Universidade Federal de Juiz de Fora
Programa de Pós-Graduação em Biodiversidade e Conservação da Natureza

BRUNA PAGLIANI SIMONATO

BIOACÚSTICA DO GOLFINHO-COMUM-DE-BICO-CURTO *Delphinus delphis* NO ATLÂNTICO SUL OCIDENTAL

[Bioacoustics of the short-beaked-common-dolphin *Delphinus delphis* on the Western South Atlantic]

Juiz de Fora, Minas Gerais - Brasil
Agosto de 2020

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Tese apresentada ao Programa de Pós Graduação em Biodiversidade e Conservação da Natureza da Universidade Federal de Juiz de Fora, como parte dos requisitos necessários à obtenção do grau de Doutor em Biodiversidade e Conservação da Natureza.

Orientador: Prof. Dr. Artur Andriolo

Coorientador: Dr. Thiago Orion Simões Amorim

Juiz de Fora, Minas Gerais - Brasil

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“Live as you were to die tomorrow. Learn as you were to live forever”

Mahatma Gandhi

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RESUMO

O golfinho-comum-de-bico-curto *Delphinus delphis* tem uma coloração lateral característica variando em tons de amarelo e cinza. São encontrados em águas tropicais e temperadas, em regiões costeiras e oceânicas. Os indivíduos são muito ativos e altamente comunicativos. O repertório dos golfinhos é muito diversificado, abrangendo assobios e sons pulsados (*clicks* e sons explosivos). Estudos sobre as emissões acústicas de *D. Delphis* tem sido registrados no mundo todo, exceto na região de talude do oceano Atlantico Sul Ocidental (ASO). Este é o primeiro estudo que aborda uma caracterização das emissões acústicas de *D. Delphis* no ASO, além de analisar a complexidade do repertório de assobios e sons pulsados. Variações intraespecíficas dos assobios foram analisadas considerando tamanho de grupo, contornos e parâmetros de tempo e frequencia. Os contornos mais frequentes foram ascendente, descendente e constante, com relação significativa entre contorno descendente e tamanho de grupo ($p < 0.05$). As frequências dos assobios variaram de 1.34-35.23 kHz e duração variou entre 0.11 e 2.16 segundos. Através do método Bonferroni verificou-se diferenças significativas na frequência mínima, duração e variação de frequência comparando-se encontros entre os anos. A análise das árvores de decisões C5.0 demonstrou corretas classificações nos assobios de 2014 e 2015 (67.0% e 54.0%, respectivamente). Ao aplicar o nMDS verificou-se um agrupamento de assobios dos oceanos Atlântico e Pacífico com base nos valores do número de inflexões e duração dos assobios, corroborados posteriormente por PERMANOVA ($p < 0.05$). A análise dos sons pulsados levou em conta os clicks de ecolocalização, sons explosivos e explosivos curtos. Apesar dos *clicks* de ecolocalização serem relativamente bem documentados, não existe um consenso acerca da ampla variedade de sons explosivos. A análise de K-means resultou em quatro clusters, cuja classificação com base nos parâmetros de tempo teve a maior acurácia (total 87%) explicada a partir dos dois primeiros eixos dimensionais (Dim1 = 70.2% e Dim2 = 17.2%). O conjunto de dados tempo-frequência combinados apresentou o melhor modelo para classificação (Acurácia = 84.6%; CI = 65.1%, 95.6%; $p < 0.01$). Intervalo entre *clicks*, taxa de repetição e frequência pico constituíram parâmetros consistentes para classificar sons pulsados de *D. delphis*. A análise de sons pulsados como um todo (clicks e sons explosivos) considera o animal como uma identidade acústica e enfatiza a importância de certos parâmetros nessa identidade, consequentemente refletindo na otimização do custo energético na produção do som. O último tópico deste estudo procurou identificar possíveis padrões de contornos dos assobios produzidos durante um evento de encalhe em massa (EEM) de *D. delphis* documentado na costa sudeste do Brasil. Das nove categorias, os contornos mais frequentes foram ascendente (35.2%), prevalecente ascendente (29.6%) e côncavo (9.9%). Do total, 33.8% continham *steps* e 14.1% continham *breaks*. As proporções de contornos comparadas entre EEM e ASO pela correlação de Kendal não demonstraram concordância. O distinto padrão em situação de estresse reforça o conceito de que cada categoria de contorno pode carregar informações diferentes associadas ao comportamento. Golfinhos são primariamente dependentes do som para viverem. Estudos abrangendo a complexidade do repertório de golfinhos e os fatores que influenciam na sua estrutura são essenciais para a manutenção da diversidade das populações e, consequentemente, para a conservação das espécies.

Palavras-chave: Assobios. Clicks. Odontocetos. Repertório acústico. Sons pulsados

ABSTRACT

The short-beaked-common-dolphin *Delphinus delphis* has a characteristic yellowish lateral color and gray flank and is found in tropical and temperate waters in both coastal and oceanic regions. Individuals are very active and highly communicative. Dolphin repertoires are strongly diversified, comprising whistles and pulsed sounds (clicks and burst sounds). Many studies have been described for *D. delphis* around the world, although none comprises the slope region of the Western South Atlantic Ocean (WSAO). This study presents the first characterization of the acoustic emissions of short-beaked common dolphin in the WSAO, besides analyze the complexity of whistles and pulsed sounds repertoire as a whole. Intraspecific whistle variations were analyzed considering group size, contours and acoustical parameters. No significant correlation was found between number of whistles and group size ($p > 0.322$). Upsweep, downsweep and constant were the most frequent contours. A positive association between downsweep contour and group size was detected ($p > 0.0403$). Whistle frequencies ranged from 1.34 to 35.23 kHz and duration varied from 0.11 to 2.16 seconds. Comparing encounters between years through the Bonferroni method, minimum frequency, duration, and frequency range were significantly different. A C5.0 decision tree indicated that whistles from 2014 and 2015 were correctly classified in 67.0% and 54.0%, respectively. The nMDS grouped the Atlantic and Pacific oceans based on inflection points and duration, further corroborated through PERMANOVA ($p = 0.039$). Clicks are broadband pulses whose frequencies range from 10 and 150 kHz. The analysis of pulsed sounds took into account echolocation clicks, burst, and short burst sounds. Despite the fact that echolocation clicks are relatively well documented, there is no classification consensus regarding the wide variety of short pulsed signals. K-means analysis resulted in four clusters, whose classification, based on time parameters, exhibited the highest accuracy (87% total) explained by the first two dimensional-axis (Dim1 = 70.2%, and Dim2 = 17.2%). Validation analysis showed that time-frequency dataset was the best classification model for pulsed sounds in *D. delphis* (Accuracy = 84.6%; confidence interval CI = 65.1%, 95.6%; $p < 0.01$). This result emphasizes the influence of certain parameters in that classification, possibly reflecting the energy cost optimization for sound production, considering the animal as an acoustical identity. The last topic of this study identified possible whistle contours patterns produced during the Mass-stranding events (MSE) of *D. delphis* documented on the Southeastern coast of Brazil. Whistle contours were classified into nine categories. The most frequent contours were upsweep (35.2%), prevailing upsweep (29.6%) and concave (9.9%). Of the total, 33.8% contained steps, and 14.1% contained breaks. A Kendall W test showed no concordances among the whistle contours from MSE compared to the results from the WSAO region. These results suggest that a distinct pattern may be related to stress situations, reinforcing the concept that each contour category may carry different information, thus applied in distinctive behavior situations. Dolphins are primarily dependent on sound for living. Studies comprising the complexity of dolphin repertoires and factors that influence their structure are essential in maintaining population diversity and species conservation.

Keywords: Acoustic Repertoire. Clicks. Odontocetes. Pulsed Sounds. Whistles.

CHAPTER 1: INTRODUCTION AND SYNOPSIS

1 GENERAL INTRODUCTION

The short-beaked-common-dolphin *Delphinus delphis* Linnaeus, 1758 belongs to the Delphinidae family and exhibits a very characteristic and easily recognizable color pattern. The back varies from dark gray to black, the belly is white, the thoracic stain is clearly visible laterally with coloration in yellowish tones and a flank displaying light gray tones (Lodi & Borobia 2013) (Figure 1). The species is found in tropical and temperate waters, from coastal to oceanic regions worldwide (Reeves et al. 2002). In general, *Delphinus* sp. observations in Southeastern Brazil are restricted to coastal areas (18-70m), while records between South Brazil and the central region of Argentina occur in deeper waters (Tavares et al. 2010).

Figure 1. Color pattern of the short-beaked-common-dolphin *Delphinus delphis.* Modified from Science direct.com

Common dolphins exhibit very active behavior, including jumping and aerial acrobatics. They form groups ranging from dozens to hundreds of individuals. It is believed that these groups exhibit a certain degree of segregation by sex and age. They are often observed in association with other species, especially spotted dolphins (*Stenella* sp.) and pilot whales (*Globicephala* sp.). Cases of hybrids have been reported in the literature (Jefferson et al. 2008).

This species is highly communicative (Jefferson et al. 2008) and descriptions of the acoustic parameters of *Delphinus delphis* have been studied in many areas worldwide (Oswald et al. 2003, 2007; Ansmann et al. 2007; Henderson et al. 2012; Petrella et al. 2012; Papale et al. 2014). Sound is inherent to the cetacean way of life, essential for communication, individual recognition, parental care, defense against predators, orientation, navigation, group cohesion, prey capture and mating (Tyack 2000; Dolman et al. 2004).

Vocalizations in Odontoceti can be grouped into four types: 1) tonal whistles displaying modulated frequency; 2) highly targeted pulsed sounds, the echolocation clicks; 3) burst sounds or explosive calls, with a high repetition rate and a small inter-click interval; and 4) pulsed calls (Caldwell & Caldwell 1965; Ford & Fischer 1986; Richardson et al. 1995; Au & Hastings 2008; Luís et al. 2016). Whistles and explosive sounds are believed as utilized preferentially for communication (Au 2000).

Whistles are narrow band tones lasting a few seconds with fundamental frequencies in the general range of 5 to 20 kHz. Except for killer whales *Orcinus orca* whose fundamental frequencies can reach up to 75 kHz (Samarra et al. 2010). They consist of modulated frequencies and can be described based on contour visualization through spectrograms (Richardson et al. 1995).

Pulsed sounds are fast pulses usually lasting between 100 and 2000 milliseconds. These sounds are emitted at an energy frequency ranging from approximately 1 kHz to more than 150 kHz (Ridgway & Au 2009). Burst sounds are characterized by a high repetition rate (higher than 300 pulses per second) or a low inter-pulse interval (less than 3 milliseconds), and are generally associated with an emotional or emotional media context, especially in agonistic behavior and in alarm or aggressive situations (Au & Hastings 2008). The difference between clicks and burst sounds is subtle, with the former exhibiting a wider range of inter-clicks and preferably recorded during echolocation (Au & Hastings 2008).

Pulsed calls consist in fast broadband sound pulses with high repetition rates and distinct tonal properties that frequently shift abruptly (Ford 1989). The high repetition rate is reproduced between sidebands intervals and is regularly modulated during the call (Watkins 1967; Ford 1989). They have been described for Killer Whales *Orcinus orca* (Ford & Fisher 1986; Rehn et al. 2007), False Killer Whales *Pseudorca crassidens* (Murray et al. 1998), long-finned pilot whales *Globicephala melas* (Nemiroff & Whitehead 2009), Belugas *Delphinapterus leucas* (Karlsen et al. 2002), Narwhals *Monodon monoceros* (Ford & Fisher 1978), and spinner dolphins *Stenella longirostris* (Rossi-Santos et al. 2008). Pulsed calls, as well as whistles, are highly stereotyped and are believed to be associated with cultural transmission and vocal learning (Ford 1991; Deecke et al. 2002; Foote et al. 2006; Riesch et al. 2006).

Whistle repertoires are greatly diversified, with variations between species, populations and even individuals from the same populations (Asmann et al. 2007). The diversity of whistle characteristics may be related to several factors. Some authors consider body size as one of the main morphological characters for sound emission, influencing signal frequencies (Marquet & Taper 1998, Papale et al. 2013). The inverse relationship between frequency and body size differs significantly between animals inhabiting different ocean basins (Wang et al. 1995; Papale et al. 2013). Locality also affects sound emission characteristics, since some parameters are more similar in populations from adjacent areas than in populations more distant from each other (Azevedo & Van Sluys 2005; Rossi-Santos & Podos 2006; May-Collado & Wartzok 2008).

For some species, frequency parameters can vary more conspicuously between populations, while duration and number of inflection points parameters seem to be more relevant within the same population (Morisaka et al. 2005; Azevedo & Van Sluys 2005). Within a population, whistle duration and number of inflections and steps are more diverse, and these variations can carry information regarding an individual's identity or behavior (Rendell et al. 1999; Morisaka et al. 2005). Species that tend to present a more stable group composition do not generally display large variations in the structure of each individual's repertoire, but exhibit a specific group repertoire, called a dialect, whereas species whose groups are more fluid display a more variable individual whistling repertoire (Tyak 1986). Understanding acoustic repertoire variations within and between populations can assist in knowledge concerning displacement patterns and cultural transmission of a certain species (Samarra et al. 2014).

Behavior is another major driver of repertoire characteristic variations, with differences observed during mother-calf interactions and nursing (Mello & Amundin 2005; Weib et al. 2005); feeding events (Acevedo-Gutiérrez & Stienessen 2004) and cutting or agonistic behaviors (Herzig 1996). Directly interfering with the behavior, the main threat to dolphin communication is noise pollution. Noise can influence vocalization patterns and cause changes in acoustic characteristics (Morisaka et al. 2005; de Andrade et al. 2015; Pirotta et al. 2015; Erbe et al. 2016).

Ocean noise has been intensively studied in the past decades and Passive Acoustic Monitoring (PAM) techniques have become more accessible and widely used for behavioral studies (Cantor et al. 2016; Andriolo et al. 2015; Hildebrand et al.

2015), assess occurrence patterns, identifying critical habitats for different cetaceans (Moron et al. 2015) and characterize repertoires in different species, populations and individuals (Azevedo & Van Sluys 2005; Asmann et al. 2007; Papale et al. 2013).

Only two species belonging to the *Delphinus* genus are currently recognized, namely the short-beaked-common-dophin *D. delphis* and the long-beaked-common-dophin *D. capensis* (Heyning & Perrin 1994). Recently, Cunha et al. (2015), considering both morphological samples and molecular data with cytochrome b sequences, suggested the existence of *D. delphis* only as a valid species in the Western South Atlantic. Based on this assumption, we tested the hypothesis that acoustic parameters (whistles and pulsed sounds - clicks and explosives) display certain intraspecific variation between dolphin encounters, but with a similar bioacoustic pattern at the population level.

Therefore, this study aimed to characterize the acoustic emissions of *D. delphis* composed of whistles, clicks and burst sounds. Comparison of the whistle analyzed possible intra and/or inter-population variations between the six encounters registered in different locations in the slope region of the Western South Atlantic Ocean. Also, those variations were analyzed through time and frequency parameters. Clicks, burst and short burst sounds were investigated according to specific parameters in order to classify the pulsed sounds produced by *D. delphis*. The click trains were categorized and compare with literature for a better understanding, specially regarding the complexity of burst sounds.

This thesis consists of five chapters and, except for the first ('Introduction and Synopsis') and last ('Final conclusions'), each was elaborated in article format. The second chapter covered a general characterization of the whistles and intraspecific variations among the repertoires of the six registered *D. delphis* encounters. In addition, common dolphin whistling recorded in the slope region of the Western South Atlantic was compared to those from other locations. The third chapter dealt with the general characterization of pulsed sounds (echolocation clicks and burst sounds) from *D. delphis* and classifications based on specific parameters. The fourth chapter described aspects related to whistle contours in an atypical massive stranding situation of this species. This chapter included the results of whistle characterization of the chapter 2 as a baseline to compare the whistles patterns in order to improve the knowledge in those critic events. Also, this chapter highlighted the importance of information registered by people in general, which can be used for the benefit of scientific knowledge and dolphin conservation. The fifth and last chapter presents the final conclusions of this thesis.

