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Group Sizes, Activity Patterns and Acoustic Production of Three Delphinid Species in Azores

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Resumo

Os repertórios acústicos de três espécies de delfinídeos com distribuição simpátrica sazonal, que são geralmente avistadas durante o Verão no arquipélago dos Açores, foram estudados e comparados. Os assobios de *Tursiops truncatus*, *Delphinus delphis* e *Stenella frontalis* foram gravados através de um hidrofone conectado a um gravador com uma frequência de amostragem de 96 kHz. Esta recolha de dados ocorreu entre 24 de Agosto a 14 de Outubro de 2019 ao redor das ilhas do Faial e do Pico. Para além das gravações acústicas efetuadas, foram também registados diferentes padrões de atividade e o tamanho dos grupos para cada espécie.

O número de assobios nas gravações acústicas foi contado através do programa Raven Pro 64 1.4 de modo que as suas taxas de emissão pudessem ser calculadas. Do número total de assobios foram selecionados 484 para *Tursiops truncatus* (n = 175), *Stenella frontalis* (n = 282) e *Delphinus delphis* (n = 27) onde foram medidos e comparados os parâmetros acústicos e os perfis de modulação de frequência entre as três espécies.

Ao analisar estatisticamente os resultados, *Tursiops truncatus* apresentou uma taxa de emissão média significativamente maior do que *Delphinus delphis, Stenella frontalis* não obteve diferenças significativas nos valores das taxas de emissão entre os padrões de atividade e para cada espécie não houve correlação entre o tamanho dos grupos e as taxas de emissão. No entanto, as três espécies de golfinhos apresentaram diferenças significativas tanto nos parâmetros acústicos como nos perfis de modulação de frequência dos assobios.

Palavras-chave: Delfinídeos, Açores, Assobios, Padrões de atividade, Tamanho dos grupos

Abstract

The whistle repertoires of three seasonally sympatric delphinid species, that are commonly sighted during the summer in the Azores Archipelago, were study and compared. *Tursiops truncatus*, *Delphinus delphis* and *Stenella frontalis* whistles were recorded using a hydrophone connected to a recorder with a 96 kHz sampling frequency. These data collection occurred from August 24th to October 14th, 2019 around the Faial and Pico islands. Besides the acoustic recordings performed, it was also registered different activity patterns and the groups size for each species.

The Raven Pro 64 1.4 program was used to count the number of whistles in the acoustic recordings so that the whistle emission rates could be calculated. From the total number of whistles 484 were selected for *Tursiops truncatus* (n = 175), *Stenella frontalis* (n = 282) and *Delphinus delphis* (n = 27) where acoustic parameters and frequency contours were measured and compared among the three species.

When the results were statistically analyzed, *Tursiops truncatus* exhibited an average emission rate significantly higher than *Delphinus delphis*, *Stenella frontalis* did not present significant differences in the emission rate values between the activity patterns and for each species there was no correlation between the groups size and the emission rates. However, the three dolphin species presented significant differences in both whistle acoustic parameters and frequency contours.

Keywords: Delphinids, Azores, Whistles, Activity patterns, Group sizes

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1. Introduction

Cetaceans are a diversified and monophyletic group of aquatic mammals that are commonly known as dolphins, porpoises, and whales. Due to their molecular and morphological traces they can be grouped into two uneven parvorders: the Mysticeti containing 14 species of baleen whales and the Odontoceti which includes 75 species of toothed cetaceans (Lisney & Collin, 2019; Sayigh, 2013). One of the key features that distinguish these two groups is that mysticetes retain their food through baleen plates while odontocetes have teeth to catch their prey (Lisney & Collin, 2019; Sayigh, 2013).

Underwater sound plays a very important role for cetaceans as visibility is highly limited due to the absence of light penetration at greater depths and even in more shallow areas when the water is turbid (Au & Lammers, 2007). Furthermore, the reduced attenuation of sound with distance and its fast propagation, as compared with air, allow efficient broadcasting of acoustic signals (Pavan & Borsani, 1997). Aquatic mammals use acoustic signals for several reasons: communication, cohesion, and coordination of the group, foraging, and navigation. In addition to the sound being used at different spatial scales, for example, dozens of meters for one species and dozens of kilometers for others, it is also used differently depending on the species (Au & Lammers, 2007). Odontocetes have developed an advanced sense called echolocation which allows them to locate food and navigate, however baleen whales do not have this ability, except in a highly wide sense (Au & Lammers, 2007; Lisney & Collin, 2019).

Toothed whales have specialized hearing for exceptionally high frequencies which in some species can extend to 180 kHz. Most species of odontocetes can also discriminate fine-scale frequencies and process the sounds quickly which, in a way, offsets not only the complex demands for echolocation but also for the high speed of sound underwater (Mooney, Yamato & Branstetter, 2012).

1.1. Dolphin Vocalizations and Activity Patterns

Many of the processes that are crucial for reproductive success imply communication between animals, such as finding a mate, forming societies, parent-offspring relationship and foraging (Ruxton & Schaefer, 2011). Dolphins have developed a specialized system of acoustic communication. The sounds used by delphinids were classified into three types: clicks, whistles and burst-pulsed sounds that can be utilized for distinct purposes and situations (Morisaka, 2012).

Clicks are short-lasting broadband sounds which are used mostly in echolocation (Frasier et al., 2017). These sounds have frequencies with ultrasonic peaks and high sound pressure levels (Figure 1.1B) (Morisaka, 2012). Wild bottlenose dolphins can present frequency peaks between 120 and 130 kHz (with source level higher than 200 dB re 1 µPa), and free Atlantic spotted dolphins can have peaks between 110 and 130 kHz, while wild common dolphins can exhibit frequency peaks between 23 and 67 kHz (Au, 1993; Au et al., 1974; Au & Herzing, 2003).

The utilization of sound in echolocation is different from what is produced in communication, where significant information is included in the sound signals of the individuals that will cause the receiver to respond in a determined way (Gridley et al., 2015). The sounds that are known to have an essential function in social contexts and communication are the burst-pulsed sounds and whistles (Gridley et al., 2015; May-Collado & Wartzok, 2008).

Burst-pulsed sounds are short broadband impulses emitted with a high repetition rate and which manifest in the spectrograms as harmonic bands (Figure 1.1C) that are heard by humans as a steady sound (Gridley et al., 2015). Although there is increasing evidence that these sounds play a major function in communication, the exact role of burst-pulsed sounds is still not clear and might change with context and species (Gridley et al., 2015; Janik, 2009; Morisaka, 2012).

Whistles are narrowband, frequency-modulated sounds (Figure 1.1A) that can last around 0.1 and 4 seconds (dos Santos et al., 2005). They usually have a predominant frequency range from 5 to 14 kHz (dos Santos et al., 2005). The features of whistles may change with the noise level, environmental characteristics, intra and interspecific relationships (Janik, 2009; Papale et al., 2016). These sounds are normally defined by their relative modification in frequency through time and can be classified by their form (Azevedo et al., 2007; McCowan, 1995).

The frequency parameters of these sounds vary according to the dolphin species, and some factors have been suggested to explain this variability, such as socialization, phylogeny, morphological restrictions, and geographical distribution (May-Collado & Wartzok, 2008). It is thought that in cetaceans, the development of minimum frequency can be influenced by the size of the group and the body, while the complexity of whistling, which is measured by the average number of inflection points, can be affected by the social structure. In the same species, whistles vary mainly in duration and the average number of inflection points (May-Collado & Wartzok, 2008).

In some species of dolphins, the individuals have their distinguished signature whistle which is emitted mainly when individuals are segregated from conspecifics. These whistles are frequency modulation patterns and can be learned by copying and then changing the sounds in the individuals' environment (Janik, 2009). The only way an animal can be identified by its signature whistle is if the conspecifics have been able to learn the distinct modulation pattern of the whistle. In species like *Tursiops truncatus*, *Stenella frontalis,* and *Delphinus delphis* there is evidence of signature whistles (Janik, 2009).
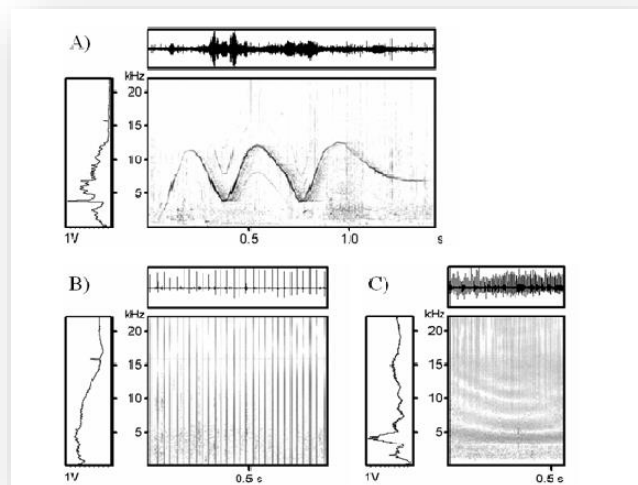
Figure 1.1- Spectrogram of a whistle (A), clicks (B) and a burst-pulsed sound (C) from wild Indo-Pacific bottlenose dolphins (*Tursiops aduncus*). The top panel of each spectrogram demonstrates the shape of the wave and the left panel shows the power spectrum of the sound (Morisaka, 2012).

Dolphins are generally known to build complex coalitions among populations (Randic et al., 2012). These communities require the development of social strategies that involve the contribution of several individuals (Connor, 2007). The superficial behavior of delphinids can be classified into aerial behaviors, in other words, jumps that can occur individually or synchronously, and percussive behaviors which are any kind of surface pattern that results in a sound made by slapping a part of the body on the surface of the water (Lusseau, 2006). All events that happen on the surface, such as leaps, breaches, chin slapping, and tail slapping, are a non-vocal communication in which the sound that is produced underwater probably transmits a communicative signal (Dudzinski et al., 2009).

Through observation of these behaviors, it is possible to categorize the activity patterns of these animals. The most mentioned activity patterns are: travelling, resting, foraging, feeding, socializing and milling (Ballance, 1992; Díaz López, 2011; Henderson & Würsig, 2007; Herzing, 2015; Lusseau, 2006; Wells et al., 2013).

Delphinid communication by sound has many roles in its behavior (Dolman et al., 2004). One part of a dolphin's behavior that can be used as a benefit for protection against predators or feeding is the cohesion of the group. During feeding and in the presence of tourism operations, like swimming with dolphins, an increase in whistles has already been observed to keep the group cohesion (Acevedo-Gutiérrez & Stienessens, 2004; Scarpaci et al., 2000; Stockin et al., 2008). There has also been an increase in whistle rates when there is a separation between a calf and its mother (Guerra et al., 2014). The mother-calf relationship implies a bond of cohesion which is maintained through the whistles that are utilized as contact calls (Guerra et al., 2014; Smolker, Mann & Smuts, 1993). If communication between the calf and the mother is disturbed by noise, it may result in a decrease in cohesion and coordination between them (Guerra et al., 2014).

1.2. Delphinids in the Azores

The Azores archipelago is a region that presents a great diversity of cetaceans, with more than 20 species, and where several species of delphinids inhabit (Gannier et al., 2019; Silva et al., 2013). Species such as the common bottlenose dolphin, *Tursiops truncatus*, and the short-beaked common dolphin, *Delphinus delphis*, can be sighted throughout the year and are generally abundant, while other species occur seasonally such as the Atlantic spotted dolphin,

Stenella frontalis, which is sighted during the late spring to the late summer and is one of the most observed species in the summer season (dos Santos et al., 2016; Gannier et al., 2019). These three species are often sighted in the Azores in mixed pods that can normally be related to foraging activities (Quérouil et al., 2008). Bottlenose dolphins normally have a small group size of fewer than 20 individuals, although pods of many hundreds can be seen (Wells & Scott, 1990), on the other hand, common dolphins usually associate in large groups with 30 or even more animals (Palomares & Pauly, 2019). In the case of Atlantic spotted dolphins, they usually form moderate-sized groups with fewer than 50 individuals. Nevertheless, large pods with hundreds of dolphins have been sighted in the Azores (Jefferson, Webber & Pitman, 2015).

The whistles of bottlenose dolphins are known to have a frequency range between 0.8 and 28.5 kHz (Gridley et al., 2015). The whistle acoustic parameters of this species have been studied and compared in several regions, for example, in the Western and Eastern Atlantic Ocean, where different values were obtained for whistle duration, frequency, and number of inflection points (May-Collado & Wartzok, 2008). For instance, the average initial frequency values ranged from 5.80 and 11.26 kHz, mean final frequency values varied from 6.40 and 13.15 kHz (dos Santos et al., 2005; May-Collado & Wartzok, 2008). As for the average values of the minimum and maximum frequencies the wild bottlenose dolphins can present between 5.32 and 17.61 kHz, respectively (dos Santos et al., 2005; May-Collado & Wartzok, 2008). In the case of duration and the number of inflection points in whistles, mean values have been recorded from 0.38 s to 1.30 s; and from 0.89 to 3.78 (dos Santos et al., 2005; May-Collado & Wartzok, 2008).

Common dolphins usually emit whistles with an average frequency range between 5 and 20 kHz but can reach about 1-50 kHz (Griffiths, 2009). For this species, the whistle variables have already been recorded and compared in the Eastern Atlantic (where data from the Azores was included) and Mediterranean region, in which the initial and final average frequencies varied respectively from 11.9 to 13.0 kHz and from 11.8 to 12.5 kHz (Gannier et al., 2019). Regarding the values of the minimum and maximum mean frequencies, this species can present between 8.1 kHz to 16.7 kHz, respectively (Gannier et al., 2019). The mean values for duration and number of inflection points were also recorded respectively from 0.65 s to 0.95 s; and from 0.6 to 2.3 (Gannier et al., 2019).

The whistle frequency range of the Atlantic spotted dolphins was recorded between 1.15 and 23.44 kHz (Azevedo et al., 2010). In this species, the acoustic parameters of the whistles were compared and identified for the Eastern (including Azores data) and Western Atlantic Ocean where the average values of the initial and final frequencies ranged from 8.8 to 10.2 kHz

and from 12.3 to 14.6 kHz, respectively (Gannier et al., 2019). The mean minimum and maximum whistle frequency values for this species are 7.1 and 17.9 kHz (Gannier et al., 2019). The whistles of Atlantic spotted dolphins can have an average duration and a number of inflection points from 0.36 s to 0.65 s and 0.7 to 2.7 (Gannier et al., 2019).

To analyze the vocalizations of marine mammals, passive acoustic techniques can be used and have advantages such as improving knowledge about density, habitat use and occurrence of cetaceans and the possibility of obtaining data from greater distances than through visual monitoring (Azzolin et al., 2014). Bioacoustics studies are also important to analyze activity patterns in dolphins since the acoustic signals detected in a particular activity pattern can provide valuable insights about that behavior (Cascão et al., 2020). For example, in a study conducted by Cascão et al., (2020), through the detection of signals mostly utilized by dolphins while foraging (bray calls and buzzes) it was possible to understand the foraging activity of different dolphin species in the Azores seamounts.

The study of cetacean acoustics in the context of species is an essential stage in the long-term processing of passive acoustic data recorded in the marine habitat. As the production patterns of dolphins' acoustic signals change with their behavioral condition, the spatial distance of conspecifics and their geographical localization it is important to study acoustically different populations of these animals in several places (Amorim et al., 2019).

1.3. <u>Objectives</u>

The main objective of this study is to examine if it is possible to observe differences in the whistle repertoire of three delphinid species in the Azores: *Tursiops truncatus*, *Delphinus delphis* and *Stenella frontalis*. More specifically, the whistle acoustic parameters of these three species will be studied and compared. This comparison will be conducted with the purpose of testing the hypothesis that these species, which share a common habitat during the summer, will exhibit interspecific variations in the whistle acoustic parameters.

Furthermore, the results obtained in this study, for each species, will be compared with previous published research conducted in different regions.

Other purposes of this work are to compare the whistle production among the species and in each activity pattern, to understand if the whistle emission rate depends on the group's size and to compare the whistle frequency contours among the three delphinid species.

2. Materials and Methods

2.1. Study Area

The Azores Archipelago was formed through volcanic activity in the east of Portugal about 900 miles from the mainland (Ellis, 2009). This archipelago, located in the North Atlantic Ocean, is composed by nine islands that extend over a triple junction area of the Eurasian, American and African lithospheric plates (Annex 2, Figure 2) (Cruz & Soares, 2018). The tectonic context of these plates is at the origin of the intense volcanic and seismic activity on the islands (Cruz & Soares, 2018). There are also two important structures that separate these three lithospheric plates: the Azores-Gibraltar fracture zone and the Mid-Atlantic Ridge (Annex 2, Figure 2) (Luís et al., 1994). These two structures are connected by an oblique rift system, the Terceira Rift (Weiß, Hübscher & Lüdmann, 2015).

