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Influence of dolphin-watching tourism vessels on the whistle emission pattern of common dolphins and bottlenose dolphins.

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Faculdade de Ciências e Tecnologia

2022

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Mestrado em Biologia Marinha

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Declaração de autoria de trabalho

Influence of dolphin-watching tourism vessels on the emission pattern of whistle of common dolphins and bottlenose dolphins.

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

(Ana Alves da Silva)

Dedico este trabalho a meus pais, pelo amor incondicional, pelo exemplo de garra e humildade, e pelo carinho e paciência, sem os quais nenhuma das minhas escolhas teria sido possível.

Agradecimentos

Ao meu orientador e Professor Sérgio Jesus pela sua disponibilidade e apoio constante, e por me ter dado a conhecer o fascinante mundo da bioacústica.

Ao meu orientador externo Doutor Fábio Emmanuel Lopes de Matos, pela sua simpatia e incansável ajuda.

À extraordinária equipa de trabalho AIMM – Associação para Investigação do Meio Marinho, por todo o apoio e amizade, e pelos almoços no melhor restaurante do alto mar.

À Daniela, por todo o apoio, longas conversas e amizade, e por me ensinar a ultrapassar os problemas com otimismo.

À Joana, ao André e ao Guilherme, por terem partilhado comigo todos os seus conhecimentos sobre mamíferos marinhos, e pela paciência e simpatia com que me ensinaram tudo ao longo deste percurso.

A minhas irmãs de alma, por serem esses seres humanos tão maravilhosos, e por estarem sempre presentes (mesmo quando distantes), escutando com todo o carinho e paciência as minhas muitas reclamações ao longo de todo o percurso do mestrado.

Aos colegas de mestrado, obrigada pela amizade e por tornarem os tempos em Faro muito mais divertidos.

À minha mãe, pelo apoio incansável, por nunca duvidar de mim e das minhas capacidades (mesmo quando eu própria duvidei), por me ter ensinado a nunca desistir, e por ser a minha inspiração.

Abstract

Over the last few years there has been a significant increase in the number of dolphin-watching boats in the Algarve (Portugal), which my lead to short- and long-term impacts on the target species (e.g., common dolphin, bottlenose dolphin). In recent decades there has been a greater interest in the potential effects of anthropogenic noise on marine mammals, given the important role that sound plays in the vital functions of this organisms. Several changes in the behavior and energy expenditure of cetaceans have been documented, including impacts in the vocalization parameters of dolphins, reduction in the communication range of whistles and increase energy expenditure. In this study, the whistles characteristics of common dolphin (*Delphinus delphis)* and bottlenose dolphin (*Tursiops truncates)* were analyzed in the presence and absence of dolphin-watching tour boats to detect potential impacts in the vocalization of dolphins. Field recordings of common dolphin and bottlenose dolphin whistles were made from June to September 2022, using a calibrated system. Dolphin behavior and group size were recorded, as well as the number of boats in a 300 m radius. A total of 15h of acoustic recording was analyzed. Overall, these results showed a significant increase in start, low and high frequency on both species when exposed to the presence of one or more dolphin-watching observation vessels. However, when analyzing the whistles, it was possible to observe a reduction in the number of inflection points in the presence of the same vessels. These changes can be a dolphin strategy to avoid sound masking and increase of energy expenditure. These findings indicate that anthropogenic impact in the form of dolphin-watching tour vessels can influence the vocalization parameters of dolphins and such changes could have an impact if they reduce the communication range of whistles or increase energy expenditure.

Keywords: *Tursiops truncatus*, *Delphinus delphis*, acoustic behavior, underwater noise, vocal signals, acoustic parameters

Resumo

Nas últimas décadas que tem havido um maior interesse pelos potenciais efeitos do ruído antropogénico nos mamíferos marinhos, dado o importante papel que o som desempenha nas funções vitais destas espécies. Várias mudanças no comportamento e gasto energético dos cetáceos têm sido documentadas. O impacto antropogénico resultante de tráfego marítimo pode influenciar, por exemplo, os parâmetros de vocalização dos golfinhos podendo ter efeitos a longo prazo nestes animais, nomeadamente através da redução do alcance de comunicação dos assobios ou aumento de gasto de energia. Todas as espécies de cetáceos que ocorrem em Portugal continental estão protegidas pela legislação nacional e também por regulamentação europeia (Diretiva Habitats), bem como por convenções e acordos internacionais (Berna, Bona, CITES, ACCOBAMS). Entre as medidas de proteção implementadas, inclui-se a obrigatoriedade de, nas proximidades de cetáceos, reduzir os ruídos que possam atraí-los ou perturbá-los. Em relação ao número de embarcações, não são permitidas mais de 3 plataformas num raio de 100 metros em torno de cetáceos. No entanto, o turismo dirigido a cetáceos tem vindo a crescer, em particular no Algarve, e por sua vez o número de embarcações de turismo existentes com o objetivo de observação de golfinhos. Este aumento dificulta o cumprimento das regras, e nem sempre estas são cumpridas. Atualmente, 83 empresas de turismo estão licenciadas para operar barcos de observação de golfinhos em águas continentais, das quais 49 operam ao largo da costa sul de Portugal (ICNF 2022). Em relação a 2010, há mais 35 empresas operando na região. O número de embarcações por empresa em operação varia entre 1 e 15, sendo que no total existem 131 embarcações a operar no Algarve, cada uma fazendo em média três viagens por dia. Os barcos turísticos de observação de golfinhos seguem os golfinhos por longos períodos, e muitas vezes em grande número (1-13 barcos). Com base em trabalhos anteriores, os golfinhos em tais circunstâncias são suscetíveis a ficarem stressados e esgotados. Estas alterações comportamentais podem ser detetadas, por exemplo, através da análise de padrões de frequência de assobios.

A área de estudo alvo desta tese corresponde à zona costeira ocidental do Algarve (região sul de Portugal). Compreendida entre Faro e Sagres. Todos os verões, a população algarvia triplica, devido aos milhares de turistas que escolhem este destino de férias. Este aumento leva a uma maior pressão costeira nas grandes cidades, em particular em Albufeira, onde as atividades de embarcações turísticas de observação de golfinhos são muito procuradas.

O objetivo deste estudo foi investigar os padrões de frequência dos assobios de golfinhos comuns *(Delphinus delphis)* e roazes (*Tursiops truncados)* na ausência e presença de barcos de turismo de observação de golfinhos. Mais especificamente, examinaram-se as características dos assobios (por exemplo, a duração, a frequência máxima e mínima, a extenção da frequência e os pontos de inflecção), de forma a perceber se ocorrem mudanças nos padrões de emissão na presença e ausência de embarcações. Os assobios dos golfinhos-comuns e roazes são fundamentais para a comunicação entre elementos do grupo pelo que identificar e descrever potenciais efeitos nos padrões de assobios é fundamental para a conservação destas espécies. Para além do registo acústico, o comportamento dos golfinhos e o tamanho do grupo foram registados, bem como o número de barcos em um raio de 300 m. Um CTD Ruskin Concerto foi usado durante o estudo para registo da temperatura, salinidade e velocidade do som entre outros parâmetros. Os dados foram colhidos aleatoriamente, mas sempre na presença de animais, a fim de avaliar a influência das condições do mar no comportamento acústico dos golfinhos.

As gravações de vocalizações de golfinhos comuns e roazes foram realizadas através de dois hidrofones calibrados, com variação de ± 1dB no intervalo de 1Hz a 28kHz. As sessões de gravação acústica ocorreram entre junho e setembro de 2022, tendo sido analisadas 5h de registos acústicos num total de 15 horas de gravações. Um total de 234 acústicas com assobios foram identificadas.

As gravações recolhidas foram analisadas preliminarmente através da observação dos espectrogramas e submetidas à avaliação auditiva e visual usando o Audacity 2.4.2 a fim de identificar, categorizar e contar todos os “assobios” presentes em cada registo. Um assobio foi definido como um sinal tonal, de banda estreita, modulado com duração de 0.1 s ou mais com, pelo menos, parte da frequência fundamental acima de 3 kHz. As bandas harmônicas não foram consideradas devido às limitações das frequências superiores (26.36 kHz). Apenas a frequência fundamental de cada assobio selecionado foi medida. A plasticidade do repertório e as mudanças temporárias nas variáveis de assobio, como frequência inicial, frequência final, duração, frequência mínima, frequência máxima e número de inflexões foram depois medidas usando Raven Lite 2.0.4.

Como o ruído de baixa frequência pode mascarar o componente de frequência mais baixa dos assobios, foi determinada uma estimativa do erro nas frequências medidas, tendo os assobios sido separados em três categorias de qualidade: i) má (assobio visível no espectrograma, mas muito fraco, ou sobreposto a outros sons); ii) média (apito bem visível do início ao fim); iii) boa (assobio proeminente e dominante). Apenas assobios médios e bons foram analisados.

Com base na qualidade das gravações e na relação sinal-ruído, um total de 1239 assobios foram selecionados para análise. Tanto para o golfinho comum como para o golfinho roaz, o teste de Kruskal-Wallis mostrou diferenças estatisticamente significativas para a frequência inicial, baixas e altas frequências, bem como para os pontos de infeção. A frequência inicial, baixa e alta aumentaram significativamente na presença de barcos, enquanto os pontos de inflexão diminuíram com o aumento do número de barcos de observação de golfinhos. As diferenças observadas podem resultar de uma estratégia por parte dos golfinhos para evitar a dominância do ruido das embarcações (i.e., masking), bem como o aumento do dispêndio de energia. Estes resultados indicam que o impacto antropogénico resultante da atividade de embarcações de turismo de observação de golfinhos pode influenciar os parâmetros de vocalização dos animais e tais mudanças podem vir a ter impacto negativo se houver uma redução no alcance da comunicação dos assobios ou se os mesmos aumentarem os gastos de energia.

Palavras-chave: *Tursiops truncatus*, *Delphinus delphis*, comportamento acústico, ruído subaquático, sinais vocais, parâmetros acústicos

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Chapter 1: Introduction, state of the art and objectives

1.1. Introduction

Noise pollution in marine environment has been increasing in recent years (Sèbe M. *et al*. 2022). In coastal regions, anthropogenic pressure and demand for sea-related tourism activities, in particular maritime traffic, has led to a growing concern worldwide. Notable efforts to estimate, document and demonstrate the extent of the effects of such impacts have been made, particularly on aquatic mammals, considering the importance of sound for these animals (Erb C. *et al.* 2019, Holt M. *et al.* 2009, Luís *et al.* 2014, Nowacek *et al.* 2007, Parsons 2012, Scarpaci *et al*. 2000). Sound in cetaceans is used for a series of vital processes such as navigation and exploration of the environment, feeding and detection of predators (Au 1993, Deecke *et al.* 2005), as well as for communication with conspecifics (Tyack 1998). The acoustic-auditory system of marine mammals is highly specialized, and the increase in underwater noise of anthropogenic origin can interfere with it causing damage (Wartzok and Ketten 1999).

All cetaceans species occurring in mainland Portugal are protect by national legislation (Decree-Law nr 263/1981 from 3 September) and also by European regulations (Habitats Directive) and international conventions and agreements (Bern, Bonn, CITES, ACCOBAMS), being one of the main general rules to avoid making noise in the vicinity of cetaceans that could attract or disturb them, not more than 3 platforms being allowed in an area with a radius of 100 meters around a cetacean or a group of cetaceans. However, tourism has been growing, and in turn the number of dolphin-watching tourism vessels, and the rules are not always followed, which leads to the question whether the current guidelines did not need to be adjusted.

Tourism activities associated with the nautical sector play an important role in the socio-economic development of the coastal regions. However, other important factors must be taken into account, namely the impact on marine ecosystem, since human activities with socio-economic relevance can translate into negative impacts on biological communities and habitats (Parsons E. 2012). According to the last version of the Portuguese decree-law n. º 9/2006 from 6 January updated on December 2021, 83 tourism companies are currently licensed to operate whale watching vessels in mainland Portuguese waters, of which 49 operate just off the South Coast of Portugal. Compared to 2010, there are 35 more companies operating in the region (Castro 2010). The number of boats per company in operation goes from 1 to 15, being in total 131 boats operating in the Algarve. On average, each boat makes three trips per day (André Cid, personal communication).

Short-term behavioral responses of schools of cetaceans to boat traffic are reported in several studies, such as increases in group cohesion, dive duration and travel behavior (Nowacet *et al.* 2001, Miller *et al.* 2008), changes in breathing and surface patterns (Janik and Thompson 1996, Hastie *et al.* 2003), and reduction of aerial behaviors and cessation of eating, social and resting events (Papale *et al.* 2012). In the following sections, I will describe the current knowledge about the acoustic biology and ecology of common dolphins and bottlenose dolphins, communication behaviour on these species and impacts of anthropogenic sources of underwater noise

1.2. Biology and ecology of the common and bottlenose dolphins

This study will focus on common dolphins (*Delphinus delphis*) and bottlelnose dolphins (*Tursiops truncatus*). The species under study belong to the order of cetaceans and the family Delphinidae, both having a wide geographic distribution (**Figures 1.1. and 1.2.**). These species often experience high habitat overlap with human activities, occupying habitats from freshwater rivers to coastal estuaries and the open ocean (Bencatel *et al*. 2017).

