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First acoustic evidence of signature whistle production by spinner dolphins (*Stenella longirostris*)

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Abstract

A dolphin's signature whistle (SW) is a distinctive acoustic signal, issued in a bout pattern of unique frequency modulation contours; it allows individuals belonging to a given group to recognize each other and, consequently, to maintain contact and cohesion. The current study is the first scientific evidence that spinner dolphins (*Stenella longirostris*) produce SWs. Acoustic data were recorded at a shallow rest bay called "Biboca", in Fernando de Noronha Archipelago, Brazil. In total, 1902 whistles were analyzed; 40% (753/1,902) of them were classified as stereotyped whistles (STW). Based on the SIGID method, 63% (472/753) of all STWs were identified as SWs; subsequently, they were categorized into one of 18 SW types. SWs accounted for 25% (472/1,902) of the acoustic repertoire. External observers have shown near perfect agreement to classify whistles into the adopted SW categorization. Most acoustic and temporal variables measured for SWs showed mean values similar to those recorded in other studies with spinner dolphins, whose authors did not differentiate SWs from non-SWs. Principal component analysis has explained 78% of total SW variance, and it emphasized the relevance of shape/contour and frequency variables to SW variance. This scientific discovery helps improving bioacoustics knowledge about the investigated species. Future studies to be conducted in Fernando de Noronha Archipelago should focus on continuous investigations about SW development and use by *S. longirostris*, expanding individuals' identifications (Photo ID and SW Noronha Catalog), assessing long-term whistle stability and emission rates, and making mother–offspring comparisons with sex-based differences.

Keywords Acoustic repertoire · Fernando de Noronha · Signature whistles · Spinner dolphin · *Stenella longirostris* · Whistles

Introduction

In animal cognition context, a label is a kind of shorthand for a concept, which can be used to refer to it either in thinking or in communication (Bruck et al. 2022). Dolphins and parrots are the only nonhuman animals that have been successfully trained to copy novel acoustic signals and then use them in vocal labeling (Pepperberg 1990; Richards et al. 1984). Similar to what has occurred in the evolution of human languages, animals that are capable of vocal learning, which are potentially able to create novel labels in their communication (Bruck et al. 2022).

Odontocetes (dolphins and porpoises) are able to live in challenging environments by maintaining complex social structures; this is facilitated by their diverse and, in some cases, individually specific vocalizations. The complexity of these vocalizations is impressive; they are typically divided into broadband echolocation clicks, broadband burst pulses and frequency-modulated narrowband whistles (Jones et al. 2020a). Pulsed vocal signals, such as clicks, burst pulses and "buzzes", are known to have echolocation and social functions (Baumann-Pickering et al. 2015), whereas whistles play an important role in communication and social cohesion (Jones et al. 2020a).

Individuals living in dynamic delphinid societies often form long-lasting associations with another individual (or

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individuals). Sometimes, they show extended maternal relationships with calves (Grellier et al. 2003; Smolker et al. 1993), develop mating alliances (Connor et al. 2006; Connor and Smolker 1995) or form male–male foraging groups (Connor and Wells 2000). In all these cases, individual detection and recognition mechanisms can be particularly important whenever individuals need to locate one another after they get temporarily separated from their group (Janik and Slater 1998; Kaplan et al. 2014). It is so, because they provide a system to enable these individuals to remember who they have shared mutual investments with (Shapiro 2006).

An individually distinct vocalization—which prevails in whistle repertoire and is especially produced by bottlenose dolphins (*Tursiops truncatus*) when they are isolated from, or out of visual contact of, their conspecifics—was identified and described for the first time, more than half a century ago (Caldwell and Caldwell 1965), when it was properly named signature whistle (SW).

Nowadays, it is known that signature whistles (SWs) are frequency-modulated vocalizations that remain stable throughout dolphins' life (crystalized). These vocalizations are developed during animals' first months of life, through a vocal production learning process based on their auditory social experiences; moreover, they are issued in a repetitive pattern capable of transmitting senders' identity to their surroundings. Whistle copies of SWs, eventually produced by conspecifics, are rare and can be recognizable as such because copiers consistently modify some acoustic parameters of a signal when copying it (Caldwell et al. 1990; Esch et al. 2009; Fripp et al. 2005; Harley 2008; Heiler et al. 2016; Janik 1999, 2009; Janik and Sayigh 2013; Janik and Slater 1998; Janik et al. 2006; Jones et al. 2020b; King et al. 2013; Kriesell et al. 2014; Longden et al. 2020; Luís et al. 2016; Papale et al. 2015; Rio et al. 2022; Sayigh et al. 1995, 2007, 2017; Terranova et al. 2021; Watwood et al. 2005).

Although SWs are the best example of a designed individual acoustic label within the animal kingdom (Sayigh et al. 2007), scientific knowledge about their function and use is mostly documented in bottlenose dolphins (*Tursiops truncatus*) (Caldwell et al. 1990; Cones et al. 2022; Gridley et al. 2014; Janik and Sayigh 2013). Their use and presence in other delphinid taxa remain poorly investigated and understood (Cones et al. 2022; Fearey et al. 2019). Nowadays, eight delphinid species are known to produce individually distinctive SWs in order to transmit identity information: (Cones et al. 2022): (1) bottlenose dolphins (*Tursiops truncatus*); (2) Indo-Pacific bottlenose dolphins (*Tursiops aduncus*); (3) common dolphins (*Delphinus delphis*); (4) Atlantic spotted dolphins (*Stenella frontalis*); (5) Pacific white-sided dolphins (*Sagmatias obliquidens*); (6) Atlantic white-sided dolphins (*Lagenorhynchus acutus*); (7) Pacific humpback dolphins (*Sousa chinensis*); and (8) Guiana dolphins (*Sotalia guianensis*) (Caldwell and Caldwell 1965, 1968, 1971; Caldwell et al. 1973; Cones et al. 2022; de Figueiredo and Simão 2009; Fearey et al. 2019; Gridley et al. 2014; Janik and Sayigh 2013; van Parijs and Corkeron 2001).

