

Whistle structure and geographical variations in three delphinid species recorded in eastern Algarve Coast

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Declaração de autoria de trabalho

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Whistle structure and geographical variations in three delphinid species recorded in eastern Algarve coast

Declaro ser a autora deste trabalho, que é original e inédito. Autores e trabalhos consultados estão devidamente citados no texto e constam da listagem de referências incluída.

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Susana Gil

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Susana Gil

Abstract

This study aims to characterize and compare the whistle repertoires of three odontocete species, *Delphinus delphis* (short-beaked common dolphin), *Tursiops truncatus* (common bottlenose dolphin), and *Pseudorca crassidens* (false killer whale), in the coast of Faro. This knowledge is essential for their monitoring and conservation, particularly for this region. Especially because this is an area with high anthropogenic pressure and limited prior research.

These species were chosen due to their presence in the region and high vocal activity, with special emphasis on the poorly studied false killer whale (*Pseudorca crassidens*), recently observed using the area as a seasonal feeding ground.

Recordings were obtained between April 2022 and June 2024 using an opportunistic platform during dolphin and whale watching surveys. A total of 36 acoustic events were analyzed. Visual analysis and manual whistle extraction of ten acoustic parameters were conducted using Raven Pro: beginning, end, minimum, and maximum frequency, frequency range, duration, initial and final slope, number of inflection points, and number of steps. In total 327 whistles were analyzed and used in this study. Whistle contours were also categorized into six standard types according to previous studies (Constant, Upsweep, Downsweep, Convex, Concave and Sine).

Statistical analyses were conducted to describe and categorize the repertoire of the three species. A non-parametric Kruskal-Wallis' test was used to determine overall differences in whistles parameters among species, pairwise with Dunn's test and adjusted with sequential Bonferroni. A non-metric multidimensional scaling (NMDS) was used to visualize acoustic separation between species. The whistles contours were also compared using a correlation matrix. Additionally, a Random Forest model measured the classification performance and the importance of each acoustic variable.

Results showed significant acoustic differences in whistle parameters between species, especially between *Pseudorca crassidens* and the two other

dolphin species. *Delphinus delphis* and *Tursiops truncatus* showed higher variability in frequency and contour type, potentially related to behavioral context or social structure.

To explore potential geographic variation, Faro data was compared with published values from other geographic regions to evaluate possible geographic divergence using mean values of frequency parameters available in literature.

Geographic comparisons revealed that the population of Faro exhibits distinct whistle characteristics when compared to populations from other regions. The results also showed that geographic distance alone does not predict acoustic similarity or divergence. Instead, environmental context plays a key role in shaping dolphin's repertoire, likely driving specific acoustic adaptations.

These findings contribute to baseline knowledge of local cetacean acoustic repertoires and highlight the importance of passive acoustic monitoring for population, behavioral, and conservation studies. This work reinforces the importance of regional characterization to detect alterations related to human activity and environmental change, and it supports future efforts to implement effective mitigation and management strategies for cetacean conservation in southern Portugal.

Keywords: Passive acoustic monitoring; geographic acoustic variation; dolphins whistles; Odontocetes; Algarve, Portugal

Resumo em Português

Compreender o repertório acústico dos cetáceos é essencial para a monitorização e conservação, especialmente em regiões com elevada pressão antropogénica e pouco estudadas. Este estudo caracteriza e compara a estrutura dos assobios de três espécies de odontocetos, *Delphinus delphis* (golfinho-comum), *Tursiops truncatus* (roaz-corvineiro) e *Pseudorca crassidens* (falsa-orca), recolhidos na região de Faro, no sotavento algarvio, Portugal. Estas espécies foram selecionadas devido à sua presença na área e elevada atividade acústica, com especial destaque para *Pseudorca crassidens*, uma espécie pouco estudada em Portugal continental, mas recentemente observada a utilizar a região sazonalmente como área de alimentação.

As gravações foram recolhidas entre abril de 2022 e junho de 2024, utilizando uma plataforma de oportunidade durante saídas de observação de golfinhos e baleias. No total, foram analisados 36 eventos acústicos. A extração manual dos assobios e a análise visual foram realizadas no software Raven Pro, onde foram medidos dez parâmetros acústicos: frequência inicial, final, mínima e máxima, amplitude da frequência, duração, inclinação inicial e final, número de pontos de inflexão e número de quebras. No total, foram analisados 327 assobios. Os contornos dos assobios foram também classificados em seis tipos, segundo estudos anteriores: constante, ascendente, descendente, côncavo, convexo e sinusoidal.

Foram aplicadas análises estatísticas para descrever e categorizar o repertório das três espécies. O teste de Kruskal-Wallis foi utilizado para identificar diferenças nos parâmetros acústicos entre espécies, seguido do teste de Dunn para comparações entre pares, com correção de Bonferroni. NMDS (Non-Metric Multidimensional Scaling) permitiu visualizar a separação acústica entre espécies. Os contornos dos assobios foram também comparados através de uma matriz de correlação. Adicionalmente, foi aplicado um modelo de Random Forest para avaliar a capacidade de classificação e a importância de cada parâmetro acústico.

Para explorar variações geográficas, os dados de Faro foram comparados com valores médios de parâmetros de frequência disponíveis na literatura,

provenientes de outras regiões, com o objetivo de avaliar uma possível divergência acústica regional.

Os resultados revelaram diferenças acústicas significativas entre as espécies, especialmente entre *Pseudorca crassidens* e as duas outras espécies de golfinhos. *Delphinus delphis* e *Tursiops truncatus* apresentaram maior variabilidade nos parâmetros de frequência e nos tipos de contorno, possivelmente relacionada com o contexto comportamental ou estrutura social. As comparações geográficas revelaram que a população de Faro apresenta características acústicas distintas quando comparada com populações de outras regiões. Os resultados mostraram ainda que a distância geográfica, por si só, não é um fator preditivo de semelhança ou divergência acústica. Pelo contrário, o contexto ambiental desempenha um papel fundamental na forma como os golfinhos estruturam o seu repertório, promovendo adaptações acústicas específicas.

Estes resultados contribuem para o conhecimento de base sobre os repertórios acústicos das populações locais de cetáceos e reforçam a importância da monitorização acústica em estudos populacionais, comportamentais e de conservação. Este trabalho sublinha a relevância de uma caracterização regional para detetar alterações relacionadas com a atividade humana e com mudanças ambientais, apoiando esforços futuros na implementação de medidas eficazes de mitigação e gestão para a conservação de cetáceos no sul de Portugal.

Palavras-chave: Monitorização acústica passiva; Variação geográfica; Assobios de golfinhos; Odontocetos; Algarve, Portugal

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List of abbreviations

DD	<i>Delphinus delphis</i> – short beaked common dolphin
FFT	Fast Fourier transform
NMDS	Nonmetric Multidimensional Scaling
NT	Near threatened
PAM	Passive acoustic monitoring
PC	<i>Pseudorca crassidens</i> – False killer whale
RF	Random Forest
SC	<i>Stenella coeruleoalba</i> – Striped dolphin
TT	<i>Tursiops Truncatus</i> – Common bottlenose dolphin
UICN	International Union for Conservation of Nature

Chapter 1

State of the Art

1. Bioacoustics

In recent years, marine bioacoustics has emerged as a prominent interdisciplinary field, attracting significant attention from scientists due to its capacity to explore a diverse range of questions regarding the reception and utilization of sound by various aquatic organisms (Au & Hastings, 2008). Acoustic energy, unlike other forms such as electromagnetic, thermal, or light energy, propagates four to five times faster in water than in air (Lurton, 2002), with low attenuation over distance (Au & Hastings, 2008). This phenomenon highlights the crucial role of acoustics in the underwater environment, where sound serves as the most effective method for animals to perform various functions associated with their life cycles, including communication, navigation, obstacle avoidance, and prey detection (Au & Hastings, 2008). Fundamental parameters of acoustic waves, including intensity, duration and frequency, are key to understanding sound in marine environments (Saheban & Kordrostami, 2021).

Key to this study is passive acoustic monitoring (PAM), which is increasingly utilized by the scientific community to study, survey, and census marine mammals. Cetaceans can be detected acoustically, not only by listening for sounds emitted by the animals (passive acoustic detection) but also by using whale-finding sonar to listen for echoes reflecting from the animal (active acoustic detection) (Zimmer, 2011). PAM is a non-intrusive method that replaces techniques which may involve physical disturbances that negatively impact the animals (Gregoriotti et al., 2021; Zimmer, 2011).

In particular, visual observation of cetaceans can be challenging, as these animals spend most of their lives underwater. PAM offers a highly effective and cost-efficient method to acquire information on different cetacean populations, even in situations where animals are difficult to approach or observe due to

factors such as, inclement weather, reduced visibility, and logistical constraints (Oswald et al., 2007). The study of sound emitted by cetaceans represents a field of research with numerous applications, including the investigation of social interactions, behavioral patterns, and the identification of these animals (Lehnhoff et al., 2025).

Moreover, the specificity of sound signals at the species level is crucial, as it serves as the first requirement for applying acoustic techniques in studying population distribution and abundance (Matthews et al., 1999). This specificity is vital for long-term monitoring and effective conservation actions, particularly for endangered species (Frainer et al., 2023). However, the vocal production structures of different species often exhibit similar morphological adaptations, resulting in overlapping frequency characteristics that may affect the accuracy of species identification (Oswald et al., 2003)

2. Cetaceans

Cetaceans are divided into two groups, Odontocetes (toothed whales) and Mysticetes (baleen whales), and are found in a wide range of ocean habitats (Jefferson et al., 2007). This adaptation is closely tied to their ecological roles and the ways they communicate.

Cetaceans play crucial roles in marine ecosystems, serving as apex predators that influence the life history strategies of their prey (Balance, 2009). Cetaceans also function as umbrella species, meaning that the protection and conservation of these species result in positive effects for many other species (Perrin et al., 2009). However, their populations have faced significant declines. Today, fishing nets are the leading cause of incidental death in cetaceans. Other major threats to these animals include, habitat degradation and loss, environmental contamination, ship strikes, and noise pollution (Richardson et al., 1995). Human-generated sounds have been shown that can severely disrupt cetacean communication and behavior, highlighting the need for continued research and conservation efforts (Jefferson et al., 2007).

Cetacean distribution and social behavior are further shaped by environmental conditions. Some species are confined to shallow depths, while others, like the sperm whale and many beaked whales, can dive thousands of meters. Their geographic distribution varies from cosmopolitan to extremely local, influenced by factors such as major ocean currents, which affect productivity (Jefferson et al., 2007), and promote highly selective adaptations to their environments (Balance, 2009). They are highly gregarious animals, often form aggregations for feeding and protection. Most cetaceans are found in groups, although some species may occur solitarily. Group size correlates with environmental openness and predation pressure, influencing social dynamics (Perrin et al., 2009). Living in a fully aquatic environment, cetaceans have evolved to rely primarily on sound for communication and navigation, adapting their physiological structures for optimal auditory processing (Zimmer, 2011).

2.1. Communication in Cetaceans

Cetaceans developed specialized auditory systems that allow them to perceive a wide range of frequencies. This adaptation emphasizes the critical role of acoustic signals in their social interactions (Frankel, 2009). Communication is particularly complex, characterized by variability that conveys specific information about identity, activity, sex, and age within their social groups (Gregoriotti et al., 2021). Understanding how these sounds are produced is essential.

Odontocetes produce sound in the nasal system. Dolphins pressurize their nasal cavities by muscular action and control airflow past left or right phonic lips, as air passes through these lips, generates vibrations that are transmitted through the melon, a lipid-rich structure that focuses and directs sound into the water (Frankel, 2009). This directional sound emission enhances echolocation and navigation efficiency, while omnidirectional emissions facilitate communication detection (Herzing, 2014). Fine motor controlled by the nasal muscles allows dolphins to produce many sounds and often make different sounds simultaneously (Jones et al., 2019). In parallel, cetaceans have also evolved unique adaptations for sound reception.

Cetaceans lack external ears, and their auditory meatus is thin and partially occluded. In odontocetes, the lower jaw acts as an outer ear, capturing sound

energy and transmitting it to inner ear structures through a fat pad (Nummela, 2009). A recent multipath model suggests that sound may enter through multiple locations, particularly from the ventral side of the head, which appears to facilitate more effective sound transmission (Kuczaj & Winship, 2015). Odontocetes exhibit remarkable hearing abilities, with sensitivity to frequencies from a few hundred Hz to over 100 kHz, with optimal hearing occurring between 20 and 70 kHz (Hanke & Erdsack, 2015). These adaptations support the production of a variety of sound types.

2.2. Type of sounds of cetaceans

Odontocete sounds can be categorized into pulsed sounds (such as clicks and burst-pulsed signals) and narrowband tonal sounds, primarily whistles (Moore, 1997; Oswald et al., 2007). These sound types serve different ecological and social functions.

Clicks facilitate navigation and obstacle detection through echolocation, as dolphins emit these sounds and interpret returning echoes (Moore, 1997; Oswald et al., 2007). Burst-pulsed signals are primarily used during social interactions, characterized by high pulse train repetition rates. Pulsed signals are high frequency signals ranging from 20 kHz to 100 kHz, dependent on species (Dudzinski et al., 2009).

Narrowband tonal sounds, or whistles, are continuous frequency-modulated signals that exhibit diverse contours (Frankel, 2009), and often include harmonic components (Frankel, 2009). Whistles typically range from 5-20 kHz in frequency (Au & Hastings, 2008) with some extending beyond this range (Herzing & Johnson, 2015). Their duration varies from tenths of a second to several seconds (Oswald et al., 2007) and, while not produced by all odontocetes, they are critical for communication - *Physeteridae* and *Phocoenidae* rely solely on pulsed sounds (Au & Hastings, 2008).

Compared to pulsed sounds, whistles can travel longer distances being less directional yet easily localized by cetaceans (Dudzinski et al., 2009). High frequency echolocation signals can reach up to 350 meters, while modulated whistles, with frequencies generally below 25 kHz, can be detected over

distances of 1-5 km (Pavan & Borsani, 1997). The fundamental frequency of whistles is primarily in the sonic or audible range of humans, that ranges from 20 Hz to 15 kHz, with a maximal sensitivity between 1 and 6 kHz (Lammers & Oswald, 2015) making them easier to assess and measure (Herzing, 2014). In contrast, burst pulse sounds tend to have broader bandwidths and contain significant ultrasonic frequency components, requiring equipment with larger frequency bandwidth capabilities (Au & Hastings, 2008).

Furthermore, Dolphins developed a unique signal, called signature whistles, which are a notable feature of dolphin communication, representing individually distinctive learned vocalizations established during infancy (Sayigh & Janik, 2009), remaining stable over many years (Au & Hastings, 2008). Those vocalizations facilitate individual recognition and group cohesion (Sayigh & Janik, 2009).

2.3. Variations on repertoire of Cetaceans

Acoustic variations in cetaceans can be influenced by a multitude of ecological and evolutionary factors. The whistle repertoires of odontocetes show great variability between different species, different geographically separate populations, different groups within populations, or even between individuals. The characteristics of vocal repertoires not only differ between species but also to varying degrees within species (Ansmann et al., 2007). This acoustic diversity can result from various selective pressures acting on communication systems.

One key factor shaping this diversity is social structure, selection pressures on sympatric species can lead to sound signals diverging, a fact particularly evident in species with a fission-fusion social structure, such as the common bottlenose dolphin (Steiner, 1981; Mason et al., 2021). In such species, acoustic variations are often associated with group size and composition (Antichi et al., 2023). Some groups of dolphins may whistle less as group sizes increase in order to reduce the number of competing acoustic signals (Hernandez et al., 2010).

Beyond social organization, geographic distance and population genetic structure further play vital roles in acoustic geographical variation, with studies indicating that the greater the separation between populations, the more

pronounced the differences in their acoustic repertoire (Ford, 2009). Additionally, dolphins may modify their whistle characteristics in response to specific environmental conditions (Papale et al., 2021, Antichi et al., 2023). Multiple studies have suggested that both behavioral patterns and environmental context play key roles in shaping these differences. How dolphins use a particular area, whether for feeding, resting, or social interaction, can lead to distinct acoustic repertoire (May-Collado & Wartzok, 2008; Gridley et al. 2015)

While nearby groups may retain some acoustic similarities due to potential gene flow or habitat overlap, May-Collado & Wartzok (2008) noted that geographical distance alone doesn't always predict how similar two populations sound. Ding et al. (1995) found population-level variation in whistles parameters. In fact, dolphins from far-apart regions may show more acoustic resemblance than those living closer together, especially when they inhabit environments with similar acoustic constraints.

Moreover, variations in sound transmission and ambient noise can also lead to geographical acoustic divergence among populations with distinct acoustic environments (Van Ginkel et al., 2017; May-Collado & Wartzok, 2008). According to the Social Complexity Hypothesis for Communication, animals that live in more complex social environments require more elaborate communication systems to regulate interactions and relations among group members (Luís et al., 2021).

