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Influence of Dolphin-Watching Tourism Vessels on the Whistle Emission Pattern of Common Dolphins and Bottlenose Dolphins

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Abstract: Recent years have seen a notable rise in dolphin-watching boat activities along the Algarve coast in Portugal, potentially affecting the common dolphin (*Delphinus delphis*) and bottlenose dolphin (*Tursiops truncatus*) local populations. This study examines the impact of increasing underwater noise levels from these boats on dolphin vocalizations. Field recordings were conducted from June to September 2022, analyzing dolphin whistles in various boat presence scenarios. The results indicate significant changes in whistle-frequency characteristics with boat presence, including increased start, low, and high frequencies, alongside a decrease in the number of inflection points in modulated whistles. The changes might negatively impact dolphin populations viability, underscoring the need for further research. Additionally, improved mitigation strategies may be necessary to reduce the potential negative effects of dolphin watching on cetacean communication and behavior in the Algarve region.

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

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1. Introduction

Tourism activities associated with the maritime sector play an important role in the socio-economic development of the coastal regions [1]. However, negative impacts resulting from marine traffic (e.g., underwater noise, chemical pollution) are known to disturb biological communities and marine habitats—for instance, by reducing biodiversity and inducing changes in animals' behavior [2–4]. Among the different forms of marine pollution, underwater noise generated by human activities and its potential negative effects on marine ecosystems has gained ever more attention [5], particularly when concerning impacts on marine mammals (e.g., ref. [6]).

Dolphins produce a wide range of vocal and non-vocal sounds [7], which they use, for instance, for navigation and exploring the environment, social interactions [8], feeding, and detecting predators [9,10]. Most dolphin species can produce two primary types of sounds considered relevant in social interactions: (i) tonal frequency-modulated whistles and (ii) rapid-repetition-rate "burst-pulse" click trains [11]. Tonal vocalizations such as whistles are considered to be cohesion calls and communication signals to recognize members in a social group and maintain physical and vocal contact [12,13]. This type of vocalization is longer than 100 milliseconds and is emitted in frequencies varying between 0.8 and 38 kHz [14–17]. Whistles can be catalogued by visually inspecting the spectrograms and shape of the whistle contour [16,18–20]. Despite difficulties in classifying whistles due to the lack of a standard classification technique [21], ref. [16] classified whistles into (i) ascendant (initial frequency less than final frequency, without inflection points); (ii) descending (initial frequency greater than final frequency, without inflection points), (iii) modulated (more than one inflection point, descending to ascending, or vice versa); and (iv) flat (no frequency variation).

Short-term behavioral responses of cetaceans to underwater noise resulting from anthropogenic sources include an increase in group cohesion, dive duration and travel behavior [22–24]. Changes in breathing rate and in surface behavioral patterns were also reported, with a decrease in the time that animals spend at the surface after the approaches of dolphin-watching boats [25,26], including a reduction of aerial behaviors and the interruption of feeding, social and resting activities [27]. Another important behavioral response of cetaceans to underwater noise comprises changes in the frequencies of the acoustic signals and number of vocalizations as an attempt to overcome the "masking" effects of background noise [28]. For instance, in Almirante and Dolphin Bays (Panama), bottlenose dolphins (*Tursiops truncatus*) modify the frequencies of the whistles to maintain acoustic contact depending on the type of boat traffic [29]. Ref. [30] also found that the common bottlenose dolphins inhabiting La Paz Bay, Mexico, changed the characteristics of whistles in the presence of vessels: oceanic ecotypes decrease the whistles frequencies in the presence of small vessels, while coastal ecotypes show an opposite trend.

In the Algarve region, on the southern coast of Portugal, at least fourteen species of cetaceans have been reported [31,32]. Common dolphin (*Delphinus delphis*) and bottlenose dolphin (*Tursiops truncatus*) are among the species most frequently observed and are one of main target species of dolphin-watching tour boats operating in the region [33,34]. Currently, 90 tourism companies are licensed to operate tour boats in mainland Portuguese waters, of which 52 operate on the southern coast of Portugal [35]—38 more companies than in 2010 [33]. The number of vessels operating per company in the Algarve varies between 1 and 15, representing a total of 131 boats, on average taking three trips per day (André Cid, personal communication). Portuguese law regulating dolphin-watching activities (Decreto-Lei n. ° 9/2006) defines that vessels are considered inside the perimeter of a group of dolphins when present at less than 300 m of dolphins. To minimize anthropogenic impacts on dolphins' health and behavior, the code of conduct establishes a limit of three boats within a 100 m radius of the group of dolphins.

Marine traffic and underwater noise contribute to changes in whistle structure and increased energy expenditure among dolphins [36–38]. The spatial overlap of dolphins' habitat with dolphin-watching activities in the Algarve is high, hence assessing the potential impacts of this activity on the conservation of common dolphins and bottlenose dolphins is of utmost importance [33]. In this study, the whistle characteristics and potential changes in the emission patterns of common and bottlenose dolphins were investigated in the presence and absence of dolphin-watching boats. Hypotheses were formulated positing that whistle characteristics would vary according to the number of boats, due to the increase in the intensity of underwater noise. The results presented herein may contribute to improving the mitigation measures currently applied in the Algarve, such as maintaining minimum distances, limiting boat numbers, and regulating observation time, aiming to reduce the negative effects of anthropogenic activities on cetaceans inhabiting the region.

2. Materials and Methods

2.1. Study Area

The study area was located in the Algarve region, south coast of Portugal, extending from Cape St. Vicente—Sagres (37°1.35′ N, 8°59.81′ W) to Faro (37°1.07′ N, 7°56.10′ W) at a maximum distance of 25 nautical miles (nm) from shore (Figure 1). The Algarve is situated in the transitional waters between the Atlantic Ocean and the Mediterranean Sea, serving as a passage and habitat for several cetacean species (e.g., ref. [39]).