In addition, some delphinid and non-delphinid species are also known to produce stereotyped call types, such as short-finned pilot whales (*Globicephala macrorhynchus*) (Sayigh et al. 2013), melon-headed whales (*Peponocephala electra*) (Kaplan et al. 2014), narwhals (*Monodon monoceros*) (Shapiro 2006) and belugas (*Delphinapterus leucas*) (Morisaka et al. 2013). However, it is necessary to conduct further research based on reliable and comparable detection methods to help broaden knowledge about the function of these repeated calls in individual recognition processes.

The SIGID (SIGnature IDentification) method (Janik et al. 2013) has provided the easiest and most reliable way to identify SWs based on using bout analysis approach. It was initially designed for bottlenose dolphins, but, later on, it was applied to other odontocete species (Cones et al. 2022; Fearey et al. 2019; Gridley et al. 2014; Longden et al. 2020; Luís et al. 2016; Matsushiro et al. 2022; Panova et al. 2021; Papale et al. 2015; Rio et al. 2022; Terranova et al. 2021, 2022), likely due to its conservative criteria; 50% success rate and lack of false positives (Janik et al. 2013).

The current study was the first to investigate, identify and describe individually distinctive SWs in oceanic spinner dolphins (*Stenella longirostris*) from Fernando de Noronha Archipelago, Brazil.

Materials and methods

Study site

Fernando de Noronha is a volcanic archipelago located at geographic coordinates 3°52'S and 32°26'W. It represents a relatively flat top of a seamount within Fernando de Noronha Ridge in tropical Southwestern Atlantic Ocean (Fig. 1). The archipelago encompasses the main island (also called Fernando de Noronha) and another 21 smaller islands and islets that total ~27 km^2, and ~190 km^2 of insular shelf (< 80m) (Matheus et al. 2019). Although its estimated population comprised 3140 individuals, in 2021 (unofficial data), human occupation is limited to 35% of the main island territory, whose economic activity is mostly linked to tourism (Mendes 2006).

Acoustical recordings

Acoustic data were recorded in the shallow waters (< 20m) of "Biboca" bay (3°50′13.5″S 32°24′27.0″W) (Fig. 1), in the morning, on August 24th, 2022. The aforementioned bay is often visited by spinner dolphin groups;

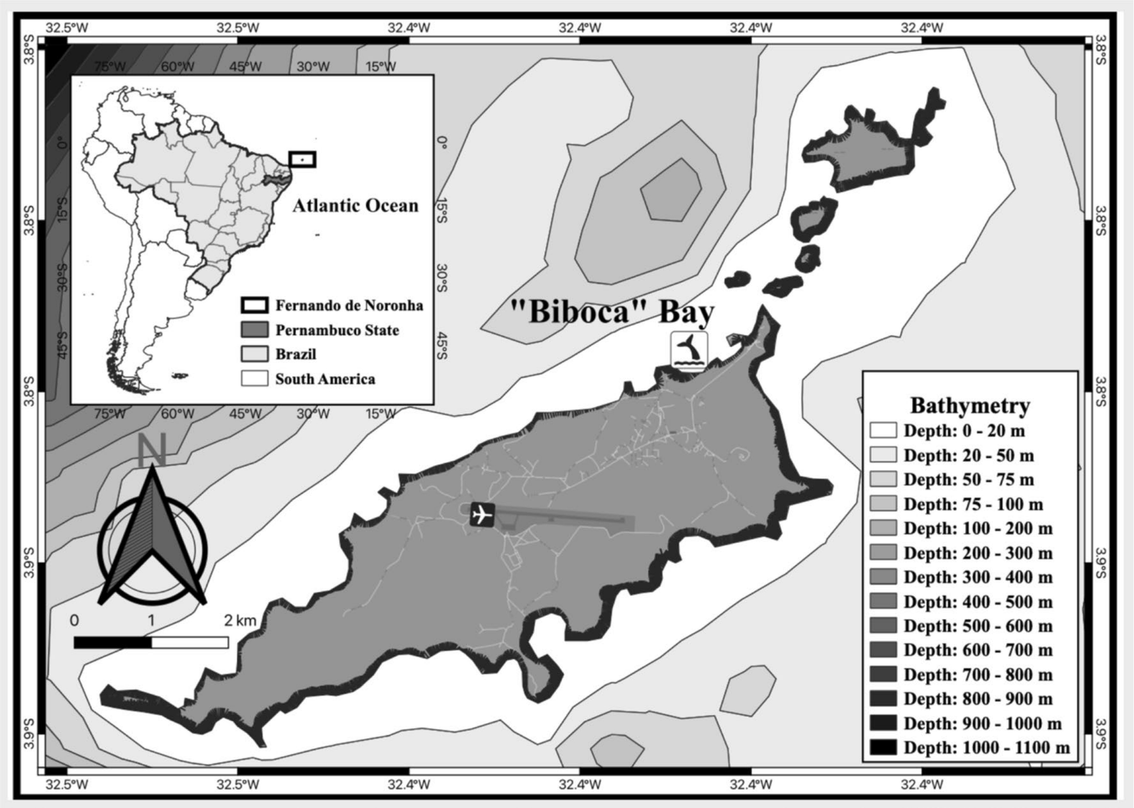

Fig. 1 Map depicting the study site in Fernando de Noronha Archipelago islands, Brazil, tropical Southwestern Atlantic Ocean. Acoustic recordings of sounds produced by oceanic spinner dolphin (*Stenella longirostris*) populations were obtained at "Biboca" bay; they are represented by the caudal fin icon

thus, it provides an important site for acoustic studies about this species. Data were collected while the equipment was installed in a Kayak (3.9-m length, 0.9-m width, 2-people places; Orca Duplo Model, Lontras™). Acoustic recordings were continuously taken after a group of approximately 50 individuals had arrived at 7:00 am. The daytime visual confirmation of *S. longirostris*' presence in the study site was obtained through continuous visual observations.

