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The Acoustic Repertoire of Bottlenose Dolphins (*Tursiops truncatus*) in the Cres-Lošinj Archipelago (Croatia): Site Dependent Diel and Seasonal Changes

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Abstract: Describing the acoustic repertoire of cetaceans is necessary to understand the functionality of their sounds and the effect anthropogenic pressures have on animals living in a marine environment. This study provides a description of the acoustic repertoire of bottlenose dolphins (*Tursiops truncatus*) in the Cres-Lošinj archipelago based on continuous 24-h recordings collected from two monitoring stations, both inside and outside the Natura 2000 Site of Community Importance, during an 8-day period in March/April 2020 and a 13-day period in July/August 2020. A total of 1008 h were visually and aurally analyzed to identify vocalizations and investigate diel and seasonal patterns in their parameters. Furthermore, sound pressure levels were calculated for the low (63 Hz–2 kHz) and high (2 kHz–20 kHz) frequency range. Bottlenose dolphins in the Cres-Lošinj archipelago were found to produce whistles, chirps, low frequency narrow-band sounds, burst pulse sounds, and echolocation clicks showing that dolphins are present at both monitoring stations, during both diel and seasonal periods, in a comparable manner. This paper also provides evidence that whistles, chirps, and low frequency narrow-band sounds change their parameters in relation to the background noise in the area, that varies according to diel and seasonal patterns. This suggests a vocal plasticity in the species and a coping strategy to avoid masking of relevant acoustic signals for the local population in the Cres-Lošinj archipelago.

Keywords: bottlenose dolphin; protected species; vocalization; bioacoustics; passive acoustic monitoring; sound pressure level; underwater noise; Adriatic Sea

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1. Introduction

Bottlenose dolphins (*Tursiops truncatus*) have been studied extensively in the Mediterranean Sea [1], which improved the knowledge on their ecology and distribution. In the Adriatic Sea, the bottlenose dolphin represents the only cetacean species inhabiting the whole basin year-round, indicating also patterns of genetic connectivity to the whole north-eastern Mediterranean metapopulation [2–4]. The east coast of the Cres-Lošinj archipelago (north-eastern Adriatic Sea, Croatia) was designated a Natura 2000 Site of Community Importance (SCI) in 2014 due to the importance of these waters to the local animals [5]. It represents critical feeding and nursing grounds for a local bottlenose dolphin population [5]. This population is estimated at approximately 200 individuals [6] which would categorize it as 'Endangered' according to the IUCN criteria. Based on a diurnal photo-identification study initiated in this area in 1987 [6,7], this is the longest monitored population in the Mediterranean Sea.

Human activities in the coastal waters often shape the underwater soundscapes. Commercial shipping was generally found to increase underwater noise levels over the low-frequency range; however, the contribution of small recreational boats to the higher frequencies may also be substantial as indicated by [8,9]. Since most of the bottlenose dolphin distribution overlaps with coastal waters, the populations are generally exposed to substantial human pressure related to coastal development, ports, and boat traffic [10–12]. The underwater noise generated by diverse human activities has been proven to adversely affect the bottlenose dolphins. This acoustically active species relies on sound to communicate [13], forage, and navigate [14]. Noisy coastal waters were found to induce habitat displacements [15,16] along with shifts in vocalization frequencies [17,18], increased duration, and vocalization rate [19,20]. An earlier study in the Cres-Lošinj archipelago, based on acoustic samples obtained using a non-continuous recording setup on ten monitoring stations from 2007 to 2009 [21,22], corroborates these results. Boat noise has been identified as the primary source of disturbance to bottlenose dolphins inhabiting the coastal area of the Cres-Lošinj archipelago where leisure boating, particularly intense in the summer, was found to trigger a displacement of the dolphins along the east side of Cres-Lošinj archipelago and changes in their whistle structure [21–23].

Recently, the Cres-Lošinj archipelago was part of the SOUNDSCAPE project (https://www.italy-croatia.eu/web/soundscape, accessed on 1 February 2023), funded through the EU Interreg Italy-Croatia Program, which continuously monitored the underwater noise for 15 consecutive months at nine monitoring stations in the Northern Adriatic Sea along the coast of Italy and Croatia between 2020 and 2021. Two out of those nine monitoring stations were located in the Cres-Lošinj archipelago, one inside and another outside the Natura 2000 SCI (see [24] for details). Based on data collected at the inner station only, the first general description of the local soundscape has been provided, along with a detailed identification of fish sounds [25]. The same study gave an overview of bottlenose dolphin vocalizations, but without a detailed description of their parameters [25].

Bottlenose dolphins are known to have an acoustic repertoire including broadband and frequency modulated narrowband sounds [26–29]; however, most of the research has focused on single sound types as part of the repertoire, mainly on whistles and echolocation clicks [30–32]. This paper aims to provide for the first time a comprehensive description of the whole acoustic repertoire of bottlenose dolphins in the Cres-Lošinj archipelago based on continuous 24-h recordings collected from two monitoring stations, both inside and outside the Natura 2000 SCI. This study included, for the first time, nocturnal recordings and data collected outside the Natura 2000 SCI. Furthermore, parameters of all the identified vocalizations here have been compared between diel and seasonal periods per monitoring station.

2. Materials and Methods

2.1. Study Area

The data were collected through the SOUNDSCAPE project and this study focused on two monitoring stations (MS) located in the Cres-Lošinj archipelago, MS5 Susak and MS6 Lošinj, characterized by distinct environmental settings and exposure to different anthropogenic pressures (Figure 1). The MS5 Susak (14.28821° E, 44.49241° N) is located on the west side of the Cres-Lošinj archipelago, near the island of Susak. The MS5 Susak is relatively close to one of the main shipping lanes to Rijeka and thus exposed to high commercial shipping but further away from the recreational boating hot spots that are closer to the coast. The MS6 Lošinj (14.57469° E, 44.54597° N) is situated on the east side of the Cres-Lošinj archipelago, in the core of the Natura 2000 SCI, near the island of Oruda (Figure 1). In addition, the MS6 Lošinj falls inside one of the two hub areas used by the resident bottlenose dolphins [33]. The area is subject to intense nautical tourism between June and September (the Tourist Season; [23]) and there were also some commercial fishing activities in the area mainly related to bottom trawling. However, the area is isolated from major commercial shipping lanes.