1.2.1. Distribution and habitat

<u>Common dolphin</u>

The common dolphin is an abundant top predator with a wide geographic distribution, generally concentrated in regions of intense coastal upwelling (*Bencatel et al.* 2017). In Europe, it is particularly abundant in the Iberian Peninsula and the Bay of Biscay (**Figure 1.1.**). In Portugal it is clearly the most abundant cetacean species, mainly on the mainland coast (Moura *et al.* 2012, Castro *et al.* 2020). However, despite being observed along the entire coast throughout the year (Ball *et al.* 2017), the patterns of seasonal and geographic occupation are not yet well known.

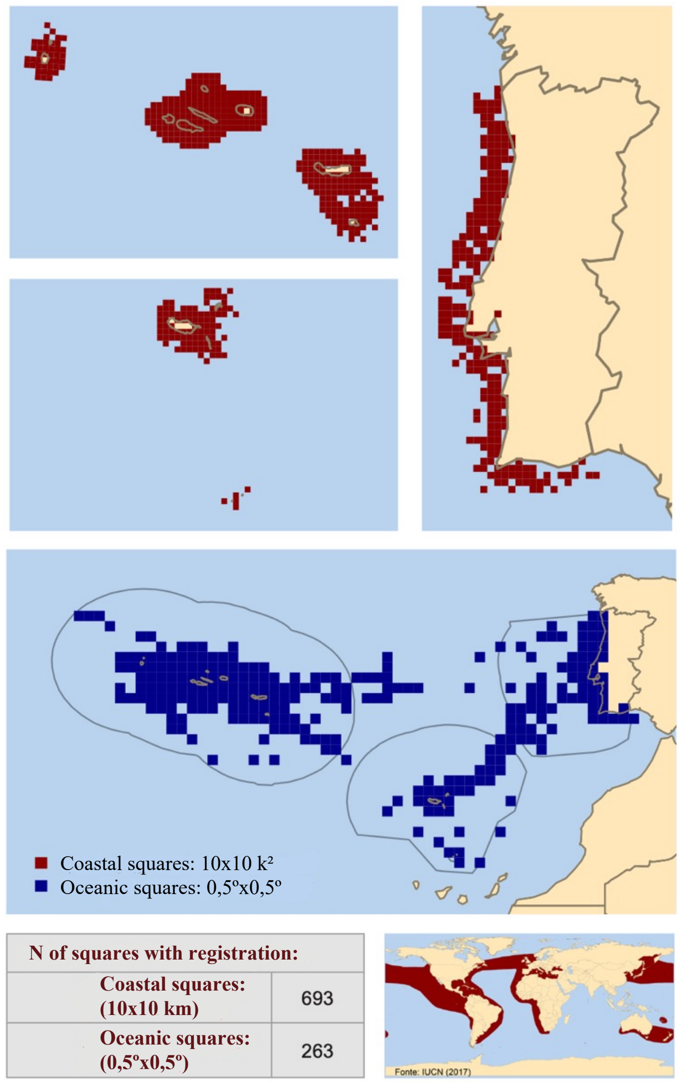

Figure 1.1. *Geographical distribution of Common dolphin, Delphinus delphis. (Adapted from Bencatel et al, 2017)*

Bottlenose dolphin (*Tursiops truncatus*)

The bottlenose dolphin can be observed in deeper waters however, it is also known to enter river systems (dos Santos *et al.* 2007). This species has a varied and flexible ecology, adapting well to the region and environmental conditions in which it is found (Louis *et al.* 2014).

It occurs in tropical and temperate waters (**Figure 1.2.**). In Europe, the bottlenose dolphin forms a metapopulation composed of several subpopulations, both coastal and oceanic (*Bencatel et al.* 2017). Several communities living in semi-enclosed waters are known, such as the Shannon Estuary (Ireland), Moray Firth (Scotland), Norman-Breton gulf (France), Galicia (Spain), Sado Estuary (Portugal), Gulf of Trieste (Slovenia) and Amvrikakos Gulf (Greece).

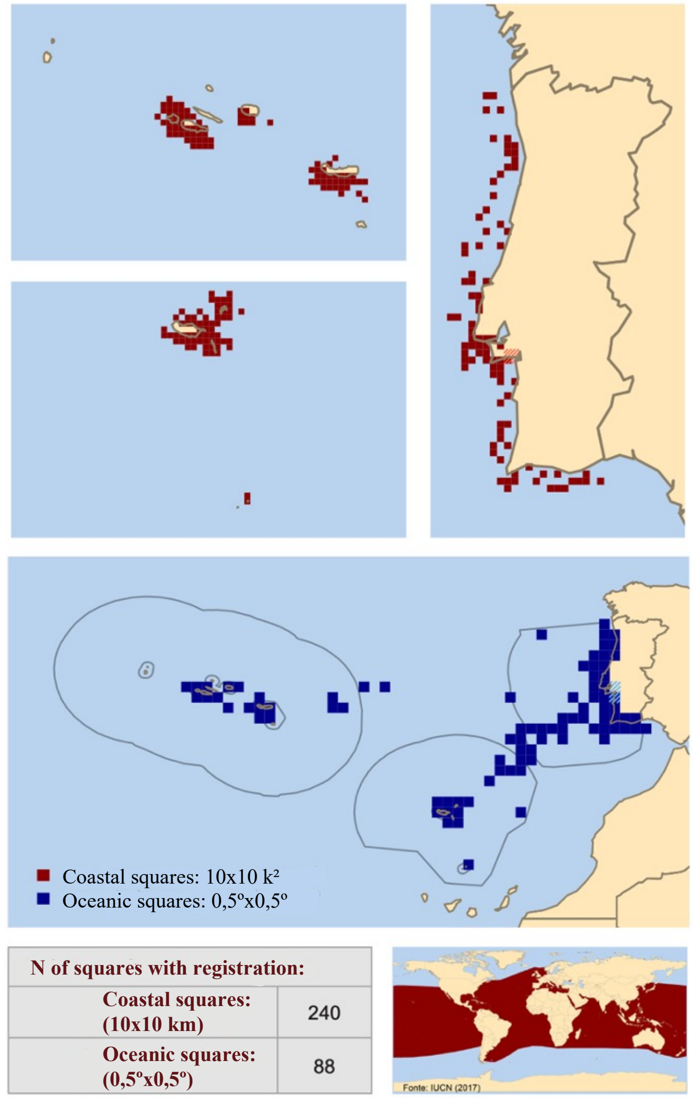

Figure 1.2*. Geographical distribution of Bottelnose dophin, Tursiops truncatus. (Adapted from Bencatel et al, 2017)*

In Portugal, the bottlenose dolphin is particularly known by the resident community of the Sado Estuary whose members are well catalogued (Augusto *et al.* 2012), however, the species can be observed throughout the continental coast, although less frequently than the common dolphin.

1.2.2. Behaviour and activity patterns

The common dolphin and bottlenose dolphin behaviour is complex and flexible, derived mainly from learning and social interaction (dos Santos 1998). Individuals tend to organize themselves in complex and dynamic groups, with a fission-fusion pattern (Tsai & Mann 2012, Castro *et al.* 2022). Individuals associate with groups for a certain period, which may vary in its composition and size through time. In these situations, communication plays a crucial role, providing information about each other in order to facilitate interaction between them (Smolker *et al.* 1992, Connor *et al.* 2000, Gibson & Mann 2008). For instance, males are grouped in trios or pairs of the same age, forming stable alliances that can last more than a decade, based on mating strategies, defence against predators and search for resources (Connor *et al.* 2000). Females are usually grouped according to their reproductive condition, with the offspring being associated with the mother until the birth of a new offspring (dos Santos 1998, Wells & Scott 1999). The frequency and nature of male-female associations depend primarily on the female's reproductive status, with stronger relationships being established during mating seasons (Connor *et al.* 2000). Hierarchical dominance relationships are also formed, based on the size of individuals but independent of sex, which are maintained through agonistic interactions (dos Santos 1998, Wells & Scott 1999).

Table 1.1. *Definitions of behavioural states for Delphinus delphis and Tursiops truncatus. (Adapted from Castro et al. 2021).*

Behavioural State	Definition
Foraging	Rapid directionless movements at the surface searching for or consuming prey; deep dives followed by loud exhalations; During this activity it is common to observe "burst swims" (rapid bursts of speed), "clean" noiseless headfirst re-entry leaps, coordinated clean leaps and tail slaps.
Resting	Low activity level, close to the surface, with slow movements at speeds of < 3 knots
Socialising	Interaction behaviors usually with directionless movement. May include chasing, body rubbing, belly-up swimming, splashing at the surface, mating, rolling, spyhops, leaping, or playing with seaweed.
Travelling	Group move with steady movement in one direction with speeds of > 3 knots

Studies focused on the behavioural ecology of these species, categorized the behaviours into global patterns of activity, with the most referenced patterns represented in **Table 1.1.** (Chilvers & Corkeron 2001, Lusseau 2006; Miller *et al.* 2010, Castro *et al.* 2021). The frequency and duration of occurrence of each activity pattern depend both on environmental factors such as habitat physiography, seasons, time of day, tidal state, and physiological factors such as reproductive seasonality (Shane *et al.* 1986, Ballance 1992).

1.3. Sensory system

Despite the similarities between the sensory systems of marine mammals with the terrestrial counterparts, the sensory system of marine mammals evolved into a system capable of detecting and processing the signal in the water. In both cases, the sensory system acts as highly selective filters (Mann *et al.* 2000). These filters cause the brain to select and attend only to signals that, evolutionarily, have proven to be important. (Wartzok, D & Ketten, D. R. 1999).

Animals can perceive stimuli from the external and internal environment. These stimuli are captured through highly specialized cells, called sensory cells. These cells can be found scattered throughout the body and in the sense organs (smell, taste, touch, vision, and hearing), forming the sensory system ((Wartzok, D & Ketten, D. R. 1999) The sensory mechanisms used by organisms are a direct consequence of the selective pressures that an environment can generate (Rodrigues, 2010). These selective pressures led the sensory system to evolve in a way that allows animals to receive and process information from their surroundings. To understand how the sensory system operates, we must also study how the physical characteristics of the environment may affects the propagation and reception of a stimulus (Wartzok, D & Ketten, D. R. 1999).

1.4. Communication and cognition

A definition of communication between living animals consists on the exchange of information between a sender and a receiver using a code of specific signals that generally serve to meet common challenges (e.g. reproduction, foraging) and to promote group cohesiveness (Vauclair 1996). In acoustic communication, there is transmission of information between individuals through sound signals, to influence the behavior of others for their own benefit. (Dawkins & Krebs 1978, Slater 1983).

Acoustic cues are affected differently in water and in air due to physical aspects, which must be taken into account when analysing the communication of marine mammals (Wartzok, D & Ketten, D. R. 1999). Because water is denser than air, sound travels faster and with less attenuation in water than in air. The sound speed in moist air is approximately 340 m/sec., while the speed of sound in seawater averages 1,530 m/sec, depending of the water conditions (e.g., density) (Ingmanson & Wallace 1973). However, these factors act synergistically, causing the ocean to have a highly variable sound profile that can change both seasonally and regionally an as they are primarily sound intensity detectors, marine mammals are affected by these physical differences (Tyack 2000).

1.4.1. Mechanisms of production of acoustic signals

There has been a great controversy in the way vocalizations are produced in bottlenose dolphins. Studies carried out in 1983 by Purves & Pilleri, argued that acoustic signals were produced through the vibration of the laryngeal folds. However, later this theory was replaced by other authors and currently the most accepted theory argues that the generation of acoustic signals takes place inside the skull, in the nasal region, through the forced passage of air between the various nasal sacs, which causes the vibration of a structure called *museau de singe* or phonic lips (**Figure 1.3.**) (Cranford *et al.* 1996). The signals produced are focused through the fat tissues of the forehead (Figure 5) and led to the water (dos Santos 1998). According to Dormer (1979) and Cranford *et al.* (1996), there are two systems of nasal sacs, that correspond to two distinct sound generators, that enables the production different types of vocalizations simultaneously (e.g., a train of clicks and a whistle). The different types of vocalizations may result in the emission of sounds with unequal functions: communication and orientation. Recently, studies have shown that echolocation clicks are produced by the right pair of phonic lips, and that whistles are mostly produced by the left pair (Madsen *et al.* 2013). Then, the sound signals are focused through the melon (acoustic lens), located on the forehead, and are propagated through the water (dos Santos 1998).

