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Little samplers, big fleet: eDNA metabarcoding from commercial trawlers enhances ocean monitoring

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Abstract

The global need to monitor the status of marine resources is a priority task in marine management, but most ocean surveys still rely on costly and time-consuming capture-based techniques. Here we test a novel, easy-to use device to collect eDNA on board of bottom trawl fishing vessels, during normal fishing operations, quickly and easily: custom-made rolls of gauze tied to a hollow perforated spherical probe (the ‘metaprobe’) that placed inside the fishing net aims to gather traces of genetic material from the surrounding environment. We collected six samples from three central Tyrrhenian sites. Using an established fish-specific metabarcoding marker, we recovered over 70% of the caught species and accurately reconstructed fish assemblages typical of the different bathymetric layers considered. eDNA metabarcoding data also returned a biodiversity ‘bonus’ of mostly mesopelagic species, not catchable by bottom trawls. Further investigation is needed to upscale this promising approach as a powerful tool to monitor catch composition, assess the distribution of stocks, and generally record changes in fish communities across the oceans.

Keywords

Marine biodiversity; fish communities; fisheries; Mediterranean Sea; environmental DNA

1. Introduction

It is widely accepted that human activities and climate changes triggered by them are contributing substantially to the loss of biodiversity worldwide (Bálint et al., 2011). In marine ecosystems, one of the main causes affecting biodiversity and species distribution is unsustainable fishing activity, which has caused a steep decline of wild populations at a global scale. While several management policies are being implemented to reverse stock depletions, there is an increasing need to move towards more sustainable fishing practices, by refining assessment methods, improving habitat protection spatially and temporally, reducing bycatch, and strengthening enforcement (FAO, 2020). Long-term sustainability of fishery resources ultimately depends on ecosystem health and the availability of powerful and accurate monitoring tools that can rapidly assess the effects of human activities on the oceans (Bradley et al., 2019).

Data collection from the oceans represents a major logistic and financial challenge, hindering our understanding of the spatial distribution of stocks, species, and key habitats. To maximise knowledge acquisition, marine research also relies on fisheries-dependent information, which is still largely based on traditional approaches, such as logbook data, visual inspection and sorting of species. These are usually performed by fisheries observers or the fishers themselves and, given that they require time, are consequently limited to subsets of the fleet, compromising the accuracy and representativeness of the results (Vilas et al., 2019). Promisingly, technological innovations are offering solutions to update and modernize fishery data collection (Bradley et al., 2019; Plet-Hansen et al., 2019). Among these, environmental DNA (eDNA) metabarcoding is bound to establish itself as a primary source of biodiversity information in every habitat (Sigsgaard et al., 2020; Thomsen and Willerslev, 2015; West et al., 2020); yet, collecting and concentrating DNA from large water volumes can be challenging, especially in open seas and/or deep waters. To circumvent these limitations, various sampling solutions have been proposed so far, which include automated underwater high-tech vehicles and robots (Hansen et al., 2020; McQuillan and Robidart, 2017), passive and artificial collectors (Bessey et al., 2021; Verdier et al., 2021), natural samplers (Mariani et al., 2019), and even marine litter (Ibabe et al., 2020). These molecular detection approaches, however, have not found a significant place in the context of monitoring fisheries activities. Building on recent evidence demonstrating that community composition inferred from eDNA metabarcoding of water draining from the net cod end largely matched with those retrieved by visual sorting of the catches (Russo et al., 2021), we devised an improved solution to leverage commercial trawling activities. We designed a bespoke, low-cost, 3D-printed plastic probe that, placed inside the trawl net, works as a container for rolls of gauze that are poised to capture DNA from the surrounding environment during fishing operations. We compared eDNA metabarcoding results from the probe with catch compositions from trawl hauls spanning depths of 600m along the continental slope in the central Mediterranean Sea. Results strengthen the idea that eDNA-based biomonitoring can become embedded in fishery-dependent surveys, at negligible additional cost and effort, to study catch composition and the broader faunal features of the ecosystems that sustain commercial fishing.

