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Acoustical Behavior of Delphinid Whistles in the Presence of an Underwater Explosion Event in the Mediterranean Coastal Waters of Spain

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Abstract: Underwater noise is a significant pollutant produced by anthropogenic activities carried out in the sea. Several types of acoustic sources can potentially have adverse impacts on marine fauna, especially on cetaceans. The vocalization response of cetaceans to underwater noise varies depending on noise characteristics such as duration, bandwidth, and intensity, as well as the species being insonified. Some studies report changes in vocalization properties due to continuous noise, but there is a lack of knowledge regarding impulsive noise sources, especially those related to explosive events. It is known that underwater explosions represent a serious threat to marine fauna because it produces one of the highest sound pressure levels introduced by anthropogenic activities. In this communication, an opportunistic study related to changes in the dolphin vocalizations was performed by considering two scenarios (i.e., before and after a detonation event). The acoustic raw data were recorded by a passive acoustic device installed in a mooring line deployed in the Mediterranean coast of Spain. The objective of the experimental installation was to monitor the underwater sound pressure level in the framework of the development of the Marine Strategy Framework Directive (MSFD) in Spain. A detonation event of unknown origin was recorded during the monitoring period while Delphinids were vocalizing, allowing for the observation of their acoustic reaction to the explosion. The study considers the number of vocalizations, morphology of whistles, and spectral characteristics before and after the explosion. The results obtained indicate that the number of whistles, their complexity in terms of morphology, and spectral components vary due to the explosive event, showing significant differences that will be presented and discussed in this communication.

Keywords: underwater noise; detonation; bioacoustics; dolphin vocalization whistle

PACS: 43.30.+m; 43.60.−c; 43.30.Sf; 92.20.Ny

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1. Introduction

Many types of human activities can lead to an increase in underwater sound pressure levels (SPLs) in the sea. An underwater explosion (UNDEX) is a human activity that poses a high risk to marine fauna living in the vicinity of the areas where detonation takes place. This is mainly due to the amount of energy released into the aquatic medium in a very short period of time. The SPL may exceed 250 dB re 1 μPa at 1 m [1,2], creating shock waves followed by a subsequent loading effect (known as the bulk effect) [3,4]. Marine fauna can be affected in different ways and severity depending on the distance from the source and the amount of energy released in the explosion. The effect of the explosions on fish has been studied and reported to point out that the presence of swim bladder plays an important role in terms of the vulnerability of fish species with respect to the distance from them to the explosive source [5]. Regarding marine mammals, explosions represent a significant threat to their life. The conditions caused by explosions depend on the amount of energy reaching the animal. Animals located close enough to the explosion can receive the impact of the shock wave in their membranes, soft tissues, and cavities filled with air, suffering blast traumas together with other conditions, such as brain damage or intestinal hemorrhaging, among others [6,7]. As the distance increases, the amount of energy traveling through the medium decreases, and other types of conditions can be produced in marine mammals, such as permanent or temporary shifts in hearing sensitivity [8,9], behavioral changes [2], and an increased probability of entanglement [10], to name a few. In recent years, several studies have investigated the effects of underwater noise on the vocalization characteristics of cetaceans and the behavioral changes of animals living in noisy areas [11–13]. Some publications have focused on the continuous noise generated by ship traffic and its potential impact on the acoustic behavior of odontocetes. Some authors have reported variations in the vocalization characteristics of Bottlenose dolphins, such as start frequency, end frequency, minimum frequency, maximum frequency, duration, or the number of inflection points, in response to the presence of vessels near the studied groups [14,15]. The scientific literature highlights the importance of also considering the morphology of vocalizations, with studies reporting a simplification in the whistles of Bottlenose dolphins due to the increasing SPL of underwater noise, mainly caused by ship traffic [16]. However, there is limited information available regarding the effects of impulsive sound sources, such as explosive events, on the acoustic behavior of cetaceans. Lammers et al. [17] studied the potential impacts of naval mine neutralization exercises on Delphinids through the implementation of a long-term passive acoustic monitoring campaign.

This communication presents results related to the whistle activity of Delphinids when an UNDEX event occurs, considering the distance between animals and the location of the explosive source, and inferring possible patterns in their vocalization rate. However, aspects related to the morphology of whistle vocalizations, their spectral components, and duration remain unknown with respect to the impact of the UNDEX event on them. Specifically, whistle vocalizations in the 20 minutes before and after the detonation were analyzed using a passive acoustic recorder installed on a mooring line, as part of the Marine Strategy Framework Directive (MSFD) (https://www.miteco.gob.es/es/costas/temas/proteccion-medio-marino/estrategias-marinas/default.aspx, accessed on 16 February 2023) implementation in Spain (details in funding section), related to the monitoring of underwater noise in the Spanish Mediterranean marine demarcation. An UNDEX event was recorded opportunistically during the monitoring period, with the acoustical presence of Delphinid whistles observed at the same time as the explosion. A bioacoustic analysis of the recorded signals was performed considering both scenarios: before and after the detonation occurred. Each detected whistle was manually segmented by an acoustic operator to deduce the number of vocalizations, their spectral characteristics, morphology, and duration in both situations. The details of the site, data acquisition system used, and acoustic analysis developed will be provided in the Section 2. The variations in the acoustic behavior of vocalizations will be shown and discussed in Sections 3 and 4, respectively.