All cetacean species in mainland Portugal are protected by national legislation (Decree-Law nr 263/1981 from 3 September), European regulations (Habitats Directive), and international conventions (Bern, Bonn, CITES, ACCOBAMS). The national laws restrict the production of noise near cetaceans that could attract or disturb them. Additionally, no more than three touristic platforms are allowed within a 100 m radius of a cetacean or a group of cetaceans, and it is considered that platforms are in proximity to a cetacean or group of cetaceans if in less than a 300 m radius (Decreto-Lei n. ° 9/2006).

During the summer, the Algarve experiences a threefold increase in its human population due to thousands of tourists choosing this holiday destination. Consequently, coastal pressure intensifies, particularly in localities like Albufeira, where dolphin-watching tourism vessel activities are in high demand.

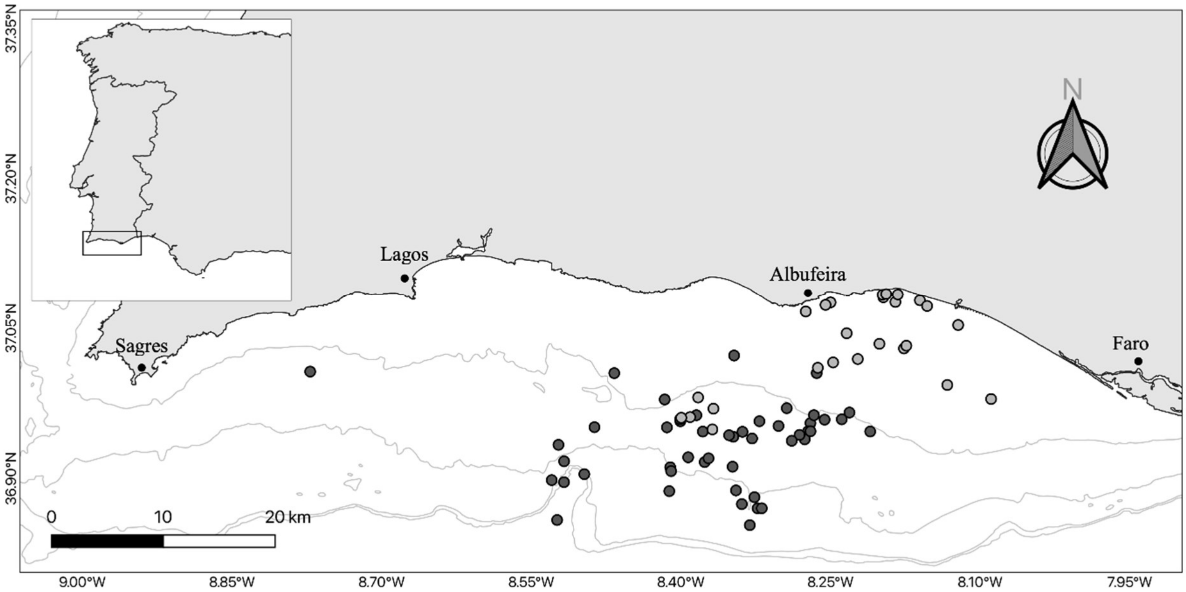

Figure 1. Map of the study area in the Algarve—southern coast of Portugal. Dots indicate the start locations of the acoustic surveys for the two species studied: light grey—*Tursiops truncatus*, dark grey—*Delphinus delphis*.

2.2. Data Collection

Vocal recordings were collected from groups of common dolphins and bottlenose dolphins from June to August 2022 using the focal follow technique [40]. We used group focal follows instead of individual focal follows considering the difficulties of identifying individual dolphins or determining which individual is vocalizing [41]. A group of dolphins was defined as an aggregation of individuals swimming in a coordinated manner within 100 m of each other while displaying the same type of behavior [42].

The surveys for acoustic records were conducted using a 7 m RHIB with a four-stroke 135 hp outboard engine departing from the port of Albufeira (37°04′53.4″ N 8°15′38.2″ W). These surveys took a random course until encountering a group of dolphins. Depending on the sea state conditions (≤3 according to the Beaufort scale, swells < 1.5 m, good visibility (>5 km), and no precipitation), surveys were conducted for approximately 6 h per day (09.00–16.00) at an average speed of 12 knots. Acoustic records were collected on board a 6.7 m long rigid-hull inflatable research boat powered by a single 135 hp outboard engine. During the surveys, more than four observers were positioned on the boat to observe in all directions while scanning the water. When a group of dolphins was located, the acoustic recording started using a calibrated autonomous hydrophone. We used a digitalHyd SR-1 autonomous hydrophone (Marsensing, Faro, Portugal), a compact acoustic recorder equipped with a SQ-26 transducer (SensorTech, Dartmouth, Canada) (sensitivity is −194 dB re 1 V/1 μ Pa with a variation within ±1 dB in the 1 Hz to 28 kHz interval), coupled with a high-pass filter of 50 Hz to decrease the effect of noise generated by the recording platform and low-frequency vibrations. All recordings were captured continuously at a sampling rate of 52.734 kHz and a 24-bit resolution. The data was then saved in 2 min acoustic sample files to ensure manageable file sizes. The system was operating in autonomous mode with an integrated battery and internal SD card for data storage and was deployed 2 m below the sea surface. Data on the group size and dominant behavior (i.e., travelling, socializing and foraging) were registered, as well as the number of tour boats whenever these conditions changed. Group size was estimated based on the maximum number of individuals surfacing. A group was no longer tracked if it changed behavior, split, or merged with another group. After completing the focal follow, a new random transect line was adopted.

