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MULTI-REGIONAL ACOUSTIC REPERTOIRES OF BOTTLENOSE DOLPHINS:

COMMON THEMES, GEOGRAPHICAL VARIATIONS AND ECOLOGICAL FACTORS

Ana Rita Francisco Luís

This thesis is submitted in partial fulfilment for the degree of PhD in

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FCT
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MINISTÉRIO DA CIÊNCIA, TECNOLOGIA E ENSINO SUPERIOR

À pequena Carolina: que continues sempre a ser aventureira,

destemida e a inventar muito.

"*On ne découvre pas de terre nouvelle*

sans consentir à perdre de vue, d'abord et longtemps, tout rivage."

André Gide

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Palavras-chave

Golfinhos-roazes; Comunicação animal; Repertório acústico; Variação geográfica

Keywords

Common bottlenose dolphins; Animal communication; Acoustic repertoire; Geographic variation

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RESUMO

Os golfinhos-roazes (*Tursiops truncatus*) são conhecidos por produzirem uma multiplicidade de sons, tanto para comunicação como para ecolocalização. O seu repertório acústico inclui sinais tonais com modulação de frequência – assobios, emissões curtas, de banda-larga e alta-frequência, os cliques de ecolocalização, e "pacotes" de pulsos de banda-larga e elevada taxa de repetição – sons pulsados.

Esta tese centra-se no repertório acústico alargado da espécie *T. truncatus* e disponibiliza descrições detalhadas dos temas comuns, tanto a uma escala local com a uma escala geográfica alargada. Através de um estudo comparativo multi-regional, as (dis)similaridades no repertório foram avaliadas e as variações acústicas foram documentadas para nove populações de golfinhos-roazes do oceano Atlântico e do mar Mediterrâneo. A ocorrência de vocalizações universais em populações geograficamente distantes, mas também a variabilidade intraespecífica observada em grupos simpátricos que apresentam diferentes características eco-etológicas, sublinha a importância de fatores ambientais na modelação das emissões acústicas destes golfinhos.

Ao estudar a estabilidade a longo-termo de assobios estereotipados, e a produção abundante de assobios não estereotipados em contextos alimentares específicos, foi possível corroborar o papel dos assobios como sinais de identidade.

De modo a contribuir para um melhor entendimento acerca dos diferentes tipos de sinais que são usualmente nomeados como "Sons pulsados", uma combinação de representações gráficas – sonogramas e dados quantitativos são aqui apresentados. Esta abordagem revelou que "rangidos", "chorincos" e "*buzzes*", vulgarmente agrupados em estudos de repertórios acústicos, são sinais acústicos distintos com diferenças significativas ao nível das suas características temporais e de frequência.

Sequências rítmicas são também uma componente importante do repertório acústico dos golfinhos. Neste estudo, os zurros gravados no estuário do Sado, em Portugal, foram utilizados como base para a caracterização estrutural dos diferentes elementos acústicos que compõem estas sequências repetitivas, e técnicas de teoria da informação foram aplicadas para analisar a ordem dos elementos e a complexidade das sequências. Características-chave dos zurros mostram que estas vocalizações são emitidas de modo não-aleatório, o que sugere a presença de conteúdo informativo relevante, uma nova perspectiva sobre estas emissões acústicas mal conhecidas.

Finalmente, a influência do tráfego marítimo, especialmente o ruído gerado pelos navios, nos repertórios acústicos de golfinhos-roazes não deve ser subvalorizado, uma vez que as embarcações contribuem marcadamente para a paisagem acústica subaquática atual. Decréscimos significativos nas taxas de emissão e alterações temporárias nas características espectrais dos assobios, aqui documentadas, revelam a existência de respostas acústicas à proximidade de embarcações, e adaptações locais a um ambiente mais ruidoso.

Uma descrição enquadrada dos diferentes elementos vocais que fazem parte do repertório acústico da espécie golfinho-roaz, bem como da influência de fatores ecológicos, nomeadamente o ruído produzido por embarcações, aqui apresentados, pretendem ser contributos substanciais para o conhecimento do sistema de comunicação acústica desta espécie que deveriam ser utilizados em esforços de conservação.

ABSTRACT

Common bottlenose dolphins (*Tursiops truncatus*) are known to produce a multiplicity of sounds, both for communication and echolocation. Their acoustic repertoire includes tonal, frequency modulated signals – whistles, short broadband high-frequency emissions, echolocation clicks, and packets of high-repetition, broadband pulses – burst-pulses.

This thesis focuses on the extended acoustic repertoire of *T. truncatus* and provides detailed descriptions of the common themes, both at a local scale and at a broad geographical scale. Through a comparative multi-regional study, repertoire (dis)similarity was assessed and acoustical variation was documented for nine bottlenose dolphin populations, across the Atlantic Ocean and the Mediterranean Sea. The occurrence of vocal universals in geographically distant populations, but also the observed intraspecific variability in sympatric groups that exhibit different eco-ethological characteristics, highlight the importance of environmental factors in shaping the acoustic emissions of these dolphins.

By studying the long-term stability of stereotyped whistles, and the abundant production of non-stereotyped whistles in specific food-related contexts, the role of whistles as identity calls was given further support.

In order to contribute to a better understanding of the different signal types often labelled as “BP-sounds”, a combination of graphical representations - spectrograms and quantitative data is here presented. This approach revealed that “creaks”, “squawks” and “short-burst-pulses”, usually lumped together in acoustic repertoire studies, are distinct signal types with significant differences in their time-frequency features.

Rhythmic call sequences are also important components of dolphin vocal behaviour. Here, bray series recorded in the Sado estuary, Portugal were used to provide a structural characterization of the discrete elements that compose these repetitive sequences, and information theory techniques were applied to analyze order of elements and explore the complexity of sequences. The key features of brays show that these vocalizations are emitted in non-random ways, which suggests the presence of relevant information content, a novel perspective for these poorly known vocal emissions.

Finally, the influence of maritime traffic, especially the noise it generates, on the acoustic repertoires of bottlenose dolphins should not be overlooked, as vessels are major contributors to the current ocean soundscape. Significant decreases in the mean overall call rates and temporary shifts in whistles characteristics, here documented, reveal vocal responses to the proximity of operating vessels, and local adaptations to a noisier environment.

The comprehensive description of the different vocal elements that comprise the acoustic repertoire of *T. truncatus* and the influence of ecological factors, such as the habitat type and vessel noise, here presented, aim to be substantial contributions to the knowledge of this species’ acoustic communication system that should be used in conservation efforts.

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CHAPTER 1

GENERAL INTRODUCTION

1.1. BACKGROUND AND OBJETIVES

Bottlenose dolphins, as other cetaceans, are acoustically adapted animals, with specialized cognitive and communicative skills (Delfour & Marten, 2006; Herman, 2010; Pack & Herman, 2004). Their vocal learning and mimetic abilities have been the focus of research in the last decades (Janik, 2000a; Janik & Slater, 2000; McCowan & Reiss, 1995, King, Sayigh, Wells, Fellner & Janik, 2013; Tyack, 1998). Much less is known about the influence of eco-ethological factors on the global acoustic repertoire of this species, or regarding the occurrence of intraspecific repertoire varieties in different sympatric and allopatric populations.

The first studies on bottlenose dolphins' acoustic communication were published in the early 1960s and described vocal exchanges (Lilly & Miller, 1961; Caldwell & Caldwell, 1965). Since then, vocal repertoires of different populations have been documented worldwide, both in captivity and in the wild. However, most of the research effort has been focused in the study of tonal signals - whistles, rather than the extended repertoire of the species. Although whistles play a crucial role in group cohesion, individual recognition and coordination during foraging activities (Caldwell, Caldwell, & Tyack, 1990; Janik, 2000a), the importance of non-tonal signals should not be overlooked. Other difficulties related with methodological aspects, namely the frequent use of spectrally limited acoustic equipment and softwares, the lack of detailed descriptions of signal types, the lump of different acoustic emissions under the same designation or the use of numerous terminologies for similar signal types, are responsible for the currently incomplete assessment and understanding of the bottlenose dolphins' global repertoire.

The multiplicity of studies available on this species, and on its congeneric Indo-Pacific form (*T. aduncus*), have shown considerable geographic variation in signal emissions, made more difficult to understand due to variable terminologies used by the different authors. An assessment of these regional repertoires and the patterns of signal use would be necessary and important, and that is one of the purposes of this thesis.

The goal of this study will be to investigate the extended acoustic repertoire of bottlenose dolphins, through a novel perspective combining the analysis of published and unpublished data, observational studies, acoustic recordings, and different statistical methods.

This thesis aims to provide a detailed description the common themes in bottlenose dolphins' acoustic repertoire and address the influence of eco-ethological factors in signal emission. Additionally, new insights regarding the issues of geographical variation, signal stability through time, and acoustic complexity will be present.

1.2. ANIMAL COMMUNICATION

Communication is a key aspect of life in animal societies considering that signaling mechanisms are an essential part of social interactions, and may often significantly affect survival and reproductive success.

In general, communication can be defined as the exchange of information between a sender and a receiver (or multiple receivers) that leads to a behavioural response (Simmons, 2003). This interaction between individuals is mediated by signal emission in different sensory modalities. In the specific case of acoustic communication, signals are received through the auditory channel and can be produced either using special sound production structures, or by interacting with a resonant substrate.

Acoustic forms of communication are widely spread across taxa, likely due to the efficiency of sound signals (e. g. transmission over long distance, transitory nature of the signal, ability to convey information in low visibility environment, time-frequency features that enable variability and complexity). However, studies on the mechanisms and functions in acoustic communication systems are mostly based in insects, anurans, birds and primates (Janik, 2009).

Acoustic communication is an integral part of animal behaviour, and acoustic signals have been described in a broad range of behavioural contexts, namely in interspecific, kinship and individual recognition (e.g.: anurans' choruses, sperm whales' codas, and bats' isolation calls), mate attraction and courtship (e.g.: birds' duets, humpback whales' songs), alarm/depredation responses (e.g. vervet monkeys' alarm calls), and agonistic encounters (e.g. chimpanzees' or lynxes' screams, damselfishes' pops and chirps).

Although anatomical structures (specialized or not) are the basis of sound production and reception, acoustic communication is strongly influenced by ecological and/or social factors. When a sound is produced, its spectral properties are limited by physiological and anatomical constrains (e.g. body size, often correlated with gender). However, in their transmission path, acoustic signals undergo structural changes, and environmental conditions will determine how far and how clear signals will propagate. To communicate the sender has to produce a signal that can be perceived by the receiver. Several studies suggest that selection has favored signals that maximize transmission in the species-typical environment and the idea that communication signals' features reflect the acoustic habitat of a species is known as the Acoustic Adaptation Hypothesis (see Ey & Fisher, 2009 for a review).

Despite these intrinsic and extrinsic constrains to communication, the sender has some control over the signal emission and may adjust it to the audience. According with the Social Complexity Hypothesis for Communication (Freeberg, Dunbar, & Ord, 2012), more elaborate communication systems are required for animals that live in more complex social environments. Social aspects such as group size and composition, or the specific behavioural context of emission, are known to influence acoustic emissions (e.g., in Indo-Pacific bottlenose dolphins, whistle production begins to decrease in group sizes of 10 or more (Hawkins & Gartside, 2010), and killer whales exhibit distinct vocal behaviour depending their dietary specialization (Barrett-Lennard, Ford, & Heise, 1996; Deecke, Ford, & Slater, 2005). For cetacean groups, there is a clear link between communication signal types and the types of social bonds: stable groups or clans (e.g. killer whales and sperm whales) exhibit group-specific repertoires (Tyack & Clark, 2000), whereas some fission-fusion groups (e.g. bottlenose dolphins) present individual-specific signals.

Overall, the production of acoustic signals presents some flexibility, and can be fine-tuned according to the eco-ethological scenario. For some species, sound production has been taken to another level, as individuals are able modify their acoustic repertoires based on auditory stimulus/references (Janik & Slater, 2000). This auditory specialization – vocal production learning - allows imitation and improvisation, and may add new elements to a species acoustic repertoire. Vocal learning has been documented for only a few taxa, namely birds, bats and cetaceans (Janik & Slater, 1998; Tyack & Clark, 2000), which suggests that this degree of flexibility in the auditory-vocal system may be a rare process. Another particularly specialized form of acoustic communication is present only in microchiropteran bats and cetaceans, which use high-frequency sounds echo to produce images of their surrounding environment. Echolocation enables prey detection (Au, 1993) but it may also act as a food-related cue for conspecifics or alarm signals for preys (Fichtel & Manser, 2010). Both vocal learning and echolocation are rare sensory-motor skills that require feedback between neural centers responsible for vocal apparatus control and auditory input. Moreover, the ability to discriminate short time intervals and the sensitivity to high frequencies, which are key requirements for echolocation, might be advantageous features for vocal learners, such as the common bottlenose dolphin, since these characteristics about fine-grid discrimination and provide a wider spectral range of stimuli.

1.3 THE COMMON BOTTLENOSE DOLPHIN

The common bottlenose dolphin *Tursiops truncatus* (Montagu, 1821) is mid-sized delphinid that inhabits temperate and tropical waters worldwide (Evans, 1987, Wells & Scott, 2018). This species is listed as Least Concern in the The IUCN Red List of Threatened Species, although it is recognized that distinct populations/sub-species are under threat (e.g. Sado estuary, Portugal; Black Sea).

Currently, three subspecies of common bottlenose dolphins are proposed, based on morphological and genetic differences (Wells, Natoli, & Braulik, 2019; IWC, 2018): the bottlenose dolphins that occur in the Black Sea - *Tursiops truncatus ponticus* (Barabash-Nikiforov, 1940), a larger form from the coastal waters of the western South Atlantic Ocean - *Tursiops truncatus gephyreus* (Lahille, 1908), and the remaining common bottlenose dolphins worldwide - *Tursiops truncatus* (Montagu, 1821).

Additionally, two distinct ecotypes of *Tursiops truncatus* have been recognized, according with morphological and eco-ethological specificities: i) coastal or inshore ecotype is smaller and with lighter coloration, inhabits coastal and inshore waters, and forms small populations that often exhibit residency patterns, ii) oceanic or offshore ecotype is larger, darker in coloration, occurs in offshore habitats, such as open ocean and oceanic islands, and forms large pods up to thousands of individuals that spread over broad areas (see Wells & Scott, 2018). Genetic analyses support this classification for North Atlantic and South-West Atlantic populations (e.g., Hoelzel, Potter, & Best, 1998; Fruet et al., 2017). Furthermore, regional studies have reported marked differences in the eastern and western basins of the Mediterranean Sea (Natoli, Peddemors, & Hoelzel, 2004), as well as between coastal populations of the Gulf of Mexico and the Northern Bahamas (Parsons et al. 2006). The taxonomy of the genus *Tursiops* is under revision and new species might be recognized in a near future (Wells et al., 2019).

Typically, *Tursiops truncatus* have a fusiform body shape with light to dark gray coloration on their dorsal region that fades into white to light pink along their ventral side. Their dimensions vary between two to four meters width and 275 to 600 kg of weight, with smaller individuals observed in warmer waters (Wells & Scott, 2018).

Common bottlenose dolphins live in fission-fusion societies characterized by fluid interactions – units and association patterns are dynamic and inter-changeable, and vary according with numerous factors such as the activity patterns but also sex, age, reproductive state and kin relationship (Scott, Wells, & Irvine, 1990; Connor, Wells, Mann, & Read, 2000). Typically, group size ranges between two and fifteen individuals (Shane, Wells, & Würsig, 1986). Females aggregate in social networks that include other females, juveniles and calves; whereas males may develop strong bonds and form long-term alliances (Connor et al., 2000). Their mating system can be defined as promiscuous or polygynandrous, with dominance hierarchy based on size (Connor et al., 2000).

Females give birth to a single calf, with 90 to 130 cm at birth and about 30 kg of weight (Leatherwood & Reeves, 1990), after a 12-month gestation period. Calves nurse up to 18 months, and maternal care may last up to six years (Wells & Scott, 2018). During this period, calves are highly dependent of their mothers and the strong bond they develop plays an important role in the learning process of several fundamental skills, through play and imitation (Krützen, Mann, Heithaus, Connor, Bejder, & Sherwin, 2005).

These long-lived marine mammals have a usual 20 to 50-year lifespan, and may live to more than 63 years (Wells, 2014). Interestingly, common bottlenose dolphins have the potential for lifelong memory, as social recognition may last for decades (Bruck, 2013). Throughout their lifetime, individuals acquire and develop a set of complex behaviours through social learning, such as communicative abilities and foraging specializations (Janik & Slater, 2000; Kuczaj, Yeater, & Highfill, 2012; Krützen et al., 2005).

Common bottlenose dolphins are generalist predators that exhibit a variety of feeding strategies and foraging specializations in pursuit of numerous preys, including fish, cephalopods and crustaceans (Walker, Potter, & Macko, 1999). Prey availability and habitat characteristics influence their diet and may determine foraging tactics (e.g. Gannon & Waples, 2004; Leatherwood, 1975). Generally, common bottlenose dolphins locate prey items in opportunistic encounters or by passive listening, chase them and use a ram-suction approach to capture prey, which involves forward swimming with it open mouth and prey engulfment (Leatherwood, 1975). Still, other hunting strategies have been documented worldwide, namely strand feeding (Rigley, VanDyke, Cram, & Rigley, 1981), crater feeding (Rossback & Herzing, 2006), sponge feeding (Smolker, Richards, Connor, Mann, & Berggren, 1997), “kerplunking” (Connor, Heithaus, Berggren, & Miksis, 2000) and mud plume feeding (Lewis & Schroeder, 2003).

Intraspecific variation is also observed in ranging patterns. Some populations exhibit strong residency patterns, with individuals living in small area (e.g. 15–65 km2; Gubbins, 2002) for many years (Connor et al., 2000), while other groups are formed by hundreds of individuals that show low site-fidelity and spread through long geographical areas (e.g. Defran, Weller, Kelly, & Espinosa, 1999).

Intraspecific variation can be noted in several biological traits and has been associated, primarily, with the ability to adapt to local conditions (Wells et al., 2019).

Despite its wide range of distribution, information regarding eco-ethological aspects, such as acoustical intraspecific variability is still scarce. Behavioural complexity and flexibility, in addition to their auditory specialization, are traits that foresight the existence of a wide acoustic repertoire, where local vocal variants are to be expected.

1.3.1 Auditory system (sound production, reception and auditory abilities)

Common bottlenose dolphins rely mostly on the auditory channel to communicate, but also to perceive their surroundings, to navigate, to forage and to detect predators. As for all cetaceans, the auditory system of this species is highly adapted to underwater sound production and reception, and presents relevant morphological adaptations to underwater life, namely the absence of external structures or air-filled external channels (Ketten, 2000), and the presence of a specialized nasal complex (Reidenberg & Laitman, 1988).

The structures and mechanisms involved in sound collection and transmission are still matter of debate (Cranford et al., 2011; Wartzok & Ketten, 1999). The opening of the outer ear canal is minimal, the external auditory meatus is vestigial and, although a residual ear channel is present, this structure is not truly linked with the tympanic membrane or the middle ear, and its functionality remains unclear (Ketten, 2000; Au, 1993).

Norris (1968) was the first to suggest that the auditory meatus is not functional and that sound enters the auditory system via the posterior portion of the mandible. Electrophysiological studies support Norris' theory: sound is collected in the lower jaw region and guided through specialized fatty tissues located at the inner side of the thin mandibular bony plate - the pan bone. This multilobed structure appears to have specific tuning properties: the anterior section appears to be tuned for ultrasonic, echolocation related signals, while lower frequency communication signals have been associated with the lateral or inferior channels (Ketten, 2000).

The fatty-filled channel extends to the tympanic bulla, where its ventrolateral wall - the tympanic plate - acts as a tympanic membrane. Acoustic signals pass on to the middle ear over a small channel in the mandible (Brill, 1988) and are conducted to the oval window, at the inner ear. Vibrations in the middle ear ossicular chain stimulate the cochlea and are perceived in the basilar membrane and hair cells; then information is transmitted via the auditory nerve, to the auditory centers of the brain.

The auditory cortex of dolphins is complex and presents specialized areas. Physiological studies indicate that dolphins' brain might have two separate auditory systems – one specialized for temporal resolution of successive brief, fast-rising signals, such as echolocation clicks, and other dedicated to longer, slower-rising signals, such as whistles (Ridgway & Au, 2010).

Common bottlenose dolphins are well known for their auditory capacities, namely the ability to detect very high frequencies and their exceptional temporal resolution aptitude.

The hearing sensitive ranges from 75 Hz up to 150 kHz, with a maximum sensitivity between 65 and 75 kHz (Johnson, 1967). The audiogram for this species is, typically, U-shaped, with sensitivity loss both at the low and high ends (see figure 1.1.).

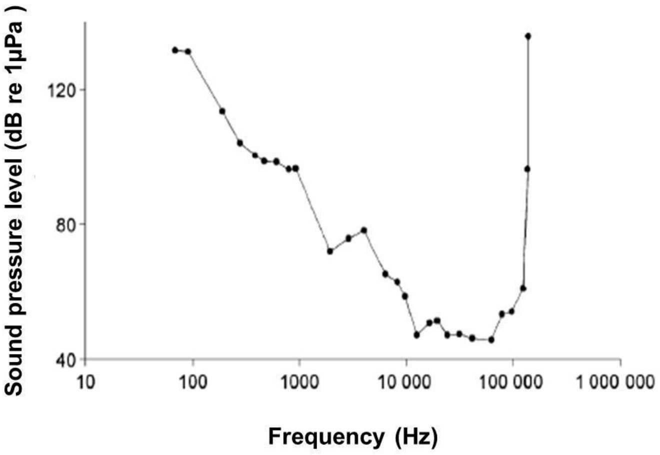

Figure 1.1. - Common bottlenose dolphin (*Tursiops truncatus*) audiogram.
Source: Johnson, 1967.

Dolphins are able to discriminate sound sources both in vertical and horizontal axis, and can distinguish between frequency modulated signals and pure tones (Au, 1993), with acute frequency discrimination, down to 0.2% signal differences (Thompson & Herman, 1975).

Their hearing abilities also include an integration time of 100-200 ms for tonal signals (Johnson, 1986) and an auditory time resolution as short as 0.02-0.03 ms for high-frequency echolocation clicks (Zaslavski, 2008).

Regarding signal emission, common bottlenose dolphins' sound production occurs in a specialized structure, which includes a nasal opening in the respiratory tract, the "phonic lips" located within the nasal passages, nasal air sacs, and fat compartments on the rostrum (Norris, 1968; Cranford, Amundin, Norris, 1996; Cranford & Amundin, 2003).

Sound production in cetaceans has been described as a pneumatic process – pressurized air circulates past the "phonic lips" to produce both pulsed and tonal signals (see Cranford et al., 1996, 2011). Interestingly, the specific structures involved in the production of different signal types are distinct. Functional studies have demonstrated that the two pairs of phonic lips (left and right) have different roles in sound generation: the larger right phonic lips are responsible for echolocation pulses' production, while tonal signals that require more air and higher air pressure are produced with the left pair (Madsen, Lammers, Wisniewska, &

Beedholm, 2013). Therefore, dolphins can produce, simultaneously, two signal types using both phonic lips at the same time.

After being produced at the phonic lips, acoustic signals interact with the skull and air sacs, and are transmitted to the melon located in the forehead. This multi-layered lipidic structure acts like an acoustic lens and sound is emitted into the water in a narrow, forward-directed beam (Aroyan, Cranford, Kent, & Norris, 1992; Au et al. 2010).

In the water, acoustic signals travel from the sender to the receiver, and as sound propagates it is filtered and suffers attenuation, an effect that is stronger in the higher frequencies (Ulrich, 1983). Thus, effective acoustic signaling depends on the auditory system characteristics, but also on the environmental constrains. The acoustic repertoire of a species reflects such influences.

1.3.2 Acoustic repertoire

The acoustic signals produced by the common bottlenose dolphin have been the focus of numerous studies, worldwide, in the last fifty-years (e.g. Lilly & Miller, 1961; Caldwell & Caldwell, 1965; Au, 1993; dos Santos, Ferreira, & Harzen, 1995; Diáz-López, 2011; Gridley, Nastasi, Kriesell, & Elwen, 2015). Based on the accumulated knowledge it is clear that the acoustic repertoire of this species is vast and includes highly specialized vocalizations.

Identifying and describing an acoustic repertoire is a challenging process, especially considering that repertoires may be ‘graded’, ‘discrete’, or a combination of the two (Bradbury & Vehrencamp, 2011; Hauser, 1997; Marler, 1976). By definition, a graded signal system includes, gradual acoustic variation between and/or within signal types, whereas discrete repertoires comprise distinct acoustic units (Marler, 1976).

In the common bottlenose dolphins’ acoustic repertoire it is possible to identify distinct acoustic units, however within these signal types gradual acoustic variation occurs.

General classifications divide the acoustic emissions of these dolphins in three groups, according with their time-frequency characteristics, namely whistles, echolocation clicks and burst-pulses.

Whistles are the best studied signal type in the acoustic repertoire of this species. These vocalizations are frequency-modulated, narrow-band, omnidirectional signals, with most of their energy between 3.5 and 14.5 kHz (May-Collado & Wartzok, 2008). Whistles are produced at lower frequencies than echolocation clicks, and propagate farther (Lammers, Au, & Herzing, 2003). The source level of these emissions have been estimated to be as high as 169 dB re 1μPa and the distance at which whistles’ can be perceive by conspecifics may exceed 20 km (Janik, 2000b). The active space (i.e. communication range) of a whistle is highly variable and is influenced by, both, internal and external factors, such as the source level, habitat structure or depth (e.g. 7-13 kHz whistle with 165 dB re 1µPa source level will be audible at 487 m in a shallow seagrass meadow, whereas 13–19 kHz whistles could be potentially detectable over 20 km in depth water channels) (Quintana-Rizzo, Mann, & Wells, 2006).