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CHAPTER 2. INTRASPECIFIC VARIATION IN SHORT-BEAKED-COMMON-DOLPHIN'S WHISTLE REPERTOIRE

Abstract: Dolphins' whistles are strongly diversified mainly related to physiology and behavior. We present the whistles characteristics and intraspecific variations of short-beaked common dolphins *Delphinus delphis* of the Western South Atlantic Ocean (WSAO). A total of 1630 whistles from six encounters were analyzed considering group size, contours and acoustical parameters. No significant correlation was found ($p > 0.322$) between number of whistles and the group size. Upsweep, downsweep and constant were the most frequent contours. A positive relation between the downsweep contour and group size was detected ($p > 0.040$). The frequencies ranged from 1.34–35.23 kHz and the duration varied from 0.11–2.16 seconds. Comparing encounters between years through Bonferroni method, minimum frequency, duration, and frequency range were significantly different. C5.0 decision tree showed that whistles of 2014 and 2015 had correct classifications (67.0% and 54.0%, respectively). The nMDS comparison with literature grouped the Atlantic and Pacific oceans based on inflection points and duration, further corroborated through PERMANOVA ($p = 0.039$). Our results emphasize the influence of certain parameters related to intraspecific variation. Whistles comparison with other localities corroborate with differences in geographical scale.

Keywords: Atlantic Ocean, Brazil, *Delphinus delphis*, Odontoceti, slope region

1 INTRODUCTION

Sound is inherent to cetacean life - it is fundamental to communication, individual recognition, parental care, protection against predators, orientation, navigation, group cohesion, prey capture, and mating (Tyack 1986; Herzing 2000; Laiolo 2010). Diversity in terms of life history, social structure, and mobility in cetaceans influences patterns of communicative signals (Moron et al. 2015; Amorim et al. 2019). These patterns vary from individual signals to group-specific repertoires, and from signals shared among animals from wide geographic areas to endemic dialects (Tyack 2000).

Whistles are strongly diversified in Odontoceti and can vary among species, between geographically separated populations, and even among individuals of the same population (Ansmann et al. 2007). Variations in the characteristics of the whistles between different species or populations appear to be related to physiological aspects (Rendell et al. 1999; Moron et al. 2015, 2018), behavioral biology (Tyack 1986; Herzing 1996; Andriolo et al. 2015), and environmental factors (Andrade et al. 2014; Morisaka et al. 2005).

Whistles are frequency modulated characterized by narrowband tonal calls with durations lasting a few seconds and fundamental frequencies generally reaching up to 20 kHz (Richardson et al. 1995). Although high frequency whistles of killer whales *Orcinus orca* might range up to 75 kHz (Samarra et al. 2010). The whistles contours constitute a qualitative characterization of the whistle (Richardson et al. 1995; Ansmann et al. 2007; Petrella et al. 2012), in which the most common categories are upsweeps, downsweeps, concave (or U-shapes), convex (or inverted U-shapes), or sinusoidal whistles. Intermediate shapes between those categories, as well as particularities on the contour view (breaks, steps, frequency jumps), are also presents and represent the complexity of the repertoires (Richardson et al. 1995).

Studies of the acoustic repertoires of *Delphinus* spp. have been reported worldwide (Ansmann et al. 2007; Oswald et al. 2007; Petrella et al. 2012; Papale et al. 2014). The first studies to analyze acoustic emissions from common dolphins were carried out in the late 1960s using captive animals and free-living dolphins in the Mediterranean Sea (Caldwell & Caldwell 1968). Repertoire studies have expanded since the development of bioacoustics research in the last decades, which has increased the number of studies focused on common dolphin vocalizations. This study is the first to evaluate intraspecific variation between encounters of the short-beaked common dolphins *Delphinus delphis* Linnaeus, 1758 recorded in the slope region of the Western South Atlantic Ocean (WSAO).

Common dolphins are found in tropical and temperate waters in coastal and oceanic environments (Reeves et al. 2002). Despite significant morphotype variation concerning the genera *Delphinus* in several oceanic basins (Heyning & Perrin 1994; Jefferson et al. 2009; Tavares et al. 2010), only two species (*D. delphis and D. capensis*) are recognized nowadays (Heyning & Perrin 1994). Recently, Cunha et al. (2015) suggested the occurrence of only one species, the short-beaked common dolphin *D. delphis*, in the South Atlantic Ocean.

Based on that assumption, this study considered only one species occurring in the SWAO. We assumed that the characteristics of *D. delphis* acoustic records in the study area present a certain degree of variation consistent with intraspecific variation. The goal of this study is to describe whistle parameters of *D. delphis* in the Slope Region of the Atlantic Ocean and provide insights of intraspecific whistle variation. Additionally, we compared our results with findings on whistles repertoire from different localities of the world.

2 MATERIAL AND METHODS

The present study was carried out in partnership with the "Projeto Cetáceos do Talude" onboard the R/V Atlântico Sul. It covered the region of the continental shelf break between Chuí, Rio Grande do Sul and Florianópolis, Santa Catarina. The area is located in the Pelotas Basin (28° and 34° S) (Santos 2009) and was limited by the isobatimetric of 2,000 meters. Researches of the Projeto Cetáceos do Talude carried out visual monitoring, identification and group size estimations of cetacean species in the studied area (Figure 2, Table 1), thus registering any other relevant information about the sighting context.

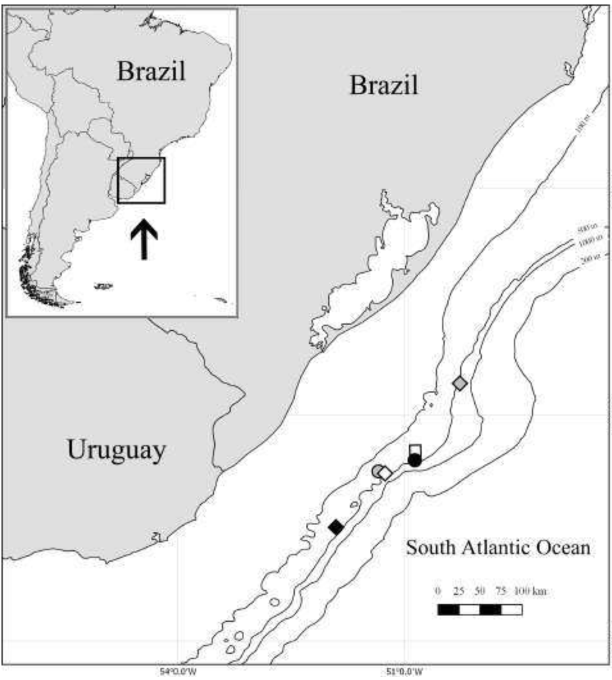

Figure 2. Localities of the six encounters of *Delphinus delphis* recorded on the slope region of Western South Atlantic Ocean. The groups are demarcated as Cruise VII-a (grey circle), Cruise VII-b (grey diamond), Cruise VIII-b (white diamond), Cruise X-a (black diamond), Cruise X-b (black circle), and Cruise X-c (white square).

Table 1. Information of the six encounters of *Delphinus delphis* recorded on the slope region of Western South Atlantic Ocean.

Encounter	Date	Lat (S)	Long (W)	Duration of the emissions (min)	Sampling Frequency
Cruise VIIa	08May2014	33.756	51.349	49.43	48kHz
Cruise VIIb	13May2014	32.562	50.293	47.92	48kHz
Cruise VIIIb	14Nov2014	33.763	51.337	40.31	48kHz
Cruise Xa	18Oct2015	34.473	51.955	63.32	192kHz
Cruise Xb	22Oct2015a	33.603	50.873	34.59	192kHz
Cruise Xc	22Oct2015b	33.463	50.885	51.75	192kHz

The acoustic recordings were obtained continuously during daylight (spring: 5:00 a.m. to 6:00 p.m., and fall: 5:30 a.m. to 6:00 p.m.) using passive acoustic equipment composed by an acoustic towed array (AUSET®). Three types of matrices were used: (Cruise VII) 250 m of a linear matrix composed of three omnidirectional elements (1.592 Hz high-pass filter) arranged equidistantly five meters apart and one meter from the end of the cable to maintain the stability of the system; (Cruise VIII) 300 m of a linear matrix composed of three omnidirectional elements (0.499 Hz high-pass filter) five and three meters apart, respectively, and arranged five meters from the end of the cable; and (Cruise X) 300 m of a linear matrix composed of three omnidirectional elements (0.499 Hz high-pass filter) five and 10 meters apart, respectively, and arranged five meters from the end of the cable.

The recorded signals were coupled to a digital recorder with two-channel input (Fostex® FR-2 LE, Fostex Company, United States of America) on board the ship and recorded in a digital file (.wav file) (Andriolo et al. 2018). An additional system consisting of a Roland Octa-Capture® digitizer plate (Roland Company, Japan) connected to an on-board computer was adopted during the last cruise, as well as the data acquisition performed using the PAMGuard program (Heriot-Watt University, United Kingdom). The whistle parameters were extracted using Raven Pro software 1.5 beta (Cornell Laboratory of Ornithology) using spectrograms with 1024 samples of DFT, 50% overlap, and 512 points on a Hann window. Only one channel was used for the analyses. Boat engine consisted the main noise source present on the recordings. Visual delimitation of whistles was established through the spectrogram to separate signature whistles from the others (variants). Signature whistles consist in a repetition of determined basic whistle contour (Caldwell et al. 1973), characteristically produced by an individual (Caldwell & Caldwell 1965; Sayigh et al. 1990). In order to detect a greater diversity of whistles that compose the WSAO common dolphins repertoire, the identified as signature whistles (Caldwell & Caldwell 1965) were selected once and kept in the analysis. All the subsequent similar whistles were excluded. The exclusion of signature whistles copies minimizes the chances of over-sampling specific whistles of an individual, group, or a given behavior (Azzolin et al. 2019). Acoustic emissions with low visual signal-to-noise ratios or overlapped with background noise were not included in the analyses.

The visual analysis of the contour of each whistle considering the frequency modulation was classified according to Ansmann et al. (2007). The following parameters were extracted from the fundamental frequency: minimum frequency, maximum frequency, frequency range, peak frequency, center frequency, initial and final frequencies, duration, and frequency gradient (the ratio between the delta frequency and duration in kHz/s). The peak frequency was extracted for contours that were not overlapped by other signals. The sampling frequencies did not affect the delimitation of the contours or the extraction of the acoustic parameters for the analysis of the general characterization of the whistles.

During the recording period, no other species were seen during the sighting. The correct species identification was confirmed visually by researchers, as well as the best group size estimation.

The statistical analyses were conducted using "R" (R Development Core Team 2015) in the 'vegan' package (Oksanen et al. 2019) and followed the sequence below:

2.1 GENERAL DATA ANALYSIS

Spearman rank correlation coefficient was used to test if the number of whistles varied according with the estimated group size of the six encounters recorded on the slope region of the WSAO in 2014 and 2015. The mean and standard deviation were calculated for each parameter.

2.2 WHISTLE CONTOUR ANALYSIS

A contingency table was created regarding the number of contour categories for each group. Then, whistle contour categories were analyzed through Matrix Correlation test (in the 'psych' package; Revelle 2020) that verifies the similarity between the orders of the data when classified by each of the quantities of whistles contours. Additionally, we tested if contours variability is related to the group size through Spearman rank correlation coefficient.

2.3 WHISTLE PARAMETERS ANALYSIS

Shapiro-Wilk test was executed to verify the normality of the whistles parameters. A non-parametric Kruskal-Wallis test was applied to determine if the acoustic parameters of whistles are different among the encounters. Multiple comparison values were analyzed through Dunn's Multiple Comparison Test and adjusted with sequential Bonferroni. A second analysis was performed considering different encounters grouped in the same year. Mann-Whitney test followed by a C5.0 decision tree algorithm analysis (Quinlan 1993) to classify intraspecific variation of significantly different whistles parameters within encounters between years.

2.4 WORLD LOCALITES COMPARISON

A fourth set of the analysis was performed to compare the results of the WSAO with the whistle parameters from other localities around the world based on previously published data (Oswald et al. 2003, 2007; Ansmann et al. 2007; Griffiths 2009; Gannier et al. 2010; Figueiredo 2014; Papale et al. 2014). The comparison was visualized by a non-metric multidimensional scaling – nMDS using Euclidian distance, followed by a permutational analysis of variance – PERMANOVA (Anderson & Braak 2003). The nMDS test was chosen due to its flexibility once the data acquired from other localities were restricted to the mean values of the whistle parameters.

3 RESULTS

Six encounters of *Delphinus delphis* were recorded between 2014 and 2015, resulting in approximately 4.8 hours of acoustic recordings. The encounters ranged from 80–250 individuals. The shortest recording period lasted 34.59 minutes, while the longest lasted about 63.32 minutes (Table 1).

3.1 GENERAL DATA ANALYSIS

No significant correlation was found ($r = 0.564$; $p > 0.322$; $n = 1377$) between number of whistles and the group size. We remove the CXa for this analysis, since the group size could not be estimated for that encounter.

3.2 WHISTLE COUNTOUR ANALYSIS

A total of 1,920 whistles were delimited. The signature whistles were identified and only the first contour of each replica was used in the analysis, totaling 290 repeated copies from different signature removed. A total of 1,630 whistles remaining were classified into six contour categories. In general, contours classified as upsweep ($n = 528$; 32.4%), downsweep ($n = 357$; 21.9%), and constant ($n = 218$; 13.4%) were the most frequent (Table 2). The whistle contours exhibited high concordance, as all categories showed high correlation among encounters (Table 3). Also, a positive relation between the downsweep contour category and group size was detected ($r = 0.895$; $p > 0.05$; $n = 1630$).

Table 2. Group size estimated and whistle contours extracted from the six encounters (CVIIa, CVIIb, CVIIIb, CXa, CXb and CXc) of *Delphinus delphis* recorded on the slope region of Western South Atlantic Ocean - WSAO.

Encounter	Group size	n Whistle	Upsweep	Downsweep	Constant	Concave	Convex	Sine
CVIIa	80	261	97	55	39	34	19	17
CVIIb	230	250	82	67	43	25	11	22
CVIIIb	250	308	83	78	42	35	35	35
CXa	?	253	66	50	36	41	18	42
CXb	200	274	106	40	32	45	19	32
CXc	230	284	94	67	26	48	28	21

Table 3. Results of the correlation matrix test comparing the six contour categories among each encounter of *Delphinus delphis* (CVIIa, CVIIb, CVIIIa, CXa, CXb and CXc) recorded on the slope region of the Western South Atlantic Ocean.

	CVIIa	CVIIb	CVIIIb	Cxa	CXb	CXc
CVIIa	1.00	0.94	0.90	0.85	0.93	0.94
CVIIb	0.94	1.00	0.96	0.87	0.78	0.87
CVIIIb	0.90	0.96	1.00	0.80	0.73	0.90
CXa	0.85	0.87	0.80	1.00	0.87	0.83
CXb	0.93	0.78	0.73	0.87	1.00	0.88
CXc	0.94	0.87	0.90	0.83	0.88	1.00

3.3 WHISTLE PARAMETERS ANALYSIS

Regarding the descriptive statistics, the frequencies ranged from 1.34–35.23 kHz (minimum-maximum), and the duration varied from 0.11–2.16 seconds. The results of the Shapiro-Wilk test showed that the parameters were not normally distributed. In general, intraspecific variation among encounters was significant for all parameters. Except for the CXc comparisons with CVIIa (p.adj < 0.01) and CVIIIb (p.adj < 0.01), multiple comparison p-values adjusted with the Bonferroni method indicated that duration did not varied significantly among encounters. When comparing encounters between years of 2014 and 2015, minimum frequency (p-value < 0.01), duration (p-value < 0.01), and frequency range (p-value < 0.01) were significantly different.

Subsequently a C5.0 decision tree algorithm was created based on the significant parameters reported above (minimum frequency, duration and frequency range). The results showed that whistles of the year 2014 had correct classification of 67.0%, while whistles of the year of 2015 had correct classification 54.0%; total accuracy was 60% and the respective Areas Under the Curve (AUC) are presented in the Figure 3. The algorithm computed the most used parameters in the classification that were minimum frequency (37.5%) and duration (37.5%). The tree plot is showed in the Figure 4.

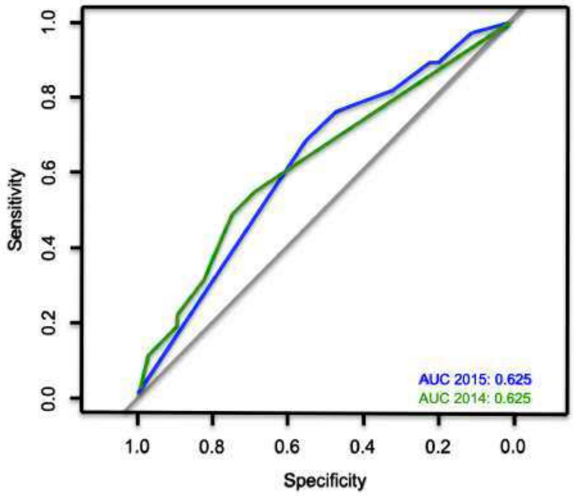

Figure 3. ROC (Receiver Operating Characteristics) curve of the tree algorithm analysis showing the intraspecific variation of whistles parameters (minimum frequency, duration and frequency range) between the encounters of *Delphinus delphis* recorded in the years of 2014 (Area under the curve – AUC colored in green) and 2015 (Area under the curve – AUC colored in blue).