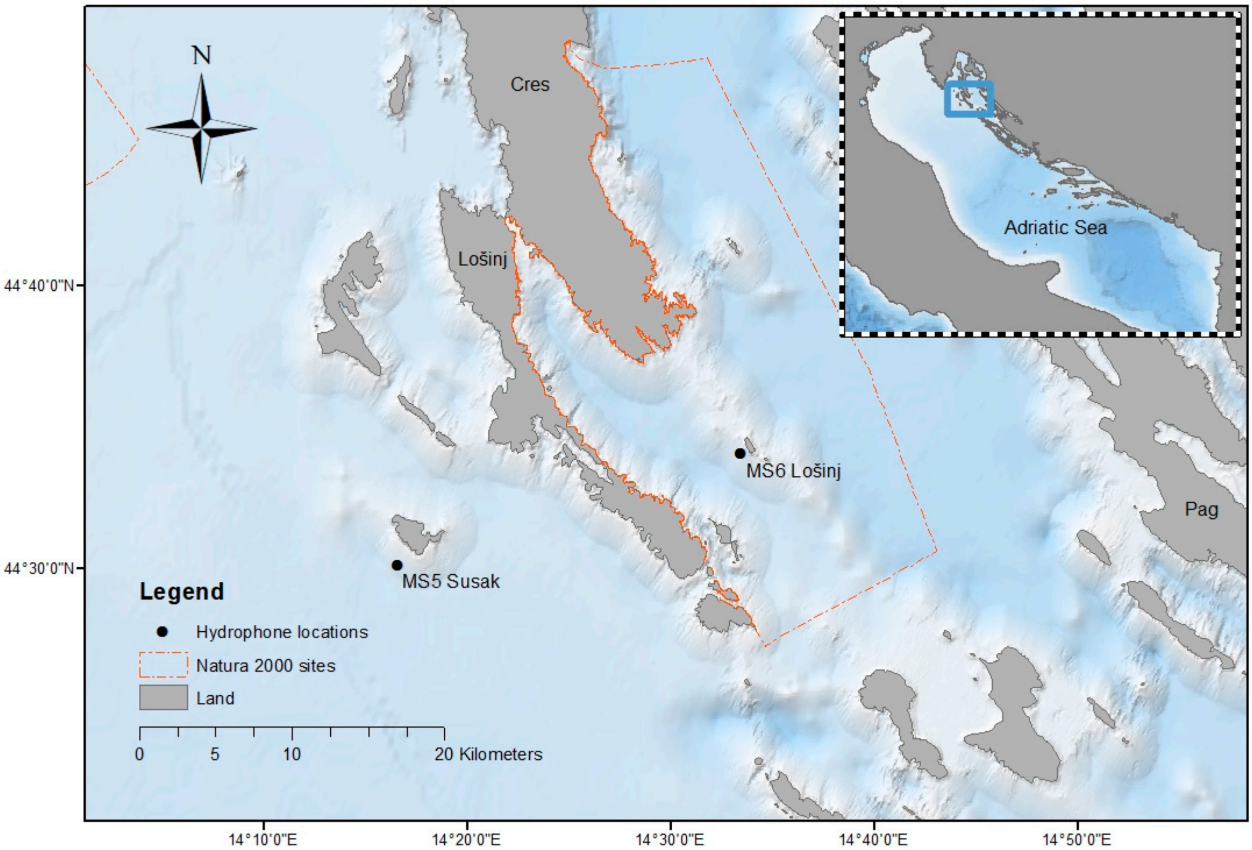

Figure 1. Map showing the locations of the MSs: MS5 Susak and MS6 Lošinj.

2.2. Data Collection

Acoustic recordings were collected with Sono.Vault autonomous passive underwater acoustic recorders (APUARs) from Develogic. The APUARs were equipped with an omnidirectional D60 hydrophone from Neptune Sonar with a sensitivity around −193 dB re 1 V/µPa and a flat frequency response over the frequency range 10 Hz–20 kHz (±3 dB). They were programmed to record continuously at a sampling rate of 48 kHz, providing a recording bandwidth of 24 kHz, with a 16-bit resolution and gain 6. A sampling rate of 48 kHz was chosen as the SOUNDSCAPE project focused on assessing the underwater noise. At the MS5 Susak and MS6 Lošinj, the same two buoy-type arrays were deployed (Figure 2). The APUAR array consisted of subsurface buoys and an anchor weight, with the APUAR being secured through a rope five meters above the seabed. The other array included a surface and subsurface buoy which were connected by rope that was tied to an anchor weight. The seabed at the MS5 Susak was rocky and sandy with a water depth of 40 m whereas the MS6 Lošinj had a water depth of 37 m and a sandy seabed. Divers were used for the deployment and recovery of the APUARs.