2. Material and Methods

2.1 Collection of samples

Samples were collected between July and August 2020 from three sites in the central Tyrrhenian Sea (FAO Geographical Sub Area 9 – Western Mediterranean Sea) (Fig. 1A), on board of a commercial bottom trawl fishing vessel. Sampling locations covered two bathymetric layers: two deep slope hauls (H1 and H8), with average depths of ~ 600m, and a shelf edge haul (H4), operating at ~130 m.

For the collection of DNA, we realized a bespoke 3D-printed hollow perforated plastic spherical probe (radium 8 cm), hereafter termed ‘metaprobe’. We built two custom-made rolls of gauze, rolling 1g of pharmacy sterilized cotton in 3 10x10cm sterile gauzes compresses (mesh-size: 1mm). Gauze rolls were tightly fixed by plastic cables tied inside the ‘metaprobe’ and dropped inside the fishing net at the beginning of each haul (Fig. 1B). At the end of fishing operations, during the sorting of catches, the ‘metaprobe’ was retrieved, and the rolls of gauze were gathered and placed in separate 50ml sterile tubes containing 99% ethanol and silica gel grains, respectively. Both were frozen on board then stored in the laboratory at -20°C until DNA extraction. At the same time, we determined the qualitative species composition of each haul. Referring to dichotomic keys and identification guides, individuals in the net were identified at the species or genus level by visual inspection of external morphology.

2.2 Laboratory procedures

DNA extraction was performed following an extraction protocol for the recovery of extremely low concentration fragmented DNA (Malmström et al., 2009) in a high containment room, specifically designed for the management of small copy number DNA such as ancient DNA (De Angelis et al., 2021). Ethanol preserved gauzes were blotted to dry before DNA extraction procedures. Half of each gauze roll was cut into small pieces and then soaked in 400 µl of extraction buffer (0.5 M EDTA pH 8, 1 M Urea) with 20 µl of proteinase K (100 µg/ml). Samples were incubated at 37°C for at least 8 h and centrifuged at 4000 rpm to separate lysate from the residual sediment particles. The supernatant was transferred to an Amicon ultra-4 30K centrifugal device to concentrate DNA. Subsequently, around 150 µl of solution was transferred into QIAQuick Spin Columns and the DNA was purified by the QIAQuick PCR Purification Kit (Qiagen). Two negative extraction controls (i.e. 400 µl of extraction buffer with 20 µl of proteinase K), one for each storage method, were included to account for possible contamination linked with extraction procedures.

eDNA metabarcoding was performed using the fish-specific Tele02 primers which target a ~167 bp fragment of the mitochondrial 12S ribosomal RNA gene and achieved >98% teleost species detection when tested *in silico* (Taberlet et al., 2018). To ease sample demultiplexing and mitigate cross-contamination and/or tag switching during sequencing, each sample was PCR amplified using the locus primers attached to a unique 8 bp tag shared by the forward and reverse primer. Each tag differed by at least three base pairs from other tags and included 2-4 degenerate bases (Ns) at the beginning of the tag sequence to improve clustering during initial sequencing. To monitor for possible contamination sources, both a positive and a negative control were amplified along with all the other samples.

Each sample was PCR amplified in triplicate using 20 µl reactions consisting of 10 µl MyFi™ Mix (Meridian Bioscience), 1 µl of each forward and reverse primer (10 µM, Eurofins), 0.16 µl of Bovine Serum Albumin (20 mg/ml, Thermo Scientific), 5.84 µl of UltraPure™ Distilled Water (Invitrogen), and 2 µl of template DNA. PCR was performed on a T100 Thermal Cycler (Bio-Rad Laboratories Ltd) with the following profile: 95°C for 10 mins, 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 mins. PCR products were stored at 4°C until replicates for each sample were pooled, and 1 µl of pooled PCR product was added to 1 µl of Gel Loading Buffer (Invitrogen) for visualisation on 2% agarose gels to ensure the amplification of the target fragment stained with SYBRsafe (Invitrogen), which were imaged using Image Lab Software (Bio-Rad Laboratories Ltd). PCR products were stored at -20°C until they were purified with Mag-Bind® TotalPure NGS magnetic beads (Omega Bio-tek Inc), following the double size selection protocol established by Bronner et al., 2009. Ratios of 1x and 0.6x magnetic beads to 30 µl of PCR product were used. Eluted DNA (20 µl) was stored at -20 °C until quantification using a Qubit™ 4.0 fluorometer with a Qubit™ dsDNA HS Assay Kit (Invitrogen). Samples (*N*=8) were normalised and pooled in equimolar concentration alongside samples for another project (total *N* = 121).