Figure 4. Decision tree algorithm showing the classification of whistles parameters of duration, minimum frequency (MinF), and frequency range (FRange) between the encounters of *Delphinus delphis* recorded in the years of 2014 and 2015.

3.4 WORLD LOCALITIES COMPARISON

Initially, all parameters were used to compare data from other localities (Table 4) with the results obtained from the slope region of the WSAO (present study). The nMDS was consistent by grouping the Atlantic and Pacific oceans, and Mediterranean Sea repertoires based on only two whistle parameters: mean inflection points and duration (Figure 5). These results were corroborated through PERMANOVA test using 1000 permutations (*pseudo-F* $_{(2,\ 8)}$ = 1.4303, $p < 0.039$), being significantly different also when localities were compared among each locality (*pseudo-F* $_{(5,\ 1624)}$ = 2.2608e+09, $p < 0.01$).

Table 4. Whistle parameters of the principal localities of *Delphinus delphis* recorded worldwide and its respective references. Mean values are listed. Frequency parameters are presented in kHz.

	South Western Atlantic Ocean, Brazil (slope region)	Atlantic Ocean, Azores, Canary Island, and Biscay Bay	Mediterranean Sea	South Western Atlantic Ocean, Brazil (coastal region)	Mediterranean Sea	North Altantic Ocean, Celta Sea, UK
n (whistles)	2761	514	193	473	120	1835
Initial Frequency	11.7	13.0	11.1	13.1	10.9	12.0
Final Frequency	12.1	11.8	12.6	12.8	11.9	12.0
Minimum Frequency	10.1	8.14	8.2	8.7	8.5	9.4
Maximum Frequency	13.6	16.7	15.7	16.4	13.2	14.7
Frequency Range	3.5	8.6	6.5	7.7	4.6	5.2
Duration (s)	0.7	0.9	0.8	0.7	0.5	0.6
Inflection Points	1.3	1.0	2.0	1.1	1.1	0.6
Reference	**present study**	Papale et al. 2014	Papale et al. 2014	Figueiredo 2014	Gannier et al. 2010	Ansmann et al. 2007

Table 4 (continue). Whistle parameters of the principal localities of *Delphinus delphis* recorded worldwide and its respective references. Mean values are listed. Frequency parameters are presented in kHz.

	North Atlantic Ocean, English Chanel, UK	Pacific Ocean, Tropical East	Northeast Atlantic Ocean, Deep Celta Sea	Pacific Ocean, Tropical East, USA, Mexico e Peru	Pacific Ocean, Tropical East, USA, Mexico e Peru
n (whistles)	435	1012	1443	314	88
Initial Frequency	12.6	11.9	12.0	11.6	9.8
Final Frequency	12.5	12.7	12.0	12.1	11.4
Minimum Frequency	9.8	9.1	9.6	8.3	7.4
Maximum Frequency	15.8	15.1	14.5	15.0	13.6
Frequency Range	6.0	6.0	4.3	6.7	6.3
Duration (s)	0.6	0.6	0.6	0.7	0.8
Inflection Points	0.6	1.8	0.5	1.6	1.2
Reference	Ansmann et al. 2007	Griffthis 2009	Griffthis 2009	Oswald et al. 2007	Oswald et al. 2003

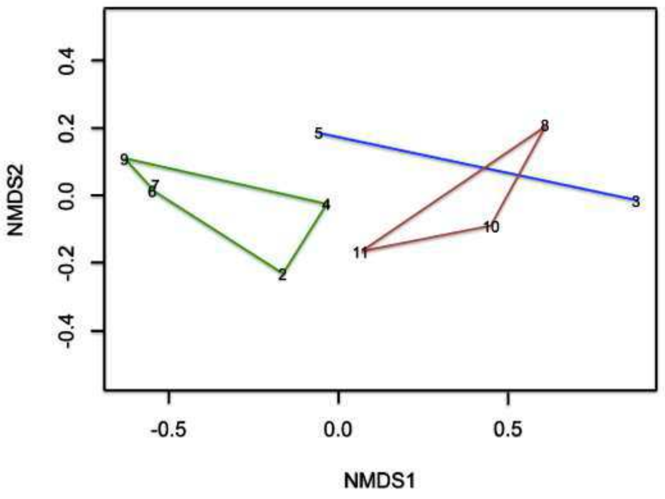

Figure 5. The nMDS analysis regarding the 11 localities of *Delphinus delphis* whistles repertoire around the world based on duration and mean inflection point parameters. The green, red and blue lines represent the Atlantic Ocean, the Pacific Ocean and the Mediterranean Sea records, respectively.

4 DISCUSSION

Intraspecific variation in the dolphin repertoire of the same population is expected due to several factors, including group size composition, behavior, body size and social structure (Azevedo et al. 2007; May-Collado & Wartzok 2008; Díaz-López 2011). Although no behavior or social structure data was registered in the present study, group size was not correlated to whistle emission rate either. Other aspects may be related to the whistle emission rate such as the level of dolphins' activity, being higher when animals are more active, (Wood 1953). Henderson et al. (2012) found that whistle emission rate was greater in more active behaviors, as fast traveling. Information contained in whistles can influence the group's timing to organize and perform a certain function (Norris et al. 1994; Janik & Slater 1998).

Upsweeps, downsweeps, and constant are the three most usual types produced by common dolphins reported in literature (Wakefield 2001; Ansmann et al. 2007; Griffthis 2009; Petrella et al. 2012; Papalle et al. 2014). The same pattern was found in this study (Table 2). The high concordance of whistle categories among the different encounters analyzed in this study demonstrates the consistency in the use of categories for this species. Griffiths (2009) analyzed whistles according to contour classification and the acoustical parameters characteristics for *D. delphis* in the Celtic Deep and Eastern Tropical Pacific. Their results revealed that roughly a third of all emitted whistles were characterized by an upsweep contour. The variability of contour categories of whistles recorded on the WSAO related to group size corroborate with previous studies described above. Dos Santos et al. (2005) suggested that, on bottlenose dolphins, a positive relation between contour variability and group size is expected. We did find a positive relation in downsweep contour and group size. Cetaceans rely on communication to maintain group cohesion (Acevedo-Gutiérrez 2009).

Studying relation of certain types of whistle contours with aspects of behavior, as group size for example, may provide insights into the use of these categories. Each contour category comprises variable units, such as duration and emission rate, that might be responsible for carrying different information (Caldwell & Caldwell 1965). The use of different categories may play an essential communicative role as an adaptive strategy, depending on behavioral circumstances and/or environment contexts. Group size comparisons with whistle contour categories are scarce. The comparison of group size and contour categories was not significant in whistles of bottlenose dolphins (dos Santos et al. 2005). Only a few studies can be found comparing whistle contour categories with behavior aspects. For instance, belugas modify their whistle contour repertoire depending on behavioral circumstances, being the upsweep contours more frequent during social events and directional swimming (Sjare & Smith 1986). McCowan and Reiss (2001) suggested that upsweep whistle category of bottlenose dolphins acted as a contact call comprising individually distinct features.

The spectral and temporal parameters of the whistles of *D. delphis* encounters recorded during the cruises on the slope region of the WSAO were quite heterogeneous (Table 4). Significant differences in whistle parameters were found among all encounters reinforcing intraspecific variation of that population. This result was expected because dolphins in general live in fluid societies (Tyack 1986; Barzúa-Durán & Au 2002; May-Collado & Wartzok 2008). This variability might represent the dolphins' ability to modulate whistle parameters to cope with constant changes in their biotic and abiotic environment. According to Tyack (1986, 2000), greater variation in an individual's whistle repertoire can be expected in dolphins that live in fluid societies; dolphins that live in stable groups generally have group-distinct repertoires. Besides group fluidity, learning and genetic differentiation also might be responsible for intraspecific whistle variation (Azevedo & Van Sluys 2005; Camargo et al. 2007; Rossi-Santos & Podos

2006). Fission-fusion behavior of common dolphins has been reported in many areas (Neumann 2001; Bruno et al. 2004; Ansmann et al. 2007) and may be also the case for the Western South Atlantic populations of common dolphins.

The analysis comparing the two years of our study demonstrated significant differences in duration, minimum frequency and frequency range parameters. Duration, inflection points and frequency range would seem to be characteristics of the whistles influenced mainly by the specific population (La Manna et al. 2020). Considering variation within species, high differences in duration and inflection point values may be an indicative of emotional communication and reflects high inter-individual variation (Wang et al. 1995; Morisaka et al. 2005; Azevedo et al. 2007). Duration and inflection did not differ among encounters, but duration was significant different when encounters were grouped between years 2014 and 2015. These differences may indicate that inter-individual variation is less variable when considering the same year, but when comparing different years, groups of individuals can change expressively and, consequently, inter-individual variation is higher. Griffthis (2009) also reported minimum frequency as significantly different between three years of studies with *D. delphis* in the Celtic Deep and Eastern Tropical Pacific. Few studies suggest that minimum frequency might be influenced by body size and group size (May-Collado et al. 2007a, 2007b). Although further studies and longer temporal dataset are necessary to support that information, our results emphasize the influence of certain parameters that might be related to intraspecific variation.

The parameters of the SWAO were compared to other localities reported in literature in order to evaluate the geographic variation in the characteristics of common dolphin repertoires. The analysis between different localities was subjected to the availability of the data. Mean inflection points and duration of the whistles were used to compare whistles repertoire of *D. delphis* from different ocean localities. Those parameters are believed to express information on individual adaptations (May-Collado & Wartzok 2008) and may indicate information about the identity of the individuals (Steiner 1981). The plasticity to modify its repertoire structure due to local environment and socio contexts (Morisaka et al. 2005, Esch et al. 2009, Henderson et al. 2012, Andrade et al. 2014) might promote variation between populations at micro and macro geographic scales (May-Collado & Wartzok 2008). It is suggested that dolphins may increase frequency parameters overall to deal with noise disturbance (Morisaka et al. 2005; Andrade et al. 2014; La Manna et al. 2020). Increasing in frequency parameters might be related to transmission efficiency and ensures detectability of acoustic signals (Rako-Gospić & Picciulin, 2016). However, those changes in the structure of the parameters likely reflect temporary shifts in whistle production (Ansmann et al. 2007; May-Collado and Wartzok 2008). The localities of populations may interfere on the emissions, once some acoustic parameters are more similar in populations of adjacent areas than in populations farther apart (Azevedo and Van Sluys 2005; Rossi-Santos and Podos 2006; May-Collado and Wartzok 2008; La Manna et al. 2020). Based on mean inflection point and duration, our results showed consistency by grouping repertoires geographically: Atlantic, Pacific and Mediterranean populations. Inflection point and duration are characteristics that reflect frequency modulation and temporal property of whistles, respectively (La Manna et al. 2020). Many factors influence the variation in dolphins' repertoire and despite not conclusive in terms of data limitation, our studies suggest that differences in those acoustic parameters may be in response to different macro scale localities.

5 CONCLUSION

Whistles parameters of *Delphinus delphis* in the Western South Atlantic Ocean show high intraspecific variation. The high concordance of whistle contour categories among the different encounters analyzed in this study demonstrates the consistency in the use of categories for the species. We find a positive relation in downsweep contour and group size. The variability of contour categories and its use in different contexts may play an essential communicative role as an adaptive strategy, depending on the environmental and behavioral circumstances. Our studies reinforce the dynamic of fission-fusion society of short-beaked-common-dolphins from SWAO through the high intraspecific variation in spectral and temporal parameters' repertoire. Also, our results suggest that certain parameters might influence the variation in whistles repertoire of this species. Repertoire comparison between our study and other localities reinforces the pattern exhibited for the species as well as corroborate with differences in geographical scale.

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CHAPTER 3. TIME-FREQUENCY AS CLASSIFICATION PARAMETERS OF PULSED SOUNDS PRODUCED BY SHORT-BEAKED-COMMON-DOLPHINS

Abstract. Sounds produced by dolphins can be grouped into tonal (whistles) and pulsed sounds (echolocation clicks and burst sounds). The clicks are broadband pulses temporarily spaced to allow the echo processing between the sound source and the object. Echolocation is related mainly to prey detection and environment recognition. Click trains tend to present a decreasing inter-click interval due to a continuous update of the target's location when the animal approaches to capture the prey. Besides foraging and feeding contexts, burst pulsed sounds have been associated with social short communication. Despite echolocation clicks are relatively well documented, there is no consensus related to the broad variety of the burst pulsed signals. The present study analyzed time-frequency characteristic through clustering and discrimination analysis to classify pulsed sounds. A total of 64 click trains were analyzed from short-beaked-common dolphins recorded in the slope region of Western South Atlantic Ocean. Three approaches (time; frequency; and combined time-frequency parameters) were compared through k-means clustering and posterior cluster validation through Random forest analysis. K-means resulted in four clusters for all approaches in which time parameters contributed more accurately with the first two dimensional-axis corresponding to 87% (Dim1 = 70.2%, and Dim2 = 17.2%). Random forest showed that time-frequency dataset revealed to be the best classification of pulsed sounds in *D. delphis* (Accuracy = 84.6%; confidence interval CI = 65.1%-95.6%; $p < 0.01$). This result considers the animal as an acoustical identity, emphasizing the importance of certain parameters that influence this identity, thus reflecting the energy cost optimization for sound production.

Key words: Acoustics, clicks, *Delphinus delphis*, slope region, Western South Atlantic

1 INTRODUCTION

Sound emissions are essential for cetaceans' survival (Tyack 2000). Acoustic emissions produced by whales and dolphins vary between species and/or populations and are generally associated with specific behavior. In general, the emission of sounds in Odontoceti can be grouped into tonals (whistles) and pulsed sounds (pulsed calls, echolocation clicks and burst sounds) (Herman & Tavolga 1980, Popper 1980, Ford 1989, Richardson et al. 1998).

Whistles are narrowband frequency modulated signals usually lower than 20kHz (Lammers et al. 2003), being used primary in a social context in order to organize and regulate group function (Herman & Tavolga 1980, Norris et al. 1994, Janik & Slater 1998). Pulsed calls have distinct tonal properties, frequently modulated during the call emission, consisting in fast broadband sound pulses with high repetition rates reproduced between sidebands intervals (Watkins 1967; Ford 1989). It is a particular type of pulsed sound only described for few species (Ford & Fisher 1978, 1986; Murray et al. 1998; Karlsen et al. 2002; Rossi-Santos et al. 2008; Nemiroff & Whitehead 2009). Pulsed sounds that comprehend echolocation clicks and burst sounds can be differentiated roughly by their temporal pattern.

Clicks have a frequency range that varies between 10 and 150 kHz and are temporarily spaced to allow processing of the bidirectional travel time between the sound source and the object (Au 1993). That particular acoustic emission is denominated echolocation and is related mainly to detect and characterize a specific target (a prey or an obstacle, for example) and to recognize the environment (Au 1993). At the end of an echolocation sound, the clicks tend to decrease the interval between each one, giving a continuous update of the target's location when the animal approaches the prey to capture it (e.g. Madsen et al. 2005, Verfuß et al. 2009). Those faster click trains are classified as burst sounds (or burst pulses) (Herman & Tavolga 1980). Burst sounds are characterized by a high repetition rate (greater than 300 pulses per second) or at low inter-pulse intervals (less than 3ms), and are often used in social communication of short distances or emotional context, mainly in agonistic, alarm or aggressive behaviors (Caldwell & Caldwell 1967, Norris et al. 1994, Lammers et al. 2006, Au & Hastings 2008). The difference between burst sounds and clicks is subtle, with the latter having a longer inter-click interval and being recorded preferentially during echolocation (Au & Hastings 2008). Other categories of pulsed sounds including buzzes, squeaks, yelps, and squawks are fast broadband pulses, usually ranging from 100 to 2000 ms. Such emissions are emitted in an energy range from around 1kHz to over 150kHz (Ridgway 2009). In this study the term ‘pulsed sounds’ will be related to echolocation clicks and burst pulses, once there is no evidence so far of pulsed calls for the genus *Delphinus* reported in literature.

