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THE IMPACT OF TOURISTIC WHALE-WATCHING ON *DELPHINUS DELPHIS* AND *TURSIOPS TRUNCATUS* ON THE ALGARVE COAST:

COMBINING ACOUSTIC ANALYSIS AND LAND OBSERVATIONS

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Faculdade de Ciências e Tecnologia

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Mestrado em Biologia Marinha

Trabalho efetuado sob a orientação de:

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Declaração de autoria de trabalho

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

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Resumo

A observação turística de cetáceos (WW) é uma atividade que tem crescido mundialmente nos últimos 50 anos. Esta atividade é comumente incluída no ramo do ecoturismo e considerada como uma alternativa sustentável à caça de baleias. É uma atividade com potencial para trazer benefícios socioeconómicos, promover educação ambiental e contribuir para a investigação. No entanto, diversos estudos apontam para alterações comportamentais e fisiológicas a curto prazo nos cetáceos aquando da presença destas embarcações. Estes possíveis impactos da atividade sobre as espécies têm levantado um grande interesse da comunidade científica. Apesar de ser difícil extrapolar alterações de curto prazo para efeitos populacionais a longo prazo, pode-se prever que efeitos a nível populacional aconteçam se atividades biologicamente relevantes como alimentação, reprodução e repouso sejam afetados adversamente. Diferentes populações são afetadas de formas distintas que dependem de características do local onde vivem, da dinâmica populacional, do tipo e da intensidade do WW praticado e da presença de outras pressões ecológicas. Por exemplo, populações pequenas que habitem águas costeiras, lagunares ou em rios são mais afetadas do que populações grandes que habitem águas oceânicas em que cada grupo tem menos probabilidade de encontrar embarcações. A existência de outros impactos antropogênicos como a introdução de toxinas no ambiente ou o declínio de presas também influenciam o nível de WW que uma população consegue tolerar. Deste modo, é necessário estudar os efeitos desta atividade em diferentes espécies e populações sob diferentes circunstâncias, e enveredar em esforços que devem ter em conta as características específicas de cada caso. Em Portugal continental a maior parte das operações estão concentradas na costa sul (Algarve). Mas apesar do grande crescimento no número de empresas, nas últimas duas décadas, estudos sobre os possíveis impactos desta atividade nas populações de cetáceos na região são escassos.

O golfinho-comum-de-bico-curto (*Delphinus delphis*) e o roaz-corvineiro (*Tursiops truncatus*) são as duas espécies mais comumente observadas na costa algarvia. Ambas as espécies são globais e abundantes com uma ampla distribuição nos oceanos Atlântico, Pacífico e Índico. O Algarve foi identificado como uma área importante para ambas as espécies funcionando como berçário, zona de reprodução e de alimentação. Colisões, alterações comportamentais (como diminuição em socialização, repouso, alimentação), alterações em dinâmicas e coesão dos grupos e alterações no comportamento acústico são alguns dos impactos causados

por WW nestas espécies. O objetivo do presente estudo foi analisar alterações no comportamento acústico do *D. delphis* e *T. truncatus* na presença de embarcações de WW e quantificar o tempo de exposição dos animais a este impacto antropogênico.

O ruído produzido por embarcações pode interferir com as vocalizações dos animais mascarando os sons que produzem e dificultando a transmissão de sinais entre os animais ou na procura das suas presas. Diversos estudos demostram alterações nos parâmetros das vocalizações de cetáceos possivelmente como um esforço exercido pelos animais para superar esta interferência. Dois tipos de sons produzidos pelos golfinhos foram analisados utilizando o software Spectralayers Pro 10; assobios e estalidos. Assobios são utilizados pelos animais para comunicação, enquanto estalidos são utilizados para a ecolocalização e associados à alimentação e/ou localização das presas. Foram recolhidos 267 minutos de áudio recolhido a bordo de embarcações de WW, resultando em 2442 e 1418 assobios para *D. delphis* e *T. truncatus*, respectivamente. Foram individualmente analisados os parâmetros acústicos: frequência mínima (kHz), frequência máxima (kHz), amplitude de frequência (kHz), duração (s), inflecções e degraus, taxa de assobios por minuto e classificação por tipos de assobios. A taxa de estalidos por minuto foi avaliada para inferir impactos na alimentação. A espécie *D. delphis* apresentou alterações em cinco dos seis parâmetros acústicos, taxa de assobios por minuto e classificação por tipos, mas não apresentou diferenças significativas na ecolocalização. Na presença de barcos os assobios de *D. delphis* tiveram frequências (kHz) e durações reduzidas, taxa por minuto aumentada e características de um contorno simplificado que indicam um esforço para manter a comunicação em um ambiente acústico poluído. A espécie *T. truncatus*, por outro lado, apenas apresentou alterações em três dos parâmetros acústicos no que diz respeito aos assobios, com a mesma característica de simplificação e diminuição na frequência (kHz), e apresentou uma redução significativa na taxa de ecolocalização por minuto. Esses resultados sugerem que o *D. delphis* é mais afetado por embarcações de WW durante momentos de socialização enquanto *T. truncatus* é mais afetado durante alimentação. Ambas as alterações têm potencial para acarretar efeitos a nível populacional ao afetar o sucesso reprodutivo e a condição física.

Para determinar o tempo de exposição dos animais a este impacto, foram utilizadas observações a partir de terra, que funcionam como um método não invasivo e permitem a cobertura de uma vasta área de estudo. Foram utilizados cinco pontos de observação distribuídos pela costa Algarvia; Farol de Santa Maria, Farol da Alfanzina e Farol do Cabo São Vicente (onde as observações foram feitas do alto da torre dos faróis), Vilamoura e Albufeira

(onde as observações foram feitas nas falésias junto ao mar). Os pontos do Barlavento (Albufeira e Farol de Alfanzina) tiveram maior concentração de barcos e de infrações à legislação. No Barlavento os animais são expostos a embarcações até 39% das horas de luz, e em 67% deste tempo o número limite de embarcações é excedido. Isso significa que os animais podem passar até 21% das horas de luz na presença de mais de três barcos. Isto é deveras alarmante para a atividade na região, visto estudos anteriores demonstraram alterações em parâmetros acústicos consoante o aumento do número de embarcações presentes.

A disponibilidade das empresas de WW em contribuir com este e outros estudos na região, demostra preocupação e disponibilidade para cooperar na criação de um cenário mais sustentável. Já foi demostrado que os turistas que frequentam este tipo de atividade apresentam um nível de preocupação com o bem-estar dos animais e preferem operadores que zelam por uma conduta ecologicamente correta. Assim sendo, a criação de medidas para minimizar os impactos beneficiam não apenas os animais como a indústria em si. Contudo, a escassez de estudos, a regulamentação inadequada e a inconformidade por parte dos operadores tornam-se desafios na implementação de uma indústria sustentável no Algarve e ameaçam as populações de cetáceos na região. Os resultados aqui apresentados demostram a necessidade de mais investigação, um plano de monitorização e a implementação de regulamentações e códigos de conduta que levem em consideração as características individuais de cada espécie sujeita a observação turística.

Palavras-chave: *Tursiops truncatus*, *Delphinus delphis*, observação de cetáceos, comportamento acústico, observação a partir de terra

Abstract

Touristic whale watching (WW) is an important socioeconomic activity worldwide. Recently, short and long-term impacts caused by WW have been reported for several cetacean species, including *Delphinus delphis* and *Tursiops truncatus*, the two most observed species in Portugal. Most of the operations in mainland Portugal are concentrated on the South Coast (Algarve). However, despite their importance for the region, studies focused on the impact of this activity on the animals are still scarce. We used acoustic behaviour analysis coupled with land-based observations to determine the alterations in the vocalisation patterns of these animals caused by WW vessels and the length of exposure to this stressor. We found that the presence of WW leads to changes in five out of six acoustic parameters of whistles, in whistle rates, and in whistle type distribution for *D. delphis*, while for *T. truncatus,* only three acoustic parameters were affected. Conversely, *T. truncatus* significantly reduced echolocation click rates, while *D. delphis* did not show significant changes. We also found that the "Barlavento" area is the most affected, with animals exposed to WW vessels for up to 38.9% of daylight hours. Furthermore, 66.7% of the observation time in this area had several vessels that exceeded the limit established by law. These results suggest that our study species are impacted by WW, while *D. delphis* is more affected during social behaviours, and *T. truncatus* is more affected during foraging activities. Both behaviours are biologically significant, and their disturbance might, in the long term, lead to adverse effects at the population level by decreasing reproduction success and fitness. A gap in knowledge, inadequate legislation and compliance issues threaten the development of a sustainable WW industry in the Algarve and might endanger local populations, therefore needing immediate attention.

Keywords: *Tursiops truncatus*, *Delphinus delphis*, whale watching, acoustic behaviour, land-based observation

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1. Chapter 1: State of the Art

1.1. Whale Watching: History and Implications

The designation Whale Watching (WW) pertains to the commercial practice of observing cetacean species (whales, dolphins, and porpoises) in their natural environment (Hoyt, 2021). It is commonly recognised as a sustainable endeavour and integrated within ecotourism (Mustika et al., 2012; Suárez-Rojas et al., 2023). The first recorded WW activities date back to 1955 in San Diego, California, when tours to view grey whales (*Eschrichrius robustus*) were offered for 1 dollar by a local fisherman (Hoyt and Parsons, 2014). In 1975, a successful partnership between science, education, and commercial WW began in Provincetown, Massachusetts, eventually giving rise to the New England model of successful WW, teaching and research, which spread far and wide (Hoyt and Parsons, 2014). By the late 1980s, WW had gained international popularity, extending its reach to places historically engaged in whaling, such as Japan, Norway and the Azores Islands (Hoyt, 2021; Vieira et al., 2018). In contrast with consumptive whale hunting practices, WW was often promoted as a solution and sustainable alternative to the exploration of cetaceans (Mustika et al., 2012). By 2001, the total expenditures worldwide from WW activities were estimated to be 1.05 billion dollars that year alone (Hoyt, 2001). Seven years later, in 2008, they had already doubled, reaching 2.1 billion dollars and supporting about 13,000 jobs (O'Connor et al., 2009).

WW holds the potential to provide significant socioeconomic advantages while also serving as a tool for conservation efforts and fostering a perception of cetaceans as a more valuable resource, alive rather than dead (Parsons, 2012). WW tours can offer educational opportunities during encounters with cetaceans, which has been shown to trigger positive shifts in public perception regarding the marine environment and the necessity of cetacean conservation efforts (Cárdenas et al., 2021; García-Cegarra and Pacheco, 2017). The New England model consists of a partnership between the Center for Coastal Studies, which provides naturalist guides for the Dolphin Fleet, who present an informal educational lecture and answer questions while conducting their photo-ID research and collecting data (Hoyt and Parsons, 2014). As the public face of WW tours, the naturalists are central to education and can act as a bridge between the tourists and the ocean. On the other hand, the education of WW tourism operators, passengers, and recreational vessel operators who use the same waters as WW boats creates a framework for successfully managing this industry (Hoyt, 2021).

However, over time, as WW was transplanted further and further afield, the New England model, unfortunately, lost its impact; in many places, operators (sometimes with little or no knowledge of cetaceans) started using different kinds of boats and encountered new economic challenges with varying types of customers (Hoyt and Parsons, 2014). The rapid expansion of WW highlighted management challenges, particularly in areas with numerous operators offering frequent tours, overcrowding of boats in limited cetacean habitat, excessive close approaches, strain on community infrastructure, conflicts among tourism companies, ineffective regulations, and inadequate compliance with existing rules (Hoyt, 2021). In that context, several studies have tried to understand WW's dynamics, management and impact on the cetacean communities worldwide.

1.1.1. North America: The Southern Resident Killer Whales (*Orcinus orca*)

The USA, Canada, and Australia are the three major countries in the WW industry (O'Connor et al., 2009). The Salish Sea region of Washington State, U.S.A. and British Columbia, Canada, is home to the world's most studied resident orca population, the Southern Resident Killer Whales (SRKW), an endangered population subjected to WW activities and heavy vessel traffic (Lachmuth et al., 2011; Seely et al., 2017). The SRKW population declined from an estimated historical size of approximately 200 during the late 1800s to around 80 whales since 2005, with the lowest recorded population level at 67 in 1971. This is possibly due to the live capture of SRKWs for oceanarium display beginning in the late 1960s, causing an estimated 30% decrease in the population (Seely et al., 2017). Since the mid-1980s, this population has been the main attraction for a large international WW industry, drawing the attention of many stakeholders and increasing the demand for protective measures (Giles and Koski, 2012).

According to an early 2000s study, the orcas had vessels nearby for a large proportion of daylight hours; even with stewardship programs in place, over 20% of the time they had at least one vessel closer than the 100 meters allowed under the guidelines, and over 75% of the time they were within a quarter mile of boats (Bain et al., 2006). In 1990, the SRKW pods were reported to be accompanied by an average of four vessels at any time during summer daylight hours, a number which, by 1997, had grown to 25 vessels at one time (of which only ¼ were commercial WW vessels) (Baird, 1999). The SRKW was a promising case study for ascertaining the impact of WW vessels on cetaceans because every individual in the population has been identified and catalogued since 1973. There is much information about their diets, acoustics, movement patterns, social organisation, population genetics, survival and reproductive rates, and they are easily observable both by boats and from shore (Trites and Bain, 2000).

Several studies throughout the years have pointed to short-term changes in behaviour, such as a significant decrease in foraging and an increase in travelling with the presence of boats (e.g. Holt et al., 2021; Lusseau et al., 2009), habitat displacement (Morton, 2002), increase in surface active behaviour (Noren et al., 2009), changes in swimming speed (Bain et al., 2006). The behavioural patterns of orcas are altered due to the number and proximity of vessels (Trites and Bain, 2000). This leads to varying responses; when swimming in more open water, males and females employ different strategies to avoid vessels (Trites and Bain, 2000). Nonetheless, although mortality rates in the SRKW population have increased and long-term data is available, it is not possible to affirm that WW (at least alone) is the cause, given that the salmon stocks (their main summer prey) have also declined in the coastal waters of British Columbia and Washington and southern residents have very high toxin levels (Trites and Bain, 2000). Overall, changes in survival rates for this population are likely caused by a combination of reduced prey quantity and quality, disturbance by vessels and associated noise, and exposure to anthropogenic toxins (Giles and Koski, 2012).