Sample pools were purified using the aforementioned ratios and elution in 25 µl, then concentrated using a 1x ratio and elution in 45 µl. End repair, adapter ligation and PCR were performed using the NEXTFLEX® Rapid DNA-Seq Kit 2.0 for Illumina® Platforms (PerkinElmer) according to the manufacturer's protocol. An Agilent 2200 TapeStation and High Sensitivity D1000 ScreenTape (Agilent Technologies) indicated secondary product (e.g. primer dimers) remained, thus gel extraction was performed on each pool using the GeneJET Gel Extraction Kit (Thermo Scientific) with elution in 20 µl. Each pool was quantified using quantitative PCR (qPCR) on a Rotor-Gene Q (Qiagen) with the NEBNext® Library Quant Kit for Illumina® (New England Biolabs), diluted to 1 nM, and 6 µl of each pool combined into one library. The final library and PhiX Control were quantified using qPCR before the library was sequenced at 60 pM with 10% PhiX Control on an Illumina® iSeq™ 100 using iSeq™ 100 i1 Reagent v2 (300-cycle) (Illumina Inc.).

2.3 Bioinformatics: data pre-processing and taxonomic identification

Bioinformatic procedures followed the OBITOOLS pipeline (Boyer et al., 2016). We first used FASTQC to assess read quality and ILLUMINAPAIREDEND to merge paired reads with a quality score >40. Samples were demultiplexed based on their unique tags via NGSFILTER, allowing for a single base mismatch in each tag sequence. We then used OBIGREP to length-filter sequences according to the expected range (129–209 bp) and to eliminate singletons, and OBIUNIQ to dereplicate sequences. We removed chimaeras with UCHIME (Edgar et al., 2011) and clustered the Molecular Operational Taxonomic Units (MOTU) using SWARM (Mahé et al., 2015) setting a clustering threshold at d = 3. We assigned taxonomy with ECOTAG against a custom-made 12S vertebrate reference database of 26,387 sequences obtained through *in silico* PCR (as implemented with ECOPCR) from the overall EMBL vertebrate database (release 143).

Finally, we validated the taxonomic assignment and filtered out potential residual artefact (Clarke et al., 2014) through three steps: (1) ambiguous (e.g. non-Mediterranean taxa) and poorly resolved MOTUs (i.e. MOTUs that couldn't be unambiguously assigned to a genus or species level) were manually BLASTed against the NCBI database to double check and validate the taxonomic assignment; (2) only sequences showing >98% identity match were retained; (3) potential contamination noise was removed taking advantages of negative controls with the DECONTAM package in R (Davis et al., 2018), using the prevalence method with a threshold of 0.5.

2.4 Data analysis and visualization

Venn diagrams were used to compare the fish assemblages retrieved from eDNA metabarcoding of samples stored in ethanol versus silica gel grains and to inspect the influence of preservation method. We built a second Venn diagram to qualitatively compare the overall composition of taxa detected by eDNA metabarcoding (combining data obtained with silica gel and ethanol preservation) and catch data. Species detections were visualised using colouring to distinguish between pelagic and demersal taxa (Froese and Pauly, 2017), as we expected eDNA detections to include an excess of pelagic taxa not detected in the trawl due to the journey of the sampling probe across the pelagic realm as the net was deployed and subsequently hauled back. Both Venn diagrams were calculated and drawn using the VENNDIAGRAM package in R (Chen and Boutros, 2011).

To assess and visualize qualitative differences in taxon composition among sampling sites and sources (i.e. visual identification and eDNA metabarcoding), we implemented a nonmetric multidimensional scaling (NMDS) based on Jaccard distances on a binary presence-absence dataset including all taxa identified to genus or species level, detected by metabarcoding and catch data respectively. We tested differences among sampling sites with a PERMANOVA test (1,000 permutations, function 'adonis') in the R package VEGAN (Oksanen et al., 2018).

