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**Original Article** 



# Acoustic fish community in a biogeographic transition zone of the Northeast Atlantic

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#### **Abstract**

Fish sounds are a significant component of marine soundscapes. Recently, passive acoustic monitoring (PAM) arose as a promising tool for ecological monitoring, but a good characterization of fish acoustic communities is still needed. This study is the first to characterize the fish acoustic community at a biogeographic transition zone in the Northeast Atlantic. The research was conducted in a marine protected area (MPA) along the Portuguese mainland coast. Based on a literature review, we identified 29 (19.3%) sound-producing fish species present at this MPA, while 70 species (46.7%) were considered potentially soniferous. Using *in situ* acoustic recordings to detect potential fish sounds, we found 33 putative fish sounds that were categorized using a simple dichotomous classification. The temporal and spectral features of the 13 most prevalent sound types were characterized and compared among them and with available recordings to identify similarities. Finally, hydrophone recordings coupled with baited remote underwater video systems were tested as a method to identify sound sources. This study provides the first fish sound catalogue from the Portuguese mainland coast, laying the foundations to survey fish communities in coastal habitats with PAM.

Keywords: passive acoustic monitoring; marine protected areas; fish sounds; sound catalogue; acoustic communication; marine soundscape; Professor Luiz Saldanha Marine Park

#### Introduction

Biological sounds in the ocean are produced by a wide range of taxa, from small invertebrates to marine mammals (Ladich and Winkler 2017). Whales and other cetaceans are better known for their vocalizations, which they can use for both echolocation and communication, across a wide range of frequencies (0.02-150 kHz) (Jones et al. 2020, Miller et al. 2021). Nonetheless, other taxa also rely on sounds to communicate or to obtain relevant information from the environment: molluscs, crustaceans, fish, and their larvae have all been shown to use sound as a sensory cue (Lillis et al. 2015, 2016, Gordon et al. 2018, Ladich 2019). Invertebrate sounds are typically broadband pulses with frequencies between 2 and 12 kHz (Radford et al. 2008, Bittencourt et al. 2016), while fish, by contrast, predominantly produce lower frequency sounds (<2-3 kHz) (La Manna et al. 2021, Vieira et al. 2021, Raick et al. 2022, Puebla-Aparicio et al. 2024), both often dominating marine soundscapes.

Currently, more than 980 species of fish from 133 families worldwide are known to produce sounds (Looby

et al. 2022, Rice et al. 2022), with communicative functions extensively studied for some species (Amorim et al. 2015, 2023, Ladich 2019, Banse et al. 2024). Fish produce sounds through active or passive mechanisms (Fine and Parmentier 2015). Passive sounds occur inadvertently, while active (communication) sounds are deliberate, produced through specialized mechanisms, and typically associated with specific behaviours (Kasumyan 2008). Generally, active sounds are species-specific (for an exception, see Raick et al. 2022) and are usually composed of low-frequency repetitive pulses, typically below 3 kHz, but mostly below 1 kHz (Amorim 2006, Fine and Parmentier 2015). Fish sounds are commonly associated with feeding, territorial, or reproductive behaviour, and their features and production rates vary depending on the behavioural context and motivation (Amorim 2006, Parsons et al. 2022). Because fish sounds can be species- and context-specific, they can be used to monitor the abundance, behaviour, distribution, and/or diversity of species, potentially playing an important role in the monitoring and management of fish communities

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(Parmentier et al. 2018, Bertucci et al. 2020, Raick et al. 2022, Stratoudakis et al. 2024).

Passive acoustic monitoring (PAM) is a non-invasive and cost-effective method that can be used for continuous monitoring of fish sounds, with long-term and large-scale coverage, unaffected by visibility or weather conditions (Mooney et al. 2020b). Furthermore, PAM can be integrated with other monitoring methods, such as environmental DNA or visual techniques, enhancing the overall capacity for comprehensive marine ecosystem studies (e.g. Elise et al. 2022, Souza et al. 2023). Acoustic monitoring can have valuable applications in marine protected areas (MPAs), which are recognized as crucial for safeguarding and restoring biodiversity. However, the limited understanding of underwater soundscapes, including the prevalence, diversity, along with limited awareness of PAM's utility amongst society and environmental agencies hinder its widespread adoption as a conservation tool in MPAs.

This study characterizes the acoustic fish community within the Professor Luiz Saldanha Marine Park (PLSMP), situated in a biogeographic transition zone on the west coast of mainland Portugal. This MPA is a biodiversity hotspot, hosting species with north-eastern Atlantic temperate affinities but also Mediterranean and subtropical species (Henriques et al. 2007). Specifically, the objectives are to (1) list sound-producing fishes in the PLSMP based on literature, (2) catalogue fish sound types recorded *in situ*, and (3) try to identify putative sound sources using audio-video recordings. To the best of our knowledge, this study is the first to characterize open coastal acoustic communities in mainland Portugal. It will serve as a foundation for future research and as a valuable tool for monitoring fish communities through acoustic methods.

#### Methods

#### Study site

The PLSMP extends 38 km along the west coast of mainland Portugal (Fig. 1). It was established in 1998 and is composed of eight zones subject to three protection levels: a full protected area (FPA, 4.3 km<sup>2</sup>), which is a no-take no-go zone, four partial protection areas (PPA, 21 km<sup>2</sup>), and three buffer areas (BA, 27.7 km<sup>2</sup>). This MPA is an important biodiversity hotspot with more than 2000 species of flora and fauna inhabiting diverse habitats ranging from sandy bottoms with bivalve fields and seagrasses to complex nearshore rocky reefs with algae, including kelp forests and gorgonians (Henriques et al. 2007). The park's location in a biogeographic transition area between temperate and sub-tropical zones enhances its biodiversity and presents a variety of habitats (Horta e Costa et al. 2014, Gonçalves et al. 2015). Its unique geography and oceanographic conditions, which include canyons and estuaries, contribute to the region's diverse wildlife. The bathymetric features near the study area are diverse due to the Lisboa-Setúbal submarine canyon system, which allows processes such as canyon upwelling and downwelling, influencing coastal ocean circulation of nutrient-rich water, boosting food web productivity (Peliz et al. 2002). Furthermore, the coastal disposition offers varying levels of protection from the wind and water currents, with an exposed offshore area to the west and south of Cape Espichel and a sheltered area to the east. The study area is also influenced by the presence of two of the biggest estuaries in Europe: the Tagus estuary located 30 km

north of the PLSMP and the Sado estuary to the east. Under north winds, the boundary of the Tagus estuary plume may extend to the south of Cape Espichel, affecting salinity values in the protected area (Oliveira et al. 2015). Similarly, the Sado estuary plume runs parallel to the coast where PLSMP is situated and can extend to Cape Espichel, potentially connecting to the Tagus plume in spring (Campuzano et al. 2018).