Clicks can be species-specific (Amorim et al. 2019, Soldevilla et al. 2008, Madsen et al. 2005, Akamatsu et al. 1998, Kamminga et al. 1996) or group-specific (Zimmer et al. 2005) and have been used for many passive acoustic-monitoring studies (Barlow & Taylor 2005, McDonald et al. 2006, Rankin et al. 2016). Most dolphin species produce broadband clicks, while porpoises and pigmy sperm whales generally produce narrowband clicks (Au 1993). Sperm whales, beaked whales and porpoises produce distinctly different clicks due to their temporal and spectral properties (Amorim et al. 2020, Goold & Jones 1995, Madsen et al. 2005, Johnson et al. 2006, Baumann-Pickering et al. 2010). Sperm whales produce specific pattern of multi-pulsed low-frequency clicks called codas (Amorim et al. 2020, Møhl et al. 2003) and beaked whales usually produce frequency-modulated clicks (Zimmer et al. 2005). Despite clicks are relatively well documented, there is a lack of studies concerning burst pulses and other faster broadband pulses categories produced by delphinids.

Based primarily on differences in the inter-click intervals (ICI), many researchers consider clicks and burst pulses (including its sub types) as two separate categories, even though both are formed by broadband pulses (Au 1993, Au & Hastings 2008, Henderson et al. 2011, Herzing & Jonhson 2015). Since pulsed sounds are composed by a variety of time-frequency parameters (Lammers & Oswald 2015), that segregation may not reflect the real nature of the produced pulses (Luís et al. 2016). Also, burst pulses can be crucial for the effective identification of some species (Rankin et al. 2007). Multivariate classification techniques involving time-frequency properties of pulsed sounds can assist dealing with those difficulties in categorization (Roch et al. 2011, Rankin et al. 2016), thus helping elucidate the hidden information resulting from generalized designation of burst sounds (Luís et al. 2016).

Most studies related to pulsed sounds, specifically to echolocation clicks of short-beaked-common-dolphins comprises discriminant and species classification (Lu et al. 2013, Rankin et al. 2016, Amorim et al. 2019). Difficulties in categorizing burst sounds are also due to the subjectivity of the descriptions, being generally classified through aural and spectrographic characteristics. Therefore, the present study is the first to analyze and classify pulsed sounds produced by this species in the Western South Atlantic Ocean. Considering that there is still no consensus about detailed characterization of pulsed sounds, the main goal of this study was to contribute to describe and clarify the short-beaked-common-dolphin's pulsed sounds.

2 MATERIALS AND METHODS

2.1 DATA ACQUISITION AND FIELD WORK

The present study was carried out in partnership with the "Projeto Cetáceos do Talude" onboard the R/V Atlântico Sul. It covered the region of the continental shelf break between Chuí, Rio Grande do Sul and Cabo Frio, Rio de Janeiro, generally limited by the isobatimetric of 2,000 meters. The three acoustic encounters investigated in this study (one in October 14th and two in October 22nd) were recorded to the south of Florianopolis, Santa Catarina, area located in the Pelotas Basin (28 and 34 S) (Santos 2009). Researches of the Projeto Cetáceos do Talude were responsible for conduct the visual monitoring and population estimations of cetacean species in the study area (Figure 6, Table 5).

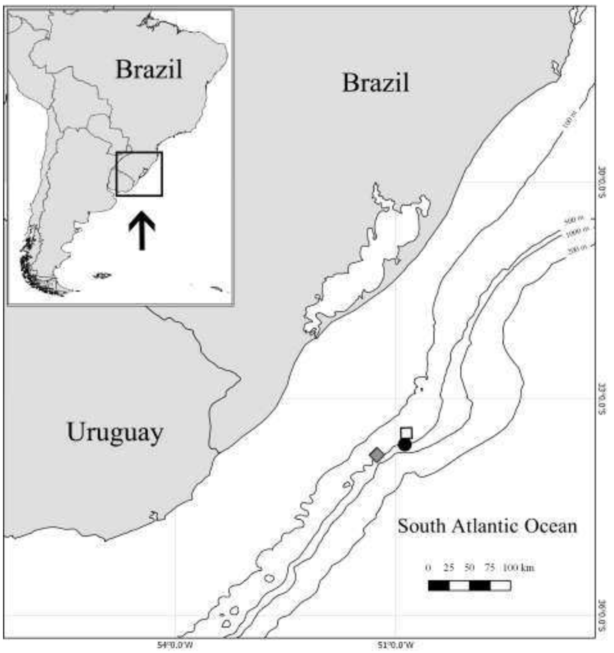

Figure 6. Localities of the three encounters of short-beaked-common-dolphins recorded on the slope region of the Western South Atlantic Ocean. ‘Cruise VIII’ (grey diamond) was registered in 2014; and ‘Cruise Xa’ (black circle), and ‘Cruise Xb’ (white square) were registered in 2015.

Table 5. Information regarding the three encounters of *D. delphis* recorded in the slope region of the Western South Atlantic Ocean.

Encounters	Date	Lat (S)	Long (W)	Recording duration (min)	Sampling Frequency
Cruise VIII	14Nov2014	33.763	51.337	40.31	200kHz
Cruise Xa	22Oct2015	33.603	50.873	34.59	192kHz
Cruise Xb	22Oct2015	33.463	50.885	51.75	192kHz

The acoustic recordings were obtained continuously during daylight (spring: 5:00 a.m. to 6:00 p.m., and fall: 5:30 a.m. to 6:00 p.m.) using passive acoustic equipment composed of a towed matrix (AUSET®). Two types of matrices were used: (Cruise VIII) 300 m of a linear matrix composed of three omnidirectional elements (0.499 Hz high-pass filter) five and three meters apart, respectively, and arranged five meters from the end of the cable; and (Cruise X) 300 m of a linear matrix composed of three omnidirectional elements (0.499 Hz high-pass filter) five and 10 meters apart, respectively, and arranged five meters from the end of the cable.

The recorded signals of the towed hydrophones were linked to a digital recorder with two-channel input (Fostex® FR-2 LE, Fostex Company, United States of America) on board the ship and recorded in a digital file (.wav file) (Andriolo et al. 2018). An additional system consisting of a Roland Octa-Capture® digitizer (Roland Company, Japan) connected to an on-board computer was adopted during the last cruise, and the data acquisition was performed using the PAMGuard (Heriot-Watt University, United Kingdom). Only one channel was used for the analyses. Boat engine consisted the main noise source present on the recordings. During the recording period, no other species were seen during the sighting. The correct species identification as well as the best group size estimation was confirmed visually by researchers onboard.

2.2 PULSED SOUND ANALYSIS

The initial analysis was performed through Raven Pro examining the spectrograms and visually detecting the pulsed sound trains (Hamming window of 512 points of FFT with 50% overlap, DFT 512 samples, resolution of 1.33 ms in time and 375 Hz in frequency). A subset of data containing the click trains of each encounter group (Cruise VIII, Cruise Xa and Cruise Xb) was created. A high pass filter of 10 kHz was implemented in order to minimize the low frequency noise influence on the audios. The duration of each pulsed sound train was also calculated directly on the spectograms. The echolocation signals were included in the analysis independent of the angle of the recorded animal's head to the hydrophone array.

A custom routine in MATLAB (Math works, Natick, MA) was used to analyze the subset data of click trains. The following parameters were automatically extracted: inter-click interval (ICI – the time between pulsed signals in a click train), repetition rate (RR – the number of clicks divided per click train duration), peak frequency (frequency of maximum energy), 3dB bandwidth (the bandwidth at 3dB points beneath from the maximum intensity), and 10dB bandwidth (the bandwidth at 10dB points beneath from the maximum intensity)

2.3 STATISTICAL ANALYSIS

For statistical analysis it was calculated the mean values for each train for all computed parameters in order to use the train as a unit representing the acoustics characteristics. Then, the non-hierarchical k-mean clustering analysis and Random forest algorithm were performed separately for time parameters (duration, ICI and RR), frequency parameters (peak frequency, 3dB and 10dB bandwidths) and for a combination of time-frequency parameters. The statistical analyses were conducted using "R" (R Development Core Team 2015) in the packages 'stats', 'MASS', 'tidyverse', 'cluster', 'factoextra', 'magrittr', 'dplyr', 'ggpubr', 'permute', 'lattice', 'vegan' for k-means analysis; packages 'randomForest', 'caret', 'e1071' and 'pROC' for Random Forest analysis; and packages 'vegan', 'dunn.test' for Dunn test adjusted with the Bonferroni.

2.3.1. Pulsed sounds K-means clustering

A non-hierarchical k-means clustering analysis was performed on each subsample of pulsed sounds previously delimitated, using the time (duration, ICI and RR) and frequency (peak frequency, 3dB and 10dB bandwidths) parameters as variables. The analysis was accomplished using the square Euclidian distance and comprises of partitioning the dataset into k clusters. Clusters consist in aggregation of data points based in certain similarities. The number k refers to the number of centroids (the center of the cluster) you need in the dataset. Each data point is distributed to every cluster by minimizing the in-cluster sum of squares. The number of bootstrap is a re-sampling to estimate how stable the clusters are and in this analysis consisted of 999. So, the k-means algorithm distinguishes k number of centroids and allocates each data point to the nearest cluster, keeping the lowest distance between the centroids (Garbade 2018). Within sum square ('wss') was the method used in the analysis. The principal objective of the K-Means analysis is to minimize the sum of distances between the points within a cluster and their corresponding cluster centroid. Optimal number of clusters (k) is based on the cluster value where the decrease in 'wss' value becomes constant, as we increase the number of clusters. The datasets were scaled in each stage.

2.3.2. Clustering validation through Random Forest

The Random Forest analysis comprises machine learning technic creating decision trees at random that will define decision-making. The algorithm will create a structure similar to a flowchart, with "nodes" where a condition is verified, and if the flow is met, it follows through one branch, otherwise, always leading to the next node, until the end of the tree. With the training data, the algorithm searches for the best conditions, and where to insert each one into the flow. The Random Forest algorithm is a strong method because in addition to classifying pulsed sounds, it also measures the degree of reliability of this classification as assignment probabilities (Rankin et al 2016).

The area under the curve (AUC) of each cluster plotted indicates the goodness of fit (1 = perfect fit) and represents how much the analysis is adjusted to the model (ROC curve). AUC provides combined measure of performance through all possible classification thresholds. ROC curves derive from the count of True Positives (Y-axis, designated as 'Sensitivity') by False Positives (X-axis, designated as '1-Specificity') as the frequencies accumulated following a rank ordering. The ROC adopts area under the curve (AUC) as the fit measure. The Out of Bag (OOB) error utilizes bootstrap aggregating (bagging) to estimate the prediction error of sub-samples of the dataset used for training. Finally, kappa is the measure of agreement between categorical variables, with values close to 1 consisting in the best kappa score. The OOB, total accuracy, balanced accuracy, model suitability and kappa were computed.

2.3.3 Multiple parameters comparison among clusters

A permutational analysis of variance – PERMANOVA was used to compare variables among the four clusters in order to support and corroborate the results of the previous classification methods. Also, multiple comparison values comprising time and frequency parameters were analyzed through Dunn's Multiple Comparison Test and adjusted with sequential Bonferroni. The significance adopted was $p < 0.05$.

3 RESULTS

A total of 86.34 min of acoustic emission containing 3037 clicks (64 click trains) were analyzed from three encounters of short-beaked common dolphins recorded.

3.1 PULSED SOUNDS K-MEANS CLUSTERING

The optimal cluster value for all the three dataset defined was $k = 4$ (Figure 7). Thus, pulsed sounds recorded in this study were successfully divided into four clusters. The results of the k-means analysis performed with time parameters dataset showed that the first two dimensions explained 87.4% of the variance of the clusters (Dim1 = 70.2%, and Dim2 = 17.2%; Figure 8). Frequency parameters dataset exhibited 81.6% of the variance explained by the first two dimensions (Dim1 = 53.7%, and Dim2 = 27.9%; Figure 9); and combined dataset of time-frequency parameters exhibited 62.7% of the variance of the clusters explained by the first two dimensions (Dim1 = 35.8%, and Dim2 = 26.9%; Figure 10).

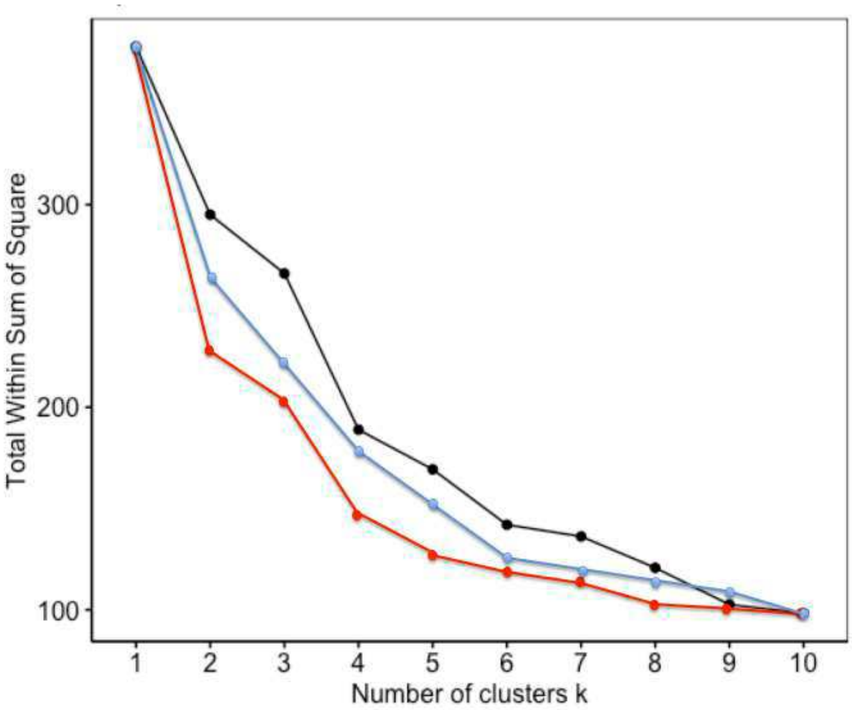

Figure 7. Optimal number of clusters of k-means analyzes using time parameters (red line), frequency parameters (blue line), and time and frequency parameters (black line) of pulsed sounds produced by *D. delphis*.

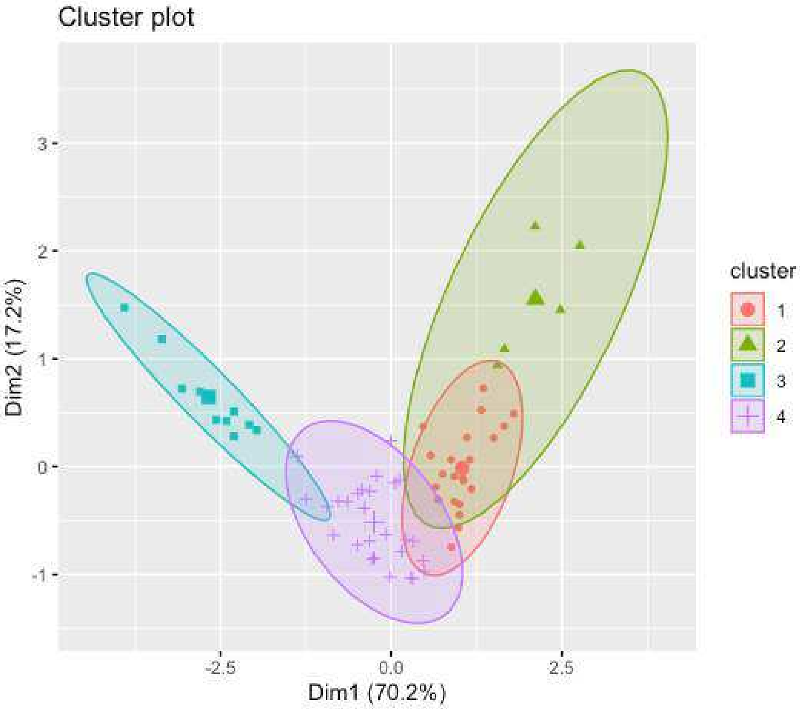

Figure 8. Cluster Plot of k-means using time parameters (duration, ICI and RR) of pulsed sounds produced by short-beaked-common-dolphins.