For metabarcoding data only, we evaluated the abundance pattern of detected taxa across sampling sites, by visualizing the fourth root of the number of reads (i.e. a putative proxy for relative eDNA abundance and, in turn, biomass (Clark et al., 2020; Russo et al., 2021; Stoeckle et al., 2021, Mariani et al., 2021)), as a heatmap.

3. Results

After bioinformatic analysis, the eight samples (two per each of the three sampling stations, plus two blanks) yielded 241,673 reads (mean per sample = 37,164 ± 7,955 SE), which allowed the detection of 63 taxa: 58 teleosts and 5 elasmobranchs. Of these, 55 taxa (50 teleosts and 5 elasmobranchs) passed the first filtering step in which we retained only sequences showing >98% of identity match. The analysis of negative blanks using the DECONTAM algorithm detected five Tyrrenhian taxa as possible contaminants among samples – *Helicolenus dactylopterus*, *Micromesistius poutassou*, *Molva* sp, *Scomber scombrus,* and *Zeus faber* – which were conservatively removed from the final dataset. We further removed two more taxa (Argentinidae and Triglidae) because they could only unambiguously be assigned to the family rank.

All but two taxa were shared between silica gel and ethanol-preserved samples (Fig. S1), hence suggesting consistency between preservation methods.

The comparison between metabarcoding and visual inspection revealed 34 taxa (56%) found by both methods; 14 (23%) were recovered only by eDNA metabarcoding and 13 (21%) only by catches (Fig. 2). Interestingly, pelagic taxa prevailed in the group of taxa that were exclusive to metabarcoding (57%), compared to the two other groups (29% pelagic taxa shared between catch and metabarcoding; 8% in catch only), even though with no statistical support (Fisher's exact test: p = 0.10 for shared taxa, p= 0.25 for only catch taxa), likely because of the small sample size.

The NMDS analysis showed an intra-site affinity and a coherent bathymetric distribution: samples were differentiated according to their sampling origin, and the 600m deep slope (H1 and H8) were clearly separated from the 130m shelf edge (H4) along the first NMDS axis (Fig. 3). This was supported by PERMANOVA results where differences between sampling sites accounted for 68.6% of the variance ($p = 0.001$).

As expected, the heatmap (Fig. 4; Table S2) depicted some differences between deep (H1 and H8) and shallow (H4) sites with respect to both taxa occurrence and their putative abundance (read counts).

4. Discussion

The progress of our society towards a sustainable use of resources depends on our ability to monitor the conditions of marine ecosystems, with an emphasis on species and habitats that are most affected by human activities. Effective large-scale monitoring is also essential to direct our management strategies in a timely manner. This is especially true for trawl fisheries, which are the most impacting fishing activities worldwide (Amoroso et al., 2018). Environmental DNA metabarcoding is expected to play an increasingly substantial role in this research field, but the process of sampling and storing samples remains a challenge, as upscaling through fishery-dependent surveys necessitates the avoidance of complex workload during fishing operations. The use of passively filtering membranes (i.e. positively charged nylon and non-charged cellulose ester) for eDNA collection from the water has already been successfully tested: Bessey et al., 2021 showed that passive filters gather DNA as effectively as active eDNA filtration. Our results demonstrate that simple and low-cost rolls of gauze encapsulated in hollow perforated spherical probes that are passively dragged within a trawl net, efficiently collect DNA from the water. Despite their non-specificity that could lead to a lower binding affinity for eDNA fragments compared with the passive filtering membranes, the gauze rolls afford a level of operational simplicity and robustness that neither capsule nor passive filters could guarantee in a context of the jolts and instability of commercial trawling operations.

Both tested storage methods appeared suitable for DNA preservation until extraction in the laboratory and can thus be conveniently employed to temporarily store collected samples on board. In fact, only two species (*Crystallogobius linearis* and *Lesueurigobius suerii*) were not shared by silica and ethanol datasets (Fig. S1). Both species were not caught by the fishing net and showed a very low number of reads. This suggests that they likely reflect traces of DNA in the surrounding environment that led to very rare templates being stochastically amplified in PCR (Alberdi et al., 2018).