Common bottlenose dolphins produce a wide variety of tonal signals that have been, traditionally, classified in basic types according with their time-frequency contour. The six fundamental categories of whistles are i) constant frequency, when overall frequency changes less than 25% over the total duration of the signal, ii) upsweep, if frequency increases over time, iii) downsweep, if frequency decreases over time, iv) concave, when an initial frequency decrease over time reaches an inflection point and is followed by a frequency increase at the ending portion, v) convex, when an initial frequency increase over time reaches an inflection point and is followed by a frequency decrease at the ending portion, vi) sinusoidal, frequency modulated whistle with, at least, two inflection points.

Whistles are used in multiple social contexts (Jones & Sayigh, 2002; Quick & Janik, 2008) and the influence of social factors, such as group size or composition, and environmental conditions, in whistles' emission patterns and signal features is widely documented (e.g., Jones & Sayigh, 2002; Watwood, Owen, Wells, & Tyack, 2005)

The occurrence of stable, stereotyped frequency contours, often emitted in bouts ("signature whistles") and their specific role in communication is also noteworthy (e.g. Caldwell & Caldwell, 1965; Janik & Sayigh, 2013; Janik, King, Sayigh, & Wells, 2013). These communication signals, developed through vocal learning, are thought to convey individual identity information and to be used to signal the presence and location of an emitter (Janik Sayigh, & Wells, 2006; Sayigh & Janik, 2013; King et al., 2013; Sayigh, Esch, Wells, & Janik, 2007). Signature whistles have been described as learned, unique, individually distinctive contact calls (Janik et al., 2006, Janik & Slater, 1998; Quick & Janik, 2008, King & Janik, 2013), and represent between 38 and 70 % of all whistle production in the wild (Buckstaff, 2004; Cook, Sayigh, Blum, & Wells, 2004).

Echolocation clicks have also been studied for several decades, but contrary to whistles' emission, most of the knowledge of dolphins' echolocation concerns its production. Echolocation clicks are highly directional, short duration (35-200 µs), broadband (0.1 a 300 kHz), pulsed emissions. The peak frequency of the typical echolocation click produced under open water conditions is between 120 and 130 kHz (Au, Floyd, Penner, & Murchison, 1974), whereas in enclosed facilities peak frequencies are much lower (30-60 kHz) (Evans, 1973;

Li, Nachtigall, & Breese, 2011). These high energy signals (greater than 210 dB re 1µPa) are usually produced in click trains, with slow repetition rate and inter-click-intervals that allow for the two-way transit time between the head of the dolphin and the target (Au, 1993). Through the emission of click trains in a directional axis and the reception of the following echo, dolphins may determine the presence, structure, composition, shape and distance of a specific target (Au & Hastings, 2008). Experimental evidences demonstrate that common bottlenose dolphins control both sound transmission and reception synchronously in a biosonar cycle to overcompensate the echo attenuation with target distance (Li et al., 2011). An echolocation click train can be considered as a discrete unit composed by multiple pulses, although the exact number of pulses, the repetition rate, the inter-click-interval and the overall duration of the train may vary according with the specificities of a task. For fine-scale tasks, the pressure level and the repetition rate are higher, and therefore the inter-click-interval is shorter. The active space of high-frequency click trains has been estimated in, approximately, 100 meters (Murchison, 1980).

Although the time-frequency characteristics, the context of emission and the functional aspects of whistles and echolocation signals are well documented, information on burst-pulses is still limited.

Burst-pulses are formed by broadband pulses often similar to echolocation signals, but with higher repetition rate (> 300 pulses per second) and shorter inter-click-intervals (< 3 ms). These vocalizations, which are emitted in sound-packages, differ greatly in duration and spectral content, but have been labelled based on the basis of what they sound like to the human ear (e.g., squawks, squeaks, creaks, buzzes, barks, grunts, moans).

This category comprises a large section of the acoustic repertoire of common bottlenose dolphins (Herzing, 2000), and their relevance as communication signals has been emphasized by several authors (e.g. Lilly & Miller, 1961; Caldwell & Caldwell, 1967; Herzing, 1996; Herman & Tavolga, 1980). Burst-pulses occur in various aroused behavioural contexts, such as agonistic interactions, fright and alarm situations, and sexual interactions. Squawks and/or squeaks, with very high repetition rates (200–1200 clicks/s), have been recorded during courtship and agonistic interactions (Caldwell & Caldwell, 1967; Herzing, 2000), but also described as “victory signals” (Ridgway, Moore, Carder, & Romano, 2014). Creaks and/or buzzes have been described as terminal buzzes, with probable echolocation value, when produced during foraging and feeding events, but also as social signals recorded during courtship (e.g., Ridgway et al., 2014; Herzing, 1996).

The acoustic repertoire of *Tursiops truncatus* also includes multi-unit rhythmic sequences - the bray series (dos Santos, Caporin, Moreira, Ferreira, & Coelho, 1990). These multi-unit acoustic signals have been reported as food-related emissions (Janik, 2000c; King & Janik, 2015) and include distinct burst-pulses, namely the low-frequency narrow-band (LFN) sounds (Simard et al. 2011), which are short-duration (0,1 s), low-frequency (<1 kHz) signals.

Although these broad categories reflect structural aspects of sound emission (pulsed *vs* tonal signals), these simple categorization does not reflect the diversity of signal types present in the global acoustic repertoire of this species. From an acoustic and behavioural standpoint, the common bottlenose dolphin global repertoire is still poorly described.

1.4 THESIS OVERVIEW

This dissertation is focuses on the common bottlenose dolphin (*Tursiops truncatus*), undoubtedly the best studied species of cetacean. Nevertheless, the global acoustic repertoire of this delphinid is still not fully described. The development of geographic variations transmitted through social learning and the long-term stability of vocal elements are standing topics, of great cognitive and ethological relevance, yet poorly investigated. Common themes in bottlenose dolphins' acoustic emissions, such as the burst-pulses and bray series, are scarcely documented. Moreover, in an increasingly noisy ocean, the effects of environmental constrains, such as maritime traffic, in the acoustic communication of resident cetacean populations only recently begun to be assessed.

A comparative perspective of the global acoustic repertoire of *Tursiops truncatus* is presented in Chapter 2. An exhaustive analysis of acoustic signals recorded in different behavioural and environmental contexts, in 9 different locations throughout the Atlantic Ocean and Mediterranean Sea, enables an overall view of the vocal emissions produced by common bottlenose dolphins. The acoustic similarity index here presented illustrates the expression of "universal" vocal behaviours (similar across populations) for this species and highlights contrasting vocal patterns. Geographic variation in the acoustic repertoire of this species is further explored through the analysis of signals emission rates and time-frequency parameters, and their link with specific social and ecological factors.

The following chapters focus on a small resident group, whose population composition and acoustic emissions have been monitored since 1980s. The accumulated knowledge provides a unique opportunity to investigate and clarify compelling aspects of bottlenose dolphins' acoustic repertoire.

Long-term stability of stereotyped whistle emissions and its context of emission are discussed in Chapter 3. Confirmed stereotypy throughout more than 20 years, supports the potential strength of signature whistle occurrence in wild populations. The possible roles of non-stereotyped whistles, which represent 68 % of all whistles recorded, are also discussed in this chapter.

The problem of repertoire definition is introduced in Chapter 4, which stresses the relevance of pulsed signal emissions' in this species repertoire. A quantitative analysis of pulsed signals emitted by wild bottlenose dolphins is here explored. The application of less subjective methods in acoustic repertoire studies is an important mark in this chapter, as quantitative signal descriptions are essential to improve classifications and to better assess the contexts of emission, geographic variation and the functional significance of the acoustic emissions.

In Chapter 5, bray series characteristics and patterns of emission are extensively described. These rhythmic sequences were firstly reported for the Sado population (dos Santos et al., 1990) and have been poorly understood, contextually and geographically. Here, the distinct elements that compose brays are detailed and the bray series are explored, in the light of information theory. The context of emission of these complex pulsed signals is also addressed and debated.

Finally, in line with European Union current risk-assessment criteria, the effects of maritime traffic in the acoustic communication of bottlenose dolphins are addressed in Chapter 6. Maritime traffic is considered the main contributor to the rise in background noise in European waters (Tasker et al., 2010). In this final chapter, the acoustic behavior of bottlenose dolphins in the presence of different types of vessels is assessed and shifts in overall call rates and whistle characteristics are discussed.

In summary, the aim of this thesis is to document the global acoustic repertoire of *Tursiops truncatus*, and to explore the specific characteristics and functional significance of different signal types. The ultimate goal of this study is to contribute for a better understanding of bottlenose dolphins' acoustic behaviour in the wild, and to clarify the role of social and environmental constrains.

CHAPTER 2

VOCAL UNIVERSALS AND GEOGRAPHIC VARIATIONS IN THE ACOUSTIC REPERTOIRE OF THE COMMON BOTTLENOSE DOLPHIN

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2.1. ABSTRACT

Acoustical geographic variation is common in widely distributed species and it is already described for several taxa, at various scales. In cetaceans, intraspecific variation in acoustic repertoires has been linked to ecological factors, geographical barriers, and social processes. For the common bottlenose dolphin (*Tursiops truncatus*), studies on acoustic variability are scarce, focus on a single signal type - whistles and on the influence of environmental variables. Here, we analyze the acoustic emissions of nine bottlenose dolphin populations across the Atlantic Ocean and the Mediterranean Sea, and identify common signal types and acoustic variants to assess repertoires' (dis)similarity. Overall, these dolphins present a rich acoustic repertoire, with 21 distinct signal sub-types including: whistles, burst-pulsed sounds, brays and bangs. Acoustic divergence was observed only in social signals, suggesting the relevance of cultural transmission in geographic variation. The repertoire dissimilarity values were remarkably low (from 0.08 to 0.4) and do not reflect the geographic distances among populations. Our findings suggest that acoustic ecology may play an important role in the occurrence of intraspecific variability, as proposed by the 'environmental adaptation hypothesis'. Further work may clarify the boundaries between neighboring populations, and shed light into vocal learning and cultural transmission in bottlenose dolphin societies.

Keywords: bottlenose dolphins, *Tursiops truncatus*, acoustic repertoire, geographic variation, signal types

2.2. BACKGROUND

For species with a wide geographic distribution, variation in behavioural traits (e.g. foraging preferences, hunting strategies, antipredatory displays or acoustic repertoires) is common, and such differences are often used to distinguish populations (Foster & Endler, 1999).

Intraspecific acoustic variants, in particular, have been described for several taxa, at various scales. Even among sympatric populations, vocal variations have long been noted in numerous bird species (see Mundiger, 1982), and a few non-human primates (e.g. Green, 1975; Hodun, Snowdon, & Soini, 1981). Variations between neighboring social groups may be considered true dialects, transmitted through learning, and have been well studied in some cetacean species, such as killer whales (Filatova et al., 2012; Ford, & Fisher, 1986), sperm whales (Gero, Whitehead, & Rendell, 2016; Rendell & Whitehead, 2003) and pilot whales (Cise, Roch, Baird, Mooney, & Barlow, 2017). On a broader scale, acoustic differences among allopatric populations have been reported in numerous species of insects, fish, anurans, birds, terrestrial mammals (e.g., Podos & Warren, 2007; Velásquez, 2014; Walker, 1962), and also in marine mammals such as Amazonian river dolphins, spinner dolphins or harbor seals (Amorim, Andriolo, & Reis, 2016; Bjørgesæter, Ugland, & Bjørge, 2004; Moron et al., 2015). Both micro and macro-geographic variations in vocal repertoires may be caused by a multiplicity of genetic, social, ecological and historical factors (Janik & Slater, 2000; Lameira, Delgado, & Wich, 2010), as selective pressures vary in different eco-ethological contexts.

In cetacean societies, vocal signaling is the primary modality of communication (Janik, 2005) and acoustic variability appears to be widespread. However, the specific causes and immediate functions of such variations still need clarification. Studies on odontocetes' acoustic divergence point to different pathways: i) for species with stable kin groups, such as killer whales and sperm whales, variations in acoustical traits have been correlated with genetic structure, but also associated with cultural identity (Deecke, Ford, & Spong, 2000; Weilgart & Whitehead, 1997); ii) for other species that live in fission-fusion groups, such as spinner dolphins and bottlenose dolphins, acoustic variations in some signal types have been linked with the variables related to the context of emission - ecological conditions, group size, group composition and activity patterns (Azevedo & Van Sluys, 2005; Bazúa-Durán & Au, 2004; Hawkins, 2015; Wang, Würsig, & Evans, 1995).

Common bottlenose dolphins (*Tursiops truncatus*) inhabit estuaries, coastal regions and open ocean ecosystems, worldwide in tropical and temperate waters, in resident or transient fission-fusion groups that may range from dozens of individuals to mega-pods of thousands (Connor et al., 2000). These acoustically specialized animals present unique cognitive and communicative characteristics (Janik & Slater, 1998, 2000; Marino et al., 2007; Sayigh et al., 1999), and their acoustic skills include the ability to modify and produce novel vocalizations as a result of experience (vocal learning) and capability to imitate sound patterns (vocal mimicry). Their vast acoustic repertoire includes click trains for echolocation (Au, 2004; Herzing & Santos, 2004; Jensen, Bejder, Wahlberg, & Madsen, 2009), narrow-band frequency-modulated whistles for communication, and a wide variety of burst-pulsed sounds, whose specific functions are still matter of debate (dos Santos et al., 1995; Herzing, 2000; Díaz-López & Shirai, 2009; Luís, Alves, Sobreira, Couchinho, & dos Santos, 2018).

Although the acoustic emissions of bottlenose dolphins are widely documented, both for *T. truncatus* and *T. aduncus*, studies on geographic variation are scarce, and mostly focused on the influence of environmental factors on whistles' emission (Hawkins, 2015; May-Collado & Wartzok, 2008; Wang et al., 1995). Acoustic divergence in bottlenose dolphins has been assessed by comparing whistles' features or emission rates in different populations, at a local scale (Jones & Sayigh, 2002; La Manna et al., 2017; May-Collado & Wartzok, 2008; Wang et al., 1995). However, geographic distance, social behavioural patterns and population genetic structure may also play important roles in acoustic geographic variation (Papale et al., 2014).

A comparative analysis of the extended acoustic repertoire of common bottlenose dolphins, at a broader scale, may shed light on the species' vocal flexibility, its social learning mechanisms and cultural transmission in bottlenose dolphin societies.

With that in mind, our goal is to identify and compare the different vocal elements that comprise the extended acoustic repertoire of several *T. truncatus* populations across the Atlantic Ocean and the Mediterranean Sea, and to highlight the expression of shared vocal elements and acoustic variants among allopatric and sympatric populations.

2.3. METHODS

2.3.1 Data collection

Bottlenose dolphins underwater vocalisations were recorded, from 2002 to 2016, at nine location (see Fig. 2.1.): Northeast Atlantic (Sado estuary, Portugal), Mid-North Atlantic (Azores, Portugal), Adriatic sea (Croatia), Central Mediterranean Sea (Sicily Channel, Italy), Southeast Atlantic (Namibia), Caribbean Sea (Bahamas), West Central Atlantic (Panama and Costa Rica), Southwest Atlantic (Brazil). See locations, dates, recording systems and publications on Table 2.1.

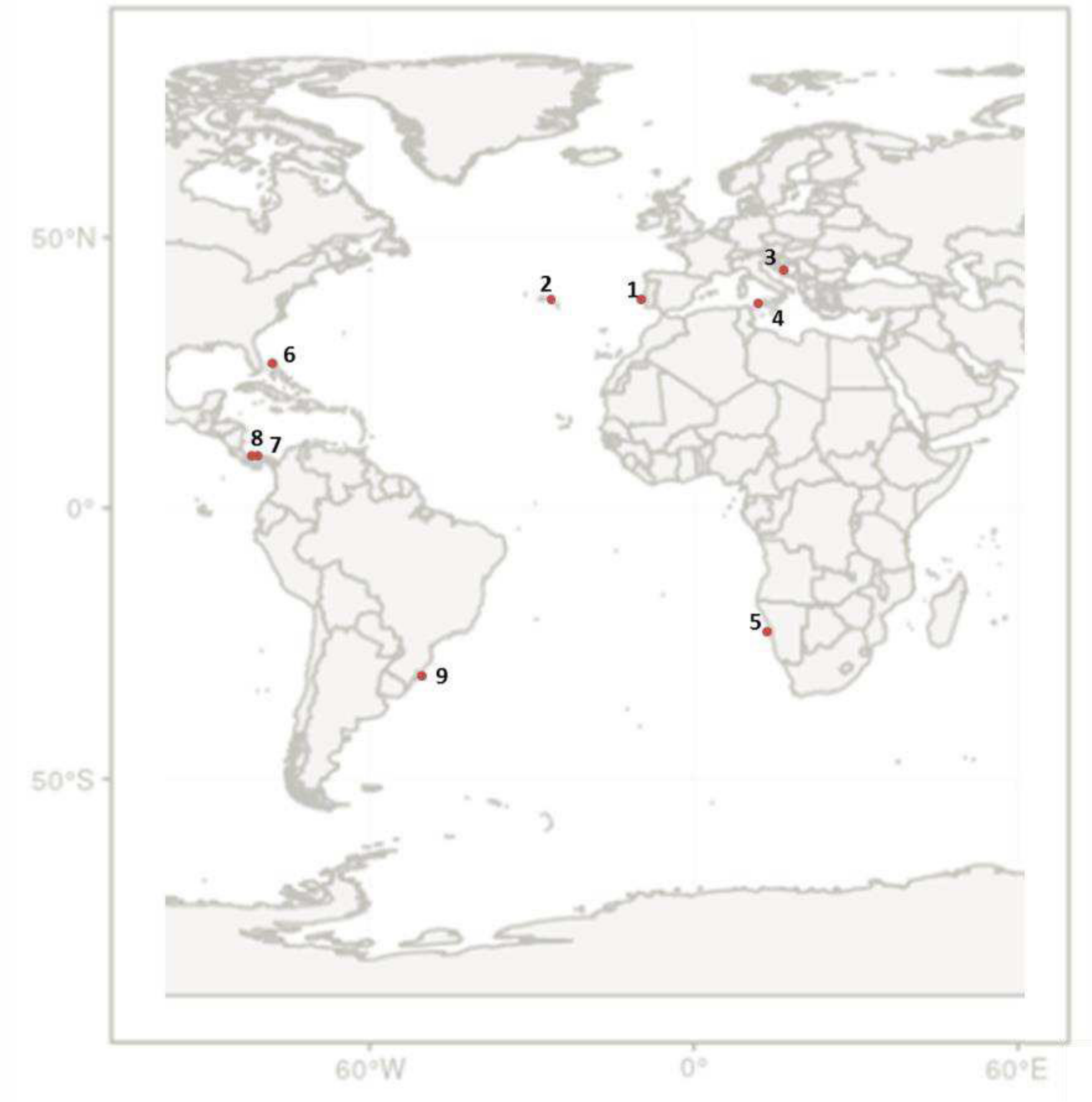


Figure 2.1. Geographic location of sampling sites.

1 - Northeast Atlantic (Sado estuary, Portugal), 2 - Mid-North Atlantic (Azores, Portugal), 3 - Adriatic sea (Croatia), 4- Central Mediterranean Sea (Sicily Channel, Italy), 5 - Southeast Atlantic (Namibia), 6 - Caribbean Sea (Bahamas), 7 - West Central Atlantic (Panama), 8 - West Central Atlantic (Costa Rica), 9 - Southwest Atlantic (Brazil)

Table 2.1. Data collection for geographical acoustic comparison

	Time frame	**Group size**	**Recording contexts**	**Hydrophone type**	**Funtional bandwidth**	**Recorder type**	**Sampling rate**
Northeast Atlantic **(Sado estuary, Portugal)**	2013, 2014	2-29	Foraging/Milling, Surface Feeding, Social interactions, Travelling	Cetacean Research Technology, model C55	20 Hz to 100 kHz (sensitivity:−165 dB re 1 V=1 μPa)	Fostex FR-2 digital recorder	192 kHz
Mid-North Atlantic **(Azores, Portugal)**	2002	14-66	Foraging/Milling, Surface Feeding, Social interactions, Travelling	omnidirectional HTI-94-SSQ, High Tech Inc.	2 Hz to 30 kHz	Tascam DA-P1	48kHz
Adriatic Sea (Croatia)	2007, 2008	-	-	RESON TC 4032	5 Hz to120 kHz (sensivity: -170dB re 1V/ μPa)	Pioneer DC-88 DAT recorder	44.1 kHz
	2016	2-29	Foraging/Milling, Surface Feeding, Social interactions, Travelling,	RESON TC 4032	5 Hz to120 kHz (sensivity: -170dB re 1V/ μPa)	SOUNDDEVICES 702	192 kHz

			Fisheries interaction				
Central Mediterranean Sea **(Sicily Channel, Italy)**	2011, 2012, 2014	2-7	Foraging/Milling, Surface Feeding, Social interactions, Travelling, Fisheries interaction, Resting	Bruel and Kjaer, model 8104	0.1 Hz –120 kHz (-205.6 dB re 1 V/1 µPa +4/-12 dB)	Digital acquisition card Avisoft Ultra Sound Gate 416HB	300 kHz
Southeast Atlantic (Namibia)	2009	1-50	Foraging/Milling, Surface Feeding, Social interactions, Travelling, Resting	High-Tec HTI-96-MIN	2 Hz to 30 kHz (sensitivity: - 170dB re 1 V=1 µPa)	Edirol UA-25 sound card to PC (2009 recordings)	96 kHz
	2011, 2012					Zoom H4n digital recorder	
Caribbean Sea (Bahamas)	2002-2005, 2009,	2-20	Foraging/Milling, Social interactions	Custom-built omni-directional hydrophone	up to 300 kHz (sensitivity of –214 dB re 1V/µPa)	Underwater Dolphin Data Acquisition System (UDDAS)	240 kHz
	2012, 2014	7-12		RESON TC 4013	1Hz to 170kHz (sensitivity: -211dB ±3dB re 1V/µPa)	Zoom H4n digital recorder	96 kHz

West Central Atlantic **(Panama)**	2004, 2007, 2008, 2012	2-10	Foraging/Milling, Social interactions, Travelling	RESON TC 4033	1 Hz to 140 kHz (sensitivity: - 203 dB re 1 V=1 µPa)	Digital acquisition cardAvisoft Ultra Sound Gate 116	384-500 kHz
West Central Atlantic **(Costa Rica)**	2004, 2007	-	Foraging/Milling, Social interactions, Travelling				
Southwest Atlantic **(Brazil)**	2014	-	-	High-Tec HTI-96-MIN (2014 recordings)	2 Hz to 30 kHz (sensitivity: - 170dB re 1 V=1 µPa)	Fostex FR-2 digital recorder	96 kHz
	2015	20	Travelling	Cetacean Research Technology, model c54 XRS	9 Hz a 100 kHz (sensitivity: -165 dB re: 1 V/µPa)		

2.3.2 Acoustic analyses

Sound recordings from all nine populations were inspected aurally, and visually using spectrograms plotted on Raven 1.4 (Cornell Lab of Ornithology, Ithaca, NY), with Hann windows of 512 points and a frequency resolution of 188 Hz and 50 % overlap. Acoustic signals were rated according to the following signal quality index (Papale et al. 2012, Gridley et al., 2015): (i) poor – signal faint and hardly visible on the spectrogram, (ii) fair – signal visible and with a clear start/end on the spectrogram, (iii) good – signal well marked and with a clear start/end on the spectrogram. Signals rated as fair or good, and with no overlapping sounds, were selected for further analysis and classified as discrete vocal units.

Signal types were labeled as whistles, burst-pulsed sounds, bray series elements or bangs, according to previous descriptions (Au & Hastings, 2008; Bázua-Duran & Bazúa-Durán, 2004; Herzing & dos Santos, 2004; Lammers, Au, & Herzing, 2003; Nowacek, 2005; Simard et al., 2011). Signal subtypes were defined using general time-frequency quantitative variation features, following (Luís et al., 2018; Luís, Couchinho, & dos Santos, 2016a; Luís, Couchinho, & dos Santos, 2016b) (see Table 2). For each population, the repertoire composition was defined based on the occurrence of different signal types and sub-types.

