

## Article

# Vocal Behaviour of a Bottlenose Dolphin Pod during a Deadly Bycatch Event in the Gulf of Catania, Ionian Sea

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**Abstract:** Cetacean bycatch is increasing worldwide and poses a threat to the conservation of several delphinids. The bottlenose dolphin (*Tursiops truncatus*) is frequently involved in bycatch incidents, due to its coastal distribution and opportunistic behaviour. The acoustic behaviour of cetaceans during death-related events is a largely unexplored topic. During an acoustic monitoring survey of the bottlenose dolphin population inhabiting the Gulf of Catania (Ionian Sea), we documented the entangling and subsequent death of a sub-adult male in a fishing net. Here, we provide a detailed analysis of the vocal behaviour of the pod of bottlenose dolphins during the event. We identified a total of 720 vocalisations in a timespan of 138 min, including 436 signature whistles, 51 non-signature whistles, and 233 burst pulses. We recorded high vocal activity, with a peak characterised by emission rates of 36 signature whistles per minute and 70 burst pulses per minute. Although future studies are required, our results show massive vocal activity during net entangling, characterised by a significant emission of signature whistles and burst pulses, including bray series of gulps and squeaks. Therefore, we suggest that developing tools for automatically detecting the peaks of these sound types could be helpful in recognising bycatch events, in order to better quantify the impact of professional fishing on small cetaceans.

**Keywords:** artisanal fishing; bioacoustics; cetaceans; entanglement; signature whistles; death



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## 1. Introduction

Bycatch events, characterised by the unintentional entrapment of non-target species, pose a significant threat for cetaceans [1–4]. Among these, the bottlenose dolphin (*Tursiops truncatus*) is highly susceptible to the risk of remaining entangled in fishing nets during depredation events (i.e., animals approach fishing nets to consume the caught fish). Indeed, in the Mediterranean Sea, the bottlenose dolphin distribution is usually confined to the continental shelf within the 200 m isobath [5], where most small-scale fisheries operate. In this context, the opportunistic feeding behaviour of bottlenose dolphins and the collapse of fish stocks [6], due to overfishing, have led to competition between fishers and dolphins for the same resource, as shown by depredation events [7]. Therefore, reports of dolphin–fishery interactions—often linked to net damage and catch loss—are becoming more consistent and widespread throughout the Mediterranean basin [8–10]. In this scenario, there is a growing risk of cetaceans becoming entangled. Thus, it is fundamental to prevent bycatch events to preserve the local bottlenose dolphin population. Indeed, a slight increase in bycatches of local bottlenose dolphin populations can result in a marked increase in the probability of local extinction [11].

There have been many attempts to mitigate depredation events in the Mediterranean basin, from modifications to nets to keep animals away, to several acoustic deterrents (e.g., pingers [12]). Nevertheless, these solutions alone have proved ineffective, with dolphins habituating to acoustic deterrents after only a few exposures [13]. Furthermore, several studies have reported cetaceans using pingers as cues to locate fisher nets [14]. The economic cost effort caused by the presence of holes in the nets, bent hooks, reduction in the amount or value of the catch caused by wild animals, and the lack of compensation for such damage (or the difficulty to obtain) make the situation critical [15,16]. There are different examples of bottlenose dolphins' injuries exerted by fishers in Sicily, in the Aeolian archipelago, where mutilations due to gunshot wounds have been reported [17]. These tensions make it difficult to only rely on fishers to estimate the real risks for cetaceans, linked to fishery activity.