By applying a standard eDNA metabarcoding pipeline and filtering procedure, we were able to molecularly identify 50 taxa based on their 12S barcode, 39 to species level, 9 to genus level, and 2 to family level. Of the five taxa conservatively removed by the DECONTAM algorithm, some (i.e. *Helicolenus dactylopterus* and *Micromesistius poutassou*) are demersal species, common in the examined sampling area, which were also detected in the catch – their presence in negative controls could be due, for instance, to cross-contaminations during extraction and/or amplification and tag jumping. Their exclusion was due to their proportional read count in the negative controls. A more extensive sampling effort in the future would allow a more nuanced spatial analysis of these detections.

Our results also lend further support to the notion that eDNA metabarcoding, once optimized, can be efficiently used as a monitoring technique for the composition of catches. Figure 2 shows that the DNA retrieved from the probe is a good proxy of catch assemblages, mirroring 72% of the composition of commercial fishing catch, consistent with recent results obtained analysing water dripping from the trawl net cod end (Russo et al., 2021). Here we show that this process can be vastly streamlined by the use of low-cost, resistant, passive sampling probes, which are robust to variation in preservation methods and do not significantly disrupt fishing operations.

Additionally, 14 species detected by the metabarcoding probe and not by the visual inspection of the catch likely represent a biodiversity ‘bonus’ that reflects the power of metabarcoding in detecting taxa that are not otherwise catchable (Nguyen et al., 2020). These can be rare and cryptic species, part of specimens (e.g. gamete, mucus, faeces, regurgitates, tissue scraps), or life stages (e.g. larvae) and/or too small/large taxa that are not catchable by bottom-trawl fishing vessels. Remarkably, we recovered a substantial fraction (57.1%) of meso-and bathypelagic species within taxa only detected by eDNA metabarcoding (Fig. 2). This is apparently counterintuitive since bottom-trawl fisheries target mostly demersal species that live close to the sea bottom (van Denderen et al., 2013). However, a possible explanation is that the metabarcoding probe can capture DNA even from the pelagic domain. Specifically, gauze may have intercepted the DNA of pelagic species while the fishing net was descending towards the sea bottom. Additionally, DNA from pelagic species sedimented on the bottom floor may have been upwelled during net trawling and captured by the metaprobe. In any case, further exploration on the time course of gauze binding capacity will be needed before this method can be routinely applied. For instance, a logical extension would be investigating the relationship between the number of pelagic taxa recovered and the time the net takes to get to the sea bottom.

On the other hand, 13 species were detected by visual inspection but not through eDNA metabarcoding; though, considering that three of the five species removed after our conservative decontamination procedure were found also in the catches, only 10 species were exclusive to the catch data. There are various non-mutually exclusive explanations for non-detection by eDNA metabarcoding. First, the incompleteness of reference databases may artifactually reduce the ability to detect some taxa, a well-known challenge in eDNA metabarcoding (Weigand et al., 2019). Second, the 12S metabarcode has been proven to deliver lower taxonomic resolution in some cases (Collins et al., 2019), as exemplified by the Argentiniformes in our data. In the catches, two Argentinid species (*Argentina sphyraena* and *Glossanodon leioglossus*) were retrieved, while in the eDNA metabarcoding dataset neither *A. sphyraena* nor *G. leioglossus* could be reliably distinguished for sequence identity >98%, and hence they were pooled in the family Argentinidae. In support of this explanation, the degree of mismatch between catch and metabarcoding assemblages decreased at a lower taxonomic resolution (i.e., at the family level; Fig. S2).