3. Region and species in the study

The study of cetacean acoustics in Portugal remains limited, with only a few publications available. Recent research has examined the impact of underwater noise from tourist vessels on dolphin vocalizations in southern Portugal (Silva et al., 2024; Forli et al., 2024). However, no studies have focused on characterizing the acoustic repertoires of local populations. There is a significant gap regarding the continental waters of Portugal, especially in the Algarve region. This lack of comprehensive research underscores the urgent need for further exploration of cetacean communication in these vital marine environments.

The Algarve coast lies on the northern margin of the Gulf of Cádiz, stretching from the eastern Strait of Gibraltar to Cape San Vicente in the west. This region

is characterized by complex hydrological dynamics driven by the Portuguese branch of the Canary Current Upwelling System, which plays a crucial role in supporting marine biodiversity. Additionally, the important water exchanges with Mediterranean Sea further contribute to the region's ecological richness (Lafuente & Ruiz, 2007; De Oliveira Júnior et al., 2022). The oceanic region off the coast of Faro is divided by Cape Santa Maria, resulting in a narrower western shelf and a broader eastern shelf, with significant differences in shelf width and depth (Mulero-Martinez et al., 2024). The dynamics of surface circulation are influenced by factors such as bathymetry, wind patterns, and river discharges, which create local upwelling conditions that are critical for the habitats of cetaceans (Mulero-Martinez et al., 2024; Llope, 2017).

The region is also subject to intense human activity. The southern region of Portugal faces substantially higher fishing effort compared to other areas of continental Portugal (Marçalo et al., 2015), making local populations of these areas more vulnerable to bycatch and high levels of low frequency noise generated by fishing vessels.

In the waters of continental Portugal, it is believed that 28 species of cetaceans occur (Mathias, et al., 2023). However, limited data is available for the Algarve region, in southern Portugal, which hosts several species of cetaceans that either reside or pass through during their migratory routes (Ferreira et al., 2023a). Most occurrence data come from opportunistic platforms and stranding records. Studies from the west coast of Portugal showed that short-beaked common dolphins (*Delphinus delphis*) and common bottlenose (*Tursiops truncatus*) dolphins as the most common cetacean (Brito et al., 2009; Brito & Sousa, 2011), with fin whale (*Balaenoptera physalus*) among the most common baleen species (Brito & Sousa, 2011). The cetacean community along the central coast of Portugal appears similar to that along the Iberian shore (Brito & Sousa, 2011). Bottlenose and common dolphins are two resident species in continental Portugal (Ferreira et al., 2023a; Ferreira et al., 2023b) and key targets for acoustic monitoring in the region, are two highly vocal animals, and the most acoustically study in previous studies.

Bottlenose dolphins have a broad distribution, occurring in both coastal and oceanic waters, favoring productive areas of the continental shelf, with different adaptations where depth can have great importance in their choice (Ferreira et al., 2023b). Two ecotypes of bottlenose dolphins (coastal and oceanic) have been identified in the region (Ferreira et al., 2023b), with distinct morphological and behavioral traits (Antichi et al., 2023), these differences in habitat use are likely to influence their acoustic communication. This species is not only protected under the Habitats Directive (92/43/EEC, May 21st, 1992), like all cetaceans in Europe, but is also granted priority protection status (Annex IV) (CetAMBICion Project, 2022). Nevertheless, it faces major threats in Portugal, including bycatch in fishing gear, underwater noise, and disturbance from recreational vessels, particularly due to the presence of resident coastal groups that share areas with intense human activity (Ferreira et al., 2023b).

Common dolphins are considered abundant and wide-ranging with wide distribution along the coast (Mason et al., 2021), they are primarily an oceanic species that show a strong preference for upwelling-modified waters and areas with steep sea bottoms, such as seamounts and escarpments (Jefferson et al., 2007). They are often found in large groups and frequently observed in mixed-species groups with other cetaceans. Although common dolphins are abundant in Portugal, they are facing a decline in population, particularly due to bycatch in fishing nets, and they are now considered near threatened (NT) by UICN (Mathias et al., 2023). The study by Marçalo et al. (2015) revealed that this species was the most observed during fishing activities along the southern coast of the Algarve, making it particularly vulnerable to bycatch in this region.

False killer whales (*Pseudorca crassidens*) were already identified as a possible species in Portugal, being considered occasional and of low abundance (Ferreira et al., 2023c). The first record of their sightings from Ocean vibes happens in April of 2023, feeding on Atlantic bluefin tuna (*Thunnus thynnus*) in the area with a high aggregation of animals spread in different subgroups. The two years after (2024 and 2025) the same pattern was registered. This species continues to use these waters as a feeding area during the same time of the year. This was, in fact, a huge discover, knowing that in continental Portugal no information about this species is described in literature, and a big opportunity to

collect data to further understand more about its distribution, dynamics, behavior and more.

Therefore, PAM can play a key role in conservation of these species in the region by identifying regularly used areas, assessing behavioral responses to noise and vessel traffic, and supporting public awareness and policy decisions. This knowledge is essential to guide the implementation of effective mitigation measures and may help identify the need for marine protected areas.

4. Methodologies in acoustic studies

A basic PAM system typically consists of a single hydrophone lowered from a boat (Zimmer, 2011). A hydrophone is an electroacoustic transducer that converts the received acoustic pressure into electrical energy (Richardson et al., 1995; Au & Hastings, 2008; Zimmer, 2011). Key parameters of a hydrophone include sensitivity, which indicates the electric voltage generated as a function of acoustic sound pressure (dB re 1V/ μ Pa), and the natural resonance frequency (f_r), which limits the useful bandwidth of the hydrophone (Zimmer, 2011). Hydrophones cover a wide frequency range, from a few Hz to over 100 kHz, and are typically omnidirectional (Pavan & Borsani, 1997). To improve sensitivity and directionality, complex hydrophone arrays are often necessary (Pavan & Borsani, 1997). Due to the generally low voltage produced by transducers, preamplifiers are commonly used to amplify these small signals (Zimmer, 2011).

However, the effectiveness of signal acquisition also depends on the surrounding acoustic environment. Signal acquisition is influenced by acoustic noise, which includes all sounds other than the target signals. The acoustic noise in the ocean is often referred to as ambient or background noise (Au & Hastings, 2008). It originates from a variety of natural or anthropogenic sources. Most natural underwater noise is generated by wind-related surface agitation and biological sources, such as other marine organisms (Au & Hastings, 2008). Anthropogenic sources, on the other hand, are primarily associated with maritime traffic, especially distant ship movements (Neighbors & Bradley, 2017). Another common source of acoustic noise is self-noise, generated by the measurement method itself, including the hydrophone or the platform used to carry it (Neighbors

& Bradley, 2017). This can also affect signal quality. For example, hydrophones lowered from moving boats may pick up noise from water flow, engine sounds, electrical noise or even from the hydrophone cable being dragged through the water (Au & Hastings, 2008).

Once signals are acquired, they can be graphically represented using an oscillogram, a time-domain plot that shows how the amplitude of a waveform changes over time, corresponding to variations in sound pressure (Frankel, 2009; Pavan & Borsani, 1997). However, spectrographic analysis is more informative, as it describes the distribution of sound power across frequencies and time (Pavan & Borsani, 1997). A standard method for obtaining frequency information is through Fourier transformation techniques (FFT) (Zimmer, 2011). These graphic representations enable comparisons of various signals, facilitating the identification of similarities or differences, classification based on structural characteristics, and correlation with observed behaviors or identified individuals (Pavan & Borsani, 1997).

Regarding data interpretation, various approaches have been employed to analyze acoustic data, the traditional method relies on visual inspection of spectrograms, with particular emphasis on the shape of the whistle contour (Au & Hastings, 2008). Whistle contours can be categorized into six fundamental types: constant frequency (signals where the frequency that stays stable for almost the entire duration), upswing (frequency increasing over time), downswing (frequency decreasing over time), concave (instantaneous frequency initially increasing with time, followed by an ending portion with the instantaneous frequency decreasing with time), convex (frequency initially decreasing with time, followed by an ending portion in which the frequency increases with time), and sinusoidal (more than one repetition of a concave or convex and the contour appearing like a sinusoidal signal with at least two inflection points) (Au & Hastings, 2008; Ansmann et al., 2007; Antichi et al., 2023; Antichi et al., 2024). However, researchers may consider contours with slight variations as belonging to a new and separate category. This qualitative technique can be highly subjective, resulting in variable outcomes among researchers and highlighting a lack of standardization in techniques and classification categories (Ansmann et al., 2007). The complexity of some calls,

which may include combinations of different individual structural units, introduces additional subjectivity in determining whether a particular contour is truly unique or merely exhibits minor variations (Au & Hastings, 2008).

To mitigate subjectivity, a more quantitative approach to describe whistles has been adopted, which involves extracting various frequency and time-based parameters (Zimmer, 2011). Common parameters include beginning frequency, end frequency, minimum frequency, maximum frequency, number of inflection points, duration, contour breaks or steps, and the presence of harmonics (Au & Hastings, 2008; Antichi et al., 2023; Antichi et al., 2024). These parameters can be processed using statistical software for further analysis (Au & Hastings, 2008).

5. Goals of study

This study aims to characterize and compare the whistle repertoires of three odontocete species, *Delphinus delphis* (short-beaked common dolphin), *Tursiops truncatus* (common bottlenose dolphin), and *Pseudorca crassidens* (false killer whale), in the coast of Faro. These species were selected due to their high vocal activity, which made them easier species to study acoustically.

Common and bottlenose dolphins are the most frequently sighted species in the region, occurring all year around and using this area for a variety of purposes. False killer whales are still poorly understood and there is still a huge gap in knowledge about their population in the region.

Acoustic studies can provide vast opportunities to better understand how these species use this habitat and to infer aspects of their population dynamics. Understanding the local cetacean baseline repertoire is a crucial first step to allow further research into acoustic impacts of vessels, sonars and other anthropogenic acoustic threats. Given the considerable differences in same-species vocalizations around the world, it is crucial to determine variable parameters for the main target species in each region in order to assess potential changes and impacts induced by human activities. For this reason, the results of the acoustic characterization carried out in Faro were also compared to studies conducted in other regions of the world. This comparative approach helps to identify

geographic variation in acoustic behavior and assess the extent to which local populations may be affected by environmental and anthropogenic factors.

Moreover, acoustic data is detrimental to evaluating potential behavioral changes, monitoring populations, and mitigate activities such as fishing with the use of deterring devices (pingers). Without this knowledge, other studies could only be assumptions if compared with distant populations.

5. References

- Ansmann, I. C., Goold, J. C., Evans, P. G., Simmonds, M., & Keith, S. G. (2007). Variation in the whistle characteristics of short-beaked common dolphins, *Delphinus delphis*, at two locations around the British Isles. *Journal of the Marine Biological Association of the United Kingdom*, 87(1), 19–26. <https://doi.org/10.1017/s0025315407054963>
- Antichi, S., Austin, M., May-Collado, L. J., R, J. U., Martínez-Aguilar, S., & ViloriaGómora, L. (2023). Differences in the whistles of two ecotypes of bottlenose dolphins from the Gulf of California. *JASA Express Letters*, 3(5). <https://doi.org/10.1121/10.0019502>
- Antichi, S., Manali Rege-Colt, M., Austin, M., & May-Collado, L. (2024). Whistle structure variation between two sympatric dolphin species in the Gulf of California. *Hydrobiologia*. <https://doi.org/10.1007/s10750-024-05675-3>
- Au, W. W. L., & Hastings, M. C. (2008). Principles of marine bioacoustics. In Springer eBooks. <https://doi.org/10.1007/978-0-387-78365-9>
- Balance, L.T. (2009). Cetacean Ecology. In *Encyclopedia of marine mammals* (pp. 196–201). <https://doi.org/10.1016/b978-0-12-373553-9.00235-2>
- Brito, C., & Sousa, A. (2011). The Environmental History of Cetaceans in Portugal: Ten Centuries of Whale and Dolphin Records. *PLoS ONE*, 6(9), e23951. doi: 10.1371/journal.pone.0023951
- Brito, C., Vieira, N., Sá, E., & Carvalho, I. (2009). Cetaceans' occurrence off the west central Portugal coast: a compilation of data from whaling, observations of opportunity and boat-based surveys. *Marine Animals and Their Ecology*, 2(1), 10–13.
- CetAMBICion Project. (2022). Results from pilot project on mitigation: Set nets and purse seine nets (Deliverable D4.3_V1). In WP4 – Effectiveness assessment of cetacean bycatch reduction strategies and fishing technical measures proposal (GA No. 110661/2020/839610/SUB/ENV.C2). Coordinated by IIM-CSIC. Leader partner: UALG. Retrieved from https://www.cetambicion-project.eu/wp-content/uploads/2023/08/CetambICion_Deliverable-WP4_Task4.3_Pilots-nets-and-purse-seine_FINAL.pdf
- De Oliveira Júnior, L., Relvas, P., & Garel, E. (2022). Kinematics of surface currents at the northern margin of the Gulf of Cádiz. *Ocean Science*, 18(4), 1183–1202. <https://doi.org/10.5194/os-18-1183-2022>
- Ding, W., Würsig, B. & Evans, W. E. (1995). Comparisons of whistles among seven odontocete species. In R. A. Kastelein & J. A. Thomas (Eds.), *Sensory Systems of Aquatic Mammals* (pp. 299–323). De Spil Publishers; and Ding, W., Würsig, B. & Evans, W. E. (1995). Whistles of bottlenose dolphins: Comparisons among populations. *Aquatic Mammals*, 21, 65–77.

- Dudzinski, K. M., Thomas, J. A., & Gregg, J. D. (2009). Communication in marine mammals. In Elsevier eBooks (pp. 260–269). <https://doi.org/10.1016/b978-0-12-373553-9.00064-x>
- Ferreira M, Eira C, López A & Sequeira M (2023a). *Delphinus delphis* golfinho-comum. In Livro Vermelho dos Mamíferos de Portugal Continental. FCIências.ID, ICNF, Lisboa.
- Ferreira M, Eira C, López A & Sequeira M (2023b). *Tursiops truncatus* roaz. In Livro Vermelho dos Mamíferos de Portugal Continental. FCIências.ID, ICNF, Lisboa.
- Ferreira M, Eira C, López A & Sequeira M (2023c). *Pseudorca crassidens* falsa-orca. In Livro Vermelho dos Mamíferos de Portugal Continental. FCIências.ID, ICNF, Lisboa.
- Ford, J. K. B. (2009). Dialects. In Elsevier eBooks (pp. 310–311). <https://doi.org/10.1016/b978-0-12-373553-9.00075-4>
- Forli, M., Santos, R. P. D., Rodrigues, A., & Castilho, R. (2024). The impact of touristic whale-watching on *Delphinus Delphis* and *Tursiops truncatus* in the Algarve Coast: Combining acoustic analysis and land observations. *Ocean & Coastal Management*, 259, 107431. <https://doi.org/10.1016/j.ocecoaman.2024.107431>
- Frainer, G., Dufourq, E., Fearey, J., Dines, S., Probert, R., Elwen, S., & Gridley, T. (2023). Automatic detection and taxonomic identification of dolphin vocalisations using convolutional neural networks for passive acoustic monitoring. *Ecological Informatics*, 78, 102291. <https://doi.org/10.1016/j.ecoinf.2023.102291>
- Frankel, A. S. (2009). Sound Production. *Encyclopedia of marine mammals* (pp. 1056– 1071). <https://doi.org/10.1016/b978-0-12-373553-9.00242-x>
- Gregorietti, M., Papale, E., Ceraulo, M., De Vita, C., Pace, D. S., Tranchida, G., Mazzola, S., & Buscaino, G. (2021). Acoustic Presence of Dolphins through Whistles Detection in Mediterranean Shallow Waters. *Journal of Marine Science and Engineering*, 9(1), 78. <https://doi.org/10.3390/jmse9010078>
- Gridley, T., Nastasi, A., Kriesell, H., & Elwen, S. (2015). The acoustic repertoire of wild common bottlenose dolphins (*Tursiops truncatus*) in Walvis Bay, Namibia. *Bioacoustics*, 24(2), 153–174. <https://doi.org/10.1080/09524622.2015.1014851>
- Hanke W & Erdsack N (2015). Ecology and Evolution of dolphin sensory systems. In *Dolphin communication and cognition: past, present, and future*. <https://lib.ugent.be/catalog/ebk01:3710000000478100>
- Hernandez, E. N., Solangi, M., & Kuczaj, S. A. (2010). Time and frequency parameters of bottlenose dolphin whistles as predictors of surface behavior in

the Mississippi Sound. *The Journal of the Acoustical Society of America*, 127(5), 3232–3238. <https://doi.org/10.1121/1.3365254>

Herzing, D. L. (2014). Clicks, whistles and pulses: Passive and active signal use in dolphin communication. *Acta Astronautica*, 105(2), 534–537. <https://doi.org/10.1016/j.actaastro.2014.07.003>

Herzing, D. L., & Johnson, C. M. (2015). Dolphin communication and cognition: past, present, and future. <https://lib.ugent.be/catalog/ebk01:3710000000478100>

Jefferson, T. A., Webber, M. A., & Pitman, R. L. (2007). *Marine Mammals of the World: A Comprehensive Guide to their identification*. <http://ci.nii.ac.jp/ncid/BA8426088X>

Jones, B., Zapetis, M., Samuelson, M. M., & Ridgway, S. (2019). Sounds produced by bottlenose dolphins (*Tursiops*): a review of the defining characteristics and acoustic criteria of the dolphin vocal repertoire. *Bioacoustics*, 29(4), 399–440. <https://doi.org/10.1080/09524622.2019.1613265>

Kuczaj II S & Winship K (2015). How do dolphins calves make sense of their world. In *Dolphin communication and cognition: past, present, and future*. <https://lib.ugent.be/catalog/ebk01:3710000000478100>

Lafuente, J. & Ruiz, Javier. (2007). The Gulf of Cádiz pelagic ecosystem: A review. *Progress in Oceanography* 74. 228-251. [10.1016/j.pocean.2007.04.001](https://doi.org/10.1016/j.pocean.2007.04.001).