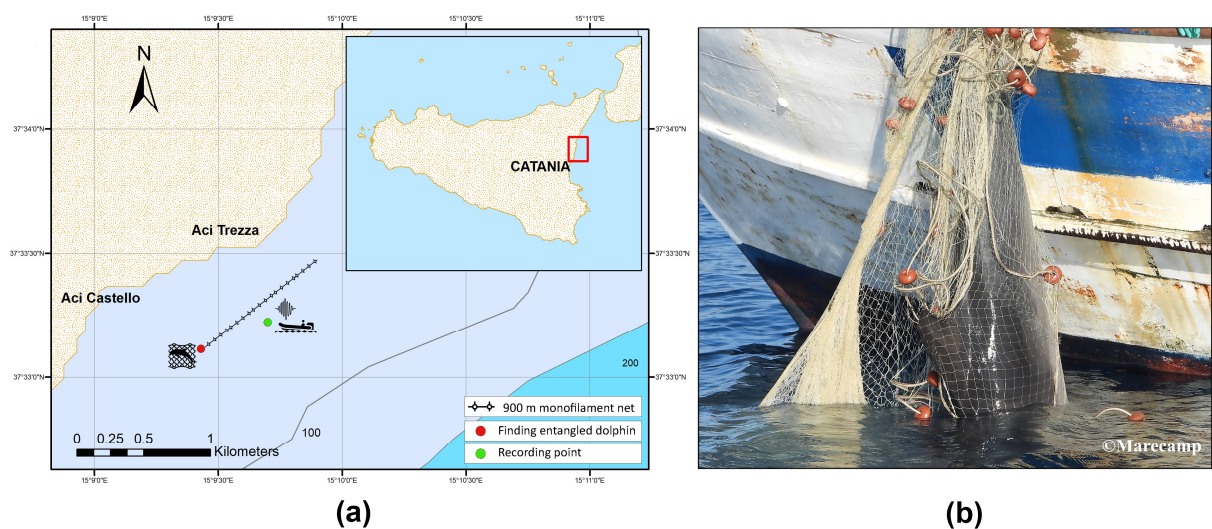
Nowadays, many studies have demonstrated that acoustic monitoring is useful to assess the presence of cetaceans [18,19] and their threats, linked to anthropic activity [20,21]. In the existing literature, only a few reports have described the vocal behaviour of cetaceans during entanglement events (i.e., [22,23]). Acoustic behaviour is a crucial element of cetaceans' phenotype and is the primary way they interact with the social, biological, and physical environment [24,25]. Accordingly, acoustic monitoring of cetaceans is an increasingly used research tool to assess the presence of animals, and represents a powerful and non-invasive alternative to traditional methods (e.g., photo identification), to study their activity and distribution [26]. Monitoring the emission of sounds during risky events is necessary for species conservation. As bycatch events are exceptional accidents, scientific reports are fundamental in improving our understanding of vocal behaviour during these contexts.

Here, we described the acoustic behaviour of a bottlenose dolphin pod involved in a death-related event of a sub-adult male, caused by bycatch. Our results show an intense use of vocalisation by the dolphins engaged in this context, with a massive emission of signature whistles and burst pulses. Accordingly, we suggest that the vocal behaviour could be instrumental in developing an acoustic monitoring protocol capable of identifying these accidents from audio recordings, and, thus, could help to prevent bycatch events of small cetaceans.

## 2. Materials and Methods

### 2.1. Study Context and Acoustic Recordings

On 19 July 2021, while performing routine monitoring of the interaction between cetaceans and small-scale fishing activities in the Gulf of Catania (Ionian Sea; Figure 1a) from a 7 m inflatable boat, we were able to document the vocal behaviour of a bottlenose dolphin pod during the entangling (and subsequent death) of a groupmate in a fishing net. The event occurred in the early morning (Table 1) under good weather conditions and in calm waters (Douglas scale 0–1 and Beaufort scale 0–1). At 04:51 a.m., the dolphins approached the fishing boat while positioning a monofilament net (900 m length, placed at 50 m depth), which had been left in place for about three hours. We confirmed the dolphins' presence from the beginning of the acoustic monitoring through passive acoustic listening. Once the sun rose (05:20 a.m.), we visually inspected the area and spotted ten adult bottlenose dolphins with one calf. At 05:27 a.m., we observed a sequence of surfacing behaviour performed by five adult individuals moving from north-east towards south-west. These individuals performed accelerations, body contacts, bows, and breaches [27]. After the surfacing behaviour, the animals disappeared from our view. We ended the acoustic monitoring at 07:25 a.m., and we then reached the fishers to interview them about the caught fish and net conditions. Intensive damage to the fishing net was reported. At 07:44 a.m., when the fishers ultimately hauled up the last part of the net (37°33.224' N–15°9.700' E), a sub-adult male bottlenose dolphin was found entangled and dead (Figure 1b).