This study contributes to a better understanding of how these animals vary their bioacoustic activity in the presence of a specific underwater explosion event, focusing on the 20 min before and after the detonation. It is well-known that understanding the effects of underwater noise on endangered species is necessary to comprehend the potential impact of human activities and to implement measures that protect the species and their habitats.

2. Materials and Methods

2.1. Study Site

A mooring line with an autonomous passive acoustic recorder was deployed within the Gulf of Vera (see Figure 1). The bathymetry of this area presents an irregular slope with escarpments [18]. The structural features contain a rich variety of sediments along the elongated highs with different trends, which favor large-scale mass transport. This area has a large presence of cetaceans, including *Stenella coeruleoalba* (striped dolphin), *Delphinus delphis* (common dolphin) [19], *Globicephala melas* (long-finned pilot whale) [20], *Grampus griseus* (Risso's dolphin), *Tursiops truncatus* (Bottlenose dolphin), etc. For this reason, the Gulf of Vera contains the Special Area of Conservation of "Valles submarinos del Escarpe de Mazarrón" (SiteCode: ES6200048) included in the (Natura2000 protected areas, https://natura2000.eea.europa.eu/Natura2000/SDF.aspx?site=ES6200048, accessed on 16 February 2023). It is important to note that some of the marine mammals mentioned earlier, such as the Bottlenose dolphin, are listed in the national red list or the Habitat Directive (Species Annex IV, V).

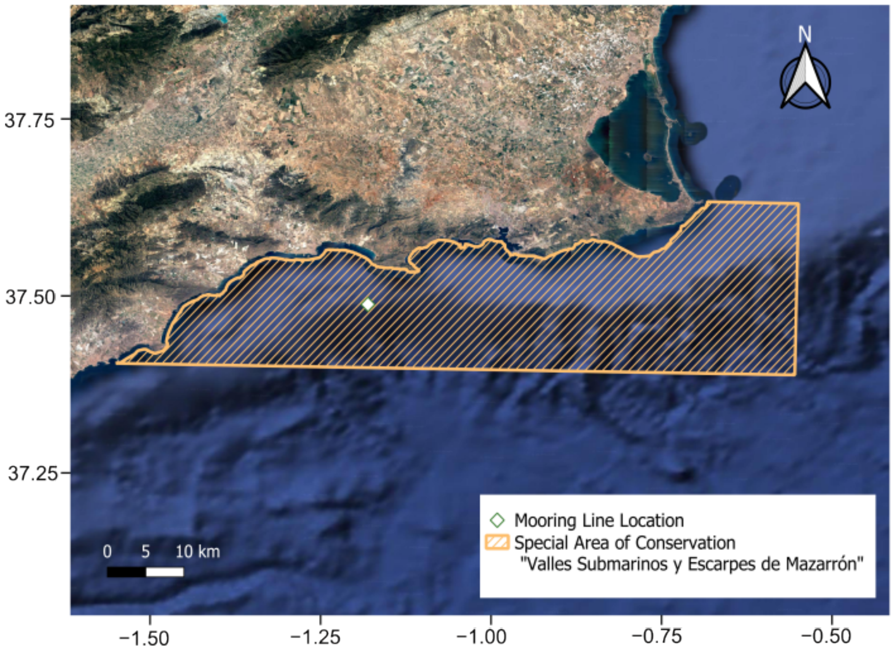

Figure 1. Detail of the mooring line location within the special area of conservation "Valles submarinos del Escarpe de Mazarrón".

2.2. Data Acquisition

Acoustic data were collected from October 28 to November 15 of 2020. Recordings were made with a passive acoustic recording device named SAMARUC (http://samaruc.webs.upv.es, accessed on 15 February 2023) [21,22]. The passive acoustic monitoring (PAM) device used in this study was equipped with a Cetacean Research C57 hydrophone with a sensitivity of −167 dB re 1 V/µPa and a flat frequency response of ±0.3 dB between approximately 10 Hz and 96 kHz. The acquisition was set to record using a sampling rate of 192 kHz at 16 bits, with an amplification of 12 dB. The duty cycle was set to 50 min on, followed by 5 min off. The technical characteristics of the acoustic recording configuration are summarized in Table 1.

Table 1. PAM recording device configuration.