To minimize the effect of the presence of the research boat on the animals' behavior, the following protocol was implemented: (i) dolphins were approached according to the Portuguese Law (Decreto-Lei n. ° 9/2006) that regulates the observation of wild cetaceans in mainland Portugal [43], and (ii) no data were collected during the first 15 min for the animals to get used to the presence of the research boat [33]. We used the 300 m radius of the group of dolphins as a reference, since this is the distance from which a platform is considered to be inside the perimeter of the group, according to the Portuguese Law (Decreto-Lei n. ° 9/2006). When no boats except the research boat (with its engine off) were present, the acoustic record was classified as control data—absence of tour boats, while for samples collected in the presence of at least one dolphin-watching boat within 300 m radius of the focal group, we identified three categories according to the number of boats observed: 1 boat, 2–3 boats and $\geq$4 boats. The classification of the acoustic records in the presence of tour boats in classes was defined to obtain a more uniform number of observations. Tour boats in the study area have approximately the same length and use outboard engines with similar power. Close to the boat (within a few meters), significant differences in noise levels and direction can be detected. However, as the distance from the boat increases (beyond 20–30 m), the sound disperses, and these differences become less noticeable. Based on this, the number of boats can be considered a reliable proxy for estimating the potential impact of underwater noise on dolphin communication.

2.3. Acoustic Analysis

The acoustic records collected with the hydrophone were first inspected as spectrograms and subjected to both acoustic and visual assessment using Audacity 2.4.2 to identify, categorize, and count all the "whistles" present on each sample. A whistle was defined as a tonal, narrow-band, modulated signals lasting 0.1 s or more, with at least part of the fundamental frequency above 0.8 kHz [15,16]. The fundamental frequency of each selected whistle contour was measured based on the most intense frequency within the signal, due to upper-frequency limitations (26.36 kHz) [44]. Every type of whistles was analyzed; however, when multiple signature whistles were identified following the Identification and Characteristics of Signature Whistles method—SIGID [45], just one whistle was considered for analyses. This rule was applied to reduce the risk of collecting many whistles from the same individual (pseudo-replication) [46].

The low-frequency noise can mask the lowest-frequency component of the whistles, producing an erroneous estimation of the measured frequencies [37]. Therefore, all whistles were separated into three different quality categories: (i) poor (whistle visible on the spectrogram but too faint or overlapping with other sounds); (ii) fair (whistle clearly visible from its start to its end); and (iii) good (prominent and dominant whistle). Only whistles scored as fair or good were used for analysis [46]. The whistles were categorized according to [16], as in Figure 2.

The plasticity of the repertoire and temporary shifts in whistle characteristics were measured according to seven parameters: duration, minimum, maximum, and frequency range, start and end frequency and the number of inflection points (Figure 3) using the software Raven (Raven Lite 2.0.4). This software provides a precise interface for measurement and automatically calculates the values of each parameter. The duration, minimum, maximum, and frequency range were automatically derived from the software using the selection tool (1024 point fast Fourier transform (FFT) and 512 window size, Hamming window, 50% overlap), while the start and end frequency and the number of inflection points were measured or counted manually (Table 1). A mean whistle rate was calculated for the control data and each class of number of tour boats by dividing, in each category, the total number of whistles by the number of minutes and then by the number of dolphins present in the focal group (number of whistles/individual/minute).

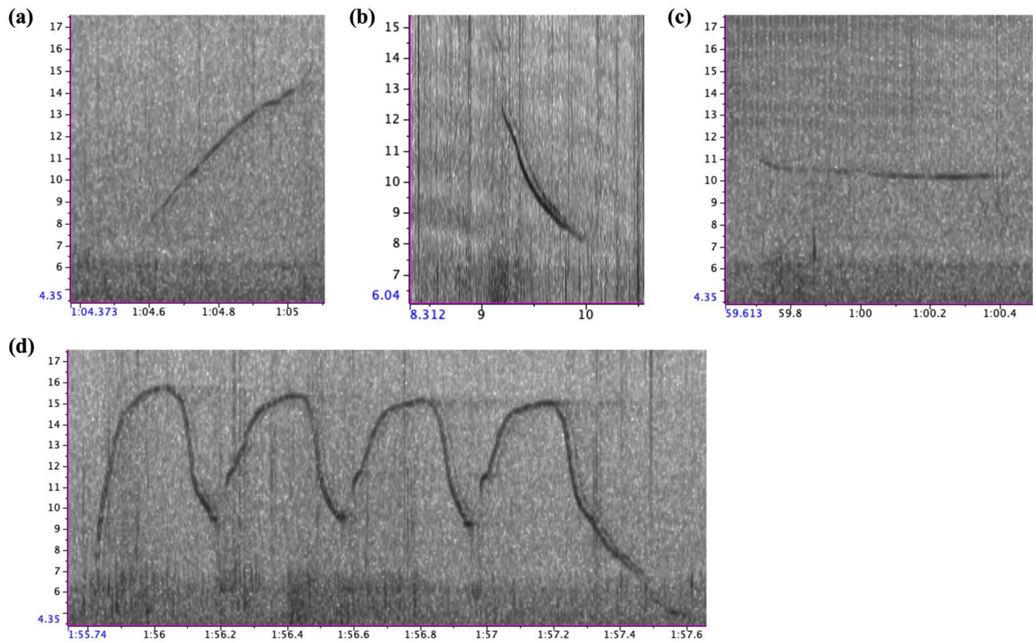

Figure 2. Spectrograms used in this study, captured from Raven software and representing the four whistle types considered in this study; (**a**) ascending, (**b**) descending, (**c**) flat, (**d**) modulated. The scale varies among spectrograms. All y-axes are in kHz and x-axes varies from milliseconds (mm:ss) to seconds (s) as samples extend beyond 1 s.