Despite the low number of sampling sites, the DNA in the ‘metaprobes’ well represented the alpha- and beta-diversity of the considered area both qualitatively and quantitatively (Figs. 3-4). The possibility of employing (transformed) read counts obtained from eDNA metabarcoding as a measure of taxa abundances is still debated (Deiner et al., 2017), yet several studies have demonstrated a strong correlation between frequencies of occurrence and read abundance, pointing out the use of DNA abundance as a valuable proxy for relative proportion of taxa among sampling sites (Mariani et al., 2021; Postaire et al., 2020; Shelton et al., 2019). Concerning eDNA metabarcoding applied to fisheries, Stoeckle et al. (2021) and Russo et al. (2021) revealed a robust association between the number of sequence reads and species abundance in the catch as expressed by biomass and number of individuals. Here we found a distribution of the most abundant species among sampling sites generally coherent to expectations (Fig. 4). For instance, the European hake (*Merluccius merluccius*), one of the key target species for demersal fisheries in the Mediterranean Sea (Russo et al., 2019), appeared amongst the most abundant species in all the hauls according to sequence read data. Similarly, other detected species showed a clear affinity with the corresponding depth layer: the shelf edge haul (H4) was dominated by the typical target species of this bathymetric level (e.g. *Trachurus trachurus* and *T. mediterraneus*, *Pagellus erythrinus*, *Spicara* spp., and *Mullus barbatus*). Conversely, the two deep slope hauls (H1 and H8) mostly shared the more abundant taxa that are typical deep-sea fish species (e.g., *Phycis blennoides*, *Gadiculus argenteus*, *Lepidorhombus whiffiagonis*, and *Hymenocephalus italicus*).

The approach illustrated herein embodies many of the features that the marine management community anticipates as essential to upscale ocean monitoring. First, the ‘metaprobe’ method appears to better mirror the catch contents: the proportion of taxa detected by both 12S metabarcoding and catches was 55.74% for ‘metaprobes’ and 23.3% for the previous approach based on net ‘slush’ by Russo et al. (2021), who also used COI metabarcoding to improve taxon detection. Second, the ‘metaprobe’ is a simpler, cheaper, and faster way of collecting eDNA samples, even by non-technical operators, as compared to all other methods proposed to date (Bessey et al., 2021, Stoeckle et al., 2021; Verdier et al., 2021). Third, the possibility to preserve rolls of gauze both in ethanol and silica gel makes them a convenient solution for sample storage and shipping, compared to the logistic constraints associated with freezing water collections aboard fishing vessels. All these advantages are valuable prerequisites for the future application of this technique on commercial fishing vessels, where the ‘metaprobe’ may establish itself as a complementary tool for stock assessment and ocean biodiversity monitoring. As a last point, it is worth noting that most trawlers (i.e. vessels > 15 m) operating worldwide are equipped with remote tracking devices such as the Vessel Monitoring System (VMS) and/or the Automatic Information System (AIS) (Amoroso et al., 2018; Russo et al., 2019). This offers the opportunity to reconstruct, with great spatial and temporal accuracy, the origin of landings and/or catches (Russo et al., 2018). It is therefore easy to foresee how the information provided by the 'metaprobe' could be integrated with the fishing footprint of trawlers to obtain powerful reconstruction of marine biodiversity at different scales. From changes in catches to whole-community composition patterns, including range shifts and invasive species detection, marine management could soon leverage these nimble, low-effort innovations to obtain a more comprehensive understanding of the distribution of species across the oceans, at speeds and scales that have so far been perceived as unfeasible. The opportunity to associate these novel methods with the activities of the fishers also provides a blueprint for a future where ocean stewardship is increasingly reflective of more inclusive engagement across all sectors that depend on ocean health.

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Figure captions

Figure 1 - Sampling with ‘metaprobe’ rolls of gauze: (A) Map identifying the three sampling sites in the central Tyrrhenian Sea (Geographic Sub Area 9); the bottom-left map indicate the geographic position of the study area (B) From left to right: model of the hollow perforated plastic spherical probe (the ‘metaprobe’) with rolls of gauze beside and inside it; loaded ‘metaprobe’ in the fishing net; rolls of gauze in the 50ml falcon tubes stored in 99% ethanol and silica gel grains; half roll of gauze on a petri dish prior to DNA extraction.

Figure 2 - Venn diagram of the taxa detected through eDNA metabarcoding of 'metaprobe' and visual inspection of catch. In blue are pelagic taxa, and in red are demersal taxa. See Table S1 for the names of taxa denoted by numbers. Diagram areas are proportional to the number of taxa.

