Received: 29 July 2022 | Revised: 9 December 2022 | Accepted: 23 December 2022

DOI: 10.1002/edn3.389

Environmental DNA
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WILEY

SPECIAL ISSUE ARTICLE

Net gain: Low-cost, trawl-associated eDNA samplers upscale ecological assessment of marine demersal communities

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Funding information
Food and Agriculture Organization of the United Nations; General Fisheries Commission for the Mediterranean; UK Natural Environment Research Council, Grant/Award Number: NE/T007028/1

Abstract

Marine biodiversity stewardship requires costly and time-consuming capture-based monitoring techniques, which limit our understanding of the distribution and status of marine populations. Here, we reconstruct catch and demersal community composition in a set of 24 fishing sites in the central Tyrrhenian Sea by gathering environmental DNA (eDNA) aboard commercial bottom-trawl fishing vessels. We collected genetic material from two sources: the water draining from the net after the end of hauling operations ("slush"), and custom-made rolls of gauze tied to a hollow perforated sphere placed inside the fishing net ("metaprobe"). Species inventories were generated using a combination of fish-specific (Tele02 12S) and universal metazoan (COI) molecular markers. DNA metabarcoding data recovered over 90% of the caught taxa and accurately reconstructed the overall structure of the assemblages of the examined sites, reflecting expected differences linked to major drivers of community structure in Mediterranean demersal ecosystems, such as depth, distance from the coast, and fishing effort. eDNA also returned a "biodiversity bonus" mostly consisting of pelagic species not catchable by bottom trawl but present in the surrounding environment. Overall, the "metaprobe" gauzes showed a greater biodiversity detection power as compared to "slush" water, both qualitatively and quantitatively, strengthening the idea that these low-cost sampling devices can play a major role in upscaling the gathering of data on both catch composition and the broader ecological characteristics of marine communities sustaining trawling activities. This approach has the potential to drastically expand the reach of ecological monitoring, whereby fishing vessels operating across the oceans may serve as opportunistic scientific platforms to increase the strength and granularity of marine biodiversity data.

KEYWORDS
environmental DNA, environmental impacts, fished communities, marine biodiversity, trawl fishery, Tyrrhenian Sea

Stefano Mariani and Tommaso Russo equally contributed to this work.

1 | INTRODUCTION

The Mediterranean Sea is a well-established biodiversity marine hotspot (Myers et al., 2000). Despite covering <1% of the global ocean surface, it hosts more than 18% of the world's known macroscopic marine species, of which ~30% are endemic (Bianchi & Morri, 2000). Nowadays, many Mediterranean ecosystems and communities are under grave threat and have been suffering from severe alterations, degradation or losses under the combined pressure of climate change and human impact (Cramer et al., 2018; Piroddi et al., 2020). One of the main causes affecting global marine biodiversity is unregulated fishing activity, especially bottom trawling: Mediterranean Sea fishing grounds are intensively trawled at depths ranging from 50 to 700 m (Amoroso et al., 2018). There is an urgent need to progressively improve fishing practices, reducing bycatch, and increasing habitat and species protection, especially endangered ones (Fiorentino & Vitale, 2021). Simultaneously, also the advancement of assessment methods is required (Cardinale et al., 2021). In fact, despite the availability of technological innovations, data collection from the oceans remains a major logistic and economic challenge, hindering our understanding of species distribution and ecosystem status. Marine monitoring, and in particular the branch pertaining to fishery management, often relies on fisheries-dependent information, which is still largely based on traditional approaches, such as logbook data, visual inspection and sorting of catches. Fisheries-dependent data are also a crucial input for widely accepted and applied assessment approaches (Pennino et al., 2016); unfortunately, the collection of this kind of data is a time-consuming activity, frequently performed by fisheries observers or even the fishers themselves, and limited to small subsets of the fleet, eventually compromising the accuracy and reliability of resulting estimates (Vilas et al., 2019).

In this context, environmental DNA (eDNA) metabarcoding is progressively playing a primary role in enhancing our knowledge of species presence and distribution in every habitat (Sigsgaard et al., 2020; Thomsen & Willerslev, 2015; West et al., 2020). Environmental DNA represents an important source of biodiversity information, easier, cheaper to obtain, and often more accurate than pre-existing methods. In recent years, eDNA-based metabarcoding approaches have been successfully applied to many fields of biological research. Various attempts have been made to integrate trawl-based surveys with eDNA metabarcoding (Salter et al., 2019; Thomsen et al., 2016; Zou et al., 2020), including in a fishery management context (Maiello et al., 2022; Russo et al., 2021; Stoeckle et al., 2021). In classical eDNA approaches, based on pumping and filtration of water, the main limitation remains the collection and concentration of DNA from large water volumes, which require specific sterile tools (such as filters, pumps or syringes) and can be challenging, especially in remote environments. Russo et al. (2021) suggested the feasibility of collecting samples directly from fishing vessels, inferring catch composition from eDNA metabarcoding of water draining from the net cod-end (hereafter "slush"). However, "slush" collection is not always practical, since it may interfere with routine fishing operations (e.g., the hauling of the net and sorting of catch). To circumvent such limitations, we leveraged commercial trawling activities by employing a bespoke 3D-printed plastic sphere (the "metaprobe"), which was deployed inside the trawl net to passively absorb traces of DNA from the surrounding environment during fishing operations (Maiello et al., 2022). Encouragingly, our initial findings suggested that community composition inferred from metabarcoding of metaprobe-collected eDNA largely reflects that retrieved by visual sorting of the catches.

Here, we examined both "slush" water and this new low-effort, non-interfering "metaprobe" approach over a broad area on board a commercial trawling vessel in the central Tyrrhenian Sea (FAO/GFCM Geographical Sub Area, GSA 9). Importantly, the study area encompasses ecologically different sites that are subjected to various extents of anthropogenic pressures and are representative of the fishing footprint exerted by trawlers in the GSA 9 area (Russo et al., 2019). Specifically, we: (1) tested differences between the two eDNA-sampling methods; (2) compared eDNA metabarcoding results with visually-reconstructed catch composition; (3) linked species distribution to environmental and anthropogenic variables that are thought to be drivers of demersal community composition; (4) evaluated the pattern of per-taxon eDNA read abundance, as a semiquantitative proxy of biomass/number of individuals (Clark et al., 2020; Russo et al., 2021; Stoeckle et al., 2021). Our results strengthen the idea that a broader usage of the "metaprobe" in association with commercial trawling activities could upscale eDNA-based biomonitoring at negligible additional cost and effort, to study community and ecosystem responses to commercial fishing.