Sensitivity of the pre-amplified hydrophone	−167 dB re 1V/µPa (Cetacean Research C57)
Programmable gain	12 dB
Storage capacity	2 TBytes
Sampling rate	192 kHz
Duty cycle (minutes)	50 ON/5 OFF
Channel	1 (mono) at 16 bits
Dynamic Range	93 dB
System bandwidth ±3 dB	10 Hz–96 kHz

The PAM system was deployed at a depth of 504 m in the location shown in Figure 1. The deployment followed a typical mooring line procedure (see Figure 2). The experimental setup consisted of several parts, including an anchor, an acoustic release for retrieving the PAM system to the surface, and a buoy installed in conjunction with the PAM device. The constituent elements of the mooring line were linked by a rope that implemented proper dimensions between them. It is worth noting that the distance between the hydrophone and the seabed was sufficient to distinguish between reflections produced in the seabed and the direct signal arrival.

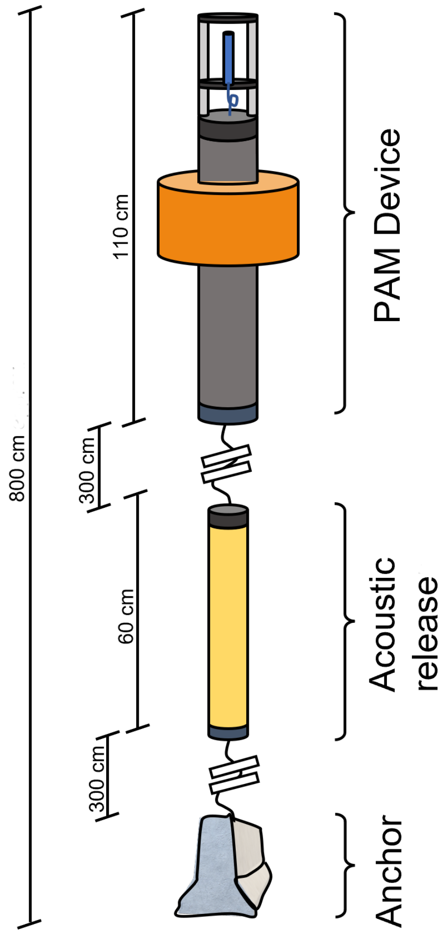

Figure 2. Details of the mooring line experimental setup. It consists of an anchor, an acoustic release, and the passive acoustic recorder integrated into a ringed buoy.

2.3. Detonation Event and Bioacoustic Analysis

Through an analysis of the acoustic data recorded during the deployment period, a detonation event was opportunistically detected. The temporal and spectral characteristics of the explosion can be observed in Figure 3.

After consulting with the competent authorities regarding the origin of the detected detonation, we were informed that an underwater explosion (UNDEX) event occurred about 1 mile from the deployment site. The recorded signal characteristics were consistent with the phenomena described in [3]. Due to the high SPL reaching the PAM recorder, the acoustic data were clipped; thus, a peak SPL greater than 150 dB ref 1 µPa occurred at the mooring line location. It can be inferred that the source level of the UNDEX event exceeded 200 dB ref 1 µPa.

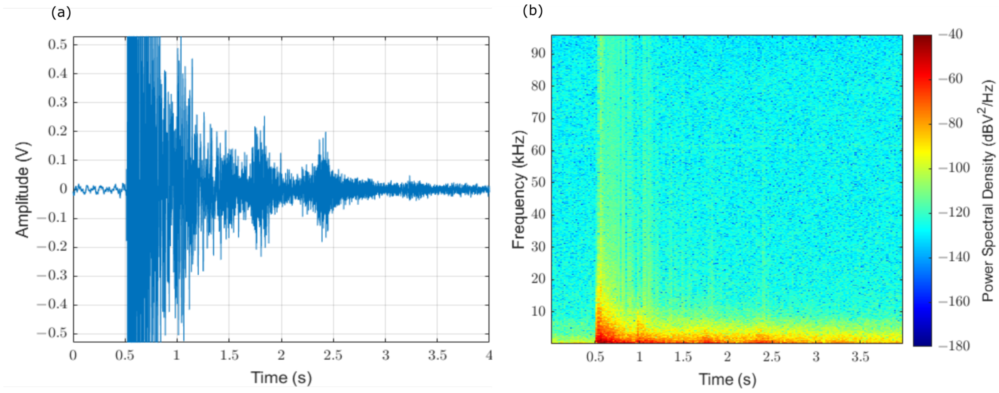

Figure 3. Details of an underwater explosion event recorded (**a**) in the temporary domain, with the Y-axis representing the output voltage of the electroacoustic chain and (**b**) in the time–frequency domain (obtained in dBV^2/Hz). It is possible to observe how the acoustical signal exceeded the allowable full-scale input ($\pm\sqrt{2} * 0.375$ V) of the SAMARUC analog-to-digital converter.

It was observed that at the time of the explosion, there was an increase in the bioacoustic activity of Delphinids in the form of whistle vocalizations. This presented an opportunity to study their acoustic behavior in the presence of an UNDEX event. The proposed analysis focuses on three aspects differentiated in relation to whistle vocalizations: rate, temporal/spectral characteristics, and morphology, considering scenarios before and after the explosion. The acoustic data analyzed included a 20-minute period before and after the detonation to observe the acoustic reaction of Delphinids inhabiting the insonified area.