Lammers M & Oswald J (2015). Analyzing the acoustic communication of dolphins. In *Dolphin communication and cognition: past, present, and future*. <https://lib.ugent.be/catalog/ebk01:3710000000478100>

Lehnhoff, Loïc & Glotin, Hervé & Gall, Yves & Menut, Eric & Peltier, Helene & Spitz, Jerome & Canneyt, Olivier & Mérigot, Bastien. (2025). High resolution acoustic recordings of wild free-ranging short-beaked common dolphins for etho-acoustical and repertoire studies. [10.5194/essd-2025-193](https://doi.org/10.5194/essd-2025-193).

Llope, M. (2017). The ecosystem approach in the Gulf of Cadiz. A perspective from the southernmost European Atlantic regional sea. *ICES Journal of Marine Science*, 74(1), 382–390. <https://doi.org/10.1093/icesjms/fsw165>

Luís, A. R., May-Collado, L. J., Rako-Gospic, N., Gridley, T., Papale, E., Azevedo, A., Silva, M. A., Buscaino, G., Herzing, D., & Santos, M. E. D. (2021). Vocal universals and geographic variations in the acoustic repertoire of the common bottlenose dolphin. *Scientific Reports*, 11(1). <https://doi.org/10.1038/s41598-021-90710-9>

Lurton (2002). *An introduction to underwater acoustics: Principles and Applications*. Springer Science & Business Media.

- Marçalo, A., Katara, I., Feijó, D., Araújo, H., Oliveira, I., Santos, J., Ferreira, M., Monteiro, S., Pierce, G. J., Silva, A., & Vingada, J. (2015). Quantification of interactions between the Portuguese sardine purse-seine fishery and cetaceans. *ICES Journal of Marine Science*, 72(8), 2438–2449. <https://doi.org/10.1093/icesjms/fsv076>
- Mason, S., Kent, C. S., & Bilgmann, K. (2021). Common dolphins form unexpected strong social bonds: insights into social plasticity of *delphinids*. *Marine Mammal Science*, 37(4), 1174–1195. <https://doi.org/10.1111/mms.12815>
- Mathias ML (coord.), Fonseca C, Rodrigues L, Grilo C, Lopes-Fernandes M, Palmeirim JM, Santos-Reis M, Alves PC, Cabral JA, Ferreira M, Mira A, Eira C, Negrões N, Paupério J, Pita R, Rainho A, Rosalino LM, Tapisso JT & Vingada J (eds.). (2023). Livro Vermelho dos Mamíferos de Portugal Continental. FCIências.ID, ICNF, Lisboa.
- Matthews, J. N. S., Rendell, L., Gordon, J., & Macdonald, D. W. (1999). A review of frequency and time parameters of cetacean tonal calls. *Bioacoustics - the International Journal of Animal Sound and Its Recording*, 10(1), 47–71. <https://doi.org/10.1080/09524622.1999.9753418>
- 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. <https://doi.org/10.1644/07-mamm-a-310.1>
- Moore, P. W. (1997). Cetacean auditory psychophysics. *Bioacoustics - the International Journal of Animal Sound and Its Recording*, 8(1–2), 61–78. <https://doi.org/10.1080/09524622.1997.9753354>
- Mulero-Martinez, R., Gómez-Enri, J., De Oliveira Júnior, L., Garel, E., Relvas, P., & Mañanes, R. (2024). Spatiotemporal variability of the coastal circulation in the northern Gulf of Cadiz from Copernicus Sentinel-3A satellite radar altimetry measurements. *Advances in Space Research*, 73(10), 4922–4936. <https://doi.org/10.1016/j.asr.2024.02.054>
- Neighbors, T. H., & Bradley, D. (2017). *Applied underwater acoustics*: Leif Bjørnø. Elsevier.
- Nummela, S. (2009). Hearing. In *Encyclopedia of marine mammals* (pp. 553–562). <https://doi.org/10.1016/b978-0-12-373553-9.00129-2>
- 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. <https://doi.org/10.1111/j.1748-7692.2003.tb01090.x>
- Oswald, J. N., Rankin, S., Barlow, J., & Lammers, M. O. (2007). A tool for real-time acoustic species identification of *delphinid* whistles. *Journal of the Acoustical Society of America*, 122(1), 587–595. <https://doi.org/10.1121/1.2743157>

- Papale, E. B., Azzolin, M. A., Cascão, I., Gannier, A., Lammers, M. O., Martin, V. M., Oswald, J. N., Perez-Gil, M., Prieto, R., Silva, M. A., Torri, M., & Giacoma, C. (2021). Dolphin whistles can be useful tools in identifying units of conservation. *BMC Zoology*, 6(1). <https://doi.org/10.1186/s40850-021-00085-7>
- Pavan, G., & Borsani, J. F. (1997). Bioacoustic research on cetaceans in the Mediterranean Sea. *Marine and Freshwater Behaviour and Physiology*, 30(2), 99–123. <https://doi.org/10.1080/10236249709379019>
- Perrin, W. F., Würsig, B. G., & Thewissen, J. G. M. (2009). Encyclopedia of Marine Mammals. In Elsevier eBooks. <https://doi.org/10.1016/b978-0-12-373553-9.x0001-6>
- Richardson, W. J., Greene, C. R., Jr, Malme, C. I., & Thomson, D. H. (1995). *Marine Mammals and Noise*. Academic Press. <https://doi.org/10.1093/icesjms/fsw165>
- Saheban, H., & Kordrostami, Z. (2021). Hydrophones, fundamental features, design considerations, and various structures: A review. *Sensors and Actuators A: Physical*, 329, 112790. doi: 10.1016/j.sna.2021.112790
- Sayigh, L. S., & Janik, V. M. (2009). Signature whistles. In *Encyclopedia of marine mammals* (pp. 1014–1016). <https://doi.org/10.1016/b978-0-12-373553-9.00235-2>
- Silva, A. A., Castro, J., Cid, A., Jesus, S. M., & Matos, F. L. (2024). Influence of Dolphin-Watching tourism vessels on the whistle emission pattern of common dolphins and bottlenose dolphins. *Oceans*, 5(4), 770–784. <https://doi.org/10.3390/oceans5040044>
- Steiner, W. W. (1981). Species-specific differences in pure tonal whistle vocalizations of five western North Atlantic dolphin species. *Behavioral Ecology and Sociobiology*, 9(4), 241–246. <https://doi.org/10.1007/bf00299878>
- Van Ginkel, C., Becker, D. M., Gowans, S., & Simard, P. (2017). Whistling in a noisy ocean: bottlenose dolphins adjust whistle frequencies in response to real-time ambient noise levels. *Bioacoustics*, 27(4), 391–405. <https://doi.org/10.1080/09524622.2017.1359670>
- Zimmer, W. (2011). Passive acoustic monitoring of cetaceans. <https://doi.org/10.1017/cbo9780511977107>

Chapter 2

Whistle structure and geographical variations in three delphinid species recorded in eastern Algarve coast

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Abstract

Cetacean's acoustic signals are species-specific to varying degrees and offer a promising tool for long-term monitoring, population assessment, and conservation. This study characterizes and compares the whistle structure of three odontocete species, *Delphinus delphis*, *Tursiops truncatus*, and *Pseudorca crassidens*, recorded in the coast of Faro, Portugal. Between April 2022 and June 2024, 327 whistles were analyzed from 36 acoustic events, collected using an opportunistic platform during dolphin and whale watching surveys. Visual inspection and manual whistle extraction of ten acoustic parameters were performed, along with whistle contours categorization. Faro data were then compared with published values from other geographic regions to evaluate possible geographic divergence. Results showed significant differences in whistle parameters between species, especially between *P. crassidens* and the two other dolphin species that showed higher variability in frequency and contour type. Geographic comparisons revealed distinct whistle characteristics in Faro populations and indicated that distance alone does not predict acoustic similarity. Instead, environmental context plays a key role in shaping dolphin's repertoire, likely driving specific acoustic adaptations. environmental context likely drives regional acoustic adaptations. These findings support the use of passive acoustic monitoring for local conservation efforts.

Keywords: Passive acoustic monitoring; geographic acoustic variation; dolphins whistles; Odontocetes; Algarve, Portugal.

1. Introduction

Passive acoustic monitoring (PAM) is a non-invasive method for studying and monitoring cetacean populations. The acoustic signals produced by cetaceans are species-specific to varying degrees and offer a promising tool for long-term monitoring, population assessment, and conservation, particularly for elusive or endangered species (Matthews et al., 1999; Frainer et al., 2023). Beyond identification, these signals provide insight into social interactions, behavioral patterns, and habitat use (Lehnhoff et al., 2025).

Among odontocetes, communication is highly developed and adapted to an aquatic lifestyle. These animals rely heavily on acoustics and have evolved specialized auditory and vocal production systems (Zimmer, 2011; Frankel, 2009). Their vocal repertoire includes pulsed sounds (e.g., clicks and burst pulses) and frequency-modulated tonal sounds (whistles) (Moore, 1997; Oswald et al., 2007). Whistles are especially relevant for social communication due to their variable contours, frequency range (typically 5–20 kHz), and ability to travel longer distances than pulsed sounds (Au & Hastings, 2008; Herzing & Johnson, 2015). A key component of dolphin communication is the use of signature whistles, individually distinctive signals that remain stable over time and help maintain group cohesion (Sayigh & Janik, 2009).

This study focuses on three odontocete species found in the Algarve region: the short-beaked common dolphin (*Delphinus delphis*), the common bottlenose dolphin (*Tursiops truncatus*), and the false killer whale (*Pseudorca crassidens*). Short-beaked common dolphin and common bottlenose dolphin are resident species commonly observed in Portuguese waters (Ferreira et al., 2023a; 2023b), while *Pseudorca crassidens* has only recently been documented in the area, offering a rare opportunity to study its vocal behavior. The oceanography dynamic of Faro coastal region, influenced by the Canary Current Upwelling System and water exchanges from Mediterranean Sea, shape a complex shelf structure characterized by a narrow continental shelf and a steep slope close to the coast (Mulero-Martinez et al., 2024). These conditions create a productive and biodiverse environment that supports local cetacean populations (Lafuente & Ruiz, 2007; De Oliveira Júnior et al., 2022; Mulero-Martinez et al., 2024).

Despite the ecological richness of this region, acoustic studies in southern Portugal remain limited, especially when compared to other areas like the Azores (Gannier et al., 2020). This research gap highlights the need to establish baseline knowledge of the acoustic repertoires of local cetaceans. Understanding regional variations is essential, as whistles can differ significantly between and within species, populations, and even individuals, driven by behavioral context, environmental conditions, and social structure (Ansmann et al., 2007; Steiner, 1981; Antichi et al., 2023). Additionally, anthropogenic noise and other environmental factors may cause acoustic divergence between populations (Van Ginkel et al., 2017; May-Collado & Wartzok, 2008).

Therefore, this study aims to describe and compare the whistle repertoires of the three target species, providing a baseline for local acoustic diversity and supporting future conservation and impact assessments. To complement the local analysis, the Faro data were also compared with studies from other regions, allowing the identification of geographic variation in acoustic behavior and the evaluation of environmental and anthropogenic impacts on local populations.

2. Methodology

2.1 Study area and data collection

This study was conducted in the offshore waters of the Ria Formosa Natural Park, located in Faro, Algarve, southern Portugal (Figure 2.1).

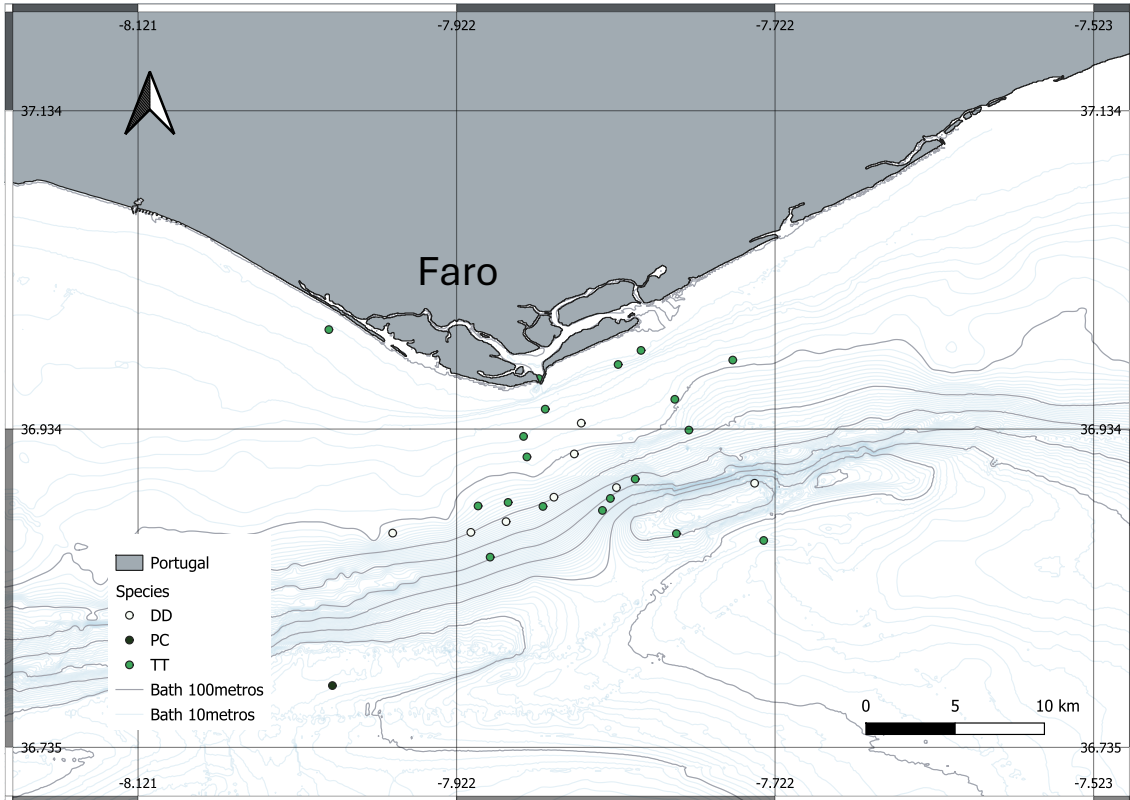


Figure 2.1 - Survey area (Faro, Portugal) showing the locations of 30 dolphin groups recorded and use in this analysis, from the 36 acoustic events (6 has no coordinates data). Image generated from QGIS software.

Between April 2022 and June 2024, data was collected using an opportunistic platform. A 6.4-meter motorized whale and dolphin watching boat was used to collect acoustic recordings under favorable weather conditions (Beaufort scale ≤ 2). During surveys, the team actively searched for the animals, and when cetaceans were sighted, they were approached for species identification in accordance with regulations on vessel behavior near cetaceans (Portuguese decree-law no. 9/2006, 2006) taking care not to directly affect their behavior.

Recordings were conducted during periods when visual contact with the animals could be maintained, with no fixed duration. The recordings were made

with the engine and the boat transducer off, using an Aquarian AS-1 Hydrophone (sensitivity: -206 dBV re 1 μ Pa, frequency range: 1 Hz to 110 kHz, omnidirectional) connected to a PA-4 Hydrophone Preamplifier (12 dB gain, 14 Hz high-pass filter, 48 kHz low-pass filter) and a ZOOM H5 Recorder (sampling rate: 48 kHz - captured frequencies up to 24 kHz; 16/24-bit resolution). When contact was lost, the engine was turned on and the group was carefully followed to attempt another recording session.

Additional data, such as species, group composition, behavior, were collected through visual observation by trained observers during encounters. Other information, including time of observation, GPS position, sea surface temperature, and depth, were also recorded using the boat plotter system. Several mixed-species groups were observed, mainly comprising *Delphinus delphis* and *Stenella coeruleoalba*. Surface behavior categories were defined according to previous studies. (Herzing, 2010).

2.2 Whistle acoustic analyses

Analysis focused on whistles of *Delphinus delphis*, *Tursiops truncatus*, and *Pseudorca crassidens*. Whistles were selected for analysis because the fundamental frequency of dolphin whistles typically falls within the human audible range, 20 Hz to 15 kHz (Lammers & Oswald, 2015) and can be recorded using the available standard equipment.