The Azores islands are located on both sides of the Mid-Atlantic Ridge and in the southern zone lies the Azores-Gibraltar fracture zone (Luís et al., 1994). These islands are divided into three groups: an eastern group consisting by Santa Maria and São Miguel, a central group composed by Terceira, Graciosa, São Jorge, Pico and Faial, a western group comprising Flores and Corvo (Gaspar et al., 2015).

It has been recognized that the Azores archipelago is an oasis in the middle of the Atlantic Ocean and an essential environment for pelagic organisms due to the interaction between the uneven topography and the occurrence of atmospheric and oceanographic events such as fronts and eddies (García et al., 2018). There is a high abundance and species richness of top predators, like whales and dolphins, in these marine productive regions (Di Tullio et al., 2016).

In the Azores there is a great diversity of cetaceans with 28 different species documented (Silva et al., 2013).

2.2. <u>Data Collection</u>

This study was conducted using the platforms of opportunity provided by a whale watching company, *Naturalist, Science & Tourism*, which is based in the Azores Archipelago, namely in Faial Island. Initially an exploratory period was carried out from July 10th to August 14th, 2019, where behavioral records of *Tursiops truncatus*, *Delphinus delphis* and *Stenella frontalis* were collected around Faial and Pico Islands (38°N, 28°W). Then from August 24th to October 14th of the same year the data from these three dolphin species used for this study were collected. This survey was carried out on a semi-rigid boat measuring 8.5 meters, in length, with a capacity for 16 people. Each trip was approximately 3 hours long and before the expeditions the cetaceans were sighted by four lookouts, through binoculars (15×80), which were positioned in the North and South of Faial and Pico Islands.

For data collection the weather conditions had to be optimal, that is absence or low wind (usually wind force between 0 and 3 on the Beaufort scale) and low swell.

The sampling of these field data was composed of two methods: behavioral registers and acoustic recordings.

2.2.1 Behavior Sampling

In the behavioral records, the species were first identified by direct observation based on group size, behavior and morphological characteristics presented both in the observer's guide (Gallagher, Porteiro & Serrão Santos, 2013), which was taken to the expeditions, and in the identification key of the cetaceans (Instituto da Conservação da Natureza e das Florestas, n.d.). These records were carried out after a 5-minute adaptation period, whenever possible, and for each species of dolphin the registers were made every 3 minutes on a table (Annex 3, Table 1). The behavioral records were collected according to the predominant activity sample that is a procedure from which states are measured in a short interval of time to collect the shortest states of relevance (Mann, 1999).

During these observations, the following data were recorded: the expedition number, the date, start and end time of the expedition, the vessel type and the weather conditions (tide time and Beaufort scale) (Annex 3, Table 1). In addition to these data it was also noted the name of the species under observation, the time of each record, the geographic coordinates and depth through the GPS (Garmin, Olathe, Estados Unidos) and the vessel's probe, the groups size and

the activity patterns (Annex 3, Table 1). The register of activity patterns was complemented with the observation of behavioral events such as the individual's dominant direction (obtained by the boat's compass), the dolphins movement speed (slow, normal, fast) and some observations, particularly if there were behaviors on the surface such as jumps, interactions with the boat, individual contact, associations with other species and offspring in the pods (Annex 3, Table 1). While these data were being registered, photographic records (Canon EFS 18-135 mm) were made.

The activity patterns were considered as: travelling (movement in a steady direction, with relatively constant and brief diving intervals), resting (slow movement in a constant course or remaining nearly stationary on the water surface with synchronized, short and relatively steady dive intervals), foraging (movement in a varied direction, fish pursuit and, in general, remaining in the same area), feeding (movement in a variable direction, direct feeding evidence e.g. a prey in the oral cavity of individuals), socializing (movement in a varied direction with different dive intervals, interactive behaviors such as physical contacts, synchronized jumps and other active surface behaviors) and milling (movement with variable directions, short diving intervals and individuals changing direction frequently) (Henderson & Würsig, 2007; Lusseau, 2006; Wells et al, 2013).

Although behavioral records have been collected since 10 July, only the registers gathered at the same time as the acoustic recordings were considered, that is, from 24th August until 14th October.

2.2.2 Acoustic Recordings

The acoustic recordings were always made with the boat engine switched off, to reduce noise and avoid disturbing the animals, and with the fewest boats in the area as possible. These recordings were performed with the dolphin's pod approximately at 10-100 m from the stationed vessel and using an omni-directional hydrophone with a 10-meter cable (LSTN2, Holsworthy, UK) which was placed in the water at a depth between 3-8 m. This hydrophone was connected by a jack audio adapter (3.5 mm-6.35 mm) to a mobile professional recorder (Zoom H4n Pro, Tokyo, Japan) with a playback and recording capacity up to 96 kHz/24 bits. This sampling frequency allows the recording of dolphin sounds up to 48 kHz, which covers the relevant sounds for this study. In order to monitor the quality of the recordings, headphones or a speaker were used, which were connected to the P2 input standard of 3.5mm on the

recorder. The recordings were, whenever possible, held during 1 minute several times in the same sighting and simultaneously with the behavioral samplings (Figure 2.1). At the end of each recording, the audio code entered on the recorder, which consisted of species name, date and recording number, was noted on the table (Annex 3, Table 1).

Figure 2.1- Areas around Faial and Pico where behavioral sampling and acoustic recordings of the three delphinid species were collected. *Tursiops truncatus-* Lozenges; *Delphinus delphis-* Circles; *Stenella frontalis-* Triangles.

2.3. Bioacoustics Analysis

All collected sound files (saved in wav format) were reproduced on a computer through the Raven Pro 64 1.4 program (Cornell Lab of Ornithology, Ithaca, NY), which displays digital audio files on a frequency vs. time plot. The sound pressure level is presented in a spectrogram, defined by a scale of color, with Hann windows (512 points of recording length and 50 % overlap). Although the human ear can only reach levels up to 20 kHz, these spectrograms display the frequencies that are above the human hearing capacity and are quite valuable in discriminating and identifying distinct sounds (Lee et al., 2012).

As this study was focused on the whistles, first a classification of the sounds was made in two categories: tonal signals, i.e., whistles, and burst pulsed sounds (López & Shirai, 2009). All the whistles of each recording were counted and classified based on the signal quality index: "poor" - weak signal and barely perceptible in the spectrogram; "fair"- signal evident and with a perceptible beginning/ending in the spectrogram; "good"- signal clearly marked and with a definite beginning/end in the spectrogram (Luís, 2019). After counting the number of whistles in each recording, emission rates were calculated, for each species, through the division of the whistle number by group size and recording time (dos Santos et al., 2005). Although all whistles were included in the counting, only the acoustic parameters of whistles considered as "fair" or "good" were analyzed.

It was also measured just the whistles that were not overlapped or presented at most two other overlapping whistles. (Bazúa-Durán & Au, 2002). Even some of these whistles were not extracted from the recordings if they had identical time-frequency contours and were repeated several times, as for example: stereotyped whistles that are potentially signature whistles (Janik & Sayigh, 2013). For similar whistle series, only a single whistle was chosen (as in Gannier et al., 2019) to prevent replication of sample.

In order to be able to compare the whistles between the three delphinid species, the following acoustic parameters were extracted from the whistles selected, through the Raven selection table, for each species: the initial and final frequency, the minimum and maximum frequency, the 1st quartile and 3rd quartile frequency, the inflection points, the center and peak frequency (Figure 2.2). Only the fundamental formant was measured, the occasional harmonic bands visible were ignored (Figure 2.2). After the whistles were measured, the selection tables of each recording were exported to *Microsoft Excel* (Microsoft Corporation, Redmond, Washington). Then it was calculated the whistle duration (by subtracting the end and begin whistle time) and the frequency range (through subtraction of the maximum and minimum frequency) (Figure 2.2).

After the calculation of whistles duration, only the ones with duration equal to or greater than 0.25 seconds were selected, since whistles with duration less than this value were considered in previous studies as chirps (Bazúa-Durán & Au, 2002).
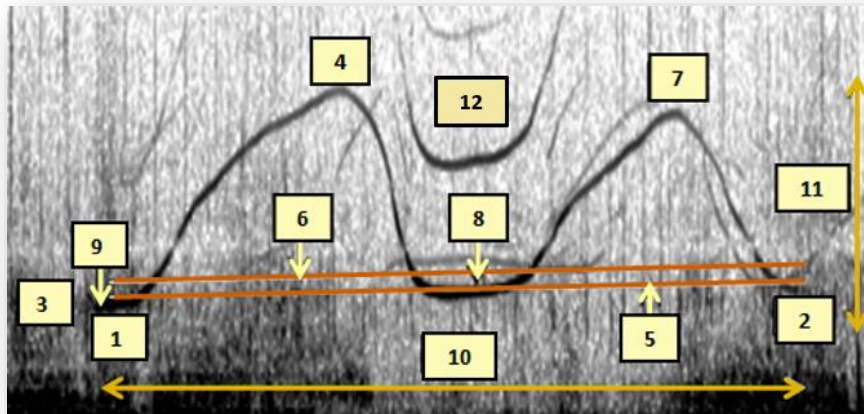
Figure 2.2- Portion of the spectrogram observed in an acoustic recording of *Stenella frontalis* and the whistle acoustic parameters extracted from the Raven Pro 64 1.4 program. 1- Initial Frequency; 2- Final Frequency; 3- Minimum Frequency; 4- Maximum Frequency; 5- 1st Quartile; 6- 3rd Quartile; 7- Inflection point; 8- Center Frequency; 9- Peak Frequency; 10- Duration; 11- Frequency Range; 12 - First Harmonic (Not measured).

In Raven's selection table was also included the whistle frequency contour which was determined through visual classification and based on the methodology adopted by Ansmann et al. (2007). In this method the whistles were divided into six categories (Figure 2.3): 1- Upsweep (no inflection point and increased frequency), 2- Downsweep (no inflection point and decreased frequency), 3- Concave (there is an initial decrease in frequency, an inflection point occurs, and the frequency increases), 4- Convex (the frequency initially rises, an inflection point occurs, and then there is a decrease in frequency), 5- Sinusoidal (several inflection points), 6- Constant (no inflection point and with a frequency variation lower than 25%) (Ansmann et al., 2007; Luís, 2019).

After the description of the whistle frequency contours, the occurrence of each whistle category was compared among the three species of delphinids.
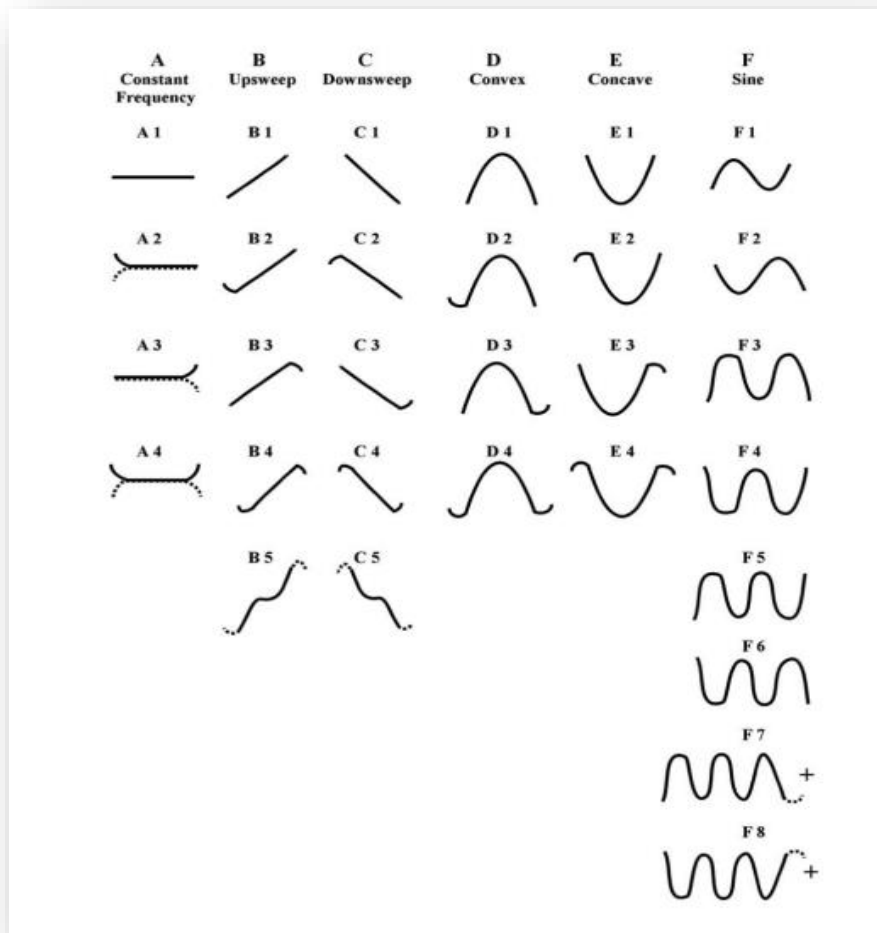
Figure 2.3- Illustrations representing each subcategory and category of whistle applied to the method of visual classification (Ansmann et al., 2007).

2.4. Statistical Analysis

The statistical procedures were all conducted in the program *IBM SPSS Statistics*, *Version 22* (International Business Machines Corporation, North Castle, New York).

In a first stage, normality tests for emission rates, the number of whistles on each recording and the group sizes were carried out in all species (Shapiro-Wilk, $p < 0.05$). The nonparametric tests of Kruskal-Wallis and Dunn *post-hoc* multiple comparisons were used to evaluate the differences in the whistles emission rates among *Tursiops truncatus*, *Stenella frontalis* and *Delphinus delphis*. Then, in order to compare the values of the emission rates in each activity pattern, a nonparametric test was applied, the Mann-Whitney. Furthermore, a

Spearman rank correlation coefficient was conducted to verify if there was any association between the number of whistles in each recording and the group sizes for each and all species.

Afterwards, another normality test was applied to each acoustic parameter of the selected whistles (Kolmogorov-Smirnov, $p < 0.05$). Then nonparametric tests, the Kruskal-Wallis, and the Dunn *post-hoc* multiple comparisons, were carried out to analyze the differences between the whistle's acoustic parameters of the three delphinid species.

Lastly, the Pearson's chi-squared test was conducted to verify if the whistles frequency contours varied according to the species.

3. Results

From 10th July to 14th October, 193 behavioral records were carried out in 52 sightings of the three delphinid species. From the total number of sightings 28 correspond to *Stenella frontalis*, 12 to *Tursiops truncatus* and 12 refer to *Delphinus delphis*.

However, as the acoustic recordings were only conducted from August 24th to 14th October, only the behavioral records carried out in that period were considered for this study. Therefore, 23 behavioral records were obtained (Table 3.1) in 13 sightings (4 from *Tursiops truncatus*, 4 of *Stenella frontalis* and 5 from *Delphinus delphis*) of the different dolphin species, simultaneously with the acoustic recordings.

In relation to the sounds emitted by these animals, 50 acoustic recordings of these three delphinid species were carried out (Table 3.1). Nevertheless, from the total number of recordings, 5 were not included in this work because they were conducted in the presence of mixed-species groups (Table 3.1). For *Tursiops truncatus* a total of 11.91 minutes of recording was obtained, and 182 whistles were measured. In the case of *Stenella frontalis*, the recording time was 17.93 minutes, and 300 whistles were measured. Regarding *Delphinus delphis*, the time of recording was 14.63 minutes and a total of 27 whistles were measured/selected (Table 3.1).

Table 3.1- Data set description utilized for this study. am- Data obtained in the morning; pm- Data collected in the afternoon. (*) - Acoustic recordings of mixed pods that were discarded.