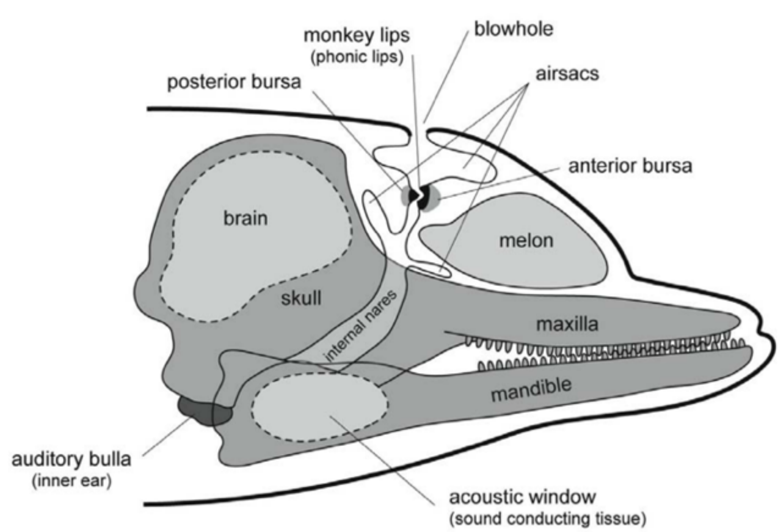

Figure 1.3. *illustration of a dolphin head showing the position of the melon and associated sound-producing structures. (Modified and adapted from Cranford et al. 1996.)*

1.4.2. Acoustic signal receiver mechanisms

As in terrestrial mammals, the auditory system of cetaceans is divided into three sections: the outer ear, the middle ear, and the inner ear (cochlea) (Wartzok, D & Ketten, D. R. 1999). However, in terrestrial mammals, the middle ear and inner ear are embedded in the skull, while in cetaceans these hearing mechanisms are contained in a bony structure (tympanic bulla), which is connected to the skull through cartilage and adipose tissue (McCormick. *et al.* 1970). Cetaceans lack the pinna, an obvious physical feature of the outer ear of their terrestrial ancestors (Ketten 1992, Ridgway *et al.* 2001, Hemila *et al.* 2010). Instead, the termination of the external auditory canal of the dolphin was characterized by a small hole on the surface of the animal, located behind the eye (Ketten 1992), which suggests the little importance of this structure in the conduction of sound to the ear (McCormick *et al.* 1970, Ridgway 2000, Bullock *et al.* 1968).

Recent studies have supported the theory of Norris (1968), in which the sound is captured by the lower jaw, being conducted to the middle ear and inner ear, through the adipose tissue existing in a small canal of the mandible (Brill *et al.* 1988, Norris 1968, Tyack 2001, Cranford *et al.* 2010, Montie *et al.* 2011). This channel runs through the mandible to the proximity of the tympanic bulla, being filled with lipid substances similar to those found on the forehead (Varanasi & Malins 1971 in Jensen 2011). The middle ear acts as an acoustic impedance device, which neutralizes the loss of transmission of sound waves between the surrounding water and the fluid contained in the inner ear (Hemila *et al.* 1999; Nummela *et al.* 1999). As in terrestrial mammals, it is in the inner ear (cochlea) that sound energy is converted into nerve impulses that are transmitted to the brain via the auditory nerve (Reynolds *et al.* 2000).

1.4.3. Dolphins vocalization

Dolphins are extremely vocal organisms, and vocal communication is extremely important in mediating social interactions (Smolker and Pepper 1999). These animals can produce a wide variety of vocal and non-vocal sounds, including sounds produced by percussive activities or produced as a by-product of bodily functions, whereas vocal sounds are generated internally (Cranford *et al.* 1996). Most dolphin species can produce two primary types of sounds thought to play a role in social interactions: (i) tonal, frequency-modulated whistles, and (ii) rapid repetition rate "burst-pulse" click train (Van der Woude 2009, Lópes *et al.* 2010, Luís *et al.* 2016). Vocalization rates are dependent on a dolphin's behavior, with feeding and socialization having the highest vocalization rates (Jones and Sayigh 2002, Acevedo-Gutiérrez & Stienessen 2004, dos Santos *et al*. 2005).

Tonal vocalizations or whistles are considered to be cohesion calls and communication signals that allow individual recognition of members in a social group and as a contact call to maintain physical and vocal contact (Janik and Slater 1998, Smolker and Pepper 1999). This type of vocalization is longer than 200 ms and have most energy between 4 and 23 kHz. (Lópes *et al.* 2010) (**Figure 1.4.**)

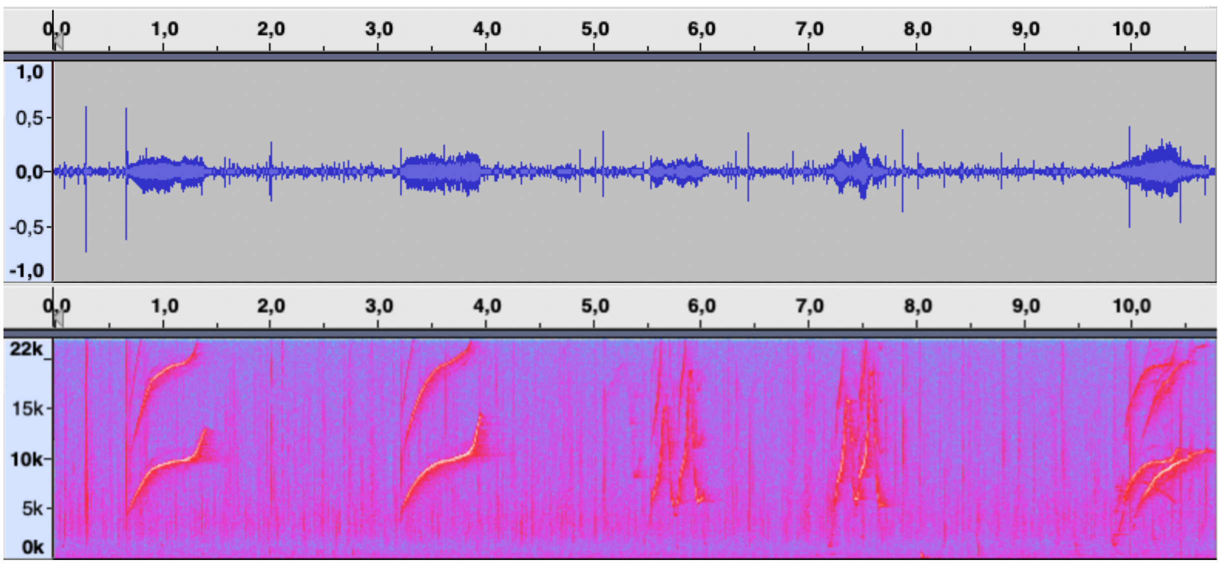

Figure 1.4. *Spectrogram (below) and waveform (above) of common bottlenose dolphin (Tursiops truncatus) whistles recorded by Bottlenose Dolphin Research Institute.*

'Burst-pulsed' click trains are used in sonar-related tasks and detection, sounds are emitted during social interactions, and during foraging/ feeding events (dos Santos *et al.* 1995) (**Figure 1.5.**). There are two types of burst pulsed vocalizations, the "short burst pulsed sounds", characterized by single burst intrinsically short (less than 200ms) and where we can detect six different signal types: chokes, gulps, coughs, brays, quacks, and croaks; and the "long burst pulsed sounds" class, that has a longer signal (longer than 200ms) and it is composed of a single or a sequence of pulses (Figure 1.5.). Within this class, six different signal types were detected: buzzes, creaks, screeches, yelps, pops, and cries. (Lópes *et al.* 2010).

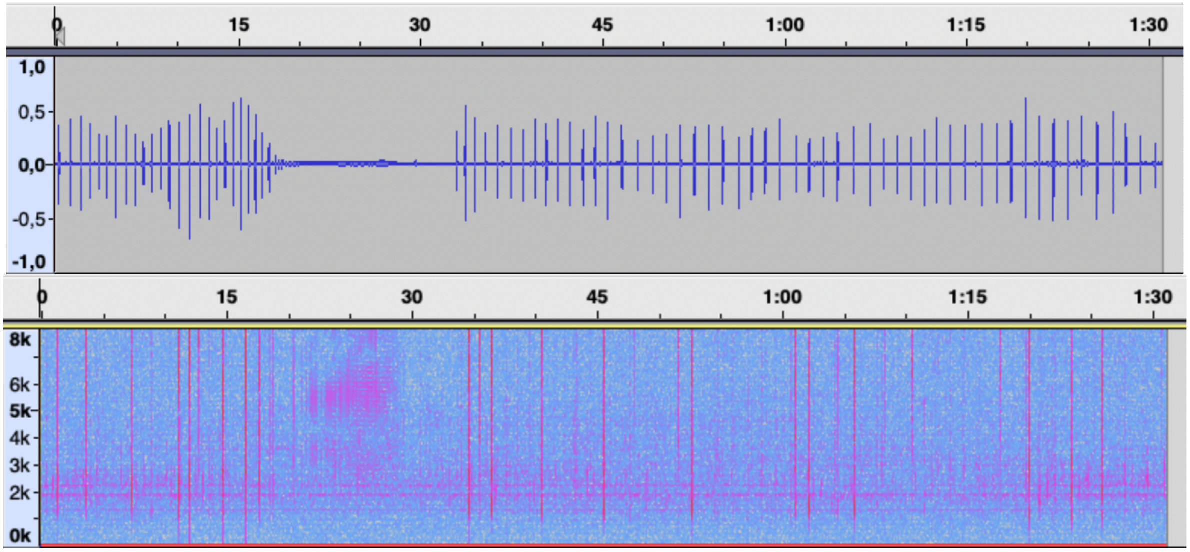

Figure 1.5. Spectrogram (below) and waveform (above) of sperm whale (Physeter macrocephalus) clicks recorded by Bottlenose Dolphin Research Institute.

Burst pulsed sounds comprise the majority of conspecific vocalizations been strongly implicated in communication (Lópes *et al.* 2010). Some authors have suggested they are related with courtship, dominance, and/or aggressive behaviors in the same species (Overstrom, 1983). However, it has received much less attention because they are recorded far less frequently than whistles and thus require high levels of field study effort to build up large samples, making their occurrence and functional significance still only poorly understood.

1.5. Whistle function

Of all types of vocalizations, whistles are the ones that have received the most attention from the scientific community, due to their relatively low frequencies, audible by humans (Díaz Lopez 2011). The study of cetacean whistles began in the 1950s with the first investigations into the acoustic emissions of dolphins. Much of the existing knowledge about the acoustic communication of dolphins derives from studies carried out on the whistle emission of the bottlenose dolphin (Cadwell& Cadwell 1965, Cadwell *et al.* 1990, Acevedo-Gutiérrez & Stienessen 2004, dos Santos *et al.* 2005, Janik *et al.* 2013, La Mamma *et al.* 2020, Rako-Gospić, N. *et al.* 2021), which is the most common species in captivity.Whistles are tonal sounds with a narrow spectral band, with fundamental frequencies ranging between 1 and 38 kHz (La Manna *et al.* 2020.), which can exceed 50 kHz when harmonics are present (Lammers *et al.* 2003). Despite having a low directionality, whistles show a series of easily variable parameters (frequency, duration, number of repetitions, acoustic pressure) and are abundantly emitted in situations of social excitement (social behaviour, mating, feeding), leading to communicative functions and the expression of emotions being attributed to them (dos Santos 1998).Whistles usually have an average duration of less than one second and can last up to three seconds (Au & Hastings 2008).

As mentioned above, dolphins may live in a complex society of fusion-fission pattern, in which the composition of groups is variable, however, the establishment of associations between certain individuals is often stable (Connor *et al*. 2000). The maintenance of these relationships, within a constantly changing social environment, requires a system of individual recognition, which in underwater conditions becomes limited due to poor visibility and reduced olfactory sensitivity (Sayigh *et al*. 1990). Acoustic communication thus acquires the most important role in transmitting identity information and maintaining group cohesion (Caldwell et al. 1990, Janik & Slater 1998). The fact that these animals produce very stereotyped whistles, that is, with the same frequency modulation profile, for long periods of time suggests the existence of acoustic signals as individual identifiers (Caldwell *et al*. 1990, Sayhigh *et al*. 1990, dos Santos *et al*. 2005). Each individual dolphin develops its unique frequency modulation pattern whistle, termed "signature whistle" that functions as an identifier (Caldwell & Caldwell 1965, Herzing 1996, Janik & King 2013) and may be mimicked by other animals (dos Santos *et al*. 1990).

1.5.1. Whistle types

Whistles can be categorized into different classes by visually inspecting the spectrograms, giving special emphasis to the shape of the whistle contour (Janik 1999, Romeu *et al.* 2017, Díaz Lopez 2011, Rako-Gospić *et al.* 2021). However, studies carried out on this topic have faced difficulties in naming whistles due to the lack of standardization of category classification techniques (Au & Hastings 2008). Rako-Gospić *et al.* 2021, classified the whistles by its contour, accord by the following types: i) ascendant (initial frequency less than final frequency, without inflection points); ii) descending (initial frequency greater than final frequency, without inflection points), iii) modulated (more than one inflection point, descending to ascending, or vice versa); iv) flat (no frequency variation) (**Figure. 1.6.**).