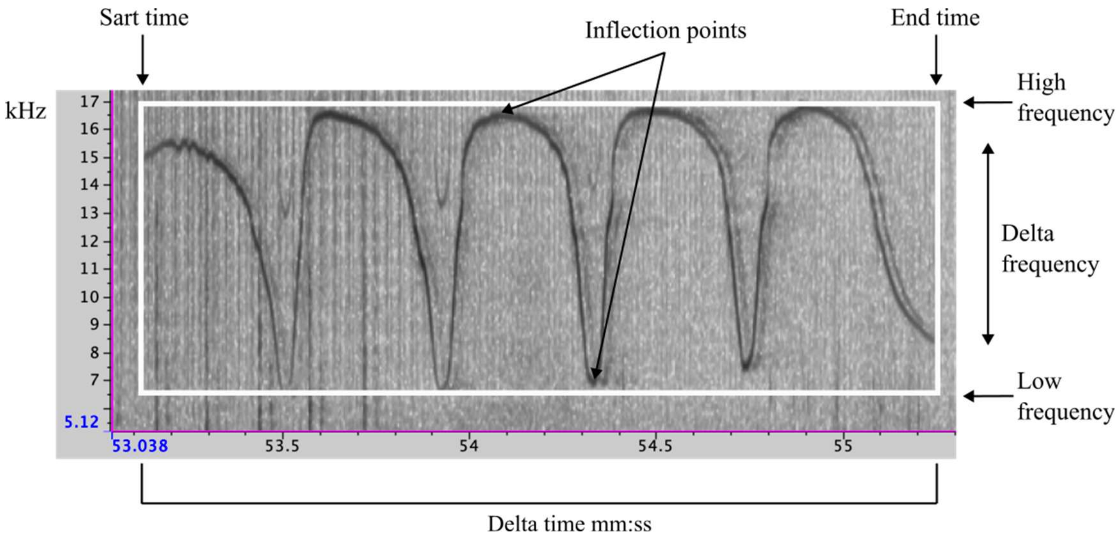

Figure 3. Example of spectrogram captured from Raven software of a whistle of bottlenose dolphins recorded in Algarve (Portugal) showing the seven variables analyzed. Fast Fourier transform (FFT) = 1024, frame duration = 2 milliseconds.

Table 1. Parameters of whistles considered in this study. Adapted from [16], with permission from Elsevier, 2024. kHz—Kilohertz.

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

2.4. Statistical Analyses

Statistical analyses were conducted to determine if the presence of tour boats (grouped in four categories: absence of boats, 1, 2–3 and ≥4 boats present) significantly affected the whistle characteristics by species. Due to the uneven number of whistle samples across the classes of number of boats and behavior types, it was not possible to include dominant behavior in the statistical analysis. As the assumptions of normality and homogeneity of variance was not valid for all data, non-parametric Kruskall–Wallis tests were used to analyze differences in the parameters of whistles according to the number of dolphin-watching boats. For significant Kruskall–Wallis results, the Conover test was applied using a Bonferroni correction to compare differences in the acoustic parameter between the four categories of number of tour boats. All statistical analyses and visualizations of results were performed using R 4.0.3 software [47] and using the packages "purrr" [48], "DescTools" [49], "rstatix" [50], "ggplot2" [51] and "ggpubr" [52].

2.5. Ethical Note

Sampling was conducted under the Instituto da Conservação da Natureza e das Florestas (ICNF) permit AOC/30/2020.

The study was entirely observational, and animals were approached according to the Portuguese Law (Decreto-Lei n. ° 9/2006) that regulates the observation of wild cetaceans in mainland Portugal (Governo de Portugal, 2006).

3. Results

From a total of 15 h of acoustic records, 149 acoustic samples of 2 min were selected (103 recordings of common dolphin and 46 of bottlenose dolphin), representing approximately 5 h of data (Supplementary Table S1). Based on record quality, a total of 1239 whistles were selected for analysis (737 whistles of *D. delphis*, 502 whistles of *T. truncatus*—Table 2). During the observation period, the maximum number of boats observed following the same group of dolphins was 13.

Table 2. Mean values (±standard deviation) of the whistles' characteristics measured for common dolphin (*Delphinus delphis*) and bottlenose dolphin (*Tursiops truncatus*) in the presence and absence of tour boats. Statistically significant differences of whistles characteristics between categories of number of boats according to the Kruskall–Wallis test are identified with Dde for common dolphin and Ttr for bottlenose dolphin. IP—inflection point; N—total number of observations; kHz—Kilohertz.

Species	Number of Boats	Start Frequency (kHz) [Dde, Ttr]	End Frenquency (kHz)	Low Frequency (kHz) [Dde, Ttr]	High Frequency (kHz) [Dde, Ttr]	Duration (Seconds) [Ttr]	Inflection Points [Dde, Ttr]	N
Delphinus delphis	0	12 ± 4.15	11.6 ± 4	8.32 ± 1.97	15.3 ± 3.35	0.862 ± 0.392	2.54 ± 2.24	340
	1	14 ± 4.29	12.3 ± 3.98	8.6 ± 1.75	17.3 ± 3.23	0.922 ± 0.4	2.41 ± 1.86	297
	2–3	12.2 ± 3.72	11.8 ± 3.84	9.35 ± 2.15	15.4 ± 3.29	0.872 ± 0.352	1.31 ± 1.29	71
	≥4	11.2 ± 3.07	13.6 ± 3.63	9.34 ± 1.1	17.4 ± 2.81	0.968 ± 0.273	1.52 ± 1.09	29
Tursiops truncatus	0	7.38 ± 2.97	11.1 ± 4.55	6.26 ± 1.84	15.2 ± 3.02	0.761 ± 0.52	2.54 ± 2.44	221
	1	7.83 ± 2.46	11.3 ± 5.45	6.76 ± 2.07	16.1 ± 3.1	0.985 ± 0.53	3.02 ± 2.51	140
	2–3	9.46 ± 4.49	12.8 ± 4.89	8.92 ± 4.09	17 ± 2.44	0.966 ± 0.627	2.43 ± 2.67	37
	≥4	9.65 ± 3.68	11.3 ± 4	7.89 ± 2.5	15.6 ± 2.43	1.13 ± 0.636	2.08 ± 2.35	104

For common dolphins, according to the Kruskal–Wallis test, statistically significant differences were found between the four categories of number of boats for the start frequency of whistles ($H_3 = 39.67$, $p \leq 0.001$). For the pair-wise comparison between the four categories of number of boats, the Conover's test revealed significant differences in the absence of boats compared to the observations made in the presence of one tour boat (Figure 4a). Moreover, the start frequency of whistles of common dolphins in the presence of one boat was significantly different from the start frequency of whistles in the presence of two or more boats.