#### Fish community and reported soniferous species

A bibliographic review was conducted to identify listed soniferous and potentially soniferous fish species i.e. fish species that belong to families containing soniferous species, within the PLSMP. The fish database available on the marine park's geoportal https://arrabidaparquemarinho.ualg.pt/ was used to identify fish present in the area. Subsequently, a literature search was conducted for each species recorded within the PLSMP: species considered soniferous by Carriço et al. (2019) and Rice et al. (2022) or by the FishSounds database (Looby et al. 2023) were classified as soniferous. For the remaining species, a search was performed on Google Scholar with the terms 'soniferous', 'sound', or 'acoustic.' Fish species not reported as soniferous were classified as potentially soniferous if belonging to families containing soniferous species, and as potentially non-soniferous if otherwise. Further information was added to every species, such as conservation status [IUCN: VU (vulnerable), LC (least concern), NT (near threatened), CR (critically endangered), EN (endangered), DD (data deficient), NE (not evaluated)], environment (cryptobenthic, benthopelagic, pelagic, demersal, bathydemersal), depth range, climate affinity and, if known, the behaviour associated with the sound of the soniferous species (FishBase, Froese and Pauly 2024).

# Acoustic recording

Autonomous acoustic data loggers (Audiomoth 1.2.0; Hill et al. 2019) equipped with custom-made hydrophones (Piezo tubes PZT-P5 with 24 × 20 × 20 mm, with signal preamplification of 50 times) were deployed in three zones with different protection levels between June 2021 and September 2022. The piezoelectric sensor has a measured response sensitivity of ca. -184.5 dB re 1 V/ $\mu$ Pa at 1 kHz and a frequency response comparable to commercial hydrophones, namely the Brüel & Kjær 8104 (8104, Brüel & Kjær, Nærum, Denmark), and reliably captures the temporal and spectral characteristics of the various sound types (Fig. S1). Note, however, that in the deployments at PLSMP there was sometimes a visible loss of acoustic energy at frequencies around 400 Hz (see sound types #1, #10, #16, and #28 in Fig. 2). This is likely because the acoustic data loggers were placed horizontally in close contact with the ballast (Fig. 1c, Fig. S1). The autonomous acoustic data loggers were deployed in: (1) the BA (buffer area) at a depth of ~8.5 m in a rocky habitat; (2) the FPA (full protected area) at a depth of ~6 m at an interface of a sandy and rocky habitat; and (3) the PPA (partial protected area) at a depth of  $\sim$ 6.9 m in a rocky habitat (Fig. 1). Deployments failed in December 2021 for the FPA and at the beginning of March 2022 for the PPA. The recordings were made with a continuous duty cycle at a sampling rate of 48 kHz. Due to battery autonomy and digital storage capacity, the data loggers were retrieved, reprogrammed, and deployed every ca. 60 days.

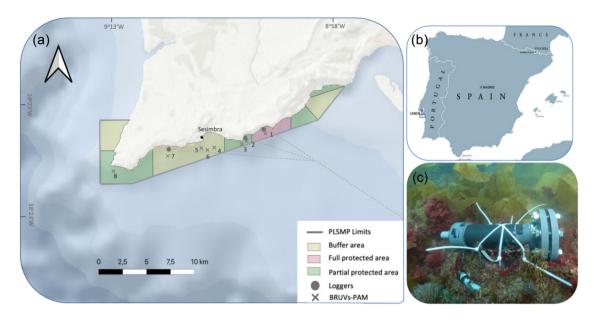


Figure 1. (a) Map of the PLSMP depicting the different protection areas, location of the acoustic loggers and of BRUV-PAM deployments; BRUVS-PAM deployments locations; (1) Derrocada, (2) Bahia do Armação, (3) Jardim das gorgonias, (4) Pedra do meio, (5) Batelão, (6) Maria Grecia, (7) 3 Milhas, (8) River. (b) Map of Europe with Portugal highlighted and location of the PLSMP; (c) Photograph of the acoustic logger deployed in the Partial Protected Area (2).

#### Detection of putative fish sounds

Acoustic recordings were analysed for the presence of putative fish sounds. Given the challenge of manually analysing large acoustic datasets, the data were subsampled. Four months were chosen to represent each of the four seasons: June for summer, September for autumn, December for winter, and March for spring. Within each month, 4 days were selected based on the moon's phases: full moon, crescent moon, waning moon, and new moon, as it may influence acoustic communities (Borie-Mojica et al. 2022). Within each selected day, 30-min recordings at four specific time periods were chosen to represent the diel period: sunrise, solar mid-day, sunset, and midnight (according to NOAA 2023).

The recordings were analysed manually through both aural and visual inspection of the spectrograms and oscillograms, using Raven Pro 1.6 (Bioacoustics Research Program, Cornell Laboratory of Ornithology; DFT size 256, contrast 80, and brightness 55; recordings down-sampled to 8 kHz). Two individuals conducted this analysis, with files randomly assigned to each to prevent biases. The manual inspection of the files allowed for the identification and categorization of putative fish sounds. Putative fish sounds were determined based on their similarity to previously reported fish calls in frequency, relative duration, and temporal patterning of their pulses. Based on categorical methods from other studies (e.g. Parsons et al. 2016, Desiderà et al. 2019, Muñoz-Duque et al. 2024, Puebla-Aparicio et al. 2024), each fish sound type was characterized using a dichotomous classification system, following the steps outlined below:

- (a) A preliminary study was conducted using Raven software to train observers to identify fish sounds in acoustic archives. Selected samples were later analysed.
- (b) Putative fish sounds were grouped and assigned to numbers (#1, #2, #3...).