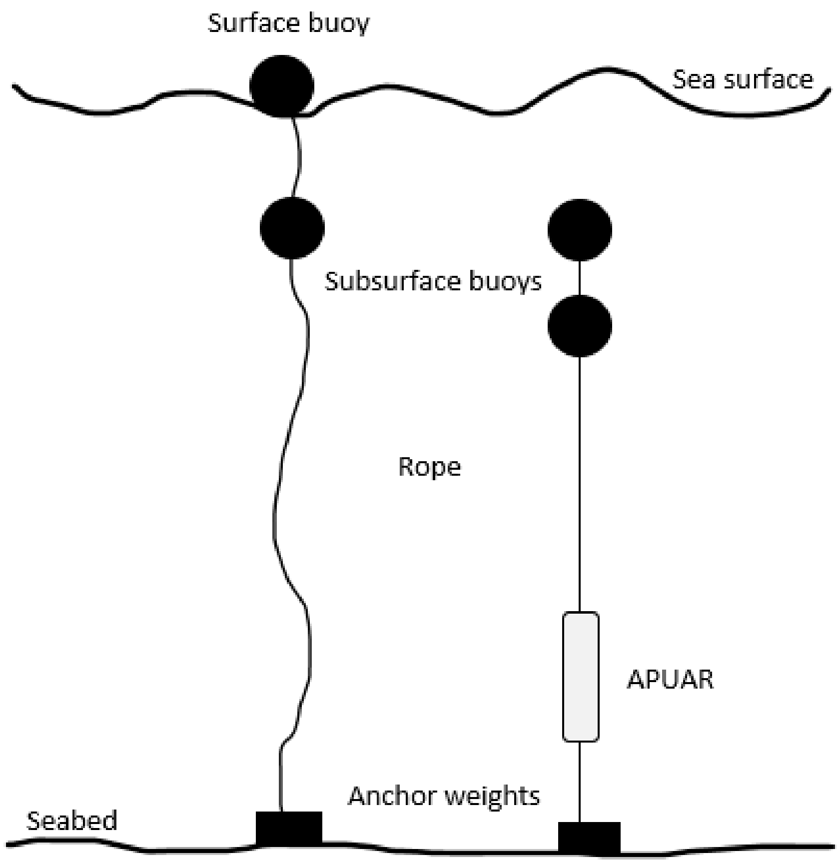

Figure 2. Schematic view of the two buoy-type arrays used at the MS5 Susak and MS6 Lošinj.

2.3. Data Analysis

2.3.1. Identifying Bottlenose Dolphin Vocalizations

To identify bottlenose dolphin vocalizations, acoustic data from an 8-day period in March/April 2020, hereafter called Non-Tourist Season (NTS), and a 13-day period in July/August 2020, the Tourist Season (TS), were considered. These two periods also include the 5 days dataset described by [25]. Here, in total 42 24-h days were manually inspected to look for vocalizations produced by bottlenose dolphins at the MS5 Susak and MS6 Lošinj. Thus, a total of 1008 h were visually and aurally analyzed using Adobe Audition 3.0 (Adobe Systems, San Jose, CA, USA). Spectrogram displays were created using a Hanning window with a FFT size of 512 points. The following description of bottlenose dolphin vocalizations was used to identify whistles, chirps, low frequency narrow-band (LFN) sounds, burst pulse (BP) sounds, echolocation clicks, and the combined sounds, the bray:

i. Whistles were narrow-band and frequency modulated tonal sounds that had part of their fundamental frequency above 3 kHz [34] and were longer than 0.1 s in duration [35,36].
ii. Chirps were narrow-band and short tonal sounds that occurred over a broad frequency range [37] and were no longer than 0.1 s in duration [29].
iii. LFN sounds were tonal sounds that had their fundamental frequency below 2 kHz [38] and were no longer than 1 s in duration [29].
iv. BP sounds were broadband discrete aural packets of pulses that appear as horizontal harmonic banded sounds where clicks were aurally and visually indiscernible in the spectrogram display [26,29,39] with an inter-click interval of less than 10 milliseconds [40].
v. Echolocation clicks were short and intense broadband sounds with ultrasonic peak frequencies that are usually produced in rapid succession [14].

vi. Brays were distinct vocal units consisting of two sound types, such as a BP sound followed by a short downsweep that resembles a LFN sound [29,41].

All bottlenose dolphin vocalizations were visually evaluated and graded based on their signal-to-noise ratio (SNR). There were three categories [29]: (i) signal was faint but still visible on the spectrogram (SNR1), (ii) signal was clearly visible on the spectrogram (SNR2), and (iii) signal was clear with no other sound in the background (SNR3). An index of vocalizations that had a SNR2 or SNR3 was created. For echolocation clicks, the percentage of clicks per hour was noted but not used for further analysis. Additionally, the presence and absence of bottlenose dolphin vocalizations was logged per hour for all 42 24-h days [42]. The presence of vocalizations included the vocalizations listed above that had a good SNR (SNR2 or SNR3).

2.3.2. Characterizing Bottlenose Dolphin Vocalization Parameters

For a more detailed analysis of bottlenose dolphin vocalization parameters, each tonal sound identified (whistle, chirp, and LFN sound) with SNR2 or SNR3 from the MS5 Susak and MS6 Lošinj was analyzed using Raven Pro 1.6 (Cornell Lab of Ornithology, Cornell University, Ithaca, NY, USA). Spectrogram displays were generated using a Hanning window with a FFT size of 512 points. Whistles, chirps, and LFN sounds were characterized by measuring the start and end frequency (whistles and chirps only), minimum and maximum frequency, peak frequency, frequency range, and duration [29]. The inflection points (IP), points at which a change in slope (+/−) occurred, of whistles and the harmonics of LFN sounds were counted by visually inspecting the spectrogram in Adobe Audition 3.0. To reduce pseudoreplication bias of signature whistles, the acoustic data were checked for multiple whistles with repetitive parameters and only a single representative whistle was used. Additionally, individual dolphins might alter their whistles in response to noise levels [17] and other factors [43], thus further reducing the concern for pseudoreplication. Avisoft-SASLab Pro 5.3 (Avisoft Bioacoustics, Glienicke/Nordbahn, Germany) was used to analyze BP sounds with SNR2 or SNR3 from the MS5 Susak and MS6 Lošinj. As the high frequency component of BP sounds was not recorded due to the APUARs sampling rate of 48 kHz, the duration and pulse repetition rate per second (p/s) was identified [29].

2.3.3. Sound Pressure Level Analysis

Based on [44], sound pressure levels (SPLs) of the background noise were calculated. One second segments were read and processed recursively (i.e., 48,000-digit data, being the sample rate equal to 48,000) with a Discrete Fourier Transform analysis for each one-hour .wav file. The SPL averaged over one second was calculated for each 1/3-octave band (see [24] for details) and 20 s averaged SPLs were then calculated from one second SPLs. Statistics on 20 s data were computed using a Python code developed within the SOUNDSCAPE project (https://anp.soundscape.ve.ismar.cnr.it/; accessed on 1 February 2022). The Python script allowed to read SPL files for diel and seasonal periods for each monitoring station and display SPLs in one-third octave (base 10) bands; descriptive statistics could be generated as, for example, percentile values (1st, 10th, 25th, 50th, 75th, 90th, and 99th percentiles) and arithmetic mean. For the present paper, in accord with [44], SPLs were calculated for a low-frequency range (SPLsLF, range: 55–2245 Hz) and a high-frequency range (SPLsHF, range: 2245–22,627 Hz) per each one-hour .wav file. SPL comparisons between diel (day and night) and seasonal (NTS and TS) periods were made for each monitoring station (MS5 Susak and MS6 Lošinj) and descriptive plots were created to highlight the principal statistics.