More than 1000 whistles were detected manually by an experienced bioacoustic operator. Once detected, the temporal and spectral whistling features were obtained. The duration of whistles, as well as the maximum, minimum, start, end, and central frequencies, were inferred from the segmented regions of the time versus frequency domain.

With the aim of studying not only the spectral and temporal features associated with both defined situations, but also the possible changes in the acoustic behavior of the Delphinid whistles, a categorization based on the morphology related to its complexity was used, considering the number of inflection points. Figure 4 shows the considered groups, encoding the vocalizations with a number followed by the typology Up (U) or Down (D), depending on the starting frequency slope.

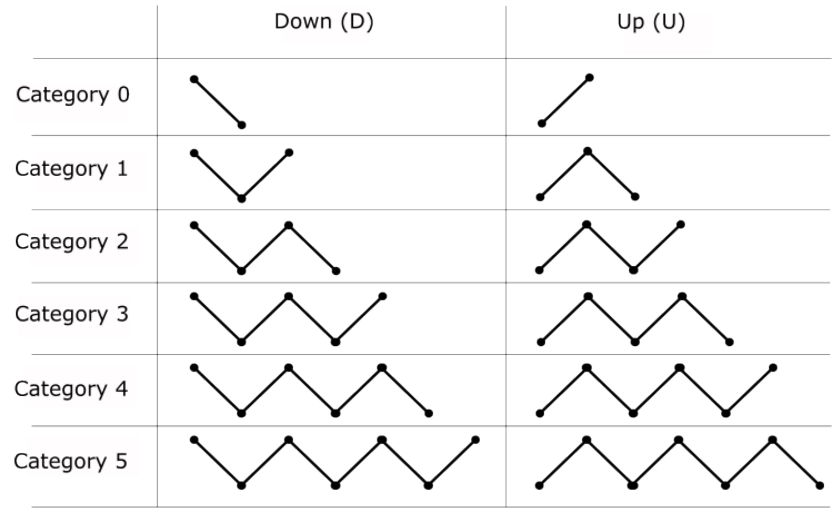

Figure 4. Classification of the whistles detected in both scenarios according to the morphology in the time–frequency (spectrogram) domain. The highest categories correspond to more complex whistles attending the morphology (number of inflection points). The nomenclature Up (U) or Down (D) corresponds to the starting frequency slope of each whistle.

The analysis consisted of several steps, which can be summarized by the scheme shown in Figure 5. The acoustic raw data were analyzed using Matlab software to obtain and inspect the associated spectrogram. A quality cut was applied to the detected whistles, neglecting harmonics or events with less than 0.2 s of duration. The dolphin vocalizations were classified based on their morphology for both scenarios. An example of a time window containing whistle events is depicted in Figure 6.

Finally, following the work of other authors [23], we extracted features related to the temporal and frequency contours of the whistle, such as duration, minimum–maximum frequencies, start–end frequencies, and central frequency. To confirm the existence of variations in the characteristics of the vocalizations after the UNDEX, a statistical analysis was carried out on these features. The Kolmogorov–Smirnov test has been used in other studies to test the normality of the acoustic features extracted from bioacoustic signals [24]. In our case, as the data were not normally distributed, the non-parametric Kruskal–Wallis test (at a significance level of $\alpha = 0.05$) was used to examine whether any of the features presented statistically significant differences between both scenarios, before and after detonation.

