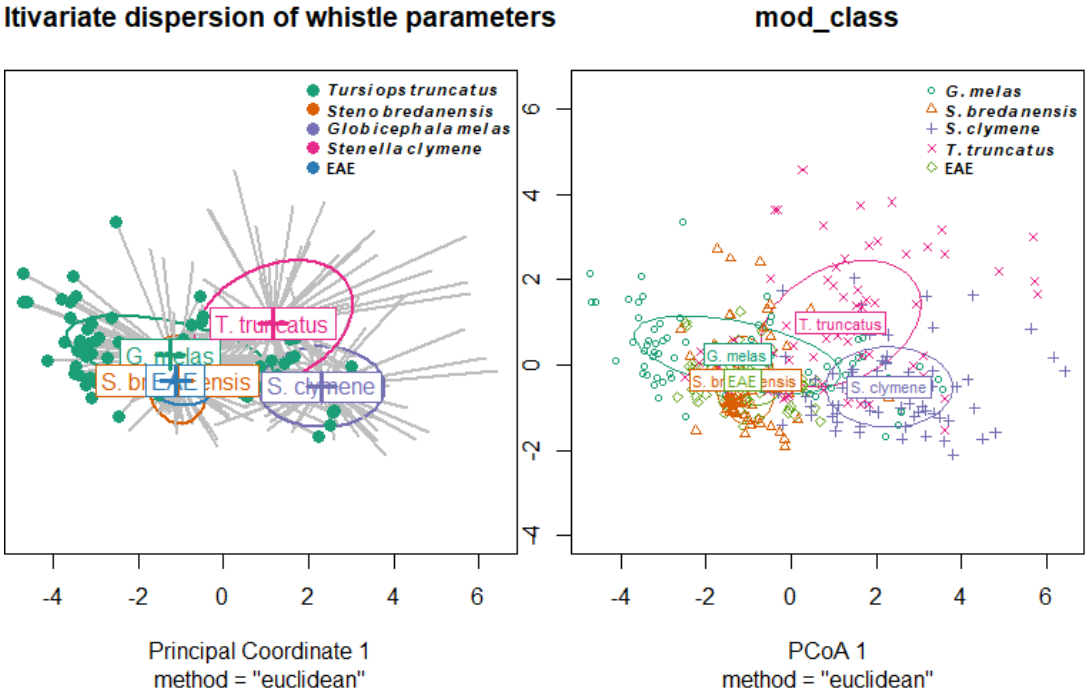


Figure 4. Multivariate scatterplots based on Euclidean distances, used to visualize the proximity between data from encounters with matched acoustic and visual records (AVM) and the exclusively acoustic encounter (EAE) in the final classification model.