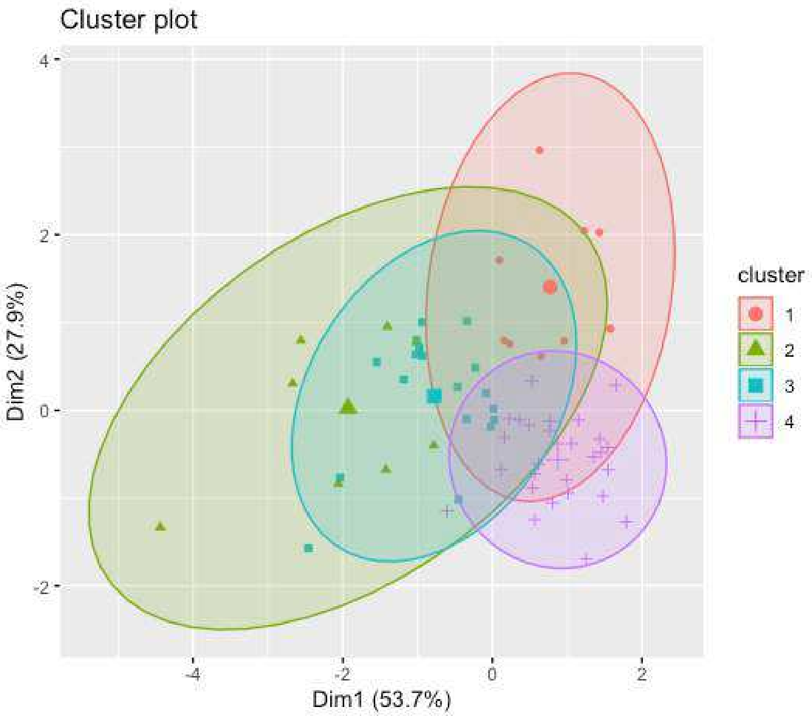

Figure 9. Cluster Plot of k-means using frequency parameters (peak frequency, 3dB and 10dB bandwidths) of pulsed sounds produced by *D. delphis*.

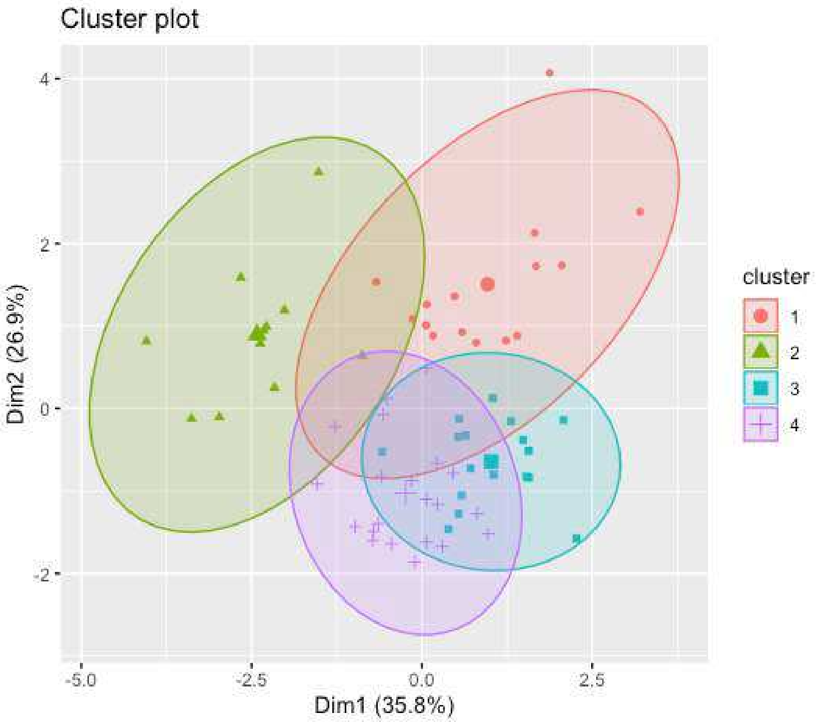

Figure 10. Cluster Plot of k-means using time (duration, ICI and RR) and frequency (peak frequency, 3dB and 10dB bandwidths) parameters of pulsed sounds produced by *D. delphis*.

3.2 CLUSTERING VALIDATION THROUGH RANDOM FOREST

The clustering validation through Random Forest algorithm followed the sequences of three-separated analyses described above (item 4.1). The train was set to 60% of the dataset, and the validation was set to 40%. The number of trees corresponded to 500 for the random forest classification. The results of OOB for each dataset: time (Figure 11), frequency (Figure 12) and all parameters (Figure 13) were 23.68%, 50.00% and 15.79% respectively.

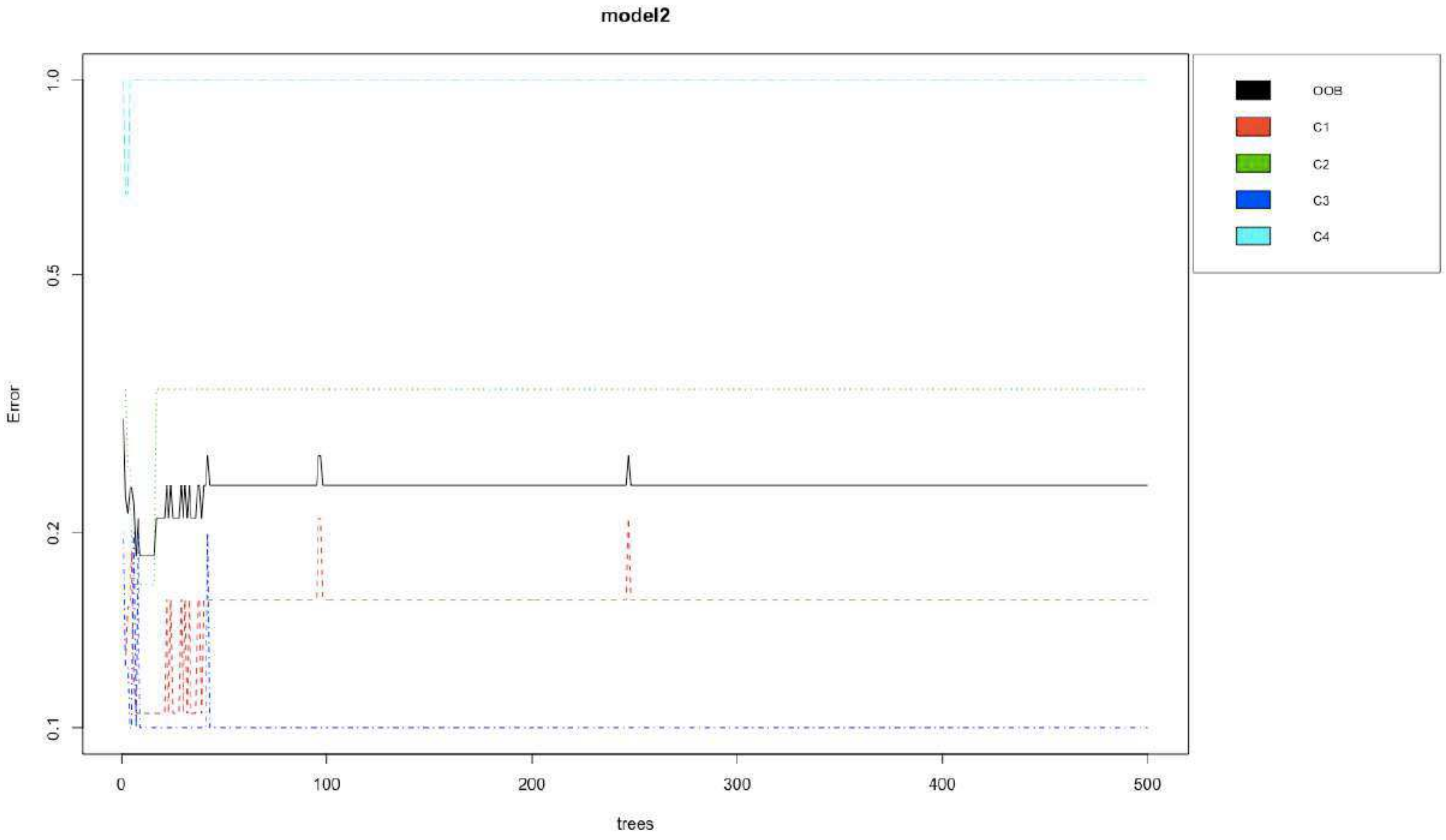

Figure 11. Errors associated to each of 500 computed trees and the Out of Bag (OOB) results regarding each cluster (C1 to C4) of the time parameters dataset (duration, ICI and RR) analyzed through Random Forest.

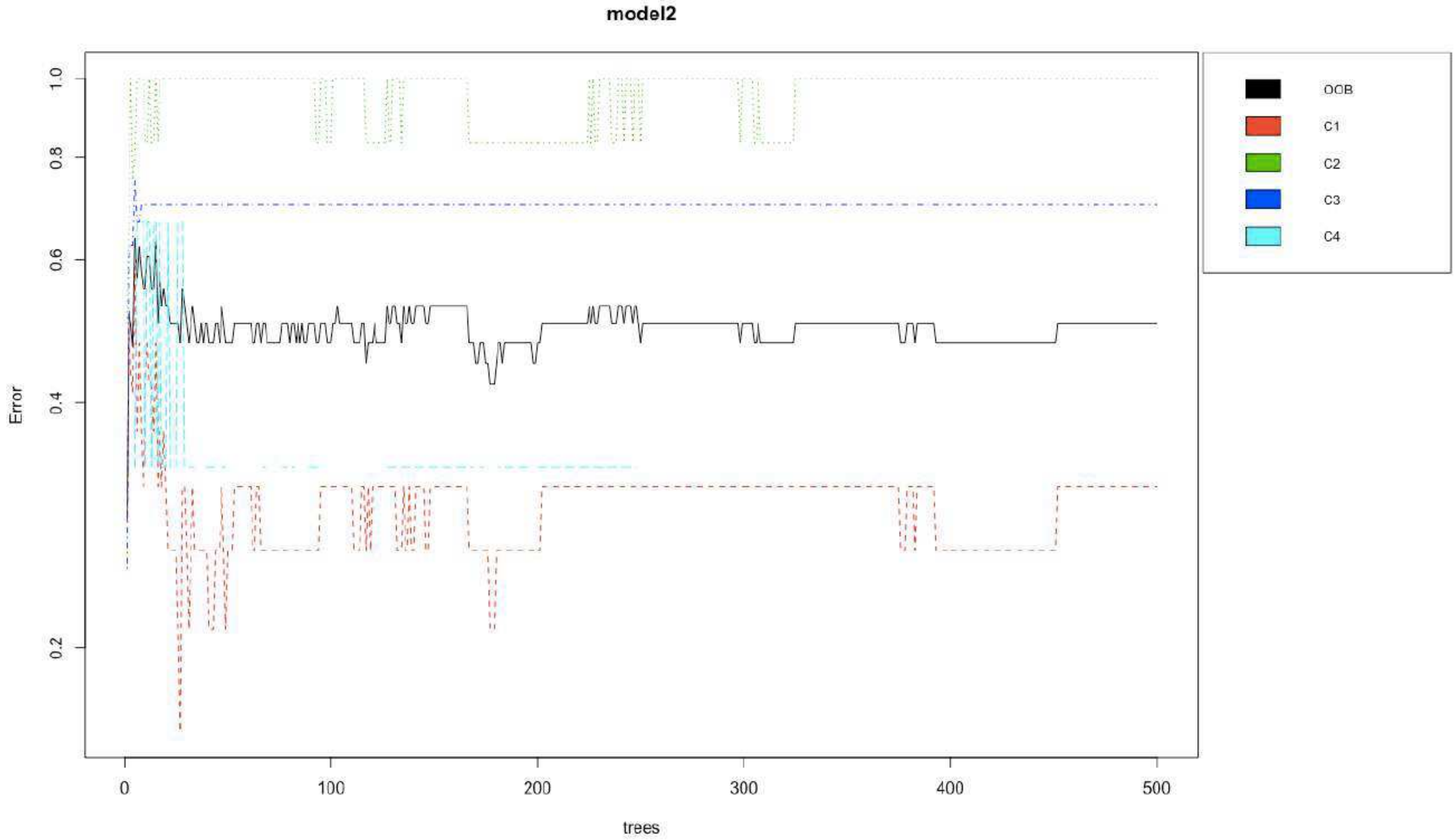

Figure 12. Errors associated to each of 500 computed trees and the Out of Bag (OOB) results regarding each cluster (C1 to C4) of the frequency parameters dataset (peak frequency, 3dB and 10dB bandwidths) analyzed through Random Forest.

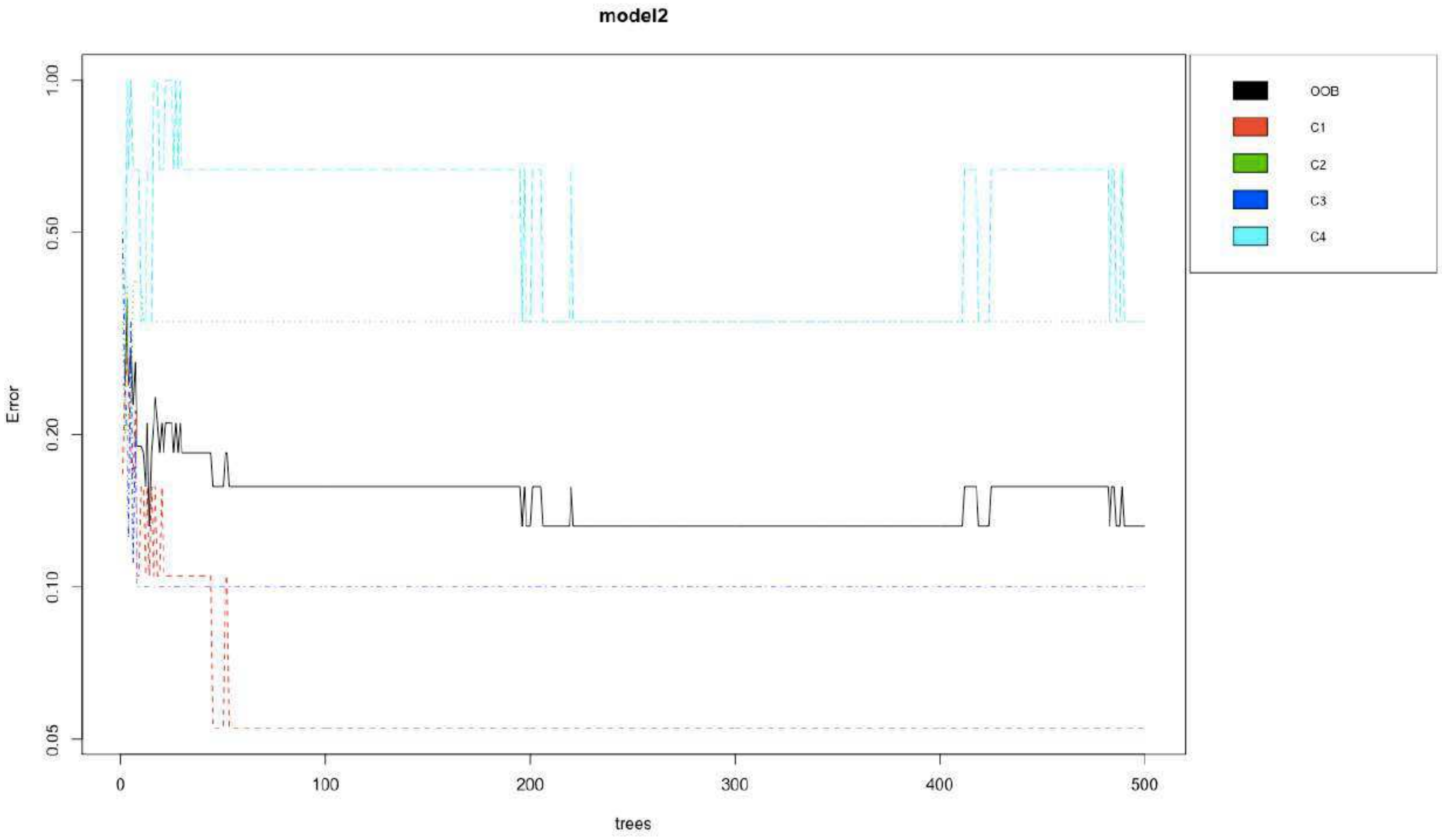

Figure 13. Errors associated to each of 500 computed trees and the Out of Bag (OOB) results regarding each cluster (C1 to C4) of all parameters dataset (duration, ICI and RR, peak frequency, 3dB and 10dB bandwidths) analyzed through Random Forest.