Underwater recordings were carried out with the aid of C75 preamplified omnidirectional hydrophone by Cetacean Research Technology™ [linear frequency range (±3dB) from 10 Hz to 170 kHz, and effective sensitivity of -186 dB, re 1V/μPa], which was placed 5-m below water surface and connected to a Tascam DR-100MKIII digital recorder by a 10-m cable. Recordings were carried out at 96-kHz sample rate and 24-bit resolution. All acoustic data were stored in 10-min-recording files (with no time interval between consecutive files) that, in their turn, were stored as time-stamped wave files in 256Gb Secure Digital Card (SD) Flash Memory Cards.

Acoustical analysis

Based on similar previous studies (Kriesell et al. 2014; Rio et al. 2022), the term whistle was used to describe a tonal sound with a fundamental narrowband frequency of over 0.1 s duration (Evans and Prescott 1962; Lilly and Miller 1961) with at least part of the fundamental frequency above 3 kHz (Simard et al. 2011; van der Woude 2009). This vocalization has been recognized as an uninterrupted (single-element or connected multi-loop) whistle or two or more repeated contours (disconnected multi-loop whistle) interrupted by very short breaks (between 0.03 and 0.25 s) (Esch et al. 2009; Kriesell et al. 2014; Rio et al. 2022). All whistles with good signal-to-noise (SNR) ratio (Papale et al. 2013) and complete, clear spectral contours were first visually and aurally demarcated; then, they were manually selected for analysis purposes. Spectrograms were plotted in the spectrogram view of Raven Pro 1.6.1 (Cornell Laboratory of Ornithology, Ithaca, NY, USA) at 1024 Fast Fourier Transform size (FFT), Hanning window and 50% overlap. To characterize the whistles, the main and classical acoustic and temporal variables were measured for all whistles: starting frequency (StaF), ending frequency (EndF), minimum frequency (MinF), maximum frequency (MaxF), frequency bandwidth (BanF), whistle duration (Dur), interwhistle interval (IWI), and the number of inflections points (InfP) (change from positive to negative aspect or vice versa) (Kriesell et al. 2014; Rio et al. 2022). All parameters were manually extracted through selection boxes in Raven Pro 1.6.1 software. Selection box boundaries were used to extract Bandwidth, MinF, MaxF and Dur, whereas StaF, EndF and InfP were additionally marked to properly represent their value.

The term ‘whistle type’ was used to ascribe all whistles of a particular/characteristic frequency modulation pattern or contour into predetermined categories based on human visual evaluations (Janik 1999; Sayigh et al. 2007). Consequently, the result of this human whistle classification was the creation of whistle categories that grouped all whistles of the same type. Whistle categories containing repeated units produced at least twice within a time period of 0.25–10 s during a recording section were classified as Stereotyped Whistles (STW) (Kriesell et al. 2014; Luís et al. 2016; Rio et al. 2022). Inter-Whistle Intervals (IWI) were calculated subtracting the end time of the first whistle from the start time of the second whistle in the recording file. Non-Stereotyped Whistles (NTW) refer to whistles emitted individually or separated by more than 10 s.

The emission sequence of whistles and its IWI were analyzed using the SIGID method (Janik et al. 2013) and the SWs were identified according to STW categories with at least four whistles. Therefore, if at least once during the sequential bout analysis 75% or more of the whistles occurred within 1–10 s of one other whistle of the same category, it was considered a SW type (Janik et al. 2013). Whistle classification was made by one experienced observer. All STW that did not pass the SIGID criteria plus NTW were defined as non-SWs for this analysis.

To confirm that different SW categories were reliably identified, a visual similarity value judgment task was performed based on the evaluation of five naive independent observers that had no experience with bioacoustics. These observers judged a randomly chosen data subset, following a methodology previously adopted (Jones et al. 2020b; Kriesell et al. 2014; Rio et al. 2022). For this task, ten SW types were previously and randomly selected from the dataset. For each one, six whistle repeats (random choice) were used; one of the replicates acted as a template whistle and the remaining five were classified by the judges. Using a Microsoft PowerPoint presentation, a sequential survey was created with each one of the 50 whistle repetitions at the slide center surrounded by the ten SW templates.

Each whistle was plotted as a spectrogram (1024 FFT, Hanning window and 50% overlap) with standardized time and frequency axis (frequency y-axis: 0–25.0 kHz; time x-axis 0–5.0 s, scales not plotted). The configuration of the template whistles did not change between slides but the order of the presentation was randomized for each observer. For the first part of the task (the preliminary phase), observers were asked to compare each whistle replicate against all 10 templates and rate the similarity of each whistle replicate on a scale from one (the whistle and the template are very different) to five (the whistle and the template are very similar). A score of 5 did not require that the whistles were identical. This resulted in a total of 500 pairwise comparisons. During the binary second part of the task, the observers were constrained to assign each whistle replicate to a single ‘most similar’ template category. The observers were instructed to ignore other details such as whistle amplitude and background noise and the presence or absence of harmonics (i.e., repetitions of the whistle contour at multiple frequency intervals above each contour). The author of this publication (RR), who created the STW catalogue and classified the SWs, also completed the tasks. Then, the Fleiss’s kappa statistic (a test for agreement above chance levels similar to Cohens Kappa but calculated when there are multiple observers: Landis and Koch 1977) was used to compare the ratings among 6 observers for 500 whistles. Two analyses were conducted, one for agreement on a 5 point scoring and the second for agreement on the fit with the most similar SW categorization.