**Figure 1.** (a) Study area and position of the recording array (green dot), the entangled dolphin (red dot), and the fishing net (dotted line); (b) carcass of the bottlenose dolphin. Adapted with permission from Marecamp association, 2021.

**Table 1.** Chronological order of the events.

Time	Event
04:21	Setting of the fishing net
04:51	Start of the acoustic recordings, detected presence of dolphins
05:05 (to 05:10)	Occurrence of the first peak of vocalisations
05:21 (to 05:23)	Occurrence of the second peak of vocalisations
05:27	Visual sighting of dolphins and surfacing behaviour
05:42	Bottlenose dolphins out of view
05:45	Recording of one single signature whistle type
06:00	End of acoustic detection of the pod
06:55	Start of hauling up the fishing net
07:25	End of the acoustic recordings
07:35	Interview of the fisherman that reported damaged fishing net
07:44	End of the net hauling and recovery of the entangled bottlenose dolphin

Recordings started at 04:51 a.m. The boat was stationary and positioned in the middle of the fishing net and parallel to it (37°33.224' N–15°9.700' E; Figure 1a). Acoustic data were collected during the event using two HTI-96-MIN (High Tech, Inc., Long Beach, MS, USA) marine-mammal hydrophones (2 Hz–30 kHz flat frequency response; sensitivity –164 dB re: 1 V/μPa) lowered at a depth of 10 m in the middle of the fishing net. One hydrophone was deployed from the stern of the boat and the other from the bow, at 7 m distance to each other, to localise the dolphin position along the net using a time-of-arrival analysis of the signals to the acoustic sensors. Additionally, a third hydrophone was deployed and connected to a laptop on-board to visualise a real-time spectrogram of the acoustic scene. The output signal from the hydrophones was digitised with a professional Zoom H5 Handy recorder (Zoom corporation, Tokyo, Japan) and saved as audio files in .wav format (16-bit resolution). Recording sessions were continuous.

2.2. Acoustic and Statistical Analyses

Spectrograms of the audio recordings were visually inspected using Praat v. 6.0.54 [28]. We generated the waveform and the fast Fourier transform spectrogram for each file with the following settings: view range = 0 to 22 kHz, window length = 0.02 s, window shape = Hamming (raised-sine squared), number of time steps = 1000, number of frequency steps = 250, and dynamic range = 70 dB. For the time-of-arrival analysis, we visually inspected and compared the dolphins’ vocalisations shown in the spectrograms of the

two .wav files recorded from the hydrophone array. More specifically, by aligning the two spectrograms and evaluating the small changes in the arrival of the sounds to the hydrophones, we were able to infer the position of the dolphins concerning the fishing net, and whether the pod approached the net from south-west or north-east. We then identified and separated whistles and burst pulse sounds [29]. Since our recording array was not set up to collect ultrasounds, we did not include echolocation clicks in the analysis. We plotted the number of vocalisations recorded by the pod during our acoustic monitoring, considering a time bin of 1 min to emphasise the bycatch event, which likely lasted no more than 5 min. We identified two different peaks of vocal activity and measured the calling rate for each peak, comparing them with each other. We further labelled signature whistles (SWs), defined as narrowband sounds with individually distinctive frequency modulations, which broadcast the identity of the emitter [30,31]. Following the SIGnature-IDentification protocol (SIG-ID; [32]), we identified stereotyped whistles, and all stereotyped whistles with at least four stereotyped contours, with 75% of the inter-whistle intervals ranging between 1 and 10 s, were categorised as SW. We considered the frequency contours interrupted by very short breaks ( $<0.03$  s) as continuous [19,33]. For each signature whistle with a high-quality contour, we extracted the fundamental frequency in MATLAB® using the “Beluga” toolbox (available for download at <https://synergy.st-andrews.ac.uk/soundanalysis/>) accessed on 15 September 2021. We then measured the following acoustic parameters on each frequency contour through a custom-built script in MATLAB®: start frequency, end frequency, minimum frequency, maximum frequency, mean frequency, frequency range (the difference between the maximum and minimum frequency), duration, and number of inflection points (i.e., any change in slope from positive to negative or vice versa).

