ACOUSTIC MIMICRY OF PLASTICS WITH THE PREY OF DEEP-DIVING CETACEANS: AN EXPERIMENTAL APPROACH

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1 INTRODUCTION

Ingestion of macroplastics by marine megafauna is a serious concern in marine conservation efforts, as it has been identified as a significant contributor to their global mortality rate[1,2,3]. The remarkable phenomena of voluntary macroplastic consumption by healthy animals[1] prompts more investigation into the sensory modalities and cognitive processes involved in such behaviour. The phenomena of marine megafauna consuming marine litter is likely linked to situations in which the distinction between natural prey and plastic items becomes blurred, leading to accidental consumption. The ability of marine visual predators to differentiate and select plastic debris as ingestible items, hinges on a multitude of factors, including species-specific ecophysiological traits governing sensory mechanisms involved in prey detection and capture, the diverse characteristics of plastic materials, and their resemblance to primary prey items[4,5,6].

Several recent studies reported the high occurrence of macroplastic ingestion by deep-diving odontocetes species[7,8,9,10]. Odontocetes, or toothed whales, are a diverse group of marine mammals belonging to the infraorder Cetacea. They inhabit oceans worldwide, ranging from coastal areas to deep pelagic waters, while exhibiting an array of specialized adaptations for underwater life[11]. Among the most remarkable features of odontocetes is their echolocation ability, a sophisticated sensory modality that allows them to navigate and explore their aquatic environment. Echolocation consists in the emission of high-frequency broadband sound pulses and their subsequent reception as echoes after they reflect on objects and organisms in the surroundings[12]. By “analysing” the timing, frequency and intensity of these returning echoes, odontocetes can construct detailed spatial maps of their environment, effectively “seeing” with sound. While echolocation is widely used by toothed whale species with different ecologies and life-styles, it becomes a crucial skill for those that dive and hunt in the deep, where vision is hindered by limited light availability[13] (as also happens for night foraging in shallow-divers[14,15]).

Stomach contents analyses suggest that deep-diving toothed whales primarily feed on cephalopods inhabiting the meso-bathypelagic realm (200 – 4000 meters), such as on members of the Histioteuthidae, Octopoteuthidae, and Cranchiidae families[16]. Cephalopods are generally considered animals with a low sound reflectivity due to their fluid-like body composition[17]. Their gladius, or pen, was proposed to be the main component of sound scattering[18], however, measurements in tanks showed that their mantle is the main reflective item, providing target strengths of -38 to -44 dB[19]. Meanwhile, plastic items that end up in the ocean can have various shapes and consistencies[20]. However, it has been shown that those that are mostly ingested by deep-diving cetaceans are characterized by a film-like consistency, such as shopping bags and packaging films[21].

Therefore, we hypothesise that deep divers’ potential misidentification of natural prey species with some types of plastic debris could be attributed to the resemblance of their acoustic signature. To test this hypothesis, a multidisciplinary approach, encompassing marine biology, ecology and acoustics, is required to comprehensively address the possibly complex interactions between deep divers and macroplastics. Therefore, we use a scientific echosounder to test and compare the acoustic signature of commonly ingested macroplastic items and natural deep-divers' prey under controlled experiments.

2 METHODOLOGY

2.1 Sonified items selection

The choice of the items to sonify was based on literature reports on both natural prey of deep-diving species and major ingested plastic items.

For the natural prey, we tested five squid species that are commonly identified in toothed whales' diet[16] or that anatomically resemble them. In particular, we included specimens of muscular squids from the Ommastrephidae family purchased at the local market, and specimens of ammoniacal squids from the Histioteuthidae and Cranchidae families, obtained from the collection of the University of La Laguna.

For the plastic items, we based our selection on the findings of Roman et al.[21]. In this recent review paper, the main types of plastic litter found in stranded cetaceans' stomachs were film-like plastics (such as plastic bags, sheeting and packaging), plastic fragments and ropes/nets. For this preliminary study, we selected a subset of different representative items of some of the most ingested plastic categories, while also including plastic types that were less ingested as a comparison. The plastic items that we included had different chemical compositions: PE, (polyethylene), PET (polyethylene terephthalate), HDPE (High-Density polyethylene), LDPE (Low-Density polyethylene) and PP (polypropylene). To replicate the likely aspect that marine debris has when floating in the ocean, we submerged our plastic items in a seawater tank exposed to sunlight for a period of a minimum one month, to allow the development of biofouling.