Effective management must respond to the cumulative impact of these threats; however, Canada and the United States continue to approve projects without adequately assessing the cumulative transboundary impact or quantifying the project's contribution to critical ecosystem-based environmental thresholds (Jefferies et al., 2021). This is a cautionary tale for managing small, enclosed populations, even more so when further complications such as food limitations and international politics constitute a delicate dynamic that requires a holistic approach. Even with abundant data, successful management can be challenging to achieve.

1.1.2. Australia: Dolphins are the Main Stars

Australia is a hotspot for WW activities, attracting over 1.6 million participants per year, out of which more than 80% are present for dolphin-oriented tours (O'Connor et al., 2009). Many studies have pointed to possible negative impacts on the dolphin populations in the area (e.g. Amrein et al., 2020; Filby, 2016). Port Stephens, in Southeast Australia, harbours the largest dolphin-watching industry in the country, which targets a small, genetically distinct population of Indo-Pacific bottlenose dolphins (*Tursiops aduncus*) (Steckenreuter et al., 2012). For this population, type of boat, number, and distances affect behaviour states and change behavioural budgets; no resting, less feeding and socializing, and more milling and travelling were registered when WW boats were present within 100 m (Steckenreuter et al., 2012). In Bunbury, Western Australia, the presence of tour boats has been shown to affect the frequency and duration of the main behaviours and change the structure of bottlenose dolphins (*Tursiops truncatus*) groups (Arcangeli and Crosti, 2009).

Indo-Pacific bottlenose dolphins of Shark Bay, Australia are particularly fascinating due to data availability before the onset of touristic WW (Bejder et al., 2006). Bejder et al. (2006) analysed three consecutive periods of approximately 4.5 years each; when there were no WW vessels, there was one licensed WW vessel, and lastly, there were 2 WW vessels, with research activities remaining more or less constant. They found that there was no significant change in abundance between the period before the onset of WW tourism and the period with only one WW vessel in operation; once two boats were in operation, there was a significant decrease in abundance, equating to a decline of approximately one in every seven individuals (Bejder et al., 2006). This local decline could not be attributed to an overall population decline because an opposite trend occurred within the adjacent control site; neither could it be explained by ecological factors, which would be similar in contiguous sites (Bejder et al., 2006).

Cetaceans are legally protected in most (albeit not all) Australian coastal waters by vessel approach regulations based on the Australian National Guidelines for Whale and Dolphin Watching 2005 (and now 2017), which maintains uniformity in marine mammal regulations across Australian jurisdictions (Puszka et al., 2021). These regulations have proved effective to some extent. For example, the *Tursiops* sp. population of Port Philip Bay has different responses according to vessel approach; during legal approaches, the dolphins are more likely to approach the vessels, while during illegal approaches, the animals display more avoidance and evading behaviours (Filby, 2016). Still severe violations of the Wildlife (Marine Mammals) Regulations during summer months, along with significant changes in behaviour caused by both vessel interaction and violation, pose a high risk of maladaptive responses to vessel disturbance, potentially leading to long-term consequences for the survival of already threatened marine mammal populations (Puszka et al., 2021; Steckenreuter et al., 2012). A serious issue regarding legislation and rules of conduct compliance is that WW boats are not the only boats interacting with the animals. Allen et al. (2023) show that WW operators have a high level of compliance with codes of conduct regarding the number of boats; however, when non-WW vessels are involved, this compliance level drops as WW operators seem to regard the code of conduct as applicable only to the number of WW boats, not taking into account the presence of recreational vessels, which in turn seem to be unaware or ignore the codes of conduct. Another point is that even when legal observation durations are respected, staggered departure times, visits to multiple schools, returning to a previously approached school, and regular 'continued interactions' involving multiple boats means the dolphins are exposed to boats for up to several hours, thus while compliance is at times acceptable, the code of conduct is still not effective in protecting the populations (Allen et al., 2023).

This case is an excellent example of how highly touristic areas where large WW industries with several operators can develop face specific management challenges. Even with compliance and collaboration between WW companies, scientists, and government, the acknowledgement, inclusion, and education of the general public and parties that might not be direct stakeholders are crucial to achieving sustainability.

1.1.3. The Azores Islands: From Whaling to Whale-Watching

The Azores islands have a long history with cetaceans; shore-based whaling was practised by the locals from the 1850s until 1987; in this type of whaling, lookouts on shore would spot the whales and guide small open boats that undertook the hunt using hand-held harpoons and lances (Prieto et al., 2013). After whaling was officially banned in 1986, there was a shift towards WW incentivised by the International Fund for Animal Welfare (IFAW), the Azores Government and the European Community; some of the lookouts previously working for the whalers immediately joined the new business and were then followed by some whalers who became skippers, thus bringing their expertise and traditional ecological knowledge to the latest activity (González García et al., 2023). In October 1998, the First Azorean Biannual Conference for Whales and Dolphins was held in Lajes do Pico, a historical juncture that local whale-watching operators, government-administrative authorities, local university scientists, and the socioeconomic elite of Lajes conceived as the "moment of transition" from whale hunting to whale watching (Neves-Graca, 2004).

Consequently, the first WW legislation was published the following year (DLR 9/99/A) (González García et al., 2023). It favoured an ecologically friendly commercial model but not a precautionary approach, as the region was not recognised as a whale nursery area; however, former whale hunters and owners of two WW companies argued against the consensus at the conference and insisted that they were dealing with resident whales who mated, reproduced, and reared their offspring in the oceanic areas adjacent to Lajes and that the whales were already showing signs of stress (Neves-Graca, 2004). Those voices had some impact (although the Azores were still not recognised as a natural sanctuary for whales and dolphins). In the following years, regulations were successively revised in 2003, 2004 and 2005. They included guidelines on platforms, approach distances, angle of approach, duration of interaction, the maximum number of boats allowed per group of animals, a limited number of licenses and specifications on the maximum size of boats (Neves-Graca, 2004; Silva, 2015).

Nonetheless, divergent views on how to design and practice WW persisted. There was no agreement regarding the WW business model and whether it should remain low volume for elite consumption or pursue higher and more popular consumption (Silva, 2015). Some of the more concerned owners created their norms of WW conduct out of precautionary principles and a strong ethical commitment to the welfare of the animals (Neves-Graca, 2004). Discussions during the 2006 "Bienal das Baleias" led to the establishment of the MONICET project, a consortium comprising a research centre and three WW companies and supported financially by the Azores Government, whose objective was to devise a methodology for scientifically reliable data collection compatible with commercial operations and implementation of a database system that allows for open access data (González García et al., 2023).

This collaboration between the WW companies and research efforts, as well as the dissemination of open-access data, has allowed for the development of several studies that are helping expand the knowledge of the cetacean populations of the Azores (e.g. García et al., 2018; Zahn et al., 2022a, 2022b). When it comes to the impacts of WW activity itself, results have been conflicting; at times, no clear pattern of short-term reactions was found, while others identified significantly reduced foraging time coupled with increased high-energy activities and changes in resting and socializing behaviour (Visser et al., 2011). In all cases, the authors agree that it is challenging to extrapolate long-term effects from short-term observations, and a precautionary approach coupled with long-term population monitoring is advisable.

The Azores case is an example of a small-sized WW industry in which stakeholders have strived to create a sustainable business model that serves education, conservation, and research, albeit with internal conflict and discussible effectiveness. This goal has been achieved to some extent; still, concern over compliance with legislation and the impacts caused to the population is very much alive. It has been reported that swim-with-dolphins operators only partially comply with current regulations, with one of the main problems being the presence of more than two swimmers in the water at any given time (Barradell and Ritter, 2007; Cecchetti et al., 2019). An early 2000s study pointed out that only 46% of the boats entirely complied with regulations during approaches and manoeuvre around the animals (Magalhães et al., 2002). However, there is a lack of more recent studies on current compliance with the rules and the effect of these violations on the animals.

1.1.4. Impacts and Implications

Several studies have indicated short-term responses to WW disturbance, such as changes in behaviour (e.g. Arcangeli and Crosti, 2009; Cecchetti et al., 2018), group structure (e.g. Toro et al., 2021), vocalisations (e.g. Fouda et al., 2018; Van Ginkel et al., 2018), habitat displacements and boat avoidance (e.g. Bejder et al., 2006; Constantine, 2001), which can negatively impact the target species. The main concerns regarding the anthropogenic impact caused by WW are the disruption of vital socialising behaviours (including reproductive behaviour), foraging, and resting because the disturbance of these behaviours may significantly impact the population's health or vitality (Sitar and Parsons, 2015). Other activities that also fall into the WW umbrella can have more direct consequences; tourist feeding of lactating mothers has been associated with a high mortality rate of bottlenose dolphin (*Tursiops* spp.) calves as hand-fed mothers were found to neglect their calves, resulting in malnourishment and death by disease and predation (Mann et al., 2000). Long-term exposure to swim-with-dolphin activities has been shown to increase avoidance of swimmers, which in turn can cause displacement of feeding and resting areas (Constantine, 2001). Filby et al. (2014) suggested that even seemingly positive swim-with-dolphin encounters might have adverse long-term effects on the populations by detracting from biologically significant behaviours such as foraging, nursing, and resting.

It is difficult to determine the long-term adverse effects of short-term behavioural changes, if any (Parsons, 2012); however, some studies have tackled this question. A long-term decline in dolphin abundance of approximately one in every seven individuals in the tourism site has been shown for the Indo-Pacific bottlenose dolphins of Shark Bay, Australia (Bejder et al., 2006). Another example was a study in Fiordland, New Zealand, which showed reduced reproduction success and long-term area avoidance, resulting in habitat displacement of bottle-nose dolphins related to unsustainable dolphin-watching tourism (Lusseau et al., 2006). It is also essential to consider that contextual information is needed to define the biological relevance of any observed short-term effects (Lusseau and Bejder, 2007). Population ecology highly influences the disturbance level that can be tolerated in any given area; small and closed populations, unable to avoid the disturbance, are the most sensitive, while open populations can withstand a higher probability of interacting with WW vessels (New et al., 2020). Varying effects can be expected from different locations depending on the species, the population traits, and the WW style. Therefore, different management strategies are required to succeed. Where feasible, studying the relationships between population traits and disturbance intensity would allow for more reliable extrapolation in data-scarce situations, aiding in identifying and prioritising populations most in need of conservation and management action (New et al., 2020).

WW is a complex and dynamic tourist activity that still faces many challenges and unanswered questions about balancing human interests and conservation (Suárez-Rojas et al., 2023). While WW operations might potentially mitigate their impacts on cetaceans sufficiently to avoid lasting or excessively adverse effects, it could be argued that most operations globally fail to do so, harming cetacean populations internationally (Parsons, 2012). In Europe, commercial WW began in 1980 with dolphin-focused tourism in Gibraltar, subsequently expanding to the UK, Ireland, and France in the mid-1980s, where various resident bottlenose dolphin (*T. truncatus*) populations were readily accessible by boat and frequently observable from shore (Hoyt, 2021). Scotland, Iceland, Spain, Portugal, the Azores Islands, and the Madeira Archipelago witnessed notable growth in WW activity from 1998 to 2008 (O'Connor et al., 2009). Portugal (mainland, Madeira and the Azores islands) claimed approximately 23% of total revenues in this period – the most significant portion for Europe (O'Connor et al., 2009). Even though Portugal claims a substantial portion of the WW activities in Europe, very little is known about the impacts on the local mainland populations. In the present study, we aim to examine this issue in the South Coast of Portugal, known as the Algarve.

1.2. Dolphin Watching in the Algarve

In mainland Portugal, WW began in 1998 with companies distributed in Peniche, Nazaré, Setúbal (Sado estuary) and along the south coast in the Algarve, with departure points mainly in Lagos, Albufeira and Portimão (Gonçalves, 2012). The first company in the Algarve was created in 2000, and the number of operations has grown since (Claro, 2009). There are 90 tourism companies licensed for WW in mainland Portuguese waters, 52 of which operate off the Algarve (ICNF, 2024). The number of vessels in operation per company varies between 1 and 15, with 131 boats operating in the Algarve, each doing, on average, three daily trips (Alves da Silva, 2022).

WW activities, be it commercial, recreational, or scientific are regulated in Portugal by the *Decreto Lei n.º 9/2006 de 6 de Janeiro do ministério do ambiente, do ordenamento do território e do desenvolvimento regional.* (2006). WW vessels must be licensed, have proper authorisation, and follow the rules of conduct. Touristic operations must have a qualified person responsible for ensuring the quality of the environmental and educational program and for the systematic and adequate collection of data. Additionally, a guide must be on-board to educate the public about the cetaceans and the region's nature, history, and culture. The rules of conduct for observation state the following:

- The active approach at less than 30 m is prohibited from any cetacean.
- The vessels must stay parallel to or behind the animals, always having a free area of 180º to the front.
- The permanence of more than three vessels within a 100 m radius of the animals is prohibited.
- The vessels must stay parallel to each other, positioning behind the animals at a 60º angle. The approach must be coordinated via radio.
- The observation is limited to 30 minutes.
- If there are signs of stress, the vessels must increase their distance.