Species	Date	Number and duration of recordings (s)	Number of measured whistles	Number of selected whistles	Mean Group size	Activity Patterns
Tursiops truncatus	25/08*	3 (97)	-	-	25	Travelling
	27/08 am	2 (100)	29	27	25	Socializing
	27/08 am	4 (177)	32	32	25	Socializing
	27/08 pm	1 (60)	21	20	35	Foraging
	30/08	1 (63)	27	27	35 (13 close to the boat)	Socializing
	30/08	1 (35)	2	2	35	Socializing
	30/08	1 (61)	30	30	35	Socializing
	30/08	2 (121)	41	37	35	Socializing
Stenella frontalis	24/08	5 (307)	78	74	90	Travelling
	29/08	5 (302)	99	90	80	Travelling
	29/08	1 (27)	0	0	80	Travelling
	09/09	2 (121)	65	62	90 (25 close to the boat)	Socializing
	09/09	1 (60)	15	14	90 (25 close to the boat)	Socializing
	09/10	2 (79)	23	23	45	Travelling
	09/10	3 (181)	20	19	45	Travelling
Delphinus delphis	02/09*	2 (80)	-	-	18	Foraging
	02/09	1 (60)	0	0	18	Feeding
	10/09	2 (120)	5	5	35	Travelling
	10/09	3 (179)	0	0	35	Travelling
	25/09	3 (181)	12	12	11	Travelling
	26/09	2 (121)	0	0	45	Travelling
	14/10	2 (121)	1	1	75 (35 close to the boat)	Travelling
	14/10	1 (77)	9	9	75 (35 close to the boat)	Travelling

3.1. Whistle Emission Rate

For the first comparison of the signal production, the global mean whistle emission rates for *Tursiops truncatus, Stenella frontalis* and *Delphinus delphis* were calculated (Figure 3.1). Then, average emission rates were calculated at each activity pattern for the three species of delphinids (Figure 3.2).
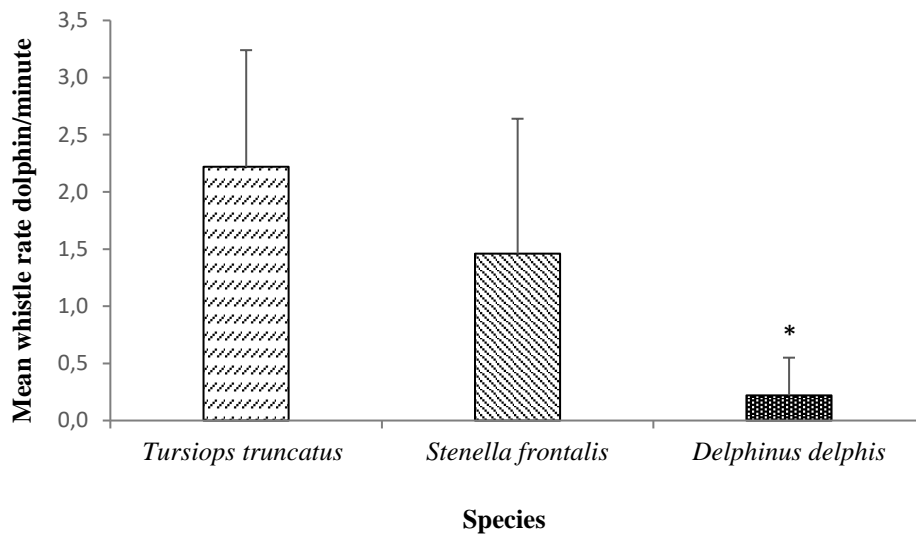
Figure 3.1- Mean rate and standard deviation of whistle emissions per dolphin per minute for the three delphinid species. The mean significantly different (Kruskal-Wallis, $p < 0.05$) is signed with *.

Bottlenose dolphins exhibited the highest average emission rate (2.22, SD = 1.02, $n = 12$) in relation to the Atlantic spotted dolphins (1.46, SD = 1.18, $n = 19$) and the short-beaked common dolphins (0.22, SD = 0.33, $n = 14$) (Figure 3.1). However, when comparing the values of the emission rates with the Kruskal-Wallis and Dunn *post-hoc* tests, *Tursiops truncatus* only presented significantly higher values than *Delphinus delphis* ($p < 0.05$) (Annex 5, Tables 2 and 3). Therefore, the average whistle rate of *Stenella frontalis* was not significantly lower than *Tursiops truncatus* ($p > 0.05$) (Annex 5, Table 3).

The short-beaked common dolphins presented a significantly lower mean emission rate both in relation to *Tursiops truncatus* and to *Stenella frontalis* ($p < 0.05$) (Annex 5, Tables 2 and 3).

In relation to signal production in each activity pattern, although a total of four different activity patterns were recorded (Table 3.1), only the mean emission rates for travelling and socializing were calculated, since there was only one recording in foraging and feeding.

The species *Stenella frontalis* was the only one where acoustic recordings were obtained both in travelling and in socializing. Therefore, for both *Delphinus delphis* and *Tursiops truncatus* it was only possible to calculate the mean emission rates for one activity pattern (Figure 3.2). In the case of *Tursiops truncatus* there were no acoustic recordings corresponding to travelling and consequently the value of the mean emission rate for this activity pattern was considered as zero (Figure 3.2). The species *Delphinus delphis* was not observed in socialization and therefore the value zero was also considered for the average emission rate in this pattern (Figure 3.2).
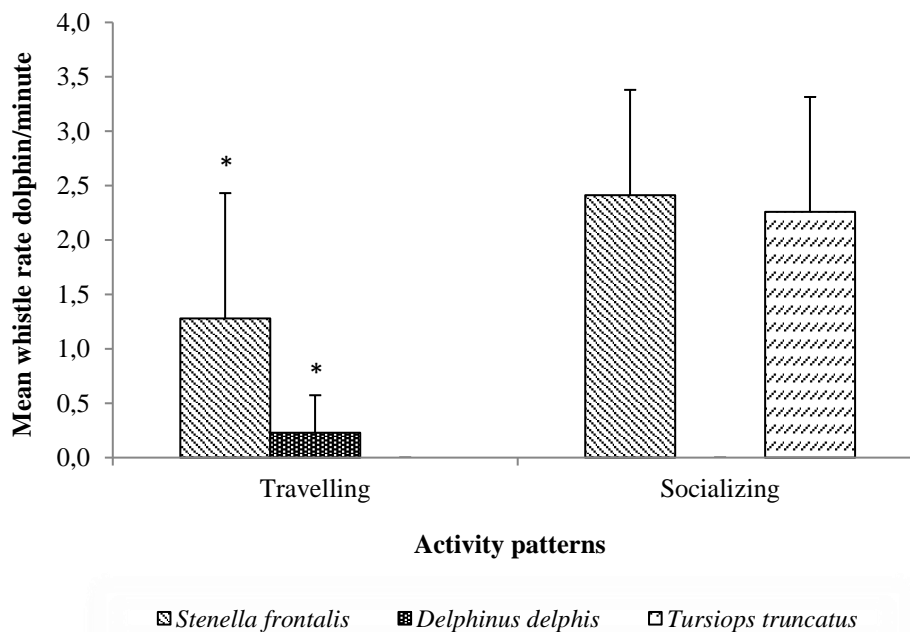
Figure 3.2- Mean rate and standard deviation of whistle emissions per dolphin per minute according to two registered activity patterns for the three delphinid species. The means significantly different (Mann-Whitney, $p < 0.05$) are signed with *.

The Atlantic spotted dolphins obtained the highest mean emission rates in both activity patterns. In travelling the average number of whistles per dolphin and per minute was 1.28 (SD = 1.15, n = 16) where one of the samples had no whistles, while during socializing the mean emission rate increased to 2.41 (SD = 0.97, n = 3) (Figure 3.2). However, despite this increase, the average whistle rate in socialization was not significantly higher than the emission rate in travelling (Mann-Whitney, $p > 0.05$) (Annex 5, Table 4).

In the case of *Delphinus delphis* the mean whistle rate during travelling was 0.23 (SD = 0.34, n = 13) where six samples did not present any whistles (Figure 3.2). When compared to *Stenella frontalis* with the Mann-Whitney test, the mean emission rate of *Delphinus delphis* in travelling was significantly lower ($p < 0.05$) (Annex 5, Table 5).

In relation to *Tursiops truncatus* the average number of whistles per dolphin and per minute in socializing was 2.26 (SD = 1.05, n = 11) (Figure 3.2). Although the mean whistle rate was higher in *Stenella frontalis*, there was no significant discrepancy between these two species in this activity pattern (Mann-Whitney, $p > 0.05$) (Annex 5, Table 6).

As for the association between the number of whistles per minute and the group's size, it was verified through Spearman's correlation coefficient that there is a significant positive correlation between these two variables, when considering the rates of all species (r = 0.338; $p < 0.05$; n = 45) (Annex 5, Table 7). However, besides this association being weak, when considering the correlation species by species the values were not significant, which means, there is no significant association between these two variables for *Stenella frontalis* (r = 0.266; $p > 0.05$; n = 19), *Delphinus delphis* (r = - 0.381; $p > 0.05$; n = 14) and *Tursiops truncatus* (r = 0.338; $p > 0.05$; n = 12) (Annex 5, Tables 8, 9 and 10).

3.2. Whistle Characterization and Comparison

To determine and compare the whistle characteristics of *Tursiops truncatus*, *Stenella frontalis* and *Delphinus delphis*, 509 whistles were measured (Table 3.1), however, only 484 were selected for analysis due to their duration being equal to or greater than 0.25 seconds (Table 3.2).

In relation to the whistle acoustic parameters, eleven were measured for the three species of delphinids. However, only nine of these parameters were used as the 1st and 3rd quartile frequencies were excluded from the analysis (Table 3.2).

Table 3.2- Comparison of whistle variables for the bottlenose dolphin, Atlantic spotted dolphin, and short-beaked common dolphin. Mean ± standard deviation and minimum-maximum values. The means significantly different (Kruskal-Wallis, $p < 0.05$) are signed with *. The species with significant differences in the acoustic parameters of the whistles are identified under each of the variables as: TTR- *Tursiops truncatus*; SFR- *Stenella frontalis* and DDE- *Delphinus delphis*.

Variable	*Tursiops truncatus* (n = 175)	*Stenella frontalis* (n = 282)	*Delphinus delphis* (n = 27)
Duration (s) (TTR-SFR) *	**0.62 ± 0.37** (0.25-2.47)	**0.69 ± 0.35** (0.26-2.29)	0.63 ± 0.23 (0.31-1.20)
Initial frequency (kHz) (DDE-TTR; DDE-SFR) *	10.14 ± 5.12 (1.34-28.44)	8.92 ± 3.76 (2.05-22.92)	**12.49 ± 4.69** (5.33-26.27)
Final frequency (kHz) (TTR-SFR; TTR-DDE) *	**10.90 ± 4.34** (1.74-22.39)	13.10 ± 5.18 (3.91-28.33)	12.96 ± 2.79 (6.93-19.20)
Minimum frequency (kHz) (DDE-TTR; DDE-SFR) *	7.51 ± 3.16 (1.34-18.44)	6.97 ± 2.17 (2.05-16.40)	**9.56 ± 3.01** (5.33-18.13)
Maximum frequency (kHz) (TTR-SFR) *	**14.76 ± 4.38** (7.64-28.44)	**17.18 ± 4.19** (6.40-28.33)	15.42 ± 3.45 (11.07-26.67)
Frequency range (kHz) (SFR-TTR; SFR-DDE) *	7.25 ± 3.77 (0.44-17.89)	**10.20 ± 4.00** (1.14-17.89)	5.86 ± 3.07 (1.12-15.07)
Center frequency (kHz) (DDE-TTR; DDE-SFR) *	9.45 ± 3.21 (3.00-22.13)	9.80 ± 2.83 (4.88-20.06)	**11.69 ± 3.33** (6.38-18.75)
Peak frequency (kHz) (DDE-TTR; DDE-SFR) *	9.12 ± 3.45 (1.88-24.94)	9.24 ± 3.24 (3.94-22.13)	**11.40 ± 3.95** (5.63-20.44)
Nº Inflection points (SFR-TTR; SFR-DDE) *	1.14 ± 1.95 (0-11)	**1.40 ± 1.45** (0-10)	0.85 ± 1.38 (0-5)

By comparing the three dolphin species with a Kruskal-Wallis test, all variables differ significantly ($p < 0.05$) (Annex 6, Table 11) (Table 3.2). However, with Dunn *post-hoc* multiple comparisons test, in all variables there was at least one combination between two species without significant differences ($p > 0.05$) (Annex 6, Tables 12, 13, 14, 15, 16, 17, 18, 19, 20).

The whistles of the Atlantic spotted dolphins were significantly longer (0.69 s, SD = 0.35) than those of bottlenose dolphins (0.62 s, SD = 0.37). However, the short-beaked common dolphin whistles (0.63 s, SD = 0.23) were not significantly shorter than those of the Atlantic spotted dolphins (Table 3.2). There were also no statistically significant differences between the whistle duration of bottlenose dolphins and common dolphins (Annex 6, Table 12).

In the case of the initial, minimum, center and peak frequencies, the *Delphinus delphis* presented values significantly higher than *Tursiops truncatus* and *Stenella frontalis* (Annex 6, Tables 13, 15, 18 and 19). However, in these four variables there were no significant differences between *Tursiops truncatus* and *Stenella frontalis* (Table 3.2).

In relation to the final frequency *Tursiops truncatus* presented values significantly lower (10.90 kHz, SD = 4.34) than *Stenella frontalis* (13.10 kHz, SD = 5.18) and *Delphinus delphis* (12.96 kHz, SD = 2.79) (Table 3.2) (Annex 6, Table 14). For this variable there were no significant differences between the whistles of *Stenella frontalis* and *Delphinus delphis* (Annex 6, Table 14).

In the maximum frequency *Tursiops truncatus* also exhibited significantly lower values (14.76 kHz, SD = 4.38) but only in relation to *Stenella frontalis* (17.18 kHz, SD = 4.19). For *Delphinus delphis* (15.42 kHz, SD = 3.45) there were no significant differences in this acoustic parameter with respect to both *Tursiops truncatus* and *Stenella frontalis* (Table 3.2) (Annex 6, Table 16).

For the Atlantic spotted dolphins, the frequency range and the number of inflection points were significantly higher compared to bottlenose dolphins and short-beaked common dolphins (Annex 6, Tables 17 and 20). In both variables there were no significant differences between the whistles of *Tursiops truncatus* and *Delphinus delphis* (Table 3.2).

The same whistles selected for characterization and comparison between *Tursiops truncatus*, *Stenella frontalis* and *Delphinus delphis* were also used to compare the whistles of each species with previous studies in different regions (Tables 3.3, 3.4, 3.5).

In the case of *Tursiops truncatus*, the average values of the whistle variables were compared with five different regions (Table 3.3). The results of the Western North Atlantic were compared twice as they were obtained in different studies (Table 3.3).

Table 3.3- Comparison of the mean whistle variables values of *Tursiops truncatus* in several regions. The values in brackets correspond to the standard deviation.

Variable	Central Azores (n = 175)	Western North Atlantic (n = 858)	Western North Atlantic (n = 894)	Sado Estuary, Portugal (n = 735)	Bocas del Toro, Panama (n = 214)	Taiji, Japan (n = 215)	Rio de Janeiro (n = 91)
Duration (s)	0.62 (0.37)	1.30 (0.63)	0.62 (0.06)	0.86 (0.396)	1.1 (0.66)	0.62 (0.34)	0.8 (0.6)
Initial frequency (kHz)	10.1 (5.12)	11.3 (3.98)	10.6 (0.55)	5.8 (1.8)	9.8 (3.7)	10.3 (2.41)	12.3 (3.7)
Final frequency (kHz)	10.9 (4.34)	10.2 (3.64)	12.4 (0.61)	12.1 (4.4)	9.1 (4.2)	8.9 (2.21)	13.3 (4.4)
Mininum frequency (kHz)	7.5 (3.16)	7.3 (1.66)	8.2 (0.24)	5.4 (1.2)	5.6 (1.8)	7.4 (1.54)	10.0 (2.9)
Maximum frequency (kHz)	14.8 (4.38)	16.2 (2.69)	15.0 (0.61)	15.0 (2.7)	15.8 (3.6)	11.6 (2.00)	17.2 (4.3)
Frequency range (kHz)	7.3 (3.77)	-	6.8 (0.59)	9.6 (-)	10.3 (3.6)	-	7.2 (3.9)
Center frequency (kHz)	9.5 (3.21)	-	11.2 (0.47)	-	-	-	-
Peak frequency (kHz)	9.1 (3.45)	-	-	9.2 (2.6)	10.4 (3.1)	-	-
Nº Inflection points	1.1 (1.95)	2.86 (2.45)	1.4 (0.21)	-	3.7 (3.8)	0.88 (0.69)	1.4 (1.6)
Reference	This study	Steiner, 1981	Baron et al., 2008	dos Santos et al., 2005	May-Collado & Wartzok, 2008	Ding et al., 1995a	Lima et al., 2020

For *Tursiops truncatus*, the values of the variables had differences or similarities depending on the regions (Table 3.3). The whistles acoustic parameters of this species that presented the greatest differences between the regions were the initial, final and minimum frequencies, the frequency range and the number of inflection points (Table 3.3).

In relation to *Stenella frontalis*, the whistle variables obtained in this study were compared with five different regions, and with the same region but in two different locations (Western North Atlantic inshore and offshore) (Table 3.4).

Table 3.4- Comparison of the mean whistle variables values of *Stenella frontalis* in different regions. The values in brackets represent to the standard deviation.