2 | MATERIAL AND METHODS

2.1 | Collection of samples

Samples were collected between July and August 2020 from 24 sampling sites in the central Tyrrhenian Sea (GSA 9—Western Mediterranean Sea), on board a commercial vessel performing bottom-trawl fishing (Figure 1a). Sampling hauls covered an area spanning from the continental shelf edge (average depth ~70 m) to the deep slope (~820 m), with a distance from the coast range of ~4–24 km (Table S1). The coast of the considered area is extremely populated and harbors large urban centers and industrial settlements, which lead to strong anthropogenic pressures in the surrounding sea. Sampling sites included the major fishing grounds of the studied location, mirroring the fishing footprint of trawlers operating in GSA 9 (Russo et al., 2019). GPS positions at a frequency of 1 min were collected, for each haul, during the sampling. For each haul, we gathered eDNA samples from two sources: the water draining from the net cod-end ("slush"; Russo et al., 2021) and rolls of gauze tied to a hollow perforated plastic sphere ("metaprobe"; Maiello et al., 2022) (Figure 1b). The "metaprobe" was custom-made and 3D-printed (the 3D project is freely accessible at: https://github.com/GiuliaMaiello/Metaprobe-2.0) and the rolls of gauze were prepared in a sterile

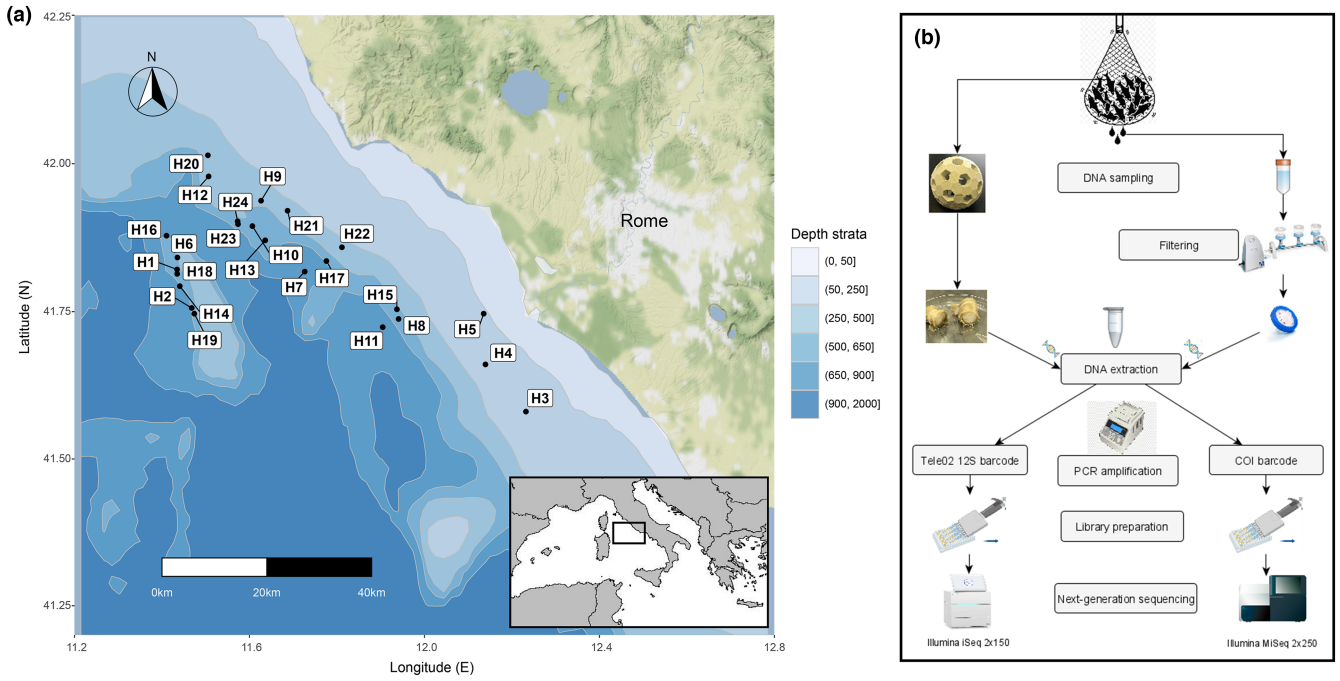

FIGURE 1 Sampling with "slush" water and "metaprobe" rolls of gauze: (a) map of 24 sampling sites in the central Tyrrhenian Sea (GSA 9); (b) graphical schematic illustrating the key steps of sample processing, from the collection on board the fishing vessel to DNA sequencing, trough the main laboratory procedures. The map was created using the R package ggmap (Kahle & Wickham, 2013).

laboratory, rolling 1 g of pharmacy sterilized cotton in 3 10×10 cm sterile gauze wrappings (mesh-size: 1 mm), and then tightly fixed to the "metaprobe" using zip-ties. On board the fishing vessel, the "metaprobe" was placed inside the net at the beginning of each haul and retrieved at the end of fishing operations during the sorting of catches. Two rolls of gauze were immediately gathered and placed in separate 50 ml sterile tubes; one gauze roll was preserved with 99% ethanol and the other one using silica gel grains. "Slush" water was collected in 50 ml sterile tubes just after the net was hauled on board, while suspended above the deck. Three samples for each sampling site (haul) were gathered (1 "slush" water, 1 ethanol and 1 silica gel preserved gauze roll), totaling 72 samples. In four of the 24 sites, we also sampled seawater nearby the vessel during hauling procedures, as field controls unaffected by the trawling activity. All samples were frozen on board and subsequently stored in the laboratory at −20°C until DNA extraction. At the same time, we determined the qualitative taxa composition of each haul: individuals in the net were identified at the species or genus level by visual inspection of external morphology.

2.2 | Laboratory procedures

For sample processing, we used specialized laboratories for DNA extraction, pre- and post-PCR procedures, in order to minimize the risk of contamination. "Slush" water samples were filtered with a vacuum pump through DNA-cellulose filters (0.2 µm) to concentrate the DNA (Figure 1b). Total DNA was then extracted from half of each filter following the Mu-DNA soil protocol (Sellers et al., 2018). DNA was lysed overnight at 37°C with 730 µl of lysis solution (1 M Tris HCl [pH 8], 0.5 M EDTA [pH 8]), 250 µl of soil lysis additive (180 mM aluminium ammonium sulphate dodecahydrate, 20% SDS) and 20 µl of proteinase K (100 µg/ml). We then extracted the DNA through the main steps of the protocol: inhibitor removal, silica binding, wash, and final elution. To extract DNA from "metaprobe" gauze rolls, we followed a procedure for the recovery of extremely low concentration fragmented DNA (Malmström et al., 2009). We dried the ethanol-preserved gauzes before DNA extraction, to avoid PCR inhibition. Half of each roll of gauze was cut into small pieces and then incubated overnight at 37°C with 400 µl of extraction buffer (0.5 M EDTA pH 8, 1 M Urea) and 20 µl of proteinase K (100 µg/ml). DNA was subsequently concentrated with an Amicon ultra-4 30 K centrifugal device and purified with the QIAQuick PCR Purification Kit (Qiagen). Four extraction negatives (two for each extraction method) were included to monitor the possibility and extent of contamination linked with extraction procedures and reagents.