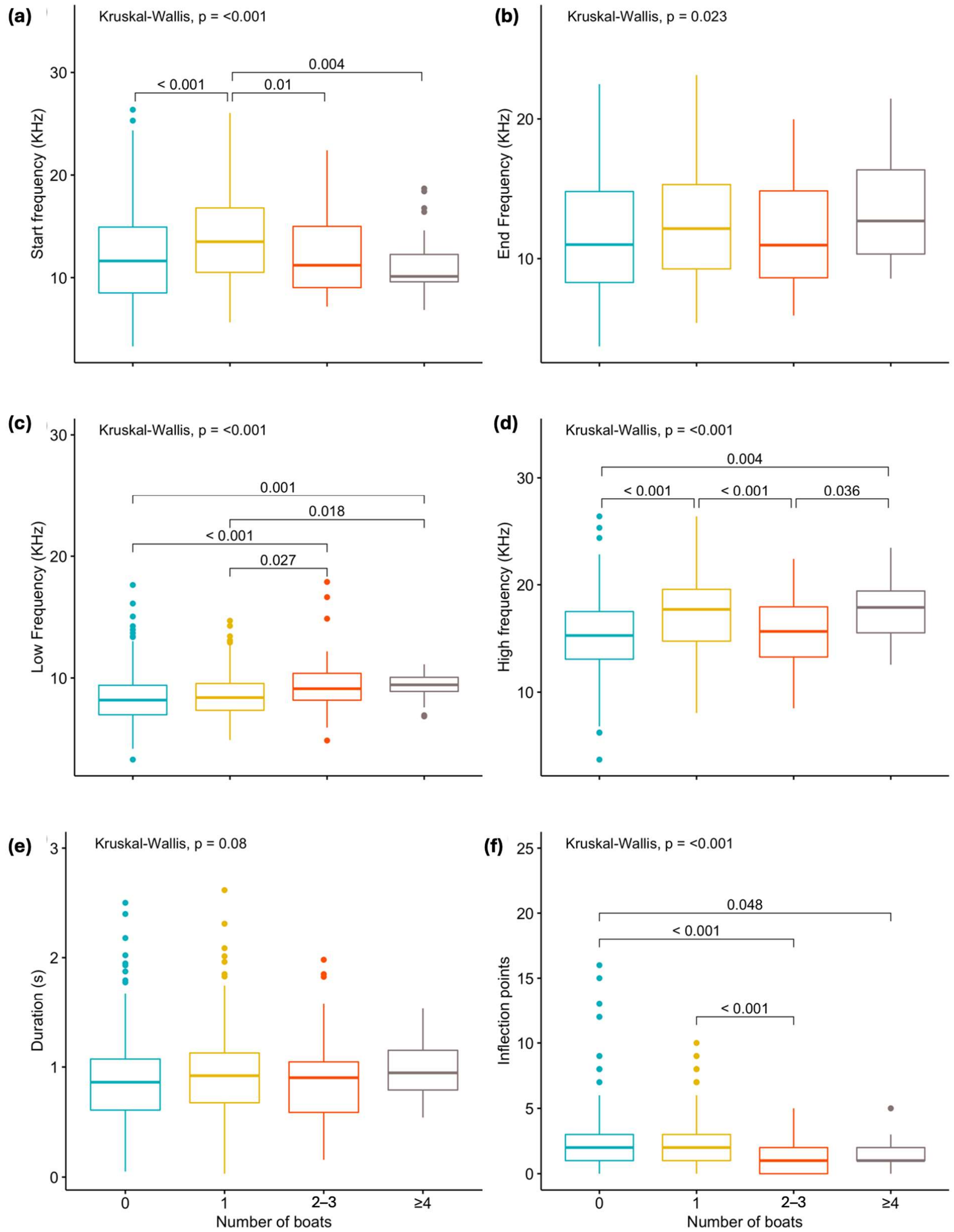

Figure 4. Boxplot with the (**a**) start frequency, (**b**) end frequency, (**c**) low frequency, (**d**) high frequency, (**e**) delta time and (**f**) inflection points of whistles for common dolphin (*Delphinus delphis*) both in the absence of tour boats and presence of 1, 2–3, and more than 4 boats. The horizontal line in the boxplots represents the median; the lower and the upper limits of the boxplot are the first and third quartiles. Whiskers show the minimum and maximum values and outliers (i.e., values within 1.5 times of the interquartile range) are represented by dots. The value of the Kruskal–Wallis test is identified at the top of each plot and the significant differences in the whistle parameter between classes of the number of boats according to the Conover's test are highlighted with the square brackets.

Likewise, significant differences were found for the low frequency of whistles between the four categories of number of boats (H_3 = 28.463, $p \leq 0.001$). For the pair-wise comparison between the four categories of number of boats, the Conover's test revealed significant differences between low frequency of whistles in the absence of tour boats compared to the presence of two or more boats, and between in the presence of one boat and two or more boats (Figure 4c).

The high frequency of whistles also showed significant differences between the four categories of number of boats (H_3 = 67.261, $p \leq 0.001$) as well as significant differences between whistles in the absence of boats and those produced in the presence of one and 2–3 tour boats. Additionally, there were significant differences between whistles in the presence of 1 and 2–3 boats, and between 2–3 and ≥4 boats (Figure 4d).