- (c) Putative fish sounds were grouped according to their temporal patterns into two main categories, continuous and pulsed sound types.
- (d) Then, pulsed sounds were categorized into lowfrequency (peak frequency < 500 Hz) pulse trains (fast and slow with pulse period under or over 50 ms) and high-frequency (peak frequency >500 Hz) pulse trains (fast and slow with pulse period under or over 50 ms). Continuous sounds included tonal sounds (frequency-non-modulated), which were further divided into low-frequency (peak frequency <500 Hz) and high-frequency (peak frequency >500 Hz), as well as noisy (wideband) sounds, which were also further divided into low (peak frequency <400 Hz, range frequency between 150 and 300 Hz) and highfrequency (peak frequency >400 Hz, range between 450 and 1000 Hz). Note that continuous sounds may be composed by pulses, but are perceived as continuos through aural and visual inspection.

These classification steps are represented in Table 2.

Note that although all putative fish sound types exhibit characteristics similar to reported fish calls, it is possible that, due to the complexity of the underwater soundscapes, not all sound types originated from fish. Additionally, the loss of acoustic energy around 400 Hz frequencies in some of the recordings, as well as the distance from the source, may influence the spectral properties of the recorded sounds. However, this did not hinder the use of a dichotomous classification system or the construction of a fish sound catalogue, as most frequencies were reasonably well represented (see Fig. 2).

# Identification of putative fish sound type sources using BRUVS

Baited remote underwater video systems (BRUVS) were used to identify potential sources of the various putative fish sound

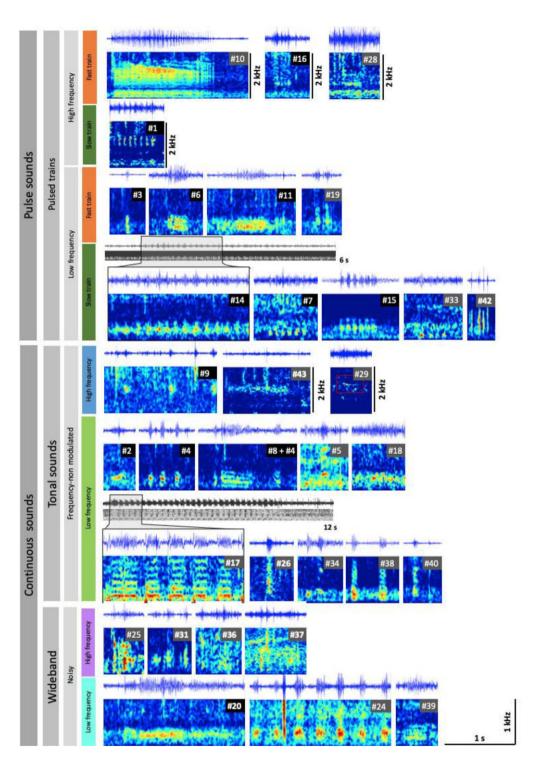


Figure 2. Catalogue of putative fish sound types registered at PLSMP. Each sound type was named numerically. The 13 most common sound types (≥20 detections) are in black. Spectrograms were produced with FFT = 256, frequency range from 0 up to 1 kHz (except when indicated).

types. The BRUVS design was adapted from Brooks et al. (2011). It comprised a PVC pipe frame equipped with a Go-Pro HERO 5 Black, housed in a SeaGIS (SeaGIS Pty Ltd https://www.seagis.com.au/) waterproof housing along with an additional external power supply, allowing for unattended video recording. A bait pole (~1 m long) was extended outwards from the frame into the field of view of the camera. In addition, the BRUVS were also fitted with an autonomous acoustic data logger (see above) that simultaneously recorded

audio, used to identify the fish sound types. For all deployments, the BRUVS were baited with 1 kg mackerel (*Scomber* spp.) to attract fish into the area. The amount of bait is consistent with standard practices of 1 kg of bait per sample hour (Harvey et al. 2007).

Deployments were made at two different periods: November 2022 and April 2023, at a depth of 2–9 m, close to the locations where the three acoustic loggers were previously deployed (see Fig. 1). The daily recordings lasted around 70 min

each, conducted at sunrise, mid-day, sunset, and early night. For the deployments conducted at night, the BRUVS were equipped with a dive torch and left to record into the night until the storage on the camera was full (~4 h) and recovered the following day.

The video was used to identify instances where sound produced by fish could be detected. This process thus aimed to test a video technique to determine the sound source or narrow down the fish species capable of producing the identified sounds. In cases where a sound source could not be determined, the fish captured in the frame were identified and included in a list of potential sound sources.

#### Multivariate analyses of putative fish sounds

To assess the similarities between the defined sound types, principal component analysis (PCA; Mardia et al. 1979) considering the 13 most frequent sound types was performed, i.e. for sounds with  $n \ge 20$  occurrences. Several acoustic features were measured, namely

- sound duration (s);
- minimum and maximum frequency (the lowest and the highest frequency of each sound in the spectrogram, Hz);
- first and third quartile frequencies (Q1, which represents the frequency at 25% of the spectral frequency range, and Q3, the frequency at 75%, Hz);
- peak frequency (frequency at which the sound presents its highest energy in the power spectrum, Hz);
- number of pulses;
- pulse duration (the mean duration of a pulse, ms);
- pulse period (mean time elapsed between the peak amplitude of two consecutive pulses within a sound, ms);
  and
- average value of the entropy (entropy measures the disorder in a sound by analysing its energy distribution).

The values obtained for each feature were standardized to zero mean and unit variance. Some sounds with indistinct pulses may be difficult to separate and recognize due to low signal-to-noise ratio or fast pulse rates, making pulse measurement challenging (#3, #6, #11, and #16) (see Fig. 2). Pulse period and pulse duration were only measured in some pulsed sound types (#1, #7, #14, and #15). Separate statistical analyses were used for sounds in which pulse period and duration could not be measured. Multivariate analyses were performed on the 13 most common sound types using 7 standardized acoustic features described above (Table S3). This led to three separate multivariate analyses: 'continuous' sound types (n = 83), 'pulsed (measured)' sound types (n = 56), and 'pulsed (not measured)' sound types (n = 65). Analyses were performed using R (R Core Team 2021) with the package 'FactoMineR' (Lê et al. 2008) and 'PCA' function (Blighe and Lun 2023). See Web Appendix 1 for further details.