Variable	Central Azores (n = 282)	Eastern Azores (n = 117)	W. Atlantic inshore (n = 328)	W. Atlantic offshore (n = 1377)	Canary Islands (n = 84)	Bahamas (n= 220)	Rio de Janeiro, Brazil (n= 1092)
Duration (s)	0.69 (0.35)	0.65 (0.35)	0.43 (0.02)	0.65 (0.04)	-	0.44 (0.30)	0.36 (0.29)
Initial frequency (kHz)	8.9 (3.76)	10.2 (4.1)	9.3 (0.36)	9.1 (0.25)	9.4 (2.3)	-	8.8 (3.2)
Final frequency (kHz)	13.1 (5.18)	14.6 (4.8)	12.3 (0.37)	13.1 (0.35)	14.6 (2.4)	-	12.8 (3.8)
Minimum frequency (kHz)	7.0 (2.17)	7.3 (2.0)	7.5 (0.21)	7.5 (0.17)	7.4 (5.2)	7.1 (1.5)	8.0 (2.5)
Maximum frequency (kHz)	17.2 (4.19)	16.2 (3.6)	14.2 (0.34)	15.8 (0.32)	17.9 (10.6)	14.5 (2.5)	13.6 (3.6)
Frequency range (kHz)	10.2 (4.00)	9.4 (3.6)	6.7 (0.38)	8.3 (0.31)	-	7.4 (2.9)	5.5 (3.5)
Center frequency (kHz)	9.8 (2.83)	11.5 (1.5)	11.2 (0.37)	11.7 (0.27)	-	10.9 (2.0)	10.8 (2.6)
Peak frequency (kHz)	9.2 (3.24)	-	-	-	-	-	-
Nº Inflection points	1.4 (1.45)	2.7 (2.4)	1.6 (0.20)	2.2 (0.14)	-	-	0.7 (1.3)
Reference	This study	Gannier et al., 2019	Baron et al., 2008	Baron et al., 2008	Papale et al., 2015	Lammers et al., 2003	Azevedo et al. 2010

As for bottlenose dolphins, the differences and similarities in the acoustic parameters of the Atlantic spotted dolphins varied according to regions. In this species the variables that showed the greatest differences between the regions were the final and maximum frequencies, the frequency range, and the number of inflection points (Table 3.4).

In the case of *Delphinus delphis* the mean values of the whistle variables were compared between five different regions (Table 3.5).

Table 3.5- Comparison of the mean whistle variables values of *Delphinus delphis* in several places. The values in brackets refer to the standard deviation.

Variable	Central Azores (n = 27)	Eastern Azores (n= 134)	Eastern Atlantic (n = 514)	Western Mediterranean (n = 188)	Celtic sea (n = 1835)	English Channel (n = 435)
Duration (s)	0.63 (0.23)	0.94 (0.32)	0.95 (0.38)	0.92 (0.52)	0.65 (-)	0.65 (-)
Initial frequency (kHz)	12.5 (4.69)	12.7 (5.2)	13.0 (4.9)	11.9 (4.4)	12.0 (-)	12.6 (-)
Final frequency (kHz)	13.0 (2.79)	11.2 (3.7)	11.8 (4.0)	12.2 (3.8)	12.0 (-)	12.5 (-)
Minimum frequency (kHz)	9.6 (3.01)	8.2 (1.9)	8.1 (1.8)	8.3 (2.3)	9.5 (-)	9.8 (-)
Maximum frequency (kHz)	15.4 (3.45)	16.7 (3.7)	16.7 (3.6)	16.1 (3.0)	14.7 (-)	15.8 (-)
Frequency range (kHz)	5.9 (3.07)	8.6 (3.3)	8.6 (3.4)	7.8 (3.3)	5.2 (-)	6.0 (-)
Center frequency (kHz)	11.7 (3.33)	12.5 (2.1)	-	-	11.9 (-)	12.7 (-)
Peak frequency (kHz)	11.4 (3.95)	-	-	-	-	-
Nº Inflection points	0.9 (1.38)	2.3 (1.9)	1.1 (1.2)	2.0 (1.6)	0.6 (1.9)	0.6 (1.9)
Reference	This study	Gannier et al., 2019	Papale et al., 2014	Azzolin et al., 2014	Ansmann et al., 2007	Ansmann et al., 2007

As in the other two species, the differences in the values of the *Delphinus delphis* acoustic parameters varied according to the regions. The whistle variables that showed the greatest differences in this species were the final, minimum, and maximum frequencies, the frequency range and the number of inflection points (Table 3.5).

3.3. Whistles Frequency Contours

In order to determine the occurrence of each whistle category in the acoustic recordings of the three delphinid species, the same 484 whistles selected for the characterization and acoustic comparison were used (Figure 3.3 and 3.4).
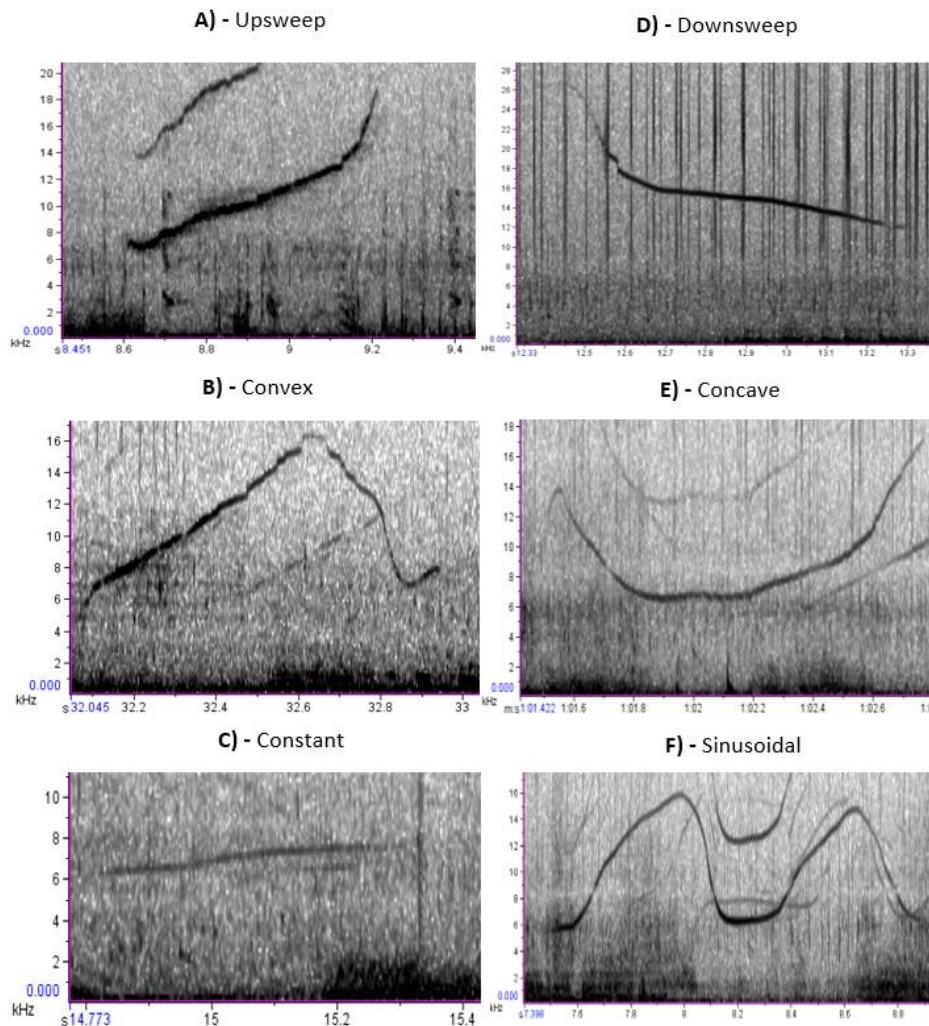
Figure 3.3- The six categories used to classify the frequency contours of the whistles selected from the three dolphin species. **A)**, **B)** and **C)-** Whistles of *Tursiops truncatus*; **D)-** *Delphinus delphis* whistle; **E)** and **F)-** Whistles from *Stenella frontalis.*
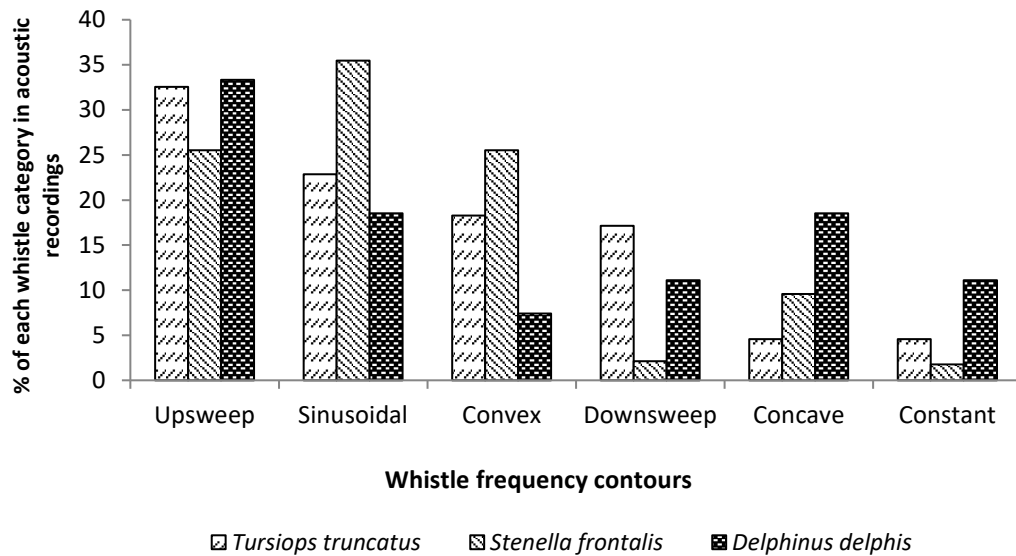
Figure 3.4- Occurrence of each whistle category (%) according to the different species.

All 6 categories were found in the three species of delphinids. The most observed whistle category in acoustic recordings was the Upsweep for both *Tursiops truncatus* and *Delphinus delphis.* However, for *Stenella frontalis* the category that occurred most in the acoustic recordings was the Sinusoidal (Figure 3.4).

Furthermore, the whistle frequency contour that occurred least in the recordings was the Constant for both *Tursiops truncatus* and *Stenella frontalis*. Nevertheless, for bottlenose dolphin the Concave category was also observed with less frequency (Figure 3.4). Unlike these two species, the least observed category for *Delphinus delphis* was Convex, followed by Downsweep and Constant (Figure 3.4).

Through the Pearson's chi-squared test it was verified that the differences in whistle frequency contours between the three dolphin species were significant ($p < 0.05$) (Annex 7, Table 21). With this test it was also observed that there is an association in which the whistle frequency contour depends on the species. For *Tursiops truncatus* there were more whistles from the categories Downsweep, Constant and Upsweep than would be expected if the variables were not related (Annex 7, Table 22). In the case of *Stenella frontalis* there was a higher number of Sinusoidal, Convex and Concave frequency contours than expected (Annex 7, Table 22). In *Delphinus delphis* the whistle categories that were more observed than expected were Downsweep, Concave and Constant (Annex 7, Table 22).

4. Discussion

As noticed in a previous study, *Stenella frontalis* was the most sighted dolphin in the summer months (from July 10th to October 14th) compared to *Tursiops truncatus* and *Delphinus delphis* (Silva et al., 2003).

In relation to global emission rates, *Tursiops truncatus* was the species that exhibited the highest mean values of the whistle rate compared to *Stenella frontalis* and *Delphinus delphis*, although these values were only significant in relation to short-beaked common dolphins. It has been observed in previous studies that the whistle emission rates in socializing have higher values than in travelling (dos Santos et al., 2005; Jones & Sayigh, 2002), and since the acoustic recordings of the bottlenose dolphins were mostly carried out in socializing while for the common dolphins were mainly conducted in travelling, it is possible that the activity patterns have contributed to the observed differences between the average emission rates. Conversely, *Delphinus delphis* presented a significantly lower mean whistle rate compared to *Tursiops truncatus* and *Stenella frontalis* which may also be related to the activity pattern since common dolphins were the only species where more than 90% of the recordings were carried out in travelling.

Regarding the emission rates in each activity pattern, *Stenella frontalis* presented the highest whistle emission rate for travelling and socializing. The mean emission rate in socializing, for this species, was higher than the rate in travelling, although this increase was not significant, it has been shown in previous studies that the whistle abundance is usually related to the activity (Azevedo et al., 2010; Cook et al., 2004; dos Santos et al., 2005; Jones & Sayigh, 2002). In these studies, it was noticed that in socializing and in feeding activities the whistle emission rate was higher than in travelling, which shows that the abundance of whistles is associated with exciting activities and supports the notion that whistles are predominantly used as a social function (Azevedo et al., 2010; Cook et al., 2004; dos Santos et al., 2005; Jones & Sayigh, 2002; Wood, 1953).

In the case of *Delphinus delphis* and *Tursiops truncatus* it was not possible to compare the emission rates between the activity patterns since recordings were only obtained in one pattern for both species. When comparing the mean emission rates of these two species with *Stenella frontalis*, although both showed lower values only for common dolphins there was a significant difference. One possibility of this difference would be the groups size; however, it was verified that the whistle emission rate for each species was not correlated with the group size. Thus, the significant lower value for the *Delphinus delphis* emission rate may be related to differences in group composition, for example presence or absence of calves, and the occurrence or lack of predators in the area (Hawkins & Gartside, 2010; Quiñones-Lebrón & May-Collado, 2011).

As mentioned previously, although in general a weak correlation between group size and emission rates was verified, this association was not observed for each species. Some previous studies verified that there was an association between these two variables (Cook et al., 2004; Jones & Sayigh, 2002; Van Parijs, Smith & Corkeron, 2002) and that in dolphins, this correlation is usually positive, which means that the number of whistles emitted increases with the number of individuals in a sampling period (Janik, 2008; Luís, Couchinho & dos Santos, 2015). However, it was also noted in other studies that this correlation has not occurred for some populations, as found in this study (dos Santos et al., 2005; Luís, Couchinho & dos Santos, 2015; Quick & Janik, 2008). As suggested before, the fact that there is no increase in whistles with higher number of individuals may be related to some social constraint mechanism that restricts emissions and due to environmental factors (dos Santos et al., 2005; Luís, Couchinho & dos Santos, 2015). In the first case if a group produces too many whistles simultaneously, recognition and perception could be constrained by overlapping or overloaded information, so not increasing the whistle emission in this case can be an improvement in communication efficiency with the increase in group size (dos Santos et al., 2005). This condition may also occur due to ambient noise, such as boat noise in the Azores, where each dolphin can restrict its whistle emissions to allow an optimal broadcast of the transmitted signals and prevent overlapping (Luís, Couchinho & dos Santos, 2015).

When comparing the whistles acoustic parameters of *Tursiops truncatus*, *Stenella frontalis* and *Delphinus delphis* it was found that they were significantly different among the species. These differences were observed for both nonfrequency and frequency features and had already been documented in previous studies for *Stenella frontalis* and *Delphinus delphis* also in the Azores (Gannier et al., 2019), for the eastern Tropical Pacific Ocean in *Tursiops truncatus* and *Delphinus delphis* (Oswald, Barlow & Norris, 2003) and in the western South Atlantic Ocean among these three species (Amorim et al., 2019). The differences in the whistle acoustic parameters varied according to the comparisons between the species, for example, between *Tursiops truncatus* and *Stenella frontalis* there were fewer differences in the frequency parameters (they only presented significant differences in initial, maximum and frequency range) compared with *Tusiops truncatus* and *Delphinus delphis* which presented differences in initial, final, minimum, central and peak frequencies. However, the bottlenose dolphins showed significant differences in duration and number of inflection points when compared with the Atlantic spotted dolphins but not with the short-beaked common dolphins. For *Stenella frontalis* and *Delphinus delphis* there were significant differences both in the frequency parameters such as the initial, minimum, central, peak and frequency range as well as in the number of inflection points.

Several factors have already been proposed to explain these differences found in the whistle characteristics of different species: taxonomic relationships, morphology, social structure, and variations in inhabited regions (Azzolin et al., 2014; Bazúa-Durán, 2004; Ding, Würsig & Evans, 1995b; Gillooly & Ophir, 2010; Herman & Tavolga, 1980; May-Collado, Agnarsson & Wartzok; Rendell et al., 1999; Steiner, 1981; Ding et al., 1995b; 2007b).