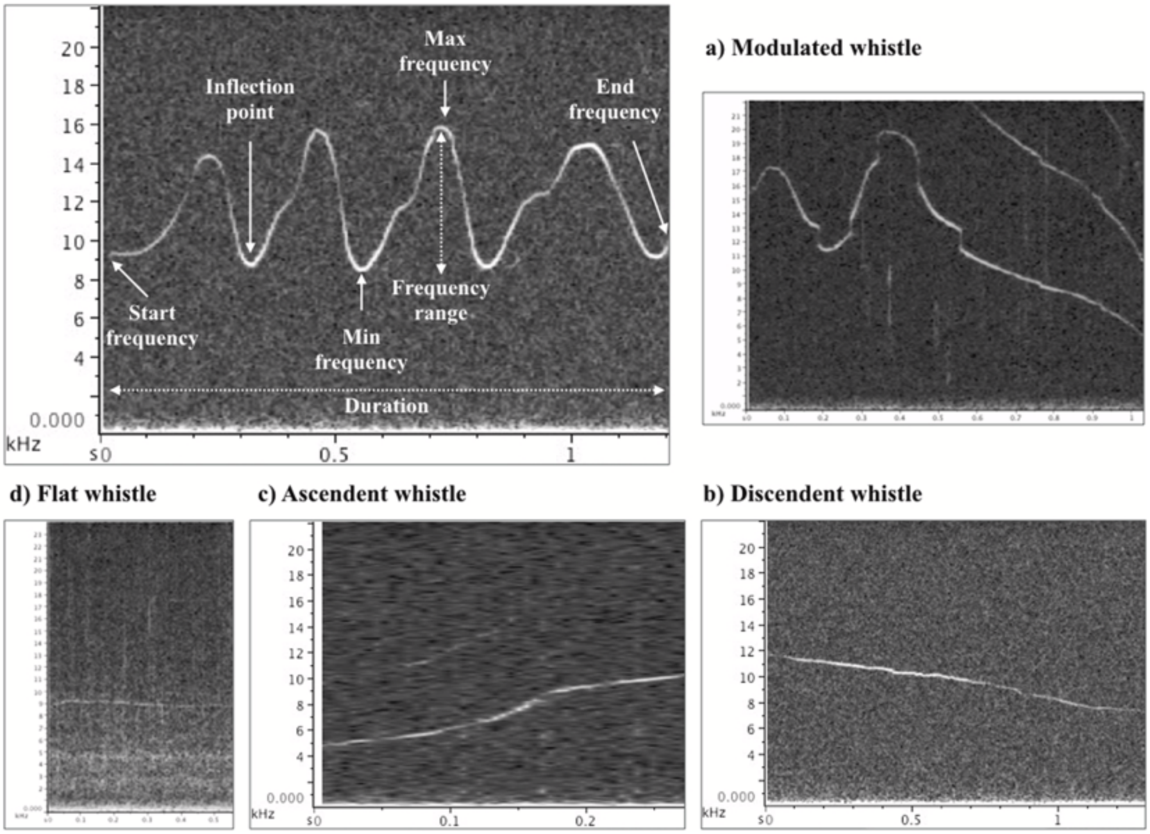

Figure 1.6. Whistle parameters measured from the contour and whistle type. Spectrogram extracted from Raven Pro 1.4 software (1024 point fast Fourier transform (FFT) and 512 window size, Hamming window, 50 % overlap). (from Rako-Gospić et al. 2021)

1.5.2. Signature Whistlers

The signature whistle hypothesis was proposed by Melba and David Caldwell (Caldwell & Caldwell 1965), after demonstrating in a group of bottlenose dolphins in captivity, that each individual produced a characteristic type of whistle with a certain pattern of frequency modulation. Other studies also carried out in captivity have found that dolphins resort to abundant whistles when temporarily separated from their conspecifics, with 80% to 100% of all whistles emitted representing signature whistles (Caldwell *et al*. 1990, Janik & Slater 1998, Sayhigh *et al*.. 2007, Janik *et al*. 2013). This leads to a strong connection between whistling and episodes of temporary separation of highly interconnected individuals, such as mother-calf associations (Smolker *et al*. 1993) and adult males that live in alliance (Watwood *et al*. 2004). Dolphin's mother and their young calves use tonal frequency-modulated whistles as signals for individual recognition (Tyack 1999). When a mother dolphin and her calf are forcibly separated in the wild, they whistle at high rates (Saigh *et al*. 1990), whereas in voluntary separations, it is usually the calf that whistles to signal a reunion (Smolker *et al*. 1993).

The bottlenose dolphin can take up to 2 years to develop an individually distinct signature whistle, but once a signature whistle is developed, it remains stable for the rest of the animal's life (Caldwell *et al.* 1990; Saying *et al.* 1990, 1995), which suggest that signature whistles may also function for individual recognition in contexts other than mother-child recognition. However, a more detailed study is needed to determine whether dolphins imitate each other's signature whistles to call another individual (Tyack 1999).

1.6. Impacts of watercraft noise on marine mammals

Most of the early work on the potential effects of ship noise on both odontocetes and mysticetes took place in the early 1980s as a result of concern over Arctic industrial development (hydrocarbons, mining and transportation) (John Richardson *et al.* 1997). For instance, experimental approaches to Greenland whales (*Balaena mysticetus*) by small boats at high speeds showed that the whales generally drifted away, interrupting their foraging, socializing, and playing behaviour, while spending less time on the surface (Richardson *et al*. 1982, Greene 1985, Richardson *et al*. 1985, Johnson *et al*. 1986). Regarding belugas (*Delphinapterus leucas*), in response to icebreakers, they lost group integrity, initiated rapid movements, asynchronous and shallow dives and changed their vocal behaviour (i.e. vocalization types) at received noise levels of 94–105 dB re 1 μPa rms (20–1000 Hz), while narwhals (*Monodon monoceros*) changed their locomotion (i.e., exhibited more directed and slower movements, became immobile, and sank) and were silent at received noise levels of around 124 dB re 1 μPa rms (20- 1000 Hz)(Finley *et al*. 1990, Richardson *et al*. 1990, Cosens and Dueck 1988). However, it was in the last 20 years that the number of publications growth more rapidly with more than 45 marine mammal species being studied. The most studied species are the bottlenose dolphin (*Tursiops truncatus*), humpback whale (*Megaptera novaeangliae*), and then beluga whale (*Delphinapterus leucas*) (Erbe *et al.* 2019).

1.6.1. Behavioural Changes

The most reported behavioural changes in populations of bottlenose dolphins resulting from boat traffic include increasing swimming speed and diving time, changing in travel route, and in the behaviour at the surface. (Evans *et al*. 1992, Nowacek *et al*. 2001, Lemon *et al*. 2006). The two factors that apparently have the greatest influence are: the change of direction of the vessels and the time of exposure. The second factor is one that raises the most concerns with

respect to dolphin-watching tourism vessels, since animals are often exposed to the tour boats presence for long periods (Nowacek *et al*. 2001).
Another factor that leads to behavioural changes in dolphins may be related to the number of vessels in circulation. Studies show that bottlenose dolphins are more susceptible when there is a greater number of vessels, especially when they are resting (Constantine *et al*. 2004). Other study reported by Lusseau 2006 show that during interactions with vessels withing a 400 m range, dolphins tend to jump laterally more often, a behaviour that is used to communicate the change of direction, useful when they cannot communicate vocally. This fact refers to signal masking situations, in which the acoustic pathway loses relevance in communication between individuals (Cruz 2012).

1.6.2. Changes in Vocalizations

Sound masking is the process that occurs when noise interferes with a marine animal's ability to hear a sound of interest. Masking can reduce the range over which signals can be heard and, consequently, reduce the quality of signal information. This may be due to the superposition of natural sounds (geophony), such as wind and ocean currents, or man- made (anthropophony), with biological sounds (Jesus 2018). Signal masking can jeopardize vital activities for individuals, such as foraging and reproduction (La Manna *et al.* 2020, Rako-Gospi ́c *et al.* 2021). Changing the frequencies of the signals and increasing the number of vocalizations may represent an attempt on the part of the animal to overcome "masking" when a sound is obscured or interfered with by background noise (Weilgart 2007). The different reactions observed in the short term in the interaction of animals with vessels can lead to significant behavioral changes, such as the avoidance of important feeding areas which, in the long term can have a biologically significant impact on the population (Lusseau 2006).
Bottlenose dolphins (*Tursiops truncatus),* for instance, produced more whistles in response to approaching boats, in order to maintain acoustic contact (Buckstaff 2004). This fact suggests that the increase in noise caused by boats can compromise group cohesion and, consequently, coordination in vital activities such as feeding and reproduction (Van Parijs & Corkeron 2001).
A study carried out by Scarpaci *et al*. (2000), at Port Phillip Bay, on the southeastern coast of Victoria - Australia, indicates an increase in whistle production during travel, feeding and social behaviour in the presence of dolphin-swim operators for bottlenose dolphins. However, the way animals react acoustically to noise varies between species and within species but also depending of the animal life stage, sex, physical status, and existence of previous contacts (Weilgart 2007).

Objectives

The effect of dolphin watching tourism vessels on dolphin populations inhabiting the Algarve are still poorly studied. There is a significant knowledge gap regarding the potential impacts of tour boats underwater noise on vocalizations of dolphins. Therefore, this work aims to:

1. Assess how the communication (i.e., whistles production and characteristics) of common dolphin and bottlenose dolphin changes depending on the presence or absence of dolphin watching tourism vessels.
2. Quantitatively analyse the patterns of occurrence and acoustically characterize the whistles produced by common dolphin and bottlenose dolphin in the Algarve region.
3. Provide scientific based evidence to support the development of conservation measures to reduce the impact of tourism boats on the population of dolphins in the Algarve.

These objectives will be addressed through the analysis and processing of acoustic data collected in field between Jun and September of 2022 in the Algarve region.
The results may contribute to the review and adaptation of the Cetacean Safeguard Action Plan, with the aim of improving the methodology for monitoring.

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Chapter 2: Scientific Manuscript

Influence of dolphin-watching tourism vessels on the whistle emission pattern of common dolphins and bottlenose dolphins.

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Abstract

Over the last few years there has been a significant increase in the number of dolphin-watching boats in the Algarve (Portugal), which my lead to short- and long-term impacts on the target species (e.g., common dolphin, bottlenose dolphin). In recent decades there has been a greater interest in the potential effects of anthropogenic noise on marine mammals, given the important role that sound plays in the vital functions of this organisms. Several changes in the behavior and energy expenditure of cetaceans have been documented, including impacts in the vocalization parameters of dolphins, reduction in the communication range of whistles and increase energy expenditure. In this study, the whistles characteristics of common dolphin (*Delphinus delphis)* and bottlenose dolphin (*Tursiops truncates)* were analyzed in the presence and absence of dolphin-watching tour boats to detect potential impacts in the vocalization of dolphins. Field recordings of common dolphin and bottlenose dolphin whistles were made from June to September 2022, using a calibrated system. Dolphin behavior and group size were recorded, as well as the number of boats in a 300 m radius. A total of 15h of acoustic recording was analyzed. Overall, these results showed a significant increase in start, low and high frequency on both species when exposed to the presence of one or more dolphin-watching observation vessels. However, when analyzing the whistles, it was possible to observe a reduction in the number of inflection points in the presence of the same vessels. These changes can be a dolphin strategy to avoid sound masking and increase of energy expenditure. These findings indicate that anthropogenic impact in the form of dolphin-watching tour vessels can influence the vocalization parameters of dolphins and such changes could have an impact if they reduce the communication range of whistles or increase energy expenditure.

Keywords: *Tursiops truncatus*, *Delphinus delphis*, acoustic behavior, underwater noise, vocal signals, acoustic parameters

2.1. Introduction

The potential effects of maritime traffic noise in coastal areas is a topic of growing concern worldwide, leading to notable efforts to document and determine the extent of such impacts, particularly on marine mammals (e.g., Nowacek *et al.* 2007). Dolphins, for instance, are extremely vocal organisms and use sound to mediating social interactions and communicate with conspecifics (Tyack 1998, Smolker and Pepper 1999),for navigation and exploration of the environment, and for feeding and detection of predators (Au 1993, Deecke *et al.* 2005). These animals can produce a wide variety of vocal and non-vocal sounds, including sounds produced by percussive activities or produced as a by-product of bodily functions (Cranford *et al.* 1996).

Short-term behavioral responses of cetaceans to boat traffic noise are reported in several studies, including increase in group cohesion, dive duration and travel behavior (Nowacet *et al.* 2001, Miller *et al.* 2008); changes in breathing and surface patterns (Janik and Thompson 1996, Hastie *et al.* 2003); and reduction of aerial behaviors and interruption of feeding, social and resting events (Papale *et al.* 2012). Another important behavioral response of cetaceans to boat noise is the changes in frequencies of the acoustic signals and number of vocalizations, as an attempt to overcome "masking" effects of background noise (Weilgart 2007).

Most dolphin species can produce two primary types of sounds considered relevant in social interactions: (i) tonal, frequency-modulated whistles, and (ii) rapid repetition rate "burst-pulse" click train (Van der Woude 2009, Lópes et al 2010; Luís *et al.* 2016). Tonal vocalizations such as whistles, are considered to be cohesion calls and communication signals to recognize members in a social group and maintain physical and vocal contact (Janik and Slater 1998; Smolker and Pepper 1999). This type of vocalization is longer than 200 ms and are emitted in frequencies varying between 4 and 23 kHz. (Lópes *et al.* 2010). Whistles can be categorized into different classes by visually inspecting the spectrograms and based on the shape of the whistle contour (Janik 1999, Romeu *et al.* 2017, Díaz Lopez 2011; Rako-Gospić *et al.* 2021). Despite difficulties in naming whistles due to the lack of a standard classification technique (Au & Hastings 2008), Rako-Gospić *et al.* 2021 classified whistles in: i) ascendant (initial frequency less than final frequency, without inflection points); ii) descending (initial frequency greater than final frequency, without inflection points), iii) modulated (more than one inflection point, descending to ascending, or vice versa); iv) flat (no frequency variation).

At least eight species of cetaceans have been reported in the southern coast of Portugal. Common dolphin (*Delphinus delphis*) and bottlenose dolphin (*Tursiops truncates*) are among the main target species by dolphin-watching tour boats. These species often experience high habitat spatial overlap with human activities, highlighting the importance of improving the current understating about the ecology, behaviour and threats to these species (Castro, 2010). Tourism activities associated with the nautical sector play an important role in the socio-economic development of the coastal regions (Parsons E. 2012). However, negative impacts on the marine ecosystem resulting from marine traffic (e.g., noise, pollution) are known, affecting biological communities and habitats, reducing biodiversity and threatening endangered species (Whitfield and Becker 2014, Nunes *et al.* 2020, Sim *et al*. 2015).