Currently, no studies are focused on evaluating WW vessels' compliance with the regulations in the Algarve. A survey about the socio-economic characterisation of the tourists who engage in WW in the Algarve reported that 50% of observations in Lagos and Sagres were made with no other vessels around and 45% with no more than two different vessels, which suggests compliance with the legislation when it comes to number of vessels; nonetheless, it was pointed out that on a few occasions up to 7 vessels were present around the animals (Claro, 2009). However, this evidence must be taken as anecdotal as it was collected through inquiries from tourists who do not understand local rules. The majority (56%) of the tourists interviewed were not aware of the code of conduct (Claro, 2009), which in itself points to a breach in compliance as Art. 15 *b)* of the Decree-Law no. 9/2006 states that one of the duties of WW operations is to offer all participants relevant information about proper code of conduct. On the other hand, Grave (2022) reported that, over ten years, 36.5% of observations on the Algarve coast were made in the presence of over three vessels; the most common number of vessels was 2 (17.63%), but a maximum of 15 vessels in a single observation was registered (0.09%). The second study covered a more extensive area and reported more vessels and infractions between Portimão and Albufeira, with fewer infractions registered for Lagos and Sagres. Neither study was focused on compliance or the impact of WW vessels on the animals. Nonetheless, those are the only reports currently available, and they indicate that there might be excessive pressure on the animals, especially during the summer months.

Every summer, the human population in the Algarve triples, with thousands of tourists choosing it as a holiday destination; this creates more coastal pressure, particularly in Albufeira, where WW activities are in high demand (Alves da Silva, 2022). Amongst the maritime tourism activities, WW is the most sought-after by tourists in the Algarve (Silva, 2022). From 2008 to 2022, there was an increase of 1000% in licensed vessels in the Algarve (ICNF, 2022). Concerns over adverse effects on the animals and their habitat led the *Conselho Diretivo do Instituto da Conservação da Natureza e Florestas* (ICNF) to establish a carrying capacity for the region at 124 vessels, therefore determining no more licenses would be issued starting from December 23rd 2022 (ICNF, 2022). However, the lack of effective oversight from national regulatory bodies, coupled with an excessive number of regulating entities that give conflicting messages to operators, and a scarcity of data contribute to a high number of unlicensed companies operating in WW activities. This problem is further exacerbated by insufficiently trained staff unable to provide accurate information about cetaceans to the public (Castro, 2010).

Establishing proper evaluation and conservation programs is of utmost importance if this activity is to be sustainable in the long run. One of the crucial pieces of any robust conservation program is a long-term monitoring program, which has never been implemented in the Algarve; existing knowledge of the cetaceans in the region is mainly published as grey literature, and peer-reviewed publications are scarce (Morais et al., 2021). The first broadscale and long-term assessment of cetaceans' diversity in the Algarve was obtained using records from WW companies' social media accounts (Morais et al., 2021). WW companies identified the following 15 species in 10 years: *Balaenoptera acutorostrata*, *B. borealis*, *B. edeni*, *B. musculus*, *B. physalus*, *Delphinus delphis*, *Globicephala macrorhynchus*, *G. melas*, *Grampus griseus*, *Megaptera novaeangliae*, *Orcinus orca*, *Phocoena phocoena*, *Physeter macrocephalus*, *Pseudorca crassidens*, *Stenella coeruleoalba* and *T. truncatus* (Morais et al., 2021). Four had never been described in the scientific literature for the Algarve (*B. borealis*, *B. musculus*, *M. novaeangliae* and *P. crassidens*) (Morais et al., 2021). The two species most observed in the Algarve are *D. delphis* and *T. truncatus* (Morais et al., 2021). For this reason, the present study focuses on the impact of WW activities on those two species.

1.3. Biology and Ecology of *D. delphis* and *T. truncatus*

Both species are global, abundant and have a wide distribution in temperate, subtropical and tropical waters of the Atlantic, Pacific and Indian Oceans (Jefferson et al., 2015). Their presence in diverse marine environments contributes to their ecological resilience, allowing them to adapt to varying sea conditions and the availability of food sources. This widespread distribution also enables them to play significant roles in their ecosystems, influencing food web dynamics and nutrient cycling. However, their broad range exposes them to numerous human-induced threats, including pollution, fishing gear entanglement, and habitat disruption. Effective management and conservation strategies are crucial to mitigate these impacts and ensure the sustainability of their populations. Ongoing research and international cooperation are key to understanding their behaviours and needs, which will aid in crafting targeted protection measures.

The short-beaked common dolphin (*Delphinus delphis*) is the most abundant in warm-temperate waters in the Atlantic and Pacific; they often come from a distance to join boats and ride the bow wave (Perrin, 2018); these characteristics make them a great target species for dolphin-focused WW tours. The species was most recently accessed for The IUCN Red List of Threatened Species in 2020, when it was classified globally as a "Least concern" (Braulik et al., 2021). Despite its wide distribution, the species is not panmictic but occurs in separate subpopulations (Jefferson and Waerebeek, 2002). Each subpopulation may be subjected to different stressors and present varying trends, sometimes requiring individual evaluation. The

Mediterranean subpopulation, for example, was once among the most common cetaceans in the area but has experienced a generalised and significant decrease, which led it to be classified separately from the rest of the species as "Endangered" in 2003 (Bearzi, 2003).

Common dolphins live and forage in herds of a few tens of individuals, which can sometimes aggregate into groups of several hundred (Pusineri et al., 2007) but are often segregated by age and sex and associations with other marine mammals are not uncommon (Jefferson et al., 2015). They feed mostly on epipelagic schooling species such as small scombroids, clupeoids, and market squids (Perrin, 2018). Still, small mesopelagic fishes and squids found in the deep scattering layer, foraging dives up to 200 m, have been recorded (Perrin, 2018). *D. delphis* presents large-scale panmixia in European waters, likely resulting from the long-distance dispersal of individuals seeking seasonal prey (Ball et al., 2017). Population structure has been detected across the North Atlantic between Western and Eastern basins but not within regions, suggesting a single population for the Eastern North Atlantic, except the Mediterranean subpopulation (Luca et al., 2009). The Eastern North Atlantic population calving period is unimodal in summer, and the mating period extends over five months from May to September, with possibly a more active period in July and August (Murphy et al., 2009).

Although *D. delphis* is a species of least concern, there are several anthropogenic threats which may lead to population reductions: by-catch in gillnet, trawl and purse-seine fisheries around the world, direct take for human consumption in some isolated cases, prey depletion is thought to be the primary threat in the Black sea and Mediterranean and sonar produced by naval vessels is a probable cause of mass strandings (Perrin, 2018). WW watching is not listed as a threat to *D. delphis* in the IUCN Red List (Braulik et al., 2021). However, several studies have shown short-term effects that might impact population health and fitness (e.g. Cecchetti et al., 2018; Sitar and Parsons, 2015). Cumulative or synergistic effects, both in terms of individual health and at a population level, especially in cases of endangered populations such as the Mediterranean and the Black Sea, must be better understood to allow for appropriate conservation measures (Vella et al., 2021).

Out of the small cetaceans, the common bottlenose dolphin (*Tursiops truncatus*) is probably the most familiar and famous because of its coastal habits, long history of captivity worldwide, and frequent media appearances (Jefferson et al., 2015). The species is cosmopolitan and demonstrates a great deal of geographical variation in morphology, which has led to discussions about its taxonomic status (Wells and Scott, 2009). The most recent IUCN Red

List assessment was in 2018, when it was listed globally as “Least concern,” with an abundance estimate of around 750,000. Still, since most species’ range has not been surveyed, the abundance is considerably higher (Wells et al., 2019). Nonetheless, as is the case for the common dolphin, specific subpopulations are threatened by human activities, such as the Fiordland subpopulation in New Zealand, which is classified as “Critically Endangered” (Currey et al., 2013), the Mediterranean subpopulation, which is classified as “Vulnerable”, (Bearzi et al., 2012) and the Black Sea subspecies which is classified as “Endangered” (Birkun, 2012).

The biology of *T. truncatus* is the most known among dolphins, and its behaviour has been studied, both in captivity and in many different coastal areas throughout the range (Jefferson et al., 2015). Their diet is composed of many fish and squid forms, with a consistent preference for sciaenids, scombrids, and mugilids; most fish prey are bottom-dwellers and especially noise-producing fish, presumably because sound helps them locate prey (Wells and Scott, 2009). Group size is commonly less than 20, but herds of several hundred are sometimes seen in offshore areas. They are widely associated with other cetaceans, including large whales and other dolphin species (Jefferson et al., 2015). The timing of birth varies significantly within the species, with local populations showing distinct birthing seasons even at the same latitudes; many interacting environmental factors likely influence the seasonality of reproduction in different populations (Urian et al., 1996). Recently, it was revealed that the structure between coastal populations in the Eastern North Atlantic suggests that given the small population sizes of the coastal populations and their isolation, they might be vulnerable to anthropogenic impacts, and management units should be established (Nykänen et al., 2019).

Several different threats have been identified for bottlenose populations; incidental catches have been reported in several different fisheries; in some cases, dolphins are purposefully killed to prevent damage to equipment, and in some countries, direct take for human consumption or bait still exists (Wells and Scott, 2009). Coastal bottlenose dolphins are susceptible to habitat destruction and degradation, vessel collisions and environmental contaminants; in recent years, several die-offs of bottlenose dolphins have been linked to poisoning from biotoxins (Jefferson et al., 2015). Reduced prey availability caused by environmental degradation, direct and indirect disturbance, and harassment, including boat traffic, commercial WW and interactive programs, have also been identified as possible threats (Wells et al., 2019). Although the species is not endangered (Wells et al., 2019), low genetic diversity and low connectivity in small coastal populations mean that any local perturbation or global change could have drastic effects, and those are precisely located where most anthropogenic impact is concentrated (Nykänen et al., 2019).

1.3.1. *D. delphis* and *T. truncatus* Populations in the Algarve Coast

D. delphis is the most abundant cetacean species on the Algarve Coast (Castro, 2010; Moura et al., 2012). On the Portuguese coast, the preferred prey item is sardine (*Sardina pilchardus*) (Silva, 1999). At the time of Silva's (1999) study, the sardine was also the most abundant small pelagic fish species, possibly indicating that *D. delphis* is an opportunistic feeder. However, despite the decline of sardine populations in recent years, sardine continues to be the preferred prey of *D. delphis,* supporting a hypothesis of prey selection based on prey fat content and most probably also net energy gain by the common dolphin (Marçalo et al., 2018). Values of chlorophyll concentration are highly predictive of joint dolphin distribution, which also may reflect a strong local dependency on sardines as a prey item, supporting the idea that the common dolphin might be an ecological specialist restricted to small pelagic schooling fish (Moura et al., 2012). In this light, it is essential to consider that the decline of sardine populations could result in the decline of local *D. delphis* populations, which, being a specialised animal with long generation times, falls in the group of organisms more likely to suffer from extinction debt (Moura et al., 2012). This marked preference for sardines also contributes to the common dolphin being the most frequent species in interactions with the Algarve purse seine fishery, which also targets sardines, raising concerns regarding accidental capture and mortality (Dias et al., 2022).

The Algarve coast is an important area as it is a potential nursery ground for common dolphins, with immature individuals appearing in 72.5% of sightings and calves/newborns appearing in 42.5% (Castro et al., 2020). The presence of mother-calf pairs seems to be the primary factor influencing party size, behavioural state, and rate of fission-fusion of common dolphins in the Algarve; mother-calf pairs were observed in larger parties that were less social and more stable as compared to parties without mother-calf pairs (Castro et al., 2022a). Although the extension of predation pressure for common dolphins in the Algarve remains unclear, the mother-calf pair dynamics seem to be most strongly influenced by predation avoidance; the presence of bottlenose dolphins in the same area might also play a role, as interspecific aggression has been recorded (Castro et al., 2024).

Bottlenose dolphin distribution in the Algarve coast is concentrated close to shore, with a particular incidence in the Albufeira area; the presence of multiple submarine cannons creates attractive spots for cetaceans due to increased food availability associated with enhanced upwelling-driven surface productivity (Vieira, 2017). Stomach content analysis and stable isotope analysis have revealed 35 different species as prey items for bottlenose dolphins in the region, with a predominance of European conger (*Conger conger*) and European hake (*Merluccius merluccius*); they can be considered a generalist predator in the area but with a diet comprised primarily of demersal and some pelagic fishes (Giménez et al., 2017). Despite being less abundant than common dolphins, bottlenose dolphins were more frequently sighted by and reported in interactions with fishermen in the area, likely due to the most used gear in the region being bottom set nets, usually associated with higher interactions with this species (Alexandre et al., 2022). The annual fishery-related bycatch rate in the Algarve for bottlenose dolphins calculated in a 2022 study was 2.6%, which may suggest an unsustainable mortality rate for the species as the cetacean removal limit recommended by ASCOBAMS (Agreement on the Conservation of Small Cetaceans of the Baltic, North East Atlantic, Irish and North Seas) is set at 1.7% (Alexandre et al., 2022).

Similarly to the common dolphins, bottlenose dolphins in the region have been observed with calves and newborns and engaging in social-sexual behaviour. This suggests that the area might be an important breeding and nursery ground (Castro, 2010; Grave, 2022; Vieira, 2017). Nurturant and succorant epimeletic behaviour (help given by one or more healthy individuals towards a sick, injured, or dead individual, designated “succorant” when directed towards adults and “nurturant” when directed towards infants) have been opportunistically described for *T. truncatus* off the coast of Algarve (Castro et al., 2022b) which further supports the hypothesis of this area being a nursery ground. The size of groups with calves is more significant than that of those without, and it has been suggested that increasing foraging efficiency might be the primary driver for this, as foraging was the most observed behaviour in larger groups with calves in the region (Grave, 2022).

Another possible concern for *T. truncatus* is the bioaccumulation of trace elements; hepatic concentrations of Hg exceeding toxic thresholds defined for evidence of liver damage in marine mammals have been recorded from stranded animals along the coast, suggesting a higher exposure to anthropogenic contaminants (Monteiro et al., 2016).. This is particularly important, considering this species has been found to occur in southern Portugal, mostly in coastal waters (Monteiro et al., 2016).