Regarding taxonomy, although these three species belong to the same family (Delphinidae), they are classified in different genera. It was suggested by Steiner (1981) that the differences in the whistle vocalizations of distinct species were related to taxonomic relations, and it was verified that the most taxonomically distinct species had the most distinctive whistles, while congeners obtained the greatest similarities in the whistles. In addition, *Tursiops truncatus*, *Stenella frontalis* and *Delphinus delphis* are seasonally sympatric species which, as observed by Steiner (1981), results in greater differences in the characteristics of whistles compared to the allopatric species. This is because delphinids use whistles for species-specific communication, so sympatric species need to have highly distinctive whistles since they share the same habitat (Steiner, 1981).

In relation to morphology, it has already been suggested by Gillooly & Ophir (2010) that body size restrict the frequency parameters of the acoustic signals of several species, including mammals. Some studies have documented a negative relationship between body size and frequency parameters in dolphins (Azzolin et al., 2014; Ding et al., 1995b), which indicates that there has been a selection of these parameters and that, throughout evolutionary history, the response to this selection has been conditioned by the size of the body (May-Collado, Agnarsson & Wartzok, 2007a).

However, in this study the two species with more similar sizes (*Stenella frontalis* and *Delphinus delphis*) presented greater differences in frequency parameters than, for example, the Atlantic spotted dolphins and the bottlenose dolphins that are larger and have a more robust body. These significant differences in the whistle frequency parameters of the two species with similar sizes may be related to the activity patterns since the analyzed whistles of *Delphinus delphis* were mostly obtained in travelling while for *Stenella frontalis* they were also collected in socializing.

Nevertheless, *Delphinus delphis* and *Stenella frontalis* presented a higher maximum frequency value than *Tursiops truncatus* (although it was only significant for Atlantic spotted dolphins) which is in accordance with the reverse correlation among maximum frequency and body size mentioned in previous studies (Azevedo & Van Sluys, 2005; Matthews et al., 1999). It would be relevant in future studies to understand whether body size really influences the differences observed in the whistle parameters of these three species in the Azores.

Regarding the social structure, it has been documented that whistles evolve according to sociability, for example the group size, and that the two features are strongly correlated (Herman & Tavolga, 1980; May-Collado, Agnarsson & Wartzok, 2007b; Rendell et al., 1999). It was suggested by Herman & Tavolga (1980) that in odontocetes the level of gregariousness was related to the production of the whistles. It was also proposed that whistles might have developed for a particular function in large pods, like keeping vocal communication while foraging (Herman & Tavolga, 1980). In this study *Tursiops truncatus*, *Stenella frontalis* and *Delphinus delphis* presented differences in group sizes, with bottlenose dolphins being associated in smaller groups, between 25 and 35 individuals, Atlantic spotted dolphins being grouped with a larger number of individuals, between 45 and 90, and common dolphins being the most varied in group sizes with associations from 18 individuals to 75. In this way the differences in the average group sizes of these three species may have contributed to the variations in the whistles acoustic parameters, however it would be important to carry out a more detailed analysis in future studies to support this possibility.

In sum, the differences observed between the whistle acoustic parameters of *Tursiops truncatus*, *Stenella frontalis* and *Delphinus delphis* may be related to taxonomy, body size and sociability. However, in some parameters there were species that did not present significant differences. The whistle variables that exhibited the lowest differences between these three delphinids species were duration and maximum frequency. Gannier et al., (2019) also did not observe significant differences in the median values of the maximum frequency between *Stenella frontalis* and *Delphinus delphis* in the Azores. The noise produced by whale watching boat operators may cause repertoire elasticity so that temporary changes in the acoustic parameters of whistles have already been documented as a result of short-term increase in anthropogenic noise due to the boats' proximity (May-Collado & Wartzok, 2008; Morisaka et al., 2005). It has already been observed, for example, an increase in the whistle's maximum frequency and duration due to the boat's presence (May-Collado & Wartzok, 2008). However, there has also been verified a decrease in the modulation and frequency of whistles in response to anthropogenic noise (Morisaka et al., 2005). As these three delphinid species are exposed to the same ambient noise and since the recordings were made in a whale watching boat and sometimes there were other vessels in the area, it is possible that the boats' noise may have contributed to these species not presenting significant differences in some whistle acoustic parameters.

In addition to the interspecific differences obtained in the whistles of these species, intraspecific variations were also observed in the acoustic parameters, when compared with previous studies, depending on the regions. When comparing the values obtained in this study for each species with other regions, there were also more similarities or differences according to the whistle variables.

For *Tursiops truncatus* the whistles were on average shorter than in four regions. However, this species obtained the same values of the mean whistle duration as in the Western North Atlantic (Baron et al., 2008) and in Taiji, Japan. In the initial and minimum frequencies this species also presented average values similar to those of Japan while in the final and maximum frequencies the bottlenose dolphins presented greater similarities with those of the Western North Atlantic documented by Steiner, (1981) and Baron et al., (2008), respectively. The whistles of this species exhibited a greater difference in the mean values of the frequency range and peak frequency with those of Bocas del Toro. For the average number of inflection points the bottlenose dolphins had the lowest number compared to all regions with the exception of Japan.

In relation to *Stenella frontalis,* this species obtained the longest whistles and the lowest average value of the minimum frequency compared to all regions. However, these dolphins had the highest mean maximum frequency value in relation to all places, apart from the Canary Islands. For the average initial and final frequency values, there was a greater similarity with Rio de Janeiro and Western Atlantic offshore, respectively. These dolphins had the widest whistles but obtained the lowest mean value for the center frequency and the number of inflection points compared with all regions (except Rio de Janeiro for the number of inflection points).

As for *Delphinus delphis*, this species presented the shortest whistles and the highest average value of final frequency in relation to all the other regions. The mean values for the initial frequency were more similar to those of the English Channel and Eastern Azores. For the average minimum, maximum and frequency range values, this species presented the lowest after those of the Celtic Sea. The mean values of the central frequency were lower in relation to all three regions while the average number of inflection points, was neither the highest nor the lowest compared to all regions.

In previous studies it has been documented that *Tursiops truncatus* (Baron et al., 2008; May-Collado & Wartzok, 2008; Ding et al., 1995a), *Stenella frontalis* (Baron et al., 2008) and *Delphinus delphis* (Papale et al., 2014) present significantly different whistle acoustic parameters in distinct regions. Some authors have already suggested hypotheses for these geographical variations in the whistle characteristics, for example, the more distant the populations, the more different the whistles would be (Azevedo & Van Sluys, 2005; Bazúa-Durán & Au, 2002; Rossi-Santos & Podos, 2006; Ding, Wursig & Evans, 1995b), since populations that are isolated differ in taxonomy and adapt differently to ambient conditions, which might affect the characteristics of the whistle (Azevedo & Van Sluys, 2005). However, some exceptions to this hypothesis have already been found in other studies (Camargo et al., 2007) as well as in this case, since for the three species some acoustic parameters presented a greater similarity with the ones from more remote than closer locations, for example in *Tursiops truncatus* the mean values of initial, minimum frequencies and number of inflection points were more similar to those of Taiji, Japan than to those of Western North Atlantic. For *Stenella frontalis* the average values of the initial frequency were more similar to those of Rio de Janeiro and the Canary Islands at maximum frequency than to those of the Eastern Azores. As for *Delphinus delphis*, the mean values of duration, initial, minimum, maximum and frequency range were more similar to the English Channel values than Eastern Azores. Other hypotheses

for delphinid whistle variability involve learning, intra-specific group fluidity variation and genetic distinctness (May-Collado & Wartzok, 2008).

Beyond these hypotheses some authors have associated the geographical variation of the whistle variables to the environmental acoustic characteristics such as background noise (Ansmann et al., 2007; May-Collado & Wartzok, 2008; Morisaka et al., 2005). As mentioned previously, generally, animals generate signals which are adjusted to their specific environment and it has been demonstrated that cetaceans acoustically respond to ambient noise in different ways, such as changes in the frequency of the signal and a decrease or increase in the duration signal (May-Collado & Wartzok, 2008). When the median values obtained in this study were compared with the results of previous studies in different regions, the greatest differences were found for the frequency parameters (initial, final, minimum, maximum and frequency range) and the number of inflection points for the three delphinid species. The number of inflection points is a very variable parameter at intra-regional and inter-regional levels where most authors claim that the variations in this parameter indicate short-term environmental effects on the whistle characteristics (Gannier et al., 2019). Despite duration was not one of the parameters with the greatest variability between the regions in this study, it is also susceptible to a wide intra-population variation since it contains information on the emotional condition and the identity of the individual (Gannier et al., 2019).

Besides the differences found in the whistle's acoustic parameters of *Tursiops truncatus*, *Stenella frontalis* and *Delphinus delphis*, significant differences were also obtained in the whistle frequency contours. In addition to these results, it was also found that the whistles frequency contours depended on the species. It was suggested that whistle categories may be associated with behavior and might differ with specific activities at the surface (Díaz López, 2011). Hawkins & Gartside (2010), for example, associated Upsweep and Constant whistles with social behaviors for Indo-Pacific bottlenose dolphins (*Tursiops aduncus*).

In this study for both *Tursiops truncatus* and *Delphinus delphis* the most observed whistle category was Upsweep, while for *Stenella frontalis* it was Sinusoidal. These results were also obtained in previous studies for bottlenose dolphins (Díaz López, 2011; McCowan & Reiss, 2001) and short-beaked common dolphins (Ansmann et al., 2007). For Atlantic spotted dolphins, the whistle category of Sinusoidal had already been documented as one of the most observed but after the Upsweep (Azevedo et al., 2010). The outcomes of this study are in accordance with previous findings that Upsweeps, and Sinusoidal whistles have an essential role in the dolphins communicating system (Díaz López, 2011). Unlike these two whistle

categories, Constant and Downsweep are not relatively common whistle frequency contours as documented in previous studies (Díaz López, 2011; Hawkins & Gartside, 2010) and as the results obtained where Constant was the least frequent whistle category for *Tursiops truncatus* and *Stenella frontalis*. In the case of *Delphinus delphis* the whistle frequency contours Constant and Downsweep were also the least observed but after Convex whistles.

In conclusion, this study investigates the whistle characteristics of three seasonally sympatric delphinid species that are frequently sighted in the Azores, both year-round and in summer (dos Santos et al., 2016; Gannier et al., 2019). It was shown that *Tursiops truncatus*, *Stenella frontalis* and *Delphinus delphis* exhibited, in general, species-specific whistle qualities (both in the acoustic parameters and in the frequency contours) and that there are several variables revealing significant differences among these species. It would be important in future studies with more data (since in this study the sample was limited) and using whistles classification methods to understand if these three delphinid species can be identified through their repertoire of whistles.

Since it is difficult to monitoring the majority of cetacean species, the capacity of recognizing these species acoustically would enable their acoustic monitoring in space and time. Therefore, it is relevant to investigate and acoustically compare several species and populations of these cetaceans in different locations especially because dolphins may present not only significant differences in the whistle characteristics between different species but also within the same species.

5. References

Acevedo-Gutiérrez, A., & Stienessen S. (2004). Bottlenose Dolphins (*Tursiops truncatus*) Increase Number of Whistles When Feeding. *Aquatic Mammals*, 30 (3), 357–362.

Amorim, T.O.S., Rezende de Castro, F., Moron, J.R., Duque, B.R., Di Tullio, J.C., Secchi, E.R., & Andriolo, A. (2019). Integrative bioacoustics discrimination of eight delphinid species in the western South Atlantic Ocean. *Plos One,* 14 (6), e0217977.

Ansmann, I.C., Goold, J.C., Evans, P.G.H., Simmonds, M., Simon, G.K. (2007). Variation in the whistle characteristics of short-beaked common dolphins, *Delphinus delphis*, at two locations around the British Isles. *Journal of the Marine Biological Association of the UK*, *87*, 19–26.

Au, W.W.L., Floyd, R.W., Penner, R.H., & Murchison, A.E. (1974). Measurement of echolocation signals of the Atlantic bottlenose dolphin, *Tursiops truncatus* Montagu, in open waters. *The Journal of the Acoustical Society of America*, 56 (4), 1280–1290.

Au, W.W.L. (1993). *The Sonar of Dolphins*. New York: Springer-Verlag.

Au, W.W.L., & Hastings, M.C. (2008). *Principles of Marine Bioacoustics*. New York: Springer.

Au, W.W.L., & Herzing, D.L. (2003). Echolocation signals of wild Atlantic spotted dolphin (*Stenella frontalis*). *The Journal of the Acoustical Society of America,* 113 (1), 598–604.

Au, W., & Lammers, M. (2007) Cetacean Acoustics. In: T. Rossing (Eds), *Springer Handbook of Acoustics* (pp. 805-837). New York: Springer.

Au, W.W.L. (2017). Echolocation. In: B. Würsig, J.G.M. Thewissen & K. Kovacs (Eds), *Encyclopedia of Marine Mammals* (Third Edition) (pp. 289–299). London: Academic Press.

Azevedo A.F., & Van Sluys, M. (2005). Whistles of tucuxi dolphins (*Sotalia fluviatilis*) in Brazil: comparisons among populations. *Journal of the Acoustical Society of America*, *117*, 1456–1464.

Azevedo, A.F., Oliveira, A.M., Rosa, L.D., & Lailson-Brito, J. (2007). Characteristics of whistles from resident bottlenose dolphins (*Tursiops truncatus*) in southern Brazil. *The Journal of the Acoustical Society of America,* 121 (5), 2978–2983.

Azevedo, A.F., Flach, L., Bisi, T.L., Andrade, L.G., Dorneles, P.R., & Lailson-Brito, J. (2010). Whistles emitted by Atlantic spotted dolphins (*Stenella frontalis*) in southeastern Brazil. *The Journal of the Acoustical Society of America,* 127 (4), 2646–2651.

Azzolin, M., Gannier, A., Lammers, M.O., Oswald, J.N., Papale, E., Buscaino, G., Buffa, G., Mazzola, S., & Giacoma, C. (2014). Combining whistle acoustic parameters to discriminate Mediterranean odontocetes during passive acoustic monitoring. *The Journal of the Acoustical Society of America,* 135 (1), 502–512.

Ballance, L.T. (1992). Habitat use patterns and ranges of the bottlenose dolphin in the Gulf of California, Mexico. *Marine Mammal Science,* 8 (3), 262–274.

Baron, S.C., Martinez, A., Garrison, L.P., & Keith, E.O. (2008). Differences in acoustic signals from Delphinids in the western North Atlantic and northern Gulf of Mexico. *Marine Mammal Science,* 24 (1), 42–56.

Bazúa-Durán, C., & Au, W.W.L. (2002). The whistles of Hawaiian spinner dolphins. *The Journal of the Acoustical Society of America,* 112 (6), 3064–3072.

Bazúa-Durán, C. (2004). Differences in the whistle characteristics and repertoire of Bottlenose and Spinner Dolphins. *Annals of the Brazilian Academy of Sciences,* 76 (2)*, 386–392.*

Camargo, F.S., Rollo, M. M., Glampaoli, V. & Bellini, C. (2007). Whistle variability in South Atlantic spinner dolphins from the Fernando de Noronha Archipelago off Brazil. *Journal of the Acoustical Society of America*, *120*, 4071–4079.

Cascão, I., Lammers, M.O., Prieto, R., Santos, R.S., Silva, M.A. (2020). Temporal patterns in acoustic presence and foraging activity of oceanic dolphins at seamounts in the Azores. *Scientific Reports*, 10**,** 3610.

Connor, R.C. (2007). Dolphin social intelligence: complex alliance relationships in bottlenose dolphins and a consideration of selective environments for extreme brain size evolution in mammals. *Philosophical Transactions of the Royal Society B: Biological Sciences,* 362, 587–602.

Cook, M.L.H., Sayigh, L.S., Blum, J.E., & Wells, R.S. (2004). Signature-whistle production in undisturbed free-ranging bottlenose dolphin (*Tursiops truncatus*). *Proceedings of the Royal Society of London, Series B: Biological Sciences*, 271 (1543), 1043-1049.

Cranford, T.W., Elsberry, W.R., Van Bonn, W.G., Jeffress, J.A., Chaplin, M.S., Blackwood, D.J., Carder, D.A., Kamolnick, T., Todd, M.A., & Ridgway, S.H. (2011). Observation and analysis of sonar signal generation in the bottlenose dolphin (*Tursiops truncatus*): Evidence for two sonar sources. *Journal of Experimental Marine Biology and Ecology*, *407*, 81–96.

Cruz, J.V. & Soares N. (2018). Groundwater Governance in the Azores Archipelago (Portugal): Valuing and Protecting a Strategic Resource in Small Islands. *Water,* 10 (4), 408.

Deecke, V.B., Ford, J.K.B., & Spong, P. (2000). Dialect change in resident killer whales (*Orcinus orca*): Implications for vocal learning and cultural transmission. *Animal Behavior*, *60*, 619–638.

Díaz López, B. (2011). Whistle characteristics in free-ranging bottlenose dolphins (*Tursiops truncatus*) in the Mediterranean Sea: Influence of behaviour. *Mammalian Biology,* 76(2), 180–189.