Extracted DNA was PCR amplified targeting two taxonomically informative mitochondrial regions (Figure 1b), namely a ~167 bp fragment of the 12S gene and a ~313 bp fragment of the COI gene. The former was amplified using the fish-specific Tele02 primers (forward: 5′-AAACTCGTGCCAGCCACC-3′; reverse: 5′-GGGTATCTAATCCCAGTTTG-3′) (Taberlet et al., 2018), while the latter was amplified using highly degenerated universal metazoan primers (forward mlCOIintF: 5′-GGWACWRGWTGRACWNTNTAYCCYCC-3′ (Leray et al., 2013); reverse jgHCO2198: 5′-TANACYTCNGGRTGNCCRAARAAYCA-3′ (Geller et al., 2013)). To account for possible contamination, we included both a positive (*Sebastes mentella*, a subarctic species absent in the Mediterranean Sea) and a negative PCR control.

To univocally identify samples and contextually reduce the risk of cross-contamination and/or tag switching during Illumina sequencing, each sample was amplified using a unique 8 bp oligo-tag attached to the forward and reverse primers. Each tag differed for at least three base pairs from other tags and was preceded by 2–4 degenerate bases (Ns) to improve sequence diversity during sequencing.

Each of the 83 samples (48 rolls of gauze, 24 "slush" water, 4 field blanks, 4 extraction blanks, 2 PCR negative controls and 1 PCR positive control) was PCR amplified in triplicate under the following thermocycling conditions: 95°C for 10 min, followed by 40 cycles of 95°C for 30 s, 60°C for 45 s, and 72°C for 30 s, and a final elongation of 72°C for 5 min for the Tele02 12S primers; and polymerase activation at 95°C for 10 min, followed by 35 cycles of denaturation and amplification (94°C for 1 min, 45°C for 1 min, 72°C for 1 min), and a final elongation of 72°C for 5 min for the COI primers. PCRs were performed in 20 µl reactions containing 10 µl MyFi™ Mix (Meridian Bioscience), 0.16 µl of Bovine Serum Albumin (20 mg/ml, Thermo Scientific), 5.84 µl of UltraPure™ Distilled Water (Invitrogen), 1 µl of each forward and reverse primer (10 µM, Eurofins), and 2 µl of template DNA. Replicates were then pooled, and samples were visualized on a 2% agarose gel stained with SYBRsafe (Invitrogen) to ensure the successful amplification of target fragments. PCR products were then purified with Mag-Bind® TotalPure NGS magnetic beads (Omega Bio-tek Inc), adding to 30 µl of PCR products the magnetic beads: a 1× ratio and a 0.8× ratio were used for 12S and COI, respectively (Bronner et al., 2009). Purified DNA was quantified using a Qubit™ 4.0 fluorometer with the Qubit™ dsDNA HS Assay Kit (Invitrogen). Based on the total DNA concentration, samples were normalized and pooled in equimolar concentration for library preparation. End repair, adapter ligation and library PCR amplification were performed using the NEXTFLEX® Rapid DNA-Seq Kit 2.0 for Illumina® platforms (PerkinElmer) according to the manufacturer's protocol. The Agilent 2200 TapeStation and High Sensitivity D1000 ScreenTape (Agilent Technologies) indicated secondary products (e.g., adaptor dimers) remained, which were removed by another magnetic bead clean-up (1x ratio for both libraries). Libraries were quantified using quantitative PCR (qPCR) on a Rotor-Gene Q (Qiagen) with the NEBNext® Library Quant Kit for Illumina® (New England Biolabs). We diluted the Tele02 library to 1 nM and the COI library to 4 nM; final libraries and PhiX Control were quantified using qPCR before sequencing. The 12S library was sequenced at 60 pM with 10% PhiX Control on an Illumina® iSeq™ 100 using the i1 Reagent v2 (300-cycle) (Illumina Inc.). COI library was sequenced at 12.5 pM with 10% PhiX control using V3 chemistry (2×250 bp paired-end) on an Illumina MiSeq platform (Figure 1b).

2.3 | Bioinformatics

Bioinformatic procedures were based on the OBITOOLS software 1.2.11 (Boyer et al., 2016). Read quality was checked with FASTQC and low-quality ends were trimmed for downstream analysis. We used ILLUMINAPAIREDEND to merge all paired reads showing a quality score >40, and NGSFILTER to demultiplex samples based on their unique barcodes, allowing for a single base mismatch. Sequences were filtered via OBIGREP to remove singletons and reads out of the expected length range (129–209 bp for 12S; 300–325 bp for COI), and dereplicated via OBIUNIQ. We removed chimeras with UCHIME (Edgar et al., 2011) and clustered the remaining sequences into Molecular Operational Taxonomical Units (MOTU) with SWARM (Mahé et al., 2015) setting the threshold to $d = 3$ for Tele02 12S (corresponding to >98% sequence identity for selected barcode) and $d = 13$ for COI (Kemp et al., 2019; Siegenthaler et al., 2019; Wangensteen et al., 2018).

Custom-made databases were created through an in-silico PCR against the EMBL database (Release version r143) implemented with ECOPCR: a 12S vertebrate database of 26,387 sequences and a COI metazoan database of 279,692 sequences were obtained for the Tele02 12S and COI data, respectively. We first assigned taxonomy with ECOTAG and then checked the taxonomic assignment of ambiguous (e.g., non-Mediterranean taxa) and poorly resolved MOTUs (i.e., MOTUs that could not be unambiguously assigned to a genus or species level), searching against the NCBI database using BLASTn. For COI data we retained only taxa belonging to the main fished taxa (i.e., teleosts, elasmobranchs, cephalopods and decapods) for subsequent analyses. Datasets were finally filtered retaining only sequences showing >98% identity match and removing potential contamination noise using blanks and negative controls with the DECONTAM package in R (Davis et al., 2018), using the prevalence method with a threshold of 0.5. The four field blanks represented the level of contamination linked with sampling procedures during trawling activities, while extraction and PCR negative controls monitored for laboratory contaminants.

