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A line with no hook: longline-associated passive eDNA samplers for deep-sea fish monitoring

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ABSTRACT

Monitoring the status of marine biological resources is a key activity in biodiversity conservation and fisheries science. While scientific surveys still largely rely on costly and labour-intensive visual and capture-based methods, sequencing DNA traces from the environment has emerged as a promising alternative and integrative tool for biodiversity assessments. The environmental DNA (eDNA) approach has recently been further boosted by the development of a variety of passive collection methods, which can considerably reduce costs and upscale sampling reach. We adopted a 3D-printed, low-cost passive eDNA sampler (the 'metaprobe') to collect data during a deep-sea demersal longline survey in the southern Adriatic Sea (depth range: 900–1147 m). eDNA samples from 12 metaprobes were metabarcoded using 12S rDNA primers targeting elasmobranchs, and taxonomic assignment was performed against an improved custom 12S Mediterranean fish reference database. eDNA detected all four cartilaginous and three of the five bony fish species captured, and additionally 11 species (three cartilaginous and eight bony fish) not caught by the gear. These taxa not caught by the longline produced a more comprehensive picture of the deep pelagic fish assemblages (e.g., large pelagic species such as tuna and swordfish, and mesopelagic lanternfishes) and the diel variation related to these species' behaviours, highlighting the potential benefit of integrating this simple sampling tool with cooperating longline fisheries operations. Further investigation should refine sampling methodologies to optimize metaprobes interaction with longlines, to expand the types of fishing activity that can contribute to next-generation marine ecosystem monitoring.

1. Introduction

The decline or abrupt changes in fish biodiversity are critical issues compounded by insufficient knowledge of species distribution and the limitations of routinely employed monitoring techniques. The costs associated with the exploration of remote marine habitats, the identification of cryptic species and juvenile stages, the gradual loss of taxonomic expertise, and inconsistent sampling practices are just a few of the challenges that make marine biodiversity assessment a mighty task (Bozec et al., 2011), (Thomsen and Willerslev, 2015) , (Boussarie et al., 2018). Monitoring fishing activities and their impacts on species has traditionally relied on fishery-dependent data, such as logbook entries and visual observations directly from fishermen, fishery observers, or scientific surveys at sea, which can be spatially and temporally skewed and incomplete. Fishery-independent surveys, albeit more accurate, are expensive, time-consuming, and also restricted in terms of spatial and

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temporal scope (Dennis et al., 2015). These challenges in monitoring fish assemblages are not limited to coastal and surface environments but are even more pronounced in the vast and remote deep sea, which represents the largest and least explored biome on Earth (Mayer et al., 2018), (Bergstad, 2013), (Webb et al., 2010), (Costello and Chaudhary, 2017). This environment faces mounting pressures from commercial exploitation and climate change (Amon et al., 2022), even while we lack robust knowledge to predict how its processes and functions will respond.

Traditional procedures, such as bottom trawling, long-lining, and acoustic monitoring, while being useful for some aspects of biodiversity analysis, come with significant limitations related to invasiveness, selectivity, habitat disruption, and feasibility across different types of deep-sea bottoms (Ramirez-Llodra et al., 2011), (Clark et al., 2016), (Jones et al., 2017). Among the most modern and non-invasive methodologies, the high cost and limited coverage of Remotely Operated Vessels (ROVs) and Autonomous Underwater Vehicles (AUVs) restrict their application in deep-sea research (Canals et al., 2021a).

Environmental DNA (eDNA) analysis, the process of capturing DNA from an environmental sample without directly isolating any target organisms (Taberlet et al., 2012), has emerged as a promising, non-invasive technique for biodiversity assessment, capable of supplementing traditional inventory methods (Boussarie et al., 2018), (Beng and Corlett, 2020a,b), (Knudsen et al., 2019), (Miya, 2022), (Stoeckle et al., 2021a), (Veron et al., 2023), (Zou et al., 2020), and suited to be incorporated into existing monitoring practices (Leese et al., 2016), (Hering et al., 2018), (Schenekar, 2022). The advantages of the eDNA approach include the detection of rare, elusive or difficult-to-identify species that are often missed by traditional methods, and the detection of non-indigenous or invasive species, which is a critical aspect of conservation and management (Flitcroft et al., 2025), (Wang et al., 2023). Furthermore, this approach enables the study of community composition across multiple marine habitats, from surface waters to deep pelagic zones and sediments (Andruszkiewicz Allan et al., 2021), (Paulus, 2021), (Brandt et al., 2021), with high spatial and temporal resolution (Cerrillo-Espinosa et al., 2025), thereby informing on horizontal and vertical migratory patterns (Andruszkiewicz Allan et al., 2021), (Feng et al., 2022), (West et al., 2024a). This makes eDNA an effective, non-destructive, taxonomically universal methodology for monitoring early changes in biodiversity (Taberlet et al., 2012), (Chevrinais et al., 2025).

However, key challenges remain, including incomplete and/or inaccurate genetic reference databases (Deiner et al., 2017)– (Blackman et al., 2023a,b), primer specificity issues (Collins et al., 2019) and the typically sterile conditions required, which may limit its application in certain remote scenarios (McClenaghan et al., 2020). To increase the spatial and ecological coverage of environmental DNA collection, various innovative sampling strategies, such as artificial passive samplers (Bessey et al., 2021)– (Verdier et al., 2022), marine filter feeders as "natural samplers" (Mariani et al., 2019), (Cunnington et al., 2024), (Jeunen et al., 2024a,b), and even marine litter items (Ibabe et al., 2020) have been proposed.

Recent advancements in the application of innovative eDNA techniques have demonstrated their potential for large-scale, practical monitoring. Maiello et al., in 2022 (Maiello et al., 2021), introduced the "metaprobe", a 3D-printed, hollow, perforated device that can be placed inside trawl nets during commercial fishing activities. This sampling tool contains gauze rolls to absorb genetic material from species that inhabit, pass through, or interact with the surrounding environment. It allows for simple, rapid, and cost-effective sampling without disrupting regular fishing operations. The metaprobe has proven effective in reconstructing catch composition and detecting species that might otherwise remain undetected (Maiello et al., 2021)– (Maiello et al., 2023), as well as characterising fish assemblages more accurately than trawl surveys (Maiello et al., 2024). The flexibility of this innovative approach is also expected to work well in association with various other types of fishing gear, opening the opportunity to transform commercial fishing vessels into platforms for extensive biodiversity monitoring.