Details of the sonified items are reported in Table 1.

Sonified item	Codename	Type	Composition	Sonification details
Shopping bag	ShoppingB	Film-like	PE	Extended, folded
Transparent film	TranspFilm	Film-like	PE	Extended
0.25 L bottle with lid	Bottle 0.25L	Fragment	HDPE	Vertical
0.5 L bottle without lid	Bottle 0.5L	Fragment	PET	Vertical
0.75 L bottle with lid	Bottle 0.75L	Fragment	PE	Vertical
Greenhouse cover	GreenH_1	Mesh-like	PP	Extended
Greenhouse cover	GreenH_2	Mesh-like	HDPE	Extended, folded

Illex argentinus	I.argentinus	Muscular	-	Vertical, horizontal
Ommastrephes caroli	O.caroli	Muscular	-	Vertical, horizontal
Histioteuthis reversa	H.reversa	Ammoniacal	-	Vertical
Leachia atlantica	L.atlantica	Ammoniacal	-	Vertical
Megalocranchia oceanica	M.oceanica	Ammoniacal	-	Vertical, horizontal

Table 1: List and details of sonified items. Codename provides the simplified name used in the following figure and table. Film-like and mesh-like plastic items were tested both extended and folded when feasible; squid specimens were tested both placed horizontally and vertically when feasible (as *H. reversa* and *L. atlantica* were particularly delicate, we only tested one position to limit their manipulation and avoid possible damages).

2.2 Experimental setup

The experiment was carried out in October 2023 at the Oceanographic Center of the Canary Islands (Santa Cruz de Tenerife), which is part of the Institute of Oceanography of the Spanish National Research Council (IEO-CSIC). This research facility housed a large-capacity circular tank with a volume of 250 meters^3, 12 meters in diameter and a maximum depth of 3 meters, suitable for this type of experiment. A portable SIMRAD EK80 scientific echosounder containing a WBT mini transceiver that generates the electrical signal was used. The echosounder was equipped with an ES38_18_200_18C transducer (Kongsberg Maritime AS, Norway), which combines a 38 kHz split beam transducer and a 200 kHz single beam transducer, both with an 18° beam width, to emit the signal underwater. This transducer was mounted on a custom-made metal frame for hanging inside the tank and installed transversally to the lateral wall of the tank, at a depth of 1.23 meters. A transversal and a longitudinal pulley rail were installed above the tank to enable the correct placement of the various items to ensonify. The tank was then filled with seawater, and the echosounder was calibrated using a 38.1 mm diameter calibration sphere of tungsten-carbide (TS = - 42 dB) following the standard procedures for discrete frequency mode (CW)[22]. Before the experiment, we measured the temperature and salinity of the seawater in the tank using a hand-held thermo-salinometer to facilitate the accurate calculation of the sound speed[23]. The items to ensonify were placed at the same depth as the main transducer's focus, at a distance of 3 meters from it, to avoid the near-field distortions. The items were suspended with vertical nylon lines, tensioned by a simple weight located at the bottom, attached to the pulley system located above the tank (Fig. 1).

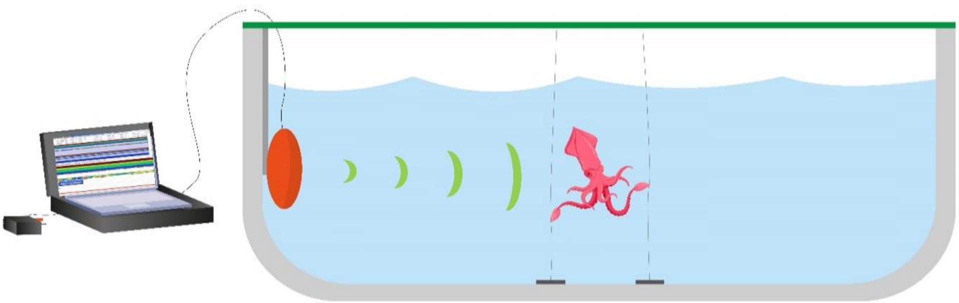

Fig. 1: Experimental design scheme. From left to right: lead-acid battery powering the echosounder, SIMRAD EK80 portable and transducer attached to a custom-built metal bar, longitudinal pulley rail (above the tank), vertical nylon lines held in place by bottom weights, item ensonified (squid, as in this case, or plastic) attached to the nylon lines.