2.4 | Data analysis

All downstream analyses were performed in R v 3.6.3 (R Core Team, 2021). Boxplots were used to compare the performance of different sampling and storage methods both in terms of number of species and number of reads. Venn diagrams were drawn using the VENNDIAGRAM package in R (Chen & Boutros, 2011) to visualize differences in taxa detections between eDNA metabarcoding of "slush" water, and ethanol- and silica-preserved gauze rolls from the "metaprobe"—the significance of comparisons was assessed by Kruskal–Wallis test and Wilcoxon post-hoc paired test, adjusting p-values for multiple testing (Bonferroni correction). Venn diagrams were also built to compare overall detections obtained by eDNA metabarcoding (combining all taxa identified by "slush," ethanol and silica gel "metaprobe" samples) versus catches, for teleosts, elasmobranchs, cephalopods, and decapods independently. We performed a Mantel test ("mantel" function in the R package VEGAN, with 9999 permutations; Oksanen et al., 2018) to assess the overall consistency between community structures (i.e., matrices of Jaccard's distance between pairs of sites) obtained by eDNA and catches. For teleosts, we also visualized (pie charts) the proportion of pelagic over demersal species (Froese & Pauly, 2022) in the three sets (i.e., only eDNA, only catch, and shared by both), as we expected

the "metaprobe" to include an excess of pelagic taxa not caught by the trawl net, as a result of its long travels up and down the water column during the deployment of the net. Binary presence-absence data from 12S and COI were combined in the above-mentioned analyses, to include taxa belonging to all the four considered groups (i.e., teleosts, elasmobranchs, cephalopods and decapods).

The Multi Response Permutation Procedure (MRPP) (Mielke & Berry, 1994) implemented in the R package VEGAN was applied to assess whether there was a significant difference in the α-diversity between the two "metaprobe" replicates (ethanol and silica gel) within the same sampling location. MRPP allowed comparing, through a permutational approach, the inter-site dissimilarities (i.e., between replicates of different sites) with the intra-site dissimilarities (i.e., between replicates of the same site) in terms of species composition. To explore relationships among communities across sampling sites, we then performed a non-metric multidimensional scaling (NMDS) using Jaccard's distance with the "metaMDS" function in the R package VEGAN. MRPP and NMDS analyses were based on a presence-absence dataset including all taxa as revealed by "metaprobes" (12S and COI data combined) and identified to genus or species level.

We contrasted the site distribution among the first two NMDS axes with environmental variables (i.e., depth and distance from the coast) and a measure of anthropogenic impact (i.e., fishing effort), which are known to influence the structure of Mediterranean demersal ecosystems. We evaluated the influence of the three considered variables through environmental fit on the NMDS using the "envfit" function in the R package VEGAN. The function fits environmental vectors or factors onto an ordination; the projections of points onto vectors are calculated in order to have maximum correlation with the corresponding environmental variables. Although depth and distance from the coast are typically correlated, we assessed the effect of these two environmental drivers separately in order to account for the morphological complexity of the sea bottom, such as shoals, seamounts or canyons. For instance, H1, H6 and H18 sites were located on shallower banks despite being located far from the coastline; conversely, H7 and H11 corresponded to a seafloor depression relatively close to the coastline (Figure 1a; Table S1). Fishing effort was calculated, for each sampling site, as the mean total hours of trawling per year over the last 5 years (2016–2020), averaged over the range of 1 km within the centroid of each haul (computed from GPS data), using Vessel Monitoring System (VMS) data (Russo et al., 2014, 2016).

Finally, we evaluated the distribution of (transformed) read abundances for taxa revealed by 12S and COI separately, eventually distinguishing between COI-detected vertebrate and invertebrate taxa. To do so, we generated barplots using the square root of the reads as a proxy of taxa abundances (Clark et al., 2020; Maiello et al., 2022; Mariani et al., 2021; Stoeckle et al., 2021).

3 | RESULTS

After bioinformatic analyses, we obtained a total of 1,389,884 reads (mean per sample = 19,036 reads) for the 12S samples, which

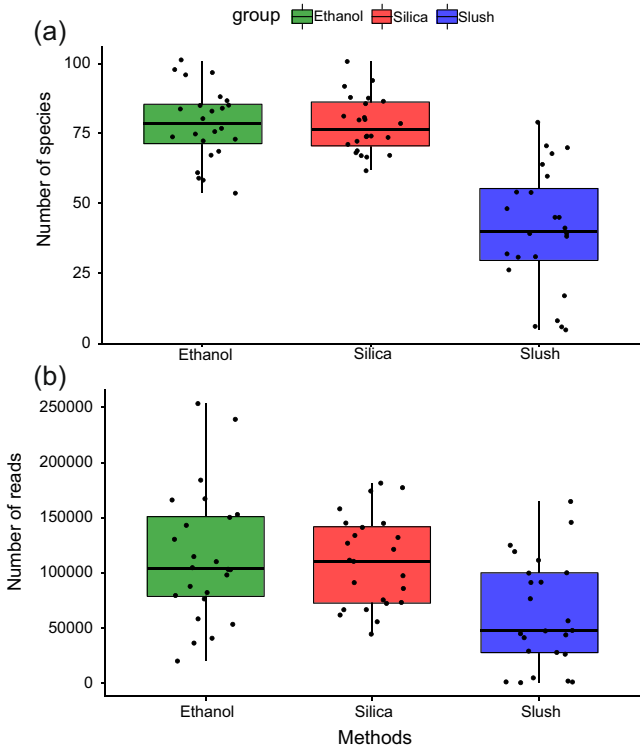

FIGURE 2 Boxplots representing (a) the number of species and (b) the number of reads detected by ethanol and silica gel grains rolls of gauze ("metaprobes"), and "slush" water, respectively. Data from 12S and COI were combined. The differences between the three methods were tested by the Kruskal–Wallis test ($p < 0.01$ both for the number of species and the number of reads).

allowed the detection of 90 taxa (84 teleosts and 6 elasmobranchs). The COI yielded overall 6,170,744 reads (mean per sample = 85,041 reads), returning 108 teleosts, 14 elasmobranchs, 28 cephalopods and 41 decapods. After data filtering and removal of potential contaminants, final datasets included 79 taxa for 12S and 187 for COI (119 vertebrate taxa and 68 invertebrate taxa). eDNA metabarcoding also revealed sequences of two typical Mediterranean cetaceans (*Delphinus delphis* and *Stenella coeruleoalba* in two and four samples, respectively), which were anyhow excluded from downstream analyses as non-target taxa.

"Metaprobe" gauze samples enabled the detection of more species than "slush" water (Kruskal–Wallis: $\chi^2 = 36.9$, df = 2, $p < 0.001$) and yielded a greater abundance of DNA sequence reads (Kruskal–Wallis: $\chi^2 = 12.4$, df = 2, $p = 0.002$) (Figure 2). Three species were recovered only by "slush" water, while 53 taxa were exclusively found in "metaprobe" samples (Figure S1). In contrast, different preservation methods of gauze rolls did not affect the efficiency of species detection (Figure 2a; Wilcoxon sign test: $W = 158$, $p = 0.82$), nor the number of reads (Figure 2b; Wilcoxon sign test: $W = 152$, $p = 0.96$). Figure S1 corroborated the consistency between storage methods, with 90% of taxa (92% when considering vertebrates only) being common across ethanol- and silica-preserved gauzes. Among the 203 taxa revealed by metabarcoding, nine were exclusive of the ethanol dataset (three of which were in common with "slush" taxa),

while 12 were found only in the silica gel data (note that seven of them were shared with "slush"-detected taxa) (Figure S1).