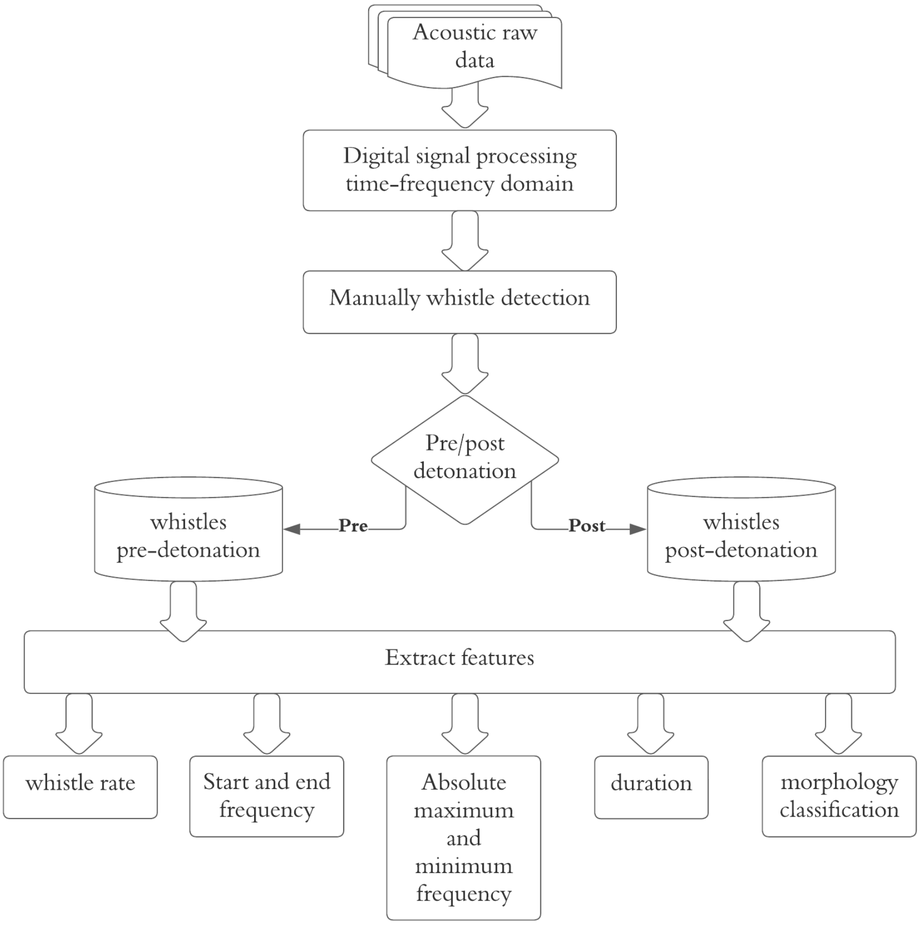

Figure 5. Analysis workflow diagram.

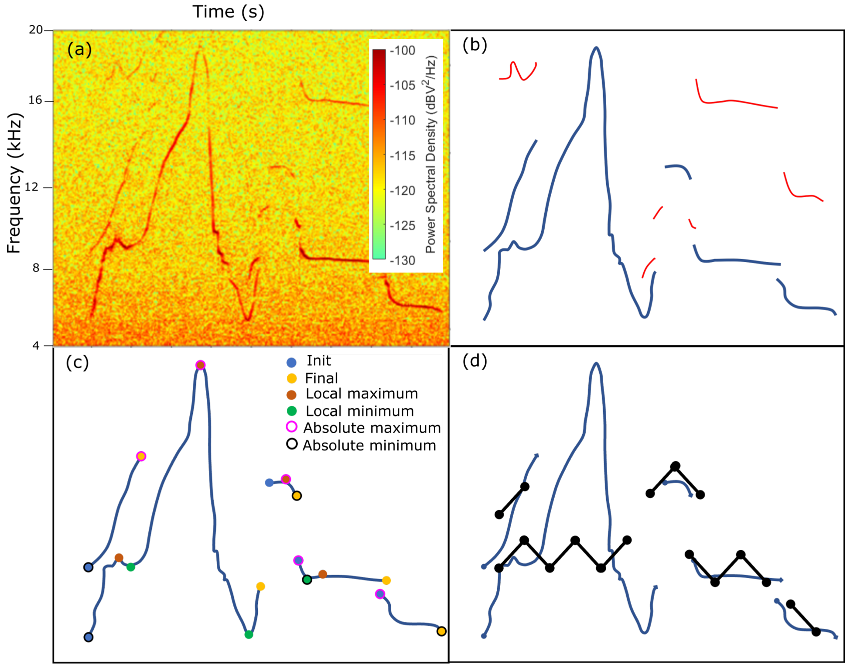

Figure 6. Acoustic analysis process exemplified by (**a**) a 2-second fragment of the spectrogram, obtained by applying a fast Fourier transform (4096 sampling windows and 50% overlap), (**b**) simplified spectrogram with quality cuts applied to harmonic and whistle durations: the blue color depicts the whistles considered in the analysis, the red color depicts the whistles that do not meet the conditions imposed by the applied quality cuts and, therefore, are neglected, (**c**) simplified spectrogram with the identification of contour properties linked with frequency properties, (**d**) simplified spectrogram overlapping the morphology classification based on the point of infection and slope determination for each whistle.

3. Results

In this section, we present the results obtained from the analysis. They are divided into two sections. The first section is related to the observed changes in acoustic behavior in terms of the whistling rate and morphology. The second section applies the Kruskal–Wallis test to the duration and spectral characteristics to study potential significant differences between the pre and post-detonation scenarios.

3.1. Acoustic Behavioral Changes of Delphinid Whistles in the Presence of Underwater Explosion Event

As mentioned earlier, the acoustic raw data analysis defines the detonation event as a boundary between two scenarios. Figure 7 shows the quantification of the acoustic activity in terms of the whistle rate in the pre- and post-detonation situations.

The bioacoustic activity is defined as the number of whistles detected in a time window of 1:50 min (110 s), calculated over 20 min before and after an explosion. After the UNDEX event, an immediate increase in vocalizations appears followed by a progressive decrease. This result is in agreement with observations carried out by other authors who reported that immediately after a detonation event occurred, the rate of whistles produced by Delphinids inhabiting the affected area increases significantly [17].

With respect to the results of the categorization of whistle morphology based on the slope and inflection points, Figure 8 presents the percentage of each type of whistle in both periods. It is important to note that the number of whistles detected after the UNDEX is almost 2.5 times greater than before the detonation. The most significant difference in morphology between the two scenarios is in Category 2 with an initial slope down. It is also worth mentioning that in both cases, the most common type of morphology is Category 0 with an initial slope down.