We configured the echosounder with a transmitted power of 400 W (for the 38 kHz) and 200 W (for the 200 kHz), a pulse duration of 0.512 milliseconds and a ping rate of 2 pps. Real-time echograms of the experiment were visualized using the EK80 user interface with settings adapted to the context (i.e.: up to 12 meters distance and applying a +30 dB gain to compensate for the reverberation within the tank). After the necessary adjustments, the setup clearly identified the item of interest and all the detectable items in the tank in the displayed echogram (Fig. 2). To analyse each ensonified item, we used open-source software ESP3[24]. We applied the calibration and environment data to visualize the ensonified object correctly. Then, we created a region covering the position of the item (i.e.: between 2.5 - 3.5 meters of the transducer). The ESP3 single target detection and target tracking algorithms were applied to this region in order to locate individual echoes of the item. We extracted and exported target strength (TS) metrics from these echoes for each single detection. This process allowed us to define the TS of each ensonified item for both the 38 and 200 kHz frequencies.

Fig. 2: Example of echogram view in the ESP3 software used during TS analyses.

2.3 Statistical analyses

Statistical analyses of TS measures were performed in R software[25]. Data were not normally and not homogeneously distributed for both tested frequencies (Anderson-Darling normality test *p-value* < 0.05; Levene's homogeneity test *p-value* < 0.05). We then performed a non-parametric test (Kruskal-Wallis) to check for significant differences in TS between groups. Both frequencies reported significant *p-values* and thus non-parametric pairwise group comparison (Dunn's test) was performed.

3 RESULTS

3.1 Target strength of sonified items

Target strength response at 38 kHz ranged between -80.03 dB and -49.44 dB for macroplastics items, between -66.14 dB and -61.01 dB for muscular squids and between -68.4 dB and -73.96 dB for ammoniacal squids. At 200 kHz, target strength ranged between -81.94 dB and -56.46 dB for plastics, between -80.44 dB and -70.10 dB for muscular squids and between -82.33 dB and -75.07 dB for ammoniacal squids (Fig. 3).

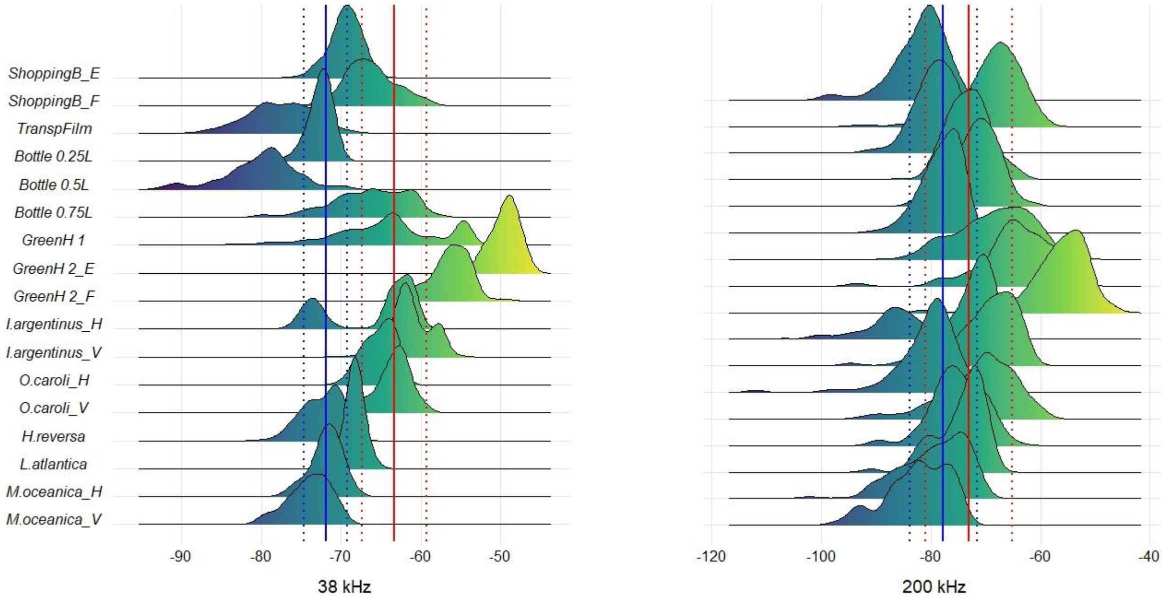

Fig. 3: Density plot displaying the TS of each of the sonified items. X-axis: TS (dB); y-axis: sonified items. The vertical solid lines represent the mean and the dotted lines the SD of muscular (blue) and ammoniacal (red) squids' TS. Some macroplastics have been sonified both extended (E) or folded (F), while some cephalopods were placed either horizontally (H) or vertically (V).