The comparison between metabarcoding and visual inspection of individuals in the net demonstrates the accuracy of eDNA in detecting caught species: more than 80% of caught taxa were identified by eDNA metabarcoding, for all examined groups (Figure 3). In total, 107 (50%) taxa were shared between eDNA metabarcoding and catches, while 96 (45%) were only found in eDNA metabarcoding data and 10 (5%) only in the catches. The Mantel test supported the consistency between community composition identified at each sampling site by eDNA metabarcoding and trawling data ($r = 0.64$, $p < 0.001$). Remarkably, pelagic teleosts largely prevailed over demersal ones in the taxa that were exclusively revealed by eDNA metabarcoding (65%). Conversely, demersal taxa were more abundant both in the group shared between metabarcoding and catch and in the group of species only detected by catch (Figure 3). The pelagic/demersal ratio was statistically different when comparing the "only metabarcoding" group with the group of shared taxa ($\chi^2 = 13.6$, df = 1, $p < 0.001$) or with the group of "only catches" ($\chi^2 = 26.1$, df = 1, $p < 0.001$).

The NMDS-based assemblage structure (Figure 4b) pointed out the consistency between ethanol and silica, showing a strong intra-site affinity. This was statistically supported by MRPP results, which established that intra-site dissimilarities (i.e., between replicates of the same site) were significantly lower than inter-site dissimilarities (i.e., between replicates of different sites) in terms of species composition (mean values of the Jaccard distance are 0.27 and 0.50, respectively, and $p = 0.001$) (Figure 4a). Both the two environmental variables considered and the fishing effort appeared to influence the distribution of samples across the first two NMDS axes; environmental fit results demonstrated that community changes lay along all three gradients (i.e., depth, distance, and fishing effort) (Figure 4b; Table 1). Environmental vectors showed a major influence of depth and distance from coast on sample distribution along the first NMDS axis, and a diagonal "top left to bottom right" gradient of fishing effort (Figure 4b). Further, vectors on the ordination plot enabled us to appreciate the subtle interplay between depth and distance from the coast: the slight difference in the depth and distance vectors orientation evident in Figure 4b probably reflects the fact that H1, H6, H18 are on a distant, yet shallower shoal.

Barplots in Figure 5 showed a different proportional abundance in terms of number of reads between the species caught by the net and those that were exclusive to eDNA data (the metabarcoding "bonus" taxa), with the latter having generally a lower number of reads. COI metabarcoding not only informed on the complex assemblages of cephalopods and crustaceans but also yielded a complement of 42% fish species in addition to the 12S detections (Figure S2). Interestingly, many of the most important target species for demersal catches in the central Tyrrhenian Sea (Russo et al., 2016; Tiralongo et al., 2021) were among the most abundant species in our eDNA metabarcoding data.

4 | DISCUSSION

Uncontrolled fishing activities, especially bottom trawling (Amoroso et al., 2018), are among the main causes affecting biodiversity and species distribution in marine habitats. Progress towards more sustainable marine exploitation practices depends on our ability to monitor the status of marine ecosystems and the composition and structure of communities, which are negatively affected by human impacts. Classical fisheries science approaches are still bound to traditional methods, such as direct capture and inspection of specimens, but the advent of environmental DNA metabarcoding is playing a

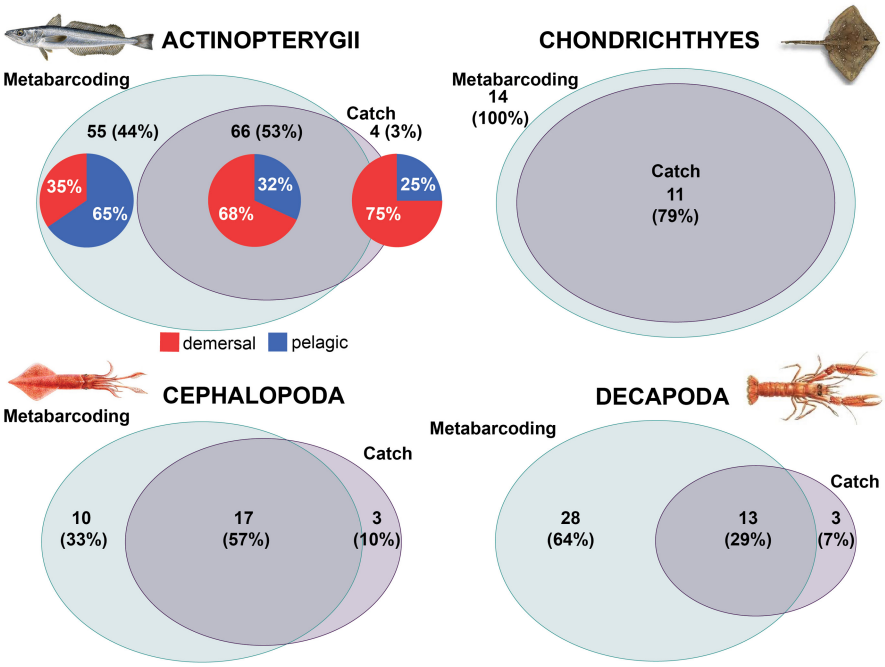

FIGURE 3 Venn diagrams of the taxa detected through eDNA metabarcoding (combining "slush" water and "metaprobe" rolls of gauze taxa detected by both 12S and COI marker) and visual inspection of catch for the four taxonomic groups considered (Actinopterygii, Chondrichthyes, Cephalopoda and Decapoda). The names of the taxa in each group are given in Table S2. Diagram areas are proportional to the number of taxa. For actinopterygians, the proportion of pelagic (in blue) over demersal (in red) taxa are indicated for each group.