2.3.4. Statistical Analysis

The number of vocalizations analyzed (n), mean, standard deviation (SD), range (minimum and maximum), and coefficient of variation (CV = SD/Mean * 100) were calculated for whistle, chirp, LFN sound, and BP sound parameters recorded at the MS5 Susak and MS6 Lošinj. More in detail, descriptive statistics of whistle, chirp, and LFN sound parameters were calculated per diurnal and nocturnal hours equal to the period of 6 am–6 pm (day) and 7 pm–5 am (night) in the NTS and 5 am–8 pm (day) and 9 pm–4 am (night) in the TS at the MS5 Susak and MS6 Lošinj.

To investigate diel and seasonal induced changes in bottlenose dolphin vocalizations, the differences in vocalization parameters were measured between diel (day vs. night) and seasonal (NTS vs. TS) periods for the MS5 Susak and MS6 Lošinj dataset separately. BP sounds, and brays were excluded from further analysis as the number of BP sounds was low, and brays were not found (Table 1).

A Test of Homogeneity of Variances was carried out for each vocalization parameter, and if the *p*-value was >0.05, which means that the assumption of homogeneity of variance was met, a one-way ANOVA test was conducted. In case the *p*-value was <0.05, meaning the assumption of homogeneity of variance was violated, a non-parametric Kruskal–Wallis test was used. All statistical tests were run through SPSS17.0 (SPSS, Inc., Chicago, IL, USA).

3. Results

3.1. Distribution and Characterization of Bottlenose Dolphin Vocalizations

Of the 504 h per monitoring station that were visually and aurally analyzed, a total of 44 h in the NTS and 55 h in the TS at the MS5 Susak included at least one type of dolphin vocalization with good SNR (SNR2 or SNR3; Figure S1). At the MS6 Lošinj, a total of 27 h in the NTS and 22 h in the TS contained at least one type of dolphin vocalization with good SNR (SNR2 or SNR3; Figure S1).

Bottlenose dolphins in the Cres-Lošinj archipelago produced different sound types: whistles, chirps, and LFN sounds were the most common ones, together with the echolocation clicks (see Figure 4a,b,d,e in [25]). A certain percentage of echolocation clicks was always present in each one-hour recording that included other bottlenose dolphin vocalizations. BP sounds have also been identified, although they were scarcer (see Figure 4c in [25]). LFN sounds often occurred in trains of either single LFN sounds with equal space between each sound or in packs of two LFN sounds close together (see Figure 4e in [25]). Brays could not be identified with certainty.

Sound production was predominant in the NTS compared to the TS for whistles and chirps at both monitoring stations. In the NTS, these sounds were more commonly found during the day at the MS5 Susak and during the night at the MS6 Lošinj (Table 1). An opposite trend occurred with LFN sounds, which were mostly produced at the MS5 Susak and had a minimum detection at the MS6 Lošinj during the night in the TS.

Table 1. Total and standardized number (*n*. sound/hour) of bottlenose dolphin vocalizations with good signal-to-noise ratio recorded at the MS5 Susak and MS6 Lošinj during the day and night in the NTS (8 24-h periods) and TS (13 24-h periods).

			MS5 Susak				MS6 Lošinj			
Seasonal Period	Diel Period	*n* of Hours Analyzed	Whistle	Chirp	LFN	BP	Whistle	Chirp	LFN	BP
NTS	Day	104	237 (2.3)	91 (0.8)	45 (0.4)	13 (0.1)	55 (0.5)	9 (0.08)	64 (0.6)	2 (0.01)
NTS	Night	88	13 (0.1)	13 (0.1)	12 (0.1)	4 (0.04)	207 (2.3)	12 (0.1)	83 (0.9)	0 (0.0)
TS	Day	208	27 (0.1)	20 (0.09)	173 (0.8)	2 (0.01)	72 (0.3)	18 (0.08)	200 (0.9)	5 (0.02)
TS	Night	104	8 (0.08)	16 (0.1)	233 (2.24)	3 (0.02)	46 (0.4)	1 (0.01)	10 (0.09)	1 (0.01)

Descriptive statistics of parameters for all bottlenose dolphin vocalizations identified at the MS5 Susak and MS6 Lošinj, are presented in Table 2. Whistles (*n* = 665) ranged from 0.87–22.46 kHz in frequency and had a mean duration of 0.79 s. Chirps (*n* = 180) occurred in frequencies between 0.51–9.02 kHz with a mean duration of 0.07 s. The frequency of LFN sounds (*n* = 820) ranged from 0.11–1.58 kHz and had a mean duration of 0.04 s. BP sounds (*n* = 30) had a mean duration of 0.17 s and a mean pulse repetition rate of 358 p/s.

Table 2. Descriptive statistics of bottlenose dolphin vocalizations produced at the MS5 Susak and MS6 Lošinj during diel (day and night) and seasonal (NTS and TS) periods.