The present study aims to assess, for the first time, the effectiveness of metaprobe-based eDNA sampling in conjunction with a deep-sea demersal longline survey in the southern Adriatic Sea. Longline fishing is a traditional commercial fishing technique that employs a long main line equipped, at regular intervals, with numerous baited hooks. The experiment took place during normal fishing activities, where the longline was equipped with baits to target mesopelagic predators and demersal fish. This experimental design allowed us to i) compare eDNA results with traditional catch data obtained through visual sorting and taxonomic identification, and ii) reconstruct deep-sea fish communities by carefully interpreting species detections while accounting for potential contamination and the influence of the bait used. Moreover, by sampling at different times of the day, our study aimed to elucidate how diel behaviours affect the detection and distribution of eDNA signals, enhancing the ecological insights derived from metabarcoding data.

The results of this study lend support to the view that these passive eDNA samplers can drastically expand the reach of participatory eDNA monitoring, even in deep and remote marine environments, without demanding unrealistic financial and logistical efforts.

2. Materials and methods

2.1. eDNA sampling

Environmental DNA sampling was implemented during the scientific demersal longline campaign "DeepSea 2022" conducted in April 2022 in the southern Adriatic Sea (General Fisheries Commission for the Mediterranean Geographical Subarea, GFCM-GSA-18 – Southern Adriatic Sea, Res. GFCM/33/2009/2). The campaign aimed at sampling deep-dwelling fish communities in six different sites at depths of more than 800 m, and piloting concomitant passive eDNA collection from the surrounding environment (Fig. 1; Table S1).

eDNA samples were collected using metaprobes according to Maiello et al. (2022) (Maiello et al., 2021). Contamination risk was reduced by preparing sampling kits in a sterile laboratory following available instructions (https://github.com/GiuliaMaiello/Metaprobe-2.1). Two rolls of pharmacy gauze filled with cotton were tightly fixed by plastic cables tied to each metaprobe, which was placed in a sterile ziplock bag together with two 50 mL sterile Falcon tubes. Kits remained closed and in a clean environment until sampling procedures.

On board, one metaprobe was attached at the beginning of the fishing line just before each haul, while a second metaprobe was attached 20 m down the branch line using a snap connector, replacing the hook. At each site, the longline was equipped with about 600 hooks (5–6.5 cm in length), consisting of 60 % Aberdeen models (sizes 3/0, 4/0, and 5/0), 30 % ringed circle hooks (sizes 8/0, 9/0, and 10/0), and 10 % beak hooks (size 9/0). Hooks were spaced every 20 m and baited with different species between bony fish (*Scomber* spp., *Sardina pilchardus* (Walbaum, 1792), *Sprattus sprattus* (Linnaeus, 1758)), and squid (*Doryteuthis gahi* (d'Orbigny, 1835)). At the end of fishing operations, the two metaprobes were immediately retrieved, wearing sterile gloves and using clean instruments. The rolls of gauze were collected and stored in separate 50 mL Falcon tubes containing 96 % ethanol for DNA preservation. A total of 12 metaprobes were deployed, with two metaprobes per longline set. Samples were kept refrigerated while on board and then stored in the laboratory at −20 °C until DNA extraction.

In parallel, for each haul, the catch composition was determined at the species level by visual inspection of the external morphology, while also recording the overall number of individuals and the total biomass of each species.

2.2. Laboratory procedures

All laboratory procedures followed high sterility standards and

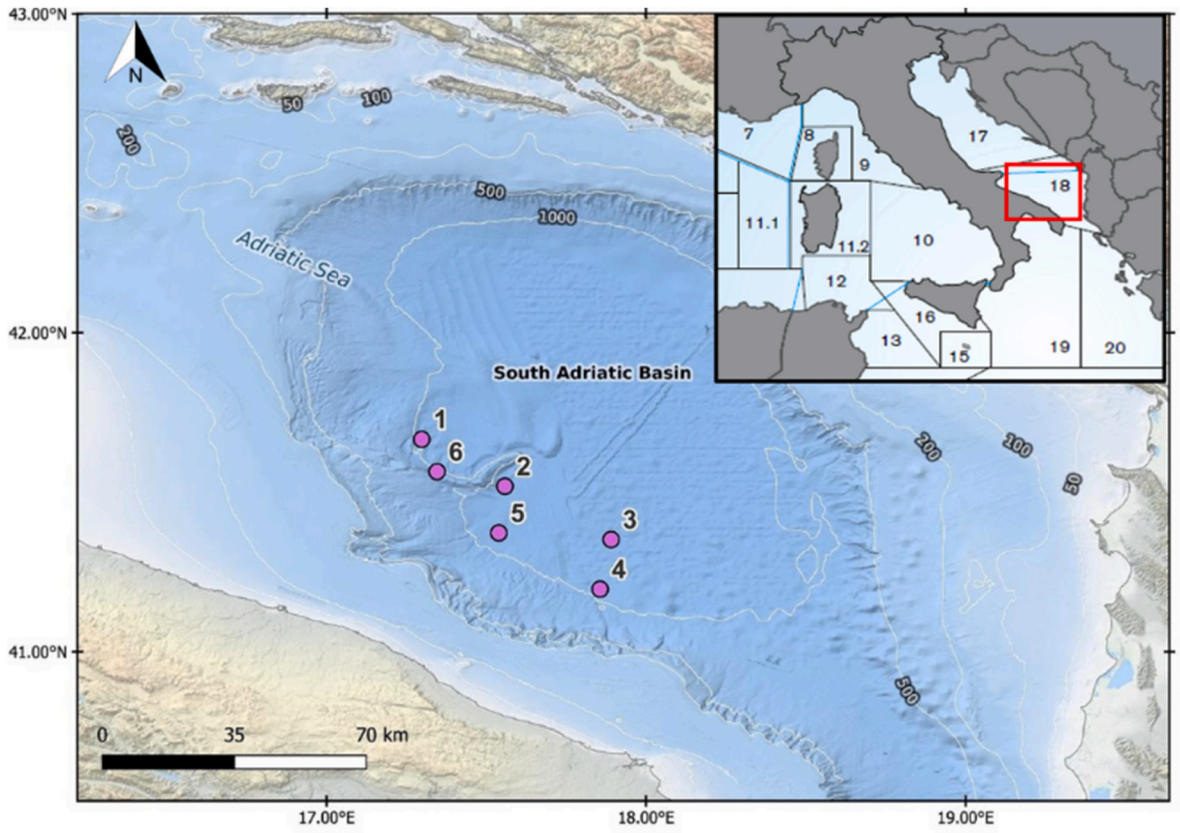

Fig. 1. Map of the six longline deployments in the Southern Adriatic Sea (GSA 18) and EMODnet bathymetry layer (https://tiles.emodnet-bathymetry.eu/; QGIS version 3.22.12).

appropriate criteria to prevent contamination by exogenous DNA (Cilli et al., 2023). DNA extraction was performed in physically separated and designated areas (pre-PCR lab) at the Laboratory of Ancient DNA of the Department of Cultural Heritage (University of Bologna), exclusively dedicated to ancient DNA analyses (Fulton, 2012). Each metaprobe contained two gauzes; and the total genomic DNA (gDNA) was extracted from just half of a gauze roll. This was previously dried to remove ethanol and cut into small pieces, then a silica-based extraction protocol was applied (Cilli et al., 2020), improved from a previously published study (Dabney et al., 2013). As a result, the final dataset consists of 12 independent eDNA samples, one for each metaprobe. The extracted gDNA was eluted in 50 μL of TET buffer (10 mMTris-HCl, 1 mM EDTA, 0.05 % Tween-20). One extraction control, containing only reagents, was included to account for possible contamination linked with extraction procedures and reagents' handling.