3.2 Statistical analyses

Non-significant relationships of Dunn's test between macroplastics and squid specimens are reported in Table 2.

Comparison	Type	Z	p-value	Frequency
ShoppingB_E – *L.atlantica*	LDPE – Ammon.	1.18	0.23	38 kHz
ShoppingB_F – *I.argentinus*_H	LDPE – Musc.	0.65	0.52	38 kHz
ShoppingB_F – *O.caroli*_H	LDPE – Musc.	1.09	0.28	38 kHz
Bottle 0.25L – *H.reversa*	PET – Ammon.	-0.68	0.50	38 kHz
Bottle 0.25L – *M.oceanica*_H	PET – Ammon.	-1.28	0.20	38 kHz
Bottle 0.25L – *M.oceanica*_V	PET – Ammon.	1.15	0.25	38 kHz
Bottle 0.75L – *I.argentinus*_H	PE – Musc.	-0.61	0.54	38 kHz
Bottle 0.75L – *O.caroli*_H	PE – Musc.	-1.09	0.28	38 kHz

GreenH 1 – *O.caroli*_H	PP – Musc.	1.65	0.10	38 kHz
GreenH – *O.caroli*_V	PP – Musc.	-1.08	0.28	38 kHz
ShoppingB_E – *M.oceanica*_V	LDPE - Ammon.	0.20	0.84	200 kHz
ShoppingB_E – *O.caroli*_H	LDPE - Musc.	1.57	0.12	200 kHz
TranspFilm – *O.caroli*_H	PE - Musc.	-1.09	0.28	200 kHz
TranspFilm – *M.oceanica*_H	PE - Ammon.	0.64	0.52	200 kHz
Bottle 0.25L – *L.atlantica*	PET – Ammon.	1.53	0.13	200 kHz
Bottle 0.5L – *O.caroli*_V	PET – Musc.	-1.01	0.31	200 kHz
Bottle 0.75L – *M.oceanica*_H	PE – Ammon.	0.77	0.44	200 kHz

Table 2: Non-significant pairwise comparisons (Dunn's test) between plastic items and squid specimens. Z score: measure of standard deviation from the mean. Some macroplastics have been sonified both extended (E) or folded (F), while some cephalopods were placed either horizontally (H) or vertically (V).

4 DISCUSSION AND CONCLUSIONS

Our study employed a multidisciplinary approach to investigate the potential misidentification of natural prey species and macroplastics by deep-diving odontocetes. By sonifying both common plastic debris and cephalopod prey items, we aimed to assess the potential similarities in TS that could contribute to voluntary ingestion. The findings of this preliminary study reveal that all tested plastics can be stronger echoic targets than all tested cephalopods, contributing to explaining why macroplastics can be selected as prey by deep-diving whales.

At 38kHz, ammoniacal squids displayed a TS similarity to those of PET (bottle 0.25L) and LDPE (shopping bag) plastic items, while muscular squids showed no significant differences to the TS of PE (bottle 0.75L), PP (greenhouse cover) as well as LDPE (shopping bag). At 200 kHz, ammoniacal squids displayed similarities with PE plastics (bottle 0.75 L), besides those already found at 38 kHz. Muscular squids again showed similarities with LDPE plastics (shopping bag), PE (transparent film) and PET items (bottle 0.5 L).

These preliminary findings suggest that specific plastic items exhibit similar TS to specific cephalopod species. From an evolutionary perspective, it is likely that whales encountered few items with a TS resembling their natural prey that were non-biological in nature. Consequently, plastic items with relatively high TS, which do not need to be chased, may appear as convenient and profitable prey options. This implies that the TS of plastics might contribute to misidentification by toothed whales, leading to their voluntary ingestion. Conversely, the broad range of prey consumed by cetaceans encompasses various TS, thus increasing the likelihood of TS overlap with plastic objects. As a result, many odontocetes, including deep divers, although predominantly feeding on cephalopods, also consume fish, crustaceans, and even gelatinous zooplankton of diverse sizes and shapes[26]. This could significantly enhance their susceptibility to acoustically mistake plastics items for potential food sources.