2.3.3. Repertoire Similarity

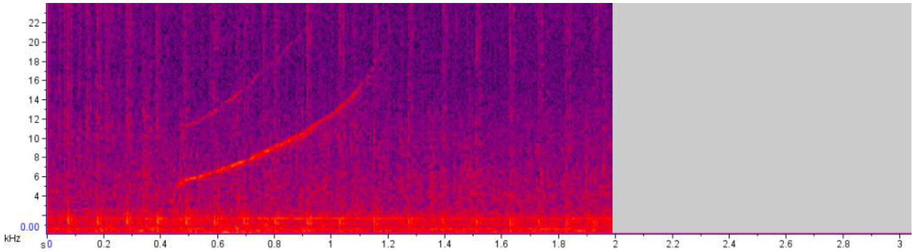
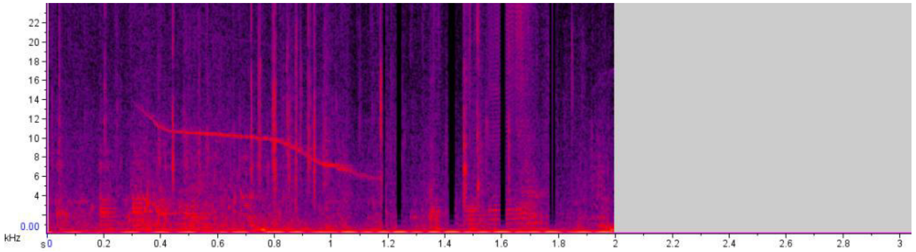
The acoustic similarity among the repertoires of different populations was calculated using an index based on the degree of signal types shared. The similarity index is derived from Dice's coefficient of association (Ford, 1991) and takes into account differences in repertoire size:

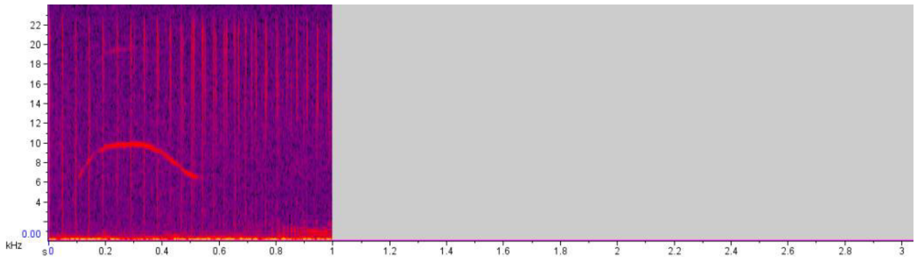
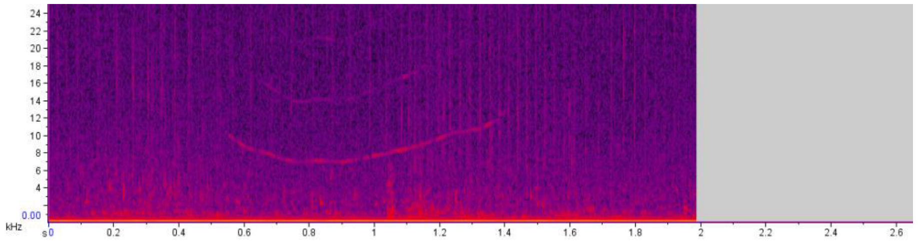
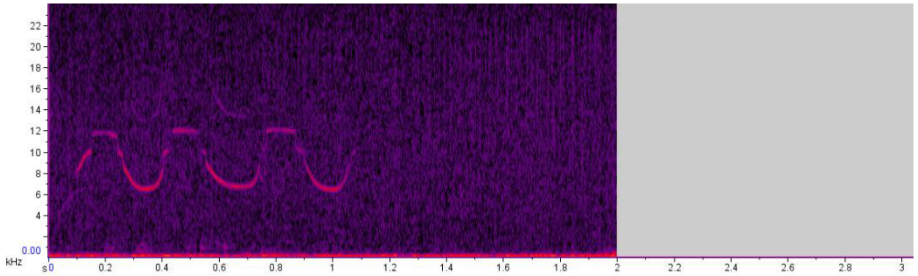
$$Index\ of\ similarity = \frac{2N_c}{R_1 + R_2}$$

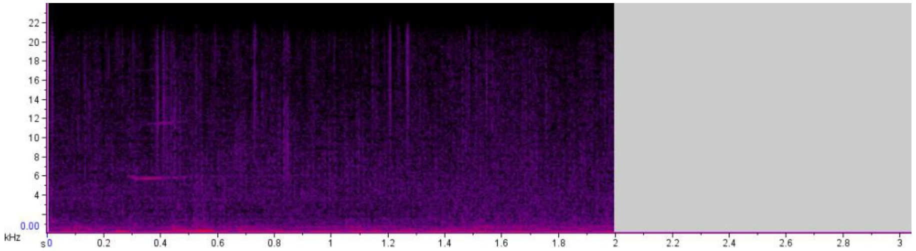
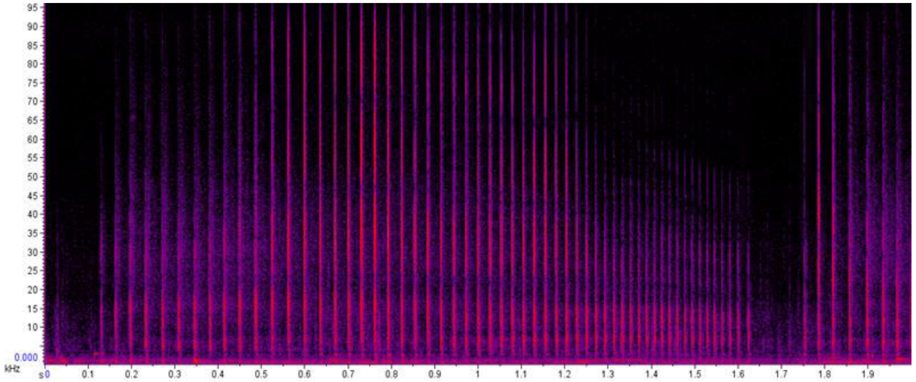
Where N_c is the total number of call types and subtypes shared, and R_1 and R_2 are the repertoire sizes (call types plus subtypes) of the two units.

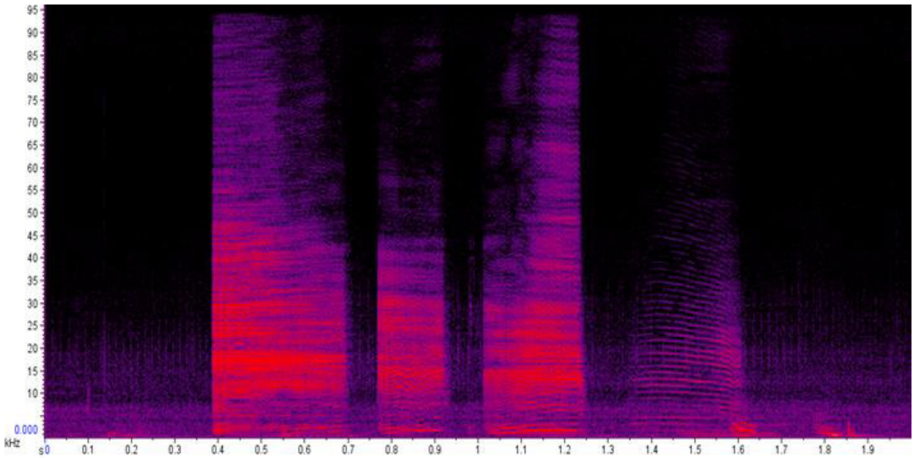
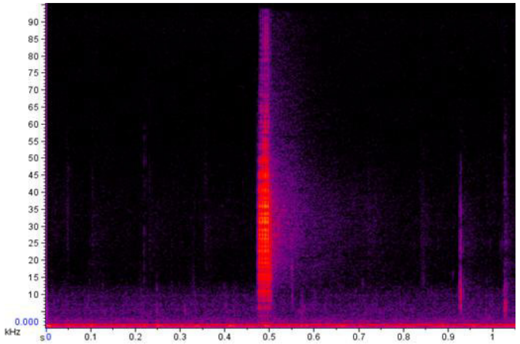
As the similarity values are distance measures, we calculated its inverse to obtain the equivalent dissimilarity value (1- Index of similarity values) and computed a dissimilarity matrix. The dissimilarity matrix was used to perform a hierarchical cluster analysis, using the average linkage method. For visual comparison, a heat map with a dendrogram was plotted. Similarity analysis was performed using R Studio software (R Development Core Team, 2018).

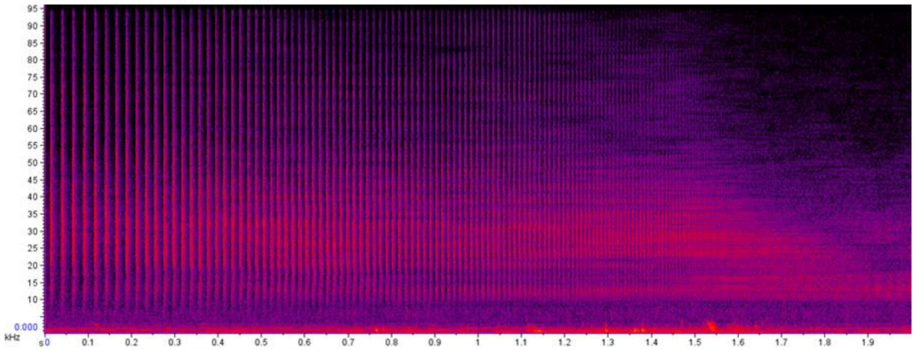
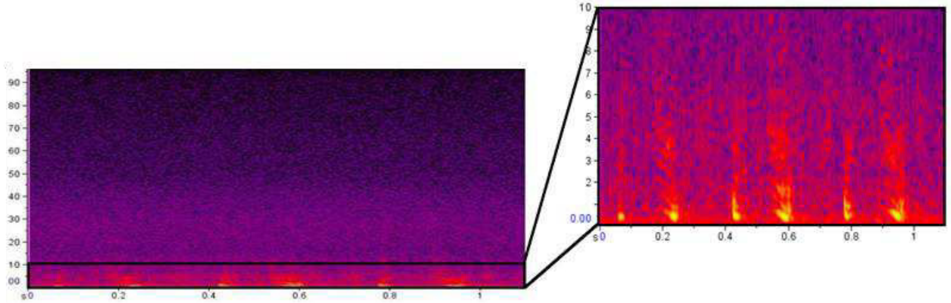
Table 2.2. Description of the general acoustic categories

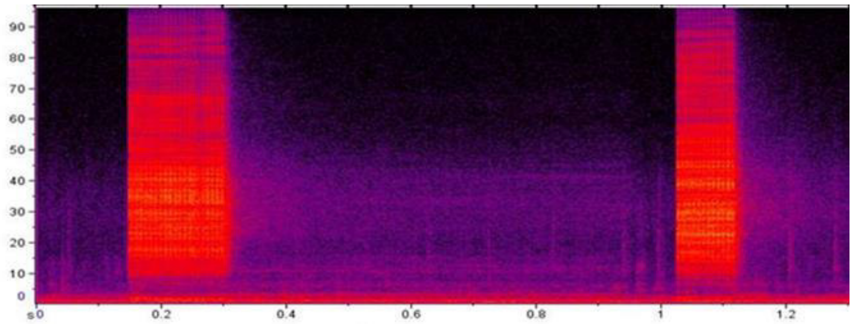
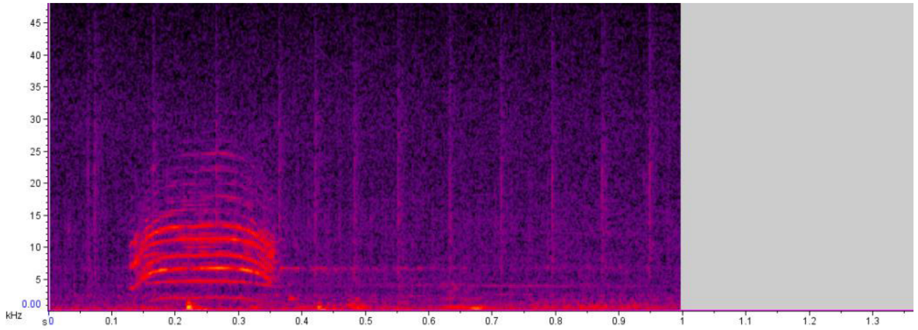
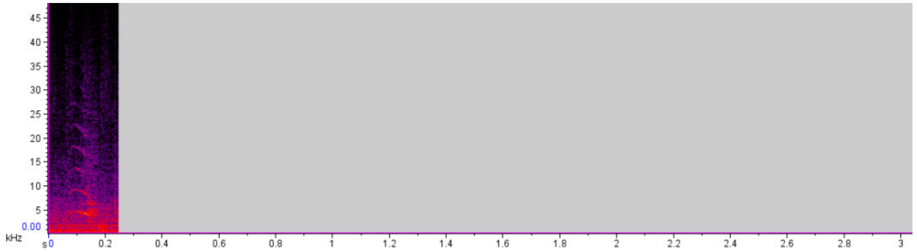
Signal type	**Signal Sub-types**	**Description**	**Example**
Whistles		Tonal, narrow-band, modulated signals	
	Upsweep	Frequency increasing over time, no inflection points	
	Downsweep	Frequency decreasing over time, no inflection points	

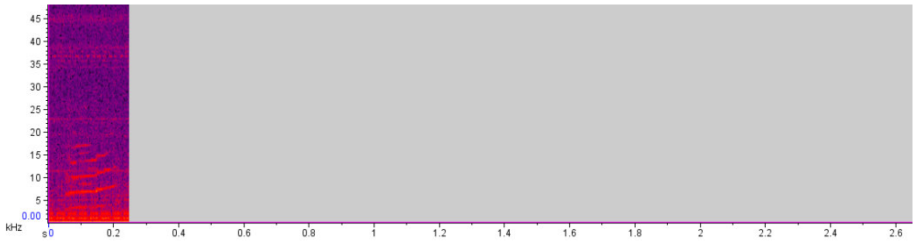
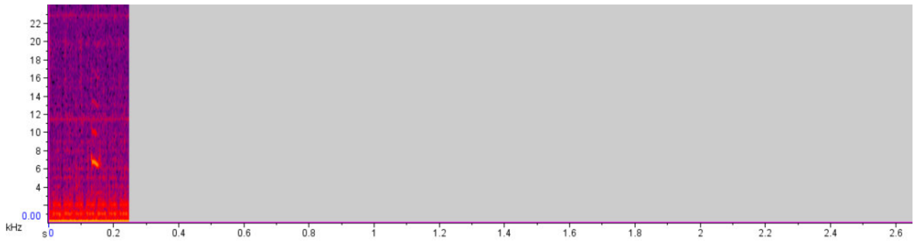
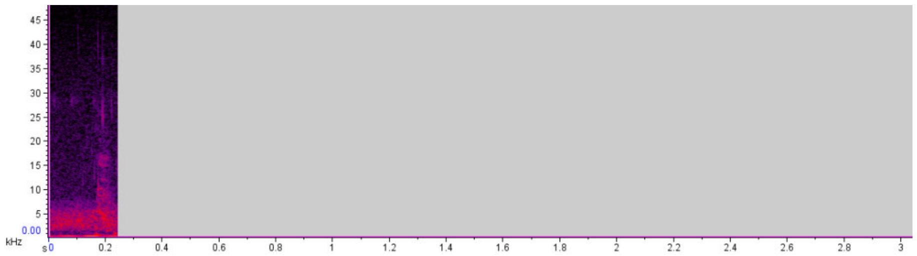
Convex	Frequency initially increasing followed by an inflection point and an ending portion decreasing	
Concave	Frequency initially decreasing followed by an inflection point and an ending portion increasing	
Sinusoidal	Multiple inflection points	

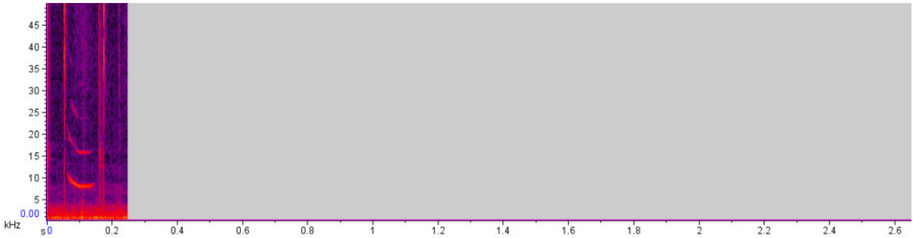
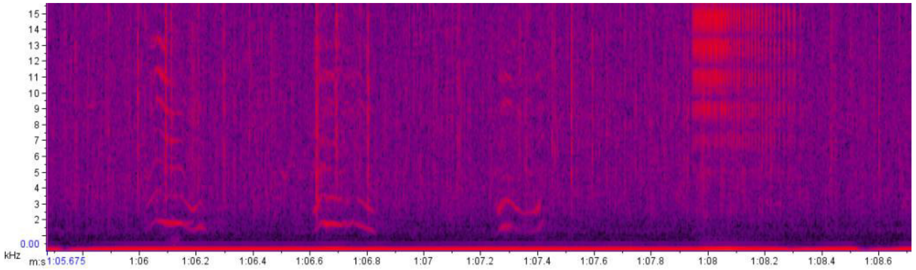
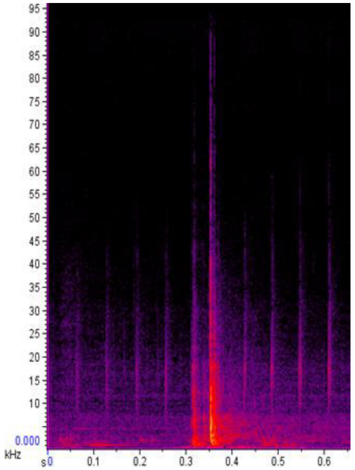
	Constant	Frequency variation less than 25%, no inflection points	
BP sounds		Packets of high repetition, broadband pulses with inter-click-intervals below 10 ms	
	Creaks (or buzzes)	Long burst-pulse (>2 ms), aurally similar to a creaking door	

Squawks	Long burst-pulse (>2 ms), with higher repetition rate than “Creaks”, reminiscent of a crying baby	
Short burst-pulse (S-BP)	Short burst-pulse (<2 ms), aurally similar to a buzzing bee but brief	

	Variable rate click train (VRCT)	Long, graded click series that include continuous creaks and squawks	
Bray series elements		Rhythmic sequences up to 30s in length, composed by short duration pulsed or tonal signals	
	Gulps (or LFN)	Low-frequency narrow-band sounds	

Grunts	Broadband burst pulses, with strong emphasis in the lower frequencies	
SC-squeaks	Short duration (<2 ms), high repetition rate harmonic structure with convex modulation	
VSC-squeaks	Very short duration (<1 ms), high repetition rate harmonic structure with convex modulation	

Up-squeaks	Very short duration (<1 ms), high repetition rate harmonic structure with upsweep modulation	
D-squeaks	Very short duration (<1 ms), high repetition rate harmonic structure with downsweep modulation	
Un-squeaks	Short duration (<2 ms), high repetition rate harmonic structure with undefined modulation	

Cc-squeaks	Short duration (<2 ms), high repetition rate harmonic structure with concave modulation	
Sin-squeaks	Short duration (<2 ms), high repetition rate harmonic structure with sinusoidal modulation	
Bangs	Isolated, high-energy, broadband pulses	

2.4. RESULTS

2.4.1. Acoustic repertoire

A total of 7048 vocal elements categorized into 21 signal sub-types were selected for analysis (see Table 2.3.), namely whistles (N=2526), BP-sounds (N=1640), bray series elements (N=2552) and bangs (N=330).

Table 2.3. Data collection and vocal elements, by location.

	Data collection		**Vocal elements**				
	Sound recordings (mins.)	Sample size	Whistles	BP-Sounds	Bray series elements	Bangs	TOTAL
Northeast Atlantic, Sado estuary, Portugal	239	156	949	590	1250	131	2920
Mid-North Atlantic, Azores, Portugal	112	17	423	93	510	5	1031
Adriatic Sea, Croatia	293	65	124	54	234	6	418
Central Mediterranean Sea, Sicily Channel, Italy	81	39	362	155	69	7	593
Southeast Atlantic, Namibia	294	108	82	60	3	4	149
Caribbean Sea, Bahamas	86	23	196	405	42	16	659
West Central Atlantic, Panama	174	102	165	92	20	139	416
West Central Atlantic, Costa Rica	93	29	157	166	91	21	435
Southwest Atlantic, Brazil	106	38	68	25	333	1	427

The average number of vocal elements recorded at each location was 15.56 ± 2.36. Repertoire size varied between 13 signal sub-types, for Namibia, Panamá, Costa Rica and Brazil, and 19 sub-types for Sado estuary, Portugal.

2.4.2. Common signal types and acoustic variants

Whistles, BP-sounds, bray series elements and bangs were recorded for all populations. A total of six signal sub-types were common across study sites, which represents 28.57% of calls shared between populations. Differences in vocal patterns were observed for specific signal sub-types, especially for bray series elements (see Table 2.4.).

Table 2.4. Differences in signal sub-units occurrence

	Whistles				**BP-sounds**	**Bray series elements**									
	Up-whistle	**D-whistle**	**Cc-whistle**	**CTF-whistle**	**S-BP**	**Gulp**	**Grunt**	**SC-Squeak**	**VSC-Squeak**	**D-Squeak**	**Up-Squeak**	**LD-Squeak**	**Un-Squeak**	**Cc-Squeak**	**Sin-Squeak**
Northeast Atlantic, Sado estuary, Portugal (Repertoire size = 19)	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓			✓		
Mid-North Atlantic, Azores, Portugal (Repertoire size = 17)	✓	✓	✓		✓	✓	✓	✓		✓		✓			
Adriatic Sea, Croatia (Repertoire size = 18)	✓	✓	✓	✓	✓	✓	✓		✓	✓				✓	
Central Mediterranean Sea, Sicily Channel, Italy (Repertoire size = 17)	✓			✓	✓	✓	✓	✓	✓	✓			✓	✓	
Southeast Atlantic, Namibia (Repertoire size = 13)	✓	✓				✓ *	✓ *	✓					✓		
Caribbean Sea, Bahamas (Repertoire size = 17)	✓				✓	✓	✓	✓	✓	✓	✓				
West Central Atlantic, Panama (Repertoire size = 13)		✓						✓	✓						✓
West Central Atlantic, Costa Rica (Repertoire size = 13)	✓		✓		✓		✓	✓		✓					
Southwest Atlantic, Brazil (Repertoire size = 13)	✓				✓	✓	✓	✓	✓						

For whistles, upsweeps were not recorded in Panama, downsweeps were absent in Sicily Channel, Caribbean Sea, Costa Rica and Brazil, concave whistles were only recorded in Sado estuary, Azores, Adriatic Sea, and Costa Rica, while constant frequency whistles were present only in the Mediterranean (Sicily Channel and Adriatic Sea) and in the Sado estuary. Differences in the occurrence of BP-Sounds were observed only for S-BP sub-type, which was not sampled in Namibia or Costa Rica.

Major variability was observed within the bray series elements, especially for squeaks' sub-types. Gulps were not recorded in Panamá or Costa Rica, and grunts were also absent in Panama.

A total of eight sub-types of squeaks were detected for all populations, however in each study site only 2 to 5 variants were recorded. The most uncommon sub-types were Up-squeak, LD-Squeak and Sin-Squeak present only in Bahamas, Azores and Panamá samples, respectively. SC-squeaks were observed in all locations, except in Adriatic.

2.4.3. Repertoire (dis)similarity

Pairwise comparisons revealed different levels of repertoire similarity across populations (see Fig. 2.2).

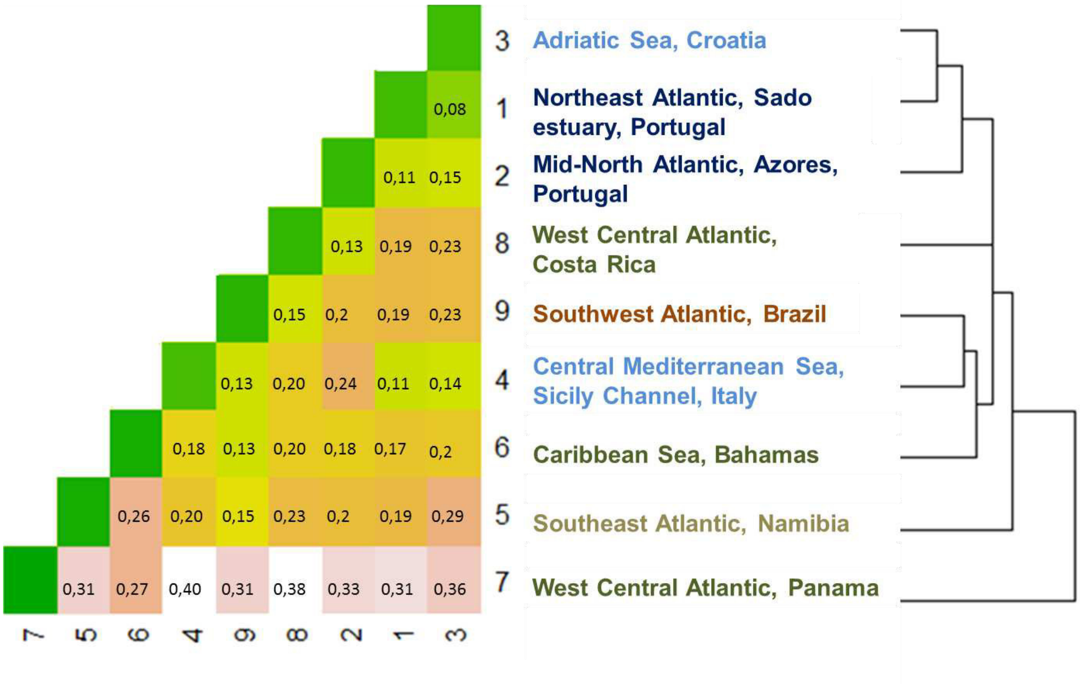


Figure 2.2. Acoustic repertoires' similarity. (a) Dendrogram of the hierarchical cluster analysis. (b) Heatmap of dissimilarity values, with pairwise comparisons values.

Sado estuary and Adriatic Sea presented the highest repertoire similarity (dissimilarity=0.08), while Panamá had the most divergent acoustic repertoire, with dissimilarity values up to 0.4. Acoustic samples from Namibia were also distinct from the majority of other repertoires (d≥0.2 for all populations, except Brazil and Sado estuary). Azores had high similarity with some of the northern hemisphere populations (Sado estuary: d=0.11, Adriatic sea, d=0.15) but also with Costa Rica (d=0.13). The highest dissimilarity value obtained for Costa Rica resulted from the comparison with Panama (d=0.38). Sicily had high similarity with coastal northern hemisphere populations (Sado estuary: d=0.11, Adriatic sea: d=0.14) but also with Brazil (d=0.13). Additionally, Brazil presented high repertoire similarity with most of the southern hemisphere populations (Costa Rica: d=0.13, Bahamas: d=0.13 Namibia: d=0.15).

2.5. DISCUSSION

This study compares acoustic signals emitted by common bottlenose dolphins from nine locations across the Atlantic Ocean and the Mediterranean Sea, with the intent of assessing multi-regional richness in the repertoires within this cosmopolitan species.

In the samples obtained from these nine populations, the repertoires included all the previously reported signal categories (whistles, BP-sounds, bray series elements and bangs), which we here classify in 21 nominal signal sub-types. Bottlenose dolphins are considered a highly vocal species, given both the diversity of calls and their often abundant emission rates. Our results validate the assumption of a rich repertoire for this species, in line with values reported for other vocal groups such as birds and non-human primates (Leighton, 2017; Mccomb & Semple, 2005).

