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## VARIABILITY OF SIGNATURE WHISTLE EMISSION IN A BOTTLENOSE DOLPHIN UNIT OFF THE EASTERN LIGURIAN COAST (ITALY)

### VARIABILITÀ DELL'EMISSIONE DI FISCHI FIRMA IN UN'UNITÀ DI TURSIOPI AL LARGO DELLE COSTE DEL LEVANTE LIGURE (ITALIA)

**Abstract** - *Tursiops truncatus* (Montagu, 1821) is a regularly present species in the Mediterranean Sea. This study aims to investigate the acoustic behaviour of this species in different contexts and group compositions. We carried out an identification of signature and non-signature whistles using the SIGID (SIGnature IDentification) method, and we compared whistles identified as primary signature whistles (necessary for the initial categorisation of signature whistles) with the secondary signature whistles (whistles with a known frequency modulation compared to previous categorisation). We used Panguard to extract the whistle contours and the SIGID criterion to identify the signature whistles. We classified 1146 non-signature whistles and 234 signature whistles. Of these, 79 were the primary signature whistles, and 155 were the secondary signature whistles. Our results suggest a more extensive use of signature whistles in our study population than that the SIGID criterion can identify.

**Keywords:** *Tursiops truncatus*, acoustic behaviour, SIGID

**Introduction** - Since 2001, Acquario di Genova and Fondazione Acquario di Genova have been conducting a long-term study program called *Delfini Metropolitan* to study cetaceans inhabiting the coasts of Eastern Liguria, within the Pelagos Sanctuary (a Specially Protected Area of Mediterranean Importance since 2002). This project revealed the presence of a geographical unit of bottlenose dolphins residing in the study area (Liguria-Tuscany), composed by around 500 individuals (Gnone *et al.*, 2022). The present study aims to investigate the acoustic behaviour of these dolphins, focusing on the identification of signature and non-signature whistles. Signature whistles are distinctive, individual and frequency-modulated sounds produced by bottlenose dolphins in specific contexts and, according to Caldwell *et al.* (1990), make up the majority of acoustic signals produced when animals are isolated. It is possible that the signature whistle evolved as an individual mother-calf recognition signal and was then reused for individual recognition between adult individuals in the dynamic fission-fusion society typical of this species (Gnone & Moriconi, 2009; Janik, 2014). To recognise these signals, Janik and colleagues (2013), created the SIGID (SIGnature IDentification) criterion, according to which a signature whistle can be identified from a spectrographic analysis when: (1) the recorded sequence has at least four stereotyped whistles in it and (2) a minimum of 75 % of the whistles, at least once, have an inter-whistle interval between 1-10 seconds (Janik *et al.*, 2013). According to the authors, the SIGID criterion can lead to the loss of an important fraction of signature whistles (47-56 %) but avoids false positives.

**Materials and methods** – The study area was between Genoa and La Spezia, in the Eastern Ligurian coast. From May to October 2023, we carried out 23 daytime surveys, each lasting 4-5 hours, under favourable weather and sea conditions. We collected the acoustic data using an HTI-96-Min hydrophone connected to a Zoom H1n Handy recorder, set up with 96 kHz/16-bit recording format. We analysed the acoustic data utilising Praat v. 6.2.23 (Boersma & Weenink, 2022), and we identified signature whistles by visual observation of the spectrograms. We classified whistles that complied with the SIGID categorisation rules (Janik *et al.*, 2013) as “primary signature whistles” (PSW), while we classified as “secondary signature whistles” (SSW) those whistles that showed a frequency modulation resembling a whistle category previously identified. We classified the remaining whistles as “non-signature whistles” (NS). Then, we used PAMGuard v. 2.02.09 CORE (Gillespie *et al.*, 2008), particularly the ROCCA module, to extract the pitch contour of the fundamental frequencies of all the whistles recognised and we ran a PCA (Principal Component Analysis) on the variables obtained. Then, we applied a t-SNE (t-Distributed Stochastic Neighbour Embedding) algorithm to plot the dimensionalities obtained from the PCA on a two-dimensional plane, using the 13 PCs obtained previously. Finally, we used a k-means clustering algorithm to group signature and non-signature whistles into 11 clusters based on the variables obtained, 10 for the

**Results** – In six months of data collection, we sighted bottlenose dolphins 26 times. In 19 sightings (73 %) we observed bottlenose dolphins in the wake of bottom trawlers. The group size ranged from 1 to 25. In eight cases (30.7 %), we sighted at least one calf in the group. Through the photo-identification technique, we identified a total of 66 individuals and the identification rate was 67 %. We collected 5 hours and 53 minutes of acoustic recordings and identified 1,380 whistles. Using the SIGID criterion, we identified 234 whistles samples as signature whistles (79 PSW+155 SSW) (Fig. 1 a - b), and we assigned these to ten stereotypical frequency modulation classes, likely representing ten different individuals. We classified the remaining 1,146 whistles as NS (Tab. 1).