We performed a principal component analysis (PCA) with varimax rotation, using the eight acoustic parameters as independent variables. We used the loadings of the PCs as a proxy for the correlation between the original variables and the unit-scaled components, and plotted the SWs in a three-dimensional space defined by the first three PCs.

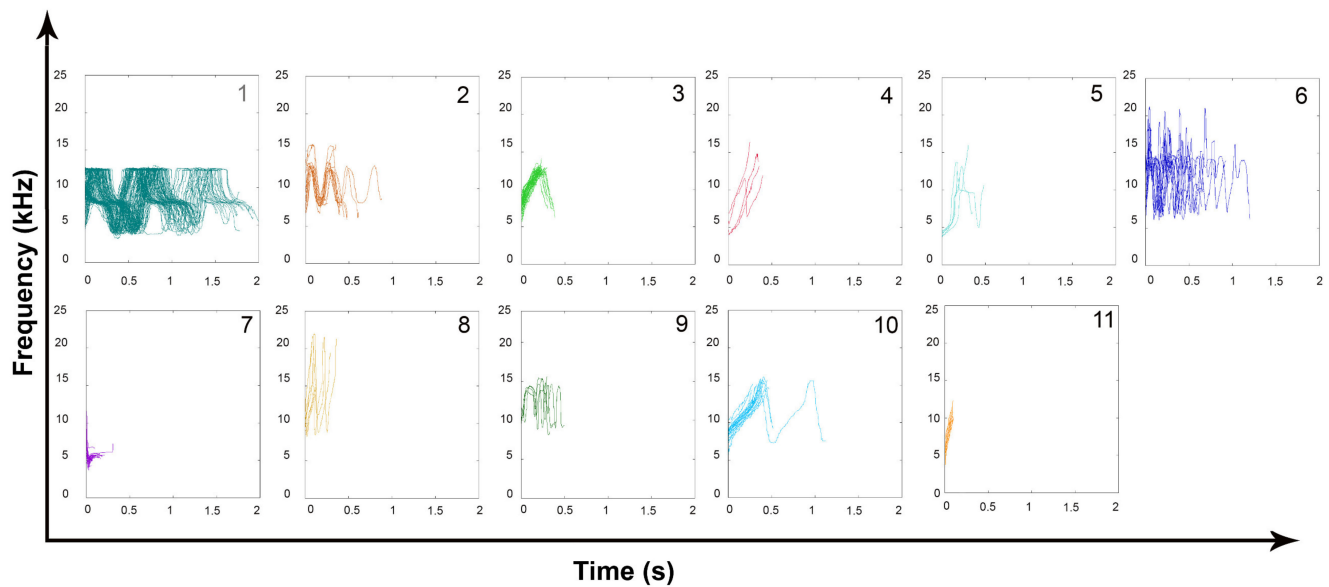
Finally, using a Wilcoxon paired-sample signed-rank test, we compared the emission rate of the six signature whistle types (i.e., SW1, SW2, SW6, SW7, SW9, and SW11) recorded during the bycatch (05:18 a.m. to 05:29 a.m.), with their emission rate pre-bycatch (04:51 a.m. to 05:15 a.m.).