#### **Results**

#### Fish community and reported soniferous species

From the 150 fish species known to occur in the study area, 29 (19.3%) species from 17 families were identified as soniferous (Table 1), 70 (46.7%) species from 17 families as potentially soniferous, and 51 (34.0%) species from 25 families as potentially non-soniferous (Table S1). The sonifer-

ous species belonged to various families, including Balistidae, Batrachoididae, Blenniidae, Carangidae, Gadidae, Gobiidae, Labridae, Lotidae, Molidae, Pomacentridae, Pomatomidae, Sciaenidae, Scorpaenidae, Serranidae, Syngnathidae, Triglidae, and Zeidae. According to the IUCN Red List of Threatened Species (IUCN 2023), three soniferous species in the study area have defined threatened conservation statuses: EN Epinephelus marginatus; VU Mola mola; and NT Pomatomus saltatrix. Four species are DD: Balistes capriscus, Hippocampus hippocampus, Hippocampus guttulatus, and Zeus faber, and two are NE: Gaidropsarus mediterraneus and Cynoscion regalis, while the other twelve were classified as LC (Table 1). The weakfish (C. regalis), although classified as NE for the north-eastern Atlantic, is an invasive species in Portugal originating from the East Coast of North America, where it is classified as EN. All sound-producing fish species present in the PLSMP partially or fully overlap their distribution depth range with our study sites. Most of the behaviours associated with sound production described in the literature were related to courtship, agonistic behaviour, feeding competition, and distress sounds resulting from manual stimulation (Table 1).

#### Characterization of putative fish sounds

Recordings from a total of 16 days were analysed, producing 2542 putative fish sound detections assigned to 33 putative sound types, with a cumulative recording time of 79.5 h. Each fish sound type was characterized and included in a catalogue (Fig. 2). Fish sounds were grouped into two main categories, continuous sound types (n = 20 sound types) and pulsed sound types (n = 13). Table S2 provides a qualitative characterization of the different sound types, while Table S3 provides a quantitative description of acoustic features for the 13 most common sound types. Together, these tables (S2, S3) and Fig. 2 provide an overview of the putative fish sounds recorded at the PLSMP.

#### Pulsed sound types

Pulsed sound types were sub-categorized as low-frequency and high-frequency sound types (Fig. 2, Table 2, and Table S2). Within the pulsed sounds, low-frequency pulsed sounds were the most common sub-category presenting a peak frequency below ca. 500 Hz. Within this subcategory, four sound types were classified as fast pulse trains (pulse period under 50 ms #3, #6, #11, and #19) and five as slow pulse trains (pulse period over 50 ms #14, #7, #15, #33, #42). Notably, within the low-frequency fast train subcategory, sound types #3 (n = 382) and #6 (n = 143) were the most abundant sounds. Within the low-frequency slow train sub-category, sound types #14 (n = 172) and #15 (n = 654) were the most abundant. Within the pulse train high-frequency sub-category, four sound types were included (#1, #10, #16, #28), all with fast trains. Sound types #1 (n = 94) and #16 (n = 92) presented the most detections in this sub-category (see description of the sound types in Table S2).

#### Continuous sound types

Continuous sound types were divided into tonal sounds and noisy sounds (Fig. 2, Table 2, and Table S2). Within the tonal sub-category, three high-frequency sounds were included (#9, #29, #43). Sound type #9 (n = 35) was the most common,

**Table 1.** Summary of the 29 confirmed soniferous fish species listed for the PLSMP.

Species <sup>a</sup>	Family	Conservationb	Environment <sup>c</sup>	Climate affinity <sup>c</sup>	Depth range <sup>c</sup> (m)	Behaviour <sup>d</sup>	Ref.d
Balistes capriscus	Balistidae	DD	Benthopelagic	Subtropical	0-100	Manual and electric stimulation	1
Halobatrachus didactylus	Batrachoididae	LC	Cryptobenthic	Subtropical	1-50	Agonistic, courtship	2, 3
Lipophrys pholis	Blenniidae	LC	Demersal	Temperate	0-8	n/a	5
Seriola dumerili	Carangidae	LC	Benthopelagic	Subtropical	1-385	n/a	1
Trachinotus ovatus	Carangidae	LC	Pelagic	Subtropical	50-200	n/a	1
Pollachius pollachius	Gadidae	LC	Benthopelagic	Temperate	40-200	Agonistic	22
Gobius paganellus	Gobiidae	LC	Cryptobenthic	Subtropical	0-15	Agonistic,courtship	18,19
Pomatoschistus pictus	Gobiidae	LC	Demersal	Temperate	0-55	Agonistic, courtship	4
Gobius cobitis	Gobiidae	NE	Demersal	Subtropical	8-35	Agonistic, courtship	18, 23
Gobius cruentatus	Gobiidae	LC	Demersal	Subtropical	15-40	Agonistic	24
Gobius niger	Gobiidae	LC	Demersal	Temperate	1–50	Agoni stic, courtship	18, 23
Pomatoschistus flavecens	Gobidae	LC	Reef associated	Temperate	0/33	Agonistic, courtship	34
Pomatoschistus marmoratus	Gobiidae	LC	Demersal	Subtropical	20–70	Agonistic, courtship	18, 25, 26, 31
Pomatoschistus minutus	Gobiidae	LC	Demersal	Temperate	4–200	Agonistic, courtship	18, 27, 28, 29, 30
Symphodus melops	Labridae	LC	Reef associated	Subtropical	1-30	Agonistic	32
Gaidropsarus mediterraneus	Lotidae	NE	Demersal	Temperate	1-450	Agonistic	5
Mola mola	Molidae	VU	Pelagic	Subtropical	30-1515	Manual stimulation	1
Chromis chromis	Pomacentridae	LC	Benthopelagic	Subtropical	1-40	Agonistic, courtship	6
Pomatomus saltatrix	Pomatomidae	NT	Pelagic	Subtropical	0-200	Escape sounds	1
Cynoscion regalis <sup>e</sup>	Sciaenidae	NE	Demersal	Subtropical	10-26	Agonistic, courtship	7, 8, 9, 10
Scorpaena porcus	Scorpaenidae	LC	Demersal	Temperate	?-800	n/a	11
Epinephelus marginatus	Serranidae	EN	Benthopelagic	Subtropical	8-300	Courtship	17
Hippocampus hippocampus	Syngnathidae	DD	Cryptobenthic	Subtropical	0-60	Agonistic, courtship	20
Hippocampus guttulatus	Syngnathidae	DD	Cryptobenthic	Temperate	0-30	Agonistic, courtship	21
Chelidonichthys lastoviza	Triglidae	LC	Demersal	Subtropical	10–150	Competitive feeding	12, 13, 14, 15
Trigla lyra	Triglidae	LC	Bathydemersal	Temperate	150-400	n/a	12, 13, 20
Chelidonichthys lucerna	Triglidae	LC	Benthic	Subtropical	20–318	Agonistic	12, 13
Chelidonichthys cuculus	Triglidae	LC	Benthic	Temperate	2–100	Distress calls	12, 13
Zeus faber	Zeidae	DD	Benthopelagic	Temperate	5-400	Agonistic	16

<sup>&</sup>lt;sup>a</sup>Marine fish of PLSMP.