Statistical analyses

Descriptive statistical analysis and the Shapiro–Wilk test were performed for all independent variables. According to the normality test, none of the acoustical or temporal data had a normal distribution.

The emission rate of non-SWs (STW and NTW) and SWs was calculated by dividing the number of whistles by the minutes of recorded whistle time. The recorded whistle time was defined as the interval between the first and last acoustic signal of dolphins (clicks or whistles) either if there was or was not visual confirmation.

The Fleiss’s kappa statistics (calculated with and without the author classification) was used to compare the ratings among observers and to determine interobserver agreement and consistency in SW categorization. If observers were in perfect agreement in their classification, then Fleiss’ Kappa statistic (k) is equal to 1.00 (Landis and Koch 1977). If agreement amongst observers was the same as would be expected by chance (i.e., the absence of agreement), then k is equal to 0.00.

A Principal Component Analysis (PCA) using a correlation matrix of the acoustic parameters and temporal patterns (StaF, EndF, MinF, MaxF, BanF, Dur, and InfP) as independent variables was performed to reduce data to a new set of independent Principal Components (PCs) and further elucidate which parameters contribute most to SWs variability. Before that, data were z-scored/normalized by subtracting averages and dividing by the standard deviation. Z-scoring is a common method used to standardize/normalize data. The matrix was rotated using Varimax rotation, which attempts to minimize the variance of squared loadings for each factor and improves interpretability of the variables (Jolliffe 2002). The factorial adequacy Kaiser-Meyer-Olkin (KMO) test was used to select the number of components (linear combinations of the original variables) to be kept for the analysis, excluding all PCs with an eigen value lower than one (Jolliffe 2002). Thus, any component that explained a lower variance than an original variable in the correlation matrix was excluded. A coefficient of correlation linking old and new variables was calculated and all variables were plotted in a three-dimensional space. The KMO measurement (> 0.5) and the Bartlett's test of sphericity ($p < 0.001$) justified the use of PCA.

All the statistical analyses were performed using IBM™ SPSS Statistics (SPSS Software Inc., Chicago, USA) and GraphPad™ 8 (GraphPad Software Inc., San Diego, USA) at a 95% level of significance.

Ethics statement

This study was entirely observational and was conducted under the following permissions: Instituto Chico Mendes de Conservação da Biodiversidade (ICMBio) SISBIO no 75705-9; Ethics Committee on the Use of Animals (CEUA-UFJF) no 026/2020]. All procedures performed followed the standards of "Guidelines for the treatment of marine mammals in field research" (Gales et al. 2009).

Results

Descriptive analyses

The recording effort time reached 02h 45m 53s, in total; all of it was taken into consideration for analysis purposes. The recorded whistle time reached 01h 46min 03s; a total number of 1902 whistles were recorded during this period-of-time and it resulted in emission rate of 17.94 whistles/min.

In total, 39.59% (753/1,902) of all recorded whistles were classified as STW, whereas the remaining narrowband signals [60.41% (1.149/1,902)] were defined as NTW.

Based on the SIGID method (Janik et al. 2013), 62.68% (472/753) of all STWs were identified as SWs; subsequently, they were ascribed to 18 SW types. Examples of spectrograms of all SW types (SW1, SW2, SW3… and SW18) are shown in Fig. 2.

SWs accounted for 24.82% (472/1,902) of all analyzed whistles, at emission rate of 4.45 SW/minute of the total recorded whistle time. Table 1 shows the number of samples per SW type, which ranged from 4 (SW17) to 61 (SW1).

Acoustic parameters

Mean values and standard deviation (SD) of acoustic parameters recorded for each SW type are described in Table 1. Overall, SW types recorded mean StaF and MinF values similar to each other; they ranged from 5.88 kHz (SW7) to 11.66 kHz (SW3), and from 5.60 kHz (SW11) to 11.23 kHz (SW18), respectively. Mean EndF value ranged from 7.93 (SW9) to 18.02 (SW16), whereas mean MaxF value ranged from 14.00 (SW11) to 18.52 (SW15). The comparison between frequencies recorded pooled mean values within less 4 kHz (EndF 12.16 kHz and MaxF 16.03 kHz). Bandwidth was the frequency acoustic parameter with the largest range, from 6.34 kHz (SW18) to 10.97 kHz (SW12); pooled mean value of 8.57 kHz was recorded for all SW types. SW type duration presented pooled mean value of 1.11 s; the shortest one was recorded for SW11 (0.38 s), whereas the longest one reached 1.74 s (SW6). The longest SW type (SW6) recorded the shortest mean for IWI (0.75 s), whereas the second shortest SW type (SW8 0.65 s) presented the longest IWI (4.05 s). All SW types recorded pooled mean value of 1.96 s for IWI. The largest number of InfP was observed in sine SW10 samples, which presented minimum 3 points and maximum 11 points of inflections in a single contour.

Visual classification

The preliminary task of rating the similarity of each repeated whistle on a scale from one to five has shown a fair/minimal interobserver agreement, using Fleiss kappa for multiple observers (Landis and Koch 1977). Specifically, the agreement on 500 whistles among 5 coders (without RR) was K-Fleiss = 0.249; $p < 0.001$ and among 6 coders (with RR) was K-Fleiss = 0.286, $p < 0.001$. On the second agreement task (the binary phase with yes or no decisions about classifying the "most similar" SW type category), the Fleiss Kappa has shown a near perfect interobserver agreement. Specifically, the agreement on 50 whistles among 5 coders was K-Fleiss = 0.949; $p < 0.001$ and agreement with 6 coders (including RR) was K-Fleiss = 0.958, $p < 0.001$.