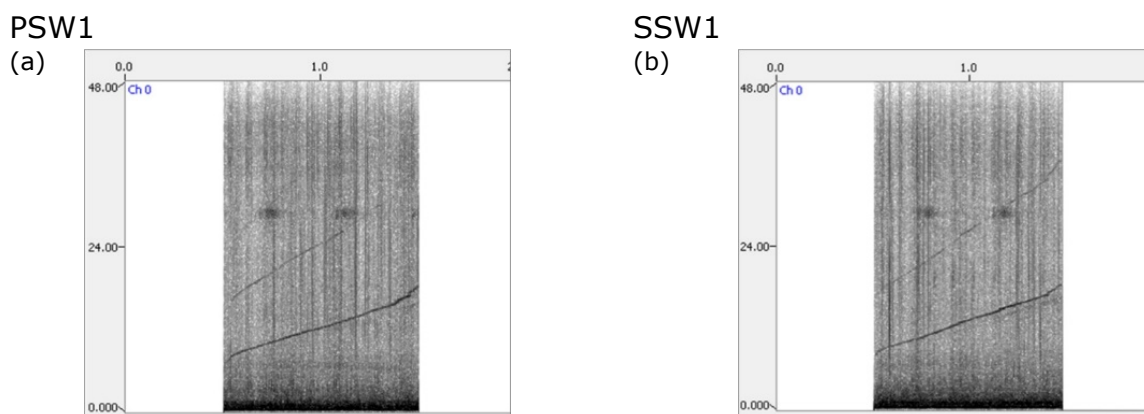


Fig. 1 – (a) Signature whistles 1 in the primary form (PSW1); (b) Signature whistle 1 in the secondary (SSW1) form. PAMGuard spectrogram settings: Hann window type, FFT Length 1024 bins, frequency resolution 93.75Hz, frequency range 0-48 kHz, time resolution 10.67 ms, time step size 5.33 ms.

(a) *Fischio firma 1 nella forma primaria (PSW1); (b) Fischio firma 1 nella forma secondaria (SSW1). Impostazioni dello spettrogramma in PAMGuard: Finestra Hann, lunghezza FFT 1024 bins, risoluzione frequenza 93,75 Hz, intervallo frequenza 0 - 48 kHz, risoluzione temporale 10,67 ms, dimensione del passo temporale 5,33 ms.*

Tab. 1 - Number of primary signature whistles (PSW), secondary signature whistles (SSW) and non-signature whistles (NS) for each identified category.  
*Numero di fischi firma primari (PSW), fischi firma secondari (SSW) e fischi non-firma (NS) per ogni categoria identificata.*

	PSW	SSW	NS
sw1	8	22	
sw2	4	8	
sw3	4	4	
sw4	14	6	
sw5	6	19	
sw6	12	47	
sw7	6	5	
sw8	16	18	
sw9	5	11	
sw10	4	15	
ns			1146
TOTAL	79	155	1146

We extracted 55 acoustic variables on which we applied the PCA, finding that 13 PCs have eigenvalues greater than 1 and explain the 85.9 % of the cumulative variance. Finally, we applied t-SNE and k-means clustering algorithms to obtain the scatter plots (Fig. 2 a - b). In Fig. 2b we selected the signature whistle 2 category (SW2), highlighting the two polygons identified by the PSW2 and SSW2.

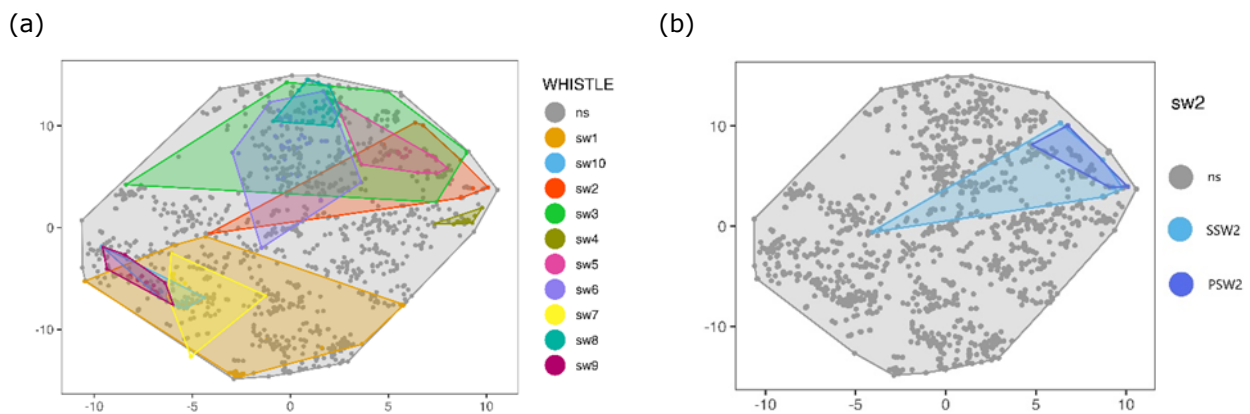


Fig. 2 - (a) Scatter plot of the 10 clusters obtained: sw=signature whistles; ns=non-signature whistles. The ordinate and abscissa axes show the positions in the space of the whistles considering the initial dimensionalities; (b) Scatter plot of the clustering of signature whistle 2: PSW2= primary signature whistle 2; SSW2= secondary signature whistle 2; ns\_s =non-signature whistles + signature whistles not considered.