A total of 13 samples (12 samples and one extraction control) along with two PCR negative controls and one positive control (i.e., *Pangasianodon hypophthalmus* Sauvage, 1878) were PCR amplified targeting a ~171 bp 12S ribosomal DNA gene fragment of the mitochondrial genome (Miya et al., 2015) using the Elas02 primer pair (Elas02_F 5′GTTGGTHAATCTCGTGCCAGC 3′ and Elas02_R 5′CATAGTAG GGTATCTAATCCTAGTTTG 3' (Taberlet et al., 2018);), which maximises elasmobranch amplification, while also detecting over >90 % of teleosts detectable with teleost-specific primers (Maiello et al., 2024). Laboratory procedures for the library preparation and Illumina iSeq™ 100 sequencing followed the ones described in Maiello et al., 2024) (Maiello et al., 2024), and are detailed in Text S1.

2.3. Bioinformatics: data pre-processing and taxonomic assignment

Bioinformatic procedures followed a customised pipeline. First, read quality was checked with FASTQC v0.12.1 (Andrews, 2010). VSEARCH v2.28.1 '*fastq_mergepairs*' function (Rognes et al., 2016) was used to merge all paired reads, and CUTADAPT v4.9 (Martin, 2011) was used to remove untrimmed sequences and demultiplex samples based on their unique barcodes. VSEARCH '*fastq_filter*' was used to remove reads containing ambiguous bases ("N"), and filtered sequences based on their expected fragment lengths (Elas02: 130–210 bp (Taberlet et al., 2018);). Dereplication was performed via '*derep_fulllenght*'. Chimeras were detected and removed with 'uchime_denovo' (Rognes et al., 2016). The remaining sequences were clustered into Molecular Operational Taxonomic Units (MOTUs) using SWARM v3.15 (Mahé et al., 2021), setting the threshold to d = 3. Finally, a frequency table was generated using the '*otutabout*' function in VSEARCH. Two different 12S reference databases were created using CRABS v1.07 (Jeunen et al., 2023), a comprehensive vertebrate sequences database, and a second one containing only Mediterranean fish species. Moreover, 12S rDNA sequences were produced ex-novo from 85 individuals of 19 Mediterranean elasmobranch and deep-sea teleost species, to enrich the public Mediterranean fish species repositories with taxa relevant to our study area (the complete list of voucher specimens processed, and NCBI/BOLD accession number of newly produced sequences are listed in supplementary materials Table S2). The 12S rDNA sequencing laboratory procedures followed Albonetti et al. (2023) (Albonetti et al., 2023a). The DNA extraction was performed using the RBCBioscience® Tissue Mini Kit (Real Genomics®) according to the manufacturer's protocol. Then, the samples were PCR-amplified with the Aa22-PheF and Aa633-12sR primers reported by Collins et al. (2021) (Collins et al., 2019). All amplicons were enzymatically purified and sequenced by Macrogen Europe BV. The use of two different reference databases, particularly a custom local reference database, alongside newly produced sequences, enhanced the accuracy and confidence of downstream taxonomic assignments by improving molecular identification and discrimination of closely related species from different geographic regions (Stat et al., 2018), (Maiello et al.,

2025), (Stoeckle et al., 2021b), (Weigand et al., 2019a).

The 'db_download' function of CRABS (Jeunen et al., 2023) was used to download sequences from three online repositories, NCBI, EMBL, and MitoFish, filtering for '12S ribosomal RNA'. In the meantime, the *in-house* sequences were imported on CRABS using the '*db_import*' function, and then all the sequences were merged using the '*db_merge*' function. The '*insilico_pcr*' function was used to perform an *in-silico* PCR on both reference databases, using the marker primers. The final Elas02 vertebrate database contained 32,717 unique sequences, while the Elas02 Mediterranean fish database contained 473 unique sequences.

Taxonomy was then assessed via VSEARCH using the Sintax algorithm (Edgar, 2016) with both 12S reference databases. For each MOTU, the final classification was determined based on the consensus among assignment methods. MOTUs receiving the same taxonomic classification across approaches were assigned accordingly. When consensus between the two databases was not achievable, priority was given to the curated Mediterranean fish reference database. Taxonomically ambiguous taxa (e.g., N/A and non-Mediterranean fish taxa) and poorly resolved MOTUs (i.e., MOTUs that could not be unambiguously assigned to a genus or species level) were manually checked by searching the NCBI database using the BLASTn v2.16 algorithm (https://github.com/asadprodhan/blastn (Gao et al., 2017);).

The dataset was filtered by retaining only sequences assigned to species or genus level showing >98 % identity match (Miya et al., 2015) and removing potential contamination noise with the microDecon package v1.0.2 in R v.4.4.2 (R Core Team, 2023) (prevalence method with 0.5 threshold (McKnight et al., 2019)), taking advantage of field blank and negative controls. Potential artefacts, i.e. spurious or low-frequency variants commonly produced by PCR or sequencing noise, were removed using tombRaider v1.0 (https://github.com/gjeunen/tombRaider). To circumvent the occurrence of low-abundance false positives resulting from tag switching, singletons for every taxon at each sampling site were removed (Maiello et al., 2024). Lastly, we applied a 10-read threshold to exclude low-abundance species.

2.4. Statistical analysis

Due to the position of the metaprobes along the longline at the end of different branchlines, each of the two samples collected in every haul was considered independent. Therefore, subsequent analyses were performed on 12 samples.

We evaluated the total number of reads per sample and assessed the composition in terms of relative read abundance across three main taxonomic categories: (i) Mediterranean target species (teleosts and elasmobranchs); (ii) no-target taxa, including humans, primates, and domestic animals; and (iii) bait species used during fishing operations. Reads distribution was visualized using bar plots generated in R. For downstream analyses, only reads assigned to Mediterranean teleost and elasmobranch taxa were retained. The bait reads were conservatively deleted as it was not possible to determine whether the DNA from this species was from naturally occurring individuals or from the bait itself, a common practice in eDNA studies (Stat et al., 2018), (Clark et al., 2024).