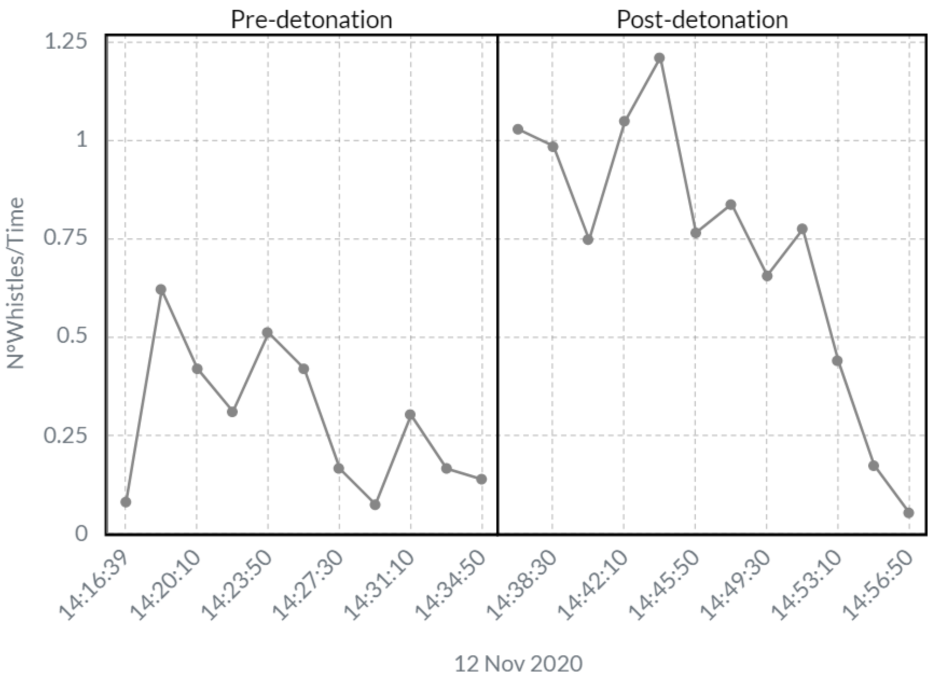

Figure 7. Evolution of bioacoustic activity in the function of time, considering a time window of 110 s and evaluated in the 20 min before and after the explosion.

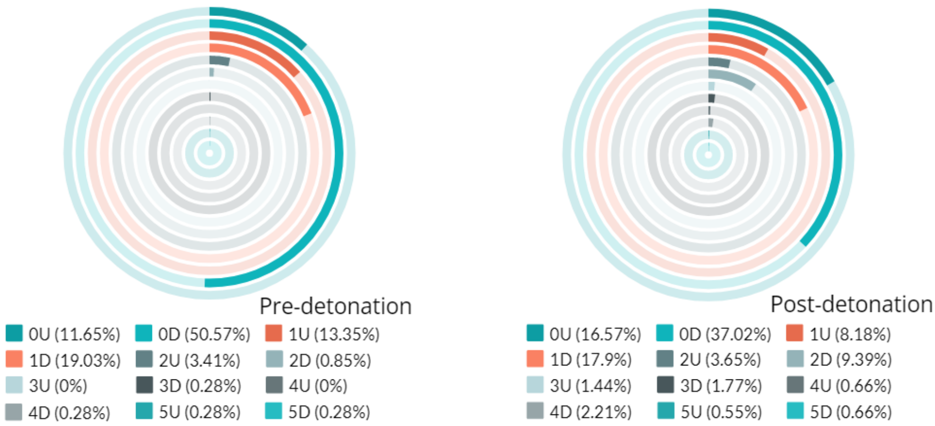

Figure 8. Percentage of whistles encoded by morphology-based categorization. The outer arcs correspond to the less complex whistles and the inner arcs correspond to the whistles with increasing inflection points.

The number of whistles attending the previously introduced categories are summarized in Table 2. It is possible to observe that after the detonation event appears, the number of whistles that belong to categories with more inflection points are higher with respect to the pre-detonation scenario.

Table 2. Number of whistles by categories according to whether they occurred before or after the detonation.

Category	0U	0D	1U	1D	2U	2D	3U	3D	4U	4D	5U	5D	Total
Pre	41	178	47	67	12	3	0	1	0	1	1	1	352
Post	150	335	74	162	33	85	13	16	6	20	5	6	905

To further explore the results based on the morphological categorization of whistles and their complexity, Figure 9 displays the percentage of whistles in both scenarios, grouped according to their category as follows: Category 0 (Up and Down), Category 1 (Up and Down), Category 2 (Up and Down), and Categories 3, 4, and 5 (Up and Down in all of them). The figure shows that post-detonation whistles are more complex (accounting for almost 20% of whistles belong to Categories 2, 3, 4, and 5) than whistles existing before the explosion (approximately 5%).