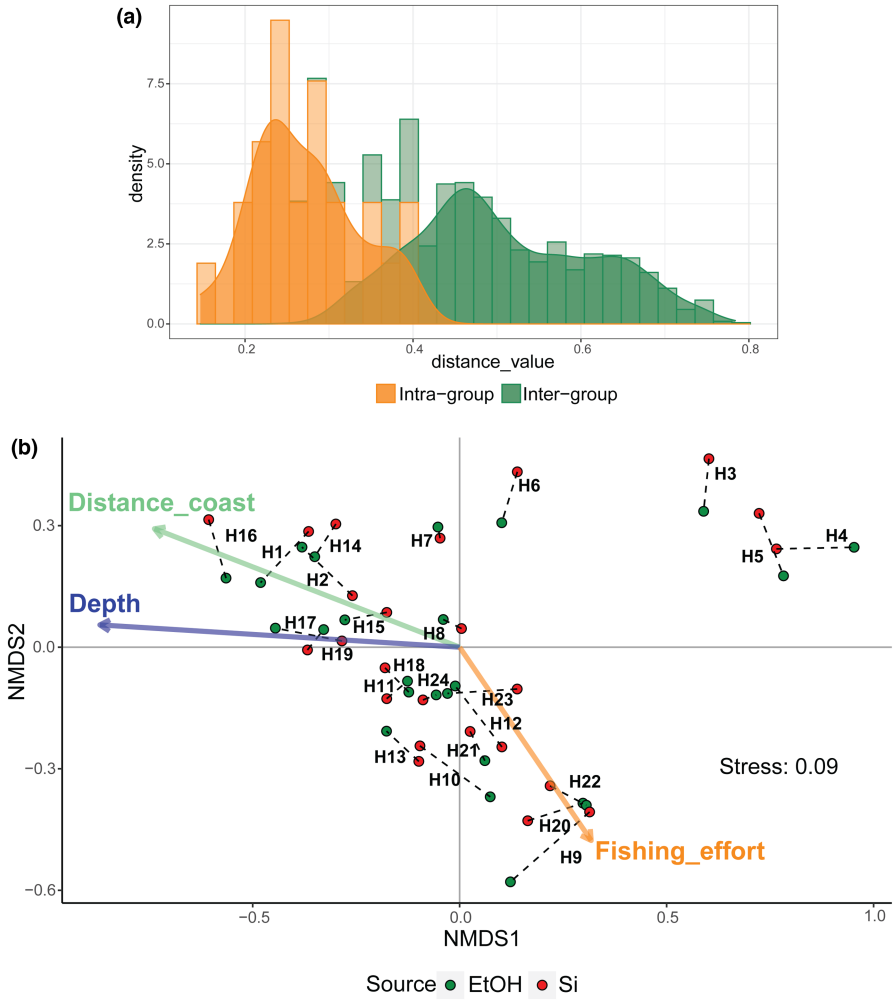

FIGURE 4 (a) Density plot representing distance values between replicates of the same sampling site (i.e. ethanol- and silica-preserved rolls of gauze) and between replicates of different sites calculated with Jaccard distance on eDNA metabarcoding data (combining species detected by 12S and COI) from "metaprobe" samples. The two density curves represent intra-site and inter-site distances, respectively. (b) Pattern of the species assemblages across the 24 sampling sites, as returned by the non-metric multidimensional scaling (NMDS) with Jaccard distance and based on eDNA metabarcoding data (combining 12S and COI) from "metaprobe" samples. Dots indicate samples, colored according to the storage method. Vectors represent gradients of the three considered variables (i.e., depth, distance from the coast and fishing effort) as returned by environmental fit on the NMDS ordination. The projections of points onto vectors is calculated in order to have maximum correlation with corresponding environmental variables.

TABLE 1 Results of the environmental fit on the NMDS carried out to explore species composition changes in the 24 sampling sites in relation to the three variable gradients considered (i.e., depth, distance from the coast, fishing effort). Values refer to Jaccard coefficients.

	NMDS1	NMDS2	r^2	Pr(>r)
Depth	−0.998	0.063	0.76	0.001
Distance_coast	−0.929	0.369	0.63	0.001
Fishing_effort	0.554	−0.832	0.33	0.001

refreshing role by eliminating stress and damage to biotas and creating opportunities for upscaling data collection (Antich et al., 2020; Gilbey et al., 2021). However, the process of collecting and storing samples still requires a complex workload (i.e., water filtration, considerable use of plastics, sterile conditions and sample freezing), which makes the integration of eDNA science with routine commercial fishing activities difficult. We recently showed that even simple, low-cost gauze rolls, encapsulated within reusable 3D-printed metaprobes, efficiently capture DNA from the surrounding water (Maiello et al., 2022). In the present study, with a broader sampling area and the use of multiple markers, we significantly strengthen earlier suggestions, by showing that these versatile low-cost methods vastly outperform previous methods, such as the collection of water from the net cod-end ("slush") (Figure 2; Figure S1). Only three taxa (i.e., *Gymnura*, *Argonauta argo* and *Munida intermedia*) were exclusively detected by "slush" water, while 53 taxa were exclusive to metaprobe samples. The three species were not caught by the fishing net and were only found in the COI dataset, at very low read numbers, likely reflecting traces of DNA in the water that led to very low templates stochastically amplified by the more universal primer set (Alberdi et al., 2018). There are inherent biases in a direct comparison between the concentrated "slush" water collected in 50 ml dripping from the cod-end, and a passive filtration approach, such as the metaprobe, which captures eDNA molecules from the moment it is deployed with the net up until its eventual haulage. Nevertheless, we specifically set out to compare these two alternative methods for integration with fishers' activities. It was important to evaluate how the metaprobe could serve its purpose compared to the previously proposed approach (Russo et al., 2021), and it was notable that its greater simplicity and implementation potential were also complemented with greater efficacy in taxon detection.

Gauze storage methods instead do not significantly affect the detection of species, as ethanol and silica proved equally suitable