Repertoires containing a large number of structurally and functionally distinct elements are often presented as a measure of complexity in communicative systems (Freeberg et al., 2012). Our results reveal a wide diversity of calls, with structurally distinct elements, each with specific time-frequency features. Moreover, it reveals substantial variability in repertoire content among nine mostly allopatric populations.

According to the Social Complexity Hypothesis for Communication (Freeberg et al., 2012), animals that live in more complex social environments require more elaborate communication systems to regulate interactions and relations among group members.It is generally assumed that complex communication systems entail a larger number of signal types. Following these notions, one might expect that populations with larger group sizes would have more signal types. Repertoire sizes in this study varied from 13 to 19 sub-types but, interestingly, the largest repertoire was obtained from the smallest population (<30 individuals, Sado estuary, Portugal). In this stable resident community, other aspects of social complexity must be considered, such as often repeated interactions with many of the same individuals, in networks, over time. In primate societies with extensive affiliative relationships, animals use diverse vocal signal types to facilitate friendly interactions (Gustison, le Roux, & Bergman, 2012; Pollard & Blumstein, 2012). The large repertoire size in the Sado estuary could be related to the very high association indices presented by this population (Augusto, Rachinas-Lopes, & dos Santos, 2012).

It should be noted that all Southern hemisphere populations had a repertoire size of 13 nominal call sub-types while all Northern hemisphere groups display slightly higher repertoire richness (an average of 17 sub-types). Our limited sample size, for some locations, precludes, at this stage, any further interpretation.

2.5.1. Common signal types and acoustic variants

Despite the differences found in repertoire size, there were common signal types recorded in all nine locations. Whistles, creaks, squawks, variable rate click trains, bangs and squeaks were recorded at all sites, although for whistles and squeaks only specific sub-types occurred at all locations. The conspicuous occurrence of several pulsed signals (creaks, squawks, variable rate click trains, bangs) may be linked to their specific food-related functions. Creaks and squawks have been described as terminal foraging buzzes and arousal calls that are emitted sequentially during feeding events (Ridgway et al., 2014). Variable rate click trains and bangs are also produced during foraging/feeding contexts and might play important roles in prey detection and startling (Luís et al., 2016b; Nowacek, 2005). Although shifts in frequency and call rate may occur as a result of local habitat adaptations (Luís, Couchinho, & dos Santos, 2014), the occurrence of these signals seems universal and may result from selective pressures related with feeding efficiency.

When it comes to social signals, such as whistles and bray series, acoustic divergence was noteworthy. While sinusoidal and convex whistles seem to be universal whistle types, other frequency modulated whistles were only recorded in some locations. Part of this variability may result from the unique contours of signature whistles, developed through vocal learning and used for long-term recognition of the individuals (Caldwell et al., 1990; Kershenbaum et al., 2013; King & Janik, 2013; Laland & Janik, 2006; Sayigh et al., 2007). Here, whistles were grouped in general frequency modulation categories, regardless of the specific contour. Frequency modulation of whistles is known to be influenced by the context of emission, namely the existence of different soundscape models and environmental constrains (Buckstaff, 2004; Luís et al., 2014; May-Collado & Quiñones-Lebrón, 2014; May-Collado & Wartzok, 2008; Morisaka, Shinohara, & Nakahara, 2005). Thus, geographic variations in whistle emission may reflect local adaptations to ambient noise backgrounds. Another relevant hypothesis is the occurrence of signal convergence/divergence for populations that exhibit interspecific associations, namely in the Bahamas, with Atlantic spotted dolphins (Herzing, 1996) and Costa Rica, with Guiana dolphins (May-Collado, 2010).

Acoustic divergence was also observed for bray series elements, which presented high variability across populations. The nature of these information-rich vocalizations has several structural similarities with syllabic emissions in humpback whales and birds' songs – sequential and timing aspects (Luís et al., 2018). In birds and whales, vocal elements in songs have a strong social basis and the expression of geographic variants can be associated with the species' vocal learning abilities – the individuals within a population learn specific vocal elements through a process of cultural transmission, horizontal or vertical (Garland et al., 2011; Podos & Warren, 2007). Bray elements may have a similar social basis, especially considering that bottlenose dolphins are also vocal learners. However, for birds and whales, songs have been linked with sexual interactions (Catchpole, 1982; Herman, 2017) whereas for dolphins brays have been associated with feeding events (Janik, 2000c; King & Janik, 2015), and social and aggressive behaviour (Herzing, 2015). One possibility is that brays might encode specific semantic content (prey-related or other) and may be produced only in certain social/cultural/environmental contexts, which would account for the geographic variability found in this study.

2.5.2. Repertoire (dis)similarity

Repertoire dissimilarity values express the acoustic divergence among populations, remarkably low in this multi-regional assessment. Our data highlight the existence of a common pool of vocal elements, possibly ancestral, shared among all the groups. Geographic variation was observed only for elements reported as rich-information calls (whistles and brays).

Numerous factors have been hypothesized as drivers of geographic variation: i) sexual selection, where acoustic innovation is primarily promoted by female preferences (Podos & Warren, 2007), ii) signaling group membership and familiarity, where acoustic convergence facilitates social integration and forms a selective advantage (Gero, Bøttcher, Whitehead, & Madsen, 2016), iii) environmental adaptation, where selective advantage arises from adaptation to specific ecological demands (Velásquez, Moreno-Gómez, Brunetti, & Penna, 2018), iv) cultural drift, where geographic variability is a by-product of learning based on local models (Xie, Sun, Jin, & Feng, 2017). In our analysis, the emission of acoustic variants could not be specifically related to female preferences or affiliative displays.

Divergence occurred only in social signals, suggesting an important role for cultural transmission.

Interestingly, acoustic similarity values did not mirror the geographic distance between groups. The highest repertoire similarity values resulted from the comparison between the Sado estuary and Adriatic Sea populations. Although not geographically close, these two groups live in similar eco-ethological contexts - small resident populations, with identical habitat constrains, such as high levels of ambient noise (Augusto et al., 2012; Luís et al., 2014; Rako-Gospić, Radulovi, Vu, Plesli, & Mackelworth, 2017).These factors could result in similar acoustic adaptation strategies to similar social and environmental challenges. In contrast, the lowest similarity value was obtained for closely located populations with distinct eco-ethological characteristics (Costa Rica – Panama). In Costa Rica, the dolphins show low site fidelity and the habitat is oceanic, with little boat traffic (May-Collado & Wartzok, 2008), while in Panama bottlenose dolphins show high degree of site fidelity (Barragán-Barrera et al., 2017) and are exposed to high levels of anthropogenic noise due to the intensive vessel traffic (May-Collado & Quiñones-Lebrón, 2014). These results strongly support the environmental adaptation hypothesis (for a review, see Ey & Fischer, 2009) – when exposed to distinct environmental pressures, individuals would produce acoustic signals with time-frequency characteristics more adapted to specific environmental situations. In fact, soundscape might be the strongest selective pressure for acoustic emissions, as it affects vocalization transmission and reception, and ultimately survival. Individuals exposed to high levels of noise are known to alter emission rates and exhibit shifts in time-frequency parameters of acoustic elements (Buckstaff, 2004; Luís et al., 2014; May-Collado & Wartzok, 2008; Morisaka et al., 2005). Long-term exposure to noise may induce acoustic divergence/convergence that eventually may result in the presence/absence of acoustic units. Moreover, the diversity of eco-ethological contexts provides numerous communication challenges but also specific environmental acoustic stimuli. Given that bottlenose dolphins are proficient vocal learners, variability in acoustic ecology among populations may well contribute to geographic variation.

The existence of acoustically distinct populations, with variant social signals, could act as a significant interaction and reproductive barrier. Combining the analyses of genetic and acoustic structure could help to clarify the boundaries and relationships between neighboring groups, and shed light into vocal learning and cultural transmission in bottlenose dolphin societies.

Geographic variation and vocal identity are aspects of biodiversity, often undervalued, and the explicit identification of acoustically distinct groups may be relevant to future conservation strategies, as recognized by the Convention on Migratory Species (CMS, 2014).

CHAPTER 3

SIGNATURE WHISTLES IN WILD BOTTLENOSE DOLPHINS: LONG-TERM STABILITY AND EMISSION RATES

Luís, A. R., Couchinho, M. N., & dos Santos, M. E. (2016). Signature whistles in wild bottlenose dolphins: long-term stability and emission rates. *acta ethologica, 19*(2), 113–122. doi: 10.1007/s10211-015-0230-z

3.1. ABSTRACT

Whistles are key elements in the acoustic repertoire of bottlenose dolphins. In this species, the frequency contours of whistles are used as individual signatures. Assessing the long-lasting stability of such stereotyped signals, and the abundant production of non-stereotyped whistles in the wild, is relevant to a more complete understanding of their biological function. Additionally, studying the effects of group size and activity patterns on whistle emission rate may provide insights into the use of these calls. In this study, we document the decades-long occurrence of whistles with stereotyped frequency contours in a population of wild bottlenose dolphins, resident in the region of the Sado estuary, Portugal. Confirmed stereotypy throughout more than 20 years, and positive identification using the signature identification (SIGID) criteria, suggests that the identified stereotyped whistles are in fact signature whistles. The potential roles of non-stereotyped whistles, which represent 68 % of all whistles recorded, are still unclear and should be further investigated. Emission rates were significantly higher during food-related events. Finally, our data show a comparatively high overall whistle production for this population, and no positive correlation between group size and emission rates, suggesting social or environmental restriction mechanisms in vocal production.

Keywords: bottlenose dolphins, *Tursiops truncatus*, signature whistles, long-term stability, activity patterns, group size.

3.2. INTRODUCTION

The exchange of information is a vital process in animal societies, and the idea that social complexity is linked to higher vocal complexity has received increasing attention (Bouchet, Blois-Heulin, & Lemasson, 2013; Freeberg, Dunbar, & Ord, 2012; Maciej, Ndao, Hammerschmidt, & Fischer, 2013).

Furthermore, the ability to remember and recognize other individuals in a group appears to be an advantage in species that live in complex social units (Casey, Charrier, Mathevon, & Reichmuth, 2015; Moreira et al., 2013; Tibbetts & Dale, 2007; Wiley, 2013). Naturally, any type of sensory label that may convey information about presence or motivational state of particular individuals is of enormous potential value in social interactions.

Bottlenose dolphins are acoustically specialized animals capable of complex cognitive processes (Bruck, 2013; Herman, 2010; Marino et al., 2007) forming fission-fusion societies (Connor et al., 2000; Wells, 2014; Wells, Scott, & Irvine, 1987). This species presents a diverse repertoire of vocalizations associated with specific behavioural contexts: click trains used in sonar-related tasks (Au, 2004; Herzing & dos Santos, 2004; Jensen, Bejder, Wahlberg, & Madsen, 2009); burst-pulsed sounds emitted during social interactions, and during foraging/feeding events (Díaz-López & Shirai, 2009; dos Santos, et al., 1995; Herzing, 2000), and narrow-band, frequency-modulated whistles used in a variety of contexts (Acevedo-Gutiérrez & Stienessen, 2004; Caldwell & Caldwell, 1965; Janik & Slater, 1998).

Whistles with stable stereotyped contours have been described as learned, unique, individually distinctive contact calls known as “signature whistles” (Caldwell & Caldwell, 1965; Caldwell et al., 1990; Janik & Sayigh, 2013; Janik et al., 2006; Janik & Slater, 1998; Sayigh et al., 1999). Several studies indicate that signature whistles are communication signals developed though vocal learning that convey individual identity information and can be used to signal the presence and location of an emitter (Janik et al., 2006; Kershenbaum et al., 2013; King, Sayigh, Wells, Fellner, & Janik, 2013; Sayigh et al., 2007). Furthermore, playback experiments have highlighted the importance of such stereotyped stable signals in kin discrimination (Sayigh et al., 1999) and long-term recognition (Bruck, 2013). As to effects of sex, Sayigh, Tyack, Wells, & Scott (1990) found greater differences between the signature whistles of females calves and those of their mothers as compared to male calves, which could be related to the matrilineal social organization reported for this species.

Although slight variations in the signature whistles or their production in multiple loops have been proximately linked to emotional state expression (Caldwell et al., 1990), the persistence of multi-year stable contours has been noted both in captivity and in the wild (Caldwell et al., 1990; Sayigh et al., 1990).

Individual recognition has been considered as a key element in social contexts such as cooperation, competition and alliance formation (Casey et al., 2015; Mumm, Urrutia, & Knörnschild, 2014; Sharpe, Hill, & Cherry, 2013; Wiley, 2013). Thus, the existence of individual identity cues encoded in long-distance communication signals may enhance the recognition of kin, mates and allies, and facilitate social interactions in a demanding underwater environment.

Bottlenose dolphins' are long-lived animals that form dynamic social units, for whom the ability to recognize 'friends or foes' during long periods of time may help to mediate interactions. Despite the relevance of signature whistles' functions, these signals have been found to represent between 38% and 70% of all whistle production in the wild (Buckstaff, 2004; Cook et al., 2004; Watwood et al., 2005). The role of non-stereotyped whistle emissions is still unclear. The identification of stereotyped and non-stereotyped whistles and its context of emission in wild populations is a critical step towards a better understanding of such distinctive calls. Still, to single out signature whistles in free-ranging dolphin groups can be challenging given the difficulty of pinpointing the emitters of each call. Recently, the application of SIGnature IDentification (SIGID) method for bout analysis has proved to be effective in the identification of signature whistles in field recordings of bottlenose dolphins (Janik et al., 2013; King & Janik, 2013; Kriesell, Elwen, Nastasi, & Gridley, 2014). Additionally, the use of the photo-identification technique in small groups of dolphins has been proposed as a possible strategy to identify the whistling animals (Lima & Le Pendu, 2014). In the Sado estuary (Portugal), the acoustic behaviour and the social structure of a well-known small resident group of approximately 30 bottlenose dolphins has been studied for more than two decades (Augusto, Rachinas-Lopes, & dos Santos, 2012; dos Santos et al., 1995) and the occurrence of stable stereotyped whistles has been previously reported (dos Santos et al., 1990; dos Santos et al., 2005).

In this study, we investigate the effects of activity pattern and group size on the emission rates of stereotyped and non-stereotyped whistles. Furthermore, we positively identify signature whistles for the first time in this population, using the SIGID method (Janik et al., 2013), and document the long-term occurrence of stable stereotyped contours.

3.3. METHODS

Field recordings were made in the Sado estuary, Portugal, and adjacent coastal waters (approximate location of the Sado mouth is 38° 29′ N, 8° 55′ W).

All data were collected from an 8.40-m-long inboard motorboat during daylight hours (1000 to 1800), from April 2011 to October 2014 with sea state ranging from 0 to 3 Beaufort. Whenever a group of dolphins was detected, and after a 15-min habituation period, the boat was positioned approximately 500 m ahead of the group's position, with the engines and all boat devices turned off, and the hydrophone was placed at a depth of at least 3 m. Acoustic samples were collected for periods of time that lasted from 1 to 10 min (mean duration=1.50 min±0.98), when the animals were within a 500- m radius.

Acoustic measurements were carried out using a factory-calibrated recording system: a Cetacean Research Technology hydrophone, model C55 (effective sensitivity of −165 dB re 1 V=1 μPa, frequency response of ±3 dB in the 0.020- to 44- kHz band and +3/−13 dB in the 0.009- to 100-kHz band, polarized by a 9-V battery) connected by a 15-m cable to a Fostex FR-2 digital recorder. High-pass filter at 100 Hz was chosen to avoid self-noise generated by the recording platform and low-frequency vibrations. Recordings were made, at a sampling rate of 192 kHz and with a 24-bit resolution, with the recording level fixed at the calibrated settings and the trim level at −26 dB. All recordings were stored on Compact Flash memory cards as time-stamped wave files. The geographic location of each recording was given by a Garmin Foretrex 301 portable GPS.

Photographs of surface behaviour and dorsal fins were collected with a digital Nikon D70S and/or a Canon EOS 400D (both with 70–300-mm zoom lenses).

Behavioural context was ascribed according to the following categories (based on dos Santos et al., 2005): (1) travelling—linear position of a tight group moving between areas, with no aerial behaviours; (2) foraging/milling—zigzag movements of a subdivided group, occasionally with dives longer than 1 min and aerial activity; (3) surface feeding— spread out individuals, groups surfacing more than 10 m apart, with only very short dives and abundant movements at the surface, including captures, prey leaping or prey toss; and (4) social interactions—active surface and aerial behaviours, with physical contacts and no prey detectable, sometimes with synchronous movements. Resting behaviour, with the animals floating at the surface in tight units, was also observed in four occasions, but these samples were excluded from the analyses.

Group size was estimated by direct counting of the animals by two observers and subsequently averaging the counts. Group composition was determined by photo-identification techniques and using the photo catalogues of the Sado population (see www.projectodelfim.pt).

3.3.1. Additional data

For the whistle classification task and long-term stability assessment, the whistles' categories created in 1990 and 2005 were used. Details of the recording equipment and methodology are given in dos Santos et al. (1990, 2005).

3.3.2. Acoustic analyses

For acoustical analyses, only recordings with no vessels within a 2-km radius of the dolphin groups were considered. In order to identify, categorize and count all the vocal elements present in each sample, recordings were first inspected by two trained independent observers, aurally and visually, using Adobe Audition CS5.5 (Adobe Systems Inc., San Jose, CA) with Hamming windows of 512 points. Tonal, narrow-band, modulated signals were classified as "whistles" and selected for further analyses.

Spectrograms of all whistles with clear and complete frequency modulation contours were plotted and labelled using Raven Pro 1.4 (Cornell Lab of Ornithology, Ithaca, NY) with Hann windows of 512 points and a frequency resolution of 93.8 Hz and 50 % overlap.

Whistles were then classified according to their fundamental frequency contour, using visual classification (a reliable method, broadly used in animal communication studies, see Janik, 1999) based on key frequency modulation aspects: start and end frequency and inflection points.

The first step of the classification process was to identify whistles with stable contours that occurred repeatedly in our dataset, which were classified as stereotyped whistles (SW). The remaining whistles, with variable contours, were classified as non-stereotyped whistles (NSW). Secondly, each SW was compared with templates of known whistle types present in a catalogue of stable whistle contours recorded in the Sado region since 1987 (dos Santos et al., 1990, 2005). Whenever a whistle matched a known category, it would be classified as such. For whistles that could not be assigned to any of the categories present in the catalogue, new mutually exclusive categories were created. Whistle classification was independently carried out by three experienced observers and subsequently discussed and cross-validated.

The SIGID method (Janik et al., 2013) was adopted in order to identify signature whistles. For each SW category, the whistles' emission sequence was analysed and inter-whistle interval measured. If 75 % or more of the whistles in each category occurred within 1 to 10 s of each other, that category would be positively identified as a signature whistle. The SIGID criteria were only applied in categories with at least five whistles.

3.3.3. Statistical analyses

For the analyses of whistle occurrence, emission rates were obtained by dividing the number of whistles by the number of minutes of each sample and by group size. Correlations between group size and the emission rates were calculated (Pearson product-moment correlation).

Lilliefors (Kolmogorov-Smirnov) tests were used to test for normality, and Levene tests were used to test for homogeneity of variances. A Mann-Whitney U test was performed to compare the emission rate of SW and NSW.

Kruskal-Wallis tests were performed to compare the emission rate according to the behavioural state identified during sampling ("surface feeding", "foraging/milling", "social interactions" or "travelling"). Post hoc tests for pairwise comparisons were used to identify the main differentiating activities (Dunn tests for SW and NSW).

All statistical analyses were performed using IBM SPSS Statistics 21 (IBM Inc.).

3.4. RESULTS

From the acoustic signals collected from 2011 to 2014, a total of 1715 whistles were extracted for further analyses (see Table 3.1.).

Of all the selected whistles, 32 % were clearly identified as stereotyped whistles (SW=550 whistles). The remaining 1165 whistles presented variable frequency-modulated contours and were considered non-stereotyped whistles (NSW).

The whistles classified as SW were ascribed to 27 contour categories (Table 3.2.).

Table 3.1. Summary of data collection

	Sample size (N)	Recordings (min)	Extracted whistles (N)
April to November 2011	127	164	923
April to August 2013	84	116	274
March to October 2014	72	123	518

Table 3.2. Contour types classified as stereotyped whistles (SW) and identified as signature whistles

	1987 & 1988 (dos Santos et al., 1990)	1992 and 1999 (dos Santos et al., 2005)	2011–2014 (this study)	Whistles with a bout interval of 1–10 s (SIGID criteria)
AP[a]	•	•	•	80 %
AP3		•	•	NA
CA1	•	•	•	44 %
CA3		•	•	68 %
CA4[a]		•	•	80 %
CE[a]		•	•	85 %
CM[a]		•	•	82 %
CM2[a]			•	78 %
CM3[a]			•	100 %
CM4			•	50 %
CO		•	•	30 %
DE[a]		•	•	90 %
DS			•	44 %
HP[a]			•	92 %
KE[a]	•	•	•	79 %
LE			•	73 %
MC		•	•	60 %
ME[a]			•	83 %
ME2			•	57 %

RA[a]	•	•	76 %
SO2		•	25 %
UL		•	50 %
UP		•	59 %
US	•	•	65 %
VU	•	•	NA
VU2[a]		•	87 %
WA		•	44 %

Following the SIGID criteria, whistles with more than 75 % of its occurrence within 1–10 s of each other were positively identified as signature whistles. ([a] Signature whistles; • Whistle type identified in the dataset).

The SW categories exhibit long-term stability: whistle types “AP”, “CA1” and “KE” were recorded during the three periods of data collection (see Fig. 3.1.), and 11 other whistle types were previously identified in the 1990s. The 13 new contour categories comprise 51 % of all SW whistles recorded from 2011 to 2014.

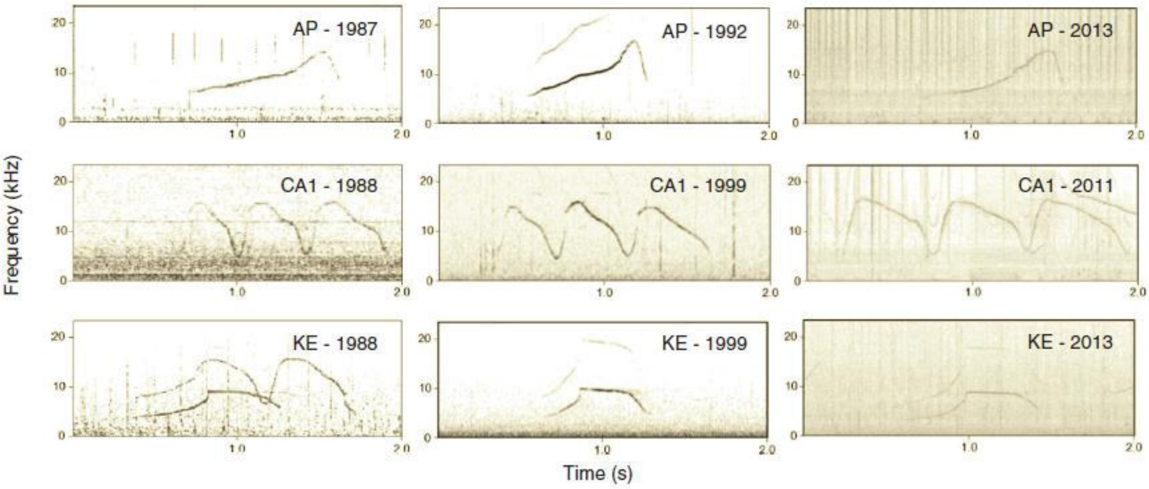


Figure 3.1. Representative spectrograms of long-term stability in stereotyped whistles recorded in the Sado region, Portugal

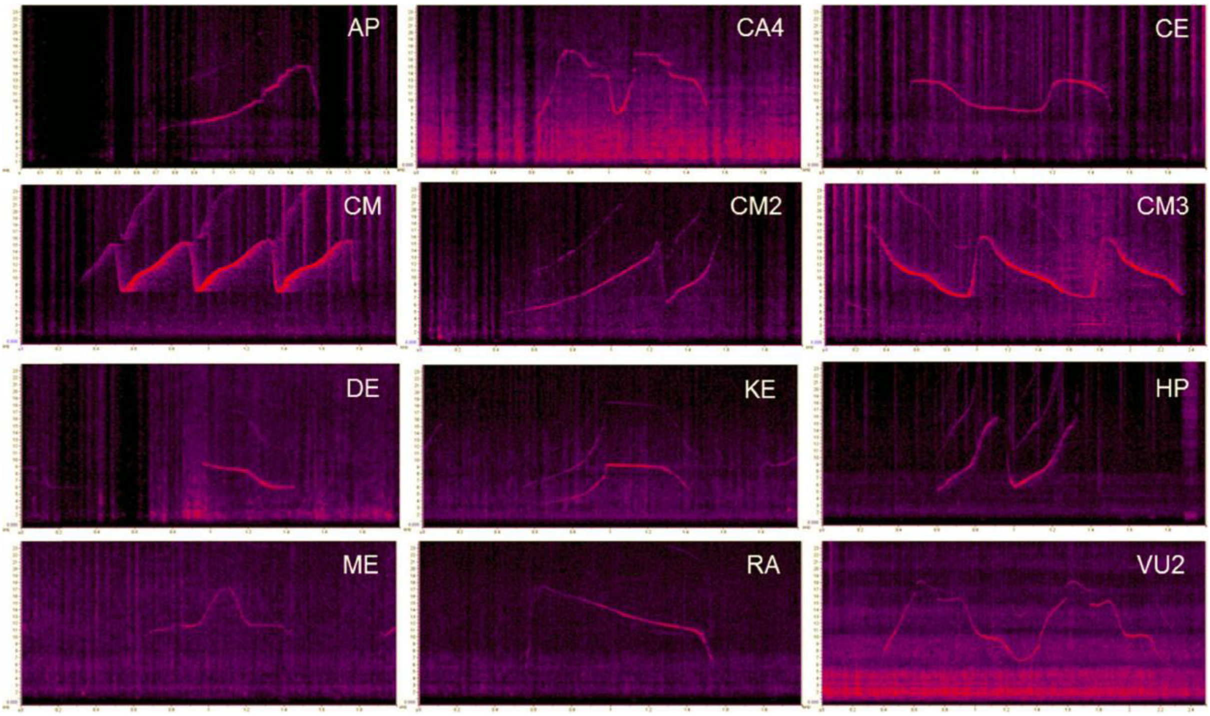


Figure 3.2. Stereotyped whistle contours positively identified as signature whistles using the SIGID method. Spectrograms with 512-point Hann windows and a frequency resolution of 93.8 Hz

The SIGID criteria for bout analysis (Janik et al., 2013) allowed a positive identification as signature whistles of 12 stereotyped whistle types in our dataset (Fig. 3.2.).