Multivariate analysis

PCA reduced the eight SW acoustic and temporal parameters to three independent PCs, which explained 77.79% of the total variance in individually distinctive whistles produced by spinner dolphins from Fernando de Noronha Archipelago, Brazil. Figure 3 shows the three-dimensional

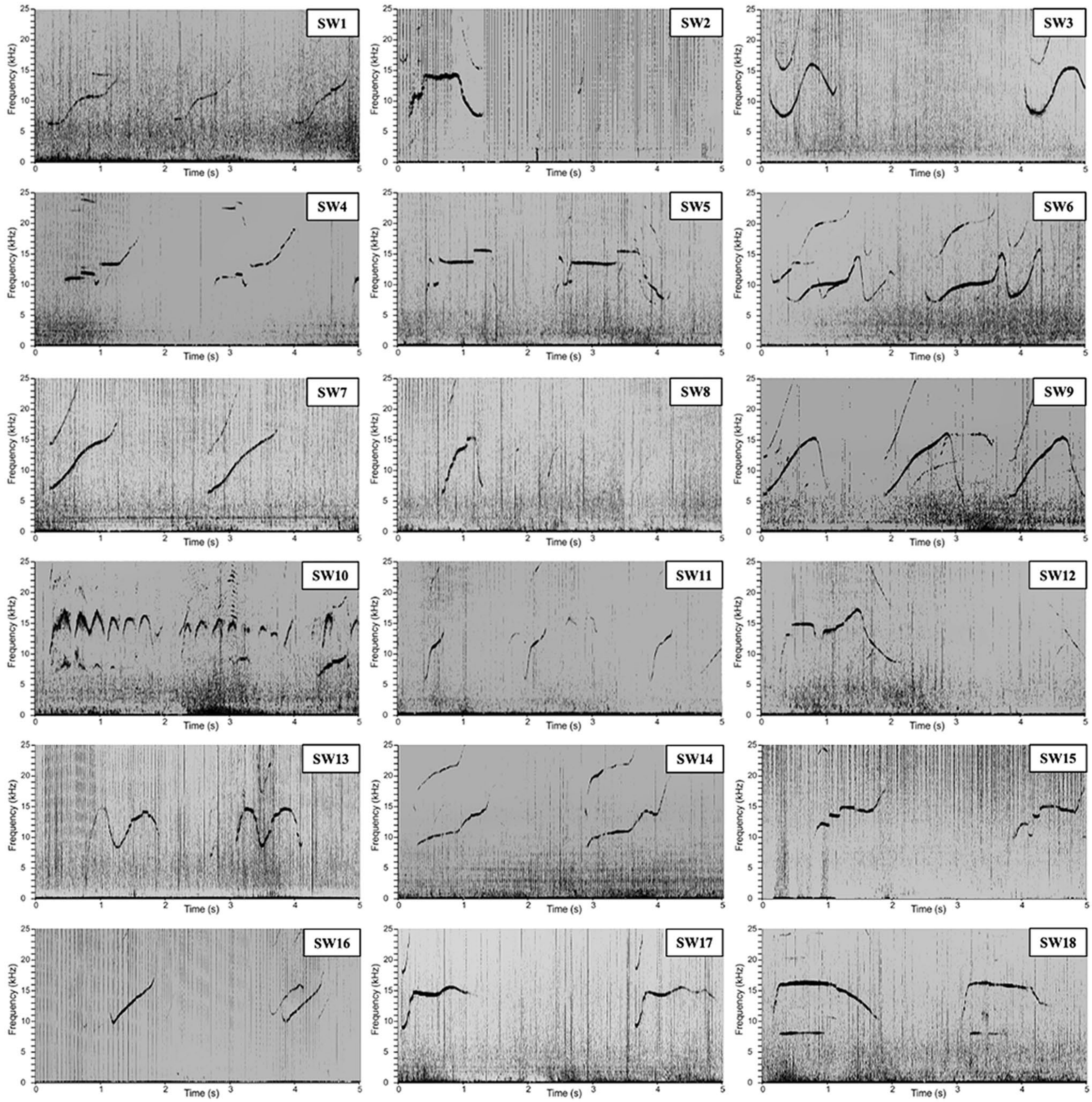

Fig. 2 Spectrogram examples of 18 signature whistle (SW) types (SW1, SW2, SW3… and SW18) identified (SIGID method) for spinner dolphins (*Stenella longirostris*) from Fernando de Noronha Archipelago, Brazil. Frequency is shown at the y-axis; it ranges from 0 to 25 kHz. Time (s) is shown at the x-axis and it represents 5 s. The same scaling was adopted for all items. Spectrogram settings: Fast Fourier Transform size = 1,024, Hanning window, overlap = 50%. The numbers at the top right corner of each item represent the identification numbers of each SW type

space identified by PCs, wherein variables were distributed in different clusters based on their correlations.

Canonical loadings have shown that BanF, MaxF, Dur and EndF were the most correlated parameters within PC 1 (variance = 39.40%), whereas StaF, InfP, and MinF were the most correlated ones in PC 2 (variance = 25.75%). IW accounted for 12.64% of total variance in PC 3. Table 2 shows the loadings of the varimax-rotation PCA of the analyzed SW acoustic and temporal variables.