### 3. Results and Discussion

The time-of-arrival analysis showed that some vocalisations (burst pulses and echolocation clicks) emitted before the second peak came from the north-eastern part of the net, and all the whistles were uttered from the south-west. In addition, all the vocalisations emitted during the second peak confirmed the presence of dolphins in the south-western part of the net. We collected 2 h, 18 min, and 54 s (138 min) of audio recordings, and identified a total of 720 vocalisations, including 51 non-signature whistles, 436 signature whistles, and 233 burst pulses. The signature whistles were assigned to 11 different signature whistle types, following the SIGI-ID method (Figure 2).

The identification of 11 signature whistle types led to confirmation of the size of the pod. Indeed, we spotted ten adult individuals and one calf during dawn. Furthermore, we provided the first acoustic characterisation of the signature whistles of the bottlenose dolphin population inhabiting the Ionian Sea (Table S1). Our results will be invaluable for future studies investigating the variability in the signature whistles in this species across the Mediterranean basin. The results of the principal component analysis supported our visual categorisation of the signature whistles. Indeed, in the three-dimensional plot defined by the first three PCs, the signature whistles were grouped into different clusters, highlighting their stereotyped individuality frequency modulation (Figure S1). In particular, the PCA combined the eight acoustic parameters measured on each frequency contour in three PCs that explained 85.89% of the total variance (PC1 = 39.52%, PC2 = 34.10%, and PC3 = 12.27%; Table S2).