3.4.1. Emission rates

Although the number of animals present during recordings varied between 2 and 24, no correlation was found between the group size and the emission rate of whistles ($r_P=-0.068$, N=271, P=0.271).

The mean overall whistle rate recorded in the Sado estuary was 0.56 whistles/min/dolphin. Non-stereotyped whistles were more abundant than stereotyped whistles in our recordings (68 % of all the good-quality whistles), and significant differences were found when comparing their emission rates (SW—0.15 whistles/min/dolphin; NSW—0.41 whistles/min/ dolphin; Mann-Whitney U test: U=46 348, N=541, P<0.01).

3.4.2. Activity patterns

For both stereotyped and non-stereotyped whistles, the lowest emission rates were observed during “travelling” (SW=0.09 whistles/min/dolphin, NSW=0.2 whistles/min/dolphin).

Significant differences were found in the emission rates of both stereotyped and non-stereotyped whistles according to the activity patterns (SW—Kruskal-Wallis test: H_3 = 19.072, P <0.01, N =272; NSW—Kruskal-Wallis test: H_3 = 39.744, P <0.01, N =269).

For the stereotyped whistles, the highest emission rates were obtained during 2social interactions” (0.29 whistles/min/dolphin). Pairwise comparisons revealed significant differences between “surface feeding” and “travelling” (P < 0.01) and “foraging/milling” and “travelling” (P=0.019) (Fig. 3.3.).

For non-stereotyped whistles, the highest emission rates were recorded during “surface feeding” (0.61 whistles/min). Pairwise comparisons revealed significant differences between “surface feeding” and “travelling” (P < 0.01) and “foraging/milling” and “travelling” (P < 0.01) (Fig. 3.4.).

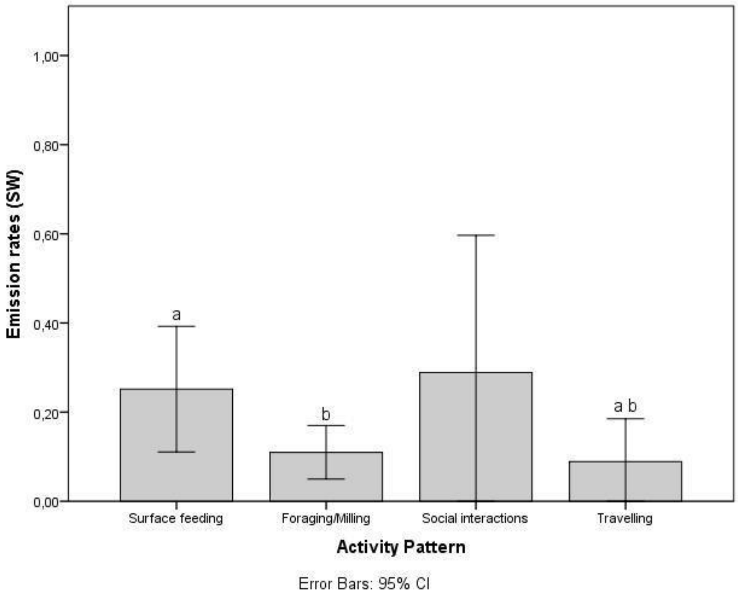


Figure 3.3. Emission rate (whistle/min/dolphin) of stereotyped whistles according to the activity pattern. Significant differences: “surface feeding” and “travelling”, (a) and “foraging/milling” and “travelling” (b). Error bars 95 % CI.

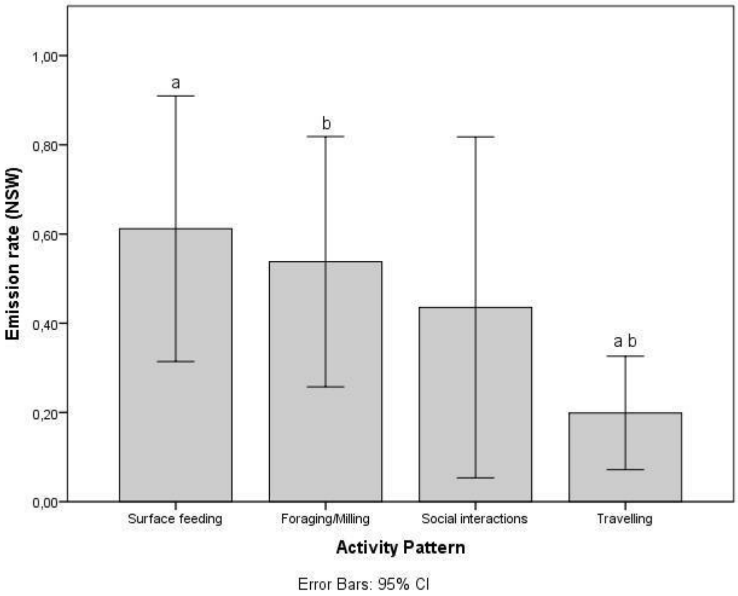


Figure 3.4. Emission rate (whistle/min/dolphin) of non-stereotyped whistles according to the activity pattern. Significant differences: “surface feeding” and “travelling” (a) and “foraging/milling” and “travelling” (b). Error bars 95 % CI.

3.5. DISCUSSION

3.5.1. Long-term occurrence of stereotyped whistles

Long-lasting, acoustically stable signals have been indicated as fundamental units for long-term individual recognition in socially complex societies (Bruck, 2013; Jouventin & Aubin, 2002; Pitcher, Harcourt, & Charrier, 2010). In this study, we document the decades-long occurrence of whistles with stereotyped frequency contours in the resident population of bottlenose dolphins from the Sado region (first recorded in 1987 and 1988, see dos Santos et al., 1990). In a context of considerable frequency contour variability, a number of stable contours were maintained during more than two decades, highlighting the importance of stereotyped whistles in the acoustic repertoire of these free-ranging dolphins. These stable acoustic signals likely serve as individual identity labels that enable long-term social recognition, as suggested by Bruck (2013) and Sayigh et al. (1990).

In our analyses, the occurrence of well-defined stereotyped contours represents approximately one-third of all whistle emissions, a figure close to the lower limit documented for the occurrence of signature whistles in other studies (Buckstaff, 2004; Cook et al., 2004; Watwood et al., 2005).

Despite the long-term occurrence of several stereotyped whistle types, only 12 contours were positively identified as signature whistles using the SIGID criteria. As its proponents admit, the SIGID criteria are conservative and therefore can miss out about half of the signature whistles in an emission dataset (Janik et al., 2013). Thus, the number of signature whistles in this population may well be underestimated.

It should be noted that during the sampling period, the resident population consisted of 27 individuals (the exact number of observed stereotyped whistle contours). Interestingly, 13 of the individuals in the current population were already present in 1999 (the exact number of stereotyped whistle contours then identified, and still observed). Hence, it is possible that all the identified stereotyped whistles are, in fact, individual signature whistles.

3.5.2. Emission rates

The overall whistle production in Sado estuary was slightly higher than the majority of the emission rates reported for other populations (Cook et al., 2004; Díaz- López, 2011; Jones & Sayigh, 2002; Quick & Janik, 2008; Watwood et al., 2005). In small resident populations, lower whistle rates have been explained by the familiarity between group members and habitat characteristics (e.g., Jones & Sayigh, 2002). In the Sado, despite the small size, the known stability of the population and its residency patterns (Augusto et al., 2012), that was not the case. As suggested by several authors (Buckstaff, 2004; Esch, Sayigh, Blum, & Wells, 2009), increase in the overall emission rate may be related to environmental disturbance. Thus, in areas with persistent human pressure, such as the Sado estuary (Luís et al., 2014), high levels of whistle emission might be an adaptive response that promotes group cohesion in the presence of multiple stressors (e.g., boat traffic, fishing activities).

For species that live in complex societies, the number of recorded calls often correlates positively with the number of individuals present during a sampling period (Borker et al., 2014; Hofmeyr-Juritz & Best, 2011; Payne, Thompson, & Kramer, 2003; Radford & Ridley, 2008). That pattern has been previously reported for bottlenose dolphins (Jones & Sayigh, 2002) but does not occur in all populations (dos Santos & Almada, 2004; dos Santos et al., 2005; Quick & Janik, 2008). In this study, high levels of whistle rates were observed both for small and larger groups, with the number of whistles per dolphin decreasing as group size increases. Similar results have been previously reported for the Moray Firth population (Quick & Janik, 2008). Environmental conditions, such as high levels of background noise, may be related to this particular pattern. In a noisy habitat, like the Sado estuary, each individual may limit its vocal emissions as the group size increases to avoid overlapping and facilitate an optimal transmission of the emitted signals, especially if a high number of communication calls are produced. It might also be a socially transmitted, variant pattern found in some populations, possibly improving communication efficiency as group size increases.

3.5.3. Activity patterns

Emission rates of both stereotyped and non-stereotyped whistles varied with ongoing dolphin activities, as found in other studies of wild bottlenose dolphin populations (Acevedo-Gutiérrez & Stienessen, 2004; Cook et al., 2004; Jones & Sayigh, 2002; Díaz-López & Shirai, 2009; Quick & Janik, 2008).

The lowest whistle rates were obtained during “Travelling”. In fact, for approximately half of our “Travelling” samples, whistle rates were zero regardless of group size. Due to its hydrographic and physiographic features, the Sado estuary is a challenging navigation area where echolocation and passive vigilance may be more important than communication during “Travelling”, especially when the groups are moving in or out. Further studies should address the role of environmental variables such as depth, estuary width and bottom topography in whistle production.

Regarding specifically stereotyped whistles, the highest emission rates were obtained during “Social interactions”. Several studies have reported higher rates of whistle emission during socialization, as a consequence of increased arousal, or to maintain contact with other group members away from the interaction (Cook et al., 2004; dos Santos et al., 2005; Quick & Janik, 2008). In the Sado estuary, social interactions occur in small groups as short sporadic events that include synchronized leaps and physical contact. Considering the strong social bonds between the individuals that compose this small, stable, resident population, the high emission of stereotyped whistles during social interactions could be seen either as affiliative, indicating a motivation to reunite or an “invitation” for other animals to join the social event, or signaling aggression.

Emission rates of stereotyped whistles were significantly higher during “Surface feeding” and “Foraging/Milling”. Recent studies highlight the role of synchronous calling during cooperative behaviour (Herzing, 2015; King & Janik, 2015). Furthermore, the matching of signature whistles has been presented as regulator of individuals spacing during intensive feeding bouts (King & Janik, 2015). At our study site, surface feeding episodes are mostly cooperative, aroused events that include prey herding. It should also be noted that in the Sado estuary visibility is usually reduced to less than 5 meters. Following the “ranging hypothesis” (Hopp & Morton, 1998), a higher emission rate of stereotyped whistles may be explained by the need of knowing the exact location of each individual during a coordinated hunting activity.

Despite the relevance of stereotyped whistles in the acoustic communication of bottlenose dolphins, we must emphasize that non-stereotyped whistles represented 68% of all the analyzed whistles and the role of such calls is still unclear. Alarm calls and food-related calls are among the most common acoustic signals produced by mammals and birds (Clay, Smith, & Blumstein, 2012; Hollén & Radford, 2009). In our study, emission rates of non-stereotyped whistles were significantly higher during “Surface feeding” and “Foraging/Milling”, suggesting a possible role of these signals as food-related cues or they may just signal an aroused context. Given the observed excitement during feeding events, non-stereotyped whistles could be simply expressions of emotion. Nonetheless, these signals may also provide information regarding food location, quantity and/or quality, as it has been reported for other species that live in complex social groups, such as ravens and chimpanzees (Clay et al., 2012). Further studies are needed to fully unravel the detailed function of non- stereotyped whistles and their relevance in the acoustic repertoire of bottlenose dolphins.

In conclusion, stereotyped whistles of bottlenose dolphins clearly have a special role as stable signature calls, with key relevance during events that require group coordination, such as feeding and foraging. Such identity labels are critical for long-term social recognition and social memory, facilitating cooperation and differential affiliation patterns based on past interactions. The decades-long occurrence of several whistle contours in this population seems to support this notion.

Although non-stereotyped whistles are particularly abundant in food-related events, their specific roles require further research.

CHAPTER 4

A QUANTITATIVE ANALYSIS OF PULSED SIGNALS EMITTED BY WILD BOTTLENOSE DOLPHINS

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4.1. ABSTRACT

Common bottlenose dolphins (*Tursiops truncatus*), produce a wide variety of vocal emissions for communication and echolocation, of which the pulsed repertoire has been the most difficult to categorize. Packets of high repetition, broadband pulses are still largely reported under a general designation of burst-pulses, and traditional attempts to classify these emissions rely mainly in their aural characteristics and in graphical aspects of spectrograms. Here, we present a quantitative analysis of pulsed signals emitted by wild bottlenose dolphins, in the Sado estuary, Portugal (2011–2014), and test the reliability of a traditional classification approach. Acoustic parameters (minimum frequency, maximum frequency, peak frequency, duration, repetition rate and inter-click-interval) were extracted from 930 pulsed signals, previously categorized using a traditional approach. Discriminant function analysis revealed a high reliability of the traditional classification approach (93.5% of pulsed signals were consistently assigned to their aurally based categories). According to the discriminant function analysis (Wilk's $\Lambda = 0.11$, $F_{3,\,2.41} = 282.75$, $P < 0.001$), repetition rate is the feature that best enables the discrimination of different pulsed signals (structure coefficient = 0.98). Classification using hierarchical cluster analysis led to a similar categorization pattern: two main signal types with distinct magnitudes of repetition rate were clustered into five groups. The pulsed signals, here described, present significant differences in their time- frequency features, especially repetition rate ($P < 0.001$), inter-click-interval ($P < 0.001$) and duration ($P < 0.001$). We document the occurrence of a distinct signal type–short burst-pulses, and highlight the existence of a diverse repertoire of pulsed vocalizations emitted in graded sequences. The use of quantitative analysis of pulsed signals is essential to improve classifications and to better assess the contexts of emission, geographic variation and the functional significance of pulsed signals.

Keywords: bottlenose dolphins, *Tursiops truncatus*, acoustic repertoire, burst-pulses, quantitative analysis

4.2. INTRODUCTION

Common bottlenose dolphins, *Tursiops truncatus*, have a complex acoustic repertoire (Caldwell et al., 1990; Herzing, 1996; Janik, 2000a; Lammers & Oswald, 2015), comprised of three major types of signals: (i) tonal, omnidirectional, frequency-modulated whistles used as cohesion calls and communication signals (Caldwell & Caldwell, 1965; Janik & Sayigh, 2013; Lilly & Miller, 1961), (ii) highly directional echolocation clicks used in biosonar tasks (Au, 1993), and (iii) a variety of other pulsed signals, with high repetition rate (above 300 pulses per second) and short inter-click-intervals (less than 3 ms), the “burst-pulses” (Au & Hastings, 2008; Herman, 2002; Herzing & Johnson, 2015).

Although burst-pulses are formed by broadband pulses often similar to echolocation signals, researchers studying acoustic production in delphinids consider these two separate categories. The distinction is supported by the notion that signals with very short inter-click-intervals (ICI) cannot be perceived individually, thus packet emission of clicks may prevent functional sonar (Au, 1993; Au & Hastings, 2008; Herman, 2002; Herzing & Johnson, 2015). However, this simplistic segregation may not reflect the true nature of these signals as the pulses within these high repetition, pulsed signals can be produced with a range of time-frequency parameters (Lammers & Oswald, 2015). During echolocation tasks, click trains are usually emitted with ICIs longer than the two-way transit time (i.e. required time for a click to propagate to the tar- get, echo back and be processed) (Au, 1993). Strikingly, in captivity experiments, it has been verified that bottlenose dolphins are able to adjust their click emission and use burst-pulses with ICIs lower than the two-way transit time during long-range biosonar tasks (Finneran, 2013).

Understanding the functional significance of such signals is still challenging, especially since burst-pulses are not yet fully described. Despite the frequent occurrence of burst-pulses both in wild and captive bottlenose dolphins (Blomqvist & Amundin, 2004; Herzing, 1996), the vast majority of dolphin acoustic repertoire studies still focus on whistles and/or echolocation clicks. While the acoustic parameters, context of emissions and functional aspects of whistles and echolocation signals are well documented (Au, 2004; Quick & Janik, 2008; Sayigh et al., 2007; Wahlberg et al., 2011), research on burst-pulses is still scarce.

Classical attempts to classify these pulsed emissions rely mainly in their aural characteristics and in graphical aspects of spectrograms, which allows for much subjectivity. As a result, various terminologies, usually onomatopoeic, have been used to report burst-pulses that occur in various aroused behavioural contexts (Herzing, 1996; Diáz-López & Shirai, 2009). “Creaks” or “buzzes” have been described as detailed echolocation signals, produced during foraging and feeding events (e.g., Herzing, 1996; Miller, Johnson, & Tyack, 2004). Burst- pulses with very high repetition rates (200–1200 clicks/s), that are often recorded during courtship and agonistic interactions, have been classified as “squawks” and “squeaks” (Caldwell & Caldwell, 1967; Herzing, 2000; Lammers et al., 2003). Additionally, there are descriptions of “barks”, “screams”, “cracks”, “pops”, “genital buzzes” and “yelps”, observed during agonistic interactions, fright and alarm situations, and courtship and precopulatory behaviours (Connor & Smolker, 1996; Herzing, 1996; Overstrom, 1983; Ridgway, Carder, & Romano, 1991). The rhythmic sequences denominated as “Bray series”, recorded during feeding activities, are also part of the diversity of burst-pulses (dos Santos et al., 1990; dos Santos et al., 1995; Janik, 2000c; King & Janik, 2015).

Although the contribution of qualitative methods to the description of dolphins' acoustic repertoires is recognized, the reach of such subjective classifications is limited. Burst-pulses are mainly ultrasonic signals (Lammers et al., 2003), with inter-click-intervals (ICI) too short to be discernible to the human ear (Au, 1993). Minor changes in ICIs may be misinterpreted or perceived differently depending on the observer, which may lead to the creation of artificial groups or incorrect classifications. Also, a signal with lower ICI, within human perception abilities, might be easier to classify using qualitative methods.

The use of graphical aspects of spectrograms in pulsed signals classification can also be problematic, due to the graphical representations of acoustic signals are highly dependent on the selected time-frequency parameters and analysis windows. High-repetition pulsed signals may be represented as horizontal bands that resemble harmonics of a tonal sound depending on the analysis windows settings (Watkins, 1968).

Distinct packets of pulses emitted by dolphins are still mostly reported under a general designation of burst-pulses, hindering comparisons among populations and diminishing our ability to document geographic variations in the acoustic repertoire of this species. These difficulties require an application for quantitative analysis to facilitate rigorous classification efforts. The use of multivariate classification techniques based on time-frequency parameters can be useful to overcome the methodological difficulties in pulsed signals categorization.

In this study, we assess the exactness of traditional classifications of pulsed signals by applying a discriminant function analysis (DFA) and hierarchical cluster analysis to a dataset of signals previously categorized using aural and visual classification.

Additionally, we present a quantitative characterization of broadband pulsed signals emitted by wild bottlenose dolphins that may contribute to clarifying the nature, context of emission and functional significance of these puzzling sounds.

4.3. METHODS

4.3.1. Data Collection

Field recordings were made in the Sado estuary, Portugal, and adjacent coastal waters (approximate location of the Sado mouth: 38° 29’ N, 8° 55’ W). All data were collected from a 8.40 m inboard motor vessel during daylight hours (1000 to 1800), on 42 days from April 2011 to March 2014, with sea state ranging from 0 to 3 Beaufort. Whenever a group of dolphins was visually detected, the research vessel was positioned approximately 500 m ahead of the group’s location, with the engines off, and the hydrophone placed at a depth of 3 m. The distance and relative position of the vocalizing individuals could not be assessed.

All acoustic measurements were carried out using a factory-calibrated recording system: a Cetacean Research Technology hydrophone, model C55 (effective sensitivity of -185 dB RMS re 1V = 1µPa, frequency response: 0.008 to 100 kHz ± 3 dB, polarized by a 9 V battery) connected by a 15 m cable to a Fostex FR-2 digital recorder. A high-pass filter of 100 Hz was chosen to avoid self-noise generated by the recording platform and low-frequency vibrations.

One-minute duration recordings were made, with a sampling rate of 192 kHz and 24-bit resolution, recording level of the Fostex at 7.5 and trim level at -26 dB. All recordings were stored on Compact Flash memory cards as time-stamped.wav files. The geographic location of each recording was provided by a Garmin Foretrex 301 portable GPS, and dolphin activities and group composition were registered by two experienced independent observers during the acoustic recordings.

4.3.2. Acoustic Analyses

Recordings were first inspected by two trained independent observers, aurally and visually, using Adobe Audition CS5.5 (Adobe Systems Inc.) with Hamming windows of 512 points, in order to identify and classify the pulsed signals present in each recording.

Pulsed signals were assigned to one of the following pre-established categories, according with graphical and aural characteristics: “Slow click trains”–discernible click trains; “Creaks”– long burst-pulse (>0.2 sec.), aurally similar to a creaking door; “Squawks”–long burst-pulse (>0.2 sec.), with higher repetition rate than “Creaks”, reminiscent of a crying baby; “Short Burst-Pulses (S-BP)”—short burst-pulse (<0.2 sec.), aurally similar to a buzzing bee but brief. Signals that were part of complex vocal sequences—Bray series were not included in these analyses due to their specific rhythmic characteristics, and will be discussed in a separate study.

All the identified sounds were rated based on signal-to-noise ratio (SNR) as follows: (i) poor–signal faint and hardly visible on the spectrogram, (ii) fair–signal visible and with a clear start/end on the spectrogram, (iii) good–signal well marked and with a clear start/end on the spectrogram. Non-overlapping signals rated as fair or good where selected for further analysis.

Raven Pro 1.4 (Cornell Lab of Ornithology) with Hamming windows of 512 points, frequency resolution of 93.8 Hz, 50% overlap was used to measure the acoustic parameters of the selected pulsed signals: minimum frequency, maximum frequency, frequency range, peak frequency and duration. The number of pulses for each pulsed signal was counted manually, using a playback rate of 0.01. In order to ensure a correct quantification of the pulses within the signal of interest, visual inspection of the spectrogram was carried out during the acoustic counting. Repetition rate (clicks/sec) and inter-click interval (ICI) were calculated based on the number of pulses and the duration of each sample.

To illustrate the variation of temporal and spectral features within each signal type the following parameters were obtained for an example of each pulsed signal: bandwidth at 3 dB and 10 dB (mean value and standard deviation based on measurements of each pulse within the signal), variation in repetition rate (difference of values between the first and the last 20% of the signal) and variation in peak frequency (difference of values between the first and the last 20% of the signal).

4.3.3. Statistical Analyses

Stepwise method of discriminant function analysis (DFA), with Wilks' Λ, was used to identify the acoustic parameters that enable the best discrimination among the selected pulsed signals. For the DFA, we used the groups Slow click trains, Creaks, Squawks and S-BPs, and the acoustic parameters: minimum frequency, peak frequency, duration, and repetition rate. As both repetition rate and ICI result from arithmetic formulas that include the number of pulses and the duration of each pulsed signals, only repetition rate was considered for the DFA analysis.

All the numeric variables were square-root transformed and multivariate outliers were removed. Based on the stepwise DFA, linear discriminant functions were computed. Classification results were used to verify the coherence between the initial labelling of sound types (based on graphical and aural characteristics) and DFA groups. A cross-validation "leave-one- out" method, with 95% confidence intervals, was used for validation.

Additionally, a hierarchical cluster analysis was carried out using the square Euclidean distance and average linkage (within groups) method, for all the pulsed signals rated as fair and good. To determine the number of clusters that best fit the data, the distance values for different stages solution, retrieved from the agglomeration schedule, were evaluated (see Tinsley & Brown, 2000). The goal was to assess how many distinct signal types could be determined based on the same acoustic parameters used for the DFA.

One-way ANOVAs (with Welch correction) were performed for the following acoustic parameters: minimum frequency, peak frequency, duration, repetition rate and ICI, using Bonferroni correction (significance level = 0.01). Dunnet's T3 post hoc tests were performed for pairwise comparisons.

All statistical analyses were performed using IBM SPSS Statistics 21 (IBM Inc.).

4.4. RESULTS

A total of 930 pulsed signals rated as fair or good were initially classified as Slow click trains (N = 393), Creaks (N = 220), Squawks (N = 252) and S-BPs (N = 65), based on aural and graphical characteristics (see Fig. 4.1., Table 4.1. and Fig. 4.2.).