Previous studies have focused on the potential impacts of dolphin-watching tourism boats on dolphin vocalizations (e.g. Scarpaci *et al*. 2000, Holt M. *et al.* 2009, Luís *et al.* 2014). A study carried out in 2004 by Buckstaf, shows that bottlenose dolphins*,* produced more whistles in response to approaching boats in order to maintain acoustic contact, potentially with deleterious effects on group cohesion and coordination of vital activities such as feeding and reproduction (Van Parijs & Corkeron 2001). Scarpaci et al. 2000 also showed an increase in whistle production in bottlenose dolphins inhabiting Port Phillip Bay, Victoria - Australia when animals were travelling, feeding and socializing in the presence of tour boats.

Currently, 83 tourism companies are currently licensed to operate whale watching vessels in mainland Portuguese waters, of which 49 operate just off the South Coast of Portugal (ICNF 2022). Compared to 2010, there are 35 more companies operating in the region (Castro 2010). The number of vessels per company in operation varies between 1 to 15, being in total 131 boats operating in the Algarve, each doing on average three trips per day (André Cid, personal communication). Dolphin-watching tourism boats follow dolphins for long periods, often in large number (1-13 boats). Based on previous work by Easch *et al.* 2009, dolphins in such circumstances are susceptible to become stressed and fatigated. These changes can be detected through changes in the whistle frequency patterns.

The aim of this study is to investigate the whistle characteristics and potential changes in the emission patterns in common and bottlenose dolphins in the presence of dolphin-watching boats operating in the southern coast of Portugal mainland – the Algarve. We hypothesized that whistle emission patterns would vary according to the number of boats, due to the increase in the intensity of underwater noise. This kind of studies can help protect dolphin populations in southern Portugal, since the anthropogenic impact of the region in the form of tourist dolphin watching boats, can influence dolphin vocalization parameters and such changes can have an impact if reduce the communication range of whistles or increase energy expenditure.

2.2. Materials and methods

Study Area

The study area corresponds to the coastal area of the western Algarve coast (souther region of Portugal) extending from Faro to Sagres. (**Fig. 2.3.**) The Algarve has a heterogeneous coastline that is characterized by various types of rock formations (e.g., boulders, low relief rocky areas, submerged rocky bottoms) and different sediment dynamics (Cúrdia et al. 2013 All these aspects have a profound effect on the biodiversity and dynamics of pelagic and bentonic communities (Fernandez-Arcaya et al. 2017), which makes this area a hotspot for the species under study. Every summer, the human population in the Algarve triples, due to the thousands of tourists who choose this holiday destination. This increase leads to greater coastal pressure in the larger cities, in particular in Albufeira where the dolphin-watching tourism vessels activities are in high demand.

Data collection

Data were collected from transients' groups of common dolphins and bottlenose dolphins in the study area from June to August 2022. The methodology used to collect the data consisted of focal-follow of dolphin groups (Mann 1999). We used group focal-follows instead of individuals focal-follow considering the difficulties of identifying individual dolphins or determine which individual is vocalising. A group of dolphins was defined as an aggregation of individuals swimming in a coordinated manner within 100 m of each other while displaying the same type of behaviour (Shane 1990).
Depending on the weather conditions (sea state < 5 according to the Beaufort scale), surveys were conducted for approximately 6h per day (09.00-16.00) following the experimental test plan (Appendix II). Recordings and observations were conducted from a semi-rigid research boat, 6.70m long and a 135hp Honda engine. Sound recordings was conducted simultaneously with data collection on the dominant behaviour of the focal group and estimates of the group size. Data was collected using: (1) continuous sampling for whistle production, and (2) five-minute scan samples of their behavioural state and group size as described by Altmann (1974).

To minimize the effect of the presence of the research boat on the animal's behaviour the following protocol was implemented: (i) dolphins were approached according to the Regulations and Guidelines for Whale Watching in Water Under Portuguese Jurisdiction and (ii) no data was collected during the first 10 minutes for the animals to get used to the presence of the research boat. When no boats except the research boat (with its engine off) were present, the acoustic record was classified as 'absence' (control data), while 'presence' refers to the occurrence of at least one dolphin-watching tour boat within 300 m of the focal group. Sound samples were recorded underwater using a calibrated autonomous hydrophone. A digitalHyd SR-1 autonomous hydrophone, a compact acoustic recorder equipped with a SensorTech SQ-26 transducer (sensitivity is −194 dB re 1 V/1 μ Pa with a variation within ± 1dB in the 1 Hz to 28 kHz interval), using a high-pass filter of 50 Hz to decrease the effect of noise generated by the recording platform and low-frequency vibrations. All recordings were made at a 52.734 kHz kHz sampling rate and with a 24-bit resolution. The system was operating in autonomous mode with the integrated battery and storage on internal SD card and deployed 2 m below the sea surface.

The CTD Ruskin Concerto, a watertight cylindrical recorder was used during the experiment to collect temperature, salinity and sound speed. Data was collected in 8 different days, always in the presence of animals, in order to evaluate the influence of sea conditions on the acoustic behavior. The CTD was lowered close the sea bottom or up to the 20 meters depth depending on the sampling location in order to make a profile of the water column.

Acoustic analysis

The acoustic records collected from both hydrophones were first inspected as spectrograms and subjected to both aural and visual assessment using Audacity 2.4.2, in order to identify, categorize, and count all the "whistles" present on each sample. A whistle was defined as a tonal, narrow-band, modulated signals lasting 0.1 s or more, with at least part of the fundamental frequency above 3 kHz. A whistles have a fundamental frequency usually below 23 kHz and harmonics up to 100 kHz. Only the fundamental frequency of each selected whistle contour was measured due to hydrophone upper-frequency limitations (26.36 kHz) (Lópes et *at.* 2010). Each signature whistle recognised by the observer was identified following the Identification and Characteristics of Signature Whistles method - SIGID (Janik *et al.* 2013), for further analysis. This rule was applied to reduce the risk of collecting many whistles from the same individual (pseudo-replication) (La Manna *et al*. 2020).

Following previous studies (Romeu *et al*. 2017, Díaz Lopez 2011, Erbe *et al.* 2020, Rako-Gospi´c *et al* 2021), the whistles were categorised in: "Rise" (whistles with initial frequency less than final frequency, without inflection points), "Fall" (whistles initial frequency greater than final frequency, without inflection points), "Flat" (no frequency variation), "Modulated" (whistles with one or more inflection point, descending to ascending, or vice versa) (Figure2.2.).

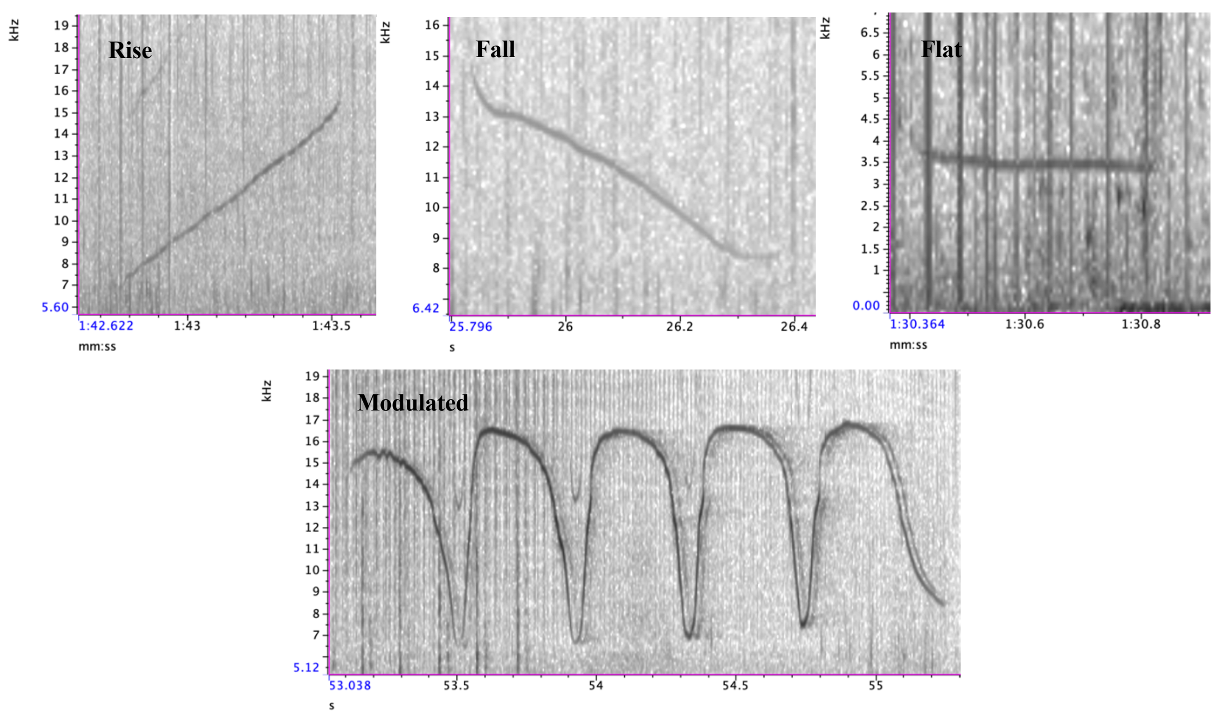

Figure 2.1. *Example spectrogram for all observed whistle types on standardized time axe. All y-axes are in kHz. The x-axes change from milliseconds to seconds as samples extend beyond 1s.*

Since low frequency noise can mask the lowest frequency component of the whistles, producing an erroneous estimation of the measured frequencies, all whistles were separated into three different quality categories: i) poor (whistle visible on the spectrogram but too faint, or overlapping with other sounds); ii) fair (whistle clearly visible from its start to its end); iii) good (prominent and dominant whistle). Only whistles scored as 2 or 3 were used for analysis (La Manna et al. 2020).

The repertoire plasticity and temporary shifts in whistle variables was measure (Fig. 2.2). Duration, minimum, maximum, and frequency range were automatically measured from the selection by Raven Lite 2.0.4 (1024 point fast Fourier transform (FFT) and 512 window size, Hamming window, 50 % overlap), while start and end frequency and the number of inflection points were measured or counted manually (Table 2.1.).

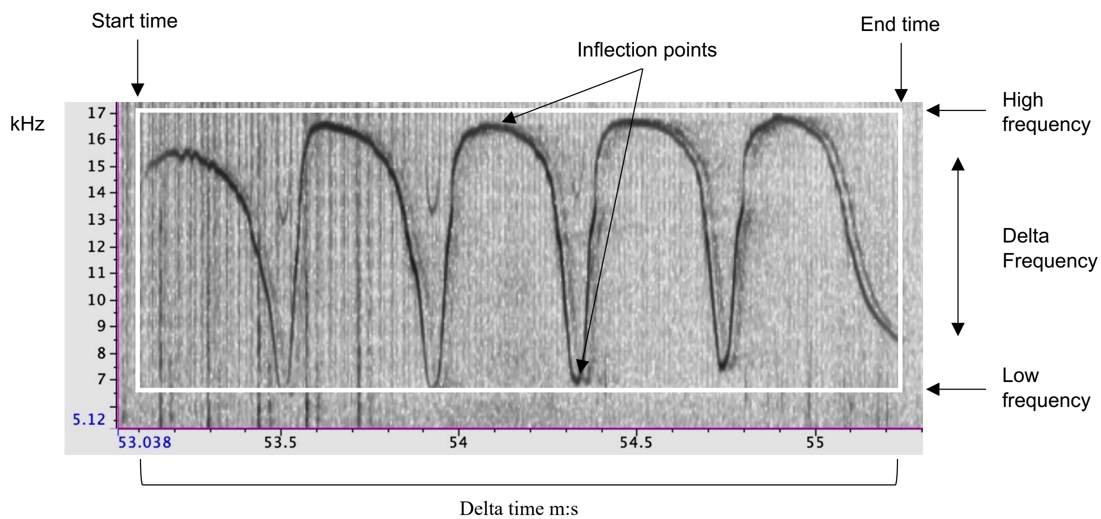

Figure 2.2. *Spectrogram of a bottlenose dolphin's whistle recorded in Algarve (Portugal) indicating the seven variables analyzed. Fast Fourier transform (FFT)= 1024, frame duration = 2 ms.*

Table 2.1. *Whistle structure is the result of seven parameters measured on the spectrogram (manually or automatically by Raven Lite software; 1024 point fast Fourier transform (FFT) and 512 window size, Hamming window, 50 % overlap). (Adapted from N. Rako-Gospi ́c et al. 2021)*

Parameter	Description
Start frequency (Hz)	The beginning frequency of the whistle.
End frequency (Hz)	The ending frequency of the whistle.
Low frequency (Hz)	The lower frequency of the whistle.
High frequency (Hz)	The upper frequency of the whistle.
Delta frequency (Hz)	The difference between the upper and lower frequency of the whistle.
Delta time (s)	The time interval between the start and the end of the whistle.
Number of inflection points	The number of inflection points defined as the change from positive to negative or negative to positive slope in the contour.

Statistical analyses

Statistical analyses were conducted to determine if tour boats presence affected the whistle production in the common and bottlenose dolphins. An approximate rate for the number of whistles in the presence of vessels was calculated by dividing the total number of whistles by the total number of minutes that the dolphin-watching tourism boat was present. This number was than divided by the number of dolphins present to remove the effect of group size on the number of whistles.