Figure 3 - Pattern of species composition of the three sampling sites from visual sorting of catches and eDNA metabarcoding of 'metaprobe', as returned by the nonmetric multidimensional scaling (NMDS) based on Jaccard distance. The colours of the sites reflect their bathymetric layer: dark blue for the deep slope hauls (H1 and H8), and light blue for the shelf edge haul (H4). The fish drawn represent typical species from each sampling site. From left to right and from the top to the bottom: *Chimaera monstrosa*, *Hymenocephalus italicus*, *Lepidorhombus whiffiagonis*, *Gadiculus argenteus*, *Mullus barbatus*, *Trachurus trachurus*.

Figure 4 - Quantitative composition of taxa in terms of read counts for each sampling site, depicted by a heatmap representing the fourth-root of the number of reads of the overall taxa detected in the metabarcoding dataset.

Figure 1 - sampling

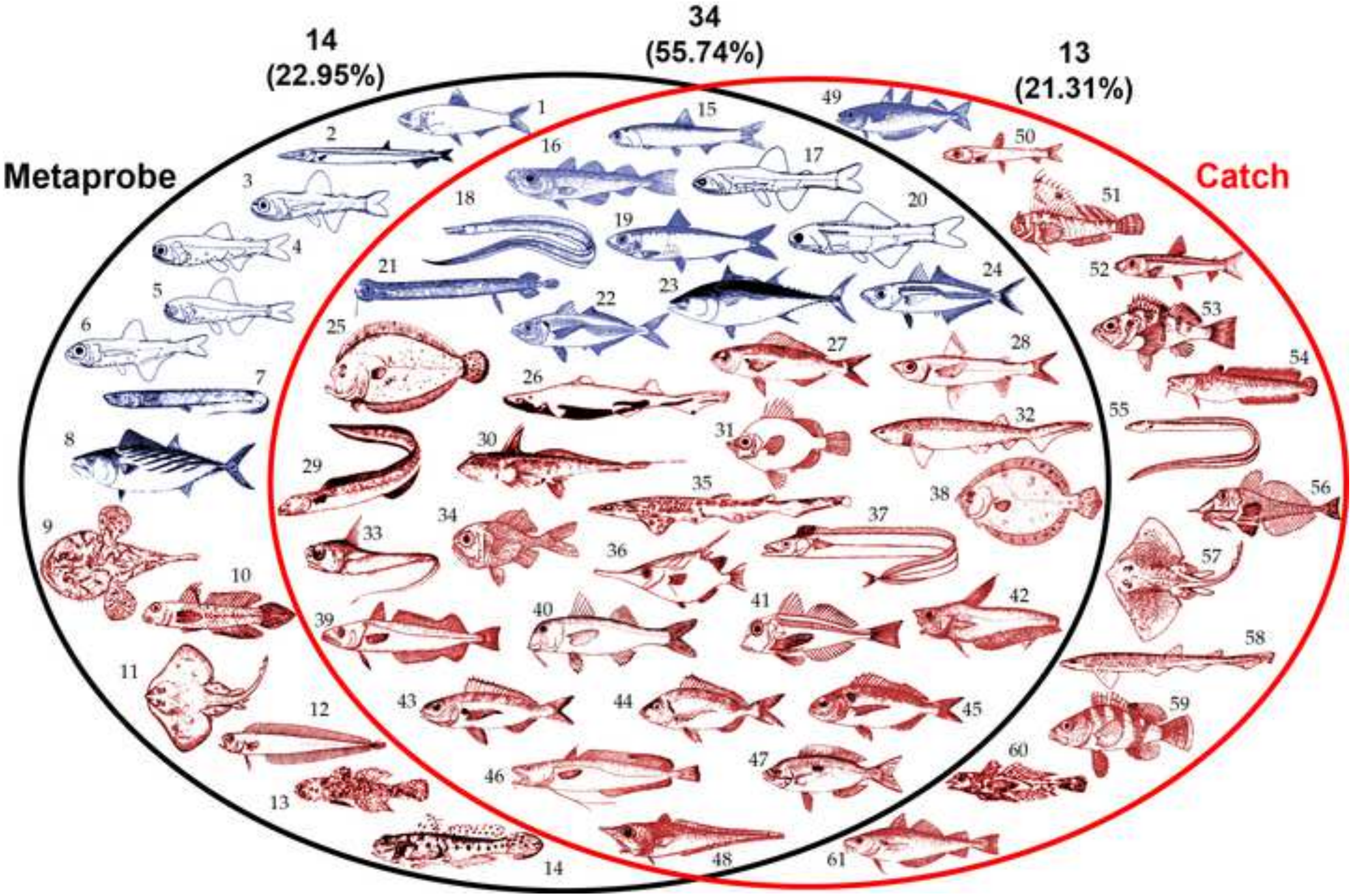
14
(22.95%)
34
(55.74%)
13
(21.31%)
Metaprobe
Catch
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Figure 3 - NMDS