Data from recordings of dolphin schools that were visually identified and confirmed to contain only one species were selected for analysis, with all mixed-species groups excluded to ensure accurate identification of species-specific sounds (Au & Hastings, 2008), however, we cannot be sure that all recordings have a single species. This is an issue that all dolphin acoustic studies face when conducted in the wild (Antichi et al. 2024).

Overlapping whistles were only included when contours could be clearly distinguished (Oswald et al., 2007). Acoustic files were visually inspected in the spectrogram view of Raven Pro (version 1.6.5) in the time-frequency, 512-point Hann window, 22 kHz maximum frequency. Each whistle was annotated with a unique ID, and several standard acoustic characteristics were extracted

manually, as automatic extraction would not work due to overlapping (Figure 2.2). The ten variables measured from each call were the following: (1) Beginning frequency (Hz), (2) End frequency (Hz), (3) Minimum frequency (Hz), (4) Maximum frequency (Hz), (5) Frequency range (Hz), (6) Duration (s), (7) Initial slope (Hz/s), (8) Final slope (Hz/s), (9) Number of inflection points, (10) number of steps (Figure 2.3). These variables were chosen for their consistency with previous studies (for example: Oswald et al., 2007; Antichi et al., 2023; Rendell et al., 1999; Ansmann et al., 2007; Pagliani et al., 2021; Ding et al, 1995), and because they could be easily measured manually from a spectrogram. Additionally, contour shapes (Figure 2.4) were categorized following Au & Hastings (2008) research by visual inspection and observer choice, when a whistle contour could include features from more than one category it was chosen the contour category that was most descriptive of the whistle.

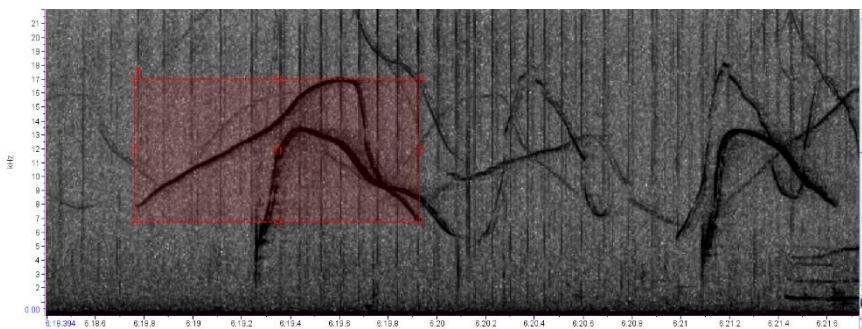


Figure 2.2 - Example of overlapping whistles during analyses in Raven Pro. Spectrogram: 512 points FFT, Hann window, x-axis: time (s), y-axis: frequency (kHz). (Whistle ID ZOOM0023, recorded on May 19th, 2022, during observation of a *Tursiops truncatus* group)

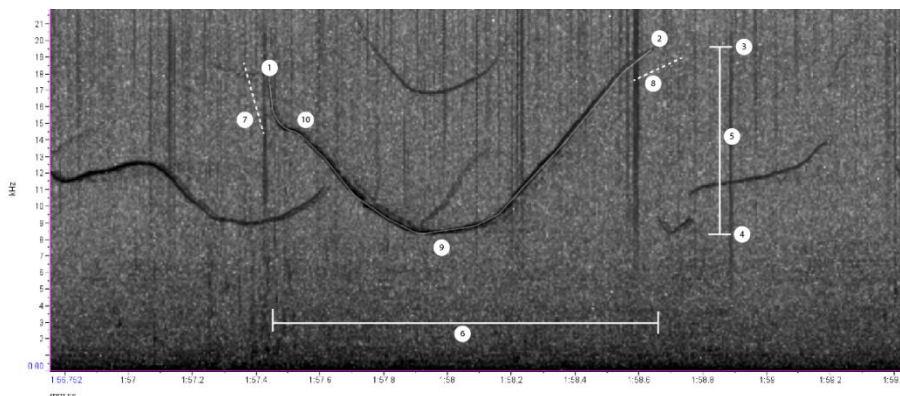


Figure 2.3 – Example of a whistle analysis process in Raven Pro. Spectrogram: 512 points FFT, Hann window, x-axis: time (s), y-axis: frequency (kHz). Whistle ID ZOOM0240, recorded on March 19th, 2024, during observation of a *Delphinus delphis* group. In the figure is represented the variables collected from each whistle: 1. Beginning frequency; 2.

End frequency; 3. Maximum frequency; 4. Minimum frequency; 5. Range frequency; 6. Duration; 7. Initial slope; 8. Final slope; 9. Inflection point; 10. Step.

2.3 Statistical analysis

Descriptive statistical analysis was calculated for each acoustic variable within each species group, obtaining the mean, standard deviation (SD), minimum and maximum values for each parameter. In addition, skewness was calculated to check asymmetry in the data distribution, and comparative violin plots were generated to visually represent the variability and distribution of each variable across species.

The contour shapes were analyzed to see how the distribution of whistle types differed among the three species (Figure 2.4). Afterwards, to test an overall association between species and whistle type usage, a Spearman correlation was calculated.

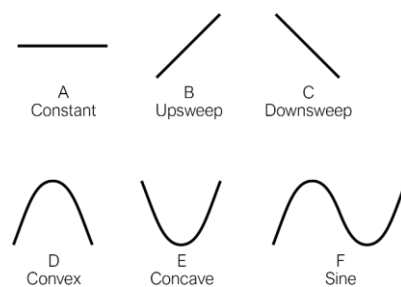


Figure 2.4 - Classification scheme of whistle contours used as a reference for categorizing dolphin whistles in this study. Adapted from Ansmann et al. (2007)

The normality of data from whistles analyzed was tested using the Shapiro-Wilk test. Since not all data followed a normal distribution, the non-parametric Kruskal-Wallis' test was used to determine whether there were overall differences among the three species in each acoustic parameter pairwise with Dunn's test for identifying which specific pairs of groups differ and adjusted with sequential Bonferroni, as done in previous studies (Pagliani et al. 2021; Antichi et al. 2024). Statistical significance was assessed with a 95% confidence interval.

A non-metric multidimensional scaling (NMDS) was performed to visualize and evaluate the separation of whistles characteristics among species, using the features that characterize the whistles. Points in the NMDS plot were coded by species, and 95% confidence ellipses were added around the points for each species group to visualize the cluster dispersion.

To further explore species differences, a Random Forest classification model was created using the `randomForest` package in RStudio (Liaw & Wiener, 2002). The model was trained to classify dolphin whistles by species using the 10 acoustic variables: duration, frequency range, minimum frequency, maximum frequency, beginning frequency, end frequency, initial slope, final slope, number of inflection points, and number of steps. The model was built using 500 decision trees (`ntree = 500`). Feature importance was computed using the mean decrease in Gini index, allowing the identification of the most contributed acoustic parameters. The dataset used for training included all labeled whistle contours with known species identity.

2.4 Geographic comparison

To understand intraspecific whistle variation of these three species across different regions, comparative geographic analysis of whistle parameters with data from this study was done with previously published studies from different locations. *Delphinus delphis* were compared with 11 different regions, *Tursiops truncatus* with 13 regions and *Pseudorca crassidens* with 4 regions. Euclidean distance between those regions and Faro were measured, similar to Kershenbaum et al. (2013) study. Also, heatmaps were created to visually analyze how close or far apart acoustics features differ within the same species.

All data processing and analyses were conducted using R (version 4.5.0) with the RStudio interface (version 2025.05.0).

3. Results

The recording effort resulted in a total of 7 hours and 41 minutes of acoustic data from 85 cetacean's groups. These included four species: *Delphinus delphis*, *Tursiops truncatus*, *Stenella coeruleoalba*, and *Pseudorca crassidens*. However, only whistles from 36 groups were analyzed (Table 3.1).

Table 3.1 – Summary of acoustic data collected, expressing time, group of animals, total files recorded, and the files analyzed for four species: *Delphinus delphis* (DD), *Tursiops truncatus* (TT), *Stenella coeruleoalba* (SC), and *Pseudorca crassidens* (PC), and a mix-group species of *Delphinus delphis* and *Stenella coeruleoalba* (DD+SC).

	DD	TT	SC	DD + SC	PC	Total
Total time recorded	02h26min	03h21min	12min	26min	29min	07h41min
Groups recorded	36	38	3	5	3	85
Total of audio files	57	64	3	8	13	145
Audio files analyzed	13	22	0	0	1	36

Delphinus delphis and *Tursiops truncatus* were the most frequent species encountered during fieldwork. Despite the rarity and seasonal presence of *Pseudorca crassidens*, enough quality data were collected to include this species in the study from one of the encounters. *Stenella coeruleoalba*, was not included in the study, due to the frequency presence in mixed species with *Delphinus delphis*, making the probability of recordings including more than one species very high.

A total of 327 whistles were obtained from the analysis with all species with equal number for a balanced data, better for comparability: *Delphinus delphis* (DD), n = 109; *Tursiops truncatus* (TT), n = 109, *Pseudorca crassidens* (PC), n= 109, providing a robust sample for comparative analysis.

Results from surface behaviors observed during encounters are described in Table S1 and Figure 3.1. Feeding and socialization were the most common behavior registered during recordings.

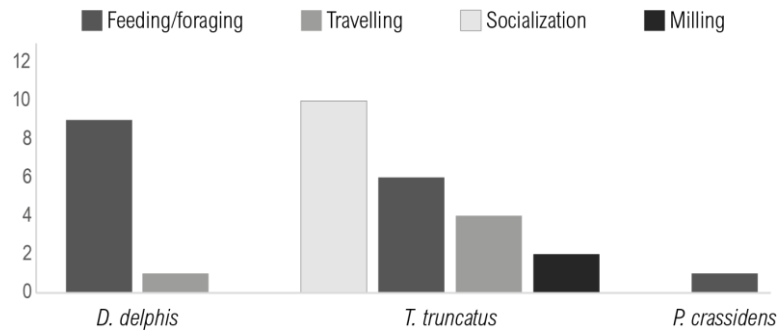


Figure 3.1 – Visually representation of behavior observed during recording by species (y-axis: Number of observed events).

Table 3.2 - Descriptive statistics of the whistle variables of the three dolphin species.

		Minimum (Hz)	Maximum (Hz)	Range (Hz)	Duration (s)	Inicial slope (Hz/s)	Final slope (Hz/s)	Beginning (Hz)	End (Hz)	Inflection	Steps
<i>Tursiops truncatus</i>	mean	6892,9	14904,8	8008,8	1	38096,2	-16993,5	8183,2	9686,1	2,2	7,8
	sd	1512,1	2453	2489,2	0,6	50587,3	56594,8	2212,9	3630,8	2,1	5
	min	3551	8965	2327	0,3	-62864,9	-242419,4	3925	4506	0	1
	max	10758	21005,5	14296	4,8	221834,9	143114,8	15609	21005,5	15	27
	skew	0,3	0	-0,1	3,1	1,1	-1,1	0,7	0,8	2,9	1,7
<i>Delphinus delphis</i>	mean	9178,8	17352,6	8173,8	1	-15464,8	2974,3	13648,5	12428,8	1,5	5,6
	sd	2256,4	2952,2	2653,7	0,3	61018,3	29063,5	4126,8	4277,9	1,1	2,4
	min	5033,7	9546	1503	0,3	-166244	-75519,5	5634,4	5033,7	0	1
	max	17842	22001	14061,7	1,7	142954,7	160091,7	22001	22000	5	14
	skew	0,6	-0,5	0,1	0,1	-0,3	1,7	0,3	0,3	0,6	0,7
<i>Pseudorca crassidens</i>	mean	6039,6	9698	3658,4	0,6	-27427,3	3030,6	7527	8426,4	1	3,8
	sd	1421,7	2031	1700,2	0,2	108389,8	19808,9	1933,4	2519,4	1	1,8
	min	2235	6249	559	0,2	-939500	-73909,1	3454	3251	0	1
	max	13870	18391	9551	1,5	132774,2	53350	14225	18391	6	10
	skew	2,1	0,9	0,4	0,7	-5,7	-0,4	1	0,7	2,1	1

3.1 Analysis of whistles from Faro populations

Results from the statistical analysis are described in table 3.2 and the violin plots in Figure 3.5 display the distributions of all variables. The results show maximum frequency values are highest for *Delphinus delphis*, mostly concentrated between 15 and 22 kHz. In contrast, the lowest values are seen in *Pseudorca crassidens*, (5 and 15 kHz), while *Tursiops truncatus* shows intermediate values, ranging from 10 to 20 kHz.

The minimum frequencies follow a similar trend, with *Delphinus delphis* again showing the highest values, typically around 9 kHz. The other two species have comparable minimum values, but *Pseudorca crassidens* displays high skewness, with a tail extending into higher frequencies. This suggests that

although most minimum values are concentrated around 6 kHz, some outliers reach much higher values.

For the frequency range, *Pseudorca crassidens* stands out with low values (mostly between 600 Hz and 5 kHz), differing significantly from the other two species, which have similar and wider ranges.

Duration values for *Delphinus delphis* clustered around 1 seconds, while *Pseudorca crassidens* shows shorter durations (around 0.6 seconds). *Tursiops truncatus* exhibits more variability, with durations similar to *Delphinus delphis*, but with several longer outliers.

Delphinus delphis and *Tursiops truncatus* tended to show positive slopes at the beginning of the whistle, whereas *Pseudorca crassidens* has a highly variable distribution with both extreme positive and negative values. *Tursiops truncatus* and *Pseudorca crassidens* showed more upward frequency toward the end of whistles, while *Delphinus delphis* often shows flatter or descending contours.

Tursiops truncatus exhibited the highest variation and complexity, with some whistles containing over 10 inflections points. In contrast, *Pseudorca crassidens* showed smoother and more stable frequency contours. A similar pattern was shown for number of steps.

The same trends are supported by the contour matrix correlation analysis (Table 3.4), which shows a strong positive correlation between *Delphinus delphis* and *Tursiops truncatus* ($r = 0.9$), indicating similar contour usage. In contrast, *Pseudorca crassidens* was negatively correlated with both species ($r = -0.5$ with DD; $r = -0.4$ with TT), confirming its distinct contour profile.

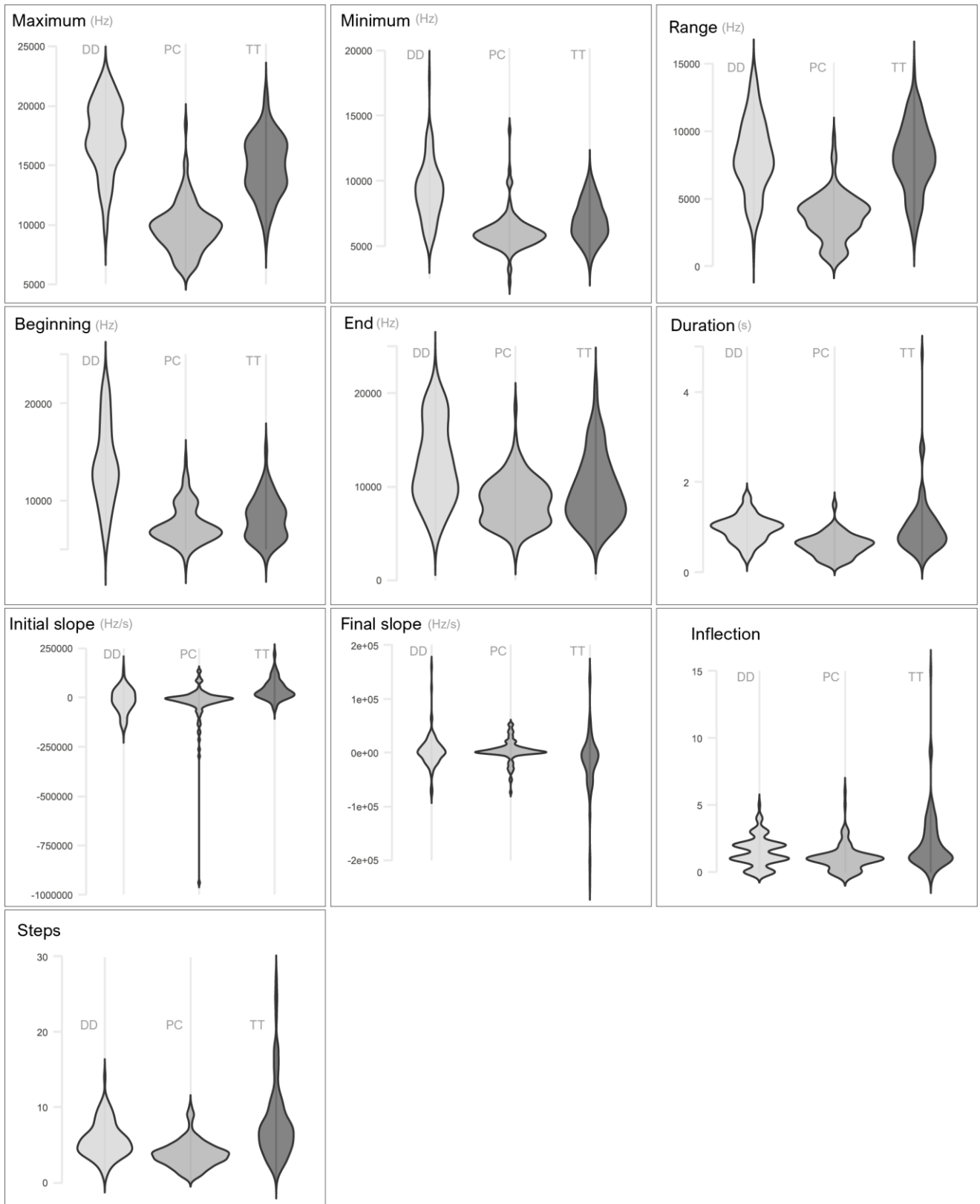


Figure 3.2 – Violin plots representing the distribution of the ten acoustic variables. From left to right: Light grey – *Delphinus delphis* (DD); grey – *Pseudorca crassidens* (PC); dark grey: *Tursiops truncatus* (TT). Each plot represents the density and spreads of values for one variable.