Table 1 Mean ± standard deviation (SD) of acoustic and temporal parameters recorded for 18 signature whistle (SW) types ($n=472$) produced by spinner dolphins (*Stenella longirostris*) from Fernando de Noronha Archipelago, Brazil

ID	*n*	FREQUENCY (kHz)					SHAPE		
		Start (StaF)	End (EndF)	Minimum (MinF)	Maximum (MaxF)	Bandwidth (BanF)	Duration (s) (Dur)	Interwhistle (s) (IWI)	Inflection (*n*) (InfP)
SW1	61	6.87 ± 0.61	15.62 ± 2.25	6.33 ± 0.33	15.59 ± 2.21	9.26 ± 2.14	1.20 ± 0.25	1.21 ± 0.60	0.00 ± 0.00
SW2	43	9.85 ± 2.64	7.96 ± 2.06	7.33 ± 1.07	15.77 ± 0.57	8.44 ± 1.11	0.86 ± 0.22	1.75 ± 1.47	1.00 ± 0.00
SW3	54	11.66 ± 1.34	12.12 ± 1.60	7.41 ± 0.30	16.03 ± 0.47	8.63 ± 0.59	1.04 ± 0.18	3.21 ± 2.67	2.44 ± 0.51
SW4	34	10.16 ± 1.11	17.37 ± 2.20	9.61 ± 0.61	17.78 ± 2.27	8.17 ± 2.42	1.12 ± 0.23	1.42 ± 0.81	2.00 ± 0.00
SW5	46	11.17 ± 2.61	10.58 ± 2.98	8.36 ± 1.30	15.38 ± 1.34	7.03 ± 2.30	1.36 ± 0.41	2.84 ± 1.98	3.04 ± 0.21
SW6	29	7.93 ± 0.44	10.53 ± 2.57	6.96 ± 0.46	15.25 ± 1.12	8.28 ± 1.28	1.74 ± 0.42	0.75 ± 0.45	2.59 ± 1.12
SW7	14	5.88 ± 0.42	16.26 ± 1.30	5.88 ± 0.42	16.24 ± 1.30	10.35 ± 1.21	1.01 ± 0.17	0.97 ± 0.33	0.00 ± 0.00
SW8	14	7.04 ± 0.87	10.16 ± 2.94	7.04 ± 0.87	15.95 ± 0.57	8.91 ± 0.84	0.65 ± 0.08	4.05 ± 1.50	1.00 ± 0.00
SW9	32	6.15 ± 1.71	7.93 ± 1.99	5.83 ± 0.72	16.61 ± 1.12	10.78 ± 0.74	1.10 ± 0.92	1.44 ± 1.53	1.00 ± 0.00
SW10	26	9.02 ± 1.77	9.89 ± 2.78	7.96 ± 1.94	16.08 ± 1.06	8.12 ± 1.81	1.06 ± 0.32	1.65 ± 2.52	6.46 ± 2.23
SW11	19	6.47 ± 1.55	14.05 ± 0.82	5.60 ± 0.59	14.00 ± 0.82	8.40 ± 0.85	0.38 ± 0.06	1.58 ± 2.08	1.00 ± 0.00
SW12	06	6.81 ± 1.48	9.91 ± 2.84	6.61 ± 1.28	17.58 ± 0.76	10.97 ± 1.45	1.37 ± 0.33	3.73 ± 3.39	5.00 ± 3.00
SW13	15	8.94 ± 2.02	11.08 ± 2.63	7.72 ± 0.80	15.32 ± 0.68	7.61 ± 0.94	0.83 ± 0.19	1.46 ± 0.62	3.07 ± 0.26
SW14	9	6.66 ± 1.52	17.60 ± 1.33	6.66 ± 1.52	17.60 ± 1.33	10.94 ± 1.57	1.12 ± 0.11	1.20 ± 0.22	0.00 ± 0.00
SW15	18	9.20 ± 0.72	17.98 ± 1.96	9.20 ± 0.72	18.52 ± 1.29	9.32 ± 1.61	1.17 ± 0.10	1.92 ± 1.31	2.00 ± 0.58
SW16	16	11.07 ± 1.26	18.02 ± 2.85	8.98 ± 0.98	17.89 ± 3.08	8.91 ± 2.37	0.72 ± 0.13	2.88 ± 1.81	1.00 ± 0.00
SW17	4	9.05 ± 0.63	14.40 ± 1.08	8.56 ± 0.34	15.75 ± 0.10	7.19 ± 0.29	1.25 ± 0.15	1.67 ± 0.75	3.00 ± 0.00
SW18	32	10.89 ± 1.25	11.63 ± 1.71	10.23 ± 0.93	16.58 ± 0.30	6.34 ± 1.01	1.24 ± 0.24	1.62 ± 1.47	1.00 ± 0.00
Total	472	8.95 ± 2.56	12.16 ± 3.81	7.45 ± 1.53	16.03 ± 1.59	8.57 ± 1.91	1.11 ± 0.39	1.96 ± 1.87	1.70 ± 1.79

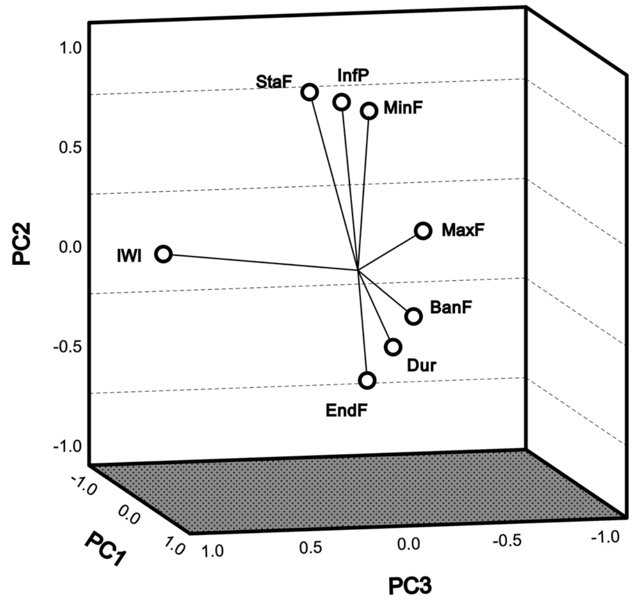

Fig. 3 Principal component analysis (PCA) results in three-dimensional space identified by PCs (PC1, PC2 and PC3), based on acoustic and temporal variables of 18 signature whistle (SW) types ($n=472$) produced by spinner dolphins (*Stenella longirostris*) from Fernando de Noronha Archipelago, Brazil. Variables are distributed to form different clusters, based on their correlations