As data were not normally distributed, the non-parametric tests Kruskall-Wallis was used to analyse the several acoustic parameters of whistles (i.e., start frequency, end frequency, minimum frequency, maximum frequency, frequency range, call duration and number of inflection points) according to the number of dolphin-watching tour boats. The number of boats were divided in four class categories (i.e., zero, one, 2-3 boats, and ≥4 tour boats present) in order to obtain a more representative number of observations in each group. For significant Kruskall-Wallis results, a pairwise comparisons using Conover test was performed to compare the acoustic parameter with between the four classes of dolphin-watching tour boat number.

All statistical analyses were performed using software RStudio, Inc. (R Core Team, 2020).

2.3. Results

From a total of 15h of acoustic records, 5h were effectively selected for analysis resulting in 149 acoustic samples. A total of 103 recordings were made for the common dolphin and 46 for the bottlenose dolphin, whose collection sites are represented on the map with green and red points respectively (Figure 2.3.).

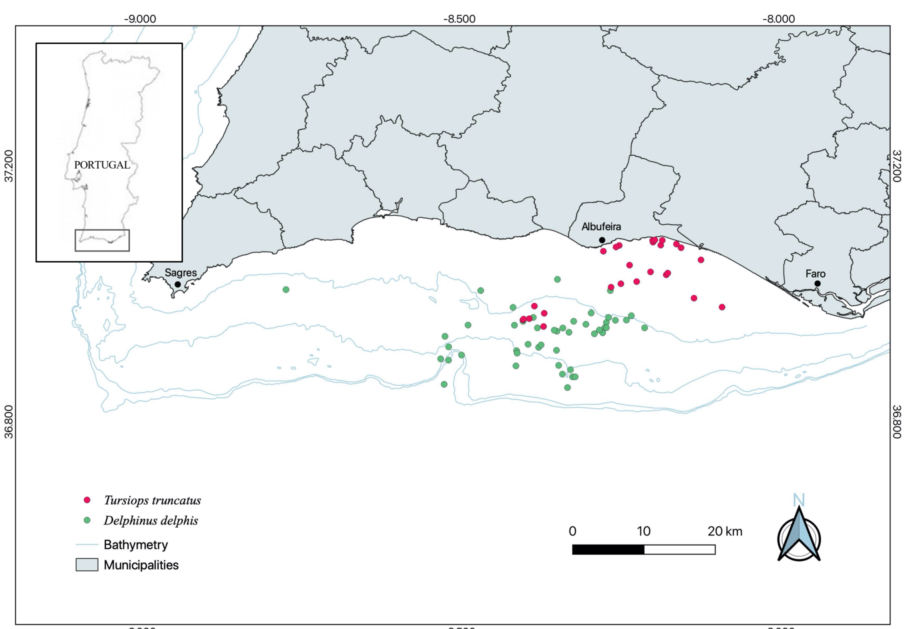

Figure 2.3. Map of study area in the coastal area of the western Algarve coast, Portugal, with the locations of the acoustic data collection (red dots:*Tursiops truncatus, green dots: Delphinus delphis*).

From the data collected by the CTD, 8 profiles were analysed. Oscillations in the speed of sound (1500-1530 m/s) were detected between the different areas where the data were collected in the thermocline in the first meters of the water column.

The rate of whistled (whistles/min/group size) varied between species and differed depending on number of dolphing-watching boats (Figure 2.4.). Bottlenose dolphins produce more whistles than the common dolphin and overall the whistle production decrease with increasing number of dolphin-watching boats for both species.

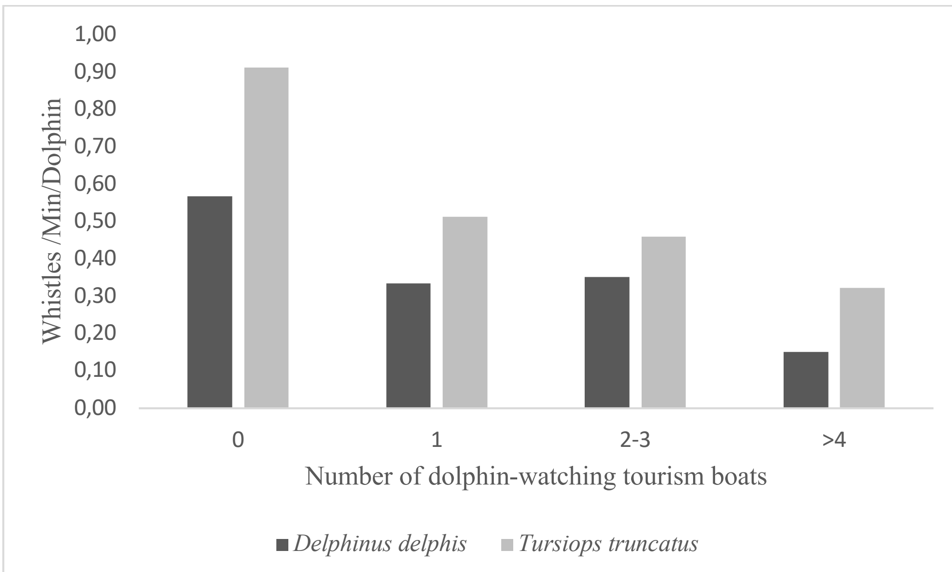

Figure 2.4. *The average number of whistles produced per minute per dolphin for common dolphin (Delphinus delphis) and bottlenose dolphin (Tursiops truncatus) in the absence and presence of tour boats.*

Based on recording quality and signal-to-noise ratio a total of 1239 whistles were selected for analysis (Table 3.1., 3.2. and 3.3.). For Common dolphin, the Kruskal-Wallis test showed statistically significant differences for the start frequency (chi-squared = 39.67, df = 3, p-value < 0.001). Conover's test revealed significant differences between whistles start frequency in the absence of boats compared to the start frequency of whistles in the presence of "1" boat, "1" boat and "2-3" boats, and "1" boat and "> 4 boats" (Fig. 2.5.a).

Significant differences among dolphin-watching tourism boats categories were also found for low and high frequency (chi-squared = 28.463, df = 3, p-value = < 0.001; chi-squared = 67.261, df = 3, p-value = < 0.001, respectively). Conover's test revealed significant differences in the low frequency of whistles between "1" boat and "2-3" boats, no boats and both "2-3" boats and ">4" boats, and "1" boat and ">4" boats. High frequency shows significant differences between

"0" boats and "1" boat, "1" boat and "2-3" boats, "2-3" boats and ">4" boats, and "0" boat and ">4" boats (Fig. 2.5.).

Table 3.1. *Mean values for the different acoustic parameters of the whistles for the Common dolphin (Delphinus delphis) and Bottlenose dolphin (Tursiops truncates) in the presence/absence of boats. A – absence of tour boats; P – presence of at leat one tour boat; IP – inflection point; N – total number of observations*

Species	Call duration (s)		Start frequency (kHz)		End frequency (kHz)		Low frequency (kHz)		High frequency (kHz)		Delta frequency (kHz)		IP		N
	A	P	A	P	A	P	A	P	A	P	A	P	A	P	
Delphinus delphis	0.86	0.84	12.02	11.93	11.61	13.09	8.32	9.22	15.27	16.36	6.95	6.94	2,54	1,80	737
Tursiops truncatus	0.75	1.10	7.44	10.47	11.14	11.88	6.36	9.06	15.09	16.43	8.74	7.37	2,58	2,38	502

Table 3.2 *Mean values of whistles' acoustic parameters for common dolphin (Delphinus delphis), in the absence and in the presence of different number of boats. IP – inflection point; N – total number of observations*

Number of Boats	Call duration (s)	Start frequency (Hz)	End frequency (Hz)	Low frequency (Hz)	High frequency (Hz)	Delta frequency (Hz)	IP	N
0	0.75	7.44	11.14	6.36	15.10	8.74	2.58	221
1	0.99	7.83	11.35	6.76	16.08	9.33	3.02	140
2-3	0.70	12.77	14.44	11.90	17.42	5.52	1.59	37
>4	1.41	10.82	11.44	9.28	16.45	7.17	2.81	104

Table 3.3 *Mean values of whistles' acoustic parameters for bottlenose dolphin (Tursiops truncatus), in the absence and in the presence of different number of boats. IP – inflection point; N – total number of observations*

Number of Boats	Call duration (s)	Start frequency (kHz)	End frequency (kHz)	Low frequency (kHz)	High frequency (kHz)	Delta frequency (kHz)	IP	N
0	0.86	12.02	11.61	8.32	15.27	6.95	2.54	340
1	0.76	13.12	11.53	8.99	15.20	6.21	1.92	297
2-3	0.67	12.25	12.86	9.45	15.59	6.14	1.81	71
>4	0.97	11.29	14.25	9.44	17.63	7.70	1.50	29

Significant differences were also found in the inflection points of whistles (chi-squared = 32.467, df = 3, p-value = < 0.001), between absence and presence of different number of dolphin-watching tour boats. Conover's test revealed significant differences in the inflection points between "0" boats and "2-3" boats, "0" boats and ">4" boats, "1" boats and "2-3" boats and, "1" boat and ">4" boats (Fig. 2.5.f).

No statistically significant differences were observed in the end frequency of whistles and their duration (delta time) between presence and absence of tour boats for the common dolphin (Fig. 2.5.b and e).

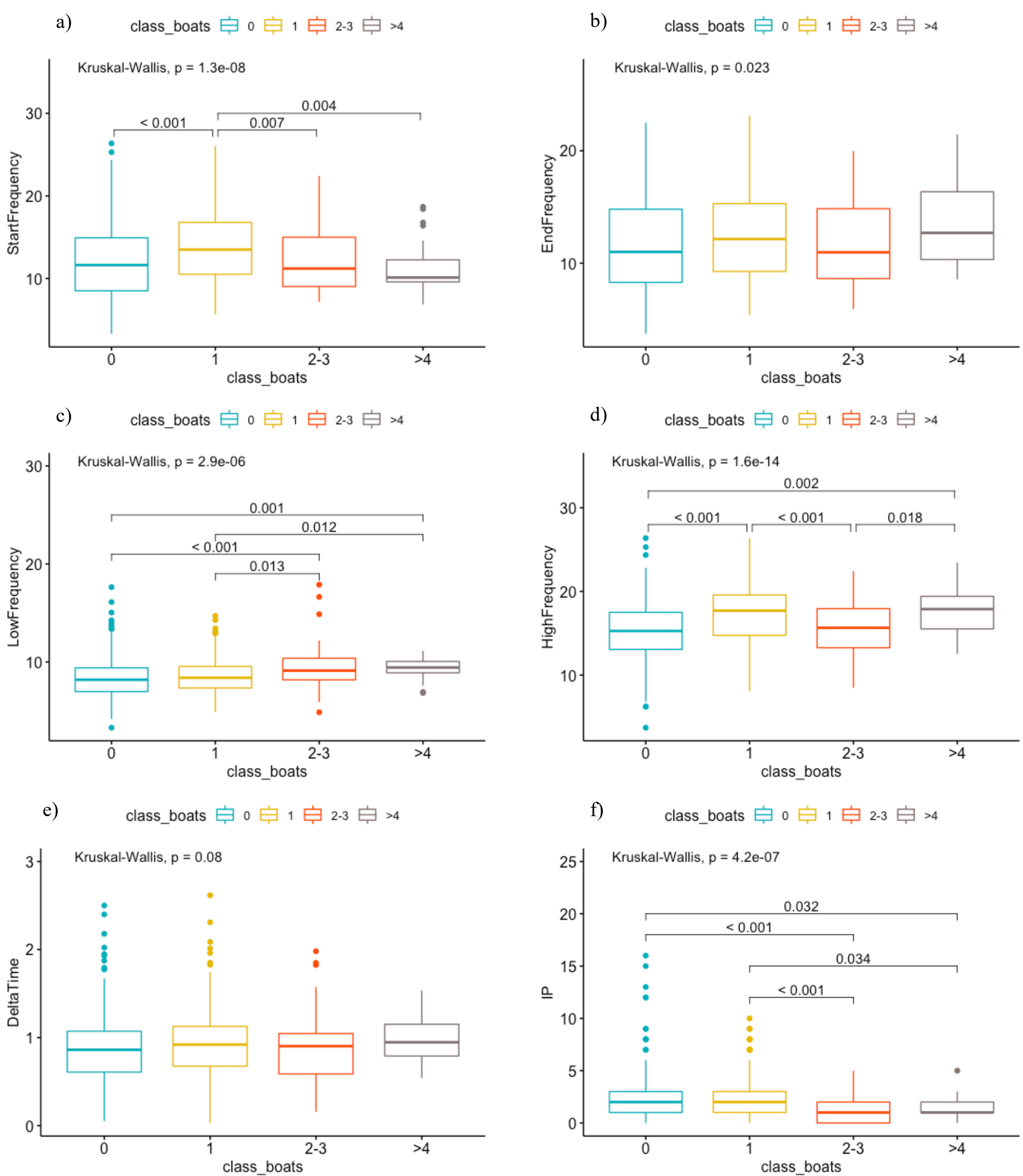

Figure 2.5. *Distribution of (a) start frequency, (b) end frequency, (c) low frequency, (d) high frequency, (e) delta time and (f) inflection points, of common dolphin (Delphinus delphis), in the absence of tour boats and presence of "1", "2-3" or >4 boats. The horizontal line in the boxplots represents the median; the lower and the upper limits of the boxplot are the first and third quartiles. Whiskers show the minimum and maximum values and outliers (i.e., values within 1.5 times of the interquartile range) are represented by dots. The value of the Kruskal-Wallis test is identified in the top of each figure and the significant differences between boat classes according to the Conover test are highlighted with the brackets.*

Regarding Bottlenose dolphin, the non-parametric test Kruskal-Wallis also showed statistically significant differences for the start frequency (chi-squared = 38.775, df = 3, p-value = < 0.001). Conover's test revealed significant differences between "0" boats and "2-3" boat, "1" boat and ">4" boats, and "0" boat and "> 4 boats" (Fig.2.6.).