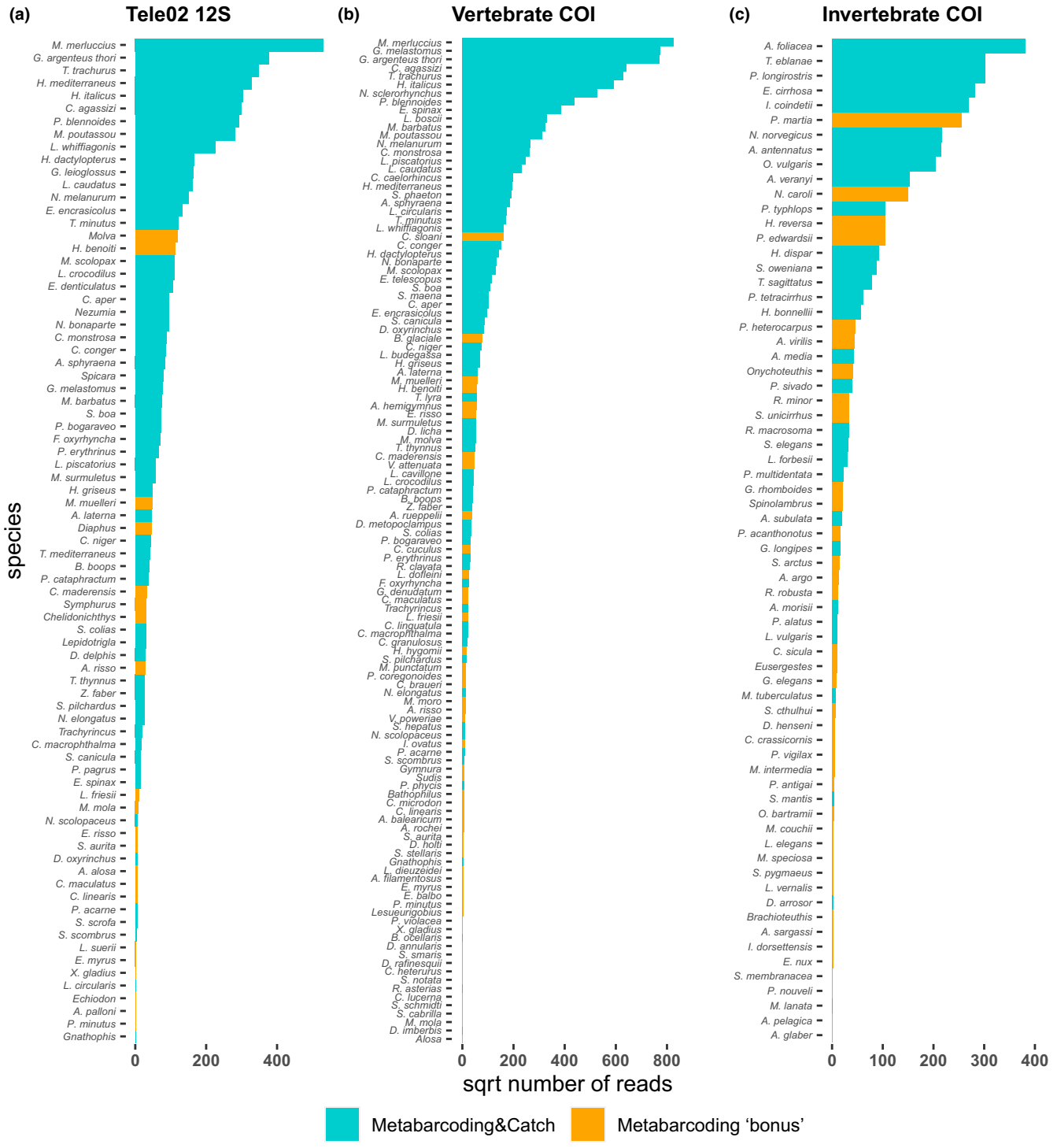

FIGURE 5 Semiquantitative composition (square root-transformed read counts) of overall taxa detected by 12S (a), vertebrate COI (b), and invertebrate COI (c) metabarcoding datasets.

for gauze preservation (Figure 2; Figure S1). Both the nine species (i.e., *Alpheus glaber*, *Auxis rochei*, *Chlorotocus crassicornis*, *Diplodus annularis*, *Mola mola*, *Scorpaena notata*, *Serranus cabrilla*, *Solenocera membranacea*, *Syngnathus schmidti*), exclusive to ethanol, and the 12 taxa (i.e., *Acanthephyra pelagica*, *Blennius ocellaris*, *Brachioteuthis*, *Callionymus maculatus*, *Ebalia nux*, *Echelus myrus*, *Macropipus tuberculatus*, *Monodaeus couchii*, *Pagurus alatus*, *Pomatoschistus minutus*, *Scyliorhinus stellaris*, *Spinolambrus*) only identified in silica gel grains data (Figure S1) had a very low number of reads. *Blennius ocellaris*, *Macropipus tuberculatus* and *Pagurus alatus* were the only ones present in the catches and *Callionymus maculatus*, *Echelus myrus*, *Mola mola* and *Pomatoschistus minutus* were the only detected by both markers, all the other non-shared species sequences were amplified only by COI. Those 21 non-shared rare

taxa mainly only amplified by the universal COI primer set, are probably the result of stochastic PCR amplification of very low templates rather than non-consistency between storage methods. Ethanol and silica can thus alternatively be used to store gauze rolls. Although we had access to an on-board freezer and could, therefore, immediately freeze our samples, both silica gel and ethanol are designed for ambient temperature storage, hence making the metaprobe a convenient solution in remote and/or logistically complex scenarios. Further investigations may be required to ascertain the impact of storage temperature on samples preserved in ethanol and/or silica gel.

Environmental DNA metabarcoding appears highly effective for catch monitoring. As shown in Figure 3, the majority of caught taxa were detected through metabarcoding: 94% for teleosts, 100% for elasmobranchs, 85% for cephalopods and 81% for decapods. Metabarcoding samples here mirrored catch composition better than the water dripping from the net cod-end in Russo et al. (2021), which already revealed a surprisingly good representation of the actual catch (71% of teleosts, 70% of elasmobranchs, 73% of cephalopods and 7% of decapods caught in the net could be identified). Furthermore, 96 species were identified by eDNA metabarcoding but not by visual inspection: this biodiversity "bonus" reflects the power of eDNA metabarcoding of detecting taxa not otherwise catchable. These can be rare and cryptic species, part of specimens (e.g., gametes, mucus, feces, regurgitates, scales), life stages (e.g., eggs and larvae) and/or too small/large taxa not catchable by bottom-trawl fishing vessels. The identification of species through the examination of external morphology can also lead to poor resolution and/or misclassification of organisms, in particular when diagnostic characters are not easy to distinguish. This is the issue with *Plesionika* shrimps in our study, which could only be visually assigned to the genus level, while eDNA was able to detect 5 different species (i.e., *Plesionika acanthonotus*, *Plesionika antigai*, *Plesionika edwardsii*, *Plesionika heterocarpus*, *Plesionika martia*). Interestingly, 65% of teleost "bonus" taxa were pelagic species, mostly bathypelagic lantern fishes (e.g., *Diaphus rafinesquii*, *Hygophum benoiti*, *Myctophum punctatum*), typical of the mesopelagic realm of the Tyrrhenian deep continental slopes. Pelagic DNA may be intercepted by the rolls of gauze in the water column while the fishing net descends towards the sea bottom or be captured by the metaprobe from pelagic DNA previously sedimented on the sea floor, upwelled during net trawling. Sediment is known to act as a temporary sink for eDNA not only for benthic organisms but also for pelagic ones: fish eDNA can even be several orders of magnitude more concentrated in sediment than in the water column (Turner et al., 2015). Realistically, the biodiversity bonus returned by the metaprobe can derive from a combination of both direct pelagic capture and sediment re-suspension, and further studies should be carried out to explore the extent to which these mechanisms shape metabarcoding results in different habitats. As expected, demersal taxa instead prevailed in the groups of organisms caught by the fishing net: bottom-trawl fishing vessels target demersal species that live close to the bottom of the oceans (van Denderen et al., 2013). By extending the area and the number of sampling sites and by using a combination of two barcodes, the number of caught species not identified by eDNA metabarcoding drastically decreased. The species accumulation curves in Figure S3 (A and B) show the importance of an extended number of sampling locations for the recovery of the whole biodiversity for both barcodes (i.e., Tele02 12S and COI) and for all the considered taxa (i.e., Actinopterygii, Chondrichthyes, Cephalopoda and Decapoda).