For the number of inflection points, according to the Kruskal–Wallis test, significant differences were also observed between the four categories of number of boats (H_3 = 32.467, $p \leq 0.001$). The Conover's test found significant differences in the number of inflection points between the absence of boats and the presence of more than two tour boats, and between whistles in the presence of 1 boat and 2–3 boats (Figure 4f).

Regarding bottlenose dolphins, the Kruskal–Wallis test indicated notable differences for the start frequency of whistles between the four categories of number of boats (H_3 = 38.775, $p \leq 0.001$). The Conover's test revealed significant differences between whistles in the absence of boats compared to the presence of 2–3 and ≥4 tour boats as well as between 1 boat and ≥4 tour boats (Figure 5a).

For the low frequency of whistles, the Kruskal–Wallis test also revealed significant differences between the four categories of number of boats (H_3 = 46.561, $p \leq 0.001$). The Conover's test indicated significant differences between whistles in the absence of boats and the presence of two or more boats, and between the presence of 1 boat and 2–3 and ≥4 boats (Figure 5c).

The high frequency of whistles showed significant differences between the four categories of number of boats (H_3 = 21.831, $p \leq 0.001$). The Conover's test identified significant differences between whistles in the absence of boats and those produced in the presence of 2–3 tour boats. Additionally, there were significant differences between whistles in the presence of 1 and 2–3 boats, and between 2–3 and ≥4 boats (Figure 5d).

For the number of inflection points, significant differences were also observed between the four categories of number of boats (H_3 = 32.467, p = 0.0071). The Conover's test found significant differences between the number of inflection points in whistles in the presence of 1 boat and ≥4 tour boats.

Finally, regarding the duration of whistles, the Kruskal–Wallis test showed significant differences between the four categories of number of boats (H_3 = 34.056, $p \leq 0.001$). The Conover's test revealed that the duration of whistles in the absence of tour boats was significantly different from the duration of whistles emitted in the presence of one tour boat as well as between whistles produced in the presence of 1 tour boat and ≥4 tour boats (Figure 5e).

The whistles rate varied between species and according to the number of dolphin-watching boats (Figure 6). Bottlenose dolphins produce more whistles than the common dolphins and the whistle production in both species tends to decrease with an increasing number of dolphin-watching boats.

For common dolphin, the majority of whistles registered were classified as modulated (87%), followed by ascendent (6%), descendent (5%) and flat (1%). No clear trend was observed in the frequency of each whistle type across different numbers of tour boats. (Figure 7a). A similar pattern was observed for the bottlenose dolphin, with modulated whistles dominating compared to other whistle types (Figure 7b). As for common dolphin, no clear trend was observed regarding the frequency of whistles by type as the number of tour boats increased.