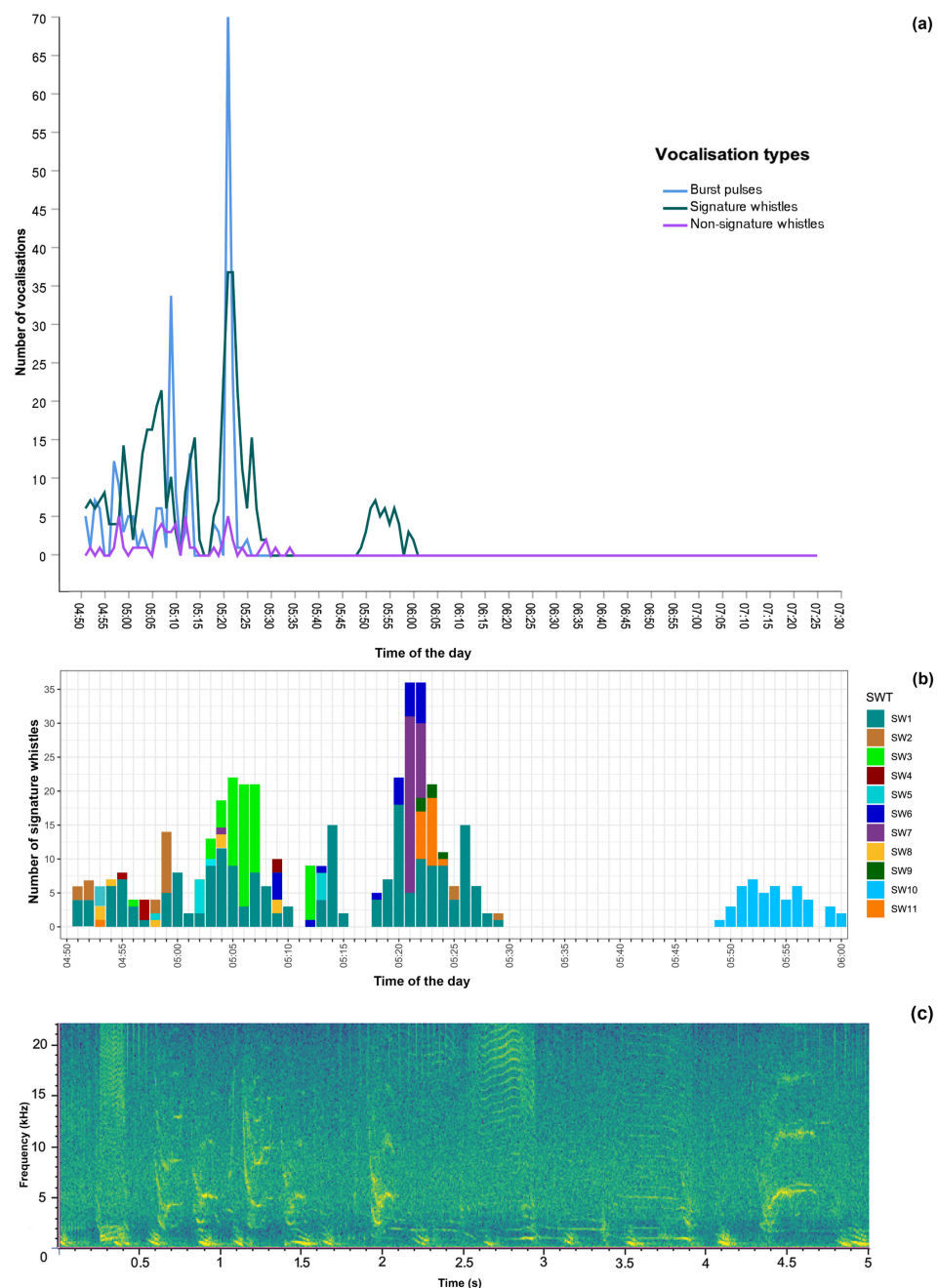


**Figure 2.** Pitch contours extracted for the 11 signature whistle types identified using the SIG-ID method [32].

The loadings showing the correlation between the acoustic parameters and PCs are presented in Table S3. We found that PC1 correlated most with the maximum frequency, mean frequency, end frequency, duration, and the number of inflections. In addition, PC2 correlated with the end frequency, duration, range frequency, minimum frequency, and inflection, and PC3 with the start frequency. Our results confirm that selected acoustic parameters (i.e., maximum frequency, duration, mean frequency, start frequency, and minimum frequency) are important for distinguishing the signature whistles of different bottlenose dolphins.

The agitated behaviour we observed at 05:27 a.m. was likely linked to the death-related event. Indeed, fast movement was observed towards the south-west, i.e., the part of the fishing net where the dolphin carcass was found, and may have represented an attempt by the pod to save their entangled groupmate. Moreover, Warren-Smith and Dunn [34] reported the same agitated behaviour in 4–5 bottlenose dolphins belonging to a pod where one juvenile member was seriously injured. Accordingly, we speculate that the unusual behaviour observed in the pod was related to the entanglement.

Two peaks of vocal activity were identifiable within the recordings (Figure 3a); the first occurred between 05:05 a.m. and 05:10 a.m., and the second peak, which was more pronounced, occurred between 05:21 a.m. and 05:23 a.m. We believe that the first peak coincided with a depredation event, characterised by bottlenose dolphins feeding and socialising by the net. Considering the massive vocal activity during the second peak, we suggest that this could correspond to the entangling and subsequent death of the dolphin. Indeed, its duration is compatible with the time a bottlenose dolphin can stay underwater without breathing (on average, 5 min, but possibly lower in a panicked/distressed event) [35,36]. We suggest that some members of the dolphin pod produced an unusual number of vocalisations in response to the high levels of stress and agitation linked to the distress calls emitted by the entangled dolphin. More specifically, the time slot from 05:18 to 05:29 was characterised by the rapid emission of signature whistles by six of the eleven different individuals (SW1, SW2, SW6, SW7, SW9, and SW11; Figure 3b). The Wilcoxon signed-rank test confirmed this hypothesis, showing that the emission rate of these six signature whistles was significantly higher during the second peak (05:18 a.m. to 05:29 a.m.; Wilcoxon test:  $n = 6$ ,  $p < 0.05$  two-tailed,  $Z = -1.992$ ). These six SW types recorded at the entanglement site (second peak) could tally with the victim plus the five individuals observed swimming towards the site.