To resolve ambiguous taxonomic assignments for genus-level identifications, a Neighbor-Joining (NJ) tree (Saitou and Nei, 1987) was constructed in MEGA X (Kumar et al., 2018) using 12S sequences generated in this study as described above, along with publicly available reference sequences from all Mediterranean species belonging to the genera in question. Node support was assessed through bootstrap analysis with 1.000 replicates (Felsenstein, 1985). Additionally, ASAP (Assemble Species by Automatic Partitioning (Puillandre et al., 2021);) analysis was conducted using the web server (https://bioinfo.mnhn.fr/abi/public/asap/asapweb.html, accessed on March 31, 2025). Both analyses were performed using the p-distance metric with pairwise deletion.

Then, the relative read abundances across the 12 samples and the relative catch data were visualized through bar plots. For the catch data, the total biomass (in grams) per species was used to facilitate comparison with the eDNA read abundances. A Venn diagram was produced using the VENNDIAGRAM package v1.7.3 in R (Chen and Boutros, 2011) to compare the overall composition of taxa detected by eDNA metabarcoding with those obtained from catch data.

To further explore the contribution of species detected by eDNA relative to those physically caught, we compared the taxa identified between the two methods (read count and total weight of catches per species, after square root transformation) across the six sites. Taxa were categorized into four groups based on their occurrence in the catches and/or their eDNA detection, as well as their presumed catchability by the considered fishing gear: i) species occurring only in the catch; ii) species detected both in the catch and via eDNA; iii) species detected exclusively through eDNA but considered compatible with the catchability of the gear; iv) species detected exclusively through eDNA and considered unlikely to be hooked by longlines (hereafter referred to as "eDNA bonus" taxa as described by Maiello et al., in 2022 (Maiello et al., 2021) referring specifically to trawl fishing vessels).

Fish assemblage variation across various groupings was tested through a non-metric multidimensional scaling (nMDS) with the '*metaMDS*' function in the R-package VEGAN v2.6-8 (Oksanen et al., 2018). eDNA metabarcoding datasets typically require normalisation. For this reason, distance matrices were calculated using two complementary methods: i) Bray–Curtis distance on the square-root transformed read abundance dataset, and ii) Bray–Curtis distance on the relative abundance dataset. The square-root transformation on the Bray–Curtis distance was applied to decrease the bias in community reconstruction due to uneven target-species sequences amounts among samples, a common and widely accepted method in community ecology (Maiello et al., 2024), (Mariani et al., 2021), (Guri et al., 2024). Because our dataset showed particularly large differences in read counts among species, applying the Bray-Curtis distance to a relative abundance matrix facilitates the consideration of the impact of dominant species and the variation in relative importance of species across samples, maintaining quantitative information (García-Machado et al., 2022).

Given that the longlines were soaked for several hours and deployed during both day and night periods, this sampling design enabled us to evaluate potential diel variation in the detected community. Based on the two matrix distances, statistical differences between the day/night period of the haul were tested via a PERMANOVA test using the '*adonis2*' function in VEGAN (9.999 permutations). The post-hoc differences were assessed through the '*pairwise.adonis*' function (9.999 permutations). Metadata are shown in Table S1. We classified as "Day" those hauls in which the longline was set around dawn and left to soak throughout daylight hours. Instead, "Night" refers to hauls in which the gear was deployed after sunset and soaked overnight. We define the start of the deployment operation as the "setting time," while the term "hauling time" refers to the retrieval of the longline.

3. Results

3.1. eDNA metabarcoding data

The full high-throughput sequencing resulted in 1.340.031 raw paired-end reads. After bioinformatic processing and taxonomic assignment, the 12 samples yielded a total of 165.154 reads.

MicroDECON R package identified five taxa as possible contaminants – the domestic buffalo (*Bubalus bubalis* (Linnaeus, 1758)), the So-iuy mullet (*Planiliza haematocheilus* (Temminck and Schlegel, 1845)), the Dusky angelfish (*Centropyge multispinis* (Playfair, 1867)), the Variable-lined fusilier (*Caesio varilineata* Carpenter, 1987) and the Pencil cardinal (*Epigonus denticulatus* Dieuzeide, 1950) - which were removed from the final dataset.

A total of 39.467 reads were assigned to non-target vertebrate sequences (humans and domestic species). The most abundant

contaminant was *Homo sapiens* Linnaeus, 1758, occurring in all samples. Contamination coming from *Bos taurus* (Linnaeus, 1758), *Sus scrofa* (Linnaeus, 1758), *Gallus gallus* (Linnaeus, 1758), *Felis catus* (Linnaeus, 1758) and *Canis lupus* (Linnaeus, 1758) was present in more than half of the samples. After the removal of contaminant and non-target reads, the dataset consisted of 125.655 reads, of which 52.058 belonged to bait species (*Scomber* spp. and *S. pilchardus*). A total of 73.597 "target reads" assigned to Mediterranean marine fishes were retained. The minimum number of total target reads was 23 for the H1a sample, and the maximum was 29.716 reads for the H6a sample. The proportion of reads of each category per sample is shown in Fig. 2. The final dataset included 18 target taxa corresponding to 11 teleosts and seven elasmobranchs (Fig. 3, Table S4). All the taxa were identified at the species level. Within Elasmobranchii, genus-level assignments to *Raja* (zOTU37) and *Dipturus* (zOTU38) were refined through the construction of a Neighbor-Joining tree and ASAP species delimitation analysis (Fig. S1), which enabled the identification of *Raja asterias* (Delaroche, 1809) and *Dipturus nidarosiensis* (Storm, 1881).

Overall, *Galeus melastomus* (Rafinesque, 1810) had the highest number of reads (29.507), followed by *Centrophorus uyato* (Rafinesque, 1810) with 18.928 reads and *Mora moro* (Risso, 1810) with 18.644 reads. The most frequently detected taxa across multiple sites were *M. moro* (detected in 11 samples), *Phycis blennoides* (Brünnich, 1768) (in seven samples), and *G. melastomus* (six samples). However, species composition was highly variable across samples. Some taxa were detected exclusively in certain samples, such as *R. asterias*, which appeared only in H1b, or *Xiphias gladius* (Linnaeus, 1758) and *Blennius ocellaris* (Linnaeus, 1758), which were found only in H3a and H3b, respectively (Fig. 3).

3.2. Comparison between eDNA data and catch data

A total of 421 individuals belonging to nine fish species (four elasmobranchs and five teleosts) were caught by demersal longlines, with *G. melastomus* and *M. moro* as the most frequent and abundant in each haul (Table S3; Fig. 3). Among these, seven species were also detected through eDNA metabarcoding, including all four elasmobranchs, resulting in a 100 % detection rate for the captured elasmobranch species, and three of the five bony fish species, for a total of 60 % of caught species.