Statistics	Start Freq (kHz)	End Freq (kHz)	Min Freq (kHz)	Max Freq (kHz)	Peak Freq (kHz)	Freq Range (kHz)	Duration (s)	IP (*n*)	Harmonics (*n*)	Pulse Rep. Rate (p/s)
Whistles										
n	665	665	665	665	665	665	665	665	-	-
Mean	7.05	9.10	6.03	10.99	7.73	4.97	0.79	1.01	-	-
± SD	2.56	3.99	1.92	4.04	2.86	3.18	0.43	1.35	-	-
Min	0.87	3.65	0.87	4.59	1.13	0.29	0.14	0.00	-	-
Max	18.17	22.46	14.84	22.46	17.34	15.09	6.10	10.00	-	-
CV	36.31	43.84	31.83	36.77	36.95	64.05	55.01	134.11	-	-
Chirps										
n	180	180	180	180	180	180	180	-	-	-
Mean	1.93	2.40	1.78	2.51	2.11	0.73	0.07	-	-	-
± SD	0.90	0.77	0.81	0.78	0.79	3.35	0.02	-	-	-
Min	0.26	0.51	0.51	1.35	1.13	0.25	0.02	-	-	-
Max	8.75	8.14	8.14	9.02	8.72	1.91	0.10	-	-	-
CV	46.50	31.95	45.42	31.12	37.24	48.04	29.51	-	-	-
LFN sounds										
n	-	-	820	820	820	820	820	-	820	-
Mean	-	-	0.33	0.70	0.50	0.37	0.04	-	0.07	-
± SD	-	-	0.10	0.14	0.10	0.12	0.02	-	0.29	-
Min	-	-	0.11	0.41	0.28	0.18	0.01	-	0.00	-
Max	-	-	0.72	1.58	1.03	1.22	0.19	-	4.00	-
CV	-	-	29.70	20.06	20.90	33.60	61.18	-	402.69	-
BP sounds										
n	-	-	-	-	-	-	30	-	-	30
Mean	-	-	-	-	-	-	0.71	-	-	358.87
± SD	-	-	-	-	-	-	0.19	-	-	104.51
Min	-	-	-	-	-	-	0.03	-	-	102.88
Max	-	-	-	-	-	-	0.69	-	-	571.07
CV	-	-	-	-	-	-	107.54	-	-	29.12

3.2. Diel and Seasonal Differences in Bottlenose Dolphin Vocalization Parameters

At the MS5 Susak, no whistle parameters differed significantly between day and night during both seasons with the exception of the end frequency, which was significantly higher during the day than the night in the TS, although the number of whistles during the night in the TS was low (Tables S1 and S4). As for seasonal changes in whistle parameters, the start frequency was significantly higher whereas the end, maximum, and peak frequency were significantly lower in the TS compared to NTS (Tables S1 and S4). In addition, the frequency range was significantly smaller and whistles had significantly more inflection points in the TS than in the NTS. Chirp parameters only differed significantly during the day in the NTS, with a significantly lower start, end, minimum, maximum, and peak frequency than during the night (Tables S2 and S5). When assessing seasonal changes, the start, end, minimum, and maximum frequency of chirps were significantly lower in the TS than NTS (Tables S2 and S5). During the day in the NTS and TS, dolphins produced LFN sounds at lower frequencies; the minimum (only in the NTS), maximum and peak frequency was significantly lower, the frequency range significantly smaller, and LFN sounds had a significantly shorter duration (only in the NTS) during the day when compared to night (Tables S3 and S6). Looking at seasonal changes, the minimum, maximum, and peak frequency were significantly lower, the frequency range significantly smaller, and the duration of LFN sounds significantly shorter in the TS than in the NTS (Tables S3 and S6).

At the MS6 Lošinj, in the NTS and TS during the day, the start, end (only in the TS), minimum (only in the TS), maximum, and peak frequency were significantly higher, the frequency range significantly wider, and whistles had significantly more inflection points when compared to night (Tables S1 and S7). Seasonal changes were observed in whistle parameters as the minimum and maximum frequency was significantly higher, the frequency range significantly wider, and the duration significantly longer in the TS than in the NTS (Tables S1 and S7). Keeping the low number of chirps in mind during the day in the NTS, chirps had a significantly wider frequency range during the day than night (Tables S2 and S8). Chirp parameters could not be compared between day and night in the TS as the sample size was too low during the night. Regarding seasonal changes, the chirp duration was significantly shorter in the TS compared to NTS (Tables S2 and S8). In the NTS, LFN sounds were produced at a significantly higher maximum frequency, lower peak frequency, with a wider frequency range, and longer duration during the day than night (Tables S3 and S9). During the day in the TS, the start, maximum, and peak frequency of LFN sounds were significantly higher when compared to the night. As to seasonal changes, LFN sounds had a significantly higher minimum, maximum, and peak frequency, and a shorter duration in the TS than in the NTS (Tables S3 and S9).

Detailed descriptive statistics of whistles, chirps, and LFN sounds are provided in Tables S1–S3, whereas statistical test outputs are presented in Tables S4–S9.

3.3. Sound Pressure Levels at the Monitoring Stations

Overall, the median SPL values were generally higher at the MS5 Susak than the MS6 Lošinj for the whole 1/3 octave frequency bands of the spectrum (Figure 3). Seasonal variations (NTS vs. TS) in median SPL values were present, although mainly evident at the MS5 Susak.

At the MS5 Susak, the median SPLs for the low (63 Hz–2 kHz) and high (2 kHz–20 kHz) frequency range were always (NTS and TS) lower during the day compared to the night (Figure 4a–d). Higher median SPL values characterized the high-frequency range during the TS compared to the NTS while the opposite trend was the case for the low- frequency range.