	<i>Delphinus delphis</i>	<i>Tursiops truncatus</i>	<i>Pseudorca crassidens</i>
Constant	4	2	31
Upsweep	11	15	25
Downsweep	10	3	14
Convex	26	42	9
Concave	19	3	14
Sine	39	44	16

Table 3.3 – Summary of whistles contour usage by the three different species.

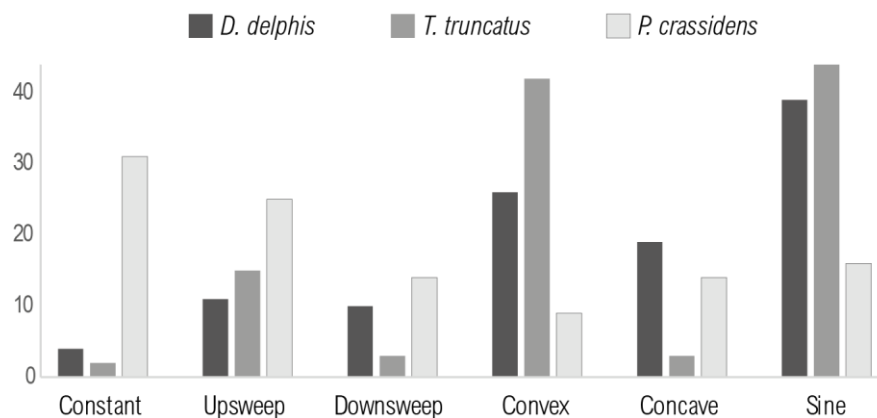


Figure 3.3 – Graphic representation of whistles contour usage by the three different species.

Table 3.4 – Matrix correlation comparing the whistles contour usage by the three different species.

<i>Delphinus delphis</i> x <i>Pseudorca crassidens</i>	-0,5
<i>Delphinus delphis</i> x <i>Tursiops truncatus</i>	0,9
<i>Pseudorca crassidens</i> x <i>Tursiops truncatus</i>	-0,4

Pairwise comparisons using adjusted p-values (from Dunn-Bonferroni correction) (table 3.5), revealed that *Delphinus delphis* and *Pseudorca crassidens* have highly significant differences in almost all measured variables ($p < 0.0001$), *Delphinus delphis* and *Tursiops truncatus* besides the significant differences in beginning, end, maximum, minimum and slopes, showed similarities in duration, range and inflection points, suggesting a more similar whistle repertoire between these two species.

Table 3.5 - Pairwise comparisons using adjusted p-values (from Dunn-Bonferroni correction). Legend: ns - Not significant; * - Significant; ** Very significant; *** Highly significant; **** Extremely significant.

Variable	<i>Delphinus delphis</i> x <i>Pseudorca crassidens</i>		<i>Delphinus delphis</i> x <i>Tursiops truncatus</i>		<i>Pseudorca crassidens</i> x <i>Tursiops truncatus</i>	
	Adj. p-value	Significance	Adj. p-value	Significance	Adj. p-value	Significance
Duration	5,92E-17	****	0,21	ns	6,96E-11	****
Range	9,58E-27	****	1	ns	1,14E-25	****
Minimum	5,38E-27	****	1,57E-11	****	2,25E-04	***
Maximum	1,89E-43	****	5,61E-05	****	1,96E-21	****
Beginning	8,20E-28	****	2,13E-20	****	0,29	ns
End	5,22E-12	****	3,18E-06	****	0,09	ns
Inicial slope	1	ns	1,27E-10	****	1,31E-13	****
Final slope	1	ns	1,35E-03	**	4,23E-05	****
Inflection	8,60E-04	***	0,21	ns	1,55E-07	****
Steps	1,83E-08	****	0,01	*	1,35E-17	****

Meanwhile, *Pseudorca crassidens* and *Tursiops truncatus* also differed significantly in most variables, particularly in range, duration, slope values, and complexity metrics (inflection points and steps), although their beginning and end frequencies did not differ significantly.

The NMDS plot (figure 3.4) illustrates distinctions among the three species in whistles feature space. *Pseudorca crassidens* forms a tight and compact cluster, located in the lower-left portion of the NMDS plot, indicating low variability and consistent acoustic repertoire. In contrast, both *Delphinus delphis* and *Tursiops truncatus* show greater dispersion, reflecting higher variability in their whistle's characteristics. These two species also exhibit substantial overlap, suggesting similar acoustic profiles for certain whistles and reduced separability in the multidimensional plot.

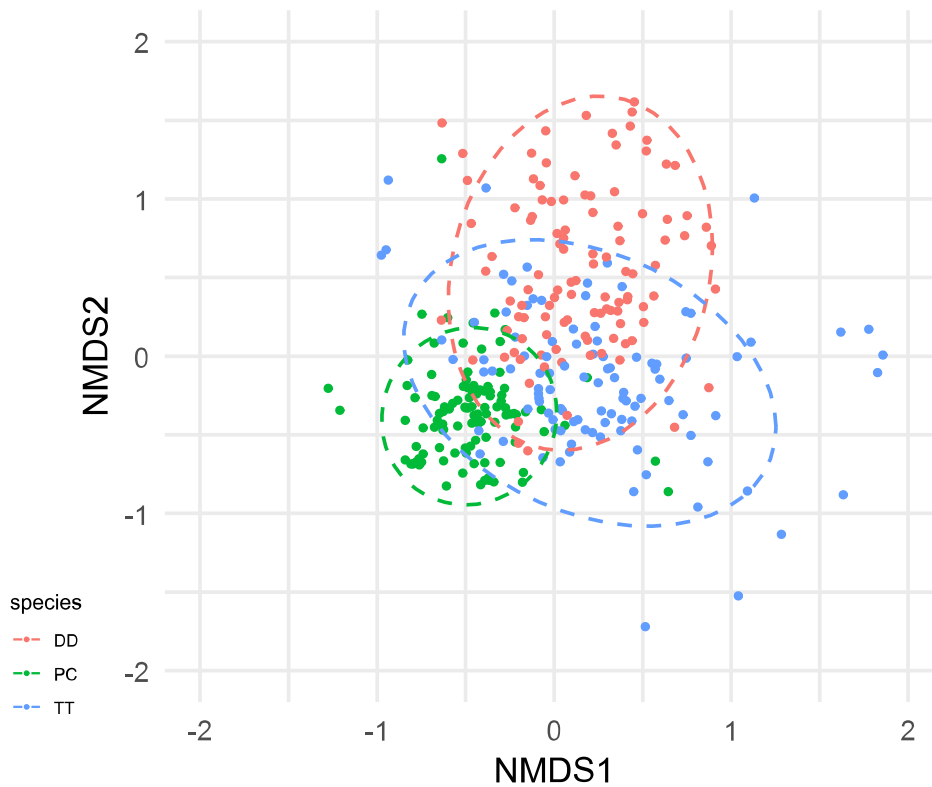


Figure 3.4 – NMDS using all variables to compare the three species analyzed in this study. DD – *Delphinus delphis*; PC – *Pseudorca crassidens*; TT – *Tursiops truncatus*

The Random Forest model achieve the highest classification accuracy for *Pseudorca crassidens*, correctly identifying 91% of its whistle, making it the best-performing group among the three species. For *Delphinus delphis* and *Tursiops truncatus*, the model correctly classified 80% and 77% of whistles, respectively. Most of the misclassifications occurred between these two species, with 19 whistles from *Delphinus delphis* misclassified as *Tursiops truncatus*, and 17 whistles from *Tursiops truncatus* misclassified as *Delphinus delphis*. In contrast, misclassification in *Pseudorca crassidens* were rare, only 3 whistles from *Delphinus delphis* and 8 from *Tursiops truncatus* were incorrectly predicted as *Pseudorca crassidens* (table 3.6).

According to the variable importance values retrieved from the Random Forest model (Figure 3.5), the top five most important variables for species classification were maximum, beginning, range, minimum and initial slope. These features reflect key aspects of whistle structure that differentiate the species.

Table 3.6 – Results from correlation matrix obtain from random forest model. DD – *Delphinus delphis*; PC – *Pseudorca crassidens*; TT – *Tursiops truncatus*. Accuracy: 82.6%; Class Error: DD=0,20, PC=0,09, TT=0,23

True species	Predicted species	Results Model RF
DD	DD	87
DD	PC	3
DD	TT	19
PC	DD	6
PC	PC	99
PC	TT	4
TT	DD	17
TT	PC	8
TT	TT	84

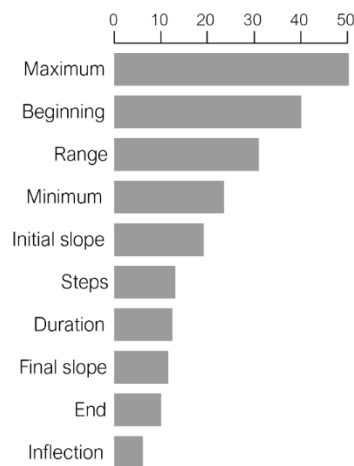


Figure 3.5 – Representation of variable importance, showing the variable with the highest importance score in the random forest model.

3.2 Acoustics characteristics comparison of Faro populations with other regions

To evaluate the geographic consistency of whistle parameters, values from the present study were compared with published data from various global regions (Figure 3.6-3.8, Table 3.7). The Faro populations of *Delphinus delphis* and *Tursiops truncatus* displayed among the highest frequency ranges and beginning frequencies. Notably, *Tursiops truncatus* in Faro exhibited one of the highest inflection counts (2,2). In contrast, *Pseudorca crassidens* showed whistle parameters with limited variation in frequency range, duration, and inflection count.

The NMDS analysis provided insight into species-level differences within the Faro dataset, however, because we only had access to regional means of whistle parameters from other regions rather than multiple observations, multivariate methods such as NMDS (that are more robust when each group has multiple observations) were not pursued further, using heatmaps and Euclidean similarity plots (Figure 3.9-3.11) as they offer direct and interpretable insights into the acoustic differences between regions.

Heatmap shows the separations between regions by variable, in the case of *Delphinus delphis* of Faro, as seen before presents high values in several variables contrasting with Tropical East Pacific (TEP-O03), that had the lowest values, however Mediterranean Sea (MS-G10) and Brazil slope region (SWA-S) were the most distinct from Faro region acoustic profile, with North Atlantic (NA-P14) and Brazil coastal region (SWA-C) being the closest, what was supported by the Euclidean distances results, however with a distance ranging 0,74-0,79 still shows some differentiation.

Tursiops truncatus showed by the heatmap high variation across all sites, Faro region dolphin whistles hadn't the highest of the values but still high compared with all regions with similarity with Western North Atlantic (WNA-BR), Sado Estuary (NA-Sado) had high values and showed Euclidean distance of 0,74 showing a degree of similarity, contrasting significantly with the low values of South Western Atlantic (SWA-UR), with 1,28 Euclidean distance.

For *Pseudorca crassidens*, similar to the other two species from Faro, showed higher values compared with other regions contrasting mostly with Mexico (TEP-Mx) (Euclidean distance of 1,94) and closer similarity with Caribbean Sea (CS) and Costa Rica (TEP-CR), however Euclidean distances ranging from 0,69-0,9.

Table 3.7 – Summary of means of whistles variables from other regions, obtain from the literature.

Delphinus delphis

Region	Code	n	Beginning	End	Minimum	Maximum	Range	Duration	Inflection	Reference
Southwestern Atlantic, Brazil (slope region)	SWA-S	2761	11,7	12,1	10,1	13,6	3,5	0,7	1,3	Pagliani et al., 2021
North Atlantic, Azores/Canary Island/Biscay	NA-P14	514	13	11,8	8,14	16,7	8,6	0,9	1	Papale et al., 2014
Mediterranean Sea	MS-P14	193	11,1	12,6	8,2	15,7	6,5	0,8	2	Papale et al., 2014
Southwestern Atlantic, Brazil (coastal region)	SWA-C	473	13,1	12,8	8,7	16,4	7,7	0,7	1,1	Pagliani et al., 2021
Mediterranean Sea	MS-G10	120	10,9	11,9	8,5	13,2	4,6	0,5	1,1	Gannier et al., 2010
North Atlantic Ocean, Celta Sea (UK)	NA-Ce	1835	12	12	9,4	14,7	5,2	0,6	0,6	Ansmann et al., 2007
North Atlantic Ocean, English Chanel (UK)	NA-EC	435	12,6	12,5	9,8	15,8	6	0,6	0,6	Ansmann et al., 2007
Tropical East Pacific	TEP-G09	1012	11,9	12,7	9,1	15,1	6	0,6	1,8	Pagliani et al., 2021
Northeast Atlantic, Deep Celta Sea	NA-DC	1443	12	12	9,6	14,5	4,3	0,6	0,5	Pagliani et al., 2021
Tropical East Pacific, USA/Mexico/Peru	TEP-O07	314	11,6	12,1	8,3	15	6,7	0,7	1,6	Oswald et al., 2007
Tropical East Pacific, Mexico/Peru	TEP-O03	88	9,8	11,4	7,4	13,6	6,3	0,8	1,2	Oswald et al., 2003
Northeast Atlantic, Faro (Portugal)	NA-Faro	109	13,648	12,428	9,178	17,352	8,173	1	1,5	Present study

Tursiops truncatus

Region	Code	n	Beginning	End	Minimum	Maximum	Range	Duration	Inflection	Reference
Southwestern Atlantic, Uruguay	SWA-Ur	4152	5,89	6,24	6,12	9,72		0,62	0,76	Tellechea, 2014
Mediterranean Sea, Croatia	MS-Cr	665	7,05	9,1	6,03	10,99	4,97	0,79	1,01	Falkner et al., 2023
Gulf of Mexico, Texas (USA)	GoM-Te	2022	8,01	8,16	5,77	11,32		0,68	2,09	Ding et al., 1995
Gulf of Mexico, Mississippi (USA)	GoM-Mi		7,48	9,83	5,94	12		0,634	3,91	Hernandez et al., 2010
Southwestern Atlantic, Brazil	SWA-Br	788	8,28	8,37	5,96	12,21		0,55	1,42	Azevedo et al., 2007
Southeast Atlantic, Namibia	SEA-Na	693	8,64	7,21	5,72	12,88	7,16	1,11	1,6	Gridley et al., 2015
Mediterranean Sea, Italy	MS-It	600	9,07	11,356	7,855	13,086		0,621	1,6	López, 2010
Southwestern Atlantic, Argentina	SWA-Ar	110	9,24	6,63	5,91	13,65		1,14	1,58	Ding et al., 1995
Tropical East Pacific, Mexico	TEP-R24	434	11,28	9,74	7,72	14,93		0,77	1,78	Rio et al., 2024
Western North Atlantic, Brazil	WNA-Br	720	9,82	8,45	6,4	15,03		0,8	1,17	Rio et al., 2025
Caribbean Sea, Venezuela	CS-Ve	518	11,01	11,44	7,52	15,87		0,73	0,88	Rio et al., 2026
Northeast Atlantic, Sado Estuary (Portugal)	NA-Sado	858	11,26	10,2	7,33	16,24		1,3	2,86	Steiner, 1981
Tropical East Pacific, USA/Mexico	TEP-O03	157	11,2	9	7,4	17,2	10	1,4	3,7	Oswald et al. 2003
Northeast Atlantic, Faro (Portugal)	NA-Faro	109	8,183	9,686	6,892	14,904	8,008	1	2,2	Present study