**Figure 3.** (a) The number of vocalisations recorded over time in a 1 min time bin visualization. (b) The number of signature whistles emitted per minute divided by signature whistle types (SWT). (c) Spectrogram (spectrogram window size: 512, Hann window, overlap 50%) of a vocal sequence extracted from the second peak of vocal activity (05:21 a.m.).

The comparison between the vocalisations emitted by the same number of individuals ( $n = 3$ ) during the first and second peaks showed that the signature whistle rate at 05:03 a.m. (first peak) was 13 signature whistles per minute, and at 05:21 a.m. (second peak), it was 36 signature whistles per minute. Regarding the burst pulses, we detected 3 burst pulses per minute at 05:03 a.m., and 70 burst pulses per minute at 05:21 a.m. Analysing the composition of the signature whistle types over the recordings (Figure 3b), we observed that during the second peak, at 05:21 a.m. and 05:22 a.m., there was a conspicuous emission of SW7. Notably, during this same temporal window, a sequence lasting 20 s, composed of 31 low-frequency bursts (gulps) and 21 long multiband bursts (squeaks), was also emitted (Figure 3c). Sequences of multi-unit vocal signals—known as bray series—may convey

different functional meanings, based on the type of sequence emitted by the animals [37]. Thus, the bray series recorded may communicate the response of the pod of bottlenose dolphins related to the bycatch event. Moreover, the conspicuous emission of SW7 could be related to the entangled dolphin emitting his signature whistle towards the other members of the pod.

We detected dolphins' vocalisations until 06:00 a.m., but after 05:30 a.m., we only recorded the presence of one signature whistle type (SW10; Figure 3b). Due to the lack of other vocalisations, SW10 was likely emitted to maintain contact between the emitter and other groupmates during movement [30]. In the chronological order of events, it is important to note that after 05:30 a.m., we did not visualise the dolphins anymore, although we remained on site until the haul of the net and the finding of the dead entangled dolphin. Given the age and sex of the entangled dolphin (i.e., sub-adult male), the abandonment of the carcass by the rest of the group shortly after the death was not unexpected. Indeed, although delphinids have been observed to demonstrate long-lasting epimeletic behaviour towards dead conspecifics, these observations relate to mother–calf pairs [38,39].

Although previous studies have described the behaviour of dolphins during death-related events [39–41], the acoustic emissions of a group of bottlenose dolphins in these situations have never been described in detail. Dudzinski et al., in 2009 [40], provided the first onomatopoeic description of the sounds produced by the bottlenose dolphin during two distinct death-related events. More recently, a description of the acoustic behaviour of a bottlenose dolphin pod during a trawl bycatch event of three bottlenose dolphins in the Adriatic Sea was reported by Corrias et al. (2021) [23]. The authors noted 23 whistles with ascending contours (upsweep), combined with an impulse signal, which they called “low-frequency”. However, the presence of signature whistles was not investigated. In contrast with these findings, we recorded many signature whistles and did not find whistles characterised by the peculiar acoustic features described by Corrias et al. (2021). The other few reports on dolphins' acoustic behaviour during death-related events mostly focussed on epimeletic behaviour towards calves [42,43]. Interestingly, Perrtree and colleagues [43] described the acoustic behaviour during birth and a likely subsequent infanticide attempt by common bottlenose dolphins (*Tursiops truncatus*). During this event, different bouts of low-frequency tonal sound (possibly gulps) occurred. The authors linked the emission of these sounds to the possible infanticide attempt. Considering the stressful situation, the sounds recorded during this event could be linked, more generally, to distress events, thus confirming our results. Kuczaj et al. [44] reported a wild bottlenose dolphin pod providing epimeletic care to a distressed conspecific, and found that signature whistles were consistently emitted when a bottlenose dolphin sought aid from others. More generally, the acoustic behaviour of bottlenose dolphins during stressful situations has been investigated during capture–release procedures in Sarasota Bay. The results showed that the whistle rates were more significant during brief capture–releases than undisturbed events. Accordingly, Esch and co-authors [45] concluded that signature whistles have the potential to be acoustic cues of stress. Furthermore, the recordings collected from a dying common dolphin (*Delphinus delphis*) showed the emission of only stereotyped whistles [46]. Moreover, Cheng and colleagues (2017) reported the acoustic behaviour of an Indo-Pacific humpback dolphin (*Sousa chinensis*) injured by fishing tackles. During this occurrence, many stereotyped whistles were recorded [47]. Therefore, our findings concerning the higher signature whistling rate observed between 05:18 a.m. and 05:29 a.m. (at 05:21 a.m., three-times higher compared to the first peak) are consistent with the previous literature, and demonstrate that the rate of emission of the signature whistles can provide information on the distress of the emitters. Thus, the SW7 that was emitted copiously in a very restricted time window (2 min) could have belonged to the entangled dolphin calling to seek aid from other group members. Moreover, the presence of bray–gulp bouts in the same temporal window of the increasing emission of signature whistles may link these vocalisations with distress events, such as bycatch.