Overall, based on their detection pattern and compatibility with the fishing gear, the identified taxa were classified into four groups (Fig. 4; Fig. S2): i) *Conger conger* (Linnaeus, 1758) and *Nettastoma melanura* Rafinesque, 1810 (10 % of the total identified species) were recorded exclusively through physical catch and not detected in the eDNA dataset; ii) seven species were detected both in the catch and via eDNA, including *G. melastomus*, *Etmopterus spinax* (Linnaeus, 1758), *D. nidarosiensis*, *C. uyato*, *M. moro*, *P. blennoides*, and *Trachyrincus scabrus* (Rafinesque, 1810); iii) eight taxa were identified exclusively via eDNA but could potentially attach to hooks, including *Lepidopus caudatus* (Euphrasen, 1788), *Micromesistius poutassou* (Risso, 1827), *Mustelus mustelus* (Linnaeus, 1758), *Pteroplatytrygon violacea* (Bonaparte, 1832), *R. asterias*, *Thunnus thynnus* (Linnaeus, 1758), *Trachinotus ovatus* (Linnaeus, 1758), and *X. gladius*; iv) eDNA bonus: species unlikely to be caught by bottom longlines due to their small size but revealed by eDNA, included *B. ocellaris*, *Ceratoscopelus maderensis* (Lowe, 1839) and *Engraulis encrasicolus* (Linnaeus, 1758).

When comparing the number of reads retrieved with eDNA (after square root transformation) and the total biomass of captured species across the six sites (12 eDNA samples; Fig. S2), a variable pattern was revealed. In some cases, longlined species had correspondingly higher read counts than species detected exclusively through eDNA, such as *G. melastomus* in H1a, H6a and H6b, and *M. moro* in H2a, H2b, H4a and H4b. In other samples, the read counts of eDNA-only detected taxa were higher than the read counts retrieved by the longlined species (e.g., H1b, dominated by *R. asterias*; H3a and H3b, dominated by *P. violacea*).

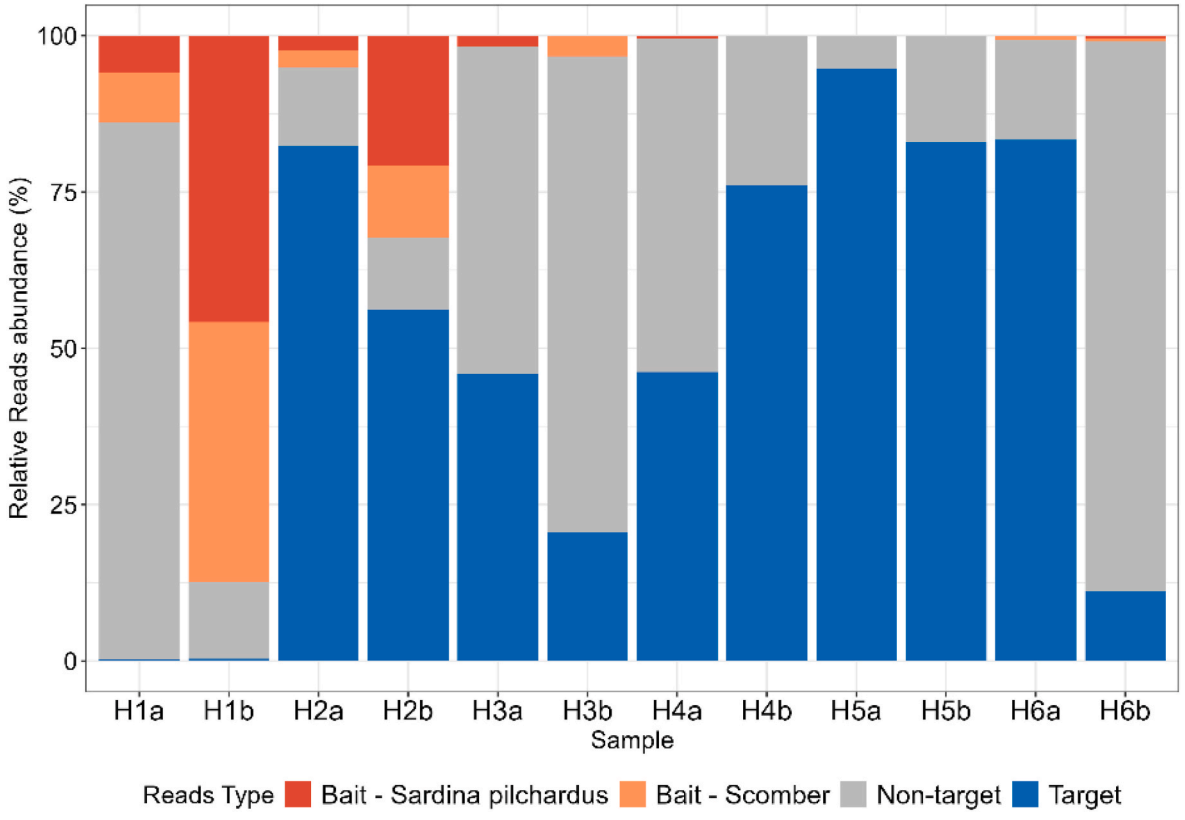

Fig. 2. – Relative abundances of read types per sample: Target (Mediterranean teleosts and elasmobranchs), bait (*Scomber* spp., *S. pilchardus*), and Non-target reads (humans and domestic species).