1.3.2. Interactions with WW Vessels

A direct and very palpable impact of cetacean-vessel interaction is the infliction of wounds by vessel collisions and propellers. Olaya-Ponzone et al. (2020) described injuries from five *D. delphis*, which were likely related to the high intensity of recreational fishing and WW activities between Gibraltar and Algeciras, an area which serves as an important breeding and feeding hotspot for the endangered common dolphin Mediterranean subpopulation. There are many other records of vessel collisions with *D. delphis* (Kemper et al., 2005; Martinez and Stockin, 2013; Waerebeek et al., 2007). This type of incident has also been repeatedly identified for *T. truncatus* (Bechdel et al., 2009; Dwyer et al., 2014; Wells et al., 2008). A recent global assessment of collisions between vessels and cetaceans found that 8.5% of reported vessel collisions happened with WW vessels, second only to sailing yachts (<25m) with 9.2% and followed closely by naval vessels with 8.2% (Winkler et al., 2020). The long-term consequences of collisions are not well understood, but several species indicate long-term locomotive impairments and possible reduced fitness; it is likely that a high mortality rate or decline in fertility could decrease population growth (Schoeman et al., 2020).

Other pressures caused by WW vessel interactions are less visible and more challenging to notice but arguably much more prevalent than collisions. Several studies have identified short-term changes in both species. There have been recorded decreases in the inter-animal distance, increases in swimming speeds and changes in heading altering course to move away from approaching ships for *T. truncatus* (Nowacek et al., 2001). Decrease in socialising and resting bouts due to the presence of boats (Lusseau, 2003; Sitar and Parsons, 2015). For both species, a significant reduction of foraging time coupled with increased travelling time has been related to the presence of WW vessels (Cecchetti et al., 2018).

Although it is hard to ascertain the extent to which these changes are harmful, short-term changes in behaviour might lead to long-term impacts by detracting from biologically significant behaviour such as foraging (Cecchetti et al., 2018); for example, if reduced foraging results in reduced prey capture, it can lead to decreased energy acquisition (Lusseau et al., 2009). WW activity in Bocas Del Toro has been linked to a decrease in three primary biologically important behaviours, reproductive, resting and foraging behaviour, in the *T. truncatus* population (Sitar and Parsons, 2015). A reduction in surface behaviour has also been correlated with increasing vessels, suggesting vessel avoidance behaviour as the animals escape to deeper layers (Toro et al., 2021). In some cases, “positive” responses have also been reported; common dolphins changed their activity in 21.2% of cases when the tour boat approached most frequently by approaching the tour boat to “bow ride” (Neumann and Orams, 2006). However, the authors also found that the animals spent more time travelling and socialising at the expense of foraging, milling and resting (Neumann and Orams, 2006).

Most studies concentrate on behaviour at the surface. However, extrapolating surface behaviour to underwater behaviour can be misleading as it only represents a tiny percentage of the animal’s lives (Janik, 2009). Considering that dolphins are highly sociable animals and very dependent on both echolocation and communication sounds (Janik, 2009), it is likely that acoustic masking by anthropogenic noises, such as engine noise from motorised vessels, has an increasingly prevalent impact on their access to crucial information for communication, navigation and prey/predator detection (Clark et al., 2009).

1.3.3. Acoustic behaviour and vessel noise

While sound travels about 4.5 times faster in water than in air, vision is often limited underwater, so it isn’t surprising that cetaceans have developed specialised acoustic tools such as echolocation and a communication system based on acoustic signals (Dudzinski et al., 2009). Odontocete sounds can be divided broadly into two signal types (fig. 1.1): pulsed and narrow-band tonal sounds; tonal sounds are called whistles and appear to have no other function than communication, some pulsed sounds called clicks are implicated in echolocation, while other burst-pulsed sounds such as barks, squawks, squeaks, blasts, buzzes, and moans, have social functions (Dudzinski et al., 2009).

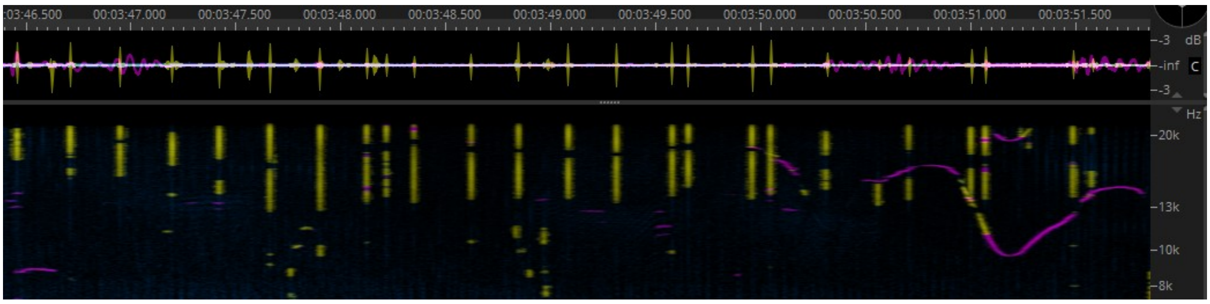


Figure 1.1 - Spectrogram of recording with T. truncatus vocalizations. Pulsed signals (clicks) can be seen in yellow. Narrow-band tonal sounds (whistles) can be seen in pink with a range from around 9 kHz to around 16 kHz; the lower end of a harmonic can also be seen around 20 kHz; the rest of the harmonic would be outside of the recording range.

Whistling dolphins, such as *T. truncatus* and *D. delphis*, produce clicks with bandwidths of tens of kilohertz with durations of 40-70 µsec, which, coupled with a remarkably sensitive auditory system, allows for very keen echolocation capabilities (Au, 2002). Whistles have durations of up to a few seconds and fundamental frequencies that typically fall between 5 and 20 kHz but might also have harmonic components at integer multiples of the fundamental reaching up to 100 kHz (fig.1.1) (Dudzinski et al., 2009; Lammers et al., 2003). Dolphin whistles are frequency modulated and can be described based on spectrogram graphs of their time-frequency contours (Ansmann, 2005). Some typical categories that are used to classify whistle contours are unmodulated constant frequency, upsweeps, downsweeps, concave, convex and wavering sinusoidal (fig. 1.2) (Ansmann, 2005; Dudzinski et al., 2009). Intraspecific variation in delphinid repertoire has been shown for all species that have been investigated, with the most commonly analysed parameters being start frequency, end frequency, lower frequency, higher frequency, frequency range, number of inflections in the frequency modulation pattern of the whistle, and duration (fig. 1.2F) (Janik, 2009).

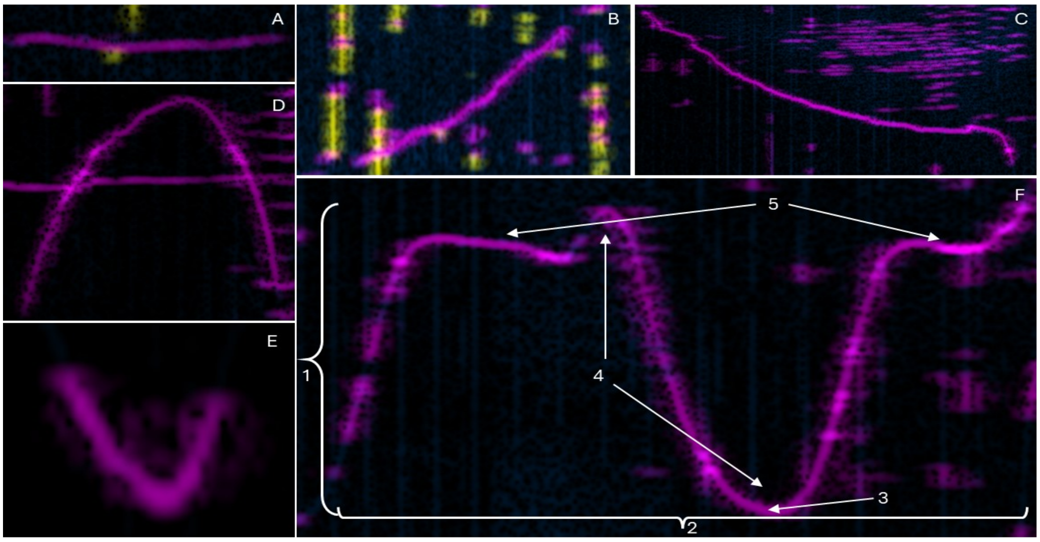


Figure 1.2 - Whistle types and parameters. A – unmodulated constant, B – upsweep, C – downsweep, D – convex, E – concave, F – Wavering sinusoidal; whistle parameters measured: 1 - range (kHz), 2 - duration (sec), 3 - lower frequency (kHz), 4 - inflection points and 5 - steps.

Acoustic masking and modifications to vocalisation patterns and characteristics because of increased environmental noise have been shown in many species, and in some cases, there is evidence of negative impacts on health and reproduction (Barber et al., 2010; Kight and Swaddle, 2011). Critical changes in marine mammals' behaviour in response to low and mid-frequency noise, including boat noise, have been shown for several species (United States

Marine Mammal Commission, 2011). *T. truncatus* is the most studied cetacean species regarding the effects of ship noise on marine mammals; with over 30 publications as of 2019, *D. delphis* counts with fewer studies while still being within the represented species (Erbe et al., 2019).

While many studies have shown how noise provokes changes in whistle parameters, results vary greatly. Many authors have found that both *T. truncatus* and *D. delphis* increase the frequency (kHz) of their whistles in the presence of noise (Alves da Silva, 2022; Ansmann, 2005; Fouda et al., 2018; Heiler et al., 2016; May-Collado and Wartzok, 2008; Papale et al., 2015; Van Ginkel et al., 2018). This shift might be a compensatory measure as the animals try to avoid masking by shifting to higher frequencies and avoiding overlap with background noise at lower frequencies (Van Ginkel et al., 2018). However, this does not seem to be a universal strategy; it was found that while whistle frequencies shifted up in the presence of low-frequency noise (63–2kHz, 63 Hz band and 125 Hz) when the noise was at a higher frequency range (2–20 kHz), dolphins decreased their whistle frequencies as well as the number of whistle inflection points (Rako Gospić and Picciulin, 2016). Different vessel types might cause different modifications; *T. truncatus* from the Sado estuary has shown increased frequency in the presence of dolphin-watching vessels, ferry boats and leisure boats but decreases in the presence of trawlers (Luís et al., 2014). Increased noise levels led to a shift to lower frequencies in groups without calves but a change to higher frequencies when calves were present (Guerra et al., 2014).

Groups with calves also produced longer whistles when tour boats were within 200m, while groups without calves produced shorter whistles when compared to no tour boats in the area (Guerra et al., 2014). Other studies have also found increases in duration (Alves da Silva, 2022; May-Collado and Wartzok, 2008), which might be a strategy to increase the probability of call detection (Brumm et al., 2004), although many studies found no difference (Buckstaff, 2004; Heiler et al., 2016; Luís et al., 2014; Van Ginkel et al., 2018) and decreases are also reported (Fouda et al., 2018). Heiler et al. (2016) did not find differences in duration between the presence and absence of boats. Still, they did find differences between different behavioural states, with whistles made during resting being the longest. They suggest that changes in duration, at least for *T. truncatus*, may be used to convey motivation rather than to counteract masking, with shorter whistles being used in states of greater emotional arousal while longer whistles are used in resting situations. This might explain the inconsistencies observed when comparing other studies.

Whistle rates tend to increase with vessel approach (Buckstaff, 2004; Heiler et al., 2016; Scarpaci et al., 2000), although this change isn't always observed (Luís et al., 2014). The whistle rate rises with the vessel's approach's onset but decreases during the interaction and after the boat goes away (Buckstaff, 2004). On the other hand, echolocation signals decrease as boats approach, suggesting that dolphins temporarily interrupt their activity when disturbed (Pirotta et al., 2015). This trend agrees with observations from behavioural studies that show a decrease in foraging behaviours in the presence of vessels.

The strategy for signal modification likely depends not only on the species, population or individual (e.g. use of signature whistles or not, level of habituation to anthropogenic noise) but also on the noise itself (Van Ginkel et al., 2018). The fact that dolphins seem to overcome masking by ambient and engine noises in different manners is evidence of how plastic these signals are and that other factors, such as habitat use, also promote significant variability to these parameters (May-Collado and Wartzok, 2008). These changes might be particularly significant in more sensitive groups such as those comprising mother-calf pairs; further understanding the changes in the overall acoustic repertoire of dolphins, the relationship between these changes, the behavioural budget and group composition in the presence of disturbance factors to assess potential implications at the population level is essential (Rako Gospić and Picciulin, 2016).

1.4. Methods for Evaluating Impact

In most situations, studies on the impact of WW, both commercial and recreational, only begin years after the establishment of the activity, the lack of pre-tourism data on target animals' behaviour, habitat use and fecundity coupled with the urgent need for information as a new WW industry is being developed, and the fact that cetaceans are long-lived, slow-breeding animals makes for a problematic research environment (Constantine and Bejder, 2008). The first challenge researchers must face is data collection and the large spatial area over which many cetacean species occur (New et al., 2015). Studies can be conducted from varying platforms, such as land-based observation (e.g. Pirotta et al., 2015), vessel-based observations (e.g. May-Collado and Wartzok, 2008), DTAGs (e.g. Kragh et al., 2019), passive acoustic monitoring (PAM) (e.g. Jensen et al., 2009), and, more recently, unmanned aerial vehicles have been gaining popularity (Castro et al., 2024). Because of logistic constraints that are so common, there is considerable interest in using platforms of opportunity such as fishing vessels, ferries and WW operations; data collection by WW operators can significantly contribute to research efforts while also satisfying their customers by involving them in science, serving as a conduit for conservation and public education and facilitating understanding and stewardship of the population that the businesses depend upon (New et al., 2015).