Pseudorca crassidens

Region	Code	n	Beginning	End	Minimum	Maximum	Range	Duration	Inflection	Reference
Caribbean Sea, Grenada	CS	1666	6,7	6,67	5,35	8,29	2,95	0,57	0,71	Rendell et al., 1999
Tropical East Pacific, Costa Rica	TEP	520	6,94	7,19	5,71	8,29	2,57	0,55	0,87	Rendell et al., 2000
Southeast Pacific, Chile	SEP				6,282	7,457	1,175	0,755	0,73	Sanino & Fowle, 2006
Tropical East Pacific, Mexico	TEP-Mx	69	5,2	5,8	4,7	6,1	1,4	0,4	0,5	Oswald et al. 2003
Northeast Atlantic, Faro (Portugal)	NA-Faro	109	7,527	8,426	6,039	9,698	3,658	0,6	1	present study

Delphinus delphis

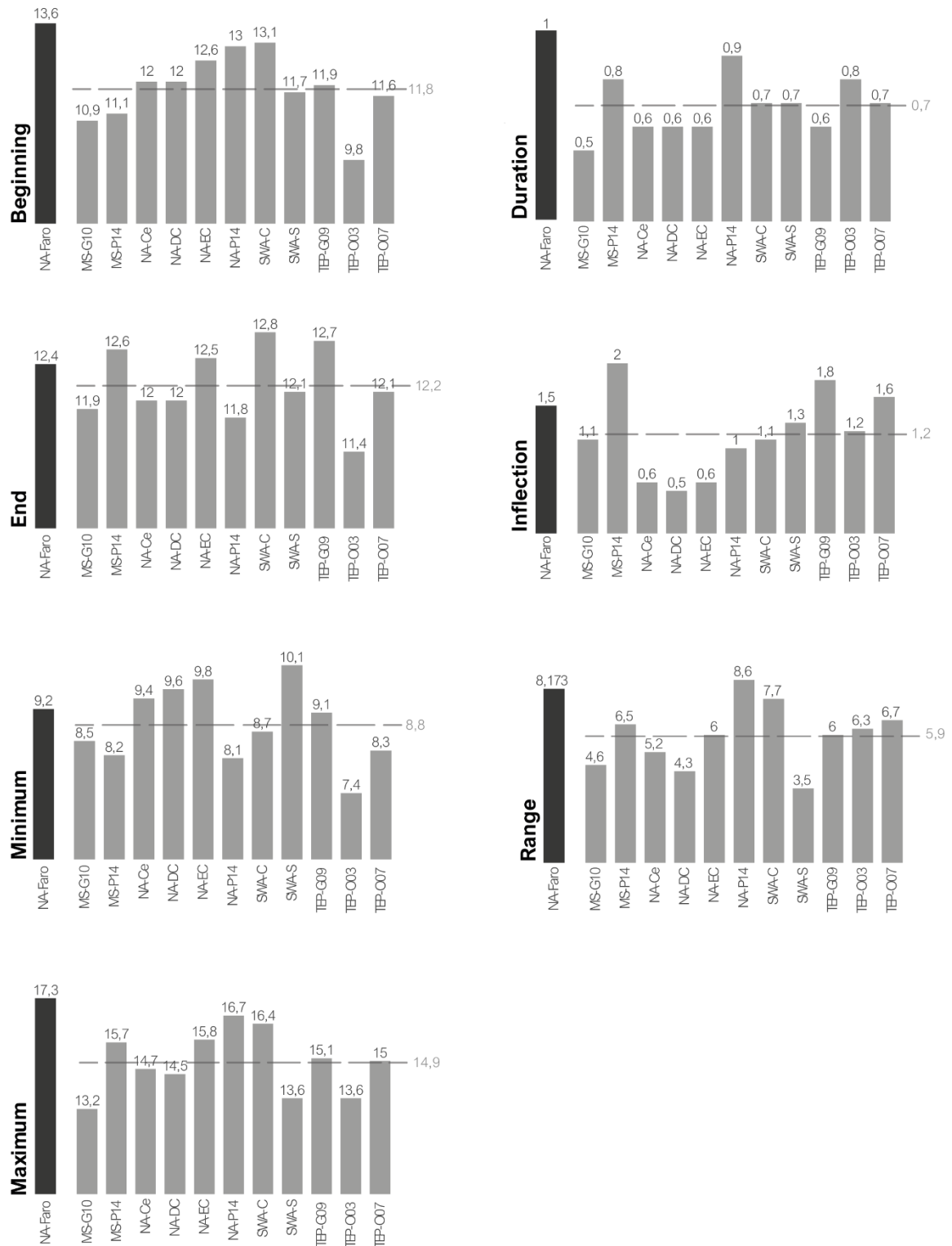


Figure 3.6 – Representation of mean values of seven acoustic variables extracted from literature from *Delphinus delphis* whistle across multiple studies of different regions. Dark Grey bars represent the mean values for the region of the present study – Faro, Portugal and light grey bars represent the mean values extracted from previous studies (Labels are on table 3.7). The horizontal dash line represents the overall average across all comparative studies for each parameter.

Tursiops truncatus

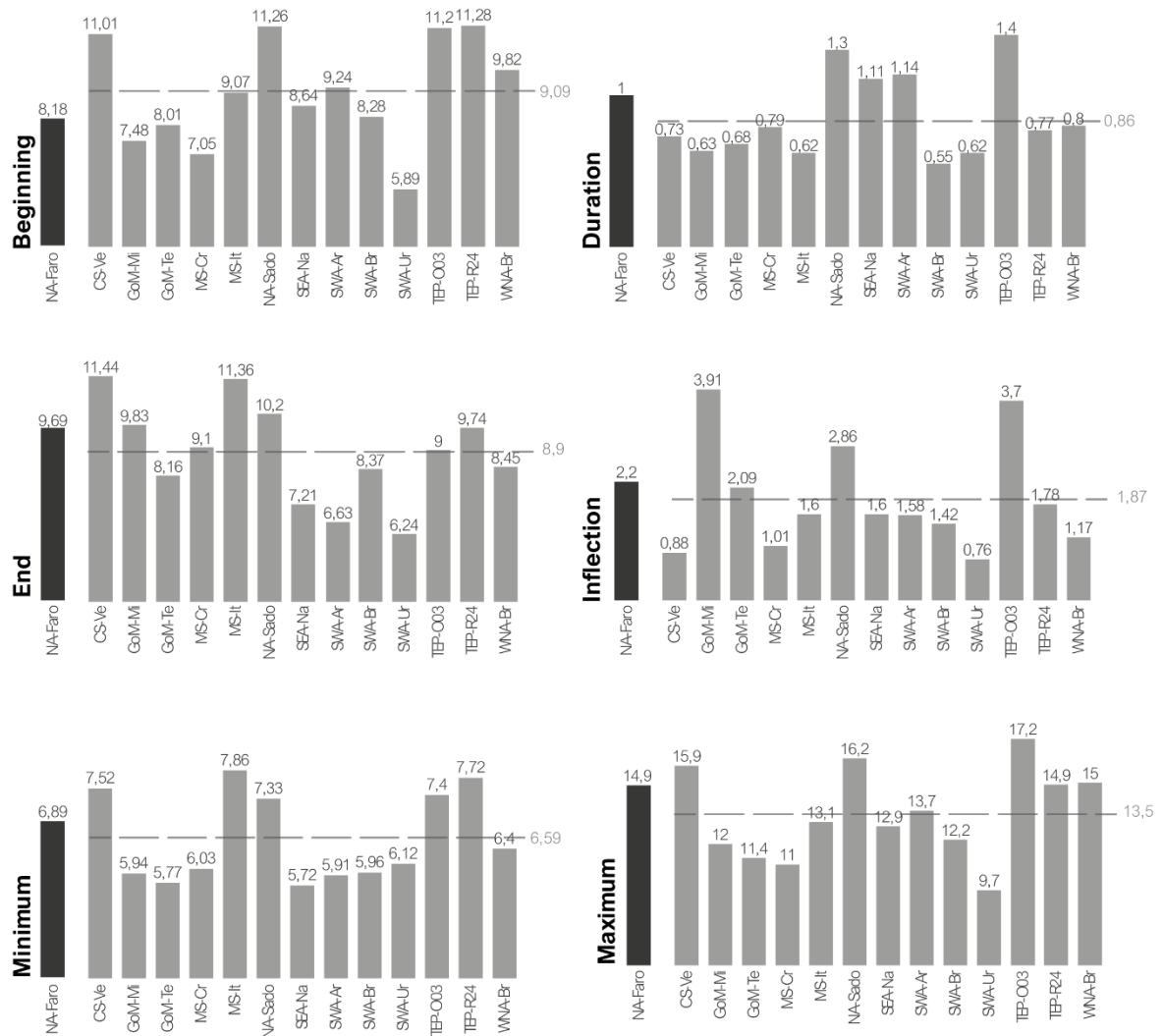


Figure 3.7 – Representation of mean values of six acoustic variables extracted from literature from *Tursiops truncatus* whistle across multiple studies of different regions. Dark Grey bars represent the mean values for the region of the present study – Faro, Portugal and light grey bars represent the mean values extracted from previous studies (Labels are on table 3.7). The horizontal dash line represents the overall average across all comparative studies for each parameter.

Pseudorca crassidens

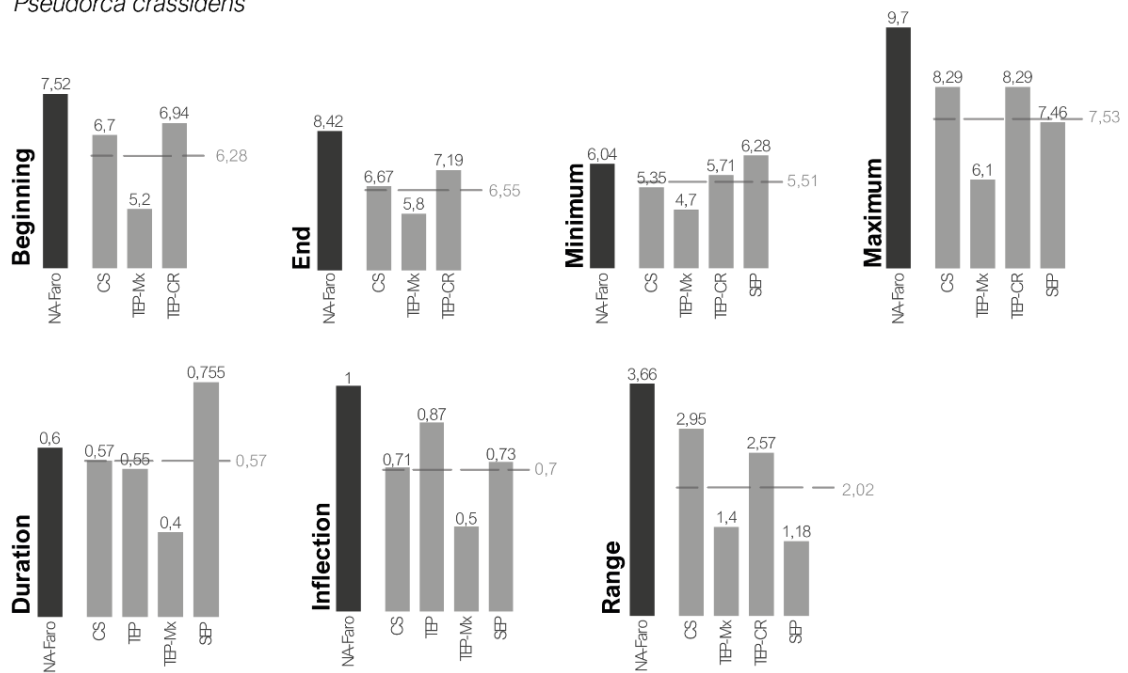


Figure 3.8 – Representation of mean values of seven acoustic variables extracted from literature from *Pseudorca Crassidens* whistle across multiple studies of different regions. Dark Grey bars represent the mean values for the region of the present study – Faro, Portugal and light grey bars represent the mean values extracted from previous studies (Labels are on table 3.7). The horizontal dash line represents the overall average across all comparative studies for each parameter.

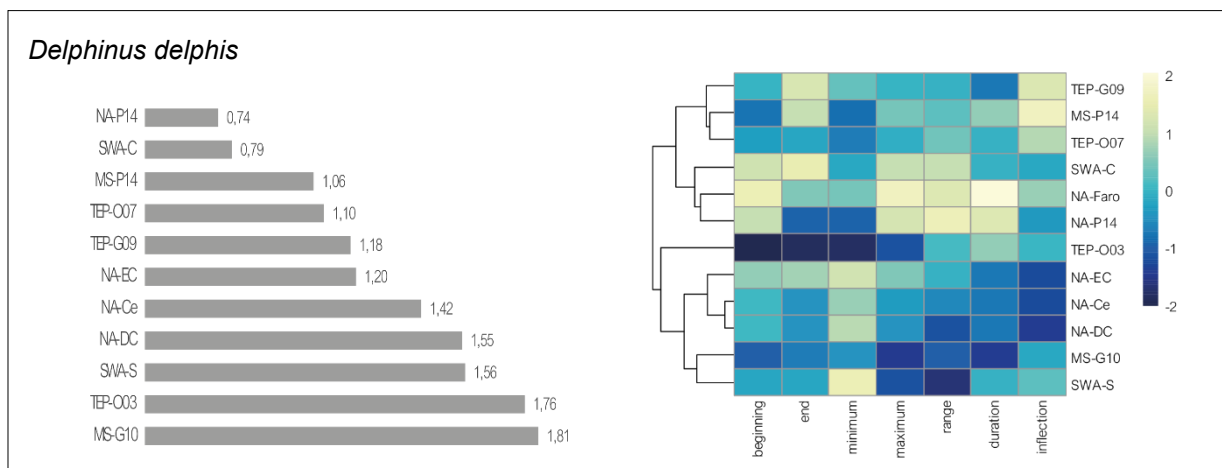


Figure 3.9 – Variation in whistle acoustic parameters of *Delphinus delphis* across different geographic populations. Left - bar plot representing the Euclidean distances referent to the population of Faro, Portugal (Present study), Lower values show high similarity. Right – Heatmap representing the comparison of standardized mean values of the six parameters for the different regional groups – darker colors (low values); lighter colors high values.

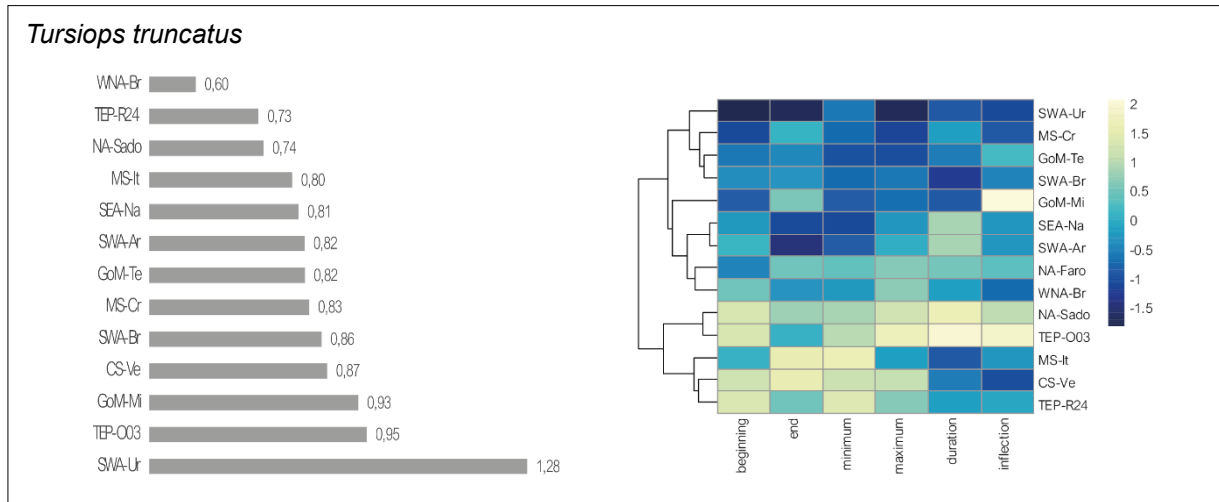


Figure 3.10 – Variation in whistle acoustic parameters of *Tursiops truncatus* across different geographic populations. Left - bar plot representing the Euclidean distances referent to the population of Faro, Portugal (Present study), Lower values show high similarity. Right – Heatmap representing the comparison of standardized mean values of the six parameters for the different regional groups – darker colors (low values); lighter colors high values.