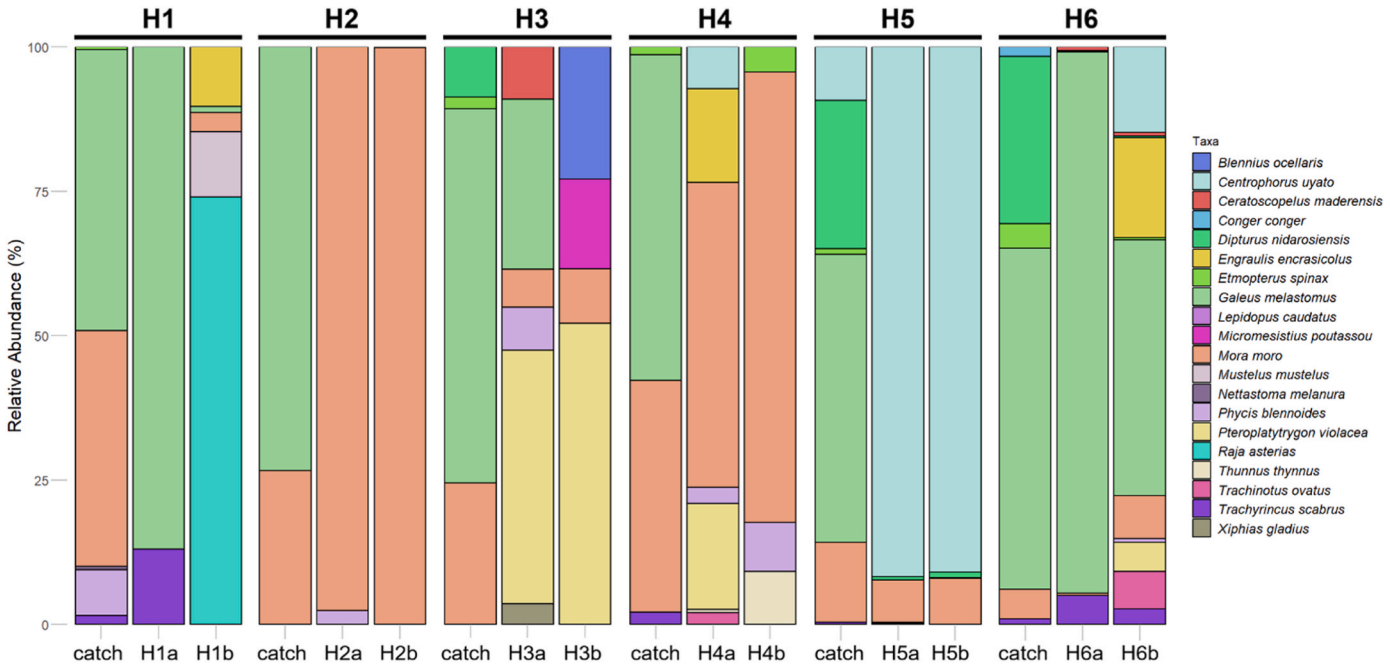

Fig. 3. – Relative catch biomass (catch) and eDNA-derived relative read abundances (samples a and b) across the six sampling sites (H1-H6). Bar plots represent the taxonomic composition, with different colours indicating distinct taxa.

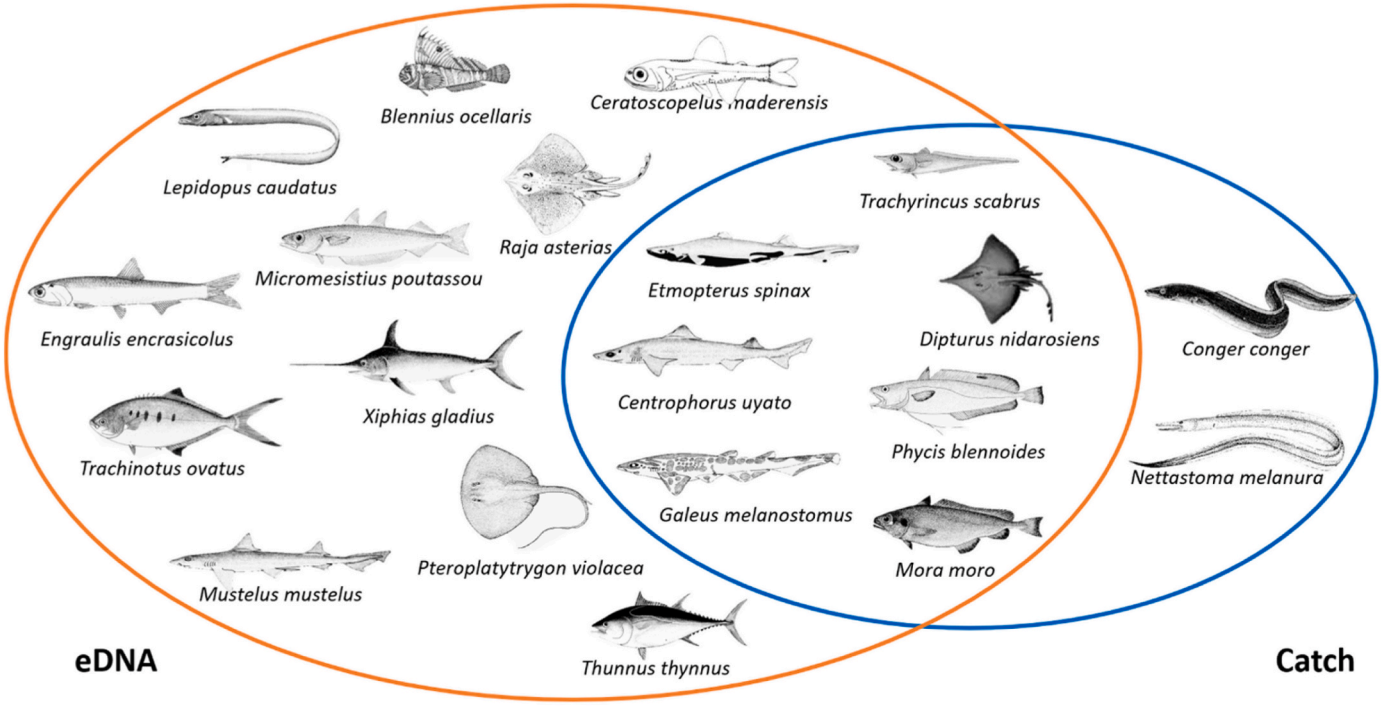

Fig. 4. Venn diagram comparing all taxa detected by eDNA metabarcoding and all species caught by longline across all samples.

Conversely, some species with the highest catch rates, such as *G. melastomus*, were not detected in some of the eDNA samples (e.g., H2a, H4a, H4b, and H5a).

3.3. Diel variation analysis

The nMDS graphs based on Bray-Curtis with square-root transformation distance matrices did not reveal a marked difference between hauls made during the day and those made at night (Fig. 5a), confirmed by the PERMANOVA analysis (p-value >0.05, Table S5a). Conversely, the Bray-Curtis distance matrix in conjunction with the relative abundance transformation delineates two distinct groups (Fig. 5b). The significant result of the PERMANOVA and post-hoc analysis corroborated this distinction (Tables S5a and S6).