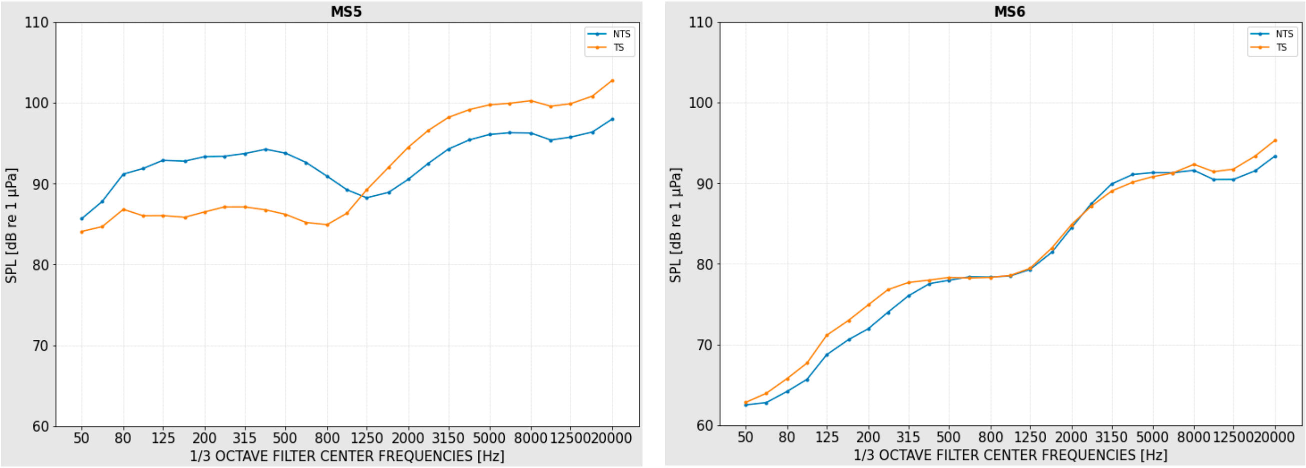

Figure 3. 1/3 octave bands SPLs (median level) for the NTS (blue line) and TS (orange line) at the MS5 and MS6.

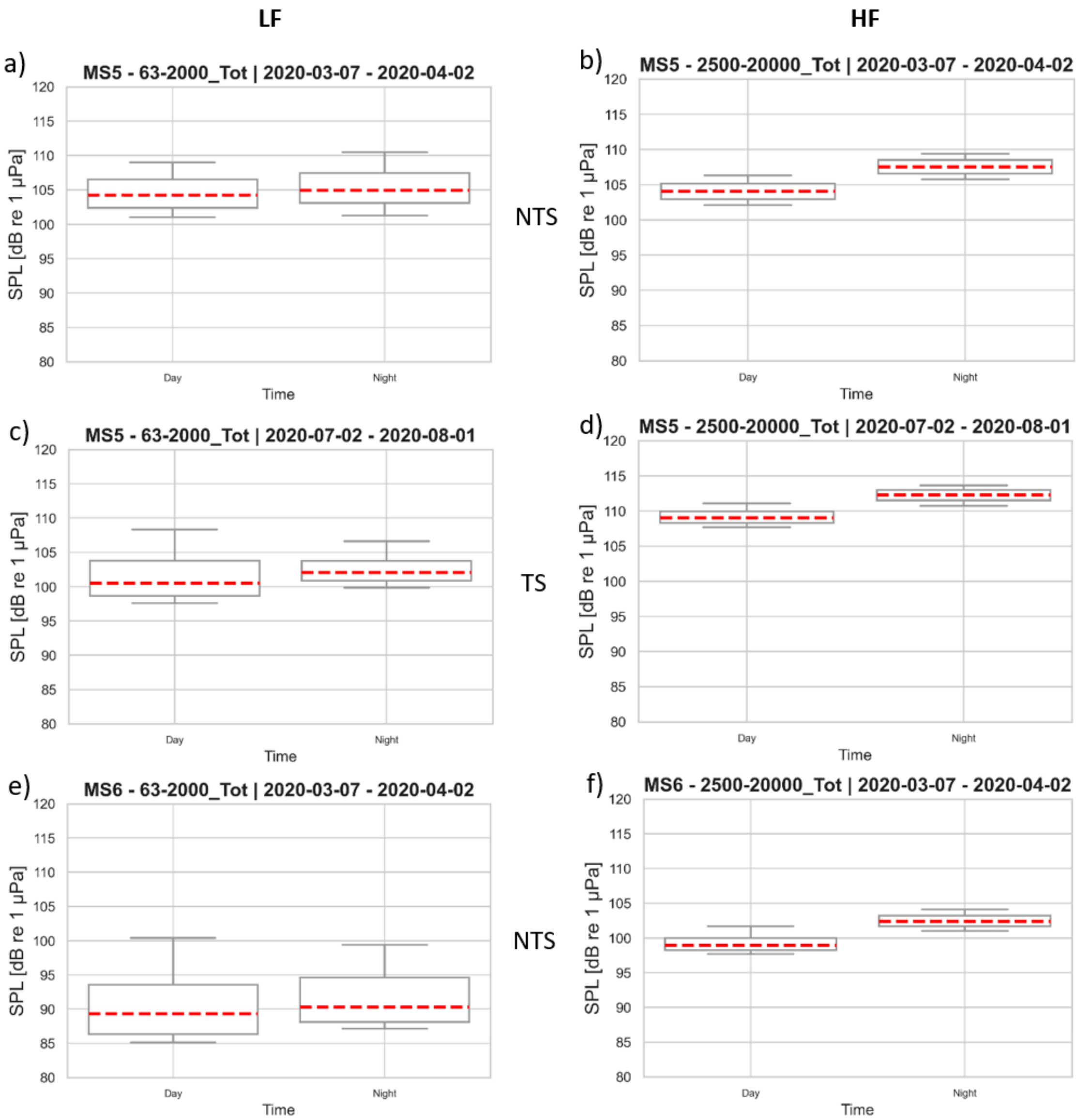

Figure 4. *Cont.*

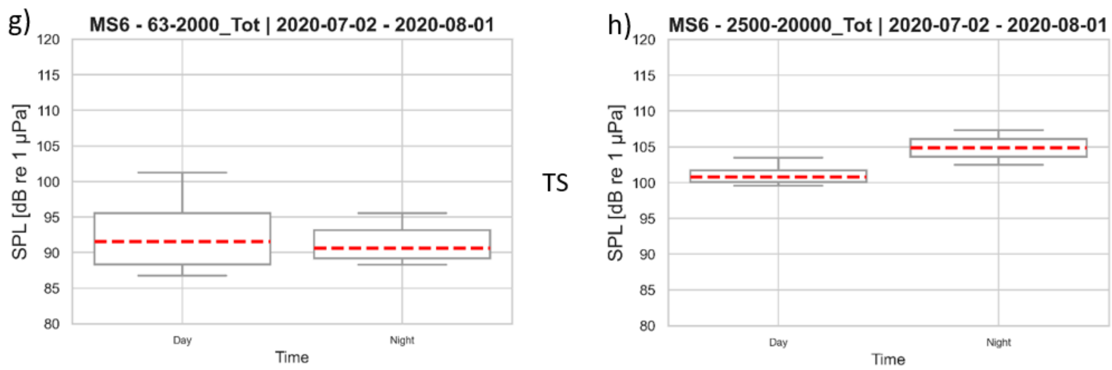

Figure 4. Distribution of SPL values for the low (63 Hz–2 kHz) and high (2 kHz–20 kHz) frequency range comparing the medians (red dotted line) for day and night in the NTS and TS at the MS5 Susak (**a**–**d**) and MS6 Lošinj (**e**–**h**).