Ding, W. (1993). *Dolphin whistles: Comparisons between populations and species*. (Ph.D. dissertation). Institute of Hydrobiology, Chinese Academy of Sciences, Wuhan.

Ding, W., Würsig, B., & Evans, W.E. (1995a). Whistles of bottlenose dolphins: comparisons among populations. *Aquatic Mammals*, *21*, 65–77.

Ding, W., Würsig, B. & Evans, W.E. (1995b). Comparisons of whistles among seven odontocete species. In R.A. Kastelein & J.A. Thomas (Eds), *Sensory systems of aquatic mammals*. Woerden, Netherlands: De Spil Publishers.

Di Tullio, J.C., Gandra, T.B.R., Zerbini, A.N., & Secchi, E.R. (2016). Diversity and Distribution Patterns of Cetaceans in the Subtropical Southwestern Atlantic Outer Continental Shelf and Slope. *Plos one,* 11 (5), e0155841.

Dolman, S., Owen, D., Parsons, C., Potter, J., Simmonds, M.P., Swift, R., & Weilgart, L. (2004). The Use of Sound by Cetaceans. In: M. Simmonds, S. Dolman & L. Weilgart (Eds), *Oceans of noise* (pp. 4–137). Chippenham: Whale and Dolphin Conservation Society.

dos Santos, M.E. (1998). *Golfinhos-roazes do Sado: estudos de sons e comportamentos*. PhD thesis. Edições ISPA, Coleção Teses, Lisboa, Portugal.

dos Santos, M.E., Louro, S., Couchinho, M., & Brito, C. (2005). Whistles of bottlenose dolphins (*Tursiops truncatus*) in the Sado Estuary, Portugal: characteristics, production rates, and long-term contour stability. *Aquatic Mammals*, 31 (4), 453–462.

dos Santos, R.P, Simião, S., Madruga, P., Mendonça, A.S., Seitre, R., & Pereira, J.N.G. (2016). Anomalously White Atlantic Spotted Dolphins (*Stenella frontalis*, Cuvier, 1892) Off the Azores. *Aquatic Mammals*, *42*, 244-252.

Dudzinski, K.M., Thomas, J.A., & Gregg, J.D. (2009). Communication in marine mammals. In: W.F. Perrin, B. Würsig & J.G.M. Thewissen (Eds), *Encyclopedia of Marine Mammals* (Second Edition) (pp. 260–269). London: Academic Press.

Ellis, R. (2009). Azorean Whaling. In W. F. Perrin, B. Würsig & J.G.M. Thewissen (Eds), *Encyclopedia of Marine Mammals* (Second Edition) (pp. 64–67). London: Academic Press.

Ford, J.K.B. (1989). Acoustic behavior of the resident killer whales (*Orcinus orca*) off Vancouver Island, British Columbia. *Canadian Journal of Zoology*, *67*, 727–745.

Frasier, K.E., Roch, M.A., Soldevilla, M.S., Wiggins, S.M., Garrison, L.P., & Hildebrand, J.A. (2017). Automated classification of dolphin echolocation click types from the Gulf of Mexico. *Plos Computational Biology*, 13 (12), e1005823.

Gallagher, L., Porteiro, F. & Serrão Santos, R. (2013). *Vida Marinha Oceânica dos Açores.* (1ª ed.). Açores : Fishpics.

Gannier, A., Fuchs, S., Quèbre, P., & Oswald, J.N. (2010). Performance of a contour-based classification method for whistles of Mediterranean delphinids. *Applied Acoustics*, *71*, 1063–1069.

Gannier, A., Fuchs, S., Gannier, A., Fernandez, M., & Azevedo, J.M.N. (2019). Dolphin whistle repertoires around São Miguel (Azores): Are you common or spotted? *Applied Acoustics, 161,* 107-169.

García, L.G., Pierce, G.J., Autret, E., & Torres-Palenzuela, J.M. (2018). Multi-scale habitat preference analyses for Azorean blue whales. *Plos One,* 13 (9), e0201786.

Gaspar, J.L., Queiroz, G., Ferreira, T., Medeiros, A.R., Goulart, C. & Medeiros, J. (2015). Chapter 4 Earthquakes and volcanic eruptions in the Azores region: geodynamic implications from major historical events and instrumental seismicity. *The Geological Society,* 44 (1), 33–49.

Gillooly, J.F., & Ophir, A.G. (2010). The energetic basis of acoustic communication. *Proceedings of the Royal Society Ser. B*, *277*, 1325–1331.

Gridley, T., Nastasi, A., Kriesell, H.J., & Elwen, S.H. (2015). The acoustic repertoire of wild common bottlenose dolphins (*Tursiops truncatus*) in Walvis Bay, Namibia. *Bioacoustics*, *24*, 153–174.

Griffiths, E.T. (2009). *Whistle repertoire analysis of the short-beaked common dolphin, Delphinus delphis, from the Celtic Deep and the Eastern Tropical Pacific Ocean*. Master's Thesis. Bangor University, North Wales, UK.

Guerra, M., Dawson, S.M., Brough, T.E., & Rayment, W.J. (2014). Effects of boats on the surface and acoustic behaviour of an endangered population of bottlenose dolphins. *Endangered Species Research*, *24,* 221-236.

Hawkins, E.R., & Gartside D.F. (2010). Whistle emissions of Indo-Pacific bottlenose dolphins (*Tursiops aduncus*) differ with group composition and surface behaviors. *The Journal of the Acoustical Society of America*, *127*, 2652–2663.

Herman L.M. & Tavolga W.N. (1980). The communication systems of cetaceans. In L.M. Herman (Eds), *Cetacean Behavior: Mechanisms and Functions*. New York: John Wiley Son Publisher.

Henderson, E., & Würsig, B. (2007). Behavior Patterns of Bottlenose Dolphins in San Luis Pass, Texas. *Gulf of Mexico Science*, 25 (2), 153-161.

Herzing, D.L. (1996). Vocalizations and associated underwater behavior of free-ranging Atlantic spotted dolphins, *Stenella frontalis* and bottlenose dolphins, *Tursiops truncatus*. *Aquatic Mammals*, 22 (2), 61–79.

Herzing, D.L. (2015). Synchronous and rhythmic vocalizations and correlated underwater behavior of free-ranging Atlantic spotted dolphins (*Stenella frontalis*) and bottlenose dolphins (*Tursiops truncatus*) in the Bahamas. *Animal Behavior and Cognition*, 2 (1), 14-29.

Instituto da Conservação da Natureza e das Florestas (s.d.). *Chave de identificação de cetáceos*. Available at: http://www2.icnf.pt/portal/pn/biodiversidade/gestao-biodiv/roazes-do-sado/identificacao

Janik, V.M. (2009). Acoustic communication in delphinids. *Advances in the Study of Behavior*, *40*, 123– 157.

Janik, V.M., & Sayigh, L.S. (2013). Communication in bottlenose dolphins: 50 years of signature whistle research. *Journal of Comparative Physiology A*, 199 (6), 479–89.

Jefferson, T.A., Webber, M.A., & Pitman, R.L. (2015). Cetaceans. In: *Marine Mammals of the World; A Comprehensive Guide of Their Identification* (Second edition) (pp. 616). London: Academic Press.

Jones, G. J., & Sayigh, L. S. (2002). Geographic variation in rates of vocal production of free-ranging bottlenose dolphins. *Marine Mammal Science*, *18*, 374-393.

Ketten, D.R. (2000). Cetacean Ears. In: W.W.L. Au, R.R. Fay & A.N. Popper (Eds), *Hearing by Whales and Dolphins* (Vol. 12, pp. 43-108). New York: Springer.

Kriesell, H.J., Elwen, S.H., Nastasi, A., & Gridley, T. (2014). Identification and Characteristics of Signature Whistles in Wild Bottlenose Dolphins (*Tursiops truncatus*) from Namibia. *Plos One*, 9 (9), e106317.

Lammers, M.O., Au, W.W.L., & Herzing, D.L. (2003). The broadband social acoustic signaling behavior of spinner and spotted dolphins. *Journal of the Acoustical Society of America, 114*, 1629–1639.

Lee, J., Dhar, S., Abel, R., Banakis, R., Grolley, E., Lee, J., Zecker, S., & Siegel, J. (2012). Behavioral Hearing Thresholds Between 0.125 and 20 kHz Using Depth-Compensated Ear Simulator Calibration. *Ear and Hearing, 33* (3), 315–329.

Lima, I.M.S., Venuto, R., Menchaca, C., Hoffmann, L.S., Rosa, L.D., Genoves, R., Fruet, P.F., Milanelli, A., Laporta, P., Tassino, B., Bueno, S.M., Freitas, T.R.O., Bittencourt, L., Lailson-

Brito, J., Azevedo, A.F. (2020). Geographic variation in the whistles of bottlenose dolphins (*Tursiops* spp.) in the southwestern Atlantic Ocean. *Marine Mammal Science*, *36*, 1058–1067.

Lisney, T.J., & Collin, S.P. (2019). Retinal Topography in Two Species of Baleen Whale (Cetacea: Mysticeti). *Brain, Behavior and Evolution, 92,* 1–20.

López, B.D., & Shirai, J.A.B. (2009). Mediterranean common bottlenose dolphin`s repertoire and communication use. In: A.G. Pearce & L.M. Correa (Eds), *Dolphins: Anatomy, Behavior and Threats* (pp. 1–20). Nova York: Nova Science Publishers.

Luís, A.R., Couchinho, M.N., & dos Santos, M.E. (2014). Changes in the acoustic behavior of resident bottlenose dolphins near operating vessels. *Marine Mammal Science,* 30 (4), 1417–1426.

Luís, A.R., Couchinho, M.N., & dos Santos, M.E. (2015). Signature whistles in wild bottlenose dolphins: long-term stability and emission rates. *Acta Ethologica,* 19 (2), 113–122.

Luís, A.R., Couchinho, M.N., dos Santos, M.E. (2016). A Quantitative Analysis of Pulsed Signals Emitted by Wild Bottlenose Dolphins. *Plos One,* 11 (7), e0157781.

Luís, A.R.F. (2019). *Multi-Regional acoustic repertoires of bottlenose dolphins: common themes, geographical variations and ecological factors*. PhD thesis. Instituto Universitário de Ciências Psicológicas, Sociais e da Vida, Portugal.

Luis, J.F., Miranda, J. M., Galdeano, A., Patriat, P., Rossignol, J. C., & Victor, L.A.M. (1994). The Azores triple junction evolution since 10 Ma from an aeromagnetic survey of the Mid-Atlantic Ridge. *Earth and Planetary Science Letters,* 125 (1-4), 439–459.

Lusseau, D. (2006). Why do dolphins jump? Interpreting the behavioural repertoire of bottlenose dolphins (*Tursiops sp.*) in Doubtful Sound, New Zealand. *Behavioural Processes,* 73 (3), 257–265.

Madsen, P.T., Lammers, M., Wisniewska, D., & Beedholm, K. (2013). Nasal sound production in echolocating delphinids (*Tursiops truncatus* and *Pseudorca crassidens*) is dynamic but unilateral: clicking on the right side and whistling on the left side. *Journal of Experimental Biology,* 216 (21*),* 4091–4102.

Mann, J. (1999). Behavioral sampling methods for cetaceans: A review and critique. *Marine Mammal Science*, 15 (1), 102–122.

May-Collado, L.J., Agnarsson, I., & Wartzok, D. (2007a). Reexamining the relationship between body size and tonal signals frequency in whales: a comparative approach using a novel phylogeny. *Marine Mammal Science,* 23 (3), 524–552.

May-Collado, L.J., Agnarsson, I., & Wartzok, D. (2007b). Phylogenetic review of tonal sound production in whales in relation to sociality. *BMC Evolutionary Biology,* 7(1), 136.

May-Collado, L.J., & Wartzok, D. (2008). A Comparison of Bottlenose Dolphin Whistles in the Atlantic Ocean: Factors Promoting Whistle Variation. *Journal of Mammalogy*, 89 (5), 1229–1240.

Matthews, J.N., Rendell, L.E., Gordon, J.C.D., & MacDonald, D.W. (1999). A review of frequency and time parameters of cetacean tonal calls. *Bioacoustics*, 10 (1), 47–71.

McCowan, B. (1995). A new quantitative technique for categorizing whistles using simulated signals and whistles from captive bottlenose dolphins (Delphinidae, *Tursiops truncatus*). *Ethology, 100*,177- 193.

McCowan, B., & Reiss, D. (2001). The fallacy of "signature whistles" in bottlenose dolphins: a comparative perspective of "signature information" in animal vocalizations. *Animal Behaviour,* 62 (6)*,* 1151–1162.

McKenna, M. F., Cranford, T. W., Berta, A., & Pyenson, N. D. (2011). Morphology of the odontocete melon and its implications for acoustic function. *Marine Mammal Science,* 28(4), 690–713.

Miller, G.S. (1923). The telescoping of the cetacean skull (with eight plates). *Smithsonian Miscellaneous Collections,* 76 (5), 1-70.

Mooney, T.A., Yamato, M., & Branstetter, B.K. (2012). Hearing in Cetaceans: From Natural History to Experimental Biology. *Advances in Marine Biology, 63,* 197–246.

Morisaka, T., Shinohara M., Nakahara, F., & Akamatsu, T. (2005). Effects of ambient noise on the whistles of Indo-Pacific bottlenose dolphin populations. *Journal of Mammalogy*, *86*, 541–546.

Morisaka, T. (2012). Evolution of Communication Sounds in Odontocetes: A Review. *International Journal of Comparative Psychology, 25*, 1-20.

Norris, K.S. (1968) The evolution of acoustic mechanisms in odontocete cetaceans. In: E.T. Drake (Eds), *Evolution and Environment* (pp. 297-324). New Haven: Yale University Press.

Oswald, J.N., Barlow, J., & Norris, T.F. (2003). Acoustic identification of nine delphinid species in the Eastern Tropical Pacific Ocean. *Marine Mammal Science*, 19 (1), 20–37.

Pavan, G., & Borsani, J.F. (1997). Bioacoustic research on cetaceans in the Mediterranean Sea. *Marine and Freshwater Behaviour and Physiology*, 30 (2), 99-123.

Palomares, M.L.D., & Pauly, D. (Eds) (2019). SeaLifeBase. *Delphinus delphis Linnaeus, 1758*. Available at: https://www.sealifebase.ca/summary/Delphinus-delphis.html

Papale, E., Azzolin, M., Cascão, I., Gannier, A., Lammers, M.O., Martin, V.M., Silva, M.A., & Giacoma, C. (2014). Macro- and micro-geographic variation of short-beaked common dolphin's whistles in the Mediterranean Sea and Atlantic Ocean. *Ethology Ecology & Evolution*, 26 (4), 392-404.

Papale E., Gamba, M., Perez-Gil, M., Martin, V.M., Giacoma, C. (2015). Dolphins adjust species-specific frequency parameters to compensate for increasing background noise. *Plos One*, 10 (4), e0121711.

Papale, E., Perez-Gil, M., Castrillon, J., Perez-Gil, E., Ruiz, L., Servidio, A., Tejedor, M., Giacoma, C., & Martín, V. (2016). Context specificity of Atlantic spotted dolphin acoustic signals in the Canary Islands. *Ethology Ecology & Evolution,* 29(4), 311–329.

Quérouil, S., Silva, M.A., Magalhães, S., Seabra, M.I., Machete, M.A., & Santos, R.S. (2008). Why do dolphins form mixed-species associations in the Azores? *Ethology*, 114 (12), 1183–94.

Quiñones-Lebrón, S.G., & May-Collado, L.J. (2011). Factors determining whistle emission rate in bottlenose dolphins of Bocas del Toro, Panama. *The Journal of the Acoustical Society of America, 129*, 2671.

Randic, S., Connor, R.C., Sherwin, W.B., & Krutzen, M. (2012). A novel mammalian social structure in Indo-Pacific bottlenose dolphins (*Tursiops* sp.): complex male alliances in an open social network. *Proceedings of the Royal Society B: Biological Sciences,* 279, 3083–3090.

Rendell, L.E., Matthews, J.N., Gill, A., Gordon, J.C.D., & Macdonald, D.W. (1999). Quantitative analysis of tonal calls from five odontocete species, examining interspecific and intraspecific variation. *Journal of Zoology*, 249 (4), 403–410.

Riesch, R., Ford, J.K.B., & Thomsen, F. (2006). Stability and group specificity of stereotyped whistles in resident killer whales, *Orcinus orca*, off British Columbia. *Animal Behavior*, *71*, 79–91.

Ridgway, S.H., & Au, W.W.L. (2009). Hearing and Echolocation in Dolphins. *Encyclopedia of Neuroscience,* 1031–1039.