However, the results also highlight the complexity of the relationships of different prey and plastic types with the sonifying frequency. While certain plastic types consistently displayed similarities to cephalopod species across both 38 kHz and 200 kHz frequencies, such as PET and LDPE, other plastics exhibited frequency-dependent similarities. This suggests that the acoustic response of plastic items may vary depending on the frequency of the sonification, potentially influenced by factors such as material composition, shape, and size. Additionally, the diverse range of plastic debris found in marine environments[16] further complicates the understanding of these acoustic interactions. Thus, there is a clear need for further research to elucidate the intricate mechanisms underlying the acoustic resemblance between plastic items and natural prey, as well as to identify specific attributes of plastic debris that may trigger similar acoustic responses.

While our experiment successfully allowed capturing the acoustic patterns of sonified items for both frequencies, it is important to acknowledge certain limitations. In particular, the simplified tank environment most likely does not fully replicate the complexities of the oceanic acoustic landscape, and additional factors such as background noise and prey behaviour require further exploration. In addition to that, it is necessary to highlight that we sonified items at narrowband frequencies (38 kHz and 200 kHz) while odontocetes' echolocation clicks typically span over broader frequency ranges[27]. Thus, it is likely that these results do not fully capture the complex acoustic discrimination abilities of these marine predators. Future research should try to expand the frequency range of sonification and to attempt experiments in the animals' natural habitat to more accurately simulate the acoustic environment experienced by deep-diving cetaceans.

To conclude, this study supports the hypothesis that deep-diving toothed whales might voluntarily ingest plastic debris due to the echoic potential of plastics, in many cases comparable to or higher than that of their natural prey. Our data provide an initial ground to advocate for a change in plastic production policies, ideally leading to a modification in their composition to prevent them from being acoustically mistaken for natural prey by cetaceans. Moreover, these data also call for an urgent need for mitigating plastic pollution to safeguard marine ecosystems and protect vulnerable marine species. Continuing research efforts are essential to deepen our understanding of the complex interactions between marine megafauna and anthropogenic debris, ensuring effective conservation measures for the world's oceans.

5 REFERENCES

1. J.F. Provencher, A.L. Bond, S. Avery-Gomm, S.B. Borrelle, E.L.B. Rebolledo, S. Hammer, S. Kühn, J.L. Lavers, M.L. Mallory, A. Trevail and J.A. Van Franeker. ‘Quantifying ingested debris in marine megafauna: a review and recommendations for standardization’, Anal.Methods. 9(9) 1454-1469. (2017).
2. S.E. Nelms, B.L. Clark, E.M. Duncan, E. Germanov, B.J. Godley, K.J. Parton, C.K. Pham, and Y. Rodríguez. ‘Plastic Pollution and Marine Megafauna: Recent Advances and Future Directions’ Plastic Pollution in The Global Ocean. 97-138. (2023).
3. M.C. Fossi, M. Baini, C. Panti, and S. Baulch. ‘Impacts of marine litter on cetaceans: a focus on plastic pollution’ in Marine mammal ecotoxicology. Academic Press. 147-184. (2018).
4. M.S. Savoca, M.E. Wohlfeil, S.E. Ebeler and G.A. Nevitt, ‘Marine plastic debris emits a keystone infochemical for olfactory foraging seabirds’, Sci.Adv. 2(11) e1600395. (2016).
5. M.S. Savoca, C.W. Tyson, M. McGill, and C.J. Slager, ‘Odours from marine plastic debris induce food search behaviours in a forage fish’, Proc.RSoc.B:Biol.Sci. 284(1860) 20171000. (2017).
6. Q.A. Schuyler, C. Wilcox, K. Townsend, B.D. Hardesty, and N.J. Marshall, ‘Mistaken identity? Visual similarities of marine debris to natural prey items of sea turtles’, BmcEcol. 14 1-7. (2014).
7. R. Brentano and M.V. Petry, ‘Marine debris ingestion and human impacts on the Pygmy sperm whale (*Kogia breviceps*) in southern Brazil’, Mar.Pollut.Bull. 150 110595. (2020).