Table 2 Principal component analysis (PCA) loadings (correlations between variables and components) applied to the Varimax rotation PCA of the acoustic and temporal variables of 18 signature whistle (SW) types ($n=472$) produced by spinner dolphins (*Stenella longirostris*) from Fernando de Noronha Archipelago, Brazil

Variables	Principal Component (PC)		
	PC 1	PC 2	PC 3
Minimum (**MinF**)	–0.144	**0.775**	–0.090
Maximum (**MaxF**)	**0.877**	0.328	–0.129
Duration (**Dur**)	**0.776**	–0.266	0.000
Bandwidth (**BanF**)	**0.902**	–0.095	–0.075
Start (**StaF**)	–0.098	**0.887**	0.223
End (**EndF**)	**0.674**	–0.445	0.108
Inflection (**InfP**)	–0.013	**0.845**	0.080
Inter-Whistle (**IWI**)	–0.062	0.106	**0.975**
% Variance	39.40	25.75	12.64
Cumulative %	39.40	65.15	77.79

Bold values are statistically significant (> absolute 0.50). Variables that are highly loaded on the same component are strongly correlated to each other

Discussion

Little is known about the occurrence of active labeling by vocal learners in natural animal communication systems (Bruck et al. 2022). Signature whistles' relative stability and prevalence in odontocetes' repertoires may be a "shortcut" to the best understanding of their communication and cognitive abilities, a fact that partly justifies researchers' growing interest in, and efforts to investigate, these distinctive acoustic signals. (Cones et al. 2022; Fearey et al. 2019, 2022; Jones et al. 2020a, 2022; La Manna et al. 2022; Morrison et al. 2020; Rio et al. 2022; Sportelli et al. 2022). The current study provides the first acoustic evidence that spinner dolphins (*Stenella longirostris*) produce SWs. This scientific discovery is a true improvement in bioacousticals knowledge about the investigated species. Moreover, it adds to important and previous studies available in the literature focused on investigating whistles [Hawaiian Islands area (Bazúa-Durán and Au 2002, 2004; Driscoll 1995; Lammers et al. 2006; Wang et al. 1995), Caribbean (Steiner 1981), Brazil (Camargo et al. 2006), Western Indian Ocean (Bonato et al. 2015), Eastern Pacific waters (Oswald et al. 2003), and Malaysia (Bono et al. 2021)] and high-frequency pulsed sounds [Hawaii (Benoit-Bird and Au 2009) and Palmyra Atoll (Baumann-Pickering et al. 2010)] produced by spinner dolphins.

On the other hand, SWs have already been described for another member of genus *Stenella*; the Atlantic spotted dolphin (*S. frontalis*) (Bebus and Herzing 2015; Caldwell et al. 1973; Caldwell and Caldwell 1966; Herzing 1996). In addition, a study conducted with *S. attenuata* has shown that they rhythmically repeated identical whistles (Pires et al. 2021). However, the adopted data collection method did not allow for assessing the SW hypothesis for Pantropical spotted dolphins.

Overall, SWs are used by Atlantic spotted, bottlenose dolphins, among other species, to coordinate movements, reunion and separation events, mainly between mothers and calves, and between allies (Bebus and Herzing 2015; Herzing 1996; Janik and Slater 1998; Smolker et al. 1993). If one takes into consideration the central role played by individual recognition mechanisms in the ecology of fission-fusion societies' members, it is likely that other species belonging to genus *Stenella,* and even other odontocetes, will have their individually distinct vocalizations revealed in the future.

Results have confirmed that SWs can account for at least one quarter (24.82%) of the overall whistle production by spinner dolphins swimming around Fernando de Noronha Archipelago, Brazil. SWs account for approximately 38–70% of all whistle production by both free-living coastal and ocean bottlenose dolphins (Buckstaff 2004; Cook et al. 2004; Rio et al. 2022; Watwood et al. 2005). The current scientific knowledge about SWs is almost entirely based on studies conducted with bottlenose dolphins (Caldwell and Caldwell 1965; Cook et al. 2004; Esch et al. 2009; Fripp et al. 2005; Janik et al. 2006; Janik and Sayigh 2013; Sayigh et al. 1990, 1995, 2007, 2017; Watwood et al. 2005). Moreover, SIGID method was developed for bottlenose dolphins and therefore may not work perfectly with spinner dolphins. Then, it is important to highlight that direct comparisons between these dolphins and spinner dolphins, among other species, must take into consideration specificities of each species, such as ecological behavior, morphological features, foraging strategies, dietary preferences, group size and used habitats. These ecological differences may lead to different pressures for SW development (Bebus and Herzing 2015) and to different ways of using it. Accordingly, *S. longirostris*' predictable daily behavior can influence its SW emission in comparison to other species. It is known that spinner dolphins tracking vertical and horizontal migrations of prey organisms in the mesopelagic boundary layer during nighttime hours (primarily myctophid fishes, small crustaceans and squid) and then moving into protected inshore areas to rest during daylight hours (Benoit-Bird and Au 2003; Thorne et al. 2012). Thus, the high-visibility and low-depth waters of Biboca bay, among other sandy resting bays, can favor the maintenance of visual contact between spinner dolphins. This factor can reduce their need to emit SWs, because cohesion calls are less necessary to maintain contact with the rest of the group, both for location and identity purposes, since non-signature whistles are mainly produced under these conditions (Janik and Slater 1998). Moreover, visual, rather than acoustic, vigilance is typical of spinner dolphins' resting state (Noris et al. 1994). However, many other factors can influence animals' whistle rate, namely: behavioral context, group size and calf presence, anthropic actions and stress level (Briefer 2012; Cook et al. 2004; Esch et al. 2009; Jones and Sayigh 2002; Kriesell et al. 2014; May-Collado and Quiñones-Lebrón 2014). Thus, it is necessary to conduct further acoustic and ecological studies to better clarify and expand this matter.