Only four of the vertebrate species detected (3%) were exclusive to trawl catches here, compared to 13 taxa (21%) in Maiello et al. (2022), where only the Tele02 12S barcode was amplified, from just three sites. The 12S metabarcode is known to have a lower taxonomic resolution (Collins et al., 2019) and less complete reference databases; the use of COI—otherwise plagued by rife non-specific amplification in aqueous eDNA studies with naturally diluted water samples (Collins et al., 2019)—clearly shows advantages in the specific context of the high DNA concentration conditions found in the trawl net. The combination of more concentrated templates and better bioinformatic resolution (greater taxonomic resolution and coverage on reference libraries) make COI useful in metaprobe-in-the-trawl applications. Indeed, the COI barcode was able to detect 57 more vertebrate taxa than the 12S (Figure S2), among those, some (i.e., *Chelidonichthys cuculus* and *C. lucerna*, *Diaphus holti*, *D. metopoclampus* and *D. rafinesquii*, *Lepidotrigla cavillone* and *L. dieuzeidei*, *Molva molva*, *Nezumia sclerorhynchus*, *Spicara maena* and *S. smaris*) were species that the 12S barcode was able to only assign to the genus level. In general, balancing between specific and non-specific amplification, greater and lower taxonomic resolution, and database representativeness, our findings support the idea that the use of a combination of barcodes could enhance eDNA metabarcoding's power of detecting species, especially in high-biodiversity environments (McElroy et al., 2020).

Despite the moderate geographic extent of the investigated area, the overall qualitative β-diversity distribution robustly discriminates between the 24 hauls and reflects patterns of community structure that fit with the expected influence of environmental variables, such as depth and distance from coast, and the anthropogenic impact, expressed by fishing effort (Russo et al., 2019). The main driver of fishing effort differences in the considered region stands in the fact the further deep sites (such as H1, H2, H6, H14, H16, H18 and H19 in our study) are reached by trawlers only during spring and summer when the weather is favorable and deep target species (e.g., *Merluccius merluccius*, *Aristaeomorpha foliacea*, *Nephrops norvegicus*) are more abundant; their fishing effort in terms of total hours of trawling per year is thus minor compared to closer to the coast sites reached all year long by trawlers. In this way, the approach presented in this paper seems to be promising in terms of sensitivity to fisheries-induced alteration of the marine community.

The semiquantitative composition of our eDNA metabarcoding data was consistent with expectations. The possibility of inferring semiquantitative estimates of species from (transformed)

number of reads has already been extensively investigated (Clark et al., 2020; Postaire et al., 2020; Zou et al., 2020). Despite skepticism, several studies found a strong correlation between species occurrence and read abundance in various environmental contexts, ranging from anadromous threatened species estimation (Shelton et al., 2019), to seasonal fish abundance patterns (Milhau et al., 2021; Stoeckle et al., 2017), shark movements (Mariani et al., 2021; Postaire et al., 2020), and invertebrate diversity (Clark et al., 2020; Nguyen et al., 2020). In a more strictly fishery-focused context, a significant association between biomass and number of species caught by the net and the (transformed) number of sequences reads has already been demonstrated (Russo et al., 2021; Stoeckle et al., 2021). Here, we found a distribution of species read abundances consistent with previous knowledge on demersal fish assemblages in the Mediterranean Sea (Russo et al., 2019). Many of the species most frequently found in landings of bottom trawlers in the central Tyrrhenian Sea (Russo et al., 2016; Tiralongo et al., 2021) were among the most abundant in terms of number of reads in our eDNA metabarcoding data; the European hake (*Merluccius merluccius*), was the most represented species in both COI and 12S data (Figure 5a,b). Concerning invertebrates, the giant red shrimp (*Aristaeomorpha foliacea*), the deep water rose shrimp (*Parapenaeus longirostris*), the Norway lobster (*Nephrops norvegicus*) and the blue and red shrimp (*Aristeus antennatus*), key target species of trawlers during spring and summer in the central Tyrrhenian Sea, were all in the 10 top species of Figure 5c. The blackmouth catshark (*Galeus melastomus*), the primary bycatch species in the Mediterranean Sea (Bradai et al., 2012), was among the most abundant species in both the 12S and the COI datasets, coherently with the available knowledge for the area of study (Sbrana et al., 2022). Interestingly, we found differences in the read abundance distribution between the species that were caught in the net and the "bonus" taxa only detected by eDNA metabarcoding, with the former being proportionally more represented than the latter ones (Figure 5). This aspect is particularly relevant for future broader use of the metaprobe aboard commercial fishing vessels without the requirement of research scientists on board. In the future, as more data become available, it may be possible to develop probabilistic models able to identify read abundance thresholds that can discriminate between the species caught in the net, and those representing the metabarcoding "bonus". Such methods could altogether remove the need for ground-truthing eDNA metabarcoding inference with the visual inspection of the catch.

The metaprobe approach embodies many of the desirable features required for upscaling data collection for ocean monitoring, with a particular emphasis on fishing activities (Russo et al., 2021; Stoeckle et al., 2021). The gauze rolls closely mirror catch composition and produce exhaustive species inventories for the marine ecosystems that sustain trawling activities. Furthermore, compared to the logistic constraints associated with standard water sampling, this novel approach is quicker, simpler, inexpensive, environmentally friendly, and robust to contamination. The prospect of availing of a nimble eDNA-sampling device that can be easily operated by the fishermen themselves, without the disruption of fishing activities, makes the metaprobe an ideal candidate for future extensive applications on commercial trawlers for fisheries and ocean biodiversity monitoring. If this vision is also embraced by the fishing community at sea, it could rapidly increase the amount and granularity of marine biodiversity data, boosting our capacity for monitoring and understanding changes in the distribution of species across the sea, in a cooperative, transdisciplinary context.

AUTHOR CONTRIBUTIONS

GM, SM, TR, and LT conceived the idea. PC, GM, TR, AS, and LT collected the samples. CB, GM, PS, and LT carried out laboratory analyses. GM led bioinformatic and ecological analyses with help from SM, TR, PS, and LT. All authors discussed and interpreted the results. GM drafted the manuscript, with contributions from SM and TR. All authors approved the final version of the manuscript.

ACKNOWLEDGMENTS

We would like to thank PhD Lorenzo D'Andrea for helping with 3D printing, and Federico Di Maio for his advice on fish morphological identification.

FUNDING INFORMATION

The present study was supported by the Food and Agriculture Organization of the United Nations ("FAO") - General Fisheries Commission for the Mediterranean (GFCM) within the project MTF /INT/943/MUL - Baby 32 - "Select Activities of the strategies towards the sustainability of fisheries and sustainable aquaculture development implemented in the Mediterranean and the Black Sea", in agreement with the Department of Biology of the University of Rome Tor Vergata and by the UK Natural Environment Research Council ["SpongeDNA" grant NE/T007028/1].

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

DATA AVAILABILITY STATEMENT

Raw data are archived in the BioProject PRJNA911173.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Giulia, M., Lorenzo, T., Chris, B., Paolo, C., Alice, S., Peter, S., Stefano, M., & Tommaso, R. (2023). Net gain: Low-cost, trawl-associated eDNA samplers upscale ecological assessment of marine demersal communities. *Environmental DNA*, *00*, 1–13. https://doi.org/10.1002/edn3.389