The Random Forest showed that all parameters combined (time and frequency parameters) had the best model (Accuracy = 84.6%; confidence interval CI = 65.1%-95.6%; $p < 0.01$) to classify pulsed sound in short-beaked-common-dolphins (Table 6). The balanced accuracy represents the accuracy of each cluster analyzed, with values ranging from zero to one, being value 1 representing 100% of accuracy (Table 7). Except for the cluster 2 (C2 = 1.00), in general our results showed that 'all parameters' combined had the best values of balanced accuracy.

Table 6. Results of the clustering validation through Random Forest analysis of pulsed sounds produced by *D. delphis*. Dataset of all parameters includes time (duration, ICI and RR) and frequency (peak frequency, 3dB and 10dB bandwidths) parameters.

Dataset	Accuracy	Confidential Interval (95% CI)	P-value	Kappa
Time parameters	0.769	0.5635 - 0.9103	$p < 0.05$	0.651
Frequency parameters	0.500	0.2993 - 0.7007	$p > 0.05$	0.309
All parameters	0.846	0.6513 - 0.9564	$p < 0.01$	0.758

Table 7. Balanced accuracy results of each cluster analyzed through Random Forest. Dataset of 'all parameters' includes time (duration, ICI and RR) and frequency (peak frequency, 3dB and 10dB bandwidths) parameters.

	Balanced Accuracy			
Dataset	C1	C2	C3	C4
Time parameters	0.769	1.000	0.727	0.790
Frequency parameters	0.692	0.425	0.716	0.978
All parameters	0.846	0.917	0.727	1.000

Predicting on Validation set was exposed in a Confusion Matrix for each dataset: time, frequency, and all parameters (Table 8). Receiver operating characteristic (ROC) from time, frequency and 'time-frequency' parameters dataset were presented in figures 14, 15 and 16, respectively. Time-frequency parameters dataset showed the best performance plotted on the AUC graphic (Figure 16), with the values very close/or equal to 1 (C1 = 0.982; C2 = 0.992; C3 = 0.977; and C4 = 1.000).

Table 8. Confusion Matrix of the predicting validation clusters for each dataset: time parameters (duration, ICI and RR), frequency parameters (peak frequency, 3dB and 10dB bandwidths), and all parameters. Values are presented in percentage (%).

	Time				Frequency				All Parameters			
	C1	C2	C3	C4	C1	C2	C3	C4	C1	C2	C3	C4
C1	76.9	0.0	15.4	7.7	77.8	22.2	0.0	0.0	80.0	6.7	13.3	0.0
C2	0.0	100	0.0	0.0	66.7	0.0	33.3	0.0	0.0	100	0.0	0.0
C3	33.3	0.0	66.7	0.0	40.0	30.0	30.0	0.0	33.3	0.0	66.7	0.0
C4	50.0	0.0	0.0	50.0	0.0	25.0	0.0	75.0	0.0	0.0	0.0	100

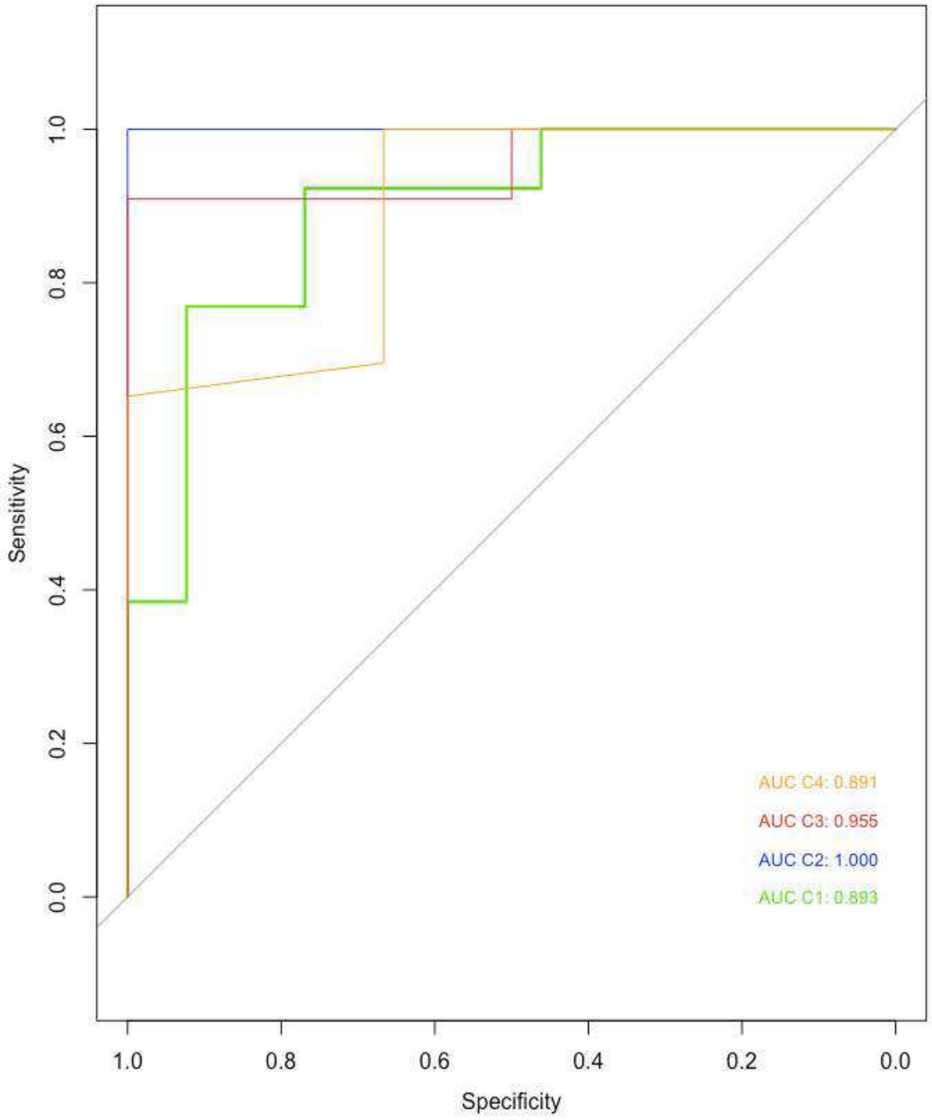

Figure 14. The Receiver Operating Characteristic (ROC) curves from time parameters (duration, ICI and RR) and the Area Under the Curve (AUC) of the four clusters analyzed through Random Forest algorithm.

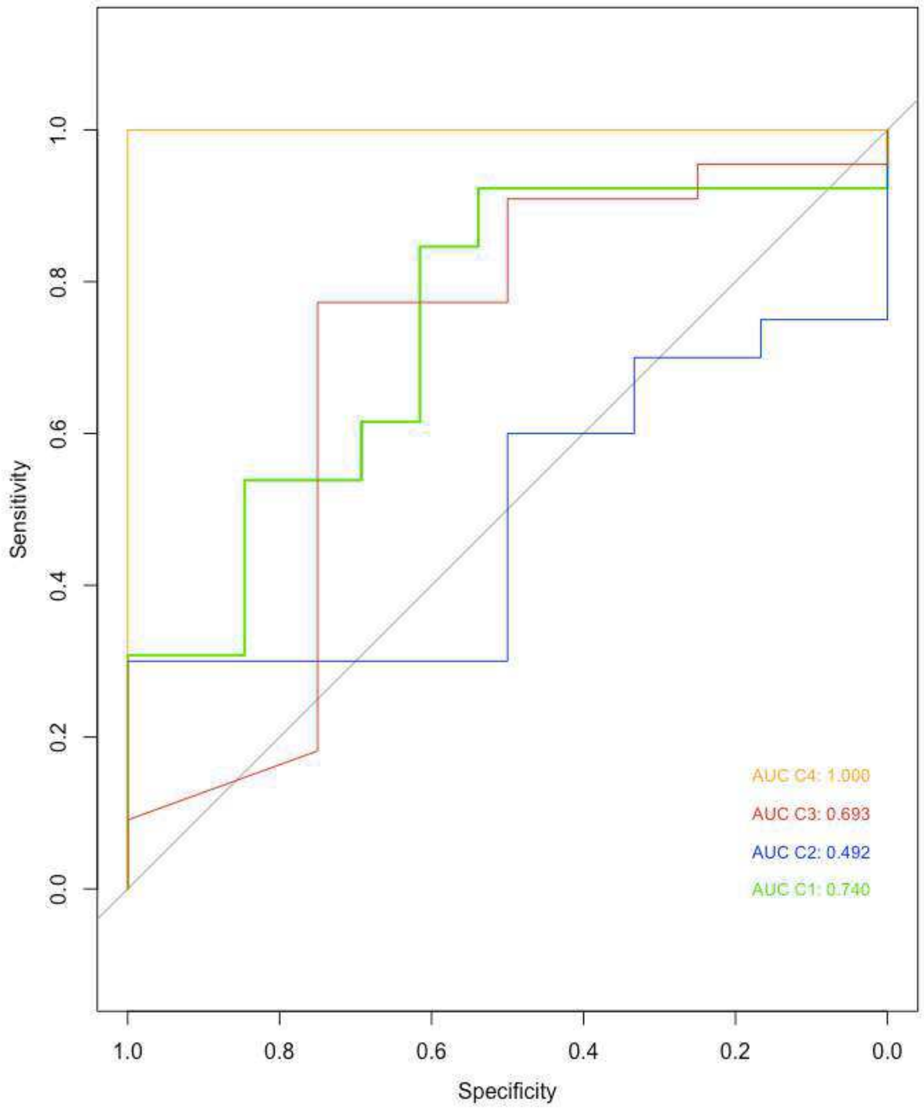

Figure 15. The Receiver Operating Characteristic (ROC) curves from frequency parameters (peak frequency, 3dB and 10dB bandwidths) and the Area Under the Curve (AUC) of the four clusters analyzed through Random Forest algorithm.

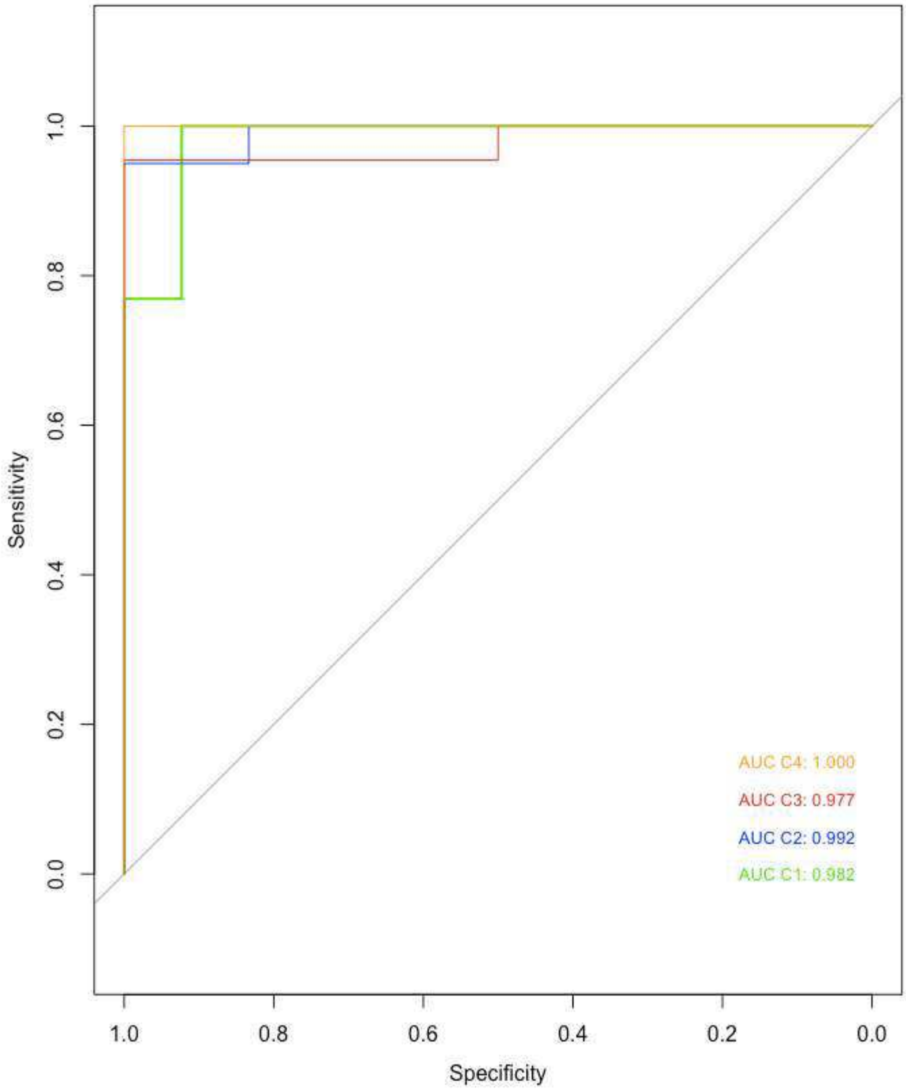

Figure 16. The Receiver Operating Characteristic (ROC) curves from all parameters (duration, ICI, RR, peak frequency, 3dB and 10dB bandwidths) and the Area Under the Curve (AUC) of the four clusters analyzed through Random Forest algorithm.

3.3 MULTIPLE PARAMETERS COMPARISON AMONG CLUSTERS

The results obtained from the previous classification analysis confirmed the four different types of pulsed signals (clusters C1 to C4) based on time and frequency parameters (Table 9), further corroborated with PERMANOVA test using 1000 permutations ($pseudo\text{-}F_{(3,\ 60)} = 5.4006$, $p < 0.05$). Regarding to click train duration and inter-click interval, the cluster C2 exhibited the highest values, while the cluster C3 showed the lowest ones. C3 cluster also comprised the highest peak frequency parameter. Multiple comparison p-values Dunn test adjusted with the Bonferroni method indicated that in general, the temporal variables (Duration, ICI and RR parameters) were significantly different comparing between pairs of clusters ($p < 0.01$). Regarding frequency variables, only peak frequency demonstrated significantly difference between some clusters ($p < 0.01$), while 3dB and 10dB bandwidths showed no significance between any of the cluster's pairs ($p > 0.05$, and $p > 0.05$, respectively).

Table 9. Parameters values of each cluster representing the types of pulsed sounds (Mean ± SD). Time parameters Duration and Inter-click Interval - ICI, are presented in milliseconds, and Repetition Rate – RR is presented in pulses per second. Frequency parameters (Peak Frequency - PF, 3dB and 10dB bandwidths) are presented in kHz.

Cluster	Duration	ICI	RR	PF	3dB	10dB
C1	384.75 ± 194.29	6.70 ± 7.73	110 ± 70	44.13 ± 2.88	12.66 ± 4.30	38.65 ± 15.05
C2	1024.50 ± 424.92	43.06 ± 15.75	20 ± 10	43.24 ± 4.20	14.45 ± 3.70	42.41 ± 17.43
C3	190.71 ± 119.37	1.99 ± 0.65	360 ± 130	48.72 ± 8.02	14.56 ± 4.20	47.20 ± 22.02
C4	373.83 ± 189.28	6.38 ± 3.25	150 ± 80	31.22 ± 4.49	13.60 ± 3.38	33.06 ± 3.96

4 DISCUSSION

Short-beaked-common-dolphins from the slope region of Western South Atlantic Ocean produce a diversity of broadband pulsed sounds, as well as have been reported for many other species of Delphinidae. However, most pulsed sound studies are based on echolocation clicks. (Au 1993, Oswald et al. 2003, Soldevilla et al. 2008, Baumann-Pickering et al. 2010, Soldevilla et al. 2010, Lu et al. 2013, Amorim et al. 2019). In general, studies covering the diversity of pulsed sounds are based mainly on spectographic and auditory aspects, being subject to the limitations of human interpretations (dos Santos et al. 1990, Rankin et al. 2017). Few studies have proposed classifications based on more judicious statistical analysis (Reyes et al. 2015, Luís et al. 2016, Rankin et al. 2016), though there is still no consensus on the standardization regarding the variety of pulsed sounds. Our study do not attempt to solve that complex question as more research has to be done with wide more species repertoire to reach that level of standardization, however we do can improve the knowledge of pulsed sounds through more meticulous investigation.