4. Discussion

Environmental DNA (eDNA) metabarcoding is becoming a

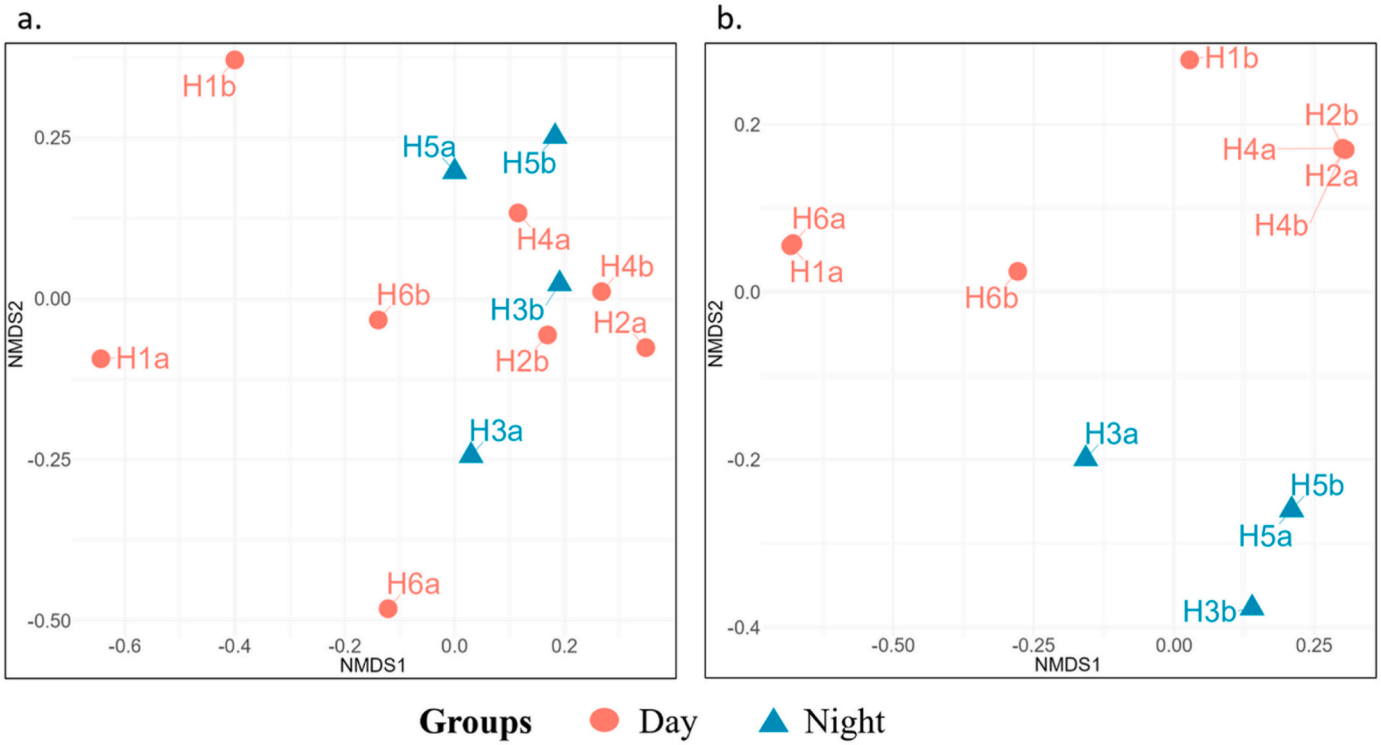

Fig. 5. nMDS plots describing the distribution of samples relative to Day/Night hauls using Bray-Curtis distances from: a) square-root transformation, and b) relative abundance preprocessing.

widespread tool for marine biodiversity monitoring, offering a non-invasive alternative to traditional visual and catch-based methods, which are often costly and limited in detecting rare or elusive species (Polanco Fernández et al., 2021), (Bernard et al., 2013). While eDNA has been previously linked to pelagic or demersal longline fisheries (Green et al., 2024), (West et al., 2024b), this study is the first to employ passive filters (metaprobe) integrated into demersal commercial longlining to specifically evaluate the composition of deep-sea fish assemblages in the deeper habitats of the Southern Adriatic Sea. The adoption of the metaprobes for the sampling procedures manages to overcome the limitations of both traditional surveys and classical eDNA approaches in deep-sea environments, such as the deployment of cumbersome or expensive gear and the logistical constraints of water filtration and pumping.

Despite the high number of discarded reads after the filtering steps, our integrated survey identified a total of 18 target fish taxa, 11 bony fishes and seven elasmobranchs. All the taxa were identified at the species level except for the genus-level assignments of *Raja* and *Dipturus*, a problem also reported in Albonetti et al., (2023) (Albonetti et al., 2023a,b). This could be linked to the lack of correct and unambiguous reference sequences in the database used for the taxonomic assignment, a common issue in highly diverse marine regions (Marques et al., 2021), (Juhel et al., 2020), (Keleman et al., 2025) and a well-documented challenge in eDNA metabarcoding (Collins et al., 2019), (Weigand et al., 2019b). We were able to reach species-level taxonomic assignment through the construction of a phylogenetic tree using 12S rDNA sequences of Mediterranean *Raja* and *Dipturus* species (Fig. S1). This allowed the correct attribution of *R. asterias* and *D. nidarosiensis*, demonstrating how the 12S metabarcoding marker (Miya et al., 2015), (Taberlet et al., 2018) has enough taxonomic power to distinguish among these specific congeneric species. Moreover, this underlines the importance of expanding online databases with the inclusion of curated data originating from vouchered reference individuals to improve the accuracy of species-level assignment, particularly for deep-sea taxa (Deiner et al., 2017), (Marques et al., 2021).

A critical, but expected, issue concerning the eDNA demersal longline data is the high level of read contamination due to human presence (the fisherman working on board) and the operation of specific fishing gear, particularly the presence of baits. Despite implementing numerous precautionary measures to mitigate the risk of contamination during the sampling and DNA extraction procedures, reads linked to human and domestic species were present in all the samples. Contamination reads linked to food, aquaculture or freshwater species are mostly connected to human activity and presence (Zhan, 2025). Concerning the bait reads, two samples out of six contained almost only bait reads (H1a and H1b). During the demersal longline survey, the fish bait *Scomber* spp., *S. pilchardus*, *S. sprattus* have been progressively substituted by the Patagonian squid *D. gahi*. The 12S Elas02 primer pair is specifically designed to amplify fish species (Taberlet et al., 2018) and is unable to amplify Cephalopods, which led to the waning of bait reads in the H2-H6 samples. Future monitoring that integrates metaprobe sampling with longline activity can therefore choose bait type accordingly to maximise target DNA amplification.

The comparison between eDNA and catch data indicated that most of the total identified species were detected only by eDNA metabarcoding. These results reflect the power of metabarcoding approaches in identifying taxa that are present in the surrounding environment but not catchable. These can be rare, elusive, and cryptic species, or result from the transport of parts of specimens (i.e., gametes, mucus, faeces, tissue scraps) or early life stages (i.e., eggs and larvae) and/or taxa that are not catchable by a certain type of fishing gear (Maiello et al., 2021), (West et al., 2024b), (Russo et al., 2021). When the specific catchability of the fishing gear is taken into account, the eDNA species constitute a biodiversity 'bonus'. This concept has been described by Maiello et al. (2021), (Maiello et al., 2023), (Maiello et al., 2024), Mariani et al., in 2021 (Mariani et al., 2021), and Albonetti et al., 2023 (Albonetti et al., 2023a). In this study, the bonus species are represented by *B. ocellaris*, *C. maderensis*, and *E. encrasicolus*, non-target species of bottom longline fisheries. Their exclusive detection through eDNA illustrates the extended ecological reach of this approach.