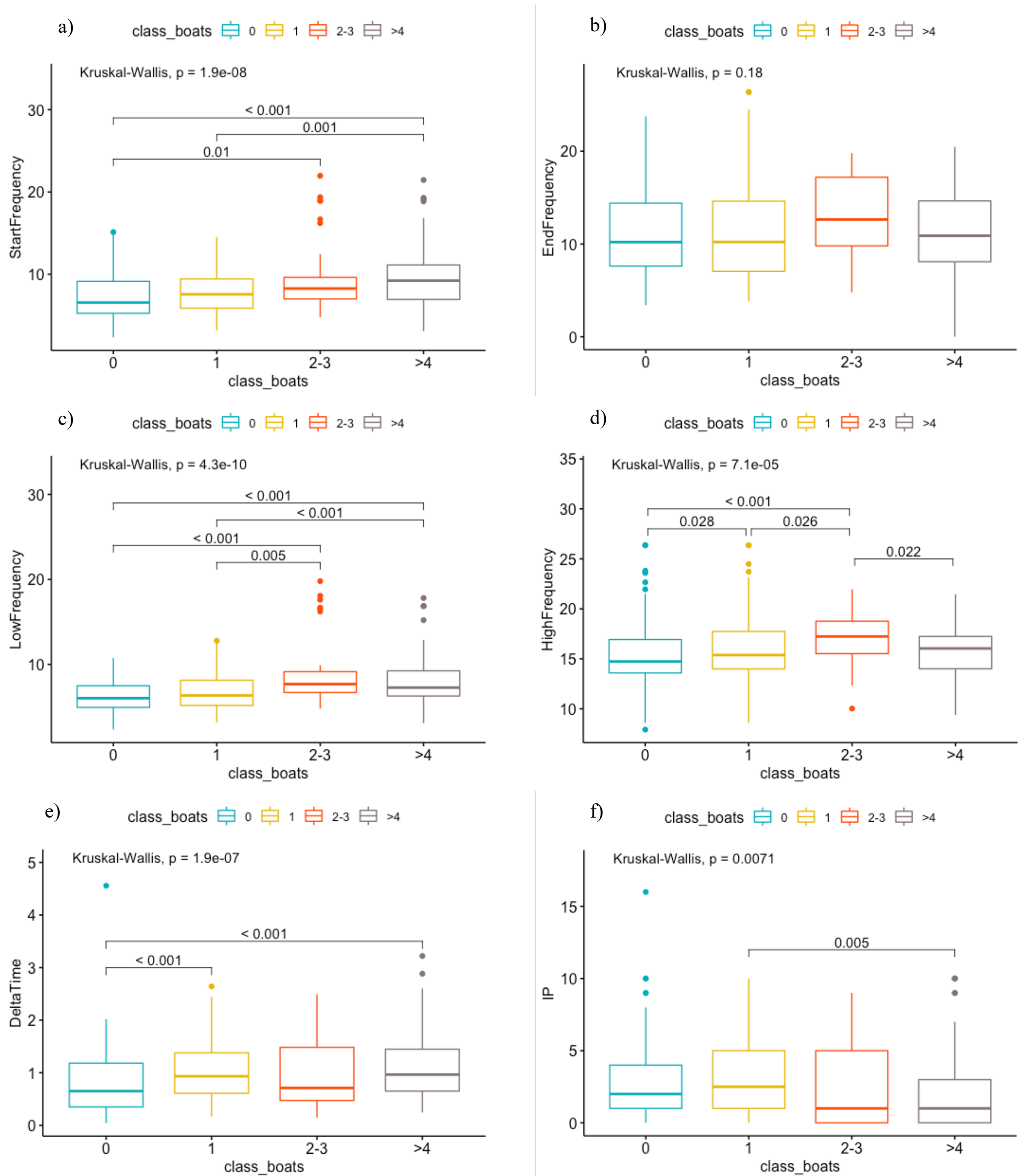

__Figure 2.6.__ Distribution of (a) start frequency, (b) end frequency, (c) low frequency, (d) high frequency, (e) delta time and (f) inflection points, of bottlenose dolphin (Tursiops truncatus), in the absence of tour boats and presence of "1", "2-3" or >4 boats. The horizontal line in the boxplots represents the median; the lower and the upper limits of the boxplot are the first and third quartiles. Whiskers show the minimum and maximum values and outliers (i.e., values within 1.5 times of the interquartile range) are represented by dots. The value of the Kruskal-Wallis test is identified in the top of each figure and the significant differences between boat classes according to the Conover test are highlighted with the brackets.

Significant differences among dolphin-watching tourism boats categories were also found for low and high frequency (chi-squared = 46.561, df = 3, p-value = 4.31e-10; chi-squared = 21.831, df = 3, p-value = 7.074e-05). Conover's test revealed significant differences in the low frequency, between "1" boat and "2-3" boats, "0" boats and "2-3" boats, "1" boats and ">4" boats, and "0" boat and ">4" boats. High frequency shows significant differences between "0" boats and "1" boat, "1" boat and "2-3" boats, "2-3" boats and ">4" boats, and "0" boat and "2-3" boats (Fig. 2.6.).Significant differences were also found in the inflection points (chi-squared = 32.467, df = 3, p-value = 4.173e-07), between absence and presence of different number of dolphin-watching tour boats. Conover's test revealed significant differences in the inflection points between "0" boats and "2-3" boats, "0" boats and ">4" boats, "1" boats and "2-3" boats and, "1" boat and ">4" boats (Fig.2.6.).In Bottlenose dolphin, significant differences were found also in delta time (chi-squared = 34.056, df = 3, p-value = 1.928e-07), between "0" and "1" boat, and "0" and ">4" boats (Fig.2.6.). No statistically significant differences were observed in the end frequency of whistles between presence and absence of tour boats for the common dolphin (Fig. 2.6. b).

2.4. Discussion

Temperature time series at different depths evidence oscillation between 17 °C and 19 °C in the thermocline layer between 2m and 16m, depending on the day, which are responsible for the modification of the vertical structure of sound speed profile. It is perfect visible that sound speed change together with the temperature. These changes can modify the sound propagation in the area between the source and the receiving array, and, in turn, are able to induce fluctuations in the received acoustic signals (Palmese *et al.* 2002). However, the amplitude associated to these oscillations may be a result of the depths at which the CTD was deployed, not being relevant for this study.

In this study, the acoustic behaviour of common dolphin and bottlenose dolphin was sampled in the presence and absence of dolphin-watching tourism boats, within 300-m radio in the Algarve region. Adjustments in acoustic behavior in the presence of dolphin-watching tour boats have been reported in several species of animals, including dolphins, in order to optimize signal transmission (Buckstaff 2004, Holt *et al.* 2009, Luís *et al.* 2014, Perez-Ortega *et al.* 2021, Scarpaci *et al.* 2000). This study shows that the number of dolphin-watching tourism boats significantly affects the whistle acoustic structure in both the common dolphin and bottlenose dolphin. Overall, both species vocalized with significantly higher low and high frequency in the presence of one or more tour boats. These results are in agreement with Perez-Ortega *et al.* (2021), were bottlenose dolphins in Dolphin bay, Panama, increased whistles frequency in ∼2–4 kHz in the presence of dolphin-watching tourism boats. Another study in Walvis Bay, Nambia, shows that bottlenose dolphins show an increase of 1.99 kHz in several whistle frequency variables, in the presence of tour-boats compared to the research boat (Heiler *et al.* 2016). In Portugal, studies carried out on bottlenose dolphins resident in the Sado estuary observed significant changes in high frequency between the dolphin-watching boats (15.33 kHz) and the trawlers (12.46 kHz) (Luís *et al.* 2014). This difference may be related to the proximity to which this type of vessel arrives from groups of dolphins.

Significant changes were also observed for the number of inflection points. Regarding common dolphin, there are no significant differences in the inflection points between “0” boats and “1” boat however, as more boats approach, the inflection points decrease significantly. Our finding contradicts results from Esch *et al.* (2009) which showed that the number of inflection points in dolphin whistles increased during stressful circumstances. Another theory from the same author is that increases in whistle or inflection points production could reflect an increased motivation to communicate rather than an increased stress level, while a decrease could result from fatigue. The increasing pressure of tour boats over the dolphin groups target may conduct to the animals exhaustion, forcing them to reduce the number of inflection points per whistles and whistle rates. However, in Esch *et al.* 2009 research they focused only on signature whistles, while in this study the whole whistle repertoire was analyzed.

Bottlenose dolphins also show a significant increase in delta time in the presence of boats, resulting in longer signals. Increases in delta time in the presence of boats have been reported for several delphinid species in different studies (Bittencourt *et al.* 2016; Guerra *et al.* 2014, La Manna *et al.* 2013) while in others no significant different were detected (Buckstaff 2004, Luís *et al.* 2014). Extending the call duration is a mechanism used by animals to increase the probability of detection in high noise conditions (Brumm *et al.* 2004).

Changes in whistle frequency are a common short-term response of marine mammals to noise in order to increase signal detection or compensate for masking effects (Esch *et al.* 2009, Luís *et al.* 2014, Perez-Ortega *et al.* 2021). The spectral overlapping of boat noise can lead to a reduction in the range at which dolphins' whistle can be heard by conspecifics, having the ability to mask the signals (Buckstaff 2004, Heiler *et al.* 2016). Hence, the increase in the delta time of the whistles observed in the bottlenose dolphins inhabiting the Algarve can be an attempt to overcome the impacts of tour boats water noise.

The vocal responses induced by the noise of maritime-tourist vessels have biological costs for dolphins. These costs include increased detection by competitors or predators, degradation of signal effectiveness in social contexts, as well as energy costs related to changes in metabolic demands or activity budgets, due to increases in the amplitude, duration, and/or repetition rate of acoustic signals (Holt *et al.* 2015). The results for the high frequency showed a significant increase with the increase in the number of tour boats. The higher frequencies could be the strategy that less energy demand from dolphins in response to dolphin-watching vessel noise (Heiler *et al*. 2016).

A study by Perez-Ortega *et al.* 2021 in the archipelago of Bocas del Toro on the Caribbean coast of Panama suggests as a mitigation strategy in order to reduce the impact of tourism boats on dolphins in the Bay of Dolphins, a reduction in the approach distance, the number of boats and contact time, as well as an increase in the time between interactions. The national guidelines for dolphin watching stipulate not more than three platforms being allowed in an area with a radius of 100 meters in the animals to avoid making noise and attract or disturb them. However, these rules are not met most of the time requiring therefore a stronger enforcement of the law by local authorities. Furthermore, if tour companies, reduce unnecessary underwater noise by switching boat engines off (whenever it is feasible and safe to do so) during encounters could lessen the overall impact of marine tourism on the dolphin community in southern Portugal.

However, additional research in acoustic behavior is required in order to improve the understanding of the causes of acoustic variability in these dolphin populations and to improve management of anthropogenic noise and dolphin-watching tourism boats traffic in southern Portugal.

Acknowledgments

These results could not have been possible without the collaboration of Associação para Investigação do Meio Marinho - AIMM. Heartfelt thanks are extended to all the AIMM volunteers and internships who gave generously of their time to help in field work.

The authors are also very grateful for the constant support of Ricardo Jesus Duarte throughout this study.

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APPENDIX

APPENDIX I. - Example of an Acoustic Record Sheet

Acoustic record sheet

NAME________________

DATA _________

CTD
Start time: Lat:
End time: Log:

	Sample	Hydrophone		Lat	Long	Prof (m)	Dolphins Distance (from hyd)	Boats Distance (From dolphins)	Nº boats	Tip. boats	Species	Group size		Dominant activity	Group Composition	Noisy leap	Notes
		Start time	End time									I	F				
1																	
2																	
3																	
4																	
5																	
6																	
7																	
8																	
9																	
10																	

Typology boats
1 - semi-rigid. **2** – Catamaran. **3** – Fibreglass boat
Size: >10 , <10.
Type of propulsion: motor **(M)** , sail boat (**V**)

*Boat distance from de dolphins to considerate- maxim 300 m

Group Size: Inicial (**I**), Final (**F**)

Group composition
Adults (**A**), Juvenils (**J**), Calves(**C**)

Dominant activity: Foragingn **(F)** Resting **(R)**
Socialising **(S)** Travelling **(T)**

APPENDIX II. - Experimental Test Plan

Experimental Test Plan

Ana Alves da Silva (a72612)

Title: 'Influence of dolphin-watching tourism vessels on the whistle emission pattern of common dolphins and bottlenose dolphins.')
Period covered: June- August 2022
Test location: Marina of Albufeira, Algarve, Portugal
Intern supervisor: Professor Doutor Sérgio Manuel Machado Jesus (UALG)
Extern supervisor: Doutor Fábio Matos (AIMM)
Participating Institutions: UALG, AIMM

1. Objectives & Relevance

The impact of underwater noise of anthropogenic origin on marine mammals has generated a growing concern worldwide. For marine mammals, acoustic communication is a vital mechanism for environmental perception, navigation, detection of predators and prey, as well as communication with conspecifics. Factors that interfere with these functions may have negative effects on the well-being and survival of these animals.
The Algarve coast has been a temporary visiting area for populations of marine mammals. The objective of this work will be to evaluate the potential impacts of the traffic of cetacean observation vessels on the whistle emission patterns.