Table 2. Dichotomy of the main sound categories found on the analysed recordings.

Categories	Sub-category		Frequency	Definition
Pulsed sounds	Pulse trains	Low frequency	Fast train	Fast pulsed sounds, with an average duration of 400–600 ms and a peak frequency below 500 Hz. Pulse period under 50 ms.
			Slow train	Serial of short slow train pulses (duration between 30 and 3500 ms) with peak frequency below 500 Hz. Pulse period over 50 ms.
		High frequency	Fast train	Series of high-frequency pulse trains with a peak frequency above 500
Continuous sounds	Tonal sounds	Frequency-non modulated	Low frequency	Hz (in this dataset it ranged 600–800 Hz). Pulse period under 50 ms. Low-frequency tonal sound with a duration between 600 and 2000 ms and a peak frequency below 500 Hz.
			High frequency	Tonal sound that has a peak frequency above 500 Hz.
	Wideband	Noisy	Low frequency	Continuous noisy sound with a duration of 1000 and 2000 ms and a peak frequency below 400 Hz.
			High frequency	Broadband, continuous sound with a duration of <1000 ms and a peak frequency above 400 Hz (in this dataset ranged between 450 and 1000 Hz).

usually composed of a sequence of 3-11 short tonal sounds with a peak frequency of 500 Hz. Ten sound types were included in the tonal low-frequency sub-category (#2, #4, #5, #8, #17, #18, #26, #34, #38, and #40). This was the subcategory with the most sound types, the most prevalent being #2, #4, and #8. Because sound type #8 often occurred after

bIUCN Red List of Threatened Species (2023): Conservation status from IUCN: LC, least concern; VU, vulnerable; NT, near threatened; EN, endangered; CR, critically endangered; DD, data deficient.

<sup>&</sup>lt;sup>c</sup>Environment, climate affinity, and depth ranges were extracted from fishbase.org.

<sup>&</sup>lt;sup>d</sup>1, Fish and Mowbray (1970); 2, Fine et al. (2001); 3, Jordão et al. (2019); 4, Amorim and Neves (2008); 5, Almada et al. (1996); 6, Picciulin et al. (2002); 7, Connaughton et al. (2000); 8, Gannon et al. (2007); 9, Ono and Poss (1982); 10, Amorim et al. (2023); 11, Bolgan et al. (2019); 12, Amorim and Hakins (1995); 13, Amorim (2006); 14, Amorim and Hawkins (2000); 15, Radford et al. (2018); 16, Onuki and Somiya (2004); 17, Bertucci et al. (2015); 18, Malavasi et al. (2008); 19, Parmentier et al. (2013); 20, Dufossé (1874); 21, Protasov (1962); 22, Wilson et al. (2014); 23, Horvatic et al. (2021); 24, Sebastianutto et al. (2008); 25, Amorim et al. (2018); 26, Lugli et al. (2008); 27, Blom et al. (2016); 28, Lindström and Lugli (2000); 29, Pedroso et al. (2013); 30, Blom et al. (2022); 31, Lugli and Torricelli (1999); 32, Bussmann et al. (2020); 33, de Jong et al. (2016), 34, de Jong et al. (2018).

<sup>&</sup>lt;sup>e</sup>This species is considered invasive in Portugal.

#4, they are probably part of a sound sequence produced by the same individual. Sound type #4 was a broadband sound presented in sequences of 3–4 sounds with an average peak frequency of 250 Hz. Sound type #8 was a low-frequency sound with a longer duration of around 500 ms and a peak frequency within 250-500 Hz. Another sub-category included four wideband noisy sound types (#25, #31, #36, #37) and three low-frequency sound types (#20, #24, #39). From these noisy sounds, sound type #20 was the most common with 61 detections.

#### Sound similarities

Three PCAs were conducted on the 13 most frequent sound types using 7 standardized acoustic features shown in Table \$3. The PCA revealed distinct patterns and separations among the sound types. For pulsed (measured) sounds, PC1 and PC2 explained 85.8% of the variance, with peak frequency, entropy, and Q3 being the most influential variables, while sound type #1 was the most distinct (see Fig. S2a). Pulsed sounds (with pulse period and duration not measured) had 77.9% variance explained by PC1 and PC2, with duration and Q1 being the most influential variables and sound type #16 showing the clearest differentiation (see Fig. S2b). For continuous sounds, PC1 and PC2 explained 79.4% of data variability, with Q1, Q3, and peak frequency driving the first component and duration influencing the second. Sound type #9 stood out among continuous sounds, while #2, #4, and #8 were more similar (see Fig. S2c). See Web Appendix 1 for a detailed description of the results obtained in these analyses.

#### Identification of putative fish sound type sources

From the 52 BRUVS deployments, 12 were discarded due to deployment conditions (e.g. unstable rig, blocked vision, strong currents). Of the 40 analysed videos, 13 included putative fish sounds. These belonged to the sound types #3, #6, #9, #16, #20, and #38 (Table S4). Several species that are known to be soniferous were also detected in these videos, namely *B*. capriscus, Coris julis, Ctenolabrus rupestris, Diplodus cervinus, Diplodus sargus, Diplodus vulgaris, Serranus cabrilla, Muraena helena, Labrus mixtus, and Trachurus spp. Sound types #6, #16, #20, and #38 only appeared in one video. The most common sound was sound type #3, which was found on six videos with eight potentially soniferous species: D. cervinus, D. sargus, D. vulgaris, C. julis, C. rupestris, M. helena, S. cabrilla, and Trachurus spp. (Table S4). As none of the species showed any obvious behaviour associated with the occurrence of sounds, source identification was not possible.