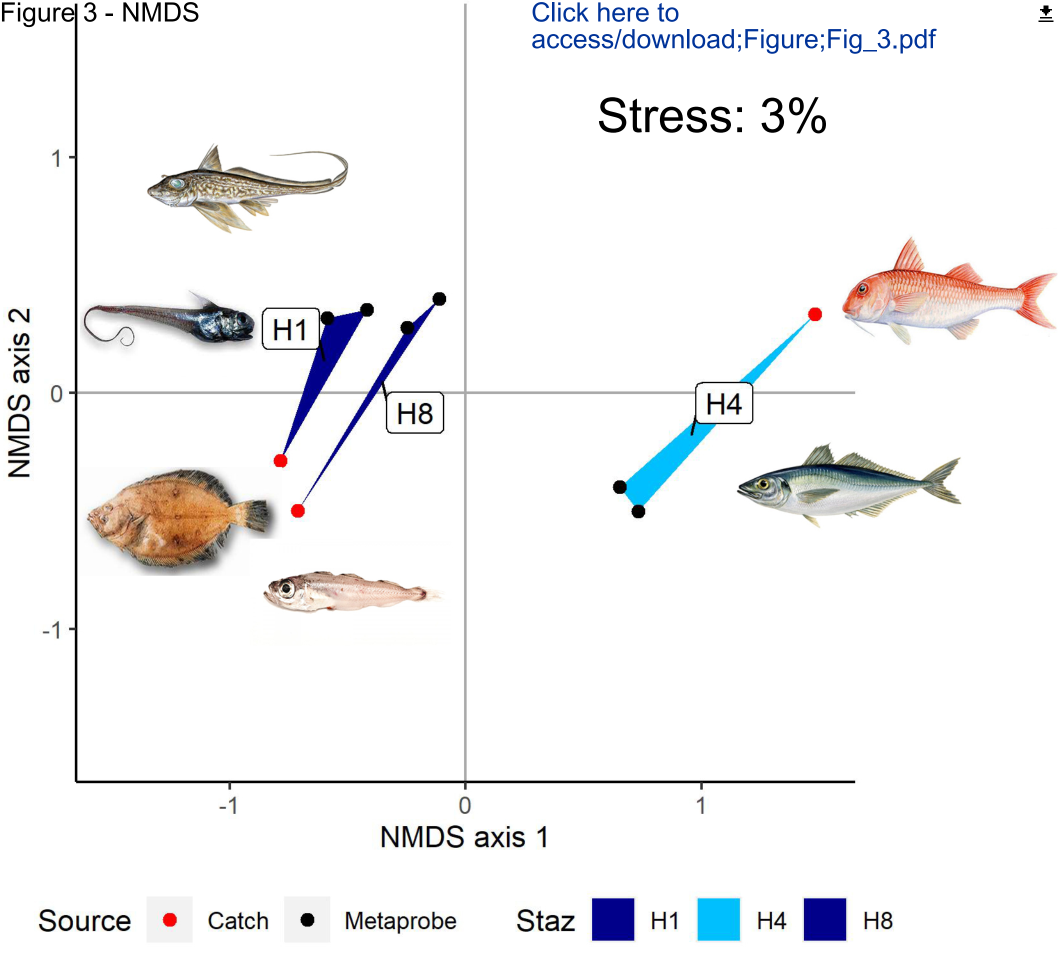

Figure 4 - heatmap