When estimating the effects of WW on the animals and populations, a wide variety of statistical approaches might be used depending on the researchers' background, research question and available data (New et al., 2015). Tools such as ANOVA (e.g. Toro et al., 2021), t-tests (e.g. La Manna et al., 2013), and non-parametric tests (e.g. Alves da Silva, 2022) are often used to compare parameters between groups with various levels of WW exposure. Markov chains and odds ratios have been used to investigate the effects of transitions between behavioural states (e.g. Lusseau, 2003). More recently, modelling approaches such as Bayesian hierarchical modelling and passive acoustic techniques to quantify the impact of boat disturbance (Pirotta et al., 2015) and population consequences of disturbance (PCoD) framework to link short-term changes in individual behaviour and physiology to the long-term effects on population dynamics (New et al., 2015) have been employed, which might help bridge the gap between the urgent need of information for conservation decisions and the lack of long-term data.

In the present study, we aimed to use land-based observations as a non-invasive approach to determining WW pressure on the Algarve coast. We coupled this with acoustic behaviour analysis using recordings provided by WW operators during touristic trips in the presence of other vessels and with no engines operating.

1.4.1. Land-based Observation

Land-based observations provide the advantage of an expanded coverage area; for example, in the Azores, using 15x or 20x binoculars, lookouts may see up to 35 km in perfect weather, amounting to 1900 km^2 of observation field (Zahn et al., 2022b). Another advantage is gathering data on more accurate “control” groups. In any vessel-based observation, while there are tactics to minimise observer bias; the effects of the research vessel on the animals cannot be avoided entirely and must be considered (Guerra et al., 2014). On the other hand, the range to which land-based observations can be used is inherently limited to a few miles off the coast, and the quality/accuracy of data collected greatly depends on the observer's skill and experience.

Land-based observation was initially an essential component of WW tourism, but with the increasing availability of vessel-based WW trips, it has become a less critical sector of the industry, which is unfortunate since land-based WW has no direct impact on the cetaceans and many species can be watched benignly from land platforms (Hoyt and Parsons, 2014). Promoting land-based WW has been recommended as an alternative to vessel-based activities to enhance the management and conservation of cetaceans (Avila et al., 2021). Land-based touristic whale watchers are significantly more concerned than boat-based respondents about the disturbance of whales caused by visitor activities, particularly from noise and boat position, which shows an important aspect of human perspectives to be considered when managing WW activities (Finkler and Higham, 2004).

1.4.2. Acoustic Behaviour Analysis

To understand the effects of WW vessels on the acoustic behaviour of cetaceans, it is essential to remember that this is an interdisciplinary problem; sound generation, propagation, measurement, and modelling are physics problems, while monitoring animals, determining impacts, and understanding biological significance are biological problems (Erbe et al., 2019). Therefore, comprehending both sides of the problem is crucial to properly assessing and interpreting these effects.

Sound is energy manifested as vibration or acoustic wave that travels through a medium such as air or water and can be characterised using different parameters; frequency refers to the rate of vibration in cycles per second measured in Hertz (Hz) or kilohertz (kHz), for underwater sound the reference pressure level is one microPascal (μPa) hence units for sound pressure level are dB re 1μPa (United States Marine Mammal Commission, 2011). Sound waves spread out and dissipate through the ocean in different ways as they travel away from their source. In contrast, low-frequency sounds (e.g. 100 Hz sound) may be detectable after propagating hundreds or thousands of kilometres, while high-frequency sounds (e.g. 100-kHz sound) may be detectable only for a few kilometres.

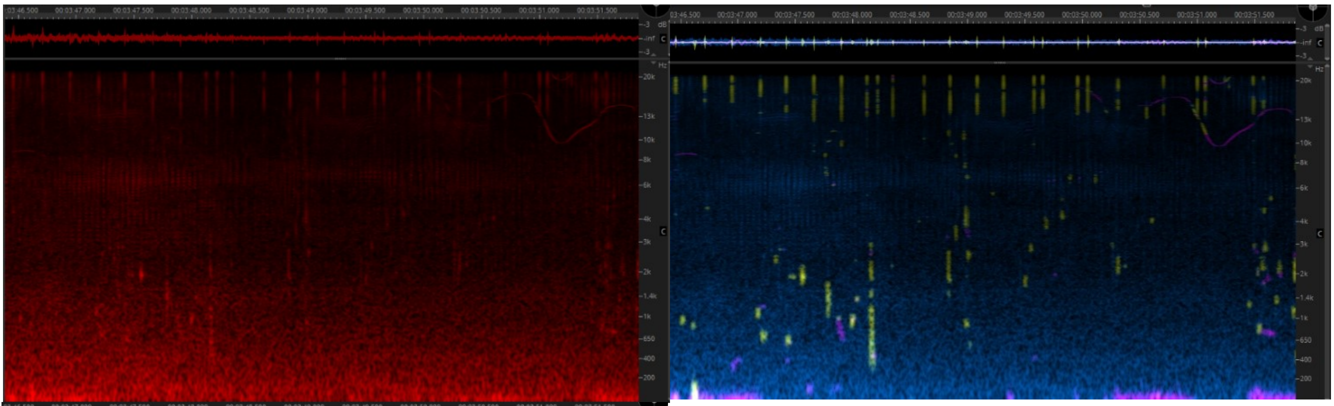
Vessels generate underwater noise from the engine and hull, but the most robust noise source is typically the propeller when it cavitates, a phenomenon whereby air bubbles form and collapse on the edge of fast-moving propeller blades, generating most of the medium and high-frequency components to the noise (Ross, 1976). WW and small research vessels produce noise received by the animals in the frequency band between 2 and 12.5 kHz and are only slightly influenced by orientation, type of boat and depth of the receivers (Jensen et al., 2009). Frequency depends primarily on speed; at speeds lower than 2.5 knots, measured noise levels are comparable to background noise (Jensen et al., 2009). These vessel sounds overlap with the audible frequency ranges of dolphins; thus, masking is one of the most commonly listed impacts (Pine et al., 2016).

There are different approaches to recording cetacean vocalisations in the presence of vessels; hydrophones might be deployed from the side of the boats, fixed to the sea floor, or deployed in buoys; tags can be used to determine the noise received by the animal; a few studies have also used playback recordings rather than the actual engine noise (Erbe et al., 2019). Each method has its artefacts; building noise-free moorings is an art, and different designs may be required for various situations (Erbe et al., 2019).

On the biological side of the discussion, there is the necessity of establishing “control” groups when there are few environments globally which have not experienced anthropogenic stressors and frequently little to no previous information is known for the species (Erbe et al., 2019). For the present study, we choose two species for which more extensive knowledge is available, both because they are the most targeted species by WW in the region and because of the need to use WW as a platform of opportunity in the data collection process as we did not have access to our research vessel. Nonetheless, in the context of a poorly studied area with very few previous studies, having prior knowledge of the species and their relationship to WW activities in other regions becomes a stepping stone to developing a better understanding of the impacts of this industry locally.

SpectraLayers Pro 10

The software used to conduct the acoustic behaviour analysis in this study was the advanced audio spectrum editor SpectraLayers Pro 10 by Steinberg Media Technologies. Visual examination of spectrograms can be a taxing activity and many times it is hard to identify



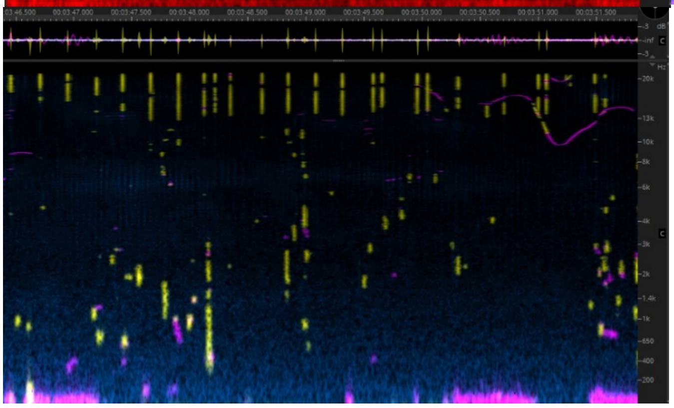


Figure 1.3 - Process of enhancing a spectrogram in SpectraLayers Pro 10. A - The audio file is loaded to the program; this is what a typical untreated spectrogram looks like. B - After "unmix - components" function has been applied. C - After individually editing on the three channels has been done to increase intensity (i.e. volume) and resolution of tonal and transient and decrease intensity of noise. Tonal sounds shown in pink, transients in yellow and noise in blue. There are clicks across the top of the spectrogram in yellow and a whistle to the far right in pink (zoomed version in Fig. 1.1).

features (fig. 1.3A), with resolution highly depending on the quality of the recording. The tool used for this study is called "Unmix" and it consists in decomposing sounds based on spectral modelling synthesis (Fierro and Välimäki, 2022; Verma and Meng, 2000) so that they can be individually analysed or edited. This method separates the original audio into 3 channels: tonal, transient and noise. Sinusoidal/tonal sounds are characterized by being slow-varying in the time-domain and impulsive in the frequency-domain, in contrast with transient signals which are impulsive in the time-domain but oscillatory in the frequency-domain (Verma and Meng, 2000). In simple terms, tonal sounds have lower amplitude in frequency and longer duration, they are perceived as a "pure tone" (i.e. melodic singing or single notes in a piano) while transients are high-amplitude and short in duration (i.e. a clap or snapping sound) (fig. 1.4).

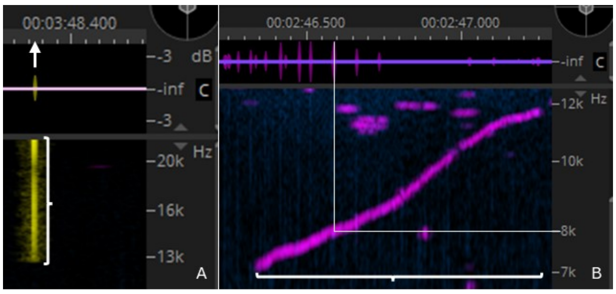


Figure 1.4 - Tonal vs transient signals. In a spectrogram time is shown in the x-axis and frequency in the y-axis. A - transient signals are short in duration as pointed by the arrow but high amplitude in frequency as shown by the bracket. B - tonal signals are long in duration as shown by the bracket, but at any given time has a single frequency as shown by the cross lines.

Dolphin whistles fit the tonal category and echolocation clicks fit the transient category, therefore the program can separate these components and allow for the creation of an enhanced spectrogram (fig. 1.3B and 1.3C). If variations in volume are to be analysed, then this data should be inferred prior to any visual editing of the spectrogram. In the case of this study no analysis to volume was conducted as we did not have a reference across different recordings, and they were therefore not comparable.

This software has not been used before to analyse cetacean vocalisations. The only instance of its use within zoology was a paper about wolf pack sizes (Papin et al., 2019).

1.5. Hypothesis and Objectives

WW activities have been shown to cause disturbances in several species of cetaceans worldwide. It is understood that different populations respond differently to this impact depending on population traits, habitat characteristics, WW style and intensity and compliance with regulations. The effect of WW activities in the Algarve is still poorly studied, and this knowledge gap impedes the development of adequate mitigation measures and conservation programs.

The present work aims to provide a quantitative analysis of the impact of WW activities in the Algarve by combining acoustic analysis and land-based observations to determine short-term changes in acoustic behaviour and the amount of time the animals are exposed to this stressor. Based on previous studies, we hypothesise that if the presence of boats affects the behaviour of dolphins, then:

1) whistle parameters will differ in the presence of WW boats;
2) clicks per minute will be different in the presence of WW boats;
3) boat pressure will vary in the “Barlavento” zone compared to the “Sotavento” areas.

These hypotheses lead to various logical expectations: whistle parameters will reflect modifications used to cope with acoustic masking in the presence of boats; clicks per minute will be lowered in the presence of ships as the dolphins detract from foraging activity in the presence of vessels; and boat pressure will be higher in the “Barlavento” zone compared to the “Sotavento” areas. Further expectations derived from the observations may lead to the conclusion that boat number limits established by law are not always followed, particularly during the high tourism season.

1.6. Significance for Conservation and Management

WW vessels spend a potentially large amount of targeted time near cetaceans; therefore, if adverse impacts are to be reduced and the industry is to be sustainable, it is of interest for regulators, stakeholders and operators to understand the levels of vessel noise and their impact (Arranz et al., 2021). The first step to creating effective management is understanding the dynamics of the populations and how they are affected by local stressors. The lack of knowledge of WW activity in the Algarve only allows for prudent decisions based on information from other locations. However, as impacts and population reactions can vary widely depending on many factors, this is no more than an educated guess. Furthermore, it is harder to argue with stakeholders and gather much-needed support when there is a lack of evidence to solve a problem that might not be as obvious to all interested parties.

WW activities have been shown to promote pro-conservation intentions and improve public knowledge (Cárdenas et al., 2021; García-Cegarra and Pacheco, 2017). Tourists have expressed concerns about the proximity to the whales, the high number of boats surrounding mother and calf groups, the speed of the boat when approaching the whales, and the fact that engine noise might disturb the animals (Finkler and Higham, 2004; García-Cegarra and Pacheco, 2017). This shows that improving WW guidelines and implementing proper management and mitigation measures benefits the species and the industry (Bentz et al., 2016).

Tourism continues to be a vital part of the Algarve's economy, and WW activities are not likely to slow down as thousands of tourists come yearly. The availability of WW operators to contribute to regional studies (e.g. Castro et al., 2020; Moura et al., 2012) shows a willingness to collaborate for a common goal of sustainability. The results of this study might give some more background on the issues revolving around WW on the south coast of mainland Portugal and help identify specific management priorities and mitigation strategies that are appropriate for this region, thus aiding in creating a more sustainable industry.