To further aid in identifying the sound sources, we also compared them with sounds reported in the literature. Figure 3 displays potential sound types linked to specific fish families/species. Sounds #4 and #8 are similar to sounds associated with the Serranidae family, identified by Bertucci et al. (2015) and Wilson et al. (2020). Sound #33 is also similar to sounds described by Bertucci et al. (2015), Wilson et al. (2020), and Vieira et al. (2024) and can be associated with the Serranidae family. Sound #10 is characteristic of the Triglidae family as described in Amorim et al. (2004). Sound #15 resembles sounds produced by the Sciaenidae family, specifically the sounds produced by the *Cynoscion regalis*, as reported in Connaughton et al. (2002) and Amorim et al. (2023). Sound #42 is similar to sounds from the Pomacentridae family, identified

by Amorim et al. (2006) and Picciulin et al. (2018). Sound #43 matches sounds from Scorpaenidae family, namely the sounds of *Scorpaena* sp. reported in Bolgan et al. (2019).

#### **Discussion**

This study highlights a wide biodiversity of putative fish sounds recorded at the PLSMP while evidencing the existing gap of information on sound sources. Moreover, it provides the first fish sound catalogue for mainland Portugal and the Atlantic Iberian coast, contributing to increasing the knowledge of fish acoustic communities, providing an initial framework for the use of PAM as a tool to monitor MPAs and coastal areas.

#### Fish community and reported soniferous species

We reviewed the literature as an approach to estimate the soniferous fish species occurring in a specific study site. Considering that only a small percentage of soniferous fish species have been reported thus far, potentially soniferous species (i.e. those closely related to known soniferous species) were also listed. This approach contributes to filling in the gaps in the current understanding of fish acoustic communities (Parmentier et al. 2021, Looby et al. 2022, Parson et al. 2022). Out of the 150 fish species listed for PLSMP, only 29 species were confirmed as soniferous, while 70 species were considered potentially soniferous, lacking documented acoustic activity studies. Similar to other regions worldwide (Parmentier et al. 2021), the number of vocal fish species identified along the Portuguese coast will likely increase, as many species have not yet been acoustically studied. The methodology of listing potentially soniferous fish species, describing their sound, and cross-referencing with recorded putative fish sound types has proven to be adaptable and applicable in various marine environments. For example, Carriço et al. (2019) listed potential soniferous species from Azores seamounts, while Puebla-Aparicio et al. (2024) applied the same methodology to the Mozambique Island coral reefs. Another example is the study conducted by Parmentier et al. (2021), which identified soniferous species on coral reefs in French Polynesia. As in our research, these studies also noted a low percentage of reported soniferous fish species.

#### Characterization of putative fish sounds

Sounds produced by fish are predominantly low-frequency, formed by pulses and usually with short duration (Amorim 2006, Parsons et al. 2016). We detected, identified, and described 33 sound types and classified them into two main categories, pulsed and continuous sounds, and then further into different sub-categories.

From all the detected sound types, only 13 were abundant enough to be characterized for different acoustic parameters and to be evaluated using multivariate analyses. Multivariate analyses indicated that some sounds were clearly differentiated within their category but highlighted similarities between some of the sound types. For example, pulsed sound types such as #1 and #16 and the continuous sound type #9 were clearly differentiated within their category. In contrast, within the pulsed sounds, #7 and #15 as well as #6 and #11 were not clearly separated by the PCA analyses. The same stands for sound #2 and #4 within the continuous sound category. The lack of distinctiveness in these sound types suggests they may

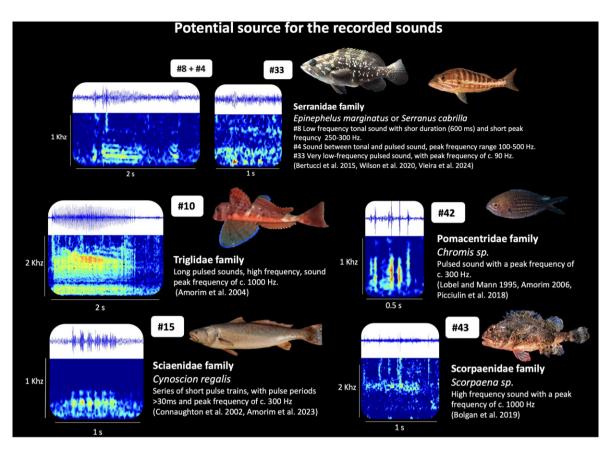


Figure 3. Potential sources (family/species of soniferous fish) of sounds recorded at PLSMP were identified by cross-referencing results from the bibliography review with field and BRUVS observations. References to articles describing the sounds produced by fish species or families are provided. Images from free sources: *E. marginatus*—Silvia Tavares iNaturalist (2019), *S. cabrilla*—Tim Camerom iNaturalist (2018), Triglidae—family Silvia Tavares iNaturalist (2020), *C. chromis*—Silvia Tavares iNaturalist (2020), *C. regalis*—Museum of Comparative Zoology, Harvard University, iNaturalist, Scorpaenidae family—Fricke (2018).

be produced by the same species, e.g. as variations of a sound type, or by different species generating similar calls. Note that the ability to use PAM to detect and properly identify each sound depends on several factors that influence the SNR. For example, quiet sounds such as the ones produced by several goby species should be detected only rarely (Parmentier et al. 2013, Amorim et al. 2018).

# Diversity of putative fish sounds

A considerable diversity of putative fish sounds contributing to the marine soundscape of PLSMP was found during the analysis of acoustic recordings. We detected, identified, and described 33 sound types. Compared to other fish acoustic communities, we found that the fish sound richness (the diversity of putative fish sounds) falls well within the range observed in other locations. For example, in the region of Macaronesia, Muñoz-Duque et al. (2024) described 43 fish sounds from a total of 188 listed fish species, including 35 soniferous and 102 potentially soniferous species. In the Mediterranean Sea, specifically at Tavolara-Punta Coda Cavallo MPA, Desiderà et al. (2019) identified 12 fish sound types from a total of 53 fish species identified through underwater visual census. Bertucci et al. (2020) identified 22 fish sounds in a coral reef from Polynesia, while a second study by Raick et al. (2023), encompassing a wider range of Polynesian islands and water depths, detected 45 fish sound types. Also in coral reefs, but, respectively, in the Mozambique Island and Hawaii, 47

and 85 sound types were identified (Tricas and Boyle 2014, Puebla-Aparicio et al. 2024). Lastly, Wang et al. (2017) found 66 fish sound types in the Pearl River estuary, a location within a global hotspot of fish biodiversity with 834 reported species. The above studies highlight that the PLSMP presents high fish acoustic diversity, comparable to other biodiversity hotspots. This is consistent with its position as a biogeographic and oceanographic transition zone between warm and cold temperate waters in the north-eastern Atlantic region (Briggs and Bowen 2012), harbouring a high diversity of fish species, from subtropical to species with more northern-temperate affinities (Henriques et al. 2007).