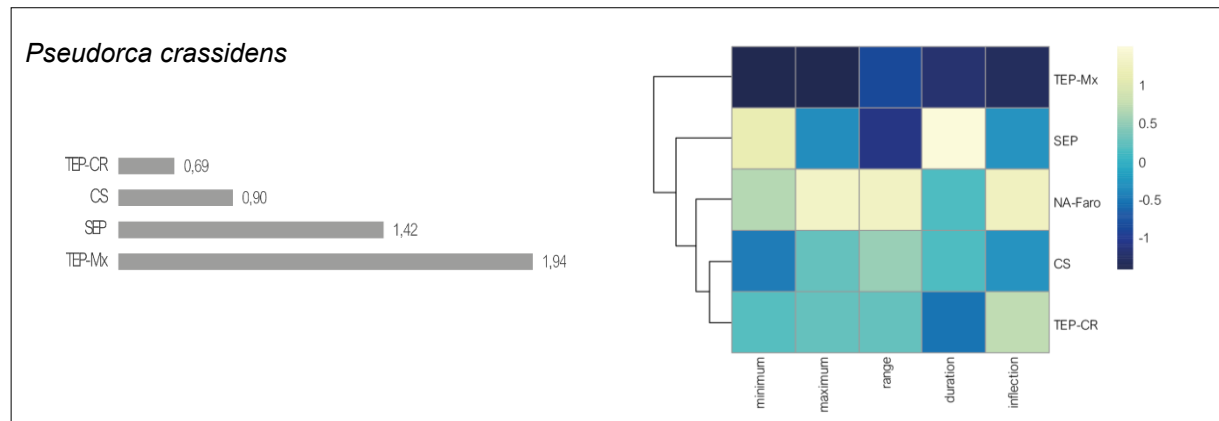


Figure 3.11 – Variation in whistle acoustic parameters of *Pseudorca crassidens* across different geographic populations. Left - bar plot representing the Euclidean distances referent to the population of Faro, Portugal (Present study), Lower values show high similarity. Right – Heatmap representing the comparison of standardized mean values of the five parameters for the different regional groups – darker colors (low values); lighter colors high values.

4. Discussion

This study aimed to characterize and compare the whistle repertoires of three odontocete species (*Delphinus delphis* - short-beaked common dolphin, *Tursiops truncatus* - common bottlenose dolphin and *Pseudorca crassidens* - false killer whale) recorded off the coast of Faro in the Northeast Atlantic. To explore geographic variation within each species, we compared the mean values of whistle parameters with published data from other regions. This approach allowed not only the local description of whistle repertoires but also the contextualization of vocalizations in terms of geographic variation and potential acoustic adaptations.

The statistical and classification analyses revealed some acoustic differences among the species. Notably, *Pseudorca crassidens* in Faro produced a consistent and acoustically distinct whistle repertoire, which the Random Forest model identified with high accuracy. In contrast, *Delphinus delphis* and *Tursiops truncatus* displayed greater variability and some overlapping features. Geographic comparisons further demonstrated significant variation in whistle features across populations, suggesting that vocal strategies are likely shaped by both evolutionary and ecological pressures.

Species-Level acoustic differences of Faro populations

In our study, the false killer whale (*Pseudorca crassidens*) population displayed a clearly distinct whistle repertoire compared to the other two species, which showed a broader variation in whistles characteristics. Short-beaked common dolphins (*Delphinus delphis*) produced whistles with comparatively higher frequencies than the other two species analyzed. In addition, the common bottlenose dolphin (*Tursiops truncatus*) exhibited the most structurally complex and varied whistle repertoire, with high occurrence of modulated whistle types such as Sine, Convex, and concave contours, while *Delphinus delphis* also revealed moderately complex structures.

This level of complexity is likely a reflection of the behavioral context. Most of our recordings were collected during feeding and social behaviors, which are described as events where communications tend to be more complex (Tellechea, 2014; Steiner, 1981; Azevedo et al., 2007). Additionally, the presence of individually distinctive vocalizations may have contributed to this complexity, reflecting a stronger presence signature whistles in our sample. A large proportion of vocal variation within cetacean

species is likely the result of vocal learning as also proposed by Ansmann et al. (2007) and Ding et al., (1995).

Supporting these assumptions, Gridley et al. (2015) and Luís et al. (2021) suggested that variability can derive from individual signature whistles and shifts in group composition or behavior. In addition, the two ecotypes of bottlenose dolphins (coastal and oceanic ecotypes), both present in the region, may have influenced whistle variation, as seen in previous studies (Antichi et al., 2023). Our results confirm this flexibility, as evidenced by the wide range of contours and structural variability observed in our recordings.

Delphinus delphis and *Tursiops truncatus* displayed some overlap in their whistle characteristics. Although some level of differentiation was present between them, these features alone were not always sufficient for perfect classification. A strong positive correlation ($r = 0.9$) between *Delphinus delphis* and *Tursiops truncatus* in the whistle contour matrix indicates a shared use of complex whistle shapes.

This similarity could reflect similar ecological adaptation strategies in these two resident species, sharing common influences, such as social structure, habitat, ecological pressures, or evolutionary traits. Besides species-specific tendencies, as previous studies also refer, considerable overlap exists among whistle characteristics of some odontocete species (Ding et al., 1995), probably also due to similarities in sound production mechanisms and local adaptations.

It is possible that certain frequency parameters are shaped by physical constraints, including the anatomy of the vocal tract or musculature, or external factors like background noise levels in their habitat (López, 2010). Previous studies have shown a negative correlation between body size and frequency. (Matthews et al., 1999; Azzolin et al., 2014) with bottlenose dolphins, which typically range from 2.5 m to 3.8 m in length (Wells & Scott, 2009), exhibiting lower overall frequencies compared to common dolphins, which are smaller, ranging from 1.6 m to 2 m (Perrin et al., 2009). Our results reflect this assumption, ranking the three species from small to large in body size correspond to a descent on whistle frequencies. Therefore, we conclude that body size was an influential factor shaping the frequency variation observed among the species analyzed.

Our results demonstrate that the acoustic behavior of odontocetes in the Faro region is shaped by a combination of species-specific traits and environmental or social contexts. These findings reinforce the importance of approaching cetacean acoustics from a region-specific point of view, recognizing that such variability is context-dependent and potentially adaptive.

Acoustics geographic Variation – Comparison within species across different regions

Our results reflect variation in dolphin whistles across different populations. For both *Tursiops truncatus*, and *Delphinus delphis*, values were highly variable across populations, suggesting a more dynamic repertoire.

Delphinus delphis, frequency parameters in this study were generally higher than those documented elsewhere, although the minimum and end frequencies were close to the mean of other regions. For *Tursiops truncatus*, there was significant variation across sites, though their overall averages in our dataset fell within the expected global average. However, geographical distance alone didn't directly determine similarities or differences in acoustic repertoire across sites. Whistles recorded in Faro from *Delphinus delphis* more closely resembled those from the North Atlantic (e.g., Macaronesia and Southwestern Atlantic) than geographically nearer populations in the Mediterranean. In contrast, *Tursiops truncatus* from Faro showed acoustic similarities not only with groups closer geographically like Sado estuary, but also with Western North Atlantic and Tropical East despite the geographic distances. This indicates that animals may adapt their signals to specific environmental conditions and also suggests some degree of population isolation. Multiple studies have suggested that both behavioral patterns and environmental context play key roles in shaping these differences. How dolphins use a particular area, whether for feeding, resting, or social interaction, can lead to distinct acoustic repertoire. More complex parameters like duration, number of inflections, and steps tend to reflect these behavioral distinctions (Rendell et al., 1999; Ansmann et al., 2007).

For example, Gridley et al. (2015) reported fine-scale variation in *Tursiops truncatus* off Namibia, while Ansmann et al. (2007) documented noticeable acoustic differences between two *Delphinus delphis* populations from the English Channel and Celtic Sea. Even though these areas are relatively close, the observed changes were

attributed to isolation. Both cases illustrate how vocal variation can emerge between populations that are geographically near, but socially or ecologically separate.

Other factors may also be at play. Group size and behavioral state have been linked to whistle variability, although Ansmann et al. (2007) found no consistent relationship and proposed that individual-level variation, possibly is due to the frequent use of signature whistles and might better explain these differences.

Noise pollution is another factor that may have shaped whistle parameters in this population's comparison and can explain why dolphin populations in Faro exhibited higher mean frequencies compared to most other populations. The southern region of Portugal encounters high fishing effort (Marçalo et al., 2015) and high pressure from touristic boat activity, making these populations exposed to high levels of vessel noise. Silva et al. (2024) observed that an increase in touristic boat presence led to significant changes in the whistle-frequency characteristics of *Delphinus delphis* and *Tursiops truncatus*, including increases in start, low, and high frequencies.

Ansmann et al. (2007) found that *Delphinus delphis* in high-noise areas produced higher-frequency whistles, observing increased frequency values in the busy English Channel compared to the quieter Celtic Sea. These findings support the acoustic niche hypothesis (Richardson et al., 1995), which proposes that dolphins adapt their signals to avoid masking by low-frequency anthropogenic noise.

Additional evidence comes from Rendell et al. (1999), who observed higher pitch and more inflections in *Globicephala spp.* from noisy regions like the Mediterranean and Tenerife. Falkner et al. (2023) also observed that dolphins exposed to high noise levels showed increased frequency values and more inflection points. Van Ginkel et al. (2017) reported that dolphins in Florida shifted both minimum and maximum frequencies by over 100 Hz per decibel of background noise. May-Collado & Wartzok (2008) further highlighted that adaptations in whistle structure may help dolphins maintain communication in noisy habitats. These studies demonstrate the remarkable vocal flexibility dolphins display in response to environmental pressures.

Methodological strengths and limitations

While this study presents clear patterns in dolphin whistle use, there are also important limitations to consider. One of the main concerns relates to the potential

sampling bias introduced during whistle selection, since longer and clearer whistles are easier to detect and analyze, they may be overrepresented in the dataset (Rendell et al., 1999). Furthermore, the traditional qualitative method used to analyze whistle contours is based on subjective visual classification and often produces highly variable results between researchers (Au & Hastings, 2008). Additionally, our whistle categorization method, based on frequency modulation, did not account for the repetition of signature whistles or their relative contribution to each species repertoire. As for the quantitative analysis, which relied on a set of acoustic features chosen for their compatibility with previous studies and ease of extraction, future work could benefit from exploring new variables that may offer lower intra-species variability and stronger inter-species discrimination.

Several other technical challenges were also encountered when analyzing dolphin sounds, that may affect our data quality. Not all recordings contained usable data, due to excessive acoustic noise, likely generated by water flow or self-noise (Au & Hastings, 2008). Low intensity dolphin whistles were often masked by this noise, which affected their clear detection and may have caused temporary interruptions in the whistle traces. These interruptions, resulting from low signal-to-noise ratios or interferences, could lead to incomplete whistle descriptions (Zimmer 2011). Improving the hydrophone-preamplifier combination could help minimize this issue, a pre-amplifier with lower input resistor reduces noise levels (Au & Hastings, 2008). Sanino and Fowle (2006) addressed the problem of self-noise by simply stabilizing their hydrophone cable during recordings, future studies should consider these technical improvements. Another challenge was the presence of multiple whistling animals, resulting in overlapping whistles which complicated the extraction process. These issues reduced the amount of data available for analysis and may have led to misinterpretations.

An alternative approach for acoustically study species is to focus on vocalizations other than whistles, such as clicks (Azzolin et al., 2014). Studies have already explored this approach (Frainer et al., 2023). However, certain acoustic features, such as echolocation clicks and burst-pulsed calls, could not be measured, because they exceeded our recording equipment's range (Gridley et al., 2015), with peak frequencies much higher than whistles, between 90 and 150 kHz (Pavan & Borsani, 1997; Oswald et al., 2007). We were also unable to estimate the distance of vocalizing individuals

from the hydrophone, which raises the possibility that some animals were outside the visual range and engaged in different behaviors than the focal group.

Another consideration is the potential for species misidentification. Although all efforts were made to ensure that no other species were present, there remains a possibility that some recordings eventually may have included unnoticed individuals belonging to other species, hydrophones can capture vocalizations over 3 km away (Oswald et al., 2003). Our own team detected distant false killer whales and bottlenose dolphins on separate occasions using the hydrophone alone (far from visual range), suggesting that some unidentified vocalizations could be present in the data.

Limited data from *Pseudorca crassidens* were obtained, all the whistles analyzed came from a single encounter limiting the extent to which we can generalize from these findings. Oswald et al. (2003) pointed out that capturing the full repertoire of a species may require recordings from multiple pods and contexts.

Bioacoustics research on wild cetaceans often faces logistical and statistical challenges. Given the high cost and difficulty of obtaining high-quality recordings in the wild, many studies, including ours, must rely on a relatively small number of opportunistic samples (Rendell et al., 1999).

The availability and consistency of data from other locations also played a role in our geographic comparisons. Differences in methodology, recording conditions, and the time periods during which data were collected in the various studies may have influenced the results. In addition, discrepancies in how whistle contours are classified, particularly when relying on subjective visual interpretation, can make comparisons across studies more difficult, potentially leading to inconsistencies or misclassification. (López, 2010; Gridley et al., 2015).

Given the limited data sample in this study, it wasn't statistically viable to separate whistles by behavior states. However, seasonal variation and behavioral context may further influence communication, as Hernández et al. (2010) and Herzing (2000) suggest. Future research could benefit from categorizing whistles by context and analyzing intra-individual variation in future research

Even within the same species, groups that share the same region but follow different life strategies might show differences in their acoustic repertoire. A good example of this is the bottlenose dolphin (*Tursiops truncatus*), which includes both coastal and oceanic ecotypes. Studies have shown that these ecotypes can differ acoustically (Antichi et al., 2023). Although they usually occupy different habitat ranges, there are areas where their distributions overlap (Ferreira et al, 2023b). In these zones, it can be difficult to tell them apart visually, which may introduce variation in the whistle data collected.

We acknowledge that while focal group sampling and random whistle selection are useful approaches, they are not without flaws. When individual identity and group dynamics cannot be tracked over time, assumptions about whistle origin or function may be speculative. Even so, this method can still offer meaningful insights when used cautiously and transparently (López, 2010).

Despite these limitations, our study offers meaningful insights and highlights the importance of combining methodological precision with technological improvements in the field of cetacean acoustic studies.

General conclusion and future directions

The results show evidence that dolphin whistles are useful for the identification of delphinid species during marine mammal surveys.

In conclusion, whistles play a central role in a variety of contexts in dolphins' lives. The factors contributing to whistle variation may differ, reflecting local conditions. Individual plasticity in dolphin whistle's structure may be adaptive when living in a continuously changing environment (e.g., changes in habitat acoustic structure). As populations become more isolated or face different environmental challenges, their acoustic repertoires are likely to evolve accordingly.

Regionally collected data are necessary to maximize detection probabilities and correct species classification, especially in passive acoustic monitoring (PAM) efforts. Software, as also suggested by Gridley et al. (2015), using local acoustic datasets greatly improves the automatic detection and accuracy in classification of species, particularly when working with closely related taxa or overlapping populations.

The frequency range used by the three species in this study, and others, overlaps considerably with sources of man-made noise (Richardson et al., 1995). This raises concerns about the potential for acoustic masking, where essential communication signals may be lost or degraded. Consequently, these species may be susceptible to masking important functional sounds from low frequency anthropogenic noise sources, including those generated by marine tourism and fishing vessels. Future management strategies must consider the possible influence of noise pollution on the communication and overall health of these coastal populations. Forli et al. (2024) found shifts in vocalizations in the presence of engine boats near bottlenose and common dolphins in the Algarve region, including Faro, with common dolphins exhibiting more pronounced changes.

This new data will contribute to the preservation of these species, which may be at risk from high maritime traffic and overfishing of fishery resources that constitute important food items for these dolphins. While this study contributes valuable data to the field, it also underscores the need for more observational and experimental work. Dolphin vocal behavior is highly dynamic, and multiple factors, such as environment, social context, and human disturbance, can influence whistle characteristics. A deeper understanding of how these variables interact will improve both species monitoring and our broader understanding of cetacean communication.

Our study highlights the importance of considering multiple influences, biological and environmental, when interpreting dolphin vocalizations. Whistle characteristics are not constant and are shaped by context, and this variability can serve as a powerful tool for monitoring species and informing conservation strategies, reinforcing the importance of combining acoustic data with ecological context to improve species knowledge and monitoring efforts, particularly in regions with overlapping dolphin populations.