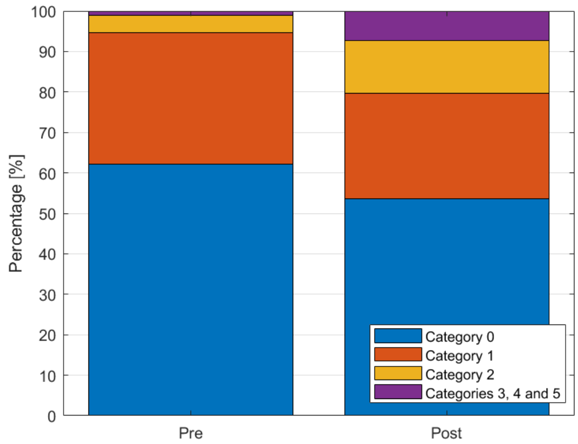

Figure 9. Bar graph with the percentage of whistles grouped by morphological categories in pre- and post-scenarios.

3.2. Statistical Analysis of Acoustic Features in Pre- and Post-Underwater Explosion Event

With the aim of studying the existence of significant differences in the acoustic vocalization of Delphinids in the presence of an explosion, the spectral and temporal characteristics of whistles were analyzed. Specifically, the variables that were analyzed using the Kruskal–Wallis test were the maximum frequency, minimum frequency, start and end frequencies, central frequency (defined as the midpoint of the bandwidth), and duration of the emitted bandwidth.

Figure 10 depicts the statistical median values of each variable considered in the analysis together with the first and third quartiles and the outliers of the distributions.

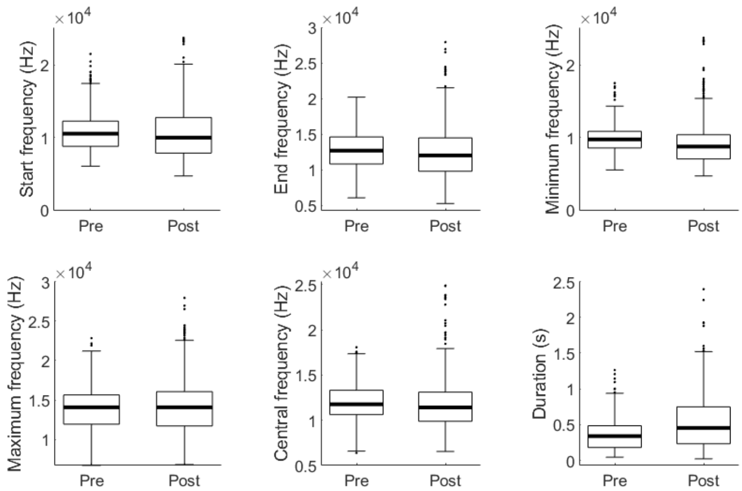

Figure 10. Box and whisker plots of whistle features before and after the detonation event. In the box plot, the horizontal thick black line indicates the median; the lower and upper box edges reflect the first and third quartiles; each whisker extends to a maximum of 1.5 inter-quartile range from the box edge. The black dots are the outliers.

The statistical analysis reveals significant differences among pre/post detonation scenarios for the spectral variables except for the maximum frequency. The Kruskal–Wallis test results are presented in Table 3.

Table 3. Whistle characteristics before and after detonation and statistical comparisons.

	Start *f* (Hz) *		End *f* (Hz) *		Min. *f* (Hz) *		Max. *f* (Hz)		Central *f* (Hz) *		Duration (s) *	
	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post
Mean	10,962	10,539	12,831	12,292	9919	9091	14,201	14,239	12,060	11,665	0.365	0.521
Median	10,552	9996	12,675	12,008	9755	8772	14,080	14,074	11,759	11,410	0.339	0.453
SD	2820	3592	2722	3502	1863	2816	2992	3506	2046	2633	0.231	0.352
X^2	8.17		10.01		58.04		0.07		11.49		47.35	
p-value	4.2×10^{-3}		1.5×10^{-3}		2.6×10^{-14}		796.1×10^{-3}		0.7×10^{-3}		5.9×10^{-12}	

* Kruskal–Wallis test, *p*-value < 0.05.

A *p*-value < 0.05 was chosen as the significance level (α) cut-off to assess the existence of significant differences between both scenarios in the study of the acoustic vocalization of Delphinids in the presence of an explosion. No lower *p*-value was considered due to uncertainties related to animal behavior in the wild. The extraction of significant differences is assumed to take into account a specific scenario evaluated in a precise environmental/animal condition, and therefore, the results should be interpreted as tentative evidence that suggests changes in some of the variables extracted from the studied whistles. Nevertheless, given the variability in the experimental monitoring conditions, the authors believe that lower *p*-values do not necessarily ensure greater reliability in the obtained results.

Among the analyzed variables, the duration and minimum frequency of the whistles showed the strongest significant differences between before and after the explosion. This is clearly reflected in Figure 11 by the statistical distribution of both variables.