Rossi-Santos, M.R., & Podos, J. (2006). Latitudinal variation in whistle structure of the estuarine dolphin *Sotalia fluviatilis*. *Behaviour, 143*, 347–364.

Ruxton, G.D., & Schaefer, H.M. (2011). Resolving current disagreements and ambiguities in the terminology of animal communication. *Journal of Evolutionary Biology,* 24(12), 2574–2585.

Sætre, G.P., Moum, T., Bureš, S., Král, M., Adamjan, M., & Moreno, J. (1997). A sexually selected character displacement in flycatchers reinforces premating isolation. *Nature*, 387 (6633), 589–592.

Sayigh, L.S. (2013). Cetacean Acoustic Communication. *Biocommunication of Animals,* 275–297.

Scarpaci, C., Bigger, S.W., Corkeron, P.J., & Nugegoda, D. (2000). Bottlenose dolphins (*Tursiops truncatus*) increase whistling in the presence of "swim-with-dolphin" tour operations. *Journal of Cetacean Research Manaement*, 2 (3), 183–185.

Silva, M.A., Prieto, R., Magalhães, S., Cabecinhas, R., Cruz, A., Gonçalves, J.M., & Santos, R.S. (2003). Occurrence and distribution of cetaceans in the waters around the Azores (Portugal), summer and autumn 1999-2000. *Aquatic Mammals*, 29 (1), 77-83.

Silva, M.A., Prieto, R., Cascão, I., Seabra, M.I., Machete, M., Baumgartner, M.F., & Santos, R.S. (2013). Spatial and temporal distribution of cetaceans in the mid-Atlantic waters around the Azores. *Marine Biology Research*, 10 (2), 123–137.

Smolker, R.A., Mann, J., & Smuts, B.B. (1993). Use of signature whistles during separations and reunions by wild bottlenose dolphin mothers and infants. *Behavioral Ecology and Sociobiology,* 33 (6), 393-402.

Steiner, W.W. (1981). Species-specific differences in pure tonal whistle vocalizations of five western North Atlantic dolphin species. *Behavioral Ecology and Sociobiology*, *9*, 241–246.

Stockin, K.A., Lusseau, D., Binedell, V., Wiseman, N., & Orams, M.B. (2008). Tourism affects the behavioural budget of the common dolphin *Delphinus* sp. in the Hauraki Gulf, New Zealand. *Marine Ecology Progress Series, 355,* 287–295.

Van Parijs, S.M., Smith, J., & Corkeron, P.J. (2002). Using calls to estimate the abundance of inshore dolphins: A case study with Pacific humpback dolphins *Sousa chinensis*. *Journal of Applied Ecology*, *39*, 853– 864.

Wartzok, D., & Ketten, D.R. (2013). Marine Mammal Sensory Systems. In J.E. Reynolds & S.A. Rommel (Eds), *Biology of Marine Mammals* (pp. 588). Washington D.C.: Smithsonian Institution.

Weiß, B.J., Hübscher, C., & Lüdmann, T. (2015). The tectonic evolution of the southeastern Terceira Rift/São Miguel region (Azores). *Tectonophysics, 654,* 75–95.

Wells, R.S., McHugh, K.A., Douglas, D.C., Shippee, S., McCabe, E.B., Barros, N.B., & Phillips, G.T. (2013). Evaluation of Potential Protective Factors Against Metabolic Syndrome in Bottlenose Dolphins: Feeding and Activity Patterns of Dolphins in Sarasota Bay, Florida. *Frontiers in Endocrinology, 4,* 139.

Wells, R.S., & Scott, M.D. (1990). Estimating bottlenose dolphin population parameters from individual identification and capture-release techniques. In: P.S. Hammond et al. (Eds.), *Individual recognition of cetaceans: use of photo-identification and other techniques to estimate population parameters. Incorporating the Proceedings of the symposium and workshop on individual recognition and the estimation of cetacean population parameters. Report of the International Whaling Commission,* Spec. Issue (12), 407-415.

Wood, F.G., Jr. (1953). Underwater sound production and concurrent behaviour of captive porpoises, *Tursiops truncatus* and *Stenella plagiodon*. *Bulletin of Marine Science of the Gulf and Caribbean*, *3*, 120-133.

Annex 1

State of the Art

The sound energy is propagated in the water with greater efficiency than nearly every energy form. Underwater sound can be used for several functions by marine animals such as navigation, communication, and detection. Thus, the most efficient way for odontocetes to carry out these functions is through the utilization of acoustics in a passive (hearing) or active (echolocation) way (Au & Hastings, 2008). Several theories already exist to explain how sounds are produced by odontocetes (Cranford et al., 2011; Madsen et al., 2013; McKenna et al., 2011).

To realize how cetaceans use sound it is fundamental to understand with which frequency range and how well an organism is able to hear. There are also other characteristics of the hearing system that are relevant, for example, the directional hearing, the discrimination of frequency and elaborated sounds like burst-pulsed sounds (Au & Hastings, 2008). Understanding these differences in auditory characteristics is fundamental to comprehend the meaning that individuals attribute to distinct sounds and how they use it (Au & Hastings, 2008).

There are certain sounds, particularly the ones emitted by predators and conspecifics, that have a unique meaning for marine organisms. Sounds emitted by conspecifics transmit a significant amount of information that varies from the alert of danger and threats in the surrounding to the producer's emotional condition (Au & Hastings, 2008; Luís, Couchinho & dos Santos, 2016). For example, whistles are narrowband sounds that can transmit an individual's behavioral condition and are important to keep social cohesion inside the groups (Azzolin et al., 2014).

Besides whistles being one of the most studied sounds in the dolphins' repertoire, its characteristics such as frequency, duration and number of inflection points have been studied and documented over the years in different species and regions (Amorim et al., 2019; Baron et al., 2008; Rendell et al., 1999; Steiner, 1981). These studies have allowed the whistles characterization of several delphinid species and as a result, the comparison of the whistle acoustic parameters between pods and populations.

1.1 A note of sound reception and production in Odontocetes

Cetaceans have an auditory system so completely adapted to the aquatic environment that they might no longer be capable of interpreting or sensing airborne signals. Nevertheless, they share ancestral hearing elements with terrestrial mammals but they had to develop morphological specializations to be able to hear underwater (Ketten, 2000). The skull of the cetaceans has been altered through a process called "Telescoping" (Miller, 1923). As described by Ketten (2000) in this process the anterior cranial structures were pushed up and back, which in turn caused one bone to slide over the other, thus modifying every aspect of the auditory periphery. These modifications occurred not only in the middle and inner ear capsules, that were fused but also in the exterior auditory channels and the pinnae which were lost or reduced (Ketten, 2000). Furthermore, Wartzok & Ketten (2013) have documented that most toothed whales have no significant bone connection between the ears and skull. However, their ears are hanging by bindings in a foam-filled cavity out of the skull, which is relevant for echolocation since they are acoustically separated from the bone conduction. They have also developed adaptations to prevent pressure-related lesions, such as some thin-walled and bony air chambers (Wartzok & Ketten, 2013).

The reception of sounds on dolphins (toothed cetaceans of the family Delphinidae), first suggested by Norris (1968), is carried out through the lower jaw which is considered to be the receiver and conductor of sound to the inner ear of these animals (Figure 1.1) (Sayigh, 2013). Through this theory, it has been demonstrated that sounds are transmitted by their entrance at the narrow posterior extremity of the mandible towards the mandibular fat body and then into the middle ear and inner ear (Ridgway & Au, 2009). In this way, sounds are then transmitted through the auditory nerve to the brain's auditory centers (Au, 2017).

Unlike terrestrial mammals, the production of sounds in dolphins is not in the larynx but inside the skull in a nasal structure known as air sacs (Madsen et al., 2013). A study by McKenna et al. (2011) described that in this sophisticated nasal system, sounds are generated inside the blowhole through the forced circulation of air between the phonic lips, resulting in the vibrations of an adjacent pair of posterior and anterior dorsal bursae (Figure 1.1). These sound vibrations are reflected in the air sacs, anterior region of the skull, where they are then directed through the melon which is a structure formed by connective and adipose tissue (McKenna et al., 2011). The major function of this structure is to focus the energy of sound,

particularly high-frequency sounds and spread it from the forehead to a specific object (McKenna et al., 2011).

It is currently supported that phonic lip pairs can act simultaneously or independently with the left and smaller pair being associated with the production of lower frequencies while the greater right pair may be the source of higher frequencies (Madsen et al., 2013). Despite the need for more research to support this theory, it has been shown that there are two sources of sound production in dolphins (Cranford et al., 2011).
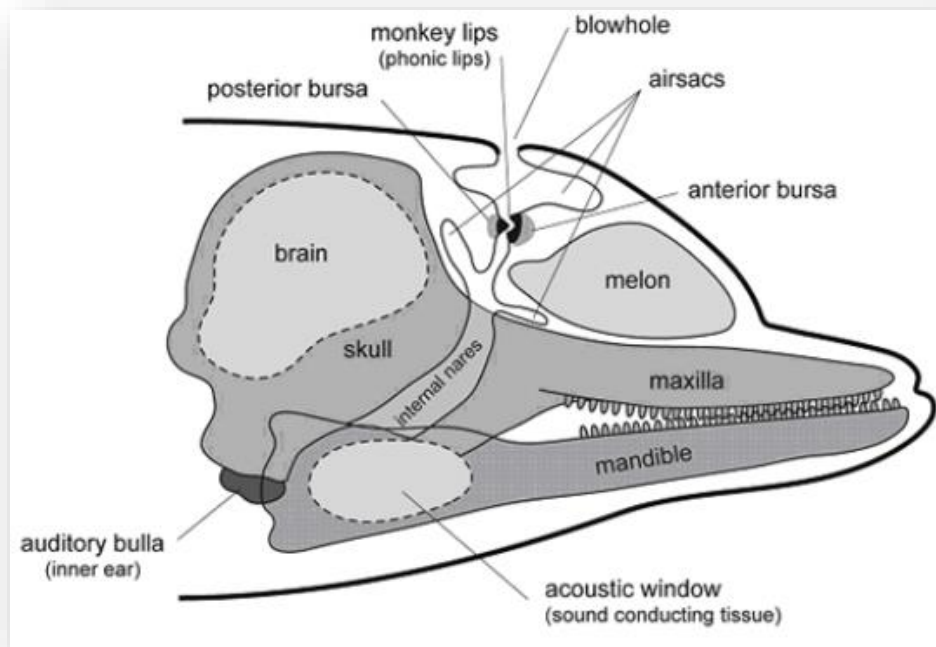

Figure 1- Diagram of a dolphin's head anatomy. Image extracted from:https://matthewhardcastle.wordpress.com/2012/09/30/hello-world/

1.2 <u>Dolphin Vocalizations</u>

As mentioned by Morisaka (2012), due to the development of technology, devices have been created that can capture toothed cetaceans sounds more easily and cheaply. As a result, in the last decade, more studies have started to report the acoustic features of different species.

There has been an interest in the interpretation of the dolphin echolocation system which is based on clicks generated on-axis (Amorim et al., 2019). The clicks that are obtained in passive acoustic monitoring might not precisely portray the whole diversity of clicks. It is considered that there are acoustic effects on clicks due to the angle between the longitudinal axis of the individual and the hydrophone. In this way, the variation observed between the clicks that are recorded and the accurate spectral representations of stable characteristics are of relevance for dolphins (Amorim et al., 2019).

Many species utilize clicks in the echolocation process, which is considered to be the case for all odontocetes. (Morisaka, 2012). In echolocation, the dolphins emit clicks on a directional beam and hear the reflected echoes of the targets that are present over a close-range (Gridley et al., 2015). In this process the number, duration and repetition rate of the emitted click trains vary according to their purpose, for example, when the aim is fine discrimination the interval between clicks will be shorter (Au, 2017; Gridley et al., 2015). Delphinids can locate objects in a three-dimensional space due to the reception beam pattern of the click trains, which in turn allows them to create an inner "picture" of their environment (Au & Lammers, 2007).

Several sounds have been known for a long time from the dolphins' repertoire, which are not the subject of so many studies, such as burst-pulsed sounds (Gridley et al., 2015). There is a great variety in these types of sounds both in the spectral components and in their duration, which may be related to the physiological and/or emotional state of the animal that produces the sounds (dos Santos, 1998). In some species of dolphins, the types of burst-pulsed sounds are known, for example, for the bottlenose dolphins (*Tursiops truncatus*). It was documented by Luís, Couchinho & dos Santos (2016) and Herzing, (1996) that these dolphins emit "squeacks" and "squawks" in aggressive and courtship behaviors, produce "barks" and "screams" in aggressive interactions, in fear and alarm situations they emit "cracks" or "pops" and in behaviors of discipline and courting they produce "genital buzzes". Besides this species, Atlantic spotted dolphins (*Stenella frontalis*) also emit "screams", "barks", "squawks" and "buzzes" in the same situations (Herzing, 1996; Papale et al., 2016).

On the other hand, whistles are the most frequently studied signs of communication in delphinids' repertories due to their multiple characteristics that are easy to measure (Azevedo et al., 2010; Gridley et al., 2015). These sounds might transmit information about population, individual and species identity, as well as the behavioral condition of the caller. It is thought that whistles are important for keeping social cohesion inside pods and for establishing the organization of groups (Azzolin et al., 2014).

Of the whistles produced by wild animals, about 52% are signature whistles that are utilized to facilitate communication between the groups, transmit identity information and approach conspecifics (Gridley et al., 2015). As referred by Kriesell et al. (2014) during the first period of life the calves learn the signature whistle which is influenced by the hearing experience. Calves' whistles are modeled by the sounds they listen to, and the majority acquires different signature whistles of their closest relatives. Nevertheless, a few male offspring may have signature whistles similar to their mothers and when the pattern of frequency modulation is formed, it may remain stable for decades. However, when alliances are formed, the types of signature whistles of males may get more alike with time, whereas identity information of the individual is maintained (Kriesell et al., 2014).

1.3 Acoustic discrimination of delphinids

Since 1981, the variability in the parameters of whistle frequency, modulation, and duration between different Odontocetes species has been studied (Amorim et al., 2019; Baron et al., 2008; Gannier et al., 2010, 2019; Oswald, Barlow & Norris, 2003; Rendell et al., 1999; Steiner, 1981; Ding, Wursig & Evans, 1995b). It was evidenced that more than just one element could explain the variability observed in the characteristics of a whistle, for example by Steiner (1981), who realized that morphology and taxonomic relationships could be related to the variations in the whistle features between species. This author also noted that the sympatric species had greater differences in the structure of the whistles than the allopatric species (Steiner, 1981). These results are in some way consistent with the ''species recognition hypothesis'', which claims that the sound structure of closely related species has developed in order to minimize hybridization (May-Collado & Wartzok, 2008; Sætre et al., 1997). In another research, it was assumed that whistle production capacity was limited by body size (Ding, 1993) while in a different study conducted by, Ding, Wursig & Evans, (1995b) was described a negative relationship among whistle frequency parameters and the size of the body.

Similarities can develop in the whistles within a dolphin pod due to the mimicking of sounds (Baron et al., 2008). These resemblances are used for interaction and recognition among individuals in the group. However, there may be intraspecific variations of whistles that evolve due to the segregation of individuals from the same species (Baron et al., 2008).

Despite the low intraspecific variation coefficient, populations differ sufficiently in frequency parameters to enable discrimination between them. This discrimination occurs both between macrogeographic populations, which are widely segregated, and among neighboring groups, namely microgeographic populations (May-Collado & Wartzok, 2008).

Differences in whistles as a result of social isolation and geographical segregation among populations have already been observed in several species such as bottlenose dolphins (Azzolin et al., 2014; Baron et al., 2008; Ding, Würsig & Evans, 1995a; Jones & Sayigh, 2002; May-Collado & Wartzok, 2008), Atlantic spotted dolphins (Azevedo et al., 2010; Baron et al., 2008; Gannier et al., 2019), short-beaked common dolphins (Ansmann et al., 2007; Azzolin et al., 2014; Gannier et al., 2019; Papale et al., 2014), killer whales, *Orcinus orca* (Deecke, Ford & Spong, 2000; Ford, 1989; Riesch, Ford & Thomsen, 2006), short-finned pilot whales, *Globicephala macrorhynchus* (Baron et al., 2008; Rendell et al., 1999) and long-finned pilot whales, *Globicephala melas* (Azzolin et al., 2014; Baron et al., 2008; Rendell et al., 1999).