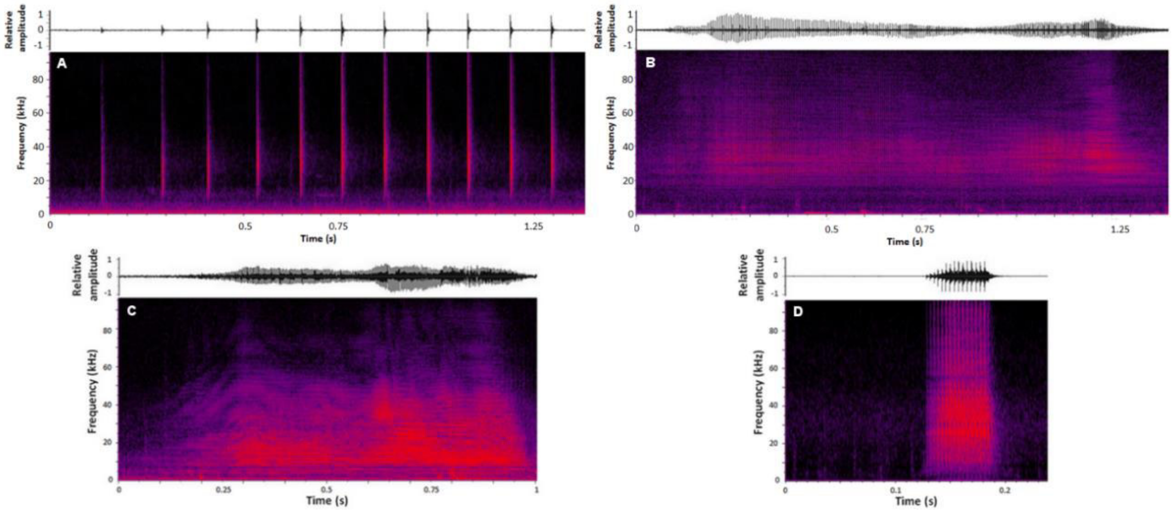

Figure 4.1. Examples of pulsed signals produced by bottlenose dolphins in the Sado region, Portugal: (A) Slow click train, (B) Creak, (C) Squawk, (D) S-BP. The upper panels show sound waveforms, with relative amplitude on the y-axis, and the bottom panels show spectrograms for each signal type, with frequency (kHz) on the y-axis. Time (s) is on the x-axis. Spectrogram settings: FFT 512, Hann window, overlap 50%.

Table 4.1. Temporal and spectral features within each signal type (illustrative example).

	Bandwidth at 3 dB (kHz)	Bandwidth at 10 dB (kHz)	Variation in repetition rate (clicks/sec.)	Variation in peak frequency (kHz)
S-BP	13.45 ± 4.46	41.92 ± 8.67	0	3.75
Slow click train	15.39 ± 4.79	28.29 ± 8.75	- 1.20	- 9.00
Creak	11.91 ± 6.22	33.55 ± 9.62	55.76	0.66
Squawk	9.56 ± 6.66	30.00 ± 7.15	73.20	0.66

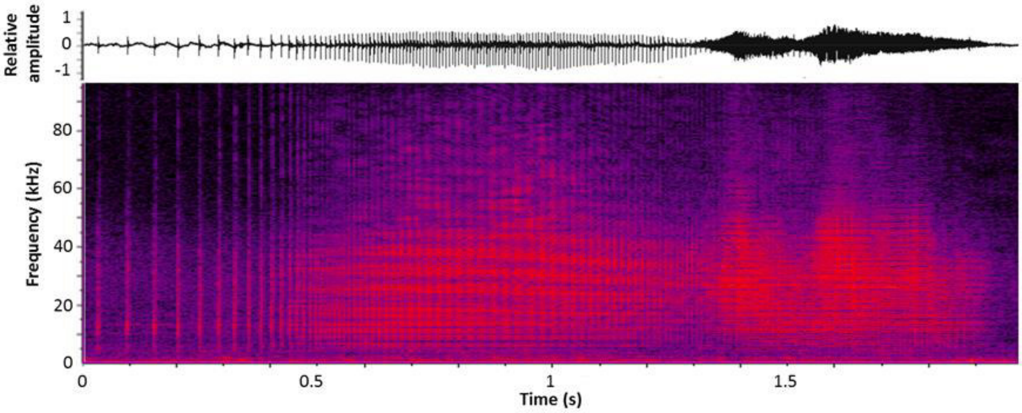

Figure 4.2. Example of pulsed signals emitted in a sequence (Slow click train–Creak–Squawk). Upper panel shows the signal waveform, with relative amplitude on the y-axis. Bottom panel shows the spectrogram, with frequency (kHz) on the y-axis and time (s) is on the x-axis. Spectrogram settings: FFT 512, Hann window, overlap 50%.

DFA extracted three functions that enable the discrimination of the selected pulsed signals (Fig. 4.3.). Function 1 ($\Lambda = 0.073$; χ^2 (12) = 2174.64; $P < 0.001$) was defined by repetition rate (structure coefficient = 0.98) and accounts for 98.9% of total variance. Function 2 ($\Lambda = 0.89$; χ^2 (6) = 98.46; $P < 0.001$) was defined by duration (structure coefficient = 0.91), and accounts for 0.8% of variance. Function 3 ($\Lambda = 0.96$; χ^2 (2) = 29.27; $P < 0.001$)

was defined by peak frequency (structure coefficient = 0.95) and minimum frequency (structure coefficient = 0.27), accounting for only 0.3% of total variance.

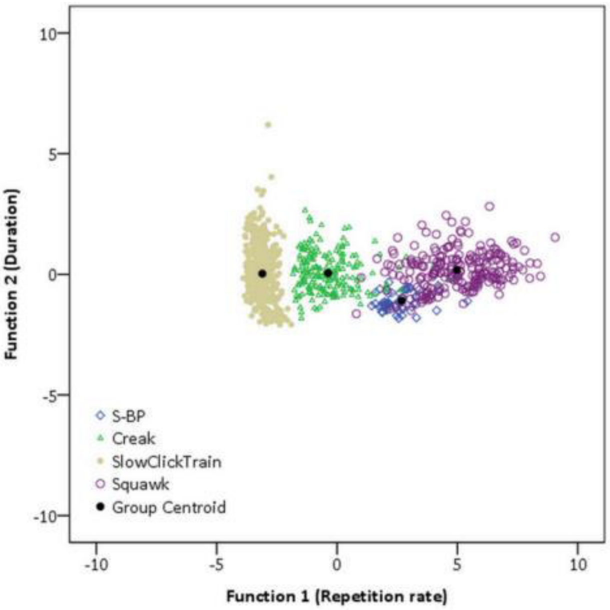

Figure 4.3. Canonical discriminant analysis plot of the four signal types. Function 1 ($\Lambda = 0.073$; $\chi^2 (12) = 2174.64$; $P < 0.001$) defined by repetition rate (structure coefficient = 0.98), on the x-axis. Function 2 ($\Lambda = 0.89$; $\chi^2 (6) = 98.46$; $P < 0.001$) defined by duration (structure coefficient = 0.91), on the y-axis. Functions 1 and 2 represent 99.7% of total variance.

The stepwise DFA correctly classified 93.5% of the analyzed pulsed signals in their predefined categories. Creaks and Slow click trains were the most consistently allocated to their original categories (93.9% and 98.1%). The few misclassified Slow click trains (N = 7) were initially named Creaks. Misclassified Creaks were initially labelled as S-BP (N= 8) or Squawks (N= 4), but not as Slow click trains. S-BPs and Squawks were also relatively well discriminated (90.7% and 86.1%), with few misclassifications of S-BPs as Squawks (N = 5), and Squawks being labelled as Creaks (N = 2) or Buzzes (N = 28). 91.3% of cross-validated grouped cases were correctly classified.

The output of the hierarchical cluster analysis revealed the existence of two possible cluster solutions (see Fig. 4.4.). For the first solution, two clusters were obtained: cluster 1 (#A) comprises all the signals previously labeled as S-BPs, and as Squawks (except for three signals); and cluster 2 (#B) combines all the signals labeled as Slow click trains, plus the large majority of signals labeled as Creaks (94%). A second possible solution with five clusters was obtained. In these solution, signals previously labeled as S-BPs, Creaks and Slow click trains were consistently clustered separately: 91% of all S-BPs were grouped in Cluster #1, 84% of Creaks were grouped in Cluster #2 and all Slow click trains were grouped in Cluster #4. Signals previously labeled as Squawks were assigned to three different clusters (#1, #2 and #3).

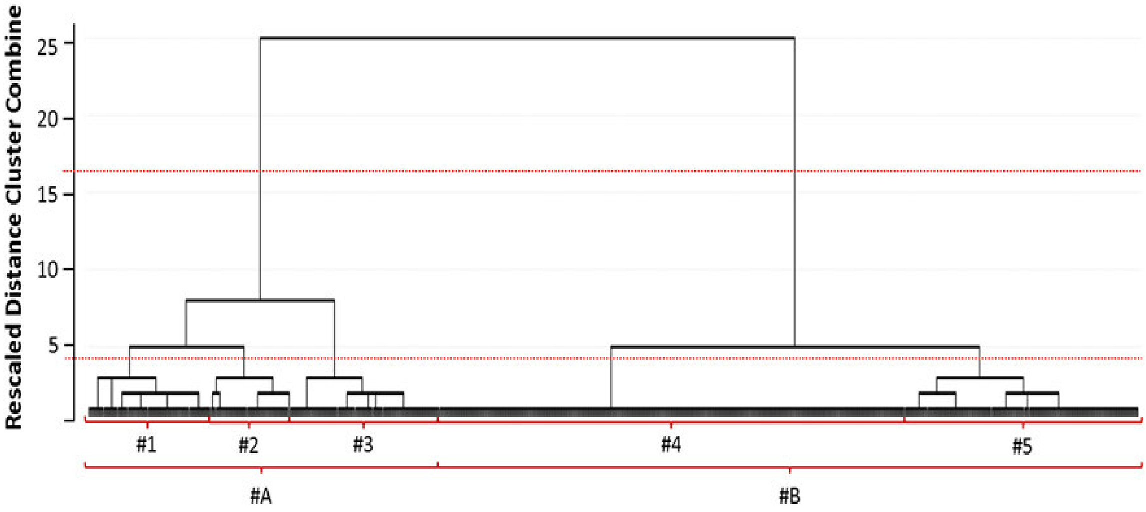

Figure 4.4. Dendrogram of the hierarchical cluster analysis using square Euclidean distance and average linkage (within groups) method. Cluster analysis was performed using square-root transformed variables (minimum and peak frequency, repetition rate and duration) of different pulsed signals produced by bottlenose dolphins in Sado region, Portugal. Two solutions with two and five clusters, respectively, are presented at X-axis with red lines. Y-axis represents the rescaled distance cluster combine, with a red line intercepting the cut-off value for the proposed cluster solution, based on the agglomeration schedule.

Different pulsed signals present specific acoustic parameters that differ significantly (Table 4.2.), especially in duration ($p<0.001$), repetition rate ($p<0.001$) and ICI ($p<0.001$).

The analyses of variance revealed significant differences for all the selected variables among the four classes of pulsed signals: peak frequency (F_{Welch} (3, 238.84) = 69.37, $P<0.001$), minimum frequency (F_{Welch} (3, 262.84) = 31.48, $P<0.001$), duration (F_{Welch} (3, 473.06) = 291.77, $P<0.001$), repetition rate (F_{Welch} (3, 207.86) = 909.29, $P<0.001$) and inter-click-interval (F_{Welch} (3, 328.64 = 671.62), $P<0.001$).

Slow click trains were the longest signals in duration followed by Creaks, Squawks and S-BPs. The highest repetition rates and lowest ICI were obtain for Squawks, followed by S-BPs, Creaks and Slow click trains. Minimum and peak frequencies were significantly higher in Slow click trains ($p<0.001$) but similar for burst-pulses: S-BPs, Creaks and Squawks.

Table 4.2. Acoustic parameters of pulsed signals produced by bottlenose dolphins in Sado region, Portugal.

	Minimum frequency (kHz)	**Peak frequency (kHz)**	**Duration (sec.)**	**Repetition rate (clicks/ sec.)**	**Inter-click-interval (sec.)**
S-BP	**4.12** ± 3.77[a]	**25.97** ± 8.57[a]	**0.06** ± 0.04[a,b,c]	**287** ± 64[a,b,c]	**0.004** ± 0.001[a,b,c]
Creak	**4.93** ± 3.33[b]	**23.73** ± 10.44[b]	**1.27** ± 0.93[a,b,c]	**83** ± 55[a,b,c]	**0. 017** ± 0.010[a,b,c]
Slow click train	**6.88** ± 3.90[a,b,c]	**31.32** ± 5.19[a,b,c]	**2.16** ± 1.63[a,b,c]	**13** ± 28[a,b,c]	**0.128** ± 0.079[a,b,c]
Squawk	**3.94** ± 3.90[c]	**21.26** ± 12.18[c]	**0.44** ± 0.40[a,b,c]	**472** ± 161[a,b,c]	**0.002** ± 0.001[a,b,c]

Values are presented as means ± standard deviation.

[a,b,c] Significant differences in pairwise comparison, using One-way ANOVAs (with Welch correction) and significance level of 0.01.

4.5. DISCUSSION

Bottlenose dolphins in the Sado region, Portugal, produce a variety of broadband pulsed signals, comparable to the repertoire of other populations that have been traditionally classified based on aural characteristics and graphical aspects (dos Santos et al., 1990). Despite the limitations of the human hearing system (essentially its frequency range and temporal processing abilities), our results show a high agreement between traditional classification and quantitative methodologies. As expected, classification based on aural and graphical features performs better with low repetition rate signals (e.g.: slow click trains and creaks), although high percentages of matching classifications were also obtained for Squawks and S-BPs. A comprehensive classification of pulsed signals is essential for a complete, detailed description of the bottlenose dolphin repertoire and its functional interpretation. The pulsed signals category remains the least understood, both structurally and functionally. All efforts to improve and assess the accuracy of categorizations, and to promote a future universal frame- work are therefore important.

Pulsed signals produced by bottlenose dolphins in the Sado region range from long, slow click trains to short burst-pulses with very high repetition rate, as it has been described for other delphinid species (e.g.: spinner, spotted and white-beaked dolphins (Lammers et al., 2003; Simard, Mann, & Gowans, 2008), false killer whales (Murray, Mercado, & Roitblat, 1998). The occurrence of signals with repetition rates that vary along a continuum (e.g., echolocation click trains and burst-pulses emitted with a continuous transition), plus the high variability observed within sound categories, supports the notion of a graded repertoire (Green & Marler, 1979; Wilson, 1975). Graded acoustic repertoires are common in species that live in complex societies, such as canids and non-human primates (e.g.: Déaux & Clarke, 2013; Keenan, Lemasson, & Zuberbühler, 2013). Furthermore, the level of gradedness of a repertoire has been linked to its potential to encode information (Hauser, 1997). The continuity here observed adds support to the possibility of a dual function for some pulsed signals, i.e., both echolocation and communication (see Herzing & dos Santos, 2004).Despite this apparent gradation, our results present statistically significant differences in temporal parameters (duration, repetition rate and ICI) of each type of pulsed signal. Also, the hierarchical cluster analysis clearly divided these signals into coherent clusters, especially Slow click trains, Creaks and S-BPs. Given the dolphins' hearing system, adapted for both high frequencies and fine-scale time resolution (Au, 1993), the observed differences in multiple temporal cues may enable the distinction between different vocal units, even if they are produced in continuous sequences. Thus, the existence of discrete pulsed vocal units with specific roles in the bottlenose dolphins' acoustic repertoire must be considered, even in the sometimes confusing cacophony of graded, pulsed and tonal emissions.

According to the DFA, repetition rate is the feature that best enables the discrimination of different pulsed signals. Repetition rate is also important for the human ability to discriminate sounds. Furthermore, fine-grade temporal discontinuities, on the order of 2–4 ms, are known to be perceived and processed by the human auditory cortex (Bidelman & Syed Khaja, 2014; Gage, Roberts, & Hickok, 2006). Thus, aural cues have a good potential in perceptual classification even when the ICI is too short for pulse discrimination by the human ear.

In our study, vocal emissions labeled as Slow click trains were markedly distinct signals, with temporal parameters typically reported for echolocation clicks produced by bottlenose dolphins in the wild (e.g., Wahlberg et al., 2011): long duration (> 1000 ms), slow repetition rate and inter-click-intervals that allow for the two-way transit time. Contrary to other studies (Akamatsu, 1998; Au,1993; Buscaino et al., 2015), Slow click trains had low peak frequency (≈30 kHz). These results may have been caused by the upper frequency limitations of our recording equipment and by the occurrence of both on- and off-axis signals, as already pointed out (Baumann-Pickering, Wiggins, Hildebrand, Roch, & Schnitzler, 2010).

Signals labeled as Creaks also appear as distinct vocal units in our DFA, and their spectral characteristics resemble those of terminal buzzes (or creaks) produced by other odontocetes and bats during prey capture efforts that require quick updates of close range scenarios (Aizpurua, Aihartza, Alberdi, Baagøe, & Garin, 2014; Akamatsu et al., 2007; Griffiths, 2013; Madsen et al., 2007; Miller et al., 2004; Van Parijs, Parra, & Corkeron, 2000). Furthermore, creaks are often produced after echolocation clicks as it is described for terminal foraging buzzes (Aizpurua et al., 2014; Akamatsu et al., 2007; Griffiths, 2013; Madsen et al., 2007; Miller et al., 2004; Van Parijs et al., 2000) and might have identical function. In our study, Creaks had time-frequency features significantly different from Slow click trains, and the inter-click-intervals, were predominantly lower than the echo-processing lag time reported for this species (Au, 1993). However, it is still not clear how dolphins perceive and process these high repetition signals if ICIs are below the limit for auditory temporal resolution (Supin & Popov, 1995) and further investigation on this topic is needed. We suggest that an echolocation function should not be excluded; especially since such signals may provide relevant motion related information (such as position shifts and velocity) when targets are at very close ranges.

As for Squawks and S-BPs, these signals fall into the definition of burst-pulses proposed for Hawaiian spinner dolphins and Atlantic spotted dolphins (pulsed signals with ICIs bellow 10 ms) (Lammers et al., 2003), and presented clearly distinct features from echolocation clicks and Creaks. While S-BPs were recorded as sporadic, single, short duration emissions, Squawks were abundant vocalizations recorded both as isolated calls, and following Creaks emissions.

Burst-pulses of short durations have been previously documented for captive and wild bottlenose dolphins (Blomqvist & Amundin, 2004; Gridley et al., 2015). Although such signals have been described as similar in duration to the S-BPs we present, their repetition rates (≈500 pps) and peak frequency (≈20 kHz) are closer to those of Squawks in our study. Furthermore, the reported burst-pulses are common vocalizations, often emitted in sequences, unlike the signals labelled by us as S-BPs. Due to the singular characteristics of S-BPs and their pattern of emission, we suggest that these burst-pulses emitted in the Sado estuary may be a new/unreported signal in the acoustic repertoire of this species.

Very high repetition rate signals (>500 ppm) such as Squawks have been documented in different social contexts (both affiliative and agonistic) and hypothesized to function as indicators of the animals' physical and emotional state during interactions (Blomqvist & Amundin, 2004; Herzing, 2000; Overstrom, 1983). Recently, burst-pulses with spectral characteristics similar to Squawks, recorded in a continuum with echolocation click trains and creaks have been described as "an acoustic signal of food reward expectation" (Ridgway et al., 2014). Synchronized squawks that resemble isolated Squawks have been reported during agonistic interactions (Herzing, 2015). In our study, Squawks had high variability in their time-frequency features and were recorded with different patterns of emission. Thus, it is possible that the category Squawks combines several variants in a range of signals with different contextual use.

Interestingly, the cluster analysis results, especially solution 1, validates a distinction between the signal types that can be linked with biosonar tasks (Slow click trains and Creaks) and other pulsed signals that probably have a communication function (Squawks and S-BPs).

4.6. CONCLUSIONS

Pulsed signals are a conspicuous component of bottlenose dolphins' repertoire and evidence supports their important role both in foraging/feeding events and intraspecific communication. Defining more correct and natural sound categories and sub-categories is indispensable to the description of a species' acoustic repertoire. We highlight the potential of combining graphical aspects of spectrograms and quantitative analysis in the process of pulsed-sounds classification.

Our results document significant differences in time-frequency characteristics of pulsed signals produced by bottlenose dolphins. These findings point to the existence of a complex repertoire of pulsed vocalizations.

Future studies should examine the patterns and contexts of production of each specific signal type, as it is likely that these emissions are determined by specific social and environmental factors.

CHAPTER 5

BRAYS AND BITS: INFORMATION THEORY APPLIED TO ACOUSTIC COMMUNICATION SEQUENCES OF BOTTLENOSE DOLPHINS

Luís, A R, Alves, I. S., Sobreira, F. V, Couchinho, M. N., & dos Santos, M. E. (2018). Brays and bits: information theory applied to acoustic communication sequences of bottlenose dolphins. *Bioacoustics, 28* (3), 286-296. doi:10.1080/09524622.2018.1443285

5.1. ABSTRACT

Acoustic call sequences are important components of vocal repertoires for many animal species. Bottlenose dolphins (*Tursiops truncatus*) produce a wide variety of vocalizations, in different behavioural contexts, including some conspicuous vocal sequences - the “bray series”. The occurrence of brays is still insufficiently documented, contextually and geographically, and the specific functions of these multi-unit emissions are yet to be understood. Here, acoustic emissions produced by bottlenose dolphins in the Sado estuary, Portugal, were used to provide a structural characterization of the discrete elements that compose bray series. Information theory techniques were applied to analyze bray sequences and explore the complexity of these calls. Log-frequency analysis, based on bout criterion interval (BCI), confirmed the bout structure of bray series. A first-order Markov model revealed a distinct pattern of emission for the bray series’ elements, with uneven transitions between elements. The order in these sequential emissions was not random and consecutive decreases in higher-order entropy values support the notion of a well-defined structure in the bray series. The key features of animal signal sequences here portrayed suggest the presence of relevant information content and highlight the complexity of the bottlenose dolphin’s acoustic repertoire.

Keywords: acoustic communication, vocal sequences, bray series, information theory, *Tursiops truncatus*, bottlenose dolphin

5.2. INTRODUCTION

The use of acoustic signals as a primary modality mode of communication is prevalent in various animal taxa (Hopp et al., 1998). Acoustically driven species have rich vocal repertoires and may produce specific sequences of acoustic signals that convey valuable information content, e.g. contextual information in chimpanzees' food-related calls (Slocombe & Zuberbuhler, 2006), great tits' alarm calls (Suzuki, 2014) or babblers' mobbing calls (Engesser et al., 2016), group-specific 'badges' in sperm whales' codas (Gero et al., 2016), killer whales' calls (Riesch et al., 2006) or skylarks' flight songs (Briefer et al., 2008), and identity cues in penguins' contact calls (Favaro et al., 2016) or dingoes' bark-howls (Deaux et al., 2016).

The vocal repertoires of cetaceans may also include multiple element types, discrete and/or combined into complex and variable sequences (see Payne & McVay, 1971, for humpback whales; Riesch et al., 2008, for killer whales; Zwamborn & Whitehead, 2017, for pilot whales). Common bottlenose dolphins (*Tursiops truncatus*) are known to produce a wide variety of acoustic signals that convey information, namely the identity cues within contact calls – the signature whistles (Caldwell & Caldwell, 1965; Janik, 2000b; Kershenbaum et al., 2013; Sayigh et al., 2007), and the contextual information encoded within food-related calls such as 'victory squeals' (Ridgway et al., 1991, 2014) or social signals like squawks, squeaks, barks, screams, pops and yelps (Connor & Smolker, 1996; Herzing, 1996).

Additionally, the vast vocal repertoire of the bottlenose dolphin includes remarkable multi-unit signals – the bray series. These conspicuous acoustic sequences were first described as occurring during social behaviour episodes in the Sado estuary, Portugal (dos Santos et al., 1990), and have been occasionally described in a few populations (e.g. Díaz-Lopez & Shirai, 2009; dos Santos et al., 1995; Gridley et al., 2015; Herzing, 2015). Also reported as food-related emissions (Janik, 2000a; King & Janik, 2015), brays are still poorly documented, both functionally and geographically. Likewise, the temporal and structural patterns of these rhythmic vocalizations are yet to be understood.

Studies of acoustic sequences and communication repertoires evaluate key features such as diversity, repetition, ordering and timing (Kershenbaum et al., 2016). These metrics, allied to the use of information theory, have been used to assess complexity and estimate the information content of cetacean acoustic sequences (McCowan et al., 1999; Suzuki et al., 2006).

Here, we applied information theory techniques to analyse the structural and temporal characteristics of the bray series and explore the complexity of these calls. This study also presents a detailed quantitative and graphical description of the discrete elements that compose the bray series, using recordings of bottlenose dolphins in the Sado estuary region.

5.3. METHODS

5.3.1. Data collection

Bottlenose dolphins' acoustic emissions were recorded in the Sado estuary, Portugal, and adjacent coastal waters (approximate location of the Sado river mouth is 38º 29' N, 8º 55' W).

Recordings were collected during daylight hours (1000 to 1800), on 40 different days, unevenly distributed from April 2014 to April 2017, from a 8.40-m long inboard motor boat. After selecting a focal group, the boat was positioned ahead of the group's location and all boat machinery turned off. Prior to acoustic sampling, a 15-min habituation period was established. All samples were collected with no vessels in a 2 km radius.

Acoustic measurements were carried out using a Cetacean Research Technology ™ hydrophone, model C55 (effective sensitivity of -185 dB re 1 V/1 µPa, frequency response of ± 3 dB in the 0.020 to 44 kHz band and +3/-13 dB in the 0.009 to 100 kHz band, polarized by a 9-V battery), placed at a depth of 5 meters. The hydrophone was connected to a Fostex FR-2 digital recorder by a 15 m cable. Recordings were made with a sampling rate of 192 kHz and a 24-bit resolution, and stored on Compact Flash memory cards as time-stamped .wav files.

5.3.2. Acoustic analyses

Bray series were initially identified through aural and visual inspection, by two trained independent observers, using Raven Pro 1.4 (Cornell Lab of Ornithology, Ithaca, NY) with Hann windows of 512 points, and frequency resolution of 375 Hz and 50% overlap. According with their graphical and aural characteristics, the varying, alternating elements in the bray series were labelled as: "Gulps", low-frequency impulsive sounds; "Grunts," broadband burst pulses, with strong emphasis in the lower frequencies and "Squeaks", short duration, pulsed signals that sound tonal to the human ear. All acoustic elements were rated based on signal-to-noise ratio (SNR) as follows: (i) poor – signal faint and hardly visible on the spectrogram, (ii) fair – signal visible and with a clear start/end on the spectrogram, (iii) good – signal well marked and with a clear start/end on the spectrogram. Non-overlapping signals in our samples rated as fair or good where selected for further analysis.