*(a) Grafico a dispersione dei 10 cluster ottenuti: sw=fischi firma; ns=fischi non-firma. L'asse delle ordinate e delle ascisse mostrano la posizione nello spazio dei fischi considerando le dimensionalità iniziali; (b) Grafico a dispersione della clusterizzazione del fischio firma 2: PSW2=fischio firma 2 primario; SSW2=fischio firma 2 secondario; ns\_s=fischi non firma + fischi firma non considerati.*

**Conclusions** - The percentage of signature whistles identified on the total (17 %) was lower than in other studies (Terranova *et al.*, 2021). This could be influenced by the context and composition of the group, such as the number of calves. In fact, we sighted at least one calf in 30.7 % of the encounters, while Terranova and colleagues' (2021), studying the bottlenose dolphins in the same area, scored a much higher percentage (83.9%). Looking at the scatter plots (Fig. 2), the polygons indicate different variability within the analysed samples. Primary signature whistles tend to occupy a subset of the total space, as shown in Fig. 3 with SW2, confirming that the variability of PSWs, identified with the rigid SIGID criterion, is lower than the variability observed when SSWs are also included in the sample. This confirms the conservativeness of the SIGID criterion. However, since the identified signature whistles were most often recorded as SSWs (Tab. 1), the probability of missing this identification using the SIGID method is rather high, as the secondary identification cannot be made without the primary identification, which must fulfil the rigid SIGID criterion. Therefore, we should assume that in the 1,146 whistles categorised as NS (non-signature whistles) there are many false negatives, i.e. many signature whistles misclassified as non-signature whistles. It seems that the SIGID criterion can only partially cover the high variability in the emission of signature whistles, which makes it a highly conservative method. Further efforts should be made to make this method less conservative, adapting it to the plasticity of the species, thus avoiding the loss of so much data.

## References

- BOERSMA P., WEENINK D. (2022) - Praat: doing phonetics by computer [Computerprogram]. URL <https://www.praat.org/>
- CALDWELL M.C., CALDWELL D.K., TYACK P.L. (1990) - Review of the signature-whistle hypothesis for the Atlantic bottlenose dolphin. In: Leatherwood S., Reeves R.R. (eds), *The bottlenose dolphin*. Academic Press, New York, NY: 199–234. URL <https://doi.org/10.1016/B978-0-12-440280-5.50014-7>.
- GILLESPIE D., GORDON J., MCHUGH R., MCLAREN D., MELLINGER D. K., REDMOND P., THODE A., TRINDER P., DENG X.Y. (2008) - PAMGUARD: Semiautomated, open source software for real-time acoustic detection and localisation of cetaceans. *J. Acoust. Soc. Am.*, **30**: 54-62. URL <https://www.pamguard.org/>.
- GNONE G., BELLINGERI M., MOLINARI Y., DHERMAIN F., LABACH H., DÍAZ LÓPEZ, B., DAVID L., DI MEGLIO N., AZZINARI G., AZZINARI C., AIROLDI S., LANFREDI C., GONZALVO J., DE SANTIS V., NUTI S., ÁLVAREZ CHICOTE C., GAZO M., MANDICH A., ALESSI J., AZZELLINO A., TOMASI N., SANTONI M., MANCUSI C., FALABRINO M., CAÑADAS A. M. (2022) - The Seabed Makes the Dolphins: Physiographic Features Shape the Size and Structure of the Bottlenose Dolphin Geographical Units. *J. Mar. Sci. Eng.*, **10** (8): 1036. URL <https://DOI.ORG/10.3390/JMSE10081036>.
- GNONE G., MORICONI T. (2009) - Use and function of distinctive whistle-like signals in bottlenose dolphin (*Tursiops truncatus*) mother-calf pair. In: Pearce A.G., Correa L.M. (eds), *Dolphins: anatomy, behaviour, and threats. Animal science, issues, and professions*. Nova Science Publishers, New York, NY: 49–167.
- JANIK V.M. (2014) - Cetacean Vocal learning and Communication. *Curr. Opin. Neurobiol.*, **28**: 60–65. URL <https://DOI.ORG/10.1016/J.CONB.2014.06.010>.
- JANIK V.M., KING S.L., SAYIGH L.S., WELLS R.S. (2013) - Identifying signature whistles from recordings of groups of unrestrained bottlenose dolphins (*Tursiops truncatus*). *Mar. Mammal Sci.*, **29** (1): 109–122. URL <https://DOI.ORG/10.1111/J.1748-7692.2011.00549.X>.
- TERRANOVA F., GNONE G., FRIARD O., BELLINGERI M., GIACOMA C., FAVARO L. (2021) - Signature whistles of the demographic unit of bottlenose dolphins (*Tursiops truncatus*) inhabiting the Eastern Ligurian Sea: characterisation and comparison with the literature. *Eur. Zool. J.*, **88** (1): 771–781. URL <https://doi.org/10.1080/24750263.2021.1936225>.