On the other hand, other eDNA-detected species could potentially interact with the longline hooks, but were not physically caught at the specific time and location of sampling. For example, the detection of the common smooth-hound shark *M. mustelus* and the Mediterranean starry ray *R. asterias* through eDNA, despite their absence from longline catches, represents a measure of the power of eDNA metabarcoding to reveal species that do not directly interact with the fishing gear. While these taxa can be targets of demersal longline fisheries operating at shallower depths (Ceyhan et al., 2010)– (Colloca et al., 2024), they are typically associated with coastal or continental shelf habitats, with *M. mustelus* occurring down to approximately 350 m and *R. asterias* rarely found below 200 m (Serena, 2005). Their detection beyond 1000 m depth suggests the ability of eDNA to capture traces of species from outside the immediate sampling area, potentially due to passive transport of genetic material, water column mixing, or transient presence in the water mass (Allan et al., 2021a). The fish assemblage detected by eDNA was composed of neritic, mesopelagic, bathypelagic and deep benthic species. This reflects the overall journey of metaprobes from the time of deployment on the surface to retrieval after several hours in proximity to the bottom. This broader ecological characterisation through eDNA is epitomised by species such as *T. thynnus*, *X. gladius*, *T. ovatus*, *L. caudatus, M. poutassou* and *P. violacea*, which are not typically expected to interact with demersal longlines deployed at depths exceeding 1000 m (Cambiè et al., 2013), (Bauer et al., 2017), (Madigan et al., 2021), (Mariño-Briceño et al., 2022), (Triay-Portella et al., 2023), (Poisson et al., 2024). It is also possible that some of the detected eDNA may reflect downwelling genetic information present at the site, which is correlated to the slower degradation rate in deep environments; though the provenance of the eDNA in diverse aquatic environments remains a subject of debate, requiring judicious interpretation of faunal detection (Jo et al., 2025a).

Our findings highlight the added ecological reach of eDNA-based monitoring in deep-sea environments, as confirmed by previous studies (Collins et al., 2019), (West et al., 2021), (Zhang et al., 2023). Due to the wider spatial distribution of baited hooks, our results further indicate that longline-associated metaprobes provide a variable eDNA signal, capturing a broad snapshot of the surrounding water column, which calls for larger, more representative sample sizes, if feasible, for a more robust biodiversity assessment. While some samples (H1a, H2b, H5a, H5b) showed a strong influence of caught species, others (H1b, H3a, H3b) presented a more balanced or even prevailing representation of species not caught (Fig. S2). These patterns suggest that longline-associated metaprobes are not strictly biased toward species interacting with the fishing gear, reinforcing their potential for detecting elusive, rare, or spatially segregated taxa.

Despite the overall efficacy of metaprobes in detecting caught species, two members of the Anguilliformes family, *C. conger* and *N. melanura*, were not detected. However, it is important to highlight that these species accounted for only three individuals out of a total of 421 captured (i.e., less than 1 %), suggesting minimal impact on the overall concordance. Conversely, a few species highly abundant in the catch were not consistently detected across all eDNA samples. For example, *G. melastomus*, which was among the most frequently caught species, was not detected in some metaprobe samples despite being physically present. These discrepancies could reflect local hydrodynamics, spatial mismatch between samplers and DNA plumes, or taxon-specific shedding and degradation rates (Sassoubre et al., 2016)– (Allan et al., 2021b). To further enhance the overlap between eDNA detections and catch records, future efforts could focus on increasing the number of samples or optimizing the spatial positioning of sampling points along the fishing line.

Taxa detected by eDNA metabarcoding included species of particular conservation and management relevance. Among threatened elasmobranchs reported in the Mediterranean IUCN Red List, the Norwegian skate *D. nidarosiensis* and the Mediterranean starry ray *R. asterias* are listed as Near Threatened in the basin, and the little gulper shark *C. uyato* and the common Smooth-hound shark *M. mustelus* are listed as Vulnerable. Among bony fish, the detection of the Atlantic bluefin tuna *T. thynnus*, listed as Endangered in the Mediterranean, and the Swordfish *X. gladius,* listed as Near-Threatened, was relevant.

The square-root transformation, by reducing the impact of the most dominant taxa did not reveal any significant difference in community composition between day and night (Fig. 5a), but the difference became significant when we considered the diel variation based on the most abundant species (Fig. 5b - relative abundance preprocessing). The same results were obtained when the fish assemblage structure was investigated through a PERMANOVA analysis; the relative abundance preprocessing with Bray-Curtis distance of raw data, revealed significant variation between day and night hauls (Tables S5 and S6). Despite the limited number of samples, it is reasonable to expect significant shifts in vertically migrating organisms, as shown in targeted investigations (Jo et al., 2025b)– (Canals et al., 2021b).

The present study demonstrates that eDNA sampling through a low-cost and low-effort sampler can be a useful supplement to scientific fishing surveys, in particular for deep-sea environments. Our results were based on only two metaprobes per longline; it is thus easy to imagine that even a moderate manifold increase of "non-hooked snoods" equipped with metaprobes, would massively increase eDNA detections, as well as reduce catch and bycatch, resulting in more sustainable surveys. Following the earliest experience of trawling (Maiello et al., 2023), it can also be envisaged that some commercial longliners could also be open or interested in engaging with marine monitoring by adding some non-hooked metaprobed branchlines to their gear, thereby increasing the spatial and temporal coverage of monitoring. In the context of climate and biodiversity crises affecting the oceans, this type of affordable solutions that also promote inter-sectoral engagement (Neave et al., 2025) can upscale data collection while reducing the considerable financial investment and the allocation of labour, which are major limitations, especially in open and deep-sea environments.

CRediT authorship contribution statement

Martina Spiga: Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation. **Valentina Crobe:** Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Data curation. **Alice Ferrari:** Writing – review & editing, Writing – original draft, Validation, Supervision, Investigation, Data curation. **Giulia Maiello:** Writing – review & editing, Writing – original draft, Validation, Methodology, Investigation, Formal analysis, Data curation. **Elia Bueloni:** Writing – review & editing, Writing – original draft, Resources, Methodology. **Elisabetta Cilli:** Writing – review & editing, Writing – original draft, Methodology, Investigation. **Tommaso Russo:** Writing – review & editing, Writing – original draft, Supervision, Methodology, Investigation, Conceptualization. **Simone Di Crescenzo:** Writing – review & editing, Writing – original draft, Data curation. **Rita Cannas:** Writing – review & editing, Writing – original draft, Supervision. **Giusy Catalano:** Writing – review & editing, Writing – original draft. **Corrado Piccinetti:** Writing – review & editing, Resources, Funding acquisition, Conceptualization. **Stefano Mariani:** Writing – review & editing, Writing – original draft, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Data curation, Conceptualization. **Alessia Cariani:** Writing – review & editing, Writing – original draft, Validation, Supervision, Resources, Project administration, Investigation, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.marenvres.2025.107823.

Data availability

The raw eDNA metabarcoding data, the raw MOTU table and the final dataset have been deposited in Zenodo and are publicly available under the DOI: 10.5281/zenodo.17914933.

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