# Identification of putative fish sound type sources Cross-reference with fish sounds from the bibliography

In our study, we observed some similarities between putative fish sounds and previously reported sound types (Fig. 3). Sound types #4 and #8 are produced consecutively, thus likely by the same species. When compared to the list of potentially soniferous species in the PLSMP, several Serranidae species were noted as possible candidates, namely Serranus atricauda, S. hepatus, S. cabrilla, E. marginatus, and Anthias anthias. Although S. atricauda is present in the marine park, it is not a frequent species and is mostly observed during summer species (Gonçalves et al. 2002). Both sound types #4 and #8 are fairly common sound types present throughout the whole year and widely distributed, decreasing the likelihood that they are produced by this species. Anthias anthias is also an improbable

source for these sound types, as the habitat range of these species is at depths greater than 40 m (Schneider 2012), and the hydrophones for this study were placed in shallower water (<10 m). Another possible candidate is the Dusky grouper (E. marginatus). These sounds are similar to what is reported for groupers (Epinephelus spp.) described by Bertucci et al. (2015) and Wilson et al. (2020), supporting the suggestion that they could be associated with the Serranidae family. The calls from the Nassau grouper (E. striatus) described by Wilson et al. (2020) show similarities with two distinct types of calls. Sound type #4 resembles the alarm call (N1) characterized by low-frequency pulses, while sound type #8 is similar to the courtship call (N2). Similar sounds have also been documented in other locations within the Madeira and Azores archipelagos where the Dusky grouper is found (Vieira et al. 2024). However, due to deeper water preferences (8–300 m) and low abundance of this species in the PLSMP (only two individuals reported by divers in a wreck ship diving spot), it is an unlikely sound source for sound types #4 and #8. Consistently, a telemetry study that released 30 E. marginatus individuals on the Portuguese coast found that three of them travelled within PLSMP between May and June 2019, highlighting their migratory behaviour and low rate of occupation in PLSMP (Silva et al. 2022). Nevertheless, we cannot exclude the possibility of S. cabrilla and S. hepatus (less frequent in shallow waters and rocky habitats) being responsible for these two sound types, as they are a common Serranidae species present year-round and also in Madeira and Azores archipelagos (Gonçalves et al. 2002, Vieira et al. 2024). Furthermore, sound type #33 was similar to the agonistic sound of E. striatus (Wilson et al. 2020) and the boom series described for E. marginatus (Bertucci et al. 2015, Vieira et al. 2024). Another sound comparable to bibliography reports is the sound type #10 that has similarities to the grunt produced by the grey gurnard, Eutrigla gurnardus, during competitive feeding (Amorim et al. 2004). Sound type #15 was one of the most common sound types, detected as part of a chorus with over 600 detections in one recording of 30 min, whose most likely source is the invasive species weakfish (C. regalis). In addition to the similarity in acoustic characteristics between sound type #15 and weakfish sounds (Amorim et al. 2023), the sound type #15 chorus was recorded in June (summer) during sunset, consistent with the reported patterns of sciaenid spawning choruses, including weakfish (Connaughton et al. 2002, Vieira et al. 2022). The chorus was also observed closer to the Sado Estuary, where weakfish are known to occur since 2014 (Morais and Teodósio 2016).

The sound type #42 bears a close resemblance to the characteristics of previous confirmed reports of the genus *Chromis* (Lobel and Mann 1995, Amorim 2006, Picciulin et al. 2018) presented in the list of potentially soniferous species in our study area (Table S2). Sound type #43 was reported at other locations within the Madeira and Azores archipelagos (Vieira et al. 2024) and is possibly produced by a species of the genus *Scorpaena*. It resembles the/kwa/recorded in the *Posidonia oceanica* meadows of the Mediterranean Sea, which is produced by species of the genus *Scorpaena*, as described by Bolgan et al. (2019). Both the/kwa/and #43 showed peak frequencies above 600 Hz and sounded alike to the human ear. Notice that this sound type was rarely detected and at a low signal-to-noise ratio. From this family, the most common species in the PLSMP are *Scorpaena scrofa* and *S. notata*,

with *S. maderensis* being less frequent but still occurring in the park.

Although the suggestions regarding the potential sources of the different sound types in our study are based on similarities with sounds of other confirmed soniferous species, it is important to note that our hypotheses remain untested. Indeed, increasing use of PAM has led to the detection of numerous unidentified fish sounds. However, the documentation of known sounds has not kept pace with these detections. Nevertheless, these unidentified sounds are valuable for assessing biodiversity and habitat health (Mooney et al. 2020a, Parsons et al. 2022).

#### Challenges in the identification of fish sound sources

As mentioned above, one important part of the effectiveness of PAM as a monitoring tool relies on the identification of sound-producing species (Mouy et al. 2018). Hence, cataloguing fish sounds using combined audio and video recordings in situ has been used to increase our knowledge and understanding of soniferous fish species (Mouy et al. 2018, Carriço et al. 2020b, Puebla-Aparicio et al. 2024). Other studies have carried out laboratory recordings to catalogue fish sounds (Bolgan et al. 2019). Although captivity studies are valuable, there is clearly a need to characterize and identify the sound sources in the natural habitat. The usage of BRUVS in this study was intended to serve this purpose. Analysis of the videos identified six distinct sound types linked to various fish sources, but determining the exact source was challenging. In this study, thirteen potential fish sounds were detected using BRUVS, belonging to the following sound types: #3, #6, #9, #16, #20, and #38. Species such as C. julis and D. sargus, which are potentially soniferous species (see Table S2), were the most abundant in the videos. However, no sounds were directly linked to a fish in the videos. It is possible that sounds could have been made by other fishes not visible on screen, as fish sounds can travel different distances, typically up to 20 meters, depending on environmental factors and source level (Carrico et al. 2020a).