At the MS6 Lošinj, in both the NTS and TS, the median SPL values for high (2 kHz–20 kHz) frequency range were lower during the day compared to the night; however, in the TS, but not NTS, slightly higher median and more variable SPL values were found during the day compared to the night for the low (63 Hz–2 kHz) frequency range (Figure 4e–h).

4. Discussion

This study assessed the acoustic repertoire of bottlenose dolphins at two monitoring stations characterized by different environmental settings (i.e., sediment and water depth) and anthropogenic pressures in the Cres-Lošinj archipelago. Dolphins were present at both monitoring stations, during both diel and seasonal periods, producing whistles, chirps, LFN sounds, BP sounds, and echolocation clicks. Vocalization parameters have been found to change in relation to the background noise which suggests vocal plasticity and a coping strategy to avoid masking of relevant acoustic signals for the local population in the Cres-Lošinj archipelago.

The present paper focused on the analysis of four broad groupings of vocalizations, whistles, chirps, LFN sounds, and BP sounds, based on clear definitions as sub-categories were not evident [29]. Whistles are mainly used in social contexts [45,46]. More specifically, signature whistles carry identity information and allow for dolphins to address each other [47,48]. Not much is known about the functionality of chirps. However, in captivity, chirps seemed to be produced more often when dolphins were positively reinforced [49]. Even though the function of LFN sounds is still unclear, they have been associated with socializing and heightened emotional contexts [50]. BP sounds are thought to play a big role in communication [51], yet their function remains unclear [52]. Different to communication vocalizations, dolphins use echolocation clicks to detect and discriminate a target [14].

In the Cres-Lošinj archipelago recordings, whistles and LFN sounds were the most common vocalizations found inside (MS6 Lošinj) and outside (MS5 Susak) the Natura 2000 SCI, whereas chirps were less frequent, and BP sounds scarce. Both MSs are known to be visited by bottlenose dolphins, although on the west side of the Cres-Lošinj archipelago, where the MS5 Susak was located, dolphins are more transient in contrast to the east side of the Cres-Lošinj archipelago, around the MS6 Lošinj, where dolphins are more resident [6,33]. Research effort on bottlenose dolphins in the Cres-Lošinj archipelago along the same timeframe as the present study highlighted diurnal animal movements inside and outside the Natura 2000 SCI, with a similar encounter rate during the winter and summer period (SOUNDSCAPE project deliverable 4.1.1 by [53]). Accordingly, dolphin vocalizations were found at both MSs during the NTS and TS. Interestingly, the presence of nocturnal vocalizations can further confirm that these areas are equally frequented by dolphins during night and daytime. The nocturnal occurrence of all vocalizations including whistles, chirps, LFN sounds, BP sounds, and echolocation clicks also suggests similar behavioral interactions, with special reference to social interactions [46,49–51].

For the first time in this area, a detailed characterization of parameters for all vocalizations produced by bottlenose dolphins in the Cres-Lošinj archipelago was provided. Vocalization parameters of whistles, chirps, LFN sounds, and BP sounds were consistent with the published data [29]. Additionally, comparisons were made considering different seasonal and diel periods for all vocalizations which has never been reported in the literature so far. Based on this analysis, it appears that most of the whistle, chirp, and LFN sound parameters differed between seasons with lower frequency values at the MS5 Susak and higher frequency values (except chirps) at the MS6 Lošinj during the TS compared to the NTS. Once considering the seasonal related dataset separately, almost no variations in the analyzed whistle parameters have been found between day and night at the MS5 Susak (both during the NTS and TS), whereas almost all whistle parameters increased during the day compared to night at the MS6 Lošinj in the TS. Chirps had lower frequencies during the day than night in the NTS at the MS5 Susak. Lower diurnal vs. nocturnal frequency values characterized LFN sounds (both TS and NTS) at the MS5 Susak, whereas at the MS6 Lošinj, the LFN sound frequencies were found to increase during the day compared to the night during the TS. Summing up, a general trend with dolphins increasing their frequency at the MS6 Lošinj, located inside the Natura 2000 SCI, and decreasing their frequencies at the MS5 Susak, outside the Natura 2000 SCI, during the day compared to the night and during the TS opposed to the NTS was evident.

Background noise at the two MSs was not similar: median SPL values were higher at the MS5 Susak than MS6 Lošinj. This could likely depend on the MS5 Susak being more exposed to continuous noise from anthropogenic sources, such as commercial shipping and fishing activities, which are ongoing throughout the entire year (see Figure 1 in [24] for details). According to the present data, the above reported changes in dolphin vocalization parameters could be related to the background noise at both monitoring stations, the MS5 Susak and MS6 Lošinj. In fact, at the MS5 Susak, median SPL values in both the low- and high-frequency ranges were always lower during the day than night and the start, end, minimum, maximum, and peak frequency of chirps was lower during the day than night in the NTS. LFN sound parameters followed the same trend with the minimum, maximum, and peak frequency being lower, the frequency range being smaller, and the duration shorter during the day than night both during the TS and NTS. SPL values at the MS5 Susak were found to increase across the high-frequency range in the TS compared to NTS. Accordingly, dolphins shifted their whistle parameters to lower frequencies—including end, maximum, and peak frequency—and shortened their frequency range in the TS compared to NTS. This result is in line with [22]: the authors found that dolphins at the Cres-Lošinj archipelago whistled at lower maximum and peak frequencies as the SPLs across the high-frequency range were elevated. Likewise, in correspondence to higher SPLs in the TS than NTS in the high-frequency range, a lower start, end, minimum, and maximum frequency of chirps was found at the MS5 Susak. Following the same trend as whistles and chirps, the minimum, maximum, and peak frequency of LFN sounds was lower, with a smaller frequency range, and shorter duration in the TS than NTS.