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2. Chapter 2: Manuscript

The Impact of Touristic Whale-Watching on *Delphinus Delphis* and *Tursiops Truncatus* in the Algarve Coast: Combining Acoustic Analysis and Land Observations

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Abstract

Touristic whale watching (WW) is an important socioeconomic activity worldwide. Recently, short and long-term impacts caused by WW have been reported for several cetacean species, including *Delphinus delphis* and *Tursiops truncatus*, the two most observed species in Portugal. Most of the operations in mainland Portugal are concentrated on the South Coast (Algarve). However, despite their importance for the region, studies focused on the impact of this activity on the animals are still scarce. We used acoustic behaviour analysis coupled with land-based observations to determine the alterations in the vocalisation patterns of these animals caused by WW vessels and the length of exposure to this stressor. We found WW presence significantly altered five out of six acoustic parameters of whistles, whistle rate, and whistle type distribution for *D. delphis*, whereas *T. truncatus* exhibited significant changes in three acoustic parameters. Conversely, *T. truncatus* significantly reduced echolocation click rates, while *D. delphis* did not show significant changes. We also found that the “Barlavento” area is the most affected, with animals exposed to WW vessels for up to 38.9% of daylight hours. Furthermore, 66.7% of the observation time in this area had several vessels that exceeded the limit established by law. These results suggest that our study species are impacted by WW, while *D. delphis* is more affected during social behaviours, and *T. truncatus* is more affected during foraging activities. Both behaviours are biologically significant, and their disturbance might, in the long term, lead to adverse effects at the population level by decreasing reproduction success and fitness. A gap in knowledge, inadequate legislation and compliance issues threaten the development of a sustainable WW industry in the Algarve and might endanger local populations, therefore needing immediate attention.

Keywords: *Tursiops truncatus*, *Delphinus delphis*, whale watching, acoustic behaviour, land-based observation

2.1. Introduction

Whale Watching (WW) refers to observing cetacean species (whales, dolphins and porpoises) in their natural environment (Hoyt, 2021). In contrast with whaling, WW is often promoted as a solution and sustainable alternative to the exploration of cetaceans; it can provide socioeconomic advantages while also serving as a tool for conservation efforts and fostering a perception of cetaceans as a more valuable resource, alive rather than dead (Mustika et al., 2012; Parsons, 2012). Several studies have indicated short-term responses to WW disturbance, such as changes in behaviour (e.g. Arcangeli and Crosti, 2009; Cecchetti et al., 2018), group structure (e.g. Toro et al., 2021), vocalisations (e.g. Fouda et al., 2018; Van Ginkel et al., 2018), as well as habitat displacements, and boat avoidance (e.g. Bejder et al., 2006; Constantine, 2001) which can negatively impact the target species. Short-term behaviour changes might lead to long-term impacts by detracting from biologically significant behaviour such as foraging, resting and reproduction (Lusseau and Bejder, 2007; Sitar and Parsons, 2015). Nonetheless, it is hard to ascertain how each short-term impact affects long-term population outcomes; contextual information is essential when evaluating the biological relevance of observed short-term changes (Lusseau and Bejder, 2007). Population ecology highly influences the disturbance level that can be tolerated in any given area; small and closed populations, unable to avoid the disturbance, are the most sensitive, while open populations can withstand a higher probability of interacting with WW vessels (New et al., 2020). In mainland Portugal, WW primarily focuses on offshore populations, except for the Sado Estuary (e.g., Luís et al., 2014). Despite the operation of many vessels targeting these diverse cetacean populations, there remains a significant gap in our understanding of the impacts of WW activities on them.

Between 1998 and 2008, WW started showing substantial growth, claiming Portugal approximately 23% of total WW revenues in Europe – the most significant portion of the continent (O'Connor et al., 2009). There are 90 tourism companies licensed for WW in mainland Portuguese waters, 52 of which operate off the south coast, known as the Algarve (ICNF, 2024). WW activities in Portugal are regulated by the Decreto Lei n.º 9/2006 which stipulates conduct rule such as the prohibition of more than three vessels to remain within a 100-meter radius of the animals and observation limits of 30 minutes. Currently, no studies have focused on evaluating regulation compliance in the Algarve. However, observations made during behavioural research show that over a ten-year period, 36.5% of observations on the Algarve coast were made in the presence of three or more vessels and that the highest number of ships and recorded infractions are concentrated between Portimão and Albufeira (Grave, 2022). The lack of effective oversight and data scarcity contribute to many unlicensed companies operating, a problem further exacerbated by insufficiently trained staff unable to provide accurate information to the public (Castro, 2010). To further complicate management, a long-term monitoring program has never been established, and most of the existing knowledge for the region is published as grey literature, with peer-reviewed publications being scarce (Morais et al., 2021). The first broadscale and long-term assessment of cetacean diversity off the Algarve was done using records from WW companies' social media accounts and identified 15 species, of which *Delphinus delphis* and *Tursiops truncatus* were the most observed (Morais et al., 2021).

These two dolphin species are global and abundant and have a wide distribution in temperate, subtropical and tropical waters of the Atlantic, Pacific and Indian Oceans (Jefferson and Waerebeek, 2002). In *The IUCN Red List of Threatened Species,* they have been classified as "least concern" (Braulik et al., 2021; Wells et al., 2019), although both have at least one subpopulation that is locally vulnerable, endangered or critically endangered (Bearzi, 2003; Bearzi et al., 2012; Birkun, 2012; Currey et al., 2013). WW has been shown to cause a series of short-term impacts on *D. delphis* and *T. truncatus*; vessel collisions (e.g. Dwyer et al., 2014; Martinez and Stockin, 2013), behavioural changes such as a decrease in socialising and resting bouts (e.g. Sitar and Parsons, 2015), reduction of foraging time coupled with increased travelling time (e.g. Cecchetti et al., 2018), changes in group cohesion and dynamics (e.g. Nowacek et al., 2001) and changes in acoustic behaviour (e.g. Rako Gospić and Picciulin, 2016; Van Ginkel et al., 2018). The Algarve coast is an essential area for both species as it functions as a nursery and breeding ground (Castro, 2010; Castro et al., 2020; Grave, 2022; Vieira, 2017). Changes in acoustic parameters might be particularly significant in more sensitive groups such as those comprising mother-calf pairs; thus, further understanding the changes in the overall acoustic repertoire of dolphins, the relationship between these changes, the behavioural budget and group composition in the presence of disturbance factors to assess potential implications at the population level is essential (Rako Gospić and Picciulin, 2016).

Studying cetaceans presents many challenges. During vessel-based observations, the effects of the research vessel on the animals cannot be avoided entirely and must be considered when establishing the "control" group (Guerra et al., 2014). Land-based observations provide an expanded coverage area (Zahn et al., 2022) without directly impacting the animals (Hoyt and Parsons, 2014). On the other hand, acoustic behaviour studies can provide insights into impacts that are not as visible at the surface. One approach to understanding acoustic impacts is to assess parameters of vocalisations such as frequency (kHz), range (kHz), duration (s), whistle rate and types, inflection points, steps and click rates in the presence and the absence of vessels (Alves da Silva, 2022; Rako Gospić and Picciulin, 2016; Van Ginkel et al., 2018). In this study, we used land-based observations as a non-invasive method to assess the environmental pressure of WW vessels in the Algarve, coupled with acoustic behaviour analysis to determine the impact of this activity on *D. delphis* and *T. truncatus*.

The impact of WW activities in the Algarve is still poorly studied, and this knowledge gap impedes the development of adequate mitigation measures and conservation programs. Tourism continues to be a vital part of the Algarve's economy, and WW activities are not likely to slow down as thousands of tourists visit every year. The availability of WW operators to contribute to studies in the region (e.g. Castro et al., 2020; Moura et al., 2012), the present one included, shows a willingness to collaborate for a common goal of sustainability. WW vessels spend a potentially large amount of targeted time near cetaceans; therefore, if adverse impacts are to be reduced and the industry is to be sustainable, regulators, stakeholders and operators must understand the levels of vessel noise and their effects (Arranz et al., 2021). Furthermore, tourists express concerns about the proximity to the whales, the high number of boats surrounding mother and calf groups, the speed of the boat when approaching the whales, and the fact that engine noise might disturb the animals (Finkler and Higham, 2004; García-Cegarra and Pacheco, 2017), which goes to show that improving WW guidelines and implementing proper management and mitigation measures benefits not only the species involved but also the industry itself (Bentz et al., 2016). In the Algarve coast, there is currently no estimative of how long the animals are exposed to WW and the extent of the impact this activity has on the animals in this region is not known.

The present work aims to provide a quantitative analysis of the impact of WW activities in the Algarve by combining acoustic analysis and land-based observations to determine short-term changes in acoustic behaviour and how long the animals are exposed to this stressor. Based on previous studies, we hypothesise that whistle parameters (lower frequency (kHz), higher frequency (kHz), range (kHz), duration (s), whistle rate and types, inflections, and steps) are different between recordings in the presence and absence of WW boats. We also hypothesise that clicks per minute are lowered in the presence of ships as the dolphins detract from foraging activity. Furthermore, we hypothesise that pressure by boats is higher around the "Barlavento" zone than the "Sotavento" and that boat number limits established by law are frequently exceeded. The results of this study might give some more background not only in the issues revolving around WW on the south coast of mainland Portugal but also on this activity worldwide, helping to identify specific management priorities and mitigation strategies that are appropriate for this region and elsewhere, thus aiding in creating a more sustainable industry.

2.2. Materials and Methods

2.2.1. Land-based observation

Five viewpoints were chosen across the Algarve Coast: two in the "Sotavento" region, Farol de Santa Maria and Vilamoura, two in the "Barlavento" region, Farol de Alfanzina and Albufeira, and one in the western side of the coast, Farol de São Vicente (fig. 2.1). In three cases the observations were made from a lighthouse, Farol Santa Maria, Farol de São Vicente and Farol de Alfanzina. For the viewpoints in Albufeira and Vilamoura observation was done from the cliffs.

Figure 2.1 - Map of the Algarve coast showing the location of the five viewpoints.

Observations were carried out between 7:30 and 20:00, from 27/09/2021 until 12/10/2023, throughout all months and lasted about 90 minutes each. Bresser 20x80 binoculars were fixed to a tripod and rotated from side to side to scan the field of view; for each observation, three scans of approximately 30 minutes were made.

For each sighting, weather conditions (sea state, cloud cover and visibility), species, behaviour, group size and number of boats per type (maritime touristic (MT) boats, fishing vessels and other vessels) were collected. Only sometimes was it possible to identify species; the closest possible level of identification was made. Also, it was not always possible to distinguish the boat type, so we just counted all boats present regardless of type.

2.2.2. Acoustic behaviour analysis

WW trips were used as a platform of opportunity for data collection for this study. Trips were two and a half hours long, and a biologist was on board responsible for acoustic sampling collected from random transects under the permit AOC/01/2024. Data was collected between April 2022 and October 2023. The audio files were recorded by deploying an Aquarian AS-1 Hydrophone; Linear range: 1Hz to 100kHz ±2dB; Receiving Sensitivity: -208dBV re 1µPa (40µV / Pascal), out the side of the boat at 5 meters depth. The control group "no boats" refers to recordings when the vessel making the recordings was the only one present and had the engine off. The species present, date and notable observations were registered during the recording. Any recordings with other species, more than one species, less than a minute or no vocalisations were discarded. The files were divided into 1-minute samples, each assigned a unique number.

Samples were analysed using the advanced audio spectrum editor SpectraLayers Pro 10 by Steinberg Media Technologies. Spectral modelling synthesis (Fierro and Välimäki, 2022; Verma and Meng, 2000) ("unmix" function set to "components") was used to separate transient sounds (targeting the pulsed sounds, i.e. echolocation clicks), tonal sounds (targeting the narrow-band tonal sounds, i.e. whistles) and noise. Since this analysis did not intend to look at volume, the three components were individually edited to enhance the clarity of the spectrogram by increasing the intensity (i.e. volume) and resolution of transient and tonal components and decreasing the noise intensity. The enhanced spectrograms were visually analysed; clicks were marked using time markers, and whistles were marked using spectral markers. Additionally, whistle type, inflections (i.e. changes in slope from negative to positive and vice versa) and steps (i.e. unmodulated or "flat" patches within a modulated whistle) were recorded in the note section of each marker. Time markers provide timestamps corresponding to each click. Spectral markers provide starting time, duration (sec), lower frequency (kHz), range (kHz) and notes (which were used for whistle types, inflections, and steps). All data was summarised in two Excel files (one for clicks and one for whistles), which also contained sample numbers, audio file references and the presence/absence of boats.

Whistle types were classified into A (unmodulated/constant), B (upsweep), C (downsweep), D (convex), E (concave), and F (sine) (Ansmann, 2005; Bazúa-Durán and Au, 2002; Taubitz, 2007). Small modulations at the start or the end of a whistle were considered “further modulation” if they had less than half the central part's frequency span and were not considered for the “type” classification. However, they affected the number of inflections and steps. For example, a B whistle with one inflection would have a further modulation at the start or end but not long enough to classify it as a D or E.

2.2.3. Data analysis

Data analysis was performed in R using RStudio 2023.12.1, packages dplyr, tidyr, readxl, tidyverse, stats and nortest were employed, ggplot2 and patchwork were used for data visualization. Statistical analysis of acoustic parameters, whistle and click rates was done through a non-parametric Wilcoxon test, as the data was not normally distributed. Whistles were individually used to analyse lower frequency (kHz), higher frequency (kHz), range (kHz), duration (sec), inflections, and steps. Higher frequency was obtained for each whistle by summing lower frequency and range. The data was summarised by sample number and boat presence to analyse click and whistle rates. For the whistle “types” distribution, a weighted chi-squared test was used as our sample sizes were significantly different for the presence and absence of boats. The three boat-type data were combined into a total boat column used to create a violin plot for land observations. To analyse the hours of impact, the total number of observations (with or without sightings) was multiplied by 90 minutes, and the number of observations with MT boats was multiplied by 30 minutes, assuming the maximum legal time of observation was respected. Observations with only non-MT boats were not considered as no duration estimative was available.