Acoustical classification of dolphins in the California Current using whistles, echolocation clicks, and burst pulses demonstrated that burst pulses are the most important variables to classify short-beaked-common-dolphins (Rankin et al. 2016). Ours results demonstrated that analyzes comprising the whole diversity of pulsed sounds (clicks and burst sounds) produced by of short-beaked-common-dolphins clearly classified in four different types of signals. As expected, K-means method pointed to time parameters as the main contributor for the clustering analysis, with the first two dimensional-axes corresponding to 87.4%. This assumption is predictable and agrees with the differentiation of pulse sounds based on subjective human perception of ICI, whose burst pulses (and their variations) are categorized as short click trains (low ICI), being roughly distinguish visually and aurally from the more widely spaced echolocation clicks (Rankin et al. 2016). In fact, clustering validation through Random Forest (Table 6) was significant when time parameters where evaluated in pulsed sound analysis (accuracy 0.769; $p < 0.05$). That was not the case for frequency parameters analysis, as the result was not significant (accuracy 0.500; $p > 0.05$). Some authors consider that spectral peaks are related to skull morphology and sound production physiology (Soldevilla et al. 2008), while time parameters (specially duration and ICI) are related to the echolocation signals and may vary due to the task or location where the animal is performing this function (Baumman-Pickering et al. 2010). The animals may tend to modify time parameters not morphological dependents with less energetic cost than it would be required for frequency parameters. That plasticity may be a strategy to use in situations where it is promptly available to detect prey movements or to short-quick statements. However, according to Baumman-Pickering et al. (2010) only time parameters are not well appropriate to classify species. In fact, our study showed that for pulsed sounds of short-beaked-common-dolphins the best categorization points towards the time-frequency parameters combined, resulting in a more accurate classification (accuracy 0.846; $p < 0.001$). Once higher time-frequency accuracy considers the acoustical emissions as an integrated process representing the animal as an acoustic unit. An integrated process of individual characteristics and its morphology. Duration and peak frequency have already been reported as the main factors that discriminate some species (Akamatsu et al. 1998, Kaminga et al. 1996). Consequently, considering the significance in the clustering validation analysis time parameters and time-frequency parameters datasets had the best balance accuracy values (Table 7).

Classification of pulsed sounds based on visual and aural characteristics may have certain degree of correctness (Luís et al. 2016) but the most realistic and accurate classification must take into account a more detailed analysis of the parameters. A more detailed non-hierarchical analysis of short-beaked-common-dolphins' pulsed sounds from the WSAO distinguished four clusters (Table 9). Comparing average values of the four clusters with literature, the cluster (C2) was evidently distinct and followed the typical aspects of echolocation clicks described for many species, including *D. delphis* (Figure 17A). Echolocation clicks consist in longer click trains, usually more than 1000 ms; slower ICI that ensures the echo time of the signal; and peak frequency usually between 40-60 kHz (Au 1993, Akamatsu et al. 1998, Wahlberg et al. 2011, Buscaino et al. 2015, Luís et al. 2016). Echolocation clicks of *D. delphis* had already been registered in the frequency band between 40-90 kHz (Yoon et al. 2017). The echolocation cluster C2 had an average duration of 1024.50 ms, ICI equivalent to 43.06 ms (the highest value in comparison with all clusters), repetition rate corresponding to 20 clicks/s (the lowest value among all clusters), and average peak frequency of 43.24 kHz.

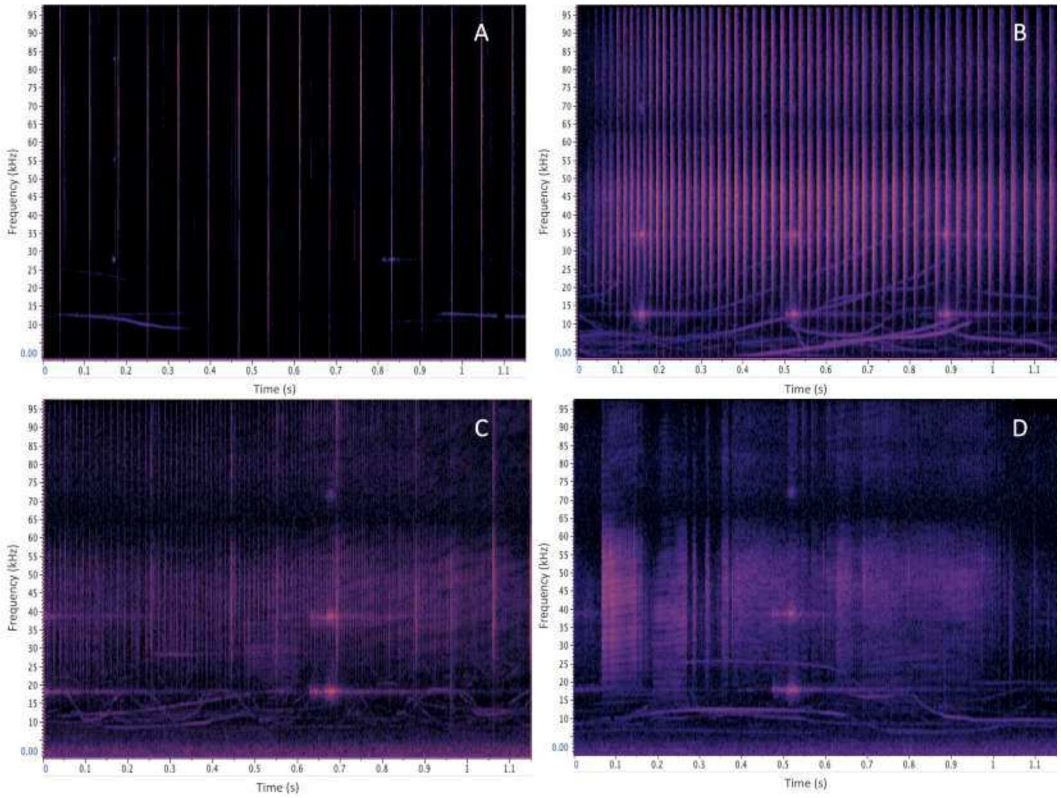

Figure 17. Pulsed sounds spectrograms showing the echolocation clicks (A), creaks (B), the decreasing rate of the click trains creak-squawk (C) and squawks (D).

The three other clusters (C1, C3 and C4) are included in the generalized category of burst sounds (or burst pulses). They refer to a series of distinct names based mainly in their aural descriptions depending on behavioral context. Following the gradation of echolocation clicks is the intermediate cluster C1, whose clicks become more rapid. It is comparable in literature with designation of ‘creaks’, ‘buzz clicks’, ‘terminal buzz’ and ‘Bray series’ (this one particularly for bottlenose dolphins), usually registered in foraging and feeding events as stage following the echolocation clicks (Herzing 1996, Miller et al. 2004, Ridgway et al. 2015, Luís et al. 2016, Cascão et al. 2020). C1 had the second highest values of duration (384.75 ms), ICI (6.70 ms) and peak frequency (44.13 kHz). The third stage of the foraging/feeding pulsed sounds is described as ‘victory squeal’, when the ‘creak’ (or ‘buzz’) turns to an emotional signal. Varying in duration, peak frequency and amplitude, the ‘victory squeal’ may be a display to affect prey making it easy to capture and/or it may function as an advert for the other dolphins to the presence of food (Ridgway et al. 2015, Cascão et al. 2020). Luís et al (2016) also reported this signals as ‘squawks’. The C3 presented the opposite extreme values from the echolocation cluster, with lowest average numbers of duration (190.71 ms) and ICI (1.99 ms); and the highest repetition rate (360 pps). The same ICI value labeled as squawks reported by Luís et al (2016). Pulsed sounds produced when animals are close to their target (<0.4m) present ICI around 2.5 ms (Evans & Powell 1967, Morozov et al. 1972). Repetition rate value of C3 was similar to the high repetition rates reported in literature. Those burst sounds with high repetition rates between 200-1200 clicks/s have been nominated as 'squawks' and 'squeaks', frequently reported also in courtship and agonistic behaviors (Caldwell & Caldwell 1967, Herzing 2000, Lammers et al. 2003, Luís et al. 2016). Signals of agonistic interactions; fright, stress and alarm situation; and courtship and pre-copulatory activities are also labeled in literature as 'cracks', 'screams', 'barks', 'pops', 'yelps' and 'genital buzzes' (Overstrom 1983, Ridgway et al. 1991, Connor & Smolker 1996, Herzing 1996, Herzing 2015). Some burst pulses are sporadic, lone, short duration emissions that exhibit ICI under 10 ms, RR around 500 pps, and peak frequency around 20kHz (Lammers et al. 2003). The C4 cluster did accounted for shorter duration with ICI under 10 ms (6.38 ms), but RR (150 pps) and peak frequency (31.22 kHz) did not fit exactly on the previous description. Discrepancies in the values of RR and peak frequency were also found by Luís et al. (2016) studying bottlenose dolphins. The authors pointed out that values suggested by Lammers et al. (2003) are close to what they classified as 'squawks'. In our study, the short burst sound cluster (C4) had values close to cluster C1 regarding duration, ICI, RR, 3dB and 10dB bandwidths. Also, C4 had the lowest average value for peak frequency (31.22 kHz) in comparison with the other clusters.

Our study clearly identified a continuum of pulsed sounds mainly related to ICI and RR parameters. The lowest ICI and the highest repetition rates were obtained for C3 ('squawks), followed by C4 ('short burst pulses'), C1 ('creaks) and C2 ('slow click trains'). Similar study comprehending multivariate classification techniques were conducted with bottlenose dolphins, in Sado, Portugal, showed that pulsed sounds differ significantly mainly in duration, repetition rate and and ICI parameters. (Luís et al. 2016).

Besides social contexts (stress/agonistic and courtship, mainly), it seems consistent in literature, particularly for bottlenose dolphins, that broadband pulsed signals (clicks and burst sounds) include three phases associated to foraging and feeding behavior (Ridgway et al. 1991, Luís et al. 2016, Cascão et al., 2020). Those three gradual phases consist in 1) the proper echolocation signals; 2) creak or buzz signal; and 3) 'squawks' or 'victory squeals'; and it might be correlated to our clusters C2, C1 and C3, respectively. However, those results should be interpreted with caution, specially related to the short-burst pulsed sounds in general and 'squawks' as the subjectivity of literature labeling and lack of quantitative studies might bias the accuracy of classification. It is difficult to detailed short burst sounds, once its characteristics have been generally classified in literature (Lammers et al 2003, Herzing 2000). 'Squawks' are also uncertain and have been described as a typical signal used in close proximity aggressive communication (Herzing et al 1996, Herzing et al. 2015) or as a 'victory squeal', the third phase of foraging behavior, expressing an emotional signal of reward (Ridgway et al 2015, Cascão et al. 2020).

Interestingly the two social signals correlated in this study with C3 ('squawks') and C4 ('short burst sounds') clusters had the extremes values of highest (48.72 kHz) and lowest (31.22) peak frequencies, respectively. The multiple variable analysis among clusters revealed that generally temporal parameters were significantly different between clusters comparison, and only peak frequency showed significantly values between some pairs of clusters. Reeves et al. (2013) believe that in peak frequency parameter, clicks show consistency in multiple peak structure and consequently many clusters would highlight any of these peaks. The authors consider peak frequency less influenced by the orientation of the dolphin's head related to the hydrophone and to differences in sound propagation paths, than parameters as duration for example. Echolocation clicks have variations on signal parameters depending on the longitudinal axis of the head of the dolphins and may be highly attenuated if clicks are received off-axis (Au 1993). Nevertheless, the off-axis alignment might transmit species-specific information in consequence of the particular effects of sound reverberation inside dolphin's head and that may have strong relevance for species classification (Soldevilla et al. 2008, Roch et al. 2011, Baumann-Pickering et al. 2015).

Based on a more detail clustering analysis the present study clearly differentiated four types of pulsed sounds, further corroborated through Permanova ($p < 0.05$). Our results demonstrated through validation method the time-frequency dataset revealed to be the best pulsed sounds classification model for *D. delphis*. This result considers the animal as an acoustic identity, emphasizing the importance of certain parameters that influence this identity. The comparison of clusters parameters with literature suggest that inter-click interval, repetition rate and peak frequency might be consistent parameters for classification. It is the first study involving the variety of pulsed sounds produced by short-beaked-common-dolphins in the WSAO, and one of the few in literature comprising this subject. Besides subjectivity in nomenclature in general, a lack of studies regarding pulsed sounds (specially burst pulses) repertoire of this species represent a big issue for the comprehension of animals that depend almost exclusively of sounds for living. Understanding mechanisms and parameters that influence cetacean's repertoires is essential for the conservation and maintenance of populations over the years.

5 CONCLUSION

Pulsed sounds produced by short-beaked-common-dolphins were clearly classified into four groups (clusters) based on time-frequency parameters. Furthermore, as expected, a validation method demonstrated the significance of time parameters to classify pulsed sounds. However, the combination of both: time and frequency parameters defined in this study are the best classification model to classify pulsed sounds in *D. delphis*. Also, our study reinforces that classification must considers the animal as an acoustical identity, and the influence of certain parameters may be a strategy for cost energy optimization in sound production.

Despite a relevant contribution attempting to clarify part of the short-beaked-common-dolphin's repertoire, there is still no consensus about detailed characterization of pulsed sounds. Our results emphasize the importance of a quantitative analysis and present additional information for *D. delphis* from Western South Atlantic Ocean. The improvement of bioacoustics knowledge of cetaceans at the species level and the factors that make up their structuring and diversification are fundamental for the conservation and maintenance of populations over the years.

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CHAPTER 4. SOUND EMISSION DURING A MASS STRANDING EVENT OF SHORT-BEAKED-COMMON-DOPLHINS

Abstract: Mass stranding events (MSE) have been documented worldwide for many species of cetaceans. Causes of stranding are difficult to detect, but are often associated to anthropogenic impacts. Cetaceans rely on acoustics signals to perform vital functions and recognize the environment in which they live. Studies on bioacoustics have increased recently, helping to elucidate aspects related to cetacean behavior and its interaction with the environment. Around 30 short-beaked common dolphins stranded alive on a beach situated on the Southeastern coast of Brazil, in March 2012. The event was filmed and the audio was extracted for acoustical analysis. This study aimed to identify possible patterns of whistles contours produced in the MSE. Seventy-one whistles were classified in nine contour categories. Whistle categories were analyzed using Kendall W test. The most frequent contours were upsweep (35.2%), prevailing upsweep (29.6%) and concave (9.9%). Of the total, 33.8% had steps, and 14.1% had breaks. The comparison between MSE and no-stranded group of *D. delphis* showed no concordance among the categories. Our results showed that a distinct pattern might be related to a stress situation and reinforced that each contour category might carry different information, which may explain its use in distinctive behavior context.

Keywords: Bioacoustics, Brazil, contours, *Delphinus delphis*, whistles.

1 INTRODUCTION

Events of stranding have been documented worldwide for many species of cetaceans and, in the majority of cases, the causes are hard to detect (Mann et al. 2010). Among the possibilities that cause cetacean stranding (e.g. Jepson et al. 2003; Fernández et al. 2005; Madsen 2005; Parsons et al. 2008; Mann et al. 2010), noise pollution is one of the main ones, putting at risk mainly coastal species (Morisaka et al. 2005a; Bittencourt et al. 2014). Anthropogenic noise acts as habitat fragmentation, as they are increasingly intense and cover gradually larger areas (Madsen et al. 2006; Williams et al. 2013). Those noises may mask cetaceans' acoustic signals and, in most cases, noisy areas tend to be avoided temporarily or permanently by many species of cetaceans, which consequently compromises the survival capabilities of the animals in a long term (Foote et al. 2004; Erbe et al. 2016).

Studies on bioacoustics have increased in the last decades, helping to elucidate aspects of the biology and ecology of cetacean species (Oswald et al. 2003, 2007; Clarck et al. 2011; Merchant et al. 2015), besides drawing attention to the impacts caused by the considerable increase of noise in the marine environment (Williams et al. 2015a, 2015b). Cetaceans rely on acoustics signals to perform vital functions and recognize the environment in which they live (Van Heel 1962; Herzing 2000; Tyack 2000). Acoustic emissions produced by whales and dolphins are generally associated with specific behavior. In a broader approach, the emission of sounds in Odontoceti can be classified into two categories: tonal (whistles) and pulsed sounds (clicks and burst sounds) (Popper 1980; Richardson et al. 1998). Whistles and pulsed sounds are often used in an emotional context or social communication (Au & Hastings 2008, Ridgway 2009).