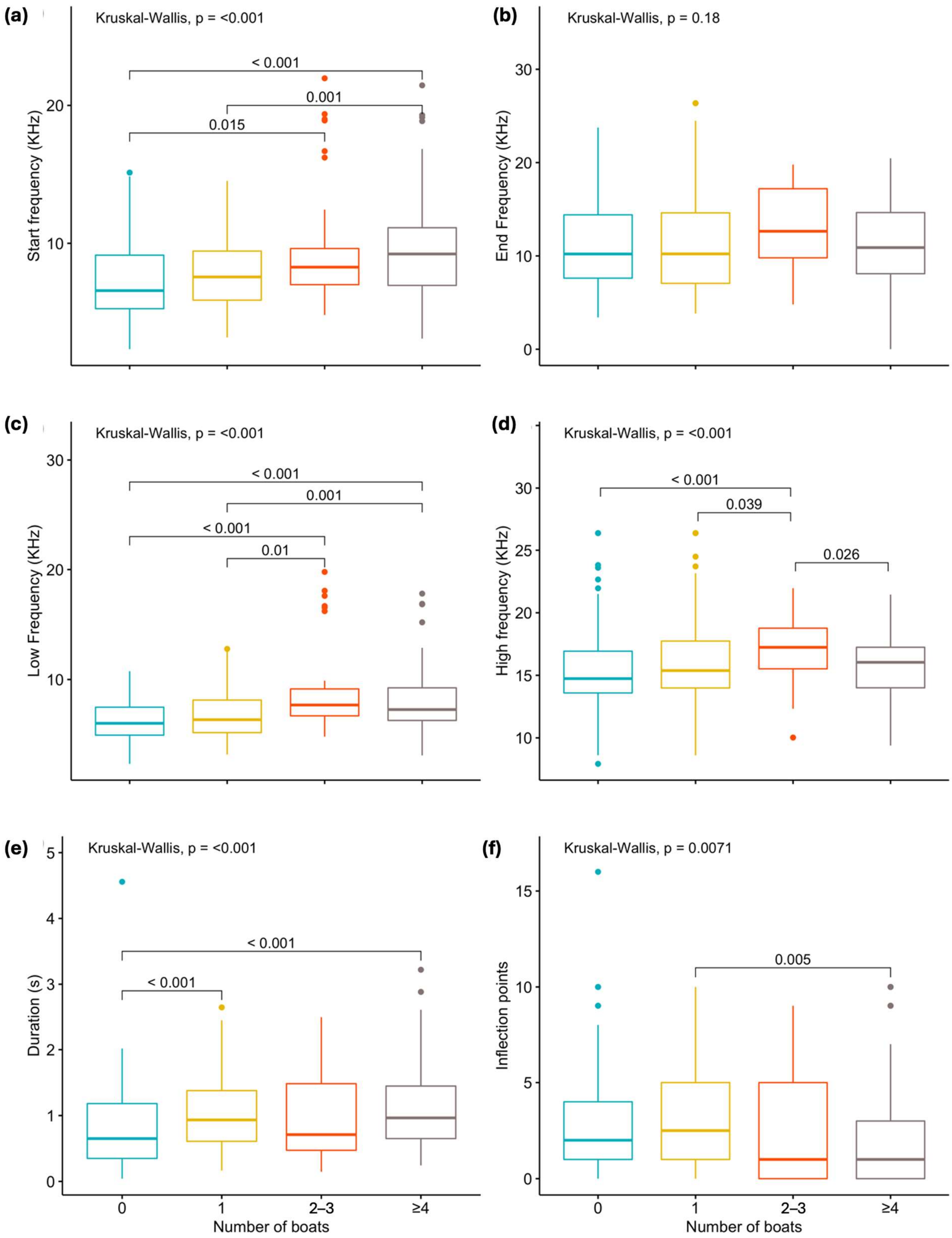

Figure 5. Boxplots with the (**a**) start frequency, (**b**) end frequency, (**c**) low frequency, (**d**) high frequency, (**e**) delta time and (**f**) inflection points of whistles for bottlenose dolphin (*Tursiops truncatus*), both in the absence of tour boats and presence of 1, 2–3 and more than 4 boats. The horizontal line in the boxplots represents the median; the lower and the upper limits of the boxplot are the first and third quartiles. Whiskers show the minimum and maximum values and outliers (i.e., values within 1.5 times of the interquartile range) are represented by dots. The value of the Kruskal–Wallis test is identified at the top of each plot and the significant differences in the whistle's parameter between classes of the number of boats according to the Conover's test are highlighted with the square brackets.

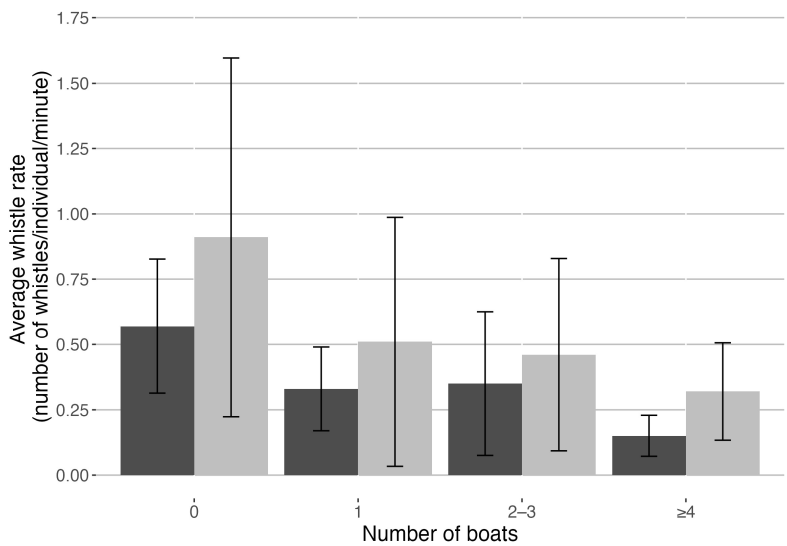

Figure 6. Average whistle rate (number of whistles/individual/minute) for common dolphin (*Delphinus delphis*, dark grey) and bottlenose dolphin (*Tursiops truncatus*, light grey) according to the number of boats. Error bars represent the standard error.

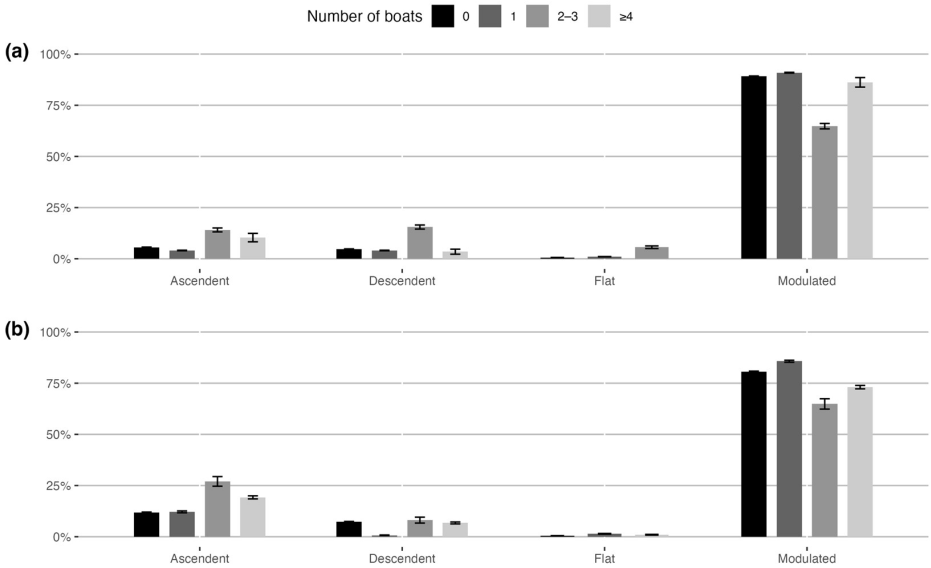

Figure 7. Percentage of ascendent, descendent, flat and modulated whistles for (**a**) common dolphin, *Delphinus delphis*, and (**b**) bottlenose dolphin, *Tursiops truncatus*, according to the number of boats. Error bars represent 95% confidence interval.