To describe each signal type, a set of acoustic parameters were extracted using Raven, with specific spectrogram display settings. Minimum, maximum and peak frequency, frequency range, and duration were extracted for all signal types (Gulps: FFT 3000 points, Grunts: FFT 256 points, Squeaks: FFT 700 points). For Squeaks, spectral features were extracted from the dominant harmonic sideband (see Watkins, 1968). To calculate repetition rate and inter-click-interval (ICI), the number of pulses within each signal was assessed and then presented as pulses per second (p/s). For Squeaks, frequency spacing between the harmonic bands was measured and used as a proxy for repetition rate; for Grunts, a supervised automatic detection function was used, in addition to manual counting of the pulses, using a playback rate of 0.01, whenever deemed necessary for precision.

5.3.3. Statistical Analysis

The temporal pattern of emission of the bray series was analyzed using silent gaps between all vocal elements (Gulps, Grunts and Squeaks). Silent intervals were measured by subtracting the end time of the first element from the start time of the second element. Silent gaps were plotted using a log-frequency analysis, and bout criterion interval (BCI) was applied (see Slater & Lester, 1982) to define each sequence.

The structural characteristics of bray sequences, namely repetition and order, were assessed using a first-order Markov model – FOMM (Gottman & Roy, 1990). To verify if transitions between elements were random, observed and expected bigrams' transition matrices were compared using a chi-squared test. To evaluate the organizational complexity within these calls, Shannon entropic orders (Shannon, 1948) were computed, as follows:

$$\text{Zero-order model: } H_0 = \log_2 N,$$

$$\text{First-order model: } H_1 = \sum_{i=1}^{N} pi \log_2 pi$$

$$\text{Second-order model: } H_2 = \sum_{i=1}^{N} pi \sum_{i=1}^{N} Pj|i \log_2 Pj|i$$

where N is the number of different vocal elements, *pi* is the probability of the i-th vocal element in a bray series and *Pj|I* is the conditional probability that the i-th vocal element being followed by the j-th vocal element in a sequence.

5.4. RESULTS

A total of 677 vocal elements, rated as fair or good, were classified as Gulps (*N* = 257), Grunts (*N* = 251) and Squeaks (*N* = 169) (see Figure 5.1.). Each signal type presents distinct time–frequency characteristics, as reported in Table 5.1.

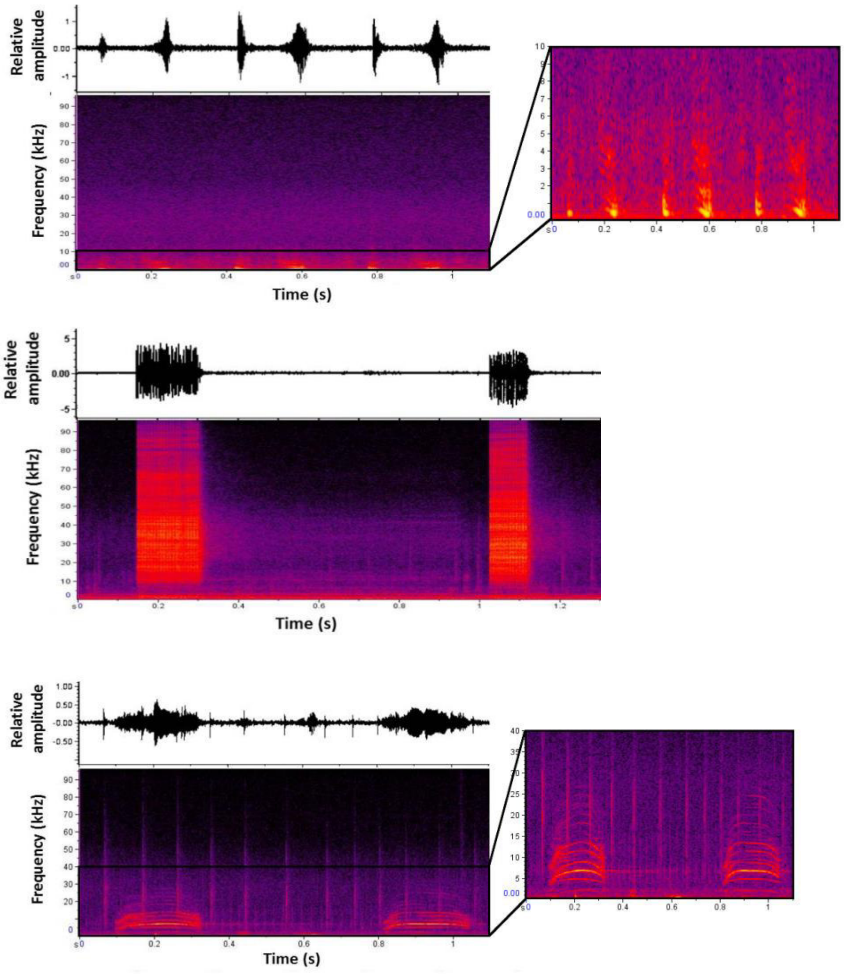

Figure 5.1. Examples of bray series' elements produced by bottlenose dolphins in the Sado region, Portugal. (A) Single-unit sequence composed by Gulps. (B) Single-unit sequence composed by Grunts. (C) Multi-unit sequence: Squeak-Gulp-Gulp-Squeak. The upper panels show sound waveforms, with relative amplitude on the y-axis, and the bottom panels show spectrograms for each signal type, with frequency (kHz) on the y-axis and ranges. Time (s) is on the x-axis.

Table 5.1. Acoustic parameters of bray series' elements produced by bottlenose dolphins in Sado region, Portugal.

	Gulps	**Grunts**	**Squeaks**
Minimum frequency (kHz)	**0.25** ± 0.20	**2.36** ± 0.53	**2.32** ± 1.23
Maximum frequency (kHz)	**0.68** ± 0.32	**93.06** ± 13.66	**14.56** ± 8.46
Peak frequency (kHz)	**4.09** ± 2.08	**10.76** ± 1.31	**4.41** ± 2.99
Duration (ms)	**50** ± 20	**320** ± 280	**140** ± 70
Repetition rate (p/s)	**-**	**300** ± 111	**3264** ± 1475
Inter-click-interval (ms)	**-**	**4** ± 2.5	**4** ± 2

The bray series' elements were identified in 26.2% of the recordings. The majority of the bray series' elements were recorded in sequences with very short silent gaps: 58% of all elements were recorded with intervals less than 300 ms in between. Signal overlapping was observed on 6.7% of the emissions. Log-frequency analysis confirms the bout structure of these emissions: the steep slope of the plot represents the short within-bouts gaps, while the flat section represents the longer between-bouts gaps (Figure 5.2.).

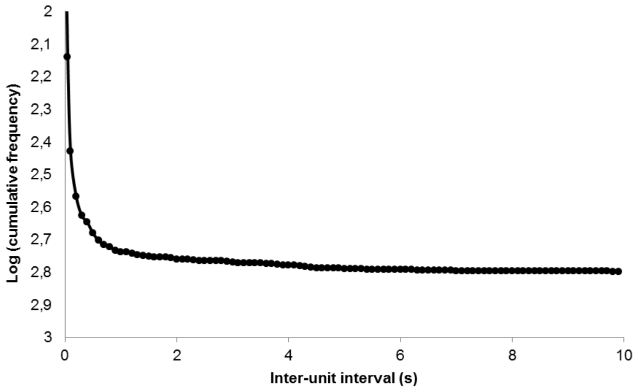

Figure 5.2. Log survivorship plot of the intervals between vocal units. The general form of the graph indicates the bout structure of bray sequences. Bout criterion interval (BCI) estimated at 0.6 seconds, which corresponds to the break point on the slope.

Based on the estimated bout criterion interval (BCI = 0.6 s), a total of 133 sequences were identified. These sequences varied in composition and length (3.98 s ± 2.92), between a minimum of 0.24 s and a maximum of 5.5 s.

Sequences were composed of 2–17 elements, starting by any of the vocal units. Grunts were the most common start element (46.2%), followed by Squeaks (31.5%) and Gulps (21.3%). Approximately, one-fourth of Squeaks or Grunts were recorded at the beginning of a sequences (24.3 and 23.9%); whereas for Gulps only, 11.3% of these vocal units were found as start element. Single-unit sequences occurred only with grunts and gulps (see examples in Figure 5.1.).

First-order Markov chain model revealed a distinct pattern of emission for bray series' elements (Fig. 5.3). According with the FOMM, the transition probability between vocal elements was uneven (Fig. 5.3.). Squeaks were highly likely to be followed by Gulps ($P_{S\text{-}Gu} = 0.76$), while Squeaks and Grunts rarely occurred sequentially ($P_{S\text{-}Gr} = 0.13$). Gulps were mainly emitted repeatedly ($P_{Gu\text{-}Gu} = 0.5$). Sequences of repeated Grunts were also highly likely to occur ($P_{Gr\text{-}Gr} = 0.58$).

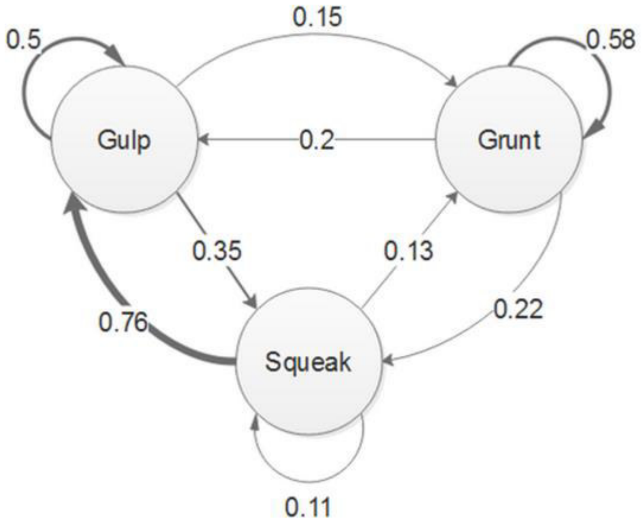

Figure 5.3. Transition diagram of bray series obtained from FOMM. Circles represent different vocal elements and values represent transition probabilities between bigrams.

The occurrence of the different bigrams was not random ($\chi^2 = 117.91$, $P<0.001$), as the transitions Gulp-Squeak, Grunt-Grunt and Squeak-Gulp were more common than expected by chance (z-scores: 4.4, 9.2, and 7.1, respectively).

Consecutive decreases in high-order entropy values were observed (H_2: 1.50 < H_1: 1.56 < H_0: 1.59), with the highest downturn between first- and second-order entropy values. These decreases in entropy clearly indicate an increase in the information content within the acoustic signal sequences.

5.5. DISCUSSION

In this study, the bray series were recorded in different behavioural contexts, as it has been previously reported for the resident bottlenose dolphins in the Sado region (e.g. surface feeding, social interactions and, less frequently, during travelling) (dos Santos et al., 1990, 1995), and quantitative information on the bray series' emissions are provided. These results illustrate the singularity and complexity of bray emissions, a call type that stands out for its oddness and conspicuousness. Bray sequences, which vary greatly in length and composition, occur frequently in recordings of these resident dolphins, and seem to be an important component of their acoustic repertoire: the bray series were present in one-fourth of the recordings. Remarkably, reports of brays elsewhere are still scarce (Janik, 2000a; Hastie et al., 2006; Herzing, 2015; King & Janik, 2015). With numerous published studies of bottlenose dolphin emissions in captivity, no brays have been reported, to our knowledge.

For a species with global distribution, such as bottlenose dolphins, the rarity of accounts for these conspicuous calls is puzzling, and raises the question of geographic specificities. However, the current lack of detailed descriptions for the discrete elements within the bray series in different populations and the limited information on the contextual use of these calls hinders further comparisons.

Sequential structure and timing aspects of the bray series may provide important insights on the functional properties of brays. In this study, the emission patterns were consistent with the bout structure reported for other cetacean acoustic sequences (e.g. killer whales' group-calling bout, (Miller et al., 2004), humpback whales' social call bouts (Rekdahl et al., 2015), bottlenose dolphins' signature whistles (Janik et al., 2013).

Although call bout structure could be demonstrated, the number of individuals involved in these emissions could not be determined, nor the behavioural interactions that were taking place underwater. However, the gaps between acoustic elements do suggest that there may have been multiple animals involved in these emissions. Sequences with very short silent gaps, as those here reported, are known to occur in animals choruses (see Henry et al., 2015). Moreover, in some social species, when vocal exchanges occur, the composite units are produced in sequences with silent gaps longer than the vocal elements to enable responses and avoid overlap (e.g. Janik, 2000b; Soltis et al., 2005). This alternation calling pattern seems compatible with the pattern of emission in the bray series. Most of the recorded sequences had silence gaps shorter than the duration of gulps, grunts or squeaks, which may suggest the communal nature of these calls. The low occurrence of overlapping (<7%) in brays also fits with the concept of vocal exchange, as overlapping may elicit the end of the exchange and, according with the ‘interference avoidance hypothesis’ (Ficken et al., 1974), overlapping is often actively avoided during interactions.

Overlapping is a relevant timing aspect within a sequence, as its prevalence may also indicate aggressive signaling (Baker et al., 2012; Naguib & Mennill, 2010). The bray series have been previously recorded agonistic contexts (Herzing, 2015) and, during this study, brays are sometimes accompanied by aerial displays, likely of an aggressive nature. However, in our recordings, acoustic signal overlapping is rare during the bray series’ emission. In complex societies with hierarchical structure, agonistic interactions may include acoustic displays without overlapping (e.g. northern elephant seals’ clap-threat calls signal incipient attack and can repeal subdominant males (Bartholomew & Collias, 1962), baboons’ threat grunts – fear barks, during dominant and lower rank female interactions (Cheney et al., 1995; Rendall et al., 1999; Bergman, 2003). For bottlenose dolphins, more detailed observations are needed to provide compelling evidence on the outright aggressive nature of bray calls.

Interestingly, the bray series’ composition suggests the existence of three typical call types: single-unit gulp sequences, single-unit grunt sequences and multi-unit sequences that include squeaks. One possibility for the observed variability in the brays series’ composition is that each vocal unit conveys different meaning and elicits the production of a specific acoustic element in response. This process occurs in duetting (both polyphonal and antiphonal calls) and requires the existence of order rules (Kershenbaum et al., 2016; Smith, 1980).

According with FOMM and Chi-square tests applied to our data, brays are in fact non-random call bouts. The observed level of order in the bray series supports the notion of a well-defined structure in these sequences, as it has been reported for other cetacean complex calls (e.g. humpback whale songs (Suzuki et al., 2006) or whistle sequences in killer whales (Riesch et al., 2008)).

Entropy values also validate this assumption: the consecutive decrease between orders suggests structural ordering, and are in line with results obtained for bottlenose dolphins’ whistle sequences (McCowan et al., 1999) or humpback whale social calls (Rekdahl et al., 2015).

In summary, the bray series do vary in structure in non-random ways, and their variability raises the possibilities that different sequences of vocal elements may convey different functional ‘meanings’, or may permit graded variation of intensity. For instance, hypothetically ‘Grunt-Grunt-Grunt …’ could signify a certain level of threat, whereas ‘Squeak-Gulp-Squeak-Gulp …’ could be a more appeasing or affiliative signalling. The possibility of relevant individual idiosyncrasies in bray production cannot be discarded either, since we are studying a small population and repeated sampling is likely.

Overall, our results have shown that the bray series are variable acoustic sequences ordered in predictable patterns of occurrence, with specific bigrams more likely to occur than others. To clarify the functional meaning embodied in these information-rich vocalizations, detailed underwater observations and further multi-regional research into the contextual use of these complex emissions are needed.

CHAPTER 6

CHANGES IN THE ACOUSTIC BEHAVIOR OF RESIDENT BOTTLENOSE DOLPHINS NEAR OPERATING VESSELS

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6.1. ABSTRACT

Maritime traffic is an issue of major ecological concern, and vessel noise may be an important source of disturbance for coastal cetaceans. In the Sado estuary, Portugal, core habitat areas of a small resident population of bottlenose dolphins (*Tursiops truncatus*) overlap with routes of intense maritime traffic, which presents an opportunity to assess vocal responses of these dolphins to specific vessel noise sources. Field recordings of dolphin vocalizations were made from April to November 2011, using a calibrated system. Dolphin behavior and group size were recorded, as well as the operating boat condition (no boats or specific boat type) in a 1,000 m radius. Spectral analyses of vocalizations allowed the categorization and quantitative analysis of echolocation click trains and social calls, including whistles. Mean overall call rates decreased significantly in the presence of operating vessels. Creaks (fast click trains) were significantly reduced in the presence of ferry boats. Significant differences were also observed in the whistles' minimum, maximum, and start frequencies. These changes in call emission rates and temporary shifts in whistles characteristics may be a vocal response to the proximity of operating vessels, facilitating communication in this busy, noisy estuary.

Keywords: *Tursiops truncatus*, bottlenose dolphin, acoustic behavior, maritime traffic, underwater noise.

6.2. INTRODUCTION

Much concern has been expressed about the effects of man-made noise on marine life (see Popper & Hawkins, 2012). Notable efforts have been made to document, demonstrate, measure, or estimate the extent of such effects, namely on aquatic mammals (e.g., Nowacek et al., 2007), especially considering that sound is important for these animals in finding prey (Au, 1993), avoiding predators (Deecke et al., 2005), and communicating with conspecifics (Tyack, 1998).

In line with current risk-assessment criteria, recent European Union documents (Dekeling et al., 2013; Tasker et al., 2010; Van der Graaf et al., 2012) highlight the importance of ambient noise monitoring, and studies of behavioral responses to specific noise sources. Some threats are considered more immediately deleterious, such as underwater explosions, air guns used in oil exploration, and mid-frequency military sonars (Dolman et al., 2011; dos Santos et al., 2010; Goldbogen et al., 2013; Hatch & Wright, 2007; Hildebrand, 2009). Acoustic deterrent devices and shipping noise are more chronic and pervasive, and have also been studied for their potential impacts on marine mammals (Aguilar de Soto et al., 2006; Hildebrand, 2009; Leeney et al., 2007).

Maritime traffic is indeed a heavy contributor to the increasing levels of noise in the oceans (Richardson et al., 1995), and it is considered the main contributor to the rise in background noise in European waters (Tasker et al., 2010). This issue is of particular concern in coastal areas, where vessel noise may be an important source of disturbance for cetaceans, especially in the case of local, resident populations.

Several studies have reported short-term behavioral responses of bottlenose dolphin schools to boat traffic, namely increases in group cohesion, dive duration, and traveling behavior (Miller et al., 2008; Nowacek et al., 2001,); changes in breathing and surfacing patterns (Hastie et al., 2003; Janik & Thompson, 1996,); and reduction of aerial behaviors and cessation of feeding, social, and resting events (Papale et al., 2012). Nevertheless, the effects of vessel noise on the acoustic behavior of cetaceans, especially in the case of resident populations, are still poorly documented.

Studies on the acoustic behavior of bottlenose dolphins when in the presence of vessels focus mainly, on the emission of whistles. Although, increases in whistling rate have been reported (Buckstaff, 2004; Scarpaci et al., 2000), changes in the acoustic behavior to the presence of vessels do not always occur (Lemon et al., 2006).

Delphinids appear to have differential responses to vessel noise. For example, Lesage et al. (1999) revealed that belugas reduced their overall call rate in the presence of vessels, but increased the emission and repetition of specific calls and shifted to higher frequency bands. Increases in the duration and source level of killer whale calls have been documented in the presence of boats (Foote et al., 2004; Holt et al., 2009), and Van Parijs and Corkeron (2001) reported increases in the whistling rate at a boat's passage but no changes in emission rates of click trains and burst pulses of *Sousa chinensis*. Jensen et al. (2009) estimated that vessels within a 50 m radius could cause a reduction of 26% in the communication range of bottlenose dolphins in shallow waters but the biological implications are still scarcely documented.

In the Sado estuary (Portugal), a small resident population of approximately 28 common bottlenose dolphins (Augusto et al., 2012) is exposed to vessel noise on a daily basis. Core dolphin habitat overlaps with zones of high maritime traffic, which presents an opportunity to assess the vocal responses of dolphins to specific noise sources. In the present study, we investigate the acoustic behavior of bottlenose dolphins in the absence and presence of different types of vessels. Specifically we examined overall call rates, whistle characteristics, and whether changes in the various burst-pulsed emissions occur in relation to boat traffic.

6.3. METHODS

Field recordings were made in the Sado estuary, Portugal, and adjacent coastal waters (Fig. 6.1.). All data were collected from an 8.40 m inboard motor vessel during daylight hours (1000–1800), on 32 days from April to November 2011, with sea state ranging from 0 to 2 Beaufort. Whenever a group of dolphins was detected, the research vessel was positioned approximately 500 m ahead of the group's displacement, with the engines off, and the hydrophone placed at a depth of 3 m. When vessels were operating within a 1,000 m radius, this was noted and vessel type categorized as "Tanker," "Cargo freighter," "Ferry boat," "Trawler," "Salt galleon" (traditional tour boats with sails), "Leisure boat" (with inboard engine), "Small boat" (with outboard engine), or "Dolphin-watching vessel Esperança." When no operating boats where present, "No boats" was noted.

All acoustic measurements were carried out using a factory-calibrated recording system: a Cetacean Research Technology hydrophone, model C55 (effective sensitiv- ity of –165 dB re 1 V = l lPa, frequency response of ±3 dB in the 0.020–44 kHz band and +3/–13 dB in the 0.009–100 kHz band, polarized by a 9 V battery) connected by a 15 m cable to a Fostex FR-2 digital recorder. High-pass filter of 100 Hz was chosen to avoid self-noise generated by the recording platform and low-frequency vibrations. One-minute duration recordings were made, with a sampling rate of 192 kHz and 24 bit resolution, recording level at 7.5 and trim level at –26 dB. All recordings were stored on Compact Flash memory cards as time-stamped wave files. The geographic location of each recording was given by a Garmin Foretrex 301 portable GPS.

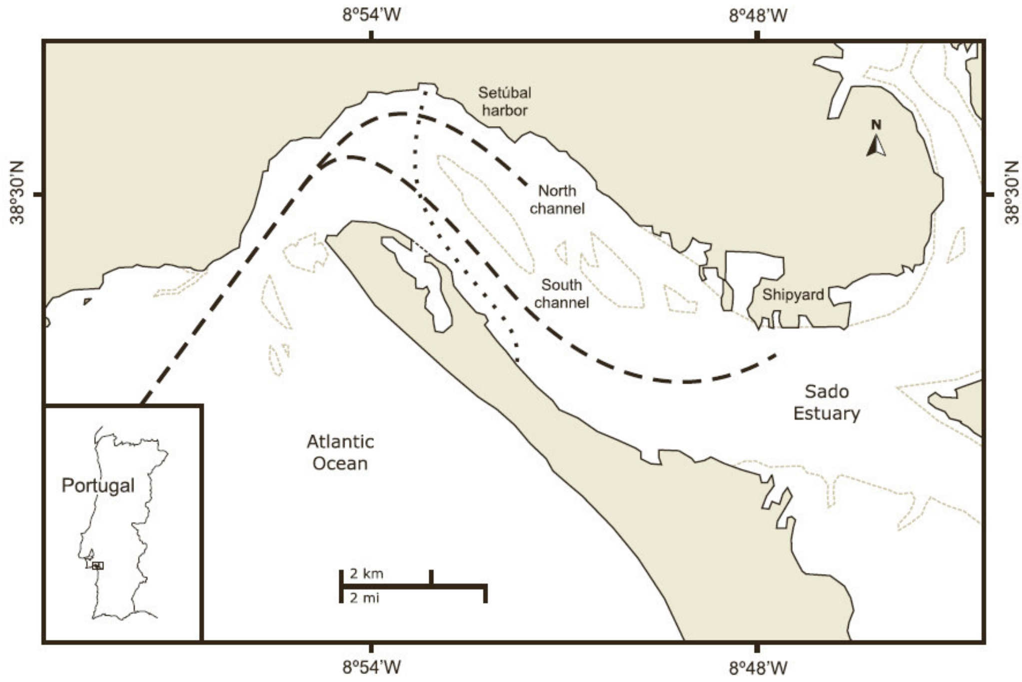

Figure 6.1. Map of study area in Sado estuary, Portugal. Ferry boat route is shown in black dashed line and ship channel is shown in black long dashed line.

Behavioral context was ascribed according with the following categories (dos Santos et al. 2005): (1) travel—rapid, linear displacement of a tight group moving between areas, with no aerial behaviors; (2) foraging—zigzag displacement of a sub- divided group, with dives longer than 1 min and occasional aerial activity or fish chase; (3) feeding—individual animals, dyads, or tryads surfacing more than 10 m apart, with only very short dives and abundant movements at the surface, including captures, prey leaping, or prey toss; and (4) socializing—dyads or tryads showing excited surface and aerial behaviors, with physical contacts and no prey detectable, sometimes with synchronous movements. Group size was determined by direct counting of the animals by two observers, averaging the counts.

6.3.1. Acoustic Analyses

Recordings were first inspected by two trained independent observers, aurally and visually, using Adobe Audition CS5.5 (Adobe Systems Inc., San Jose, CA) with Hamming windows of 512 points, in order to identify, categorize, and count all the vocal elements present in each sample.

Vocal elements were classified and counted in each sample. For slow click trains (repetition rate <40 clicks/s), presumably with a general echolocation function (Au 1993), the number of individual clicks was determined. For fast, indiscernible click trains (burst-pulses), three categories were created according to repetition rate and duration: “Creaks” (>0.2 s duration, 40–200 clicks/s); “Squawks” (>0.2 s duration, 200–600 clicks/s) and “Buzzes” (<0.2 s duration, 200–400 clicks/s). Other pulsed sounds were categorized as “Bangs,” broadband impulsive sounds; “Gulps,” low-frequency impulsive sounds; “Squeaks,” short pulsed calls that sound tonal to the human ear; and “Grunts,” broadband burst pulses, with strong emphasis in the lower frequencies. Tonal, narrow-band, modulated signals were classified as “Whistles.” The analysis of burst-pulses, other pulsed sounds and whistles was based on the number of recognizable units.