Main objectives:

4. Assess how the underwater acoustic emissions of common dolphin and/or bottlenose dolphin changes depending on the presence or absence of dolphin watching tourism vessels through the whistle emission patterns.
5. Verify whether it is possible to propose measures to reduce the negative impact of maritime traffic on the population of cetaceans in the Algarve.

Specific objectives:

- Quantitatively analyse the different types of whistles recorded, as well as their patterns of occurrence.
- Acoustically characterize the whistles produced by dolphins in the Algarve region.
- Acoustically compare the whistles emitted by dolphins when interacting with vessels and in the absence of vessels.
- Study the influence of the presence of vessels on the emission patterns of whistles of dolphins in the Algarve.

- Advance recommendations that may contribute to mitigating the impacts of maritime traffic on the dolphin population in the Algarve.

2. Experimental Site

2.1 Area & Duration

The experiment will take place, from June to August 2022, in the coastal strip of the western Algarve coast extending from Albufeira to Armação de Pêra. (Fig. 1) Every summer, the population of the southern region of Portugal (Algarve) triples, due to the thousands of tourists who choose this holiday destination. This population increase leads to greater coastal pressure in the larger cities, in particular in Albufeira.

Figure 2.1 Location of the site of the experiment.

2.2 Environmental

The Algarve has a heterogeneous coastline that is characterized by rock formations of various types (e.g., boulders, low relief rocky areas, submerged rocky bottoms) and different sediment dynamics (Cúrdia et al., 2013).
The average sea temperature in Albufeira is 17ºC, while the outside temperature varies between 19ºC and 26ºC (Fig.2.2).
The first day of sampling show high tide at 15:50 and low tide at 09:30 with wave direction W (270º)

Updated: 13.6. 22:02 WEST	We 15. 09h	We 15. 11h	We 15. 13h	We 15. 15h	We 15. 17h	We 15. 19h
Wind speed (knots)	6	7	7	12	10	11
Wind gusts (knots)	8	9	8	16	15	18
Wind direction (→)	→	↖	↖	↗	↗	→
Wave (m)	0.5	0.5	0.5	0.8	0.9	0.8
Wave period (s)	9	6	4	4	4	4
Wave direction (→)	→	→	→	→	→	→
Temperature (°C)	23	24	26	23	23	22
Cloud cover (%) high / mid / low	42	47	87	100	96	76
	36	65	71	93	88	88
*Precip. (mm/1h)					0.1	0.1
Windguru rating				★		★
Tide	09:30			15:50		

Figure 2.2 Wind and wave forecast for the experimental 24 hours (Source: Windguru)

2.3 Marine Life & Noise

At least eight species of cetaceans have been reported in these waters, with the most sighted being the common dolphin, followed by the bottlenose dolphin, which has highlighted the importance of a better knowledge of these species.
This area was selected because it is the most intensively used by dolphin-watching tourism vessels. Algarve suffer a significant increase in number of boats operating coastal waters, 49 tourism companies are currently licensed to operate whale watching vessels in the South Coast of Portugal and most commercial dolphin-watching tourism vessels depart from Marina of Albufeira, what can be an alarming situation when we talk about sensitive species such as the cetaceans.

3. Work Strategy & Equipment

3.1 Equipment/ Requirements

The follow materials are required for the experiment:

- Research vessel named Ketos with 6,70m in length and has a 135hp Honda engine. It has the capacity to take up to 10 researchers on board, is equipped with a GPS, EPIRB, VHF radio, 220V jack cold box and plank on the bow (Fig.3.1).

Figure 3.1 Research vessel

- One computer, for CTD configuration, data processing and analysis
- Technical devices:
  - DigitalHyd TP-1/4Ch Self Recording Digital Hydrophone, a 4 channel underwater acoustic signal acquisition device. The device is composed of an acquisition unit which includes an underwater connector for telemetry and power and a total of 4 hydrophones with individual cable length of 2m (Fig.3.2)

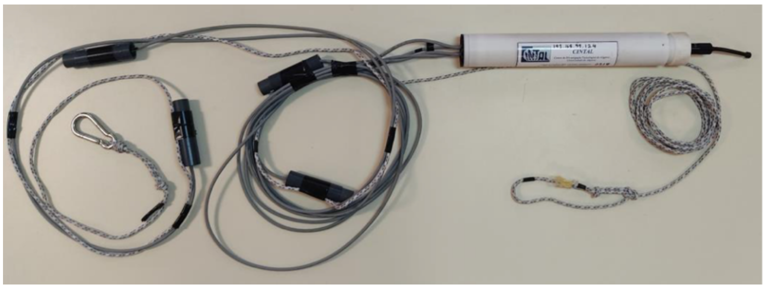


Figure 3.2 DigitalHyd TP-1/4Ch

- CTD: CTD Ruskin Concerto, a watertight cylindrical tube used in underwater measurements to evaluate the water conductivity, temperature, and depth.

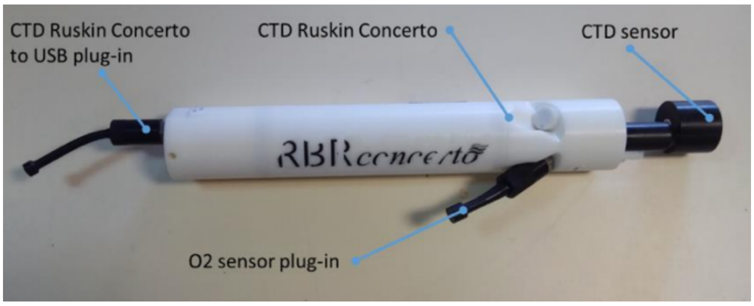


Figure 3.3 CTD Ruskin Concerto part description.

  - Batteries (6)
  - Batteries charger (1)
  - Memory card (1)
  - Memory SD card
  - Connection cable
- Nikon D5000 camera, with 55-200mm lens
- Gopro Black7
- Miscellaneous
  - Waterproof tape
  - Weights for hydrophone and CTD (≈2kg+ 2kg)
  - Buoy
  - Rope
  - Clamps
  - Towel

Figure 3.4 Extra material used do deploy the device

4. Proposed experimental set-up

DigitalHyd TP-1/4Ch

Connect a weight to the cable linked with the TP1-4 hydrophones. Then connect the buoy on the opposite site, to the TP1-4 hydrophone container. Finally, connect a safety cable between the buoy and the boat (figure 4.1).

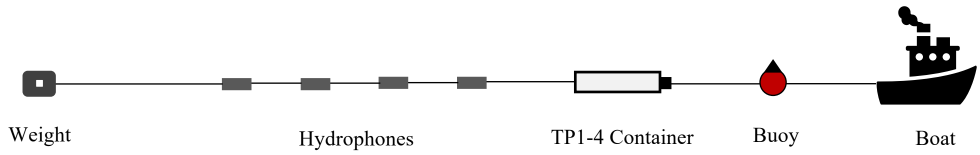
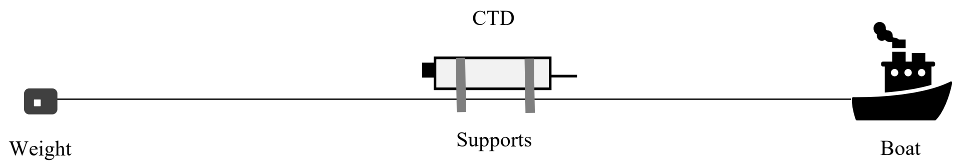


Figure 4.1 Deployment scheme.

CTD

Connect the supports to the CTD and a cable along the supports with a weight at the end of the cable on the sensor side. The opposite side will be connected to the boat.
The rope is marked every 2 meters with black waterproof tape. Place the CTD on the mark between the 4th and 6th meter.



5. Responsibilities

Field trips will be carried out between June and September along the coast of Albufeira, with the help of observers (AIMM Team) and a Skipper (Bruno), with the tasks of acoustic, behavioural, and photographic recording. Sampling will be carried out from a vessel, with the engines off.
In each sampling, the hydrophone will be placed at a depth of 2m below the surface of the water. Acoustic recordings will be performed with a resolution of 24 bit and with a sampling frequency of 50 kHz. The parameters recommended by the manufacturer of the digital recorder will always be used in order to obtain greater sensitivity.
Recording will be continuous, and files will be created every 2 minutes.

Work plan (general)

Proposal for an operations plan, weather conditions and equipment permitting.

Day	Hour	Task	Responsible
Monday		Check the material	Ana
Tuesday to Thursday	8am 9am 9.30am 4pm	Leave Faro Meet in Marina de Albufeira Go to the sea Comeback to the marina	Ana AIMM +Ana AIMM +Ana AIMM +Ana
Friday		Download and analyse data	Ana

Specific tasks before the boat:

Step 1 – arrival in Albufeira marina and meet with the rest of the team
Step 2 – connect hydrophone and CTD
Step 3 – isolate hydrophone and CTD with tape and place weights and buoy.
Step 4 - put the equipment in the container and transport to the boat.

Specific tasks on the boat:

Step 1- Spot dolphins
Step 2 - Whenever a group of dolphins is spotted, we check the behaviour of the group to see if they are traveling or not.
Step 3 – If they are traveling, we must see the direction of the animals and go with the boat forward, so that we can record longer.
Step 4 – In any of the situations (travelling or not) we have to turn off the motor of the boat before deploying the hydrophone and CTD (CTD will be deploy just ones a day).
Step 5 - deploy the hydrophone
Step 6 – While the hydrophone is recording CTD will be deploy for 1 min (just the 1st time of hydrophone deploying)
Step 7 – Notes must be taken on the logbook and acoustic record sheet during the recordings.
Step 8 – Whenever the animals leave, the hydrophone will be recollected before the motor is turn on.
Step 9 - Throughout the process, photographs will be taken by AIMM volunteers in order to provide photographic support to the project.

Specific tasks after the boat:

Step 1 – material will be taken out of the boat.
Step 2 – Hydrophone and CTD must be wash with fresh water and dried with a towel before open.
Step 3 – After washed and dried hydrophone must be open in order to be disconnected. (Ricardo)
Step 4 – After washed and dried CTD must be connected to the computer in order to stop recording. Data must be downloaded to the computer. (Ana)
Step 5 – Hydrophone and CTD must be kept in their respective boxes.

ANEXO I

tasks before the boat:

Step 1	Meeting at marina of Albufeira	All team
Step 2	Connect hydrophone and CTD	Ana
Step 3	isolate hydrophone and CTD	Ana
Step 4	Transport hydrophone and CTD to the boat	Ana + Volunteer 1

tasks on the boat:

Step 1	Spot dolphins	All team
Step 2	Check the behaviour of the group	AIMM team
Step 3	Deploy the hydrophone	Ana + Volunteer 1
Step 4	Deploy CTD	Volunteer 2
Step 5	Take notes on the logbook during the recordings/acoustic record sheet	Volunteer 3+4
Step 6	Recollect hydrophone	Ana + Volunteer 1
Step 7	Take photography's	AIMM volunteers

tasks after the boat:

Step 1	Take material out of the boat	Ana + Volunteer 1
Step 2	Wash hydrophone and CTD with fresh water and dry	Ana + Volunteer 1
Step 3	Disconnect hydrophone	Ana
Step 4	Disconect CTD	Ana
Step 5	Put hydrophone and CTD in their respective boxes	Ana

Tasks not assigned in this plan will be assigned on the first field trip. The task assigned are specific for the first day and will be adapted on the followed days according to the staff.

Notes: bring comfortable clothes, windproof jacket, sunscreen, water, lunch, and snacks.

Bibliography

Cúrdia, J., Monteiro, P., Afonso, C.M.L., Santos, M.N., Cunha, M.R. Gonçalves, J.M.S. (2013). **Spatial and depth-associated distribution patterns of shallow gorgonians in the Algarve coast (Portugal, NE Atlantic).** Helgoland Marine Research, 67, 521–534.

Table 1. Definitions of behavioural states for *Delphinus delphis* and *Tursiops truncatus*.

Behavioural State	Definition
Foraging	Searching for or consuming prey, as indicated by long, deep dives followed by loud forceful exhalations ("chuffs") and directionless movement; may include coordinated "burst swims" (rapid bursts of speed), "clean" noiseless headfirst re-entry leaps, coordinated clean leaps and tail slaps
Resting	Slow directionless movement at speeds of < 3 knots close to the surface with low activity level; often includes slow surfacing and floating near the surface
Socialising	Interacting with each other or inanimate objects; usually directionless movement and may include body and pectoral fin rubbing, rolling, belly-up swimming, spyhops, splashing at the surface, chasing, leaping, mating and playing with seaweed
Travelling slow	Steady movement in one direction at speeds of < 3 knots
Travelling average	Steady movement in one direction at speeds of 3 to 5 knots
Travelling fast	Steady movement in one direction at speeds of > 5 knots

Anexo III. – Results of CTD parameters, speed of sound (m/s) in yellow, salinity (PSU) in blue, depth (m) in pink, temperature in black (ºC).