8. H. Jerbi, W. Perez and J.S. Tellechea, ‘Ingestion of floating net debris in sperm whales and larynx strangulation with gillnet parts in bottlenose dolphins on the coast of Tunisia’, Aquat.Mamm. 47(3) 239-244. (2021).
9. S. López-Martínez, E. Giménez-Luque, J.L. Molina-Pardo, S. Manzano-Medina, H. Arribas-Arias, R. Gavara, C. Morales-Caselles and M.L. Rivas, ‘Plastic ingestion by two cetacean groups: Ziphiidae and Delphinidae’, Environ.Pollut. 121932. (2023).
10. X. Wan, J. Li, M. Ao, R.W. McLaughlin, F. Fan, D. Wang and J. Zheng, ‘The intestinal microbiota of a Risso’s dolphin (*Grampus griseus*): possible relationships with starvation raised by macro-plastic ingestion’ Int.J.Microbiol. 26(4) 1001-1007. (2023).
11. R.E. Fordyce and C. de Muizon, ‘Evolutionary history of cetaceans: a review’ in Secondary adaptation of tetrapods to life in water. Friedrich Pfeil. 169-233. (2001).
12. S.M. Brinkløv, L. Jakobsen and L.A. Miller, ‘Echolocation in bats, odontocetes, birds, and insectivores’ in Exploring Animal Behavior Through Sound: Vol.1. Springer. 419. (2022).
13. G. Jones, ‘Echolocation’, Curr.Biol. 15(13) R484-R488. (2005).
14. A. Fahlman, R.B. Tyson Moore, R. Stone, J. Sweeney, R.F. Trainor, A.A. Barleycorn, K. McHugh, J.B. Allen and R.S. Wells, ‘Deep diving by offshore bottlenose dolphins (Tursiops spp.)’, Mar.MammalSci. 39(4) 1251-1266. (2023)
15. N.H. Nielsen, J. Teilmann and M.P. Heide-Jørgensen, ‘Indications of mesopelagic foraging by a small odontocete’, Mar.Biol. 166 1-8. (2019).
16. M.R. Clarke, ‘Cephalopods as prey. III. Cetaceans’, Philos.Trans.R.Soc.Lond.B Biol.Sci. 351(1343) 1053-1065. (1996).
17. I.N. Arnaya, N. Sano and K. Iida, ’Studies on Acoustic Target Strength of Squid: Ⅲ. Measurement of the mean target strength of small live squid. Bull.Fac.Fish.Hokkaido Univ. 40(2) 100-115. (1989).
18. B.A. Jones, A.C. Lavery and T.K. Stanton, ‘Use of the distorted wave Born approximation to predict scattering by inhomogeneous objects: Application to squid’, J.Acoust.Soc.Am. 125(1) 73-88. (2009).
19. P.T. Madsen, M. Wilson, M. Johnson, R.T. Hanlon, A. Bocconcelli, N.A. De Soto and P.L. Tyack, ‘Clicking for calamari: toothed whales can echolocate squid *Loligo pealeii’,* Aquat.Biol. 1(2) 141-150. (2007)
20. K.L. Law, ‘Plastics in the marine environment’, Ann.Rev.Mar. 9 205-229. (2017).
21. L. Roman, Q. Schuyler, C. Wilcox and B.D. Hardesty, ‘Plastic pollution is killing marine megafauna, but how do we prioritize policies to reduce mortality?’ Conserv.Lett., 14(2) e12781. (2021).
22. Simrad, EK80 installation manual, https://www.simrad.online/ek80/ins/ek80_ins_en_us.pdf. (2016).
23. K.V. Mackenzie, ‘Nine-term equation for sound speed in the oceans’, J.Acoust.Soc.Am. 70(3) 807-812. (1981).
24. Y. Ladroit, P.C. Escobar-Flores, A.C. Schimel and R.L. O’Driscoll, ‘ESP3: An open-source software for the quantitative processing of hydro-acoustic data’, SoftwareX. 12 100581. (2020)
25. R Core Team, ‘R: A Language and Environment for Statistical Computing’, R Foundation for Statistical Computing, Vienna, Austria. <https://www.R-project.org/>. (2023).
26. N. Fraija-Fernández, A.A. Ramos-Esplá, M.A. Raduán, C. Blanco, J.A. Raga, F.J. Aznar, ‘Consumption of pelagic tunicates by cetaceans calves in the Mediterranean Sea’ Mediterr.Mar.Sci. 19 383–387. (2018).
27. D.S. Houser, D.A. Helweg and P.W. Moore, ‘Classification of dolphin echolocation clicks by energy and frequency distributions’ J.Acoust.Soc.Am. 106(3) 1579-1585. (1999).