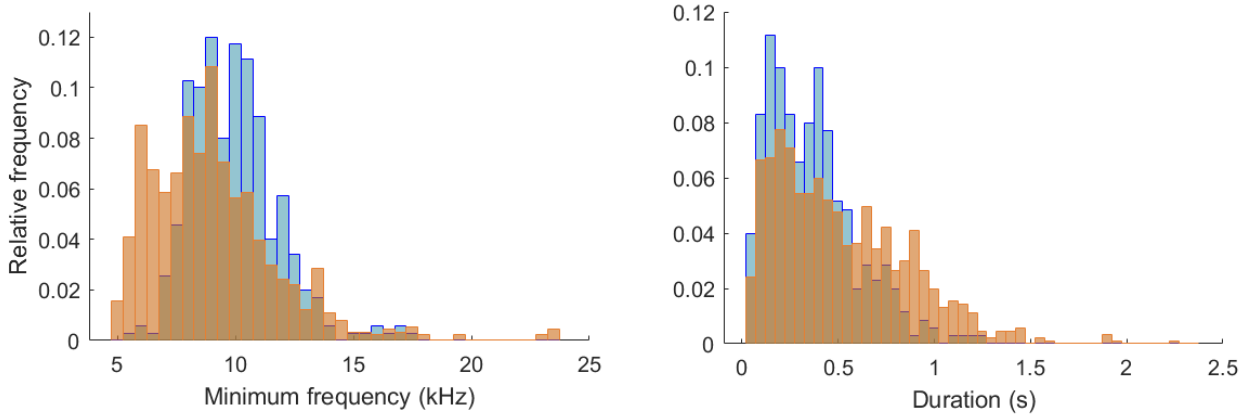

Figure 11. Histograms of the minimum frequency and duration of the whistles before (blue) and after (brown) detonation.

In order to verify that the results obtained are not influenced by the difference of pre- and post-detonation sample size, a Kruskal–Wallis test was performed considering 350 whistles of both scenarios. These whistles have been selected randomly from post detonation and the Kruskal–Wallis test was applied repeatedly 25 times inferring results collected in Table 4.

Table 4. Percentages of *p*-values below the 0.05 significance level for Kruskal–Wallis tests made with 25 different sets of post-detonation whistles.

	Start *f* (Hz)	End *f* (Hz)	Min. *f* (Hz)	Max. *f* (Hz)	Central *f* (Hz)	Duration (s)
% *p*-value < 0.05	80	84	100	4	100	100

We should note that 100% of the Kruskal–Wallis tests show a p-value < 0.05 for minimum frequency, duration, and central frequency features. The tests also show that 80% and 84% of the samples demonstrate significant differences for start and end frequencies, respectively. Finally, the only variable that does not exhibit significant differences among both scenarios is the maximum frequency. These results are in agreement with the previous ones exhibited in Table 3 applying the Kruskal–Wallis test over the whole samples for pre- and post-detonation situations.

4. Discussion

The obtained results reveal that the acoustic behavior of Delphinid whistles exposed to a specific underwater explosive event varies in terms of whistling rate, spectral features (except for the maximum frequency), duration, and morphology. This study observed a significant increase in the rate of whistling emission after the detonation, during which dolphins emitted more complex whistles.

Maximum and start frequencies present less robust differences, as noted by applying subsampling on whistle events and repeating the test 25 times. Nevertheless, these features also reject the null hypothesis of the Kruskal–Wallis test. The major significant differences are noticed in features such as the duration of whistles (longer-lasting whistles were detected in the post-detonation scenario) and minimum and central frequencies.

It is important to note that obtaining this type of acoustic datum in the wild is difficult due to the challenge of having Delphinids and an UNDEX event occur simultaneously, along with an acoustic node recording the soundscape. This study also emphasizes the importance of implementing long-term acoustic monitoring campaigns in cetacean habitats to obtain valuable results regarding the influence and potential impact of human activities on their life and fitness.

The results obtained indicate that high levels of anthropogenic impulsive noise affect the life of Delphinids. In fact, a decrease in bioacoustic activity was observed some minutes after the explosion, suggesting that the animals could have left the area affected by the explosion at that particular time. The disturbance produced may cause behavioral changes that affect the use of the habitat, reducing the available area, or provoking the displacement of dolphin groups to other locations. The repetition of explosive events could be linked to the time during which the affected area is not available with optimal conditions. However, these kinds of studies require dedicated long-term monitoring techniques, particularly considering photo ID and visual campaigns, which are beyond the scope of this publication.

This study only considers the 20-minute period before and after an explosion occurs because only one explosive event was detected using one PAM device. Therefore, no studies about potential long-term behavioral changes in relation to groups of dolphins inhabiting the area are able to be performed.

Studies on changes in the acoustic characteristics of Delphinid whistles due to UNDEX events are not available in the literature. Therefore, it is not possible to make a definitive claim that changes in vocalization features, such as increasing duration or minimum frequency, reflect stress or suffering. However, it is worth noting that the observed changes could be used for comparison with possible future studies of behavioral alterations in Delphinids related to impulsive noise events, such as the one described in this work.

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