To estimate the number of clicks per sample, the interactive detection function of Raven Pro 1.4 (Cornell Lab of Ornithology, Ithaca, NY) was used (also with Hamming windows of 512 points). Detection function parameters were adjusted to each file individually in order to optimize the signal-to-noise ratio and, subsequently, the detection probability in each recording. Detections were validated by a trained observer.

Raven Pro 1.4 was used to measure the acoustic parameters of previously selected whistles: start frequency, end frequency, minimum frequency, maximum frequency, and duration.

6.3.2. Statistical Analyses

As the abundance of emissions could be a direct function of group size, this variable had to be analyzed. To do so, correlations were calculated between group size and the mean count of each vocal element (Pearson product-moment correlation, Gravetter & Wallnau, 2000).

Analysis of variance (one-way ANOVA) was used to assess the differences in the mean call rate according to operating boat condition. Contrast analysis was used to test whether the presence of vessels could account for significant differences in the mean overall call rate. Tukey post hoc test was performed for pairwise comparisons. Due to their specific metrics and wide variance of occurrence, clicks were not included in the call rate analysis.

Analyses of variance were performed, individually, for the following vocal elements (one-way ANOVA, with Welch correction when necessary): Whistles, Creaks, Squawks, Squeaks, and Clicks, using Bonferroni correction (significance level = 0.01). Several acoustic parameters of whistles (start frequency, end frequency, minimum frequency, maximum frequency, and duration) were also analyzed using a series of one-way ANOVAs, using Bonferroni correction (significance level = 0.01). Tukey HSD and Dunnet's T3 post hoc tests were performed for pairwise comparisons. All statistical analyses were performed using IBM SPSS Statistics 21 (IBM Inc.).

6.4. RESULTS

A total of 205 samples recorded within the study area were selected for analysis (ferry boats = 24, trawlers = 12, leisure boats = 16 and dolphin-watching vessel = 18, no boats = 135). Operating boat types with fewer than 10 cases were excluded from analyses.

No correlations were found between dolphin group size and the mean overall number of vocal elements recorded in each sample ($r = 0.013$, $P = 0.853$). Therefore, group size was not included in the analysis of variance of vocal elements and acoustic parameters.

The analyses performed to assess the variance in the call rate for each boat type revealed no significant differences among the groups (F_{4}, 200 = 1.729, $P = 0.145$). Nevertheless, the contrast test revealed that, in the presence of operating vessels, the mean call rate diminished significantly ($t_{200} = 2.172$, $P = 0.031$). For the category "Ferry boat" the mean call rate was of only seven calls per minute, which is significantly lower ($P = 0.023$) than the 22 vocalizations recorded per minute in the absence of boats (Fig. 6.2.).

The analysis of variance performed for each vocal element revealed that only the mean number of creaks differed significantly according to the operating boat type (F_{Welch}, 4, 40.985 = 5.037, $P = 0.002$). For the categories "Ferry boat" and "Dolphin- watching vessel" the mean number of creaks recorded per minute was considerably lower than the mean number of creaks recorded in the absence of boats ($P = 0.01$ and $P = 0.015$*) — 1.2 and 1.4 vs. 3.5 per minute (Fig. 6.3). The mean number of whistles, squawks, squeaks, and clicks did not differ significantly between the analyzed conditions. Although not included in the analyses of variance, it should be pointed out that no "Gulps" or "Grunts" were recorded in the presence of trawlers during this study. "Bangs" were not recorded in the presence of trawlers or ferry boats and "Buzzes" were not recorded in the presence of ferry boats or leisure boats.

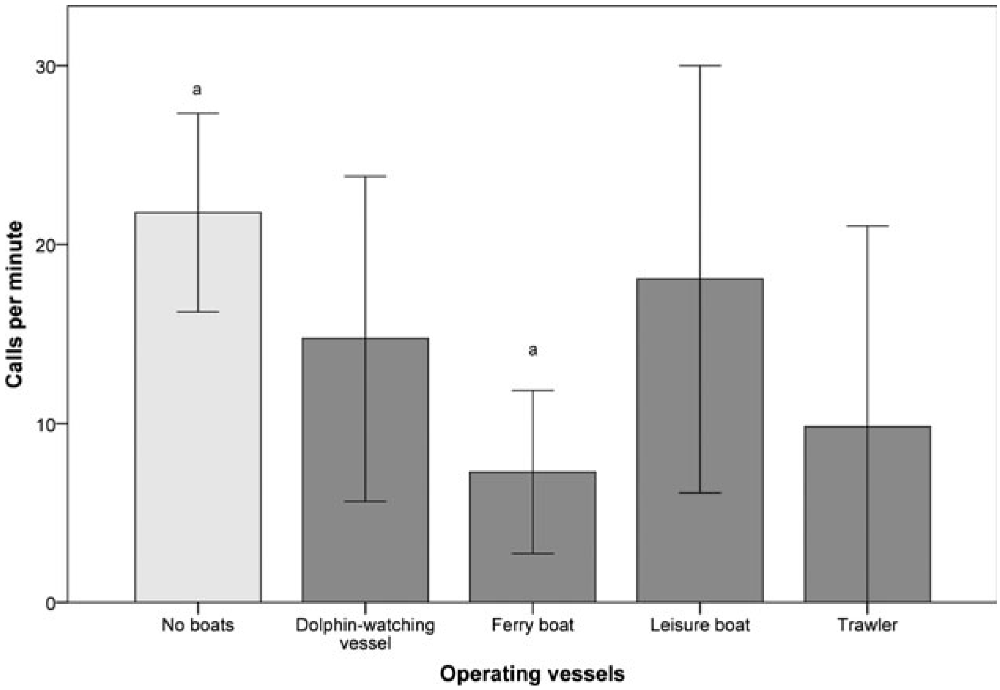

Figure 6.2. Calls per minute by type of operating vessel. Error bars 95% CI. Significant differences: a = no active vessel *vs.* ferry boat.

Based on recording quality and signal-to-noise ratio, a total of 570 whistles from the various conditions were selected for acoustic parameter analysis (ferry boats = 33, trawlers = 31, leisure boats = 37 and dolphin-watching vessel = 56, no boats = 413).

Significant differences were found for the minimum and maximum frequencies of the whistles among the various conditions ($F_{4,570} = 6.121$, $P < 0.001$; $F_{4,570} = 3.808$, $P = 0.005$). Post hoc Tukey HSD tests revealed significant differences in the minimum frequency between the “Ferry boats” (8.49 kHz), and “No boats” (6.71 kHz) and “Trawler” (6.25 kHz). For maximum frequency, significant differences were found between the dolphin-watching boat (15.33 kHz) and the trawlers (12.46 kHz) (Table 6.1).

Significant differences among vessel categories were also found for the start frequency (F4,570 = 10.759, P < 0.001). Post hoc Tukey HSD tests revealed significant differences between “Ferry boats” (10.52 kHz), and “No boats” (7.57 kHz) and “Trawler” (6.60 kHz) and between “Leisure boats” (10.52 kHz) and “No boats” and “Trawler” (Table 6.1.).

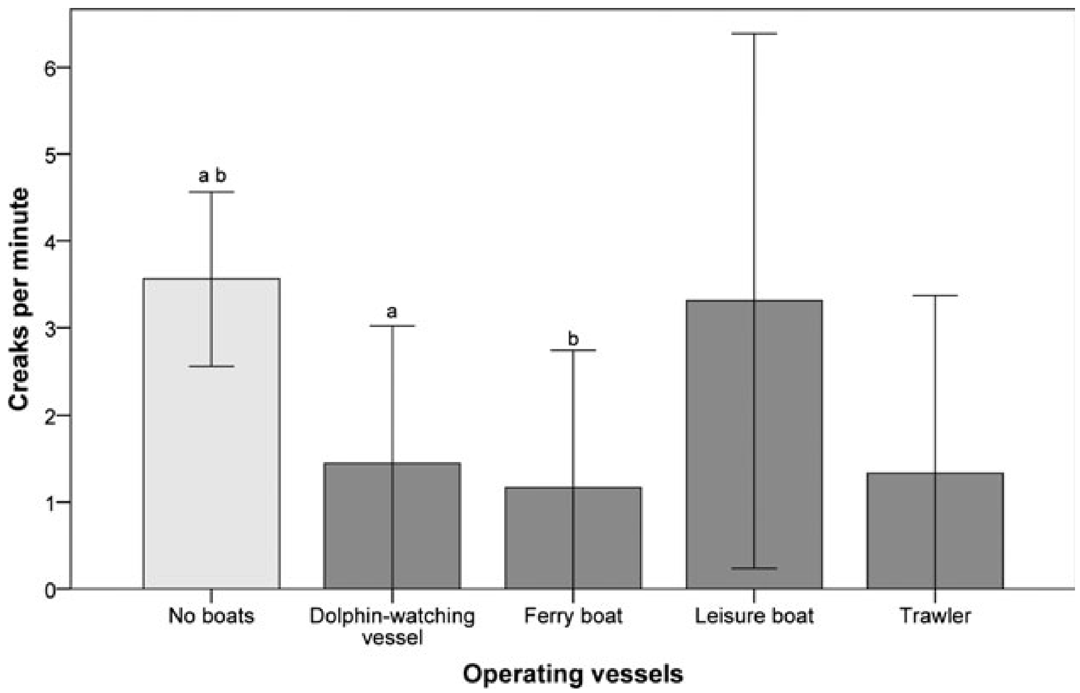

Figure 6.3. Creaks per minute by type of operating vessel. Error bars 99% CI. Significant differences: a = no active vessel *vs.* dolphin-watching vessel (P = 0.015*, marginal significance); b = no active vessel *vs.* ferry boat (P = 0.010).

Table 6.1. Mean values of whistles' acoustic parameters for each category of operating boat.

	Start frequency (kHz)	End frequency (kHz)	Minimum frequency (kHz)	Maximum frequency (kHz)	Duration (seconds)
No boats	**7.57**^{ac}	11.07	**6.71**^{a}	14.21	0.71
Dolphin-watching	7.98	11.11	6.99	**15.33**^{a}	0.82
Ferry-boat	**10.52**^{ab}	9.97	**8.49**^{ab}	13.72	0.49
Leisure boat	**10.37**^{cd}	10.74	8.10	14.70	0.59
Trawler	**6.60**^{bd}	9.55	**6.25**^{b}	**12.46**^{a}	0.75

Note: Significant differences in bold, with letters indicating the pairwise comparison results. Significance level of 0.01.

6.5. DISCUSSION

In this study, the acoustic behavior of bottlenose dolphins resident in the Sado region was sampled in the absence of nearby boats and in the proximity of various types of vessels. Several species of animals, including bottlenose dolphins, are known to adjust their acoustic behavior in order to reduce noise masking, by changing call emission rates and/or by shifting the acoustic frequency of vocal elements (Brumm & Slabbekoorn, 2005; Buckstaff, 2004; Lesage et al., 1999; Scarpaci et al., 2000; Van Parijs & Corkeron, 2001). Evidence for both responses is presented in this study: changes in the call rates and whistle characteristics were statistically significant in the presence of certain vessels.

Overall call rate was lower in the presence of operating vessels, especially ferry boats. Reducing overall emission rates is a common response of marine mammals to noise (Weilgart, 2007), perhaps to optimize communication efficiency or as an alertness response. Bird species that inhabit urban areas, with high levels of anthropogenic noise, are known to decrease singing during the noisiest periods (daytime), in order to take advantage of temporal variations in ambient noise (Fuller et al., 2007). Similarly, and since ferry boat noise is a regular, even predictable, variable on a specific section of the Sado estuary, dolphins may adjust their call rates temporally, avoiding the noisiest periods.

The decrease in the emission rate of specific vocalizations may have important biological implications depending on the calls that are affected (Weilgart, 2007). In the presence of ferry boats and the dolphin-watching boat studied, dolphins reduced their emission of creak burst-pulses associated with detailed echolocation, especially in for- aging and feeding events (Herzing, 1996; Miller et al., 2008). Similar results have been observed in sperm whales (*Physeter macrocephalus*) in response to airgun sound exposure (IWC, 2008; Tyack, 2009) and in Cuvier's beaked whales (*Ziphius cavirostris*) as a response to ship noise (Aguilar de Soto et al., 2006). In bats, another sonar-dependent predator, foraging efficiency decreases in the presence of anthropogenic noise, such as highway traffic (Siemers & Schaub, 2011).

In this study, the emission of creaks was changed in the presence of some types of vessel, which may indicate impacts in foraging efficiency, of concern given the frequent presence of ferries and the dolphin-watching boat in the dolphins' core habitat areas.

Significant changes were also observed for social calls: whistle characteristics shifted in frequency and duration, and in the presence of ferries and leisure boats, dolphins emitted whistles with higher start frequencies. The observed shifts in specific acoustic parameters of whistles may also be a vocal response that enables communication in such noisy contexts. Bottlenose dolphins are able to modify the emission ratio and the characteristics of whistles in order to avoid masking (Buckstaff, 2004; May-Collado & Wartzok, 2008; Morisaka et al., 2005). The use of higher frequencies has been reported as a masking avoidance response to low-frequency noise in several other species (Brumm & Slabbekoorn, 2005; Lesage et al., 1999; Parks et al., 2007). Nevertheless, the reported changes may limit recognition of calls by the intended receiver (Weilgart, 2007), and so it may disturb important social interactions such as mating or mother-calf communication.

This study suggests that the bottlenose dolphin residents in the Sado estuary may be moderately disturbed by the noise generated by boats in their habitat, and respond with some changes in their acoustic behavior. It was not possible to analyze differences according to habitat subarea because the animals already avoid the noisiest zones, near the Setúbal harbor, and sampling was therefore spatially biased.

The biological effects of observed changes in acoustic behavior are uncertain. Long-term implications of boat traffic must be considered and further investigated, as vessel noise may disrupt activities such as feeding or mating, and may affect residency patterns.

CHAPTER 7

GENERAL DISCUSSION

Research on marine mammals' behaviour is peculiarly challenging, including the field of bioacoustics. The marine realm is immense, three-dimensional, and visibility is often reduced. Also, the focal animals spend short periods of time at the surface, hindering detailed sampling behaviour, although their acoustic emissions may spread through large distances due the favorable underwater sound propagation conditions.

In recent years, the development of new technologies contributed, greatly, to the improvement of our knowledge on the acoustic emissions of cetaceans. The use of digital systems, unmanned aerial vehicles, radiotelemetry, accelerometry, video-tracking, passive acoustics and acoustic recording tags have provided new insights into marine mammal behaviour (Nowacek, Christiansen, Bejder, Goldbogen, & Friedlaender, 2016; Rachinas-Lopes, 2017).

Nevertheless, few advances have been made regarding the study of the global acoustic repertoires of these species. The occurrence of geographic variations on the vocal behaviour of marine mammals and the contextual use of different signal types, especially burst-pulsed emissions, continue to be topics of great interest but hardly addressed.

The overall goal of this study was to investigate the extended acoustic repertoire of the species *Tursiops truncatus*, by zooming into the specificities of their vocal behaviour. Here, distinct signal types were described in different behavioural and environmental contexts, and the factors promoting variation were assessed through a novel perspective combining the analysis of published and unpublished data, observational studies, acoustic recordings, and statistical modeling. In doing so, this study contributes to a better understanding of common bottlenose dolphins' acoustic behaviour in the wild. Furthermore, it highlights the importance of detailed acoustic behavioural studies in the identification of geographic variability and, ultimately, in the definition of conservation units.

As sound levels increase in the ocean due to human activities, it becomes clear that it is essential to understand how dolphins communicate and to assess the effects of anthropogenic noise on their acoustic emissions, and clarify its biological consequences. Moreover, the identification of acoustic distinct populations may support future conservation efforts.

7.1. Geographic variations, dialects or accents?

The occurrence of true dialects, regional accents and/or geografic variations in the acoustic repertoire of cetaceans has been a hot topic in animal communication studies in recent years (e.g. Cise et al., 2018; Cantor, Shoemaker, Cabral, Flores, & Whitehead, 2015); Gero et al., 2016). For common bottlenose dolphins, the fission-fusion social structure has been claimed as a limitation for the occurrence of dialects (Au & Hastings, 2008), although geographic variations in whistles emissions have been pointed out (Wang et al., 1995; May-Collado & Wartzok, 2008).

In Chapter 2 of this thesis, the multi-regional richness in the repertoire of common bottlenose dolphins was described with a detailed characterization of acoustic signals recorded in different behavioural and environmental contexts.

In this comprehensive comparison, it became clear that the bottlenose dolphin populations of the Atlantic Ocean and Mediterranean Sea share a common pool of acoustic signals. Interestingly, most of these “universal” signal types were pulsed sounds (creaks, squawks, variable rate click trains and bangs) that have been associated with foraging and feeding events. Acoustic divergence was only observed for social signals – whistles and bray series’ elements. Both whistles and brays have a strong social basis and have been described as information-rich signal types. Thus, the expression of these geographic variants should be discussed taking into consideration the influence of distinct social/cultural/environmental stimuli potentially afectiong the communication system and also the role of vocal learning in the prevalence of novel/different signal types.

Although the overall repertoire dissimilarity values were notoriously low in this study, the similarities in the acoustic repertoire of geographically distant populations that live in similar eco-ethological contexts are remarkable, and strongly support the ‘environmental adaptation hypothesis’ (see Ey & Fischer, 2009).

Further research on the acoustic variability in the time-frequency parameters of each signal type will provide additional information on the (dis)similarity level among acoustic repertoires.

The existence of closely located, acoustically distinct populations, with variant social signals, here portrayed, may fall into the definition of dialects. On a local scale, the documented differences between the acoustic repertoires of neighboring populations could act as a significant interaction and reproductive barrier, and avoid intermix. Eventually, acoustic divergence that affects social interactions and population structure may elicit genetic evolution, as proposed by Whitehead, Laland, Rendell, Thorogood, & Whiten, 2019 and supported by strong evidences on the link between vocal learning, culture and speciation in bird species (Lachlan & Servedio, 2004).

Future studies that combine genetic and acoustic analyses could help to clarify the boundaries and relationships between neighboring groups, and validate the existence of vocal groups, both at sympatric and allopatric.

In addition, as stated before, the explicit identification of acoustically distinct groups may be relevant to future conservation strategies, as since geographic variation and vocal identity are aspects of biodiversity recognized by the Convention on Migratory Species (CMS, 2014).

7.2. The importance of long-term studies to the understanding of acoustic repertoires

For a few dolphin populations, worldwide, information on the life history of the species, but also group dynamics and acoustic emissions have been gathered in decades-long time-series. The long-term studies provide an opportunity to investigate questions related with the calls' stability and their contextual use.

The resident population that inhabits the Sado region, Portugal, has been studied since 1981 and research efforts have made available valuable data regarding the acoustic repertoire of these dolphins. This was an ideal start-point to explore the relevance of whistles' emissions on the acoustic repertoire and clarify their link with specific activity patterns.

Whistles, especially those with stereotyped frequency contours, are extensively studied and the notion of "signature whistles", as individual calls, is now widely accepted. Their role as recognition facilitator in demanding fission-fusion underwater societies is an emerging topic and the study of whistles' emission in wild populations required new approaches.

In Chapter 3, the application of a recently developed methodology for signature whistles' identification in recordings from wild populations – SIGID (Janik et al., 2013) provided the basis to classify signature whistles for the first time in the Sado estuary.

Through the comparison of whistles' emissions from three different time-periods (1987-1988, 1992 and 1999, and 2011-2014), the link between stereotypy and signature calls become evident and long-term stability throughout more than 20 years was recognized. These results support the notion of signature whistles as identity calls that may enable social recognition even after long periods of separation among individuals.

Other findings in this study also validate the role of signature whistles as identity calls. The emission rates of signature whistles were significantly higher during activities that require group coordination, such as feeding and foraging. Common bottlenose dolphins' feeding strategies often involve group effort and cooperation, in extended foraging areas; therefore, to be able to determine who is participating in this collective task may be advantageous.

As for non-stereotyped whistles, although these calls are far more abundant than the stereotyped ones (68 % of all whistles recorded in this study), particularly in food-related events, their specific roles are largely unknown and require further research.

Long-term studies can contribute greatly to the knowledge on acoustic repertoire, especially when combined with new technologies and/or methodologies that enhance our perception of the behavioural patterns. The use of size-compatible acoustic recording tags and video technology could provide detailed information on the context of emission, namely on foraging/feeding strategies and group dynamics. Also, the detailed description of the acoustic parameters of non-stereotyped whistles and a multivariate statistical approach would be important steps to shed light on the semantic or emotional value of these calls, as well as on their functional roles.

7.3. The problem of repertoire definition

In acoustic communication studies, the problem of repertoire definition is a central question, especially taking into account that the issues raised by discrete *vs* graded signal types remain challenging. First, one must identify the distinct vocal units that comprise a repertoire, and in order to do so it is necessary to describe the vocal emissions in different behavioural and environmental contexts. Moreover, the selection of methodologies and terminologies must enable the comparison between repertoires.

In the acoustic repertoire of common bottlenose dolphins, burst-pulsed sounds have mostly been neglected, which led to a void in terms of detailed spectral descriptions and, consequently, scarce information on their functional aspects.

In Chapters 4 and 5, efforts were made to contribute to a better understanding of the different signal types that are usually lumped in the "BP-sounds" category.

The use of quantitative methods in BP-sounds analysis, in Chapter 4, aimed to improve classifications, and the results showed the validity of this approach in the identification of distinct vocal units with similar spectral characteristics. Also, the significant differences in time-frequency features of pulsed signals produced by common bottlenose dolphins were here described, which is a relevant contribute for future detailed comparisons.

Although the potential of combining graphical aspects of spectrograms and quantitative analysis in the process of pulsed-sounds classification is here emphasized, these methodologies are still very labor intensive.

The development of new acoustic software(s) with high and fast time-discrimination capacity, which could provide exact quantitative data on the spectral components of the different signal types, would be groundbreaking.

The bray series are another component of the acoustic repertoire of common bottlenose dolphins that is scarcely documented and analyzed.

Acoustic call sequences have been studied in the vocal repertoires of other cetacean species, such as humpback whales (Suzuki et al. 2006) or killer whales (Riesch et al. 2008). On the contrary, for the bray series sequences there was no information regarding key features such as diversity, repetition, ordering and timing nor available data on the acoustic characteristics of the bray series elements.

In Chapter 5, the rhythmic nature of these complex emissions, reported firstly in 1990, set the tone for an innovative approach. Once more, the accumulated information on the composition of these calls, result of the long-term studies developed in Sado region, was essential to identify the vocal units that are part of the brays.

To provide a detailed description of each signal type, which enables future comparative studies, a set of acoustic parameters and spectral representations were presented. Then, the application of information theory techniques unveiled the true nature of these calls – brays' elements are emitted in non-random ways, which could be the kick-off for further explorations on functionality of these sequences. The observed variability in order and in composition is yet to be clarified, though the presence of relevant information content seems certain. Different sequences may have distinct semantic value or convey different functional 'meanings', or could be a form of graded intensity variation.

At this point, is clear that burst-pulsed signals are a relevant component of bottlenose dolphins' communication; however, much work is still needed in this field of research. Identifying correct sound categories is a crucial step to solve the standing issue of acoustic repertoire definition, and then explore other outstanding questions such as the contexts of emission, geographic variation and the functional significance of acoustic emissions.

Future studies should aim to clarify the functional meaning of these information-rich vocalizations. In order to do so, detailed underwater observations allied with accurate acoustic recordings, that enable the emitters' identification, are required. Further multi-regional research into the contextual use of these complex emissions could also be a valuable adding to the study the acoustic repertoire of common bottlenose dolphins.

Both Chapter 4 and 5 highlight the existence of a complex repertoire of pulsed vocalizations, and stress the need of more exhaustive descriptions of these signal types in order to sort out the problem of repertoire definition and better understand bottlenose dolphins' acoustic communication.

7.4. The effects of maritime traffic in the acoustic repertoire

Acoustic emissions are undoubtedly the most efficient way to communicate underwater, and the evolution of cetaceans has exploited the advantages of sound signaling underwater. However, the ocean is becoming noisier as marine anthropogenic activities increase. Currently, vessel noise is one of the major man-made noise sources and, with the actual trends for maritime transportation, it is expected that maritime traffic will have a key presence in the future ocean soundscape.

The degradation of the acoustic environment reduces the active space of the different signal types. Thus, masking by noise has the potential to affect both communication and navigation abilities in cetacean species.

One striking question is if populations can adapt to these changes in their environment, and at what cost. In Chapter 6, the acoustic behavior of a resident population of common bottlenose dolphins was assessed in the presence of different types of vessels and the effects of maritime traffic in overall call rates and whistle characteristics were documented.

Results showed that the individuals of this population respond to vessel noise by changing their acoustic behaviour. The plasticity in the acoustic communication system of common bottlenose dolphins enables them to cope and adapt to the noise generated by boats in their habitat, through shifts in the spectral content of their calls and modifications in the emission rates. Nevertheless, the long-term biological effects of these changes in acoustic behavior are yet to be evaluated, as vessel noise may disrupt activities such as feeding or mating, and affect habitat use.

Marine noise pollution is now seen as an outstanding issue in ocean conservation and global efforts are being made in research and monitoring, but also in the application of mitigation strategies that involve technology development.

Broad assessments of the effects of vessel noise in the acoustic repertoire of the common bottlenose dolphin that included the different signal types are essential. Also, research on signal signature of different ship classes and their sound propagation profiles will enable predictions on masking zones and may serve as references for conservation strategies.

In conclusion, this thesis is an extensive study of the global acoustic repertoire of common bottlenose dolphins, both at a local scale and at a regional scale. The different vocal elements that comprise the acoustic repertoire of this species were documented and the spectral content and structural aspects of several burst-pulsed emissions are here described for the first time.

The assessment of geographic variability and the evaluation of vessel noise effects for this species are also relevant contributions for cetacean research and, ultimately, may inspire and support conservation efforts.

CHAPTER 8

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