Many studies have associated behavior with the acoustic repertoire in cetaceans. Herzing (1996) identified and classified seven types of calls associated with the underwater behavior of *Tursiops truncatus* and *Stenella frontalis*. Mello and Amundin (2005) studied bottlenose dolphins in captivity and reported changes in whistle production and behavior in pregnant dolphins, which may indicate the imminence of birth. Thomas et al. (2002) used video images and multichannel acoustic recording to link the sounds of bottlenose dolphins to their locations and behavior when held in captivity in a natural marine Lagoon on Ireland Island, Bermuda. Andriolo et al. (2015) used photos to analyze orcas' surface behavior associated with high frequency whistles emitted during predation of sperm whales. Despite frequent studies comparing behavior and acoustic emissions, there is no study of this nature reported for a mass stranding event (MSE), specially related to common dolphins.

MSE of common dolphins are rare (Brownell et al. 2013). In 1962 Van Heel comprised stranding data on small cetaceans and found only three events for the species. On the following decades, isolated cases were recorded. At Pleubian France in the English Channel about 100 common dolphins stranded in February 2002 (Viricel et al. 2008). Other two cases were reported for Hauraki Gulf, New Zealand, in 2004 (Stockin et al. 2007, Stockin et al. 2008); and at Cornwall, England, in June 2008 (Jepson et al. 2013). Recently, on March 25th, 2018 a MSE of 68 short-beaked common dolphins was reported in El Doradillo, Península Valdés, Argentina. In that event, 21 dolphins were returned alive to the sea while the rest of the animals died despite apparently good body conditions (Uhart et al. 2019). Acoustic analysis was not measured in any of the studies listed above.

Records of *Delphinus* sp. in Brazil are relatively scarce, concentrating mainly on the South and Southeast regions. In the state of São Paulo, Schmiegelow (1990) registered a total of seven single strandings of *Delphinus* sp. along 91km during two years of beach survey (from 1986 to 1988). More than ten years later Santos et al. (2002, 2010) recorded 16 stranding events of *D. delphis* (identified as *D. capensis*) at the same region. Further South, Barros (1991) recorded the first case of stranding of a common dolphin at Cassino's beach. Recently, Tavares et al. (2010) reviewed and gathered all information of *Delphinus* in South Atlantic Ocean and recorded a total of 97 stranding events. Later on, between December 2000 and March 2012, only 10 individuals were reported at the Northern part of the coast of the state of Rio de Janeiro representing 3% of the total cetacean strandings recorded at the same period (Brownell et al. 2013). All records in Brazil reported lone animals stranding event.

Common dolphins are highly communicative. Their repertoire includes echolocation clicks, burst sounds and whistles (Caldwell & Caldwell 1968; Richardson et al. 1998; Ansmann et al. 2007). Wakefield (2001) identified 18 different types of whistles in the repertoire of short-beaked common dolphins off the coast of Ireland and the United Kingdom. The whistles were grouped into six patterns (constant, upsweep, downsweep, convex, concave and sinusoidal) and the frequency ranged between 4.7 and 20.3 kHz. On the southern coast of California, Henderson et al. (2012) demonstrated that it is possible to classify the surface behavior of common dolphins using acoustic data as a tool for passive acoustic monitoring techniques.

In Western South Atlantic Ocean, studies of common dolphin's repertoire are scarce. Figueiredo (2014) classified 75 types of whistles in a coastal region of Cabo Frio (southeastern Brazil), with frequencies varying between 3.05 and 28.04 kHz. Amorim et al. (2019) demonstrated by an integrative bioacoustics' analysis the discrimination of eight delphinid species, including *D. delphis*, and suggested species-specific properties in their repertoire. Recently, a study conducted on the slope region of the Western South Atlantic evaluated consistency and intraspecific variability in whistle repertoire of *D. delphis* (Pagliani *et al.* submitted). In general, the authors found that the estimated parameters are in accordance with the species pattern and the consistency and variability in whistle repertoire are complementary adaptive abilities.

Acoustic emissions of common dolphins have been study world wide (Oswald et al. 2003; Ansmann et al. 2007; Gannier et al. 2010; Henderson et al. 2011; Petrella et al. 2012; Papale et al. 2014; Amorim et al. 2020). The species occur in tropical and temperate waters, from coastal to oceanic regions around the world (Reeves et al. 2002). A great morphotype variation of the two species (*Delphinus delphis* and *Delphinus capensis*) has been reported in several oceanic basins (Heyning & Perrin 1994; Jefferson et al. 2009; Tavares et al. 2010). However, Cunha et al. (2015) recently suggested the occurrence of only one species, the short-beaked-common-dolphin *D. delphis*, in the South Atlantic. The present study adopted this taxonomy.

Taking advantage of the chance to obtain more detailed information about the rarity of the MSE, this study sought to systematically analyze the acoustic data extracted from the only video that recorded the episode. Therefore, based on the prior knowledge of a recognized acoustic repertoire for *D. delphis*, this study aimed to identify possible patterns of whistles produced in a stress situation, as the case of a stranding event.

2 MATERIAL AND METHODS

Acoustic data and visual information were obtained from a YouTube® video filmed opportunistically by a tourist in a mass stranding event (MSE) involving about 30 short-beaked common dolphins (Werneck 2012).

The MSE occurred at around 8:00 a.m. in Prainha shore, Municipality of Arraial do Cabo (22°57’S, 42°01’W), Southeastern coast of Brazil, on March 5th 2012 (Figure 18).

Figure 18. Locality of the mass stranding event of the short-beaked-common-dolphins, Arraial do Cabo, Rio de Janeiro, Southeastern coast of Brazil. Modified from Google Earth.

In March 2012 a group of about 30 common dolphins stranded alive on seashore in the coast of Brazil. The episode was recorded as an amateur video by a tourist from land and later released on social networks. The video had enormous visibility due to the rarity of the event, and mainly due to the unexpected return of dolphins to the sea, rescued by local people.

2.1 ACOUSTICAL ANALYSIS OF WHISTLES

A file of .wav format correspondent to the MSE acoustic recording were extracted from the video and analyzed it using Raven Pro 1.5 beta (Cornell Laboratory of Ornitology) with Hann window of 512 points, 1024 points of DFT and 60% overlap. Only one channel was used for the analysis. The whistles were visually classified according to the contour categories described by Ansmann et al. (2007) and Petrella et al. (2012).

2.2. STATISTICAL ANALYSIS

The acoustical parameters recorded from the mass stranding group were compared to the acoustical parameters obtained from short-beaked common dolphins recorded in the slope region (SR) of Western South Atlantic between 28° and 34° S (from May, 2014 to October, 2015; Pagliani et al. submitted) (Table 10). The number of whistles from the SR group of dolphins was obtained at random to match the number of the whistles extracted from the MSE group.

Table 10. Information regarding the six encounters (Cruises) of *Delphinus delphis* recorded on the slope region of the Western South Atlantic Ocean - WSAO.

Encounters	Date	Lat (S)	Long (W)	Recording time of the acoustic signals (min)	Sampling Frequency
Cruise VIIa	08May2014	33.756	51.349	49.43	48kHz
Cruise VIIb	13May2014	32.562	50.293	47.92	48kHz
Cruise VIIIb	14Nov2014	33.763	51.337	40.31	48kHz
Cruise Xa	18Oct2015	34.473	51.955	63.32	192kHz
Cruise Xb	22Oct2015a	33.603	50.873	34.59	192kHz
Cruise Xc	22Oct2015b	33.463	50.885	51.75	192kHz

Visual analysis of the contour of each whistle was performed considering the frequency modulation; this process adopted the nomenclature of Asmann et al. (2007) and Petrella et al. (2012). The statistical analyses were conducted in "R" environment (R Development Core Team 2015). Shapiro-Wilk test was applied to verify the normality of data sets. Whistle categories were analyzed using Kendall W (in the 'vegan' package; Oksanen et al. 2019) for testing the hypothesis of concordance between the contours of MSE and SR groups. Kendall rank correlation coefficient Kendall's τ coefficient), is used to measure the ordinal association between two quantities. It analyzes the orderings similarity of the data ranked by each of the quantities. High Kendall correlation rank between two variables will be close or equals to 1 (Abdi 2007). Mann-Whitney-Wilcoxon test was applied to verify the divergences of the non-linear events – inflection points and steps, adopting the null hypothesis that the non-linear events are similar between the two groups.

3 RESULTS

The audiovisual record of the MSE lasted about three minutes and 45 seconds, allowing the analysis of a total of 71 whistles. Nine contour categories were verified (Table 11). The most common contours were upsweep (35.2%), prevailing upsweep (29.6%) and concave (9.9%). For whistles of *D. delphis* of the SR, the most frequent contours analyzed of the nine categories classified were upsweep (23.9%), constant (21.1%) and downsweep (18.3%). Data set showed a non-normal distribution. We found no concordance among the whistle categories when comparing MSE and SR ($W = 0.477$, $p > 0.05$).

Table 11. Number of whistles related to the contour categories of mass stranding event (MSE) and Slope Region (SR) groups of *Delphinus delphis*. Categories are listed as upsweep (up), prevailing upsweep (p-up), concave (cv), prevailing concave (p-cv), downsweep (dw), prevailing downsweep (p-dw), convex (cx), prevailing convex (p-cx), constant (ct) and sine (sn).

	up	p-up	cv	dw	p-dw	cx	ct	p-cx	sn	p-cv
MSE	25	21	7	6	5	3	2	1	1	0
SR	17	2	10	13	0	3	15	1	6	4

Duration of whistles' contours and non-linear events were analyzed for both groups. Of the MSE whistles examined, the mean duration was 0.3 ± 0.2 (mean ± SD), inflection points totalized 74 (1.04 ± 1.37; mean ± SD), steps counted 24 (0.34 ± 1.07; mean ± SD), and breaks calculated 10 (0.14 ± 0.46; mean ± SD). From the SR group of *D. delphis* whistles analyzed, the mean duration was 0.7 ± 0.3 (mean ± SD), inflection points, steps and breaks corresponded a total of 92 (1.30 ± 1.85; mean ±

SD), 5 (0.07 ± 0.49; mean ± SD), 2 (0.03 ± 0.24; mean ± SD), respectively. The comparison between MSE and SR groups showed that duration was significant different ($W = 771.0$, $p < 0.01$). Also, non-linear events demonstrated significant differences for breaks ($W = 2770.0$, $p < 0.05$), steps ($W = 2731.5$, $p < 0.05$) and duration.

4 DISCUSSION

The present study is the first to report a mass stranding event of *D. delphis* on the coast of Brazil. From all those strandings (single or mass) reported in literature, no study had access to the acoustic emissions at the time of the event. Regarding the classification of whistles contours; the proportion found in present study diverged from previous studies of common dolphins. The present results showed upsweep, prevailing upsweep and concave as the three more frequent contours. The three most frequent contours reported are upsweep, downsweep and constant (Wakefield 2001; Ansmann et al. 2007; Griffthis 2009; Petrella et al. 2012; Pagliani *et al.* submitted). Signals emitted by whistles are performed to synchronize group organization and function (Norris et al. 1994; Janik & Slater 1998). Each contour category might be responsible for carrying different information and this may explain its use in distinctive behavior situations. Dos Santos et al. (2005) found significant variation of whistle production and whistle contours related to episodes of travel and other dynamic activities in bottlenose dolphins (*Tursiops truncatus*) from Sado Estuary, Portugal. Furthermore, the authors did not found correlation between group size and whistles contours, suggesting that the categories of whistles emitted do not depend on the number of individuals. Types of whistles produced by Beluga whales (*Delphinapterus leucas*) of Cunningham Inlet, North-West Territories, varied depending on behavioral circumstances, being the upsweep contours more frequent during social events and directional swimming (Sjare & Smith 1986). Giardino et al. (2019) analyzed whistles produced by a common dolphin in a rehabilitation tank few hours before its death. The authors reported only the upsweep contour, and low variability in frequency parameters in general, suggesting stereotyped whistles related to the debility of the animal. Being the most frequent contour category Upsweep might express predominantly information in terms of communication for common dolphins, being use in different a broad range of behaviors. Although it is an isolated case, the differences in the proportion of the whistles contours registered in MSE may be an indicative of a stress behavior as a consequence of stranding.

Inflection points in the whistles of the MSE dolphins was represented by 57.75% while the non-linear events were 12.68% of steps, and 9.86% of breaks. For SR whistles, inflection points corresponded to 59.15% and the non-linear events, steps and breaks, totalized 2.82% and 1.41%, respectively. Within populations, parameters such as steps, breaks, duration and number of inflections are usually more variable and may carry information about individual identity or behavior (Rendell et al. 1999; Morisaka et al. 2005b). In fact, the Mann-Whitney-Wilcoxon test showed significant differences for duration and the non-linear events - steps and breaks - reinforcing the distinct characteristics of the whistles contours between the two groups analyzed in this study.

Although we did not aim to investigate the cause of stranding in this event, the disorientation of dolphins may have been influenced by anthropic activity in the region (Brownell et al. 2013). The Campos Basin, in which the Municipality of Arraial do Cabo is inserted, is the main area of gas and oil exploration, accounting for most of the oil production in Brazil (Mincarone et al. 2016). In addition, fishing is one of the most important economic activities after oil exploration in the area (Mincarone et al. 2016). Although we have no evidence of the direct impact on dolphins in this case, it is important to highlight the vulnerability of marine species in coastal regions and the importance of studies on acoustical emissions in the oceans, which is a special concern on animals that use sound as the primary sense.

Dolphin repertoire is known to vary according to behavior. Communication patterns have already been described in different stress situations, but none addressed the moment of stranding. Although having technical limitations, based on careful analysis this study has shown that there are differences that may indicate a particular repertoire typical of a life-threatening situation such as a stranding event.

5 CONCLUSION

This study investigated the whistles emissions in the first mass stranding event of *D. delphis* in Brazil. In that occasion, all animals were successfully rescued and returned alive to the sea. Considering the rare chance of recording such event, this work is a baseline to better understand the relation between stranding events and acoustical emission of *D. delphis*.

Regarding the contours classification of the whistles, a distinct pattern in the contours proportion was found between the contexts (stranded and non-stranded). Each contour category might be responsible for carrying different information and the unusual pattern found on MSE may explain its use in a stress situation.

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CHAPTER 5. FINAL CONCLUSIONS

The short beaked common dolphin is highly communicative and studies describing the acoustic parameters of this species have been carried out in many areas worldwide. However, this is the first study to analyze the repertoire of the *D. delphis* population from the Western South Atlantic Ocean in its entirety. Whistle contours were highly consistent among the six evaluated encounters, confirming the proportion pattern described for the species and, thus, reinforcing that the use of the categories may be responsible for carrying different information, depending on the observed behavior. The high intraspecific whistle variations noted among the encounters demonstrate *D. delphis* repertoire plasticity, corroborating the fission-fusion way of life typically registered for many Delphinid species.

Another aspect of dolphin repertoires is related to non-modulated frequencies, i.e. broadband pulsed sounds. Pulsed sounds include echolocation clicks and burst sounds and are produced in a wide variety of contexts. While echolocation clicks are used mainly during foraging and feeding behaviors, burst pulses are usually emitted in social short-distance communication. Even though not as studied as whistles, assessments involving echolocation clicks comprise the majority of ongoing research. In general, studies concerning pulsed sounds are scarce and based on aural and graphic descriptions. This subjectivity makes it difficult to characterize these sounds, resulting in a lack of consensus on the classification of pulsed sounds to date. The present study aided in elucidating basic information concerning the repertoire of the *D. delphis* WSAO population and emphasizes the importance of quantitative analyses for the classification accuracy. Pulsed sounds (clicks and burst pulses) were characterized and classified into four types (clusters), based on specific parameters. Although predefined time parameters were significant, our study demonstrated that the combination of time and spectral parameters represents the best model for classification. The results also indicate that certain parameters (interclick interval, repetition rate and peak frequency) were more consistent and may be essential to guide the characterization of different clusters. Also, the influence of certain parameters in the classification of pulsed sounds covering both temporal and spectral characteristics considers the animal as an acoustic identity and suggests that these parameters together may represent a strategy to optimize the energy costs of sound production.

This study is a relevant contribution that attempts to clarify important aspects of *D. delphis* repertoire characterization, consisting in a baseline to elucidate the complexity of sound emission contexts for the species. Furthermore, is necessary to highlight the importance of behavioral studies and its response to the marine environment. The improvement of bioacoustics knowledge for cetaceans at the species level and the factors that make up their structure and diversifity are paramount for the conservation and maintenance of populations over time.