4. Discussion

This study found that the presence of dolphin-watching tour boats within a 300 m radius of groups of common dolphins and bottlenose dolphins inhabiting the Algarve significantly affects the acoustic structure of the whistles produced. For some whistle characteristics (e.g., start and high frequency), these changes were detected even in the presence of just one tour boat. Adjustments in the acoustic behavior of animals in the presence of tour boats have been reported in several cetaceans, possibly to optimize signal transmission [29,37,41,53,54]. Our findings demonstrate that the vocalizations of both common and bottlenose dolphins exhibit a significant increase in both low and high frequencies when one or more tour boats are present, compared to when no dolphin-watching boats are nearby. For instance, common dolphins increased the whistles' low frequency in ~1 kHz in the presence of two or more tour boats, while the high frequency increased in ~2 kHz in the presence of one or more dolphin-watching boats. Regarding bottlenose dolphins, our results show an increase in the low frequency of whistles in 1.63–2.66 kHz in the presence of two or more dolphin-watching boats and in the high frequency in ~2 kHz in the presence of 2–3 tour boats. It is important to note that the range of variation in these frequencies can sometimes span up to 10 kHz. In addition to the natural variability in these parameters, the results might also highlight significant potential shifts in vocalization patterns in response to anthropogenic noise. These results are in agreement with [29], who found that bottlenose dolphins in Dolphin Bay, Panama, increase whistle frequency by ~2–4 kHz in the presence of dolphin-watching tourism boats. A similar pattern was reported for Walvis Bay, Namibia, where bottlenose dolphins show an increase of 1.99 kHz in the frequency of several whistle characteristics when subjected to the presence of tour boats [15]. In Portugal, studies carried out on a resident population of bottlenose dolphins in the Sado estuary reported significant changes in the high frequency of whistles in the presence of tour boats from 14.21 kHz to 15.33 kHz and of trawlers from 14.21 kHz to 12.46 kHz [54]. The differences observed in the intensity of these changes may be related, for example, to the proximity of dolphins to the boats and their type. Changes in whistle frequency are a common short-term response of marine mammals to noise in order to increase signal detection or compensate for masking effects [29,54,55]. The spectral overlapping of boat noise can lead to a reduction in the range at which dolphins' whistles can be heard by conspecifics [15,37]. We also noted a reduction in the high frequency of whistles in bottlenose dolphins when more than four boats were present, contrary to the observed trend for the other classes of number of boats. This observation suggests that communication patterns are altered by the presence of boats, and that an increase in the number of boats does not necessarily imply a stronger response.

Significant changes were also observed in the number of inflection points. For common dolphins, the number of inflection points decrease significantly when in the presence of two or more tour boats. For the bottlenose dolphin, the number of inflection points suffered a slight increase in the presence of one dolphin-watching tour boat compared to the control conditions, followed by a significant decrease as the number of boats increased. These findings align with those from [56], who reported that dolphin whistles displayed simpler contour shapes under increased ambient noise conditions. Such simplifications in whistle structure might be a strategy to minimize information loss caused by noise masking. This reduction in contour complexity could potentially impact the dolphins' ability to recognize individual callers, as the frequency contours might be important for individual identification.

Bottlenose dolphins exhibited variable changes in call duration in response to the presence of boats. When no boats were present, the call duration served as the baseline. This duration increased by approximately 32% in the presence of one boat and by 88% when more than four boats were present. However, when 2–3 boats were present, the call duration slightly decreased. Although these patterns were not consistent across all situations, increases in call duration in the presence of tour boats have been reported for several delphinid species [57–59]. Extending the call duration has been proposed as a mechanism used by animals to increase the probability of detection in high noise conditions [60] in an attempt to overcome the impacts of tour boats' underwater noise.

Although bottlenose dolphins produced more whistles per minute than common dolphins, a reduction in the whistles rate was observed in both species, as the number of touristic boats increased. Different factors can influence the number of whistles detected per minute, including group size [61], behavior and group composition [57], and direct interactions between dolphins and boats [37,41,55,58]. While a reduction in whistle rates is commonly observed in marine mammals as a response to anthropogenic noise [28], potentially to reduce energy expenditure, the standard error associated with our measurements suggests a high degree of variability in the results. This variability indicates that there is significant uncertainty regarding the observed pattern, and additional studies with larger sample sizes are needed to confirm these findings.

The vocal responses induced by the noise of maritime–touristic boats may have biological costs for dolphins, including an increased risk of detection by competitors or predators, the degradation of signal effectiveness in social contexts, and energy costs related to changes in metabolic demands or activity budgets to increase the amplitude, duration, and/or repetition rate of acoustic signals [62]. For instance, the higher frequencies observed in some whistles characteristics when animals are subjected to the presence of tour boats could be a strategy to reduce masking effects, resulting in increased energy expenditure [15].

The Portuguese guidelines for dolphin watching stipulate that platforms are considered inside the perimeter of a group of dolphins when at less than 300 m, and that no more than three platforms are allowed in an area within a radius of 100 m from the animals, to avoid making noise and attracting or disturbing them. We found significant effects of underwater boat noise on the whistle structure within the 300 m distance threshold, which could suggest that a distance of 100 m may not be enough to overcome impacts from dolphin-watching tour boats and the underwater noise. Further studies should consider both the 300 m and 100 m threshold. Ref. [29] suggests as a mitigation strategy a reduction in the number of boats and duration of contact with the animals, as well as an increase in the time between interactions among boats and animals, to reduce the impact of tour boats. Since the study area is an important tourist destination, the anthropogenic pressures are expected to remain high. Consequently, further research is essential to enhance our understanding of the impacts of underwater boat noise on the studied species, including whether these impacts signify deeper effects or are minor disturbances that do not compromise dolphin population viability. This includes investigating how underwater boat noise may influence group behavior, group structure and the development of younger individuals, particularly given the study area's critical role as a nursery ground [33,63]. Additionally, while our results suggest the potential benefit of revisiting the current 100 m regulation, it is important to note that our study measured the number of boats within 300 m of the dolphin group but did not specifically calibrate the vocalizations against the exact distance of boats to individual dolphins. Given that tour boats often approach closer to the 100 m limit and may occasionally cross it, we recommend this as a topic for further research to ensure that any regulatory changes are based on comprehensive evidence. Finally, although the number of boats is an adequate proxy for assessing dolphin auditory stress, future research should consider the distinct noise emissions produced by different boat types to enhance risk mitigation strategies of underwater noise on dolphins.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/oceans5040044/s1, Table S1: Number of 2 min acoustic samples analyzed by species and number of boats.

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