2.4. Results

2.4.1. Changes in acoustic behaviour

We analysed total of 267 samples, corresponding to 82 minutes for *D. delphis* and 188 minutes for *T. truncatus*. There were 2442 and 1418 whistles for *D. delphis* and *T. truncatus*, respectively. There were recordings from each month with exception of January, February, and August, with April and May being represented the most.

Both species present statistically significant differences in range (W = 724169, p-value $< 2.2e^{-16}$ and W = 269160, p-value = $4.7e^{-07}$), which resulted from a downshift in the higher frequencies (14.6 kHz to 12.8 kHz for *D. delphis* and 14.2 kHz to 12.8 kHz for *T. truncatus*) which was also statistically significant (W = 708318, p-value $< 2.2e^{-16}$ and W = 311316, p-value = $1.5e^{-10}$). Both species also showed statistically significant differences in the number of inflections (W = 629050, p-value = $1.3e^{-12}$ and W = 250363, p-value = 0.007). In both cases,

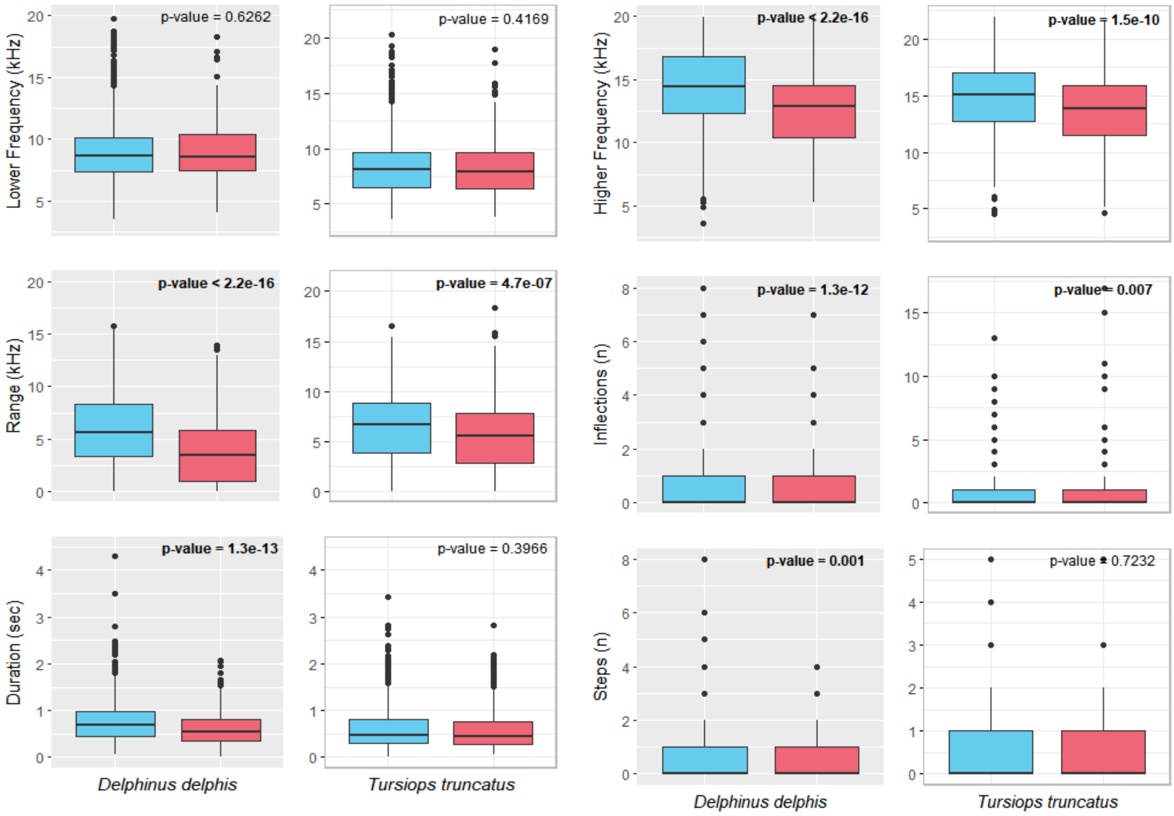

Figure 2.2 - Mean, standard deviation, standard error, and outliers for all whistle parameters. Red boxes represent the presence of boats, and blue boxes represent the absence of vessels. The background colour indicates the species: grey for *D. delphis* and white for *T. truncatus*. P-values for the non-parametric Wilcoxon test are at the top right corner; statistically significant results are bolded.

the number of inflections decreased with the presence of boats from 0.8100 to 0.4930 for *D. delphis* and 1.043 to 0.8638 for *T. truncatus*. Regarding duration and number of steps, only *D. delphis* presented statistically significant differences (W = 645554, p-value = 1.3e-13 and W = 494126, p-value = 0.001 respectively). Whistles in the presence of boats were shorter, 0.6053 seconds compared to 0.7395 seconds, and the increased number of steps was 0.5749 compared to 0.4756. *T. truncatus* did not show statistically significant differences for these parameters (W = 238612, p-value = 0.3966 and W = 234149, p-value = 0.7232).

The whistle "types" distribution only showed statistically significant differences for *D. delphis*, with simpler whistle types (A - unmodulated, B - upsweep, and C - downsweep) being much more prevalent in the presence of boats than in their absence (Fig. 2.3).

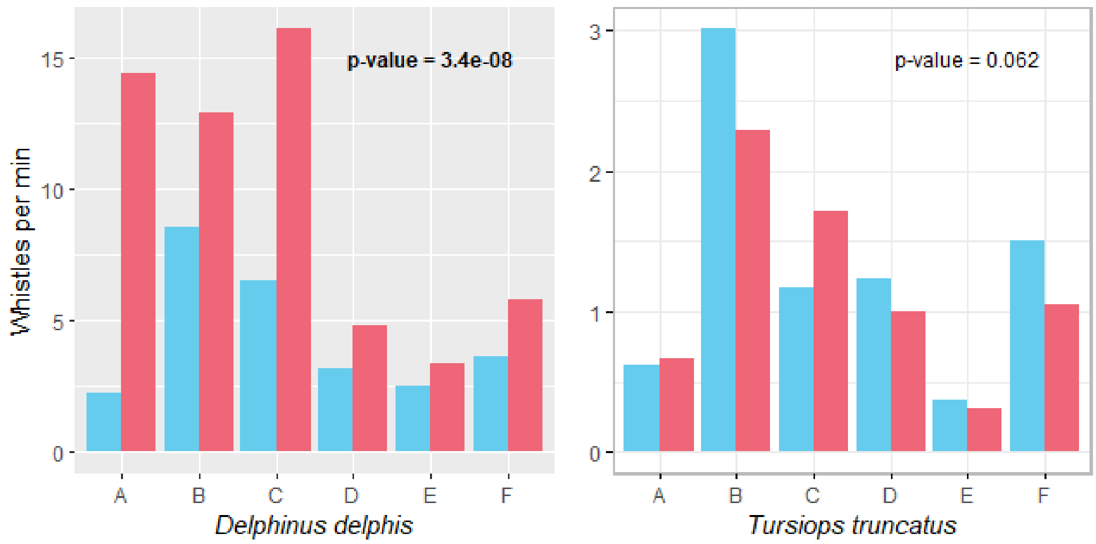

Figure 2.3 - Relative frequency of whistle types. Red bars represent the presence of boats, and blue bars represent the absence of boats. Background colour indicates species; grey for *D. delphis* and white for *T. truncatus*. P-values for non-parametric Wilcoxon test at the top right-corner, statistically significant results are bolded.

The whistle and click rates presented opposite results for the two species (fig. 2.4). *D. delphis* presented statistically significant differences in whistle rates (W = 119.5, p-value = 0.048) with an increase from 37.46 to 63.78 whistles per minute. No statistically significant differences were shown for click rate (W = 435, p-value = 0.219). *T. truncatus*, on the other hand, did not show statistically significant differences in whistle rate (W = 1267, p-value = 0.600) but did show statistically significant differences in click rate (W = 5361, p-value = 0.001) with a decrease from 327.81 to 218.63 clicks per minute.

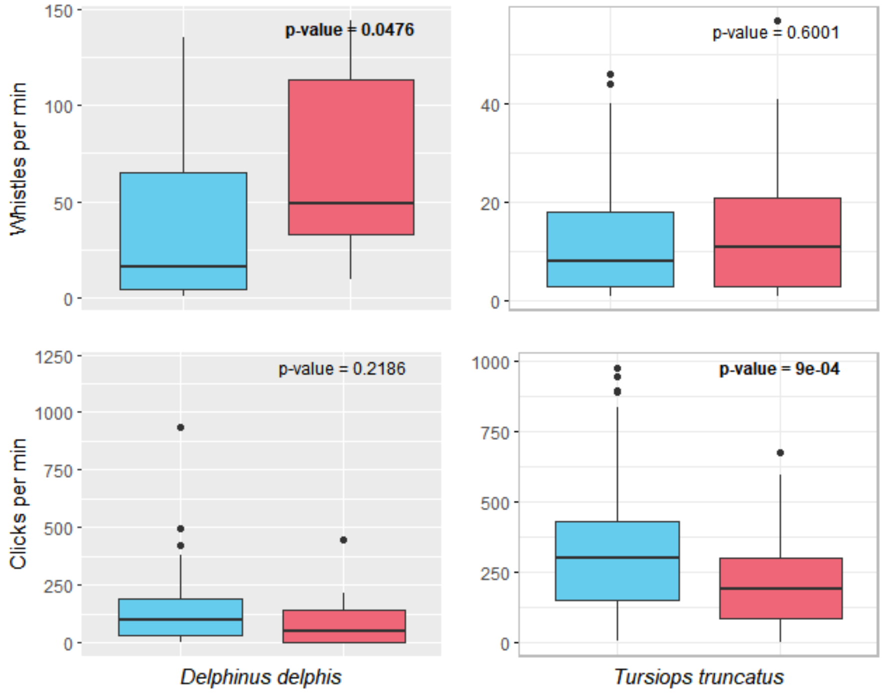

Figure 2.4 - Mean, standard deviation, standard error, and outliers for whistle and click rates. Red boxes represent the presence of boats, and blue boxes represent the absence of boats. Background colour indicates species; grey for *D. delphis* and white for *T. truncatus*. P-values for non-parametric Wilcoxon test at the top right-corner, statistically significant results are bolded.

Neither species had statistically significant differences concerning lower frequency (W = 528919, p = 0.626 and W = 238346, p-value = 0.417).

2.4.2. Land-based observation

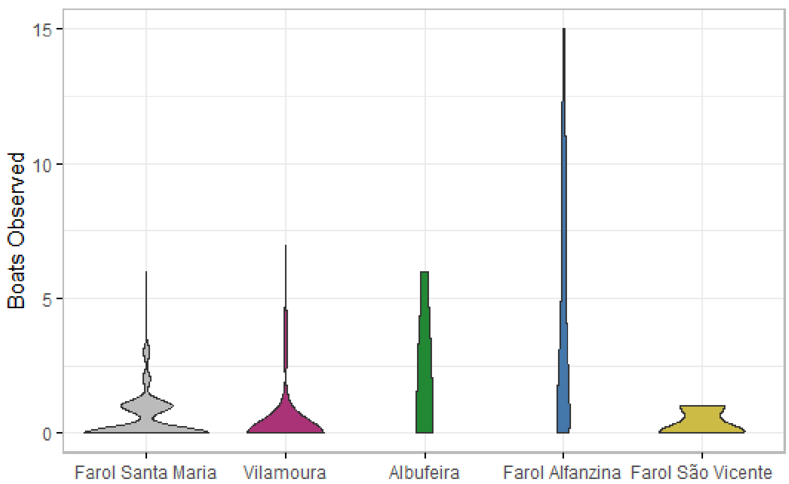

Figure 2.5 - Relative frequency of total number of boats observed for each viewpoint. The width of the shaded areas represents the proportion of observations in which that number of boats was present. Viewpoints are ordered geographically from East (left) to West (right).

We conducted a total of 178 observations, corresponding to 267 hours of effort, among the five viewpoints. Farol de Alfanzina had the most significant number of boats recorded by far, reaching a total of 15 ships observed around the animals at one time (fig. 2.5). The two viewpoints in the “Sotavento” side of the coast (Farol de Santa Maria and Vilamoura) had far more observations with no boats and under two ships. The two viewpoints on the “Barlavento” side (Albufeira and Farol de Alfanzina) had a more homogeneous distribution, from 0 to 15 ships. Farol de São Vicente had no observations with more than one boat.

From the observations, we estimated the percentage of daylight time that the animals are exposed to WW and the percentage of time when current regulations regarding the number of vessels are not followed (Table 2.1). Albufeira, Farol de Alfanzina, and Farol de Santa Maria had, respectively, the highest percentages regarding time spent in the presence of boats (38.89%, 32.14% and 30.14%). However, there is a big contrast between Farol de Santa Maria and the two Barlavento viewpoints in the percentage of time with more than three boats, which for Santa Maria was only 2.35% of the time boats were present, corresponding to 0.71% of the total time the animals were exposed to this stressor. More than three vessels were present in the Barlavento viewpoints 42.66% and 66.67% of the time during which boats were present. These results mean that the animals in this area could spend 16.67% and 21.43% of their daylight time in the presence of over three vessels.

Table 2.1 - Estimated time that the animals were in the presence of MT boats and that the number of MT boats exceeded the legal limit (three ships) for each viewpoint. Viewpoints are ordered geographically from East (left) to West (right).

	Sotavento		Barlavento		Western coast
	Farol de Santa Maria	**Vilamoura**	**Albufeira**	**Farol de Alfanzina**	**Farol de São Vicente**
Daylight time in the presence of boats (%)	30.14	8.69	38.89	32.14	22.22
Observation time with more than three boats (%)	2.35	33.33	42.86	66.67	0.00
Daylight time with more than three boats (%)	0.71	2.90	16.67	21.43	0.00

It is also worth pointing out that, although we did not record specific data for legislation breaches other than several vessels, during our observations, we often saw poorly performed approaches and vessels positioned in a circular form around the animals instead of parallel to each other, even more so when there were many boats.