Unlike the MS5 Susak, the MS6 Lošinj is mostly subject to intense nautical tourism in the TS [23], while commercial shipping remains low throughout the year. In fact, diel variations were here confirmed by higher SPL values in the low-frequency range during the day than at night in the TS, likely related to intense diurnal recreational boating. In correspondence to the higher diurnal SPLs across the low-frequency range in the TS, dolphins' whistles had a higher start, end, minimum, maximum, and peak frequency, with a wider frequency range, and more inflection points. Again, this is in accordance with [22], who also found that dolphins communicated at higher frequencies when the low-frequency range was dominated by noise in the Cres-Lošinj archipelago. A similar trend was found for LFN sounds, whose parameters had a higher minimum, maximum, and peak frequency during the day than at night in the TS, as the SPLs were higher in the low-frequency range. In the TS at the MS6 Lošinj, the SPLs for the low- and high-frequency range were higher compared to the NTS. Correspondingly, whistles had a higher minimum and maximum frequency, wider frequency range, and longer duration in the TS than NTS. Similarly, [17] reported that bottlenose dolphins in Florida (USA) increased their minimum, maximum, and peak frequency by over 100Hz for each 1dB increase in ambient noise. Following the same trend as whistles, LFN sounds had an increased minimum, maximum, and peak frequency in the TS compared to NTS.

Overall, our data suggest that at both monitoring stations, bottlenose dolphins adapt their vocalizations to the background noise, producing vocalizations at higher frequencies when noise across the low-frequency range was increased and showing the opposite pattern when the SPLs across the high-frequency range were elevated. In addition, vocalizations shifted to higher frequencies when SPLs across the low- and high-frequency range were increased, and the opposite trend occurred when noise across the low- and high-frequency range was low. Changes in the whistle structure of the resident bottlenose dolphins inside the Natura 2000 SCI in the Cres-Lošinj archipelago have already been found in relation to underwater noise and boat traffic [22]. It is the first time that these changes have been highlighted not only for whistles but also for other sound types, which similarly respond to the background noise. Adjustments to elevated noise levels have been found to have implications on the habitat range of dolphins on the east side of the Cres–Lošinj archipelago [33]. The area is recognized as an important nursing ground with a high occurrence of mother-calf pairs [5], and it is known that they are particularly sensitive to boat presence and noise [54]. The energy consumption of mothers having to take care of their offspring is already high [55], and additional energetical increases related to vocalization adjustments in a noisy environment would need to be balanced out by adequately enhanced food intake, which is not always easy to achieve. As a displacement of the bottlenose dolphins along the east side of the Cres–Lošinj archipelago has already been found in relation to increased underwater noise [21], the findings of this paper urge the need for the development of efficient regulatory measures that would contribute to bottlenose dolphin protection and preservation of the resident population in this area.

5. Conclusions

Concluding, the present paper describes in detail the acoustic repertoire of the bottlenose dolphins in the Cres-Lošinj archipelago, in two different locations inside and outside the Natura 2000 SCI, showing that dolphins are present at both monitoring stations, during both diel (day and night), and seasonal (NTS and TS) periods, in a comparable manner. This result was obtained by manually scrolling through the collected data, which assures a high detectability of all the sounds produced by the species but is extremely time-consuming and thus reduces the duration of the study period. Future studies will expand the dataset to further describe the temporal distribution of bottlenose dolphin vocalizations along both monitoring stations. Broadband and frequency-modulated narrowband sound types have been found inside and outside the Natura 2000 SCI in the Cres-Lošinj archipelago, in accord with previous research [56], which highlighted a repertoire similarity between the bottlenose dolphin population in the Sado estuary (Portugal) and east coast of the Cres-Lošinj archipelago (inside the Nature 2000 SCI) which are both resident groups inhabiting shallow waters with high levels of ambient noise. This paper also provides evidence that whistles, chirps, and LFN sounds found in this study, rather than whistles only, change their parameters in relation to the background noise in the area, which varies according to diel and seasonal patterns. In addition to being a so far unexplored topic, this suggests a vocal plasticity in the species and a coping strategy to avoid masking of relevant acoustic signals for the local population in the Cres-Lošinj archipelago.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/d15060787/s1, Figure S1: Presence (black) and absence (white) of bottlenose dolphin vocalizations at the MS5 Susak and MS6 Lošinj for all 42 24-h days during the NTS and TS; Table S1: Descriptive statistics of whistle parameters at the MS5 Susak and MS6 Lošinj during the day and night in the NTS and TS; Table S2: Descriptive statistics of chirp parameters at the MS5 Susak and MS6 Lošinj during the day and night in the NTS and TS; Table S3: Descriptive statistics of

LFN sound parameters at the MS5 Susak and MS6 Lošinj during the day and night in the NTS and TS; Table S4: Statistical tests for differences in whistle parameters between diel (day vs. night) and seasonal (NTS vs. TS) periods at the MS5 Susak (Statistically significant results are highlighted in bold); Table S5: Statistical tests for differences in chirp parameters between diel (day vs. night) and seasonal (NTS vs. TS) periods at the MS5 Susak (Statistically significant results are highlighted in bold); Table S6: Statistical tests for differences in LFN sound parameters between diel (day vs. night) and seasonal (NTS vs. TS) periods at the MS5 Susak (Statistically significant results are highlighted in bold); Table S7: Statistical tests for differences in whistle parameters between diel (day vs. night) and seasonal (NTS vs. TS) periods at the MS6 Lošinj (Statistically significant results are highlighted in bold); Table S8: Statistical tests for differences in chirp parameters between diel (day vs. night) and seasonal (NTS vs. TS) periods at the MS6 Lošinj (Statistically significant results are highlighted in bold); Table S9: Statistical tests for differences in LFN sound parameters between diel (day vs. night) and seasonal (NTS vs. TS) periods at the MS6 Lošinj (Statistically significant results are highlighted in bold).

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Data Availability Statement: The data presented in this study are available on request from the corresponding author.

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