Besides the level of isolation and spatial segregation, some studies have obtained geographical variations in whistles due to the acoustic features of the environment, for example, environmental noise (Ansmann et al., 2007; Morisaka et al., 2005). The increase in anthropogenic noise due to the proximity of vessels may cause temporary changes in whistle characteristics, such as maximum frequency or duration (Gannier et al., 2019; Luís, Couchinho & dos Santos, 2014). This type of reversible changes, also called repertory elasticity, can as well influence the results of the whistle classification for several regional species when they are recorded in different noisy contexts (Gannier et al., 2019). Changes in whistle characteristics might also occur due to interaction with other dolphin species and because of the group structure. In the presence of calves, the recording of the repertoire can be influenced, as they tend to make less modulated and longer whistles (Gannier et al., 2019).

Duration, number of inflection points and frequency are the acoustic parameters that have been used to characterize whistles of dolphin species, making it possible to compare populations and groups (Azevedo et al., 2007). The acoustic parameters of whistles from species such as bottlenose dolphins, common dolphins and Atlantic spotted dolphins have already been studied and compared in different regions (Baron et al., 2008; Gannier et al., 2019; May-Collado & Wartzok, 2008). For all three species the whistle variables were studied in the Eastern Atlantic Ocean (Gannier et al., 2019; May-Collado & Wartzok, 2008).

Previous studies have shown that these three species present significantly different whistle repertoires in distinct areas (Baron et al., 2008; Ding, Würsig & Evans, 1995a; May-Collado & Wartzok, 2008; Papale et al., 2014). It was demonstrated by Baron et al., (2008) that *Tursiops truncatus* and *Stenella frontalis* had evident inter-regional distinctions in many of the whistle variables around the Western Atlantic and the Gulf of Mexico. For the common dolphins of the Eastern Atlantic (where data from the Azores was included) and the Mediterranean Sea, differences were also found in the frequency variables of whistles (Papale et al., 2014).

Annex 2
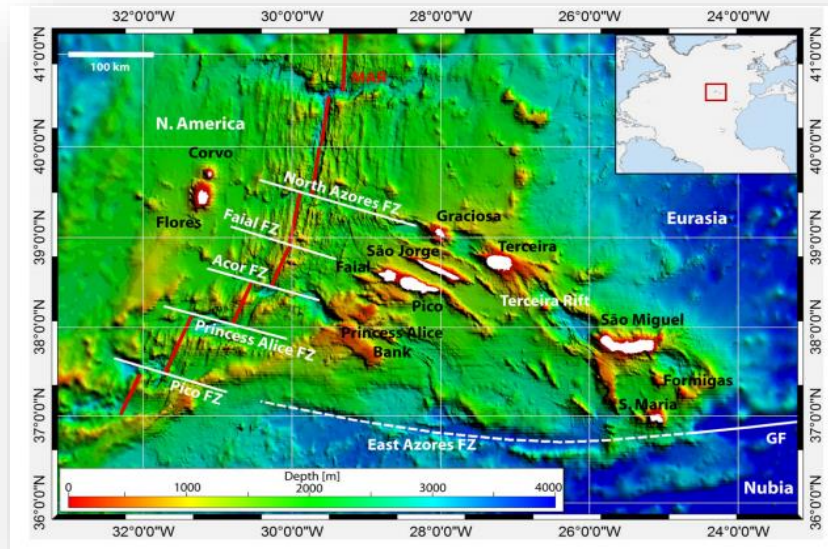
Figure 2- Map of the Azores Archipelago and respective structures: MAR- Mid-Atlantic Ridge; GF- Azores-Gibraltar fracture zone or Gloria Fault; FZ- Fracture Zone (Weiß, Hübscher & Lüdmann, 2015).

Annex 3

Table 1- Behavioral and acoustic record sheet used for the different dolphin species.

| Nº da Saída | | Hora Inicio | | Maré Baixa | | Beaufort | | | | |
| Data | | Hora Fim | | Maré Alta | | Tipologia Embarcação | | | | |

Espécie(s)	Hora	Latitude (N)	Longitude (W)	Profundidade (m)	Direção Dominante	Tamanho Grupo	*Atividade Dominante	Velocidade de deslocação	Código áudio	Obs.

Annex 4

Table 2- Result of Kruskal-Wallis test for the emission rates among the three species of delphinids. The significance level is 0.05.

	Hipótese nula	Teste	Sig.	Decisão
1	A distribuição de Whistle rate é a mesma entre as categorias de Species.	Teste de Kruskal-Wallis de Amostras Independentes	,000	Rejeitar a hipótese nula.

Table 3- Results of the Dunn *post-hoc* multiple comparisons test for the emission rates among the three dolphin species. 1,000 - *Tursiops truncatus*; 2,000 - *Stenella frontalis*; 3,000 – *Delphinus delphis*. The significance level is 0.05.

Amostra1-Amostra2	Estatística de Teste	Std. Erro	Erro Estatística de Teste	Sig.	Sig. Aj.
3,000-2,000	16,038	4,618	3,473	,001	,002
3,000-1,000	23,893	5,157	4,633	,000	,000
2,000-1,000	7,855	4,834	1,625	,104	,312

Table 4- Mann-Whitney U test results for the emission rates of *Stenella frontalis* between the two activity patterns. The significance level is 0.05.

N total	19
U de Mann-Whitney	10.000
Wilcoxon W	146.000
Estatística de teste	10.000
Erro padrão	8.940
Estatística de Teste Padronizado	-1.566
Sig. Assintótica (teste de dois lados)	.117
Sig. Exata (teste de dois lados)	.138

Table 5- Results of the Mann-Whitney U test for the emission rates between *Delphinus delphis* and *Stenella frontalis* in travelling. The significance level is 0.05.

N total	29
U de Mann-Whitney	28.000
Wilcoxon W	119.000
Estatística de teste	28.000
Erro padrão	22.643
Estatística de Teste Padronizado	-3.356
Sig. Assintótica (teste de dois lados)	.001
Sig. Exata (teste de dois lados)	.000

Table 6- Results of the Mann-Whitney U test for the emission rates between *Tursiops truncatus* and *Stenella frontalis* in socializing. The significance level is 0.05.

N total	14
U de Mann-Whitney	18.000
Wilcoxon W	24.000
Estatística de teste	18.000
Erro padrão	6.423
Estatística de Teste Padronizado	.234
Sig. Assintótica (teste de dois lados)	.815
Sig. Exata (teste de dois lados)	.885

Table 7- Spearman's correlation coefficient results between the number of whistles in each recording and the group sizes for all species. The significance level is 0.05.

			Number of whistles	Number of dolphins
rô de Spearman	Number of whistles	Coeficiente de Correlação	1,000	,338*
		Sig. (2 extremidades)	.	,023
		N	45	45
	Number of dolphins	Coeficiente de Correlação	,338*	1,000
		Sig. (2 extremidades)	,023	.
		N	45	45

Table 8- Results of the Spearman's correlation coefficient between the number of whistles in each recording and the group sizes for *Stenella frontalis*. The significance level is 0.05.

			Number of whistles	Number of dolphins
rô de Spearman	Number of whistles	Coeficiente de Correlação	1,000	,266
		Sig. (2 extremidades)	.	,272
		N	19	19
	Number of dolphins	Coeficiente de Correlação	,266	1,000
		Sig. (2 extremidades)	,272	.
		N	19	19

Table 9- Spearman's correlation coefficient results between the number of whistles in each recording and the group sizes for *Delphinus delphis*. The significance level is 0.05.

			Number of whistles	Number of dolphins
rô de Spearman	Number of whistles	Coeficiente de Correlação	1,000	-,381
		Sig. (2 extremidades)	.	,179
		N	14	14
	Number of dolphins	Coeficiente de Correlação	-,381	1,000
		Sig. (2 extremidades)	,179	.
		N	14	14

Table 10- Results of the Spearman's correlation coefficient between the number of whistles in each recording and the group sizes for *Tursiops truncatus*. The significance level is 0.05.

			Number of whistles	Number of dolphins
rô de Spearman	Number of whistles	Coeficiente de Correlação	1,000	,338
		Sig. (2 extremidades)	.	,283
		N	12	12
	Number of dolphins	Coeficiente de Correlação	,338	1,000
		Sig. (2 extremidades)	,283	.
		N	12	12

Annex 5

Table 11- Kruskal-Wallis test results for each acoustic parameter of the whistles between the three delphinid species. The significance level is 0.05.

	Duration	LowFreq	HighFreq	Frequencyrange	CenterFreq	PeakFreq	InitialFrequency	EndFrequency	Inflectionpoints
Qui-quadrado	7,446	19,684	45,200	73,283	15,309	11,560	18,194	18,590	22,275
df	2	2	2	2	2	2	2	2	2
Significância Sig.	,024	,000	,000	,000	,000	,003	,000	,000	,000

Table 12- Results of Dunn *post-hoc* multiple comparison test for the whistle duration between the three species of dolphins. 1,000 - *Tursiops truncatus*; 2,000 - *Stenella frontalis*; 3,000 – *Delphinus delphis*. The significance level is 0.05.

Amostra1-Amostra2	Estatística de Teste	Std. Erro	Erro Estatística de Teste	Sig.	Sig. Aj.
1,000-3,000	-28,094	28,918	-,971	,331	,994
1,000-2,000	-36,628	13,459	-2,721	,006	,019
3,000-2,000	8,535	28,176	,303	,762	1,000

Table 13- Results of Dunn *post-hoc* multiple comparison test between the whistle initial frequency values of the three dolphin species. 1,000 - *Tursiops truncatus*; 2,000 - *Stenella frontalis*; 3,000 – *Delphinus delphis*. The significance level is 0.05.

Amostra1-Amostra2	Estatística de Teste	Std. Erro	Erro Estatística de Teste	Sig.	Sig. Aj.
2,000-1,000	28,476	13,459	2,116	,034	,103
2,000-3,000	-113,498	28,176	-4,028	,000	,000
1,000-3,000	-85,022	28,919	-2,940	,003	,010

Table 14- Results of Dunn *post-hoc* multiple comparison test between the whistle final frequency values of the three dolphin species. 1,000 - *Tursiops truncatus*; 2,000 - *Stenella frontalis*; 3,000 – *Delphinus delphis*. The significance level is 0.05.

Amostra1-Amostra2	Estatística de Teste	Std. Erro	Erro Estatística de Teste	Sig.	Sig. Aj.
1,000-2,000	-54,619	13,459	-4,058	,000	,000
1,000-3,000	-74,047	28,919	-2,561	,010	,031
2,000-3,000	-19,428	28,176	-,690	,490	1,000

Table 15- Results of Dunn *post-hoc* multiple comparison test between the whistle minimum frequency values of the three dolphin species. 1,000 - *Tursiops truncatus*; 2,000 - *Stenella frontalis*; 3,000 – *Delphinus delphis*. The significance level is 0.05.

Amostra1-Amostra2	Estatística de Teste	Std. Erro	Erro Estatística de Teste	Sig.	Sig. Aj.
2,000-1,000	10,211	13,459	,759	,448	1,000
2,000-3,000	-124,998	28,176	-4,436	,000	,000
1,000-3,000	-114,788	28,919	-3,969	,000	,000

Table 16- Results of Dunn *post-hoc* multiple comparison test between the whistle maximum frequency values of the three dolphin species. 1,000 - *Tursiops truncatus*; 2,000 - *Stenella frontalis*; 3,000 – *Delphinus delphis*. The significance level is 0.05.

Amostra1-Amostra2	Estatística de Teste	Std. Erro	Erro Estatística de Teste	Sig.	Sig. Aj.
1,000-3,000	-23,246	28,919	-,804	,421	1,000
1,000-2,000	-89,161	13,459	-6,625	,000	,000
3,000-2,000	65,915	28,176	2,339	,019	,058

Table 17- Results of Dunn *post-hoc* multiple comparison test between the whistle frequency range of the three delphinid species. 1,000 - *Tursiops truncatus*; 2,000 - *Stenella frontalis*; 3,000 – *Delphinus delphis*. The significance level is 0.05.

Amostra1-Amostra2	Estatística de Teste	Std. Erro	Erro Estatística de Teste	Sig.	Sig. Aj.
3,000-1,000	49,956	28,919	1,727	,084	,252
3,000-2,000	151,373	28,176	5,372	,000	,000
1,000-2,000	-101,416	13,459	-7,535	,000	,000

Table 18- Results of Dunn *post-hoc* multiple comparison test between the whistle center frequency of the three dolphin species. 1,000 - *Tursiops truncatus*; 2,000 - *Stenella frontalis*; 3,000 – *Delphinus delphis*. The significance level is 0.05.

Amostra1-Amostra2	Estatística de Teste	Std. Erro	Erro Estatística de Teste	Sig.	Sig. Aj.
1,000-2,000	-27,525	13,455	-2,046	,041	,122
1,000-3,000	-109,341	28,909	-3,782	,000	,000
2,000-3,000	-81,815	28,167	-2,905	,004	,011

Table 19- Results of Dunn *post-hoc* multiple comparison test between the whistle peak frequency values of the three delphinid species. 1,000 - *Tursiops truncatus*; 2,000 - *Stenella frontalis*; 3,000 – *Delphinus delphis*. The significance level is 0.05.

Amostra1-Amostra2	Estatística de Teste	Std. Erro	Erro Estatística de Teste	Sig.	Sig. Aj.
1,000-2,000	-8,644	13,454	-,642	,521	1,000
1,000-3,000	-97,788	28,909	-3,383	,001	,002
2,000-3,000	-89,144	28,166	-3,165	,002	,005

Table 20- Results of Dunn *post-hoc* multiple comparison test between the number of inflection points in the whistles of the three dolphin species. 1,000 - *Tursiops truncatus*; 2,000 - *Stenella frontalis*; 3,000 – *Delphinus delphis*. The significance level is 0.05.

Amostra1-Amostra2	Estatística de Teste	Std. Erro	Erro Estatística de Teste	Sig.	Sig. Aj.
3,000-1,000	11,647	27,530	,423	,672	1,000
3,000-2,000	67,782	26,823	2,527	,012	,035
1,000-2,000	-56,135	12,813	-4,381	,000	,000

Annex 6

Table 21- Pearson's chi-squared test results between the whistle frequency contours and the three dolphin species.

	Valor	df	Significância Assintótica (Bilateral)
Qui-quadrado de Pearson	223,690[a]	10	,000
Razão de verossimilhança	227,082	10	,000
Associação Linear por Linear	,530	1	,467
N de Casos Válidos	1198		

Table 22- Results of the Pearson's chi-squared test between the whistle frequency contours and the three species of delphinids. Whistle shape: 1- Upsweep; 2- Sinusoidal; 3- Convex; 4- Downsweep; 5- Concave; 6- Constant. Species: 1- *Tursiops truncatus;* 2- *Stenella frontalis;* 3- *Delphinus delphis.*

			Whistle Shape						Total
			1	2	3	4	5	6	
Species	1	Contagem	57	80	96	120	40	48	441
		Contagem Esperada	50,8	106,8	117,1	57,4	73,6	35,3	441,0
		% em Species	12,9%	18,1%	21,8%	27,2%	9,1%	10,9%	100,0%
		% em Whistle Shape	41,3%	27,6%	30,2%	76,9%	20,0%	50,0%	36,8%
		% do Total	4,8%	6,7%	8,0%	10,0%	3,3%	4,0%	36,8%
		Resíduos ajustados	1,2	-3,7	-2,9	11,1	-5,4	2,8	
	2	Contagem	72	200	216	24	135	30	677
		Contagem Esperada	78,0	163,9	179,7	88,2	113,0	54,3	677,0
		% em Species	10,6%	29,5%	31,9%	3,5%	19,9%	4,4%	100,0%
		% em Whistle Shape	52,2%	69,0%	67,9%	15,4%	67,5%	31,3%	56,5%
		% do Total	6,0%	16,7%	18,0%	2,0%	11,3%	2,5%	56,5%
		Resíduos ajustados	-1,1	4,9	4,8	-11,1	3,4	-5,2	
	3	Contagem	9	10	6	12	25	18	80
		Contagem Esperada	9,2	19,4	21,2	10,4	13,4	6,4	80,0
		% em Species	11,3%	12,5%	7,5%	15,0%	31,3%	22,5%	100,0%
		% em Whistle Shape	6,5%	3,4%	1,9%	7,7%	12,5%	18,8%	6,7%
		% do Total	0,8%	0,8%	0,5%	1,0%	2,1%	1,5%	6,7%
		Resíduos ajustados	-,1	-2,5	-4,0	,5	3,6	4,9	
Total		Contagem	138	290	318	156	200	96	1198
		Contagem Esperada	138,0	290,0	318,0	156,0	200,0	96,0	1198,0
		% em Species	11,5%	24,2%	26,5%	13,0%	16,7%	8,0%	100,0%
		% em Whistle Shape	100,0%	100,0%	100,0%	100,0%	100,0%	100,0%	100,0%
		% do Total	11,5%	24,2%	26,5%	13,0%	16,7%	8,0%	100,0%