Most of the energy in the fundamental frequency of spinner dolphin's whistles was below 24 kHz; mean values ranged from MinF 8.76 kHz (Steiner 1981) to MaxF 19.63 kHz (Bonato et al. 2015). Most acoustic and temporal variables herein measured for SWs recorded mean values similar to those recorded in other studies conducted with spinner dolphins, whose authors did not differentiate SWs from non-SWs: StaF (Steiner 1981), MinF (Steiner (1981), End (Camargo et al. 2006; Oswald et al. 2003), and MaxF (Bazúa-Durán and Au 2002; Moron et al. (2015).

However, pooled and some SW type mean BanF and Dur values were the highest ones ever recorded; they were higher than the ones reported by Bazúa-Durán and Au 2002 (BanF 5.86 kHz) and Bonato et al. 2015 (Dur 0.88 s), respectively. Results in the current study presented more than twice the duration of whistles previously recorded by Camargo et al. 2006 (0.49 s) in that very same archipelago. The temporal variability of recorded whistles between studies may result from different group sizes and from different overall behavioral states (Bazúa-Durán and Au 2002). In addition, acoustic variability can be an adaptation to environmental acoustic conditions and/or to permanent acoustic changes (cultural drift or gene flow). However, although SWs accounted for part of the total acoustic whistle repertoire in the current case, their stereotyped feature may have influenced the mean value recorded for all SW acoustic and temporal parameters, depending on the number of identified whistle types (range of mean values) and on the number of samples per SW type (pooled mean values). Additional research should be conducted to enable direct comparison between SW results recorded for different spinner dolphin populations. SW frequency parameters observed for Atlantic spotted dolphins often ranged from 4 kHz to 18 kHz, and the duration of whistle bouts ranged from 0.5 to 8 s (Herzing 1996). The parameters of bottlenose dolphins' SWs usually range from 1.78 to 14.77 kHz for minimum frequency and from 6.66 to 26.92 kHz for maximum frequency (Sayigh et al. 2022).

Whistle contour plays a central role in signature information, i.e., individual recognition, (Janik et al. 2006; Kershenbaum et al. 2013; Sayigh et al. 2007); its variations based on aspects, such as frequencies and duration, may convey additional information, such as animals' emotional states (Norris et al. 1985; Steiner 1981; Wang et al. 1995). Humans are endowed with the great ability to recognize complex visual patterns, which enabled testing the reliability of the classification carried out by the author of this publication (RR) and by external observers who did not have previous experience with bioacoustics studies, based on the comparison between contours of SW types and copies. Results in the current study supported (near perfect agreement) the visual SW categorization adopted for spinner dolphin from Fernando de Noronha Archipelago. The preliminary visual agreement phase involved subjective score assignments, which naturally tend to present lower agreement between raters (Rio et al. 2022). In addition, some whistles have basic, sinusoidal structures similar to that of many other SWs; thus, appraisers could also focus on the different aspects of the analyzed spectrograms (Bebus and Herzing 2015).

Principal component analysis is a recent trend in SW assessments, as an effort to elucidate which parameters contribute most to SWs variability (Rio et al. 2022). Previous research states that the stereotype of SWs is contained in whistle contour and not in the single acoustic parameters that could change over time (Sayigh et al. 2007). Accordingly, Rio et al. (2022) showed that InfP, Dur, StaF, and IWI (PC1 = 77% of the total variance of SWs) were the most correlated acoustic values for the first principal component, indicating the importance of shape/contour variables for the variance of SWs produced by an oceanic population of *T. truncatus* from the Revillagigedo Archipelago, Mexico. On the other hand, PCA results showed that some frequency features (i.e., maximum frequency, minimum frequency and mean frequency) were more important than others to distinguish SWs emitted by a coastal bottlenose dolphin population inhabiting the Pelagos Sanctuary in Italy (Terranova et al. 2021). Based on multivariate analysis results recorded for three different PCA tests, almost 78% of the total variance observed in SWs emitted by spinner dolphin from Fernando de Noronha Archipelago (Brazil) could be explained; this finding corroborated the expected range from 70 to 90% in this rate (Jolliffe 2002). BanF, MaxF, Dur, and EndF were the most important parameters used to distinguish SWs emitted by *S. longirostris*. The herein observed canonical loads are partially comparable to these two studies aforementioned conducted with wild bottlenose dolphins; which highlighted the important role played by shape/contour variables (Rio et al. 2022) and frequency parameters (Terranova et al. 2021) in SW variance. Previous studies did not perform multivariate analyses for Atlantic spotted dolphin.

Finally, the current article is another publication resulting from a long-term research called "Ocean Sound Secrets", which started during the Covid-19 pandemic, at the same time the Non-Governmental Organization (NGO), known as Ocean Sound, was launched to monitor cetaceans from oceanic islands, based on acoustic, observational and genetic techniques. Future studies to be conducted in Fernando de Noronha Archipelago should focus on continuous investigations about SW development and use by *Stenella longirostris*, expanding individuals' identifications (Photo ID and SW Noronha Catalog), assessing long-term whistle stability and emission rates, and making mother-offspring comparisons with sex-based differences.

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Declarations

Conflict of interest The author declares no conflicts of interest.

Ethical approval This study was entirely observational and was conducted under follow permissions: Instituto Chico Mendes de Conservação da Biodiversidade (ICMBio) SISBIO no 75705-9; Ethics Committee on the Use of Animals (CEUA-UFJF) no 026/2020]. All procedures performed followed the standards of "Guidelines for the treatment of marine mammals in field research" (Gales et al. 2009).

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