As fishing activity grows, due to the diminishing of fish stock throughout the Mediterranean Sea [6], and considering the increase in depredation events [15,48], the direct consequence is an increase in bycatch events. In this context, bycatch estimates are likely to be severely underestimated for multiple reasons. Some EU countries have no dedicated observer programs, and, currently, most of the attention is being devoted to vessels over 15 m that form a minority of the fishing [3]. The economic damage caused by wild animals to fishery activity, and the lack of compensation for this damage, make it impossible to rely on fishermen to accurately report bycatch events.

Our study provides an essential contribution to the existing literature, since it quantifies—for the first time—the emission of signature whistle and burst pulses by a pod of bottlenose dolphins during the net entangling and subsequent drowning of a group-mate. Most importantly, we suggest that the acoustic detection of an unusual number of signature whistle and burst pulses (including bray series of gulps and squeaks) within a brief temporal window could be instrumental in detecting bycatch events. With the increasing use of passive acoustic monitoring (PAM) [10,19,49], our report about vocal behaviour during a bycatch event is fundamental. The use of PAM protocols in fishery zones could permit us to recognise the occurrence and quantification of bycatch events. Although further investigation is needed, our study could be the starting point for understanding how to leverage passive acoustic monitoring protocols to better estimate the impact of bycatch events during fishing activity.

**Supplementary Materials:** The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/jmse10050616/s1>, Figure S1: The 11 signature whistle types are plotted in the space defined by the first three Principal Components. Four different rotation angles are shown.; Table S1: Mean  $\pm$  standard deviation (SD) of acoustic parameters of the 11 signature whistle types.; Table S2: Variance explained by the Principal Components showing eigenvalues  $> 1$ ; Table S3: Factor loadings for the eight acoustic parameters on the Principal Components showing eigenvalue  $> 1$ .

**Author Contributions:** Conceptualization, C.M. and L.F.; visualization, F.T.; formal analysis, F.T. and L.F.; investigation, C.M., A.R., S.F. and F.T.; funding acquisition, C.M. and L.F.; supervision, C.M. and L.F.; project administration, C.M.; writing—original draft preparation, F.T.; writing—review and editing, L.F., C.M., A.R. and S.F. All authors have read and agreed to the published version of the manuscript.

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**Institutional Review Board Statement:** No ethical approval was necessary for this study.

**Informed Consent Statement:** Not applicable.

**Data Availability Statement:** Data are contained within the article or Supplementary Material.

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**Conflicts of Interest:** The authors declare no conflict of interest.

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