2.5. Discussion

The present study demonstrates that whale-watching (WW) activities influence biologically significant behaviours in two species along the Algarve coast, especially in the "Barlavento" region. Notably, there is an uneven distribution of WW activities across the Algarve coast, with a predominant concentration of vessels in one region. Consequently, cetaceans in this area are exposed to intensive WW activities during many daylight hours.

As the data collection for this study was done using WW operators as a platform of opportunity, there are some limitations. We could not measure the noise levels and acoustic parameters of the boat engines, or the noise level received by the animals. This data would have been important for a more robust acoustic analysis and recording comparison. We also did not have access to the number of boats present during the recordings and therefore could only analyse differences in “presence” and “absence”, but it is known that with an increasing number of boats, effects also increase. Furthermore, we did not have access to data on group composition and behaviour, which could have been further correlated with changes in vocalizations. Nonetheless, as this is an initial analysis in an area that lacks previous research on the subject, the information acquired is relevant to highlight a serious conservation concern that urgently needs further, more detailed studies.

2.5.1. Short-term changes in Acoustic Behaviour

The acoustic data reveals distinct impacts of WW boats on the two dolphin species studied, with *D. delphis* exhibiting more pronounced changes in vocalization patterns compared to *T. truncatus*. *D. delphis* showed alterations in five of the six whistles' acoustic parameters tested. Overall, *D. delphis* whistled more frequently in the presence of boats, and their whistles were shorter, simpler, and less modulated (increased number of A type, less inflections, and increased steps). Additionally, they reduced frequency range by downshifting the higher frequencies while the lower frequencies remained stable. These results are in agreement with prior observations of decreased frequency range and whistle simplification (Fouda et al., 2018). *T. truncatus*, on the other hand, only showed modifications in three parameters, with the same trend of decreasing frequency range by lowering higher frequencies and some simplification through decreased number of inflections. Most prior studies for these two species indicate that they tend to increase the frequency of their vocalizations in the presence of vessel noise, supposedly as a coping mechanism to counteract acoustic masking (e.g. Heiler et al., 2016; Papale et al., 2015). Nonetheless, dolphins decreasing the frequency of their vocalizations in the presence of noise is not unheard of. Decreased frequency and modulation for *Tursiops aduncus* in the presence of increased ambient noise have been reported (Morisaka et al., 2005). Rako Gospić and Picciulin (2016) showed that while dolphins significantly increase frequency when background noises are between 63 Hz to 2 kHz, they significantly reduce their whistle maximum frequencies, range, and the number of inflections when background noises are in higher frequency ranges (2 to 20 kHz). To successfully compensate for acoustic masking, dolphins may choose frequency bands which minimize background noise (Papale et al., 2015), shifting their whistle frequencies to the range with lower noise interference to increase transmission efficiency and detectability of their signals (Rako Gospić and Picciulin, 2016).

Different types of vessels have different effects on dolphin vocalization parameters (Luís et al., 2014). WW and small research vessels with outboard engines have been found to produce noise received by the animals in the frequency band between 2 and 12.5 kHz and depend primarily on speed; at speeds lower than 2.5 knots, measured noise levels are comparable to background noise (Jensen et al., 2009). For the present study, we could not evaluate the noise produced by the WW boats. Assuming the WW vessels used in the Algarve produce noise in a similar frequency range, our results agree with Rako Gospić and Picciulin (2016), and the animals could be shifting their vocalizations down to compensate for increased mid-high frequency noise.

Another factor that might influence how dolphins cope with increased ambient noise is the group composition. In the presence of tour and research vessels, *T. truncatus* groups without calves have been found to lower the frequency and duration of their whistles, while groups with calves would increase both frequency and duration when exposed to the same stressor (Guerra et al., 2014). The only previous study in the Algarve found that both species increased whistle frequencies in the presence of WW vessels (Alves da Silva, 2022). It is interesting to note that data collection for that study happened between June and August 2022 (Alves da Silva, 2022), which coincides with peak calving season for both species (Murphy et al., 2009; Wells and Scott, 2009). In the present study, we collected data over a period of 19 months, with most recordings coming from April and May, before calving season. That being said, we are more likely to have recordings of groups without calves than Alves da Silva (2022) which may explain the differences between the results.

When it comes to the whistle "types" distribution, only *D. delphis* had significant differences between presence and absence of boats. The distributions we obtained were very similar to that of previous studies with upsweeps and downsweeps being the most prevalent types (Ansmann, 2005; Griffiths, 2009; Pagliani et al., 2022; Petrella et al., 2012), which helps sustain the hypothesis that whistle repertoire is at least somewhat stable within species even across long geographical distances. We found an increase in the three less modulated whistle types in the presence of boats, particularly so on constant unmodulated whistles. Similarly, this type of whistle increased in areas with more significant vessel traffic and noise (Griffiths, 2009). These modifications may help reduce potential loss of information due to masking by simplifying the calls and were also reflected in reduced number of inflections.

D. delphis also whistled more frequently in the presence of boats. This has been observed before (Buckstaff, 2004; Heiler et al., 2016; Scarpaci et al., 2000) and could reflect an effort to keep communication despite increased noise. This change might also suggest that group cohesion is affected by the approach of WW vessels causing the animals to increase whistling to better ascertain the whereabouts of other group members (Hawkins and Gartside, 2009; Scarpaci et al., 2000). Behavioural studies have also pointed to changes related to maintaining group cohesion, such as decreasing interanimal distance and changing in heading altering course away from approaching ships (Nowacek et al., 2001). Alternatively, it may reflect the heightened levels of excitement experienced during interactive behaviours (Hawkins and Gartside, 2009). Dolphins are known to be interested in and interact with vessels (Samuels and Bejder, 2004) and increases in whistle rate have been related to interactive behaviours (Hawkins and Gartside, 2009).

T. truncatus did not show any significant differences in whistle types or whistle rates. These results seem to suggest that, at least when it comes to whistling patterns, *T. truncatus* is not as affected by the presence of WW vessels as *D. delphis*. *T. truncatus* in the Algarve coast are concentrated close to shore living near human activities (Vieira, 2017), for this reason they might show a greater level of habituation to anthropogenic impact (Bejder et al., 2009). On the other hand, *T. truncatus* did show a decrease in click rate, which was not shown for *D. delphis*, indicating that the impact for this species might be more related to foraging rather than social behaviour. *D. delphis* feeds mostly on small pelagic schooling fish (Silva, 1999) while *T. truncatus* feeds primarily on demersal fish (Giménez et al., 2017). Given the ecological niche of the two species these results might indicate that *T. truncatus* is more impacted when it comes to foraging behaviour as they might be more dependent on echolocation for prey detection and acquisition. *T. truncatus* has been shown to decrease foraging activity by 49% in short-term while in the presence of boats (Pirotta et al., 2015).

When it comes to click and whistle rates, ideally, we would have divided the number of clicks and whistles in a sample by the number of animals present to eliminate group size effects; however, we did not have access to this data. Nonetheless, the fact that for both species, whistle rate increased (although for *T. truncatus* it was not statistically significant) and click rates decreased (although for *D. delphis* it was not statistically significant) shows that these trends cannot be attributed simply to changes in group size. Previous studies have also found that the whistle rate does not relate to the number of dolphins (Hawkins and Gartside, 2009); therefore, the changes observed seem to be related to the presence of boats.

2.5.2. Implications and further research

Although it is hard to ascertain the extent to which these changes are harmful, short-term changes in behaviour might lead to long-term impacts by detracting from biologically significant behaviour such as foraging (Cecchetti et al., 2018); for example, if reduced foraging results in reduced prey capture, it can lead to decreased energy acquisition (Lusseau et al., 2009). Long-term decline in abundance has been shown for the Indo-Pacific bottlenose dolphins of Shark Bay, Australia (Bejder et al., 2006). If the click rate decrease observed for *T. truncatus* in this study translates into a decrease in prey capture, it might lead to a decline in population. Low genetic diversity and connectivity in *T. truncatus* small coastal populations mean such perturbations could have drastic effects (Nykänen et al., 2019). Resting and social behaviour can also be biologically significant, affecting fitness and reproduction success. Reduction in reproduction success and long-term area avoidance resulting in habitat displacement has been linked to unsustainable dolphin-watching tourism in Fiordland, New Zealand (Lusseau et al., 2006). As the function of the whistle is communication (Dudzinski et al., 2009), the impact observed here for *D. delphis* could impair their communication capability, affecting population dynamics by disrupting social behaviour. This becomes even more problematic when it comes to groups with infants and juveniles who are learning these communication patterns under stressful conditions. Therefore, further studies in this area must confirm the tendencies observed here. If possible, recording vessel noise under controlled conditions and having a volume reference across recordings would allow for the evaluation of volume changes which might also be relevant. Adding further data to the acoustic parameters, such as group sizes, behaviour, number of boats and time of the day will also bring new insights onto how this activity is affecting the animals.

As several studies have shown changes in behaviour in the presence of WW boats (Parsons, 2012) and whistle characteristics are significantly correlated to behaviour (Ansmann, 2005), it cannot be discarded that the changes observed might simply reflect the changes in behaviour. For example, decreased mean maximum frequency and range, coupled with increased steps have been correlated to travelling (Ansmann, 2005) and increasing in travelling has been correlated with WW vessel approach (e.g. Sitar and Parsons, 2015). However, the question of “do vocalizations change because behaviour changed, or do they change their behaviour because acoustic masking limits the behaviours they can still perform?” is not easy to answer. This is particularly relevant when discussing management approaches; if acoustic masking is the source of these changes, then minimizing noise might be a good enough solution, however if the simple presence of the boats is enough to cause changes, then different approaches such as “no go” zones might be necessary. It is likely that both factors interact with each other and that the answer to this question isn’t a clear-cut one but instead a complex dynamic. Pirotta et al. (2015) found that while noise level alone cannot explain changes in echolocation activity for *T. truncatus*, the mere presence of boats is also insufficient to elicit an activity change. Further studies focusing on behaviour as well as acoustic behaviour can help clarify some of these issues, however the big challenge of acquiring data for a true “control” in the absence of boats while also collecting behavioural data remains. Nonetheless, acoustic studies can provide a great insight into disturbances that are not so easily observed at the surface and, even if these changes are purely behavioural, can be used as a proxy which, with increasingly powerful software available, has the potential to be less subjective than behaviour observations.

2.5.3. Impact distribution in the Algarve coast and Management recommendations

It is clear from this and from previous studies (e.g. Grave, 2022) that the "Barlavento" region is the most affected area in the Algarve coast, not only by the largest number of boats but also by the highest number of law infringements. Our results indicate that *T. truncatus* and *D. delphis* have, respectively, their foraging and social behaviours potentially disturbed for up to 39% of daylight hours in the "Barlavento" area. Although we have not examined the correlation of the observed changes with the number of boats, previous studies have shown that larger number of boats increases acoustic behaviour changes (e.g. Pirotta et al., 2015). Not only are dolphins exposed to WW boats for longer in, the "Barlavento", but also, they are in the presence of over three boats for up to 66% of the time during which they experience this stress. In the area observed from Farol de Santa Maria the animals were exposed to WW vessels for 30% of daylight time, but only 2.35% of this time, the supposedly safe number of boats established by law was exceeded. Further studies are needed to establish better the correlation between the number of boats and the effects on acoustic behaviour. Furthermore, it is important to estimate the effect of this disturbance on behavioural budgets and infer the meaning of these changes for the animals. If they simply shift their activities to accommodate this 39% of time interacting with WW vessels and can compensate for this time when boats are not present, then the disturbance would not be as harmful as losing this foraging and socializing time completely.

While we can make management recommendations based on the different manners in which each species is affected, the compliance issue must be at the front of any conversation aiming to create a sustainable industry. Even when licenced WW operations present high levels of compliance, they do not consider recreational, opportunistic and unlicenced vessels which approach the animals unaware or simply choose to ignore legislation (Allen et al., 2023). Proper enforcement and monitoring of conduct, as well as public education, is of utmost importance, even before any new measures can be implemented. This is particularly true for high-demand areas such as the "Barlavento".

Staggered departure times and continued interactions involving multiple boats mean dolphins are exposed for several hours even if all operators obey the 30-minute rule (Allen et al., 2023). So aside from improvements in monitoring and enforcement, improved rules of con duct must also be developed. Our results illustrate how different species (and populations) react differently to the same stressor; therefore, effective rules should consider biologically relevant information about each target species. In the case of *D. delphis*, it seems it would be best to approach the animals during foraging activities but avoid approaching or at least reduce contact during social situations. For *T. truncatus*, on the other hand, the opposite could be recommended, avoid approaches during feeding bouts and favour interaction during social moments. Establishing "no go" zones and speed restriction zones can also help reduce impact by giving the animals a safe area to retreat to avoid disturbance and noise. Although noise reduction equipment can be used on the boats, further research needs to be conducted on the effectiveness of this strategy as it has been pointed out that changes in acoustic behaviour are not due to ship noise alone (Pirotta et al., 2015).

In conclusion, both species are affected by WW activity on the Algarve coast, especially in the "Barlavento" region, where the animals are frequently exposed to more vessels than the legal limit. While the correlation and causation between acoustic behaviour and behaviour changes are not fully understood, changes in vocalization patterns are an appropriate tool to assess disturbances that are not readily observable and give insight into what is happening underneath the surface when the animals are exposed to various stressors. Effective regulations should consider biologically relevant characteristics of each target species, as the results presented illustrate that the effects of WW are not the same across species and, therefore, neither can be the mitigation measures. This study provides one of the first assessments of WW impact on the Algarve coast, highlighting an issue that needs immediate attention if this industry is to be sustainable and successful.

2.6. Acknowledgements

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