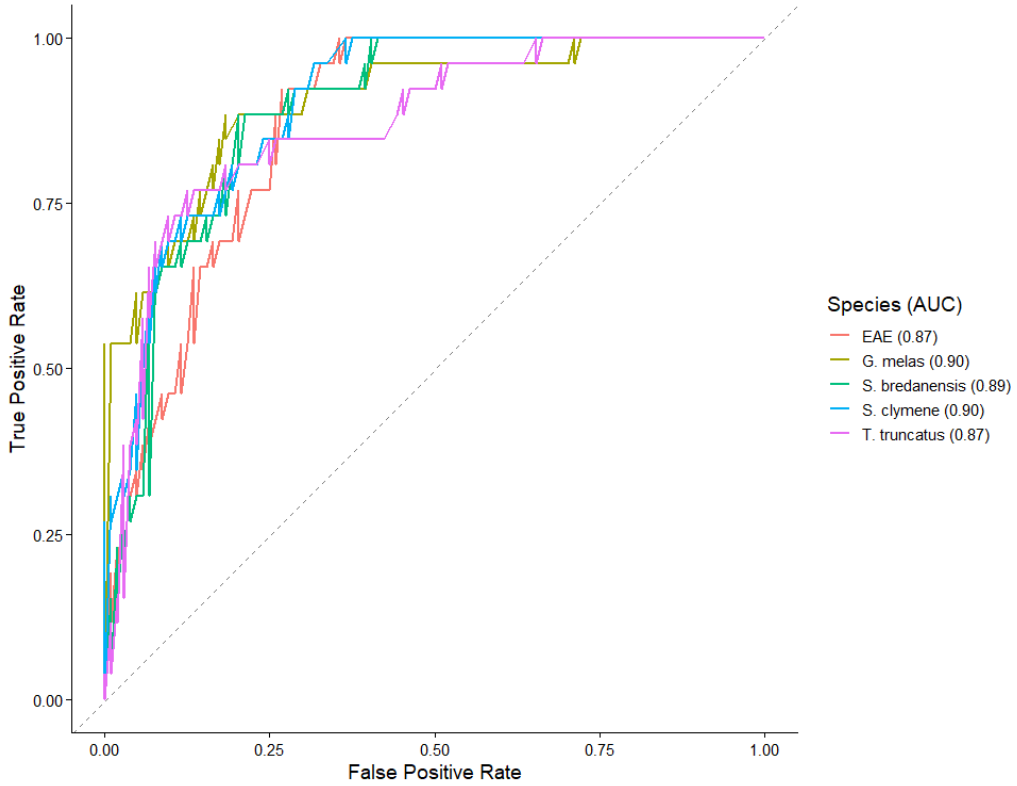


Figure 5. Receiver Operating Characteristic (ROC) curves and corresponding Area Under the Curve (AUC) values for each class in the final classification model.

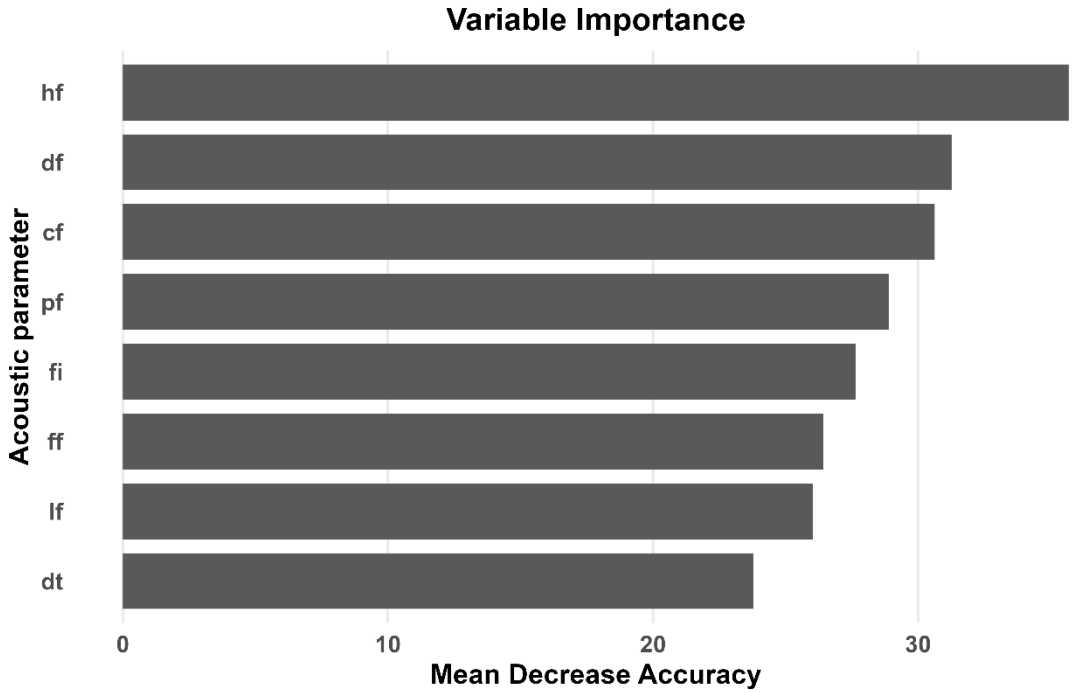


Figure 6. Mean decrease in accuracy indicating the most important variables for classifying the exclusively acoustic encounters (EAE) in the final Random Forest classification model. Abbreviations: lf = minimum frequency; hf = maximum frequency; df = bandwidth; cf = center frequency; fi = initial frequency; ff = final frequency; dt = duration.

3. Referências

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