Challenges in identifying sound sources included the presence of a bait pole and box, which added noise as fish interacted with them. Also, the detected fish sounds could not be linked to a specific species due to the presence of multiple fish species in the frame, making it difficult to associate their behaviour with the sounds. It is also likely that fish are less prone to making sounds during competitive feeding in comparison with other contexts such as agonistic interactions or reproduction (Ladich et al. 2004). To address these challenges, potential solutions include removing the bait and employing remote underwater video, as in Puebla-Aparicio et al. (2024), which can mitigate some of the additional noise. Another option could be an array of hydrophones combined with a camera to determine the position of the sound source and thus attribute sounds to individuals. This combination allows for sound localization using the time difference of arrival of the sound to the different hydrophones, complemented with video analysis to identify the soniferous fish (Mouy et al. 2018). Directional hydrophones, which are designed to detect sounds from specific directions (Mouy et al. 2023), could help to restrict the considered sound sources. Other proposed systems use 360° cameras with acoustic recorders that have been applied in etho-acoustical studies of bottlenose dolphins (Maralunda et al. 2017). Combining audio recordings with visual data

allows researchers to correlate sounds with specific species and behaviours, providing a more comprehensive understanding of the context in which sounds occur.

Nevertheless, complementing field with captivity studies could improve our knowledge on fish acoustic communication. While captivity studies allow controlled experiments, field studies capture the complexities of natural habitats where fish can express their complete acoustic repertoires. Different studies, such as Bolgan et al., Pereira et al. (2020), and Mouy et al. (2023), highlight the importance of utilizing both field and captivity approaches in understanding fish sounds. Species that breed in known spawning sites or that use nests to breed or as shelters could ease field studies, as acoustic recordings (or videos) could target these locations. For example, the acoustic repertoire of the Lusitanian toadfish was determined by placing hydrophones near nesting males (Amorim et al. 2008).

# Importance of fish sounds for research and public awareness

The field of fish bioacoustics has faced historical constraints due to the absence of an easily accessible and comprehensive inventory of known soniferous fishes, a resource readily available for other taxa such as cetaceans and birds. This limitation has hindered researchers' ability to systematically study and understand the acoustic behaviours and communication patterns of fish species (Mouy et al. 2018, Looby et al. 2022). Bioacoustics researchers have recognized the need to identify soniferous fishes and to create a comprehensive database of fish species and their sounds (Lindseth and Lobel 2018, Rountree et al. 2019), which is crucial for several reasons. These datasets serve as valuable resources for researchers in the identification of soniferous species, preventing unnecessary duplication of research efforts, and revealing general trends in fish sound production (Looby et al. 2022, Parsons et al. 2022). Moreover, the inclusion of negative results helps researchers to avoid potential biases in their analyses and may contribute to a more nuanced understanding of the conditions and contexts under which fish engage in sound production. In addition, the development of fish sound catalogues serves as reference databases for identifying vocalizations at the species or family level (Parmentier et al. 2005, Rountree et al. 2020), facilitating regional comparisons (Vieira et al. 2024), and supporting analyses across different geographical regions (Parmentier et al. 2005). Fish sounds, including those from unknown sources, can serve as ecological indicators if they occur across broad geographic areas and persist throughout the year (Di Iorio et al. 2018, Vieira et al. 2024). Unidentified sounds may also contribute to acoustic metrics such as sound richness and diversity, offering valuable insights into habitat conditions, ecosystem health, and marine biodiversity (Staaterman et al. 2017). Future research should focus on expanding these catalogues and developing tools to automate sound recognition to enhance the efficiency of PAM for fish ecology studies (Bas et al. 2017).

Furthermore, informing stakeholders about the significance of sound in the underwater environment can promote conservation efforts. Popular media often highlights soniferous marine mammals (https://www.nmmf.org/). However, over 980 fish species can produce sounds, compared to 130 marine mammals (Spriel et al. 2023). To counter this common misconception, outreach activities such as informative videos, podcasts, social media posts, and websites can help increase

ocean literacy. Sharing research findings is vital for raising awareness of fish sounds among a broad audience. Resources such as the links listed below also contribute to increasing ocean literacy on fish sounds and already include data/results from the present study: https://www.fishbioacoustics.pt, https://www.wo-pam.com/, and https://www.glubs.org/. Integrating these catalogues into citizen science programs and interactive exhibits allows visitors to engage with underwater sound-scapes, fostering marine conservation awareness and promoting environmental stewardship (Parsons et al. 2022). Indeed, organizations such as UNESCO emphasize that ocean literacy is crucial for raising awareness about the conservation, restoration, and sustainable use of our oceans.

#### **Conclusions**

This study provides a baseline for research on acoustic fish communities in PLSMP and other regions. A high diversity of fish sounds was found for this MPA, revealing the importance of the fish acoustic communities and paving the way for future monitoring programs. The potential of PAM for wildlife monitoring, habitat assessment, and acoustic pollution analvsis depends on the existence of comprehensive and reliable databases. Creating a fish acoustic catalogue and database is also crucial for improving machine learning applications in marine ecology, aiding species identification, biodiversity monitoring, and impact assessment. Machine learning is already used to detect vocalizations, identify species, and analyse acoustic data, supporting conservation and management efforts (Bermant et al. 2019, Stratoudakis et al. 2024). Furthermore, changes in fish sound type diversity can be used to monitor shifts in fish communities (Desiderà et al. 2019). This new catalogue of putative fish sounds thus constitutes an important first step in that direction and should be a part of a global effort in marine bioacoustics. Further, PAM is a low-cost, minimal-impact, and relatively low-time-consuming method (if automation methods are applied) and allows for continuous monitoring even at depths and in areas difficult to reach. These are important characteristics, especially since, in general, MPAs are reported to be understaffed and underbudgeted leading to poor law enforcement and management actions (Álvarez-Fernández et al. 2017).

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#### **Author contributions**

Noelia Ríos: Data collection, analysis and organization, manuscript writing, and imagen design, Jodanne Pereira: Data analysis and manuscript writing, Sebastian Muñoz-Duque: Data analysis, Gonçalo Silva: Manuscript review, Miguel Pessanha Pais: Manuscript and statistical review, Paulo Fonseca: Hydrophone construction and manuscript review, Manuel Vieira: Data organization, statistical and manuscript review, Maria Clara P. Amorim: Manuscript review.

# Supplementary data

Supplementary data is available at *ICES Journal of Marine Science* online.

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# Data availability

The data underlying this article will be shared on reasonable request to the corresponding author. Audio examples of all the sound types presented are available on the Fish Bioacoustics Lab website (https://www.fishbioacoustics.pt).

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