5. Acknowledgements

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6. References

- Balance, L.T. (2009). Cetacean Ecology. In Encyclopedia of marine mammals (pp. 196–201). <https://doi.org/10.1016/b978-0-12-373553-9.00235-2>
- Brito, C., & Sousa, A. (2011). The Environmental History of Cetaceans in Portugal: Ten Centuries of Whale and Dolphin Records. *Plos ONE*, 6(9), e23951. Doi: 10.1371/journal.pone.0023951
- Brito, C., Vieira, N., Sá, E., & Carvalho, I. (2009). Cetaceans' occurrence off the west central Portugal coast: a compilation of data from whaling, observations of opportunity and boat-based surveys. *Marine Animals and Their Ecology*, 2(1), 10–13.
- Dudzinski, K. M., Thomas, J. A., & Gregg, J. D. (2009). Communication in marine mammals. In Elsevier ebooks (pp. 260–269). <https://doi.org/10.1016/b978-0-12-373553-9.00064-x>
- Ferreira M, Eira C, López A & Sequeira M (2023c). *Pseudorca crassidens* falsa-orca. In Livro Vermelho dos Mamíferos de Portugal Continental. Fciências.ID, ICNF, Lisboa.
- Ford, J. K. B. (2009). Dialects. In Elsevier ebooks (pp. 310–311). <https://doi.org/10.1016/b978-0-12-373553-9.00075-4>
- Gregorietti, M., Papale, E., Ceraulo, M., De Vita, C., Pace, D. S., Tranchida, G., Mazzola, S., & Buscaino, G. (2021). Acoustic Presence of Dolphins through Whistles Detection in Mediterranean Shallow Waters. *Journal of Marine Science and Engineering*, 9(1), 78. <https://doi.org/10.3390/jmse9010078>
- Herzing, D. L. (2014). Clicks, whistles and pulses: Passive and active signal use in dolphin communication. *Acta Astronautica*, 105(2), 534–537. <https://doi.org/10.1016/j.actaastro.2014.07.003>
- Jefferson, T. A., Webber, M. A., & Pitman, R. L. (2007). *Marine Mammals of the World: A Comprehensive Guide to their identification*. <http://ci.nii.ac.jp/ncid/BA8426088X>
- Kuczaj II S & Winship K (2015). How do dolphins calves make sense of their world. In *Dolphin communication and cognition: past, present, and future*. <https://lib.ugent.be/catalog/ebk01:3710000000478100>
- Llope, M. (2017). The ecosystem approach in the Gulf of Cadiz. A perspective from the southernmost European Atlantic regional sea. *ICES Journal of Marine Science*, 74(1), 382–390. <https://doi.org/10.1093/icesjms/fsw165>
- Ansmann, I. C., Goold, J. C., Evans, P. G., Simmonds, M., & Keith, S. G. (2007). Variation in the whistle characteristics of short-beaked common dolphins, *Delphinus delphis*, at two locations around the British Isles. *Journal of the Marine Biological Association of the United Kingdom*, 87(1), 19–26. <https://doi.org/10.1017/s0025315407054963>

- Antichi, S., Austin, M., May-Collado, L. J., R, J. U., Martínez-Aguilar, S., & Viloriagómora, L. (2023). Differences in the whistles of two ecotypes of bottlenose dolphins from the Gulf of California. *JASA Express Letters*, 3(5).
<https://doi.org/10.1121/10.0019502>
- Antichi, S., Manali Rege-Colt, M., Austin, M., & May-Collado, L. (2024). Whistle structure variation between two sympatric dolphin species in the Gulf of California. *Hydrobiologia*. <https://doi.org/10.1007/s10750-024-05675-3>
- Au, W. W. L., & Hastings, M. C. (2008). Principles of marine bioacoustics. In Springer ebooks. <https://doi.org/10.1007/978-0-387-78365-9>
- 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.
<https://doi.org/10.1121/1.2713726>
- De Oliveira Júnior, L., Relvas, P., & Garel, E. (2022). Kinematics of surface currents at the northern margin of the Gulf of Cádiz. *Ocean Science*, 18(4), 1183–1202.
<https://doi.org/10.5194/os-18-1183-2022>
- Ding, W., Würsig, B. & Evans, W. E. (1995). Comparisons of whistles among seven odontocete species. In R. A. Kastelein & J. A. Thomas (Eds.), *Sensory Systems of Aquatic Mammals* (pp. 299–323). De Spil Publishers; and Ding, W., Würsig, B. & Evans, W. E. (1995). Whistles of bottlenose dolphins: Comparisons among populations. *Aquatic Mammals*, 21, 65–77.
- Falkner, R., Picciulin, M., Pleslić, G., & Rako-Gospić, N. (2023). The Acoustic Repertoire of Bottlenose Dolphins (*Tursiops truncatus*) in the Cres-Lošinj Archipelago (Croatia): Site Dependent Diel and Seasonal Changes. *Diversity*, 15(6), 787.
<https://doi.org/10.3390/d15060787>
- Ferreira M, Eira C, López A & Sequeira M (2023a). *Delphinus delphis* golfinho-comum. In Livro Vermelho dos Mamíferos de Portugal Continental. Fciências.ID, ICNF, Lisboa.
- Ferreira M, Eira C, López A & Sequeira M (2023b). *Tursiops truncatus* roaz. In Livro Vermelho dos Mamíferos de Portugal Continental. Fciências.ID, ICNF, Lisboa.
- Forli, M., Santos, R. P. D., Rodrigues, A., & Castilho, R. (2024). The impact of touristic whale-watching on *Delphinus delphis* and *Tursiops truncatus* in the Algarve Coast: Combining acoustic analysis and land observations. *Ocean & Coastal Management*, 259, 107431. <https://doi.org/10.1016/j.ocecoaman.2024.107431>
- Frainer, G., Dufourq, E., Fearey, J., Dines, S., Probert, R., Elwen, S., & Gridley, T. (2023). Automatic detection and taxonomic identification of dolphin vocalizations using convolutional neural networks for passive acoustic monitoring. *Ecological Informatics*, 78, 102291. <https://doi.org/10.1016/j.ecoinf.2023.102291>
- Frankel, A. S. (2009). Sound Production. *Encyclopedia of marine mammals* (pp. 1056–1071). <https://doi.org/10.1016/b978-0-12-373553-9.00242-x>

- Gannier, A., Fuchs, S., Gannier, A., Fernandez, M., & Azevedo, J. M. (2020). Dolphin whistle repertoires around São Miguel (Azores): Are you common or spotted? *Applied Acoustics*, 161, 107169. <https://doi.org/10.1016/j.apacoust.2019.107169>
- Gridley, T., Nastasi, A., Kriesell, H., & Elwen, S. (2015). The acoustic repertoire of wild common bottlenose dolphins (*Tursiops truncatus*) in Walvis Bay, Namibia. *Bioacoustics*, 24(2), 153–174. <https://doi.org/10.1080/09524622.2015.1014851>
- Hanke W & Erdsack N (2015). Ecology and Evolution of dolphin sensory systems. In *Dolphin communication and cognition: past, present, and future*. <https://lib.ugent.be/catalog/ebk01:3710000000478100>
- Hernandez, E. N., Solangi, M., & Kuczaj, S. A. (2010). Time and frequency parameters of bottlenose dolphin whistles as predictors of surface behavior in the Mississippi Sound. *The Journal of the Acoustical Society of America*, 127(5), 3232–3238. <https://doi.org/10.1121/1.3365254>
- Herzing, D. L. (2000). Acoustics and Social Behavior of Wild dolphins: Implications for a Sound Society. In *Springer handbook of auditory research* (pp. 225–272). https://doi.org/10.1007/978-1-4612-1150-1_5
- Herzing, D. L., & Johnson, C. M. (2015). Dolphin communication and cognition: past, present, and future. <https://lib.ugent.be/catalog/ebk01:3710000000478100>
- Kershenbaum, A., Sayigh, L. S., & Janik, V. M. (2013). The encoding of individual identity in dolphin signature whistles: How much information is needed? *Plos ONE*, 8(10), e77671. <https://doi.org/10.1371/journal.pone.0077671>
- Lafuente, J. & Ruiz, Javier. (2007). The Gulf of Cádiz pelagic ecosystem: A review. *Progress in Oceanography* 74. 228-251. [10.1016/j.pocean.2007.04.001](https://doi.org/10.1016/j.pocean.2007.04.001).
- Lammers M & Oswald J (2015). Analyzing the acoustic communication of dolphins. In *Dolphin communication and cognition: past, present, and future*. <https://lib.ugent.be/catalog/ebk01:3710000000478100>
- Lehnhoff, Loïc & Glotin, Hervé & Gall, Yves & Menut, Eric & Peltier, Helene & Spitz, Jerome & Canneyt, Olivier & Mérigot, Bastien. (2025). High resolution acoustic recordings of wild free-ranging short-beaked common dolphins for etho-acoustical and repertoire studies. [10.5194/essd-2025-193](https://doi.org/10.5194/essd-2025-193).
- Liaw A, Wiener M. (2002). Classification and regression by randomforest. *R News*, 2:18–22.
- López, B. D. (2010). Whistle characteristics in free-ranging bottlenose dolphins (*Tursiops truncatus*) in the Mediterranean Sea: Influence of behaviour. *Mammalian Biology*, 76(2), 180–189. <https://doi.org/10.1016/j.mambio.2010.06.006>
- Luís, A. R., May-Collado, L. J., Rako-Gospić, N., Gridley, T., Papale, E., Azevedo, A., Silva, M. A., Buscaino, G., Herzing, D., & Santos, M. E. D. (2021). Vocal universals and

geographic variations in the acoustic repertoire of the common bottlenose dolphin. *Scientific Reports*, 11(1). <https://doi.org/10.1038/s41598-021-90710-9>

- Marçalo, A., Katara, I., Feijó, D., Araújo, H., Oliveira, I., Santos, J., Ferreira, M., Monteiro, S., Pierce, G. J., Silva, A., & Vingada, J. (2015). Quantification of interactions between the Portuguese sardine purse-seine fishery and cetaceans. *ICES Journal of Marine Science*, 72(8), 2438–2449. <https://doi.org/10.1093/icesjms/fsv076>
- Mathias ML (coord.), Fonseca C, Rodrigues L, Grilo C, Lopes-Fernandes M, Palmeirim JM, Santos-Reis M, Alves PC, Cabral JA, Ferreira M, Mira A, Eira C, Negrões N, Paupério J, Pita R, Rainho A, Rosalino LM, Tapisso JT & Vingada J (eds.). (2023). Livro Vermelho dos Mamíferos de Portugal Continental. Fciências.ID, ICNF, Lisboa.
- Matthews, J. N. S., Rendell, L., Gordon, J., & Macdonald, D. W. (1999). A REVIEW OF FREQUENCY AND TIME PARAMETERS OF CETACEAN TONAL CALLS. *Bioacoustics - the International Journal of Animal Sound and Its Recording*, 10(1), 47–71. <https://doi.org/10.1080/09524622.1999.9753418>
- 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. <https://doi.org/10.1644/07-mamm-a-310.1>
- Moore, P. W. (1997). CETACEAN AUDITORY PSYCHOPHYSICS. *Bioacoustics - the International Journal of Animal Sound and Its Recording*, 8(1–2), 61–78. <https://doi.org/10.1080/09524622.1997.9753354>
- Mulero-Martinez, R., Gómez-Enri, J., De Oliveira Júnior, L., Garel, E., Relvas, P., & Mañanes, R. (2024). Spatiotemporal variability of the coastal circulation in the northern Gulf of Cadiz from Copernicus Sentinel-3A satellite radar altimetry measurements. *Advances in Space Research*, 73(10), 4922–4936. <https://doi.org/10.1016/j.asr.2024.02.054>
- 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), 20–037. <https://doi.org/10.1111/j.1748-7692.2003.tb01090.x>
- Oswald, J. N., Rankin, S., Barlow, J., & Lammers, M. O. (2007). A tool for real-time acoustic species identification of *delphinid* whistles. *Journal of the Acoustical Society of America*, 122(1), 587–595. <https://doi.org/10.1121/1.2743157>
- Pagliani, B., Amorim, T. O. S., De Castro, F. R., & Andriolo, A. (2021). Intraspecific variation in short-beaked common dolphin's whistle repertoire. *Bioacoustics*, 31(1), 1–16. <https://doi.org/10.1080/09524622.2020.1858449>
- Papale, E. B., Azzolin, M. A., Cascão, I., Gannier, A., Lammers, M. O., Martin, V. M., Oswald, J. N., Perez-Gil, M., Prieto, R., Silva, M. A., Torri, M., & Giacoma, C. (2021). Dolphin whistles can be useful tools in identifying units of conservation. *BMC Zoology*, 6(1). <https://doi.org/10.1186/s40850-021-00085-7>

- Pavan, G., & Borsani, J. F. (1997). Bioacoustic research on cetaceans in the Mediterranean Sea. *Marine and Freshwater Behaviour and Physiology*, 30(2), 99–123. <https://doi.org/10.1080/10236249709379019>
- Perrin, W. F., Würsig, B. G., & Thewissen, J. G. M. (2009). *Encyclopedia of Marine Mammals*. In Elsevier ebooks. <https://doi.org/10.1016/b978-0-12-373553-9.x0001-6>
- 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. <https://doi.org/10.1111/j.1469-7998.1999.tb01209.x>
- Richardson, W. J., Greene, C. R., Jr, Malme, C. I., & Thomson, D. H. (1995). *Marine Mammals and Noise*. Academic Press. <https://doi.org/10.1093/icesjms/fsw165>
- Rio, R., Romero-Mujalli, D., Cobarrubia-Russo, S., Nanduca, H. R., Vieites, F. M., De Freitas, T. R. O., & Hoffmann, L. S. (2024). Comparison of common bottlenose dolphin whistles in tropical waters. *Marine Mammal Science*, 40(3). <https://doi.org/10.1111/mms.13122>
- Sanino, G. P., & Fowle, H. L. (2006). Study of whistle spatio-temporal distribution and repertoire of a school of false killer whales, *Pseudorca crassidens*, in the eastern south pacific. *Boletín Museo Nacional De Historia Natural*, 55, 21–39. <https://doi.org/10.54830/bmnhn.v55.2006.268>
- Sayigh, L. S., & Janik, V. M. (2009). Signature whistles. In *Encyclopedia of marine mammals* (pp. 1014–1016). <https://doi.org/10.1016/b978-0-12-373553-9.00235-2>
- Silva, A. A., Castro, J., Cid, A., Jesus, S. M., & Matos, F. L. (2024). Influence of Dolphin-Watching tourism vessels on the whistle emission pattern of common dolphins and bottlenose dolphins. *Oceans*, 5(4), 770–784. <https://doi.org/10.3390/oceans5040044>
- Steiner, W. W. (1981). Species-specific differences in pure tonal whistle vocalizations of five western North Atlantic dolphin species. *Behavioral Ecology and Sociobiology*, 9(4), 241–246. <https://doi.org/10.1007/bf00299878>
- Tellechea, J. (2014). Passive Acoustic Monitoring of Bottlenose Dolphins (*Tursiops truncatus*) on the Uruguayan Coast: Vocal Characteristics and Seasonal Cycles. *Aquatic Mammals*, 40(2), 173–184. <https://doi.org/10.1578/am.40.2.2014.173>
- Van Ginkel, C., Becker, D. M., Gowans, S., & Simard, P. (2017). Whistling in a noisy ocean: bottlenose dolphins adjust whistle frequencies in response to real-time ambient noise levels. *Bioacoustics*, 27(4), 391–405. <https://doi.org/10.1080/09524622.2017.1359670>

Wells, R. S. & Scott, M. D. (2009). Common Bottlenose Dolphin. In Encyclopedia of marine mammals (pp. 255–259). <https://doi.org/10.1016/b978-0-12-373553-9.00235-2>

Zimmer, W. (2011). Passive acoustic monitoring of cetaceans. <https://doi.org/10.1017/cbo9780511977107>

7. Supplementary material

Table S1 – Information about the acoustic files used in the study featuring date of recording, species in visual contact during the recording, number of whistles analyse and extracted from the file and general behavior registered, by observation, during recordings. Species TT, DD and PC correspond respectively to *Tursiops truncatus*, *Delphinus delphis* and *Pseudorca crassidens*. Behaviour: SOC (socialization); F (Feeding/foradging); T (Travelling); M (Milling) and ND (no data available).

Filename	Day of recording	Species	n° whistles	Behavior
ZOOM0004	28/04/2022	TT	13	SOC
ZOOM0006	30/04/2022	TT	2	F
ZOOM0018	06/05/2022	TT	7	F
ZOOM0019	09/05/2022	TT	9	SOC
ZOOM0020	17/05/2022	TT	2	F
ZOOM0022	19/05/2022	TT	4	F
ZOOM0023	19/05/2022	TT	9	F
ZOOM0028	28/05/2022	TT	24	F
ZOOM0031	31/05/2022	TT	4	SOC
ZOOM0033	05/06/2022	DD	4	F
ZOOM0036	29/06/2022	DD	32	F
ZOOM0037	01/07/2022	TT	2	SOC
ZOOM0039	22/07/2022	TT	4	T
ZOOM0040	17/10/2022	DD	4	F
ZOOM0044	17/12/2022	DD	3	ND
ZOOM0047	30/12/2022	DD	5	ND
ZOOM0070	10/05/2023	TT	3	T
ZOOM0085	24/05/2023	DD	6	F
ZOOM0126	27/06/2023	DD	5	ND
ZOOM0133	02/07/2023	DD	10	F
ZOOM0148	14/07/2023	DD	20	T
ZOOM0158	10/09/2023	TT	2	T
ZOOM0159	10/09/2023	TT	1	SOC
ZOOM0160	11/09/2023	TT	5	SOC
ZOOM0162	14/09/2023	TT	4	M
ZOOM0164	19/09/2023	TT	3	SOC
ZOOM0171	26/09/2023	TT	4	T
ZOOM0185	11/10/2023	TT	3	SOC
ZOOM0199	16/11/2023	TT	1	M
ZOOM0208	11/01/2024	TT	1	SOC
ZOOM0213	11/01/2024	TT	2	SOC
ZOOM0217	07/02/2024	DD	9	F
ZOOM0236	19/03/2024	DD	4	F
ZOOM0240	19/03/2024	DD	6	F
ZOOM0262	31/05/2024	DD	1	F
F4ZM0002	06/06/2024	PC	109	F