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From sight to sequence: Underwater visual census vs environmental DNA metabarcoding for the monitoring of taxonomic and functional fish diversity

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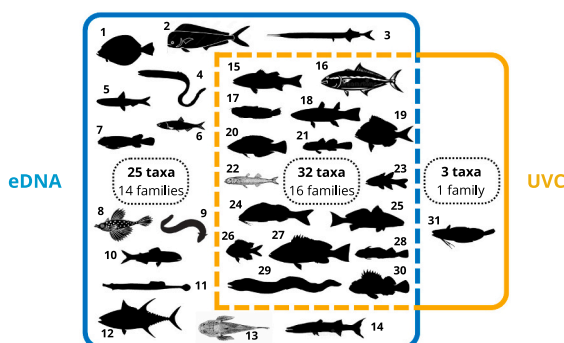
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HIGHLIGHTS

- Taxonomic and functional fish diversity were compared between eDNA and UVC.
- Almost all fish taxa identified were detected with eDNA (95 % vs 58 % with UVC).
- eDNA was even better to assess functional diversity, with little sampling effort.
- eDNA appears as a promising tool to survey complex fish communities.
- Combining eDNA/UVC in a same sampling approach could benefit monitoring programs.

GRAPHICAL ABSTRACT



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ABSTRACT

Fish monitoring is essential for assessing the effects of natural and anthropic stressors on marine ecosystems. In this context, environmental DNA (eDNA) metabarcoding appears to be a promising tool, due to its efficiency in species detection. However, before this method can be fully implemented in monitoring programs, more studies are needed to evaluate its ability to assess the composition of fish assemblages compared with traditional survey methods that have been used for decades. Here, we used both eDNA metabarcoding and Underwater Visual Census (UVC) to assess the taxonomic and functional diversity (presence-absence data) of Mediterranean fish communities. We collected eDNA samples and performed UVC strip transects inside and outside four Marine

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Protected Areas in the Mediterranean Sea. Samples for eDNA analysis were collected by filtering seawater simultaneously at the surface and the bottom, and DNA was amplified using a combination of three sets of primers. We found that eDNA alone made an outstanding characterisation of fish composition with the detection of 95 % of the 60 taxa identified in this study, whereas UVC recovered only 58 % of them. Functional diversity was better evaluated with eDNA than with UVC, with the detection of a greater breadth of functional traits. eDNA was even better at characterising functional than taxonomic diversity, providing reliable information on ecosystem functioning with little sampling effort. Together these results suggest that eDNA metabarcoding offers great potential for surveying complex marine ecosystems. Combining eDNA metabarcoding and UVC in integrated monitoring programs would therefore improve monitoring strategies and enhance our understanding of fish communities, a key step promoting their conservation.

1. Introduction

All over the world, marine species and their associated ecological functions have been impacted as a consequence of anthropogenic stressors and global changes (Carozza et al., 2019; McLean et al., 2019; Mouillot et al., 2013; Wernberg et al., 2016). This is particularly true for fish communities exposed to a global threat because of overfishing, destruction of their habitat and introduction of non-indigenous species (Goren et al., 2016; Pinsky et al., 2011; Stamp et al., 2022). Fish stocks provide major economic, cultural and recreational benefits for maritime territories (Holmlund and Hammer, 1999; Villéger et al., 2017). It is therefore crucial to ensure their long-term conservation by improving their sustainable management (Melnychuk et al., 2021). Fish communities also contribute to the functioning of marine ecosystems by carrying out many ecological functions such as regulation of nutrient cycling, control of trophic networks through predation and acting as ecosystem engineers (Allgeier et al., 2017; Coleman and Williams, 2002; Villéger et al., 2017). Their effective management requires ecological data collected through monitoring programs (Baker et al., 2016). These programs provide information on the health status of ecosystems and allow managers to adjust their conservation strategies (Cheal et al., 2021). In this context, a wide range of fish metrics and survey methods have been developed.

Most fish community surveys have conventionally focused on Taxonomic Diversity (TD), which corresponds to the number of taxonomically distinct entities and their abundances, without considering the key roles played by fishes in the ecosystem (Bosch et al., 2017). However, different fish species do not contribute equally to ecosystemic processes and the diversity of functional traits, Functional Diversity (FD), appears to be a more reliable indicator than TD to assess the impact of natural and anthropic perturbations on marine ecosystems (D'agata et al., 2014; McLean et al., 2019; Parravicini et al., 2021; Pecuchet et al., 2016). As such, trait-based studies have become more common to evaluate the FD of fish communities in various ecosystems at different spatial scales (Aune et al., 2018; M. A. Coleman et al., 2015; Parravicini et al., 2014; Riera et al., 2017).

Ecological data required to compute diversity metrics are usually collected in coastal areas using conventional methods such as underwater visual census (UVC) (Di Franco et al., 2021; Giakoumi et al., 2019), remote underwater video (RUV) that can be unbaited (Mallet et al., 2014; Zarco-Perello and Enríquez, 2019) or baited (BRUV) (Andradi-Brown et al., 2016; Sih et al., 2019) and experimental fishing (Seytre and Francour, 2009). UVC is the most common technique and has been effectively used to assess the abundance, biomass and composition of fish communities, allowing their taxonomic and/or functional description (Caldwell et al., 2016; Rincón-Díaz et al., 2018). This survey method relies on trained observers, is efficient to monitor demersal fishes and requires little post-survey processing (Aglieri et al., 2021; Cheal et al., 2021). However, UVC has several limitations such as depth and visibility restrictions, underestimation of the diversity of cryptic and large mobile species and modification of fish behaviour (i.e., attraction or flight) due to the diver's presence (Boussarie et al., 2018; Emslie et al., 2018; Figueroa-Pico et al., 2020). Alternatively, environmental DNA (eDNA) metabarcoding has emerged as a promising tool for

fish community surveys (Deiner et al., 2017; Pawlowski et al., 2021; West et al., 2020). This non-invasive method is not subject to the same limitations as UVC and fieldwork simply involves collecting environmental samples (e.g., seawater) to capture the DNA traces released by fishes into the environment (Bohmann et al., 2014). DNA is then extracted from filtered sea-water, PCR amplified using specific primer sets, sequenced and finally assigned to taxa using genetic reference databases (Miya, 2022). This method provides promising results to study the spatiotemporal composition of fish assemblages and allows the detection of rare and cryptic species (Alexander et al., 2022; Boussarie et al., 2018; Gold et al., 2023). However, each survey method has limitations and eDNA metabarcoding is no exception. For example, eDNA metabarcoding does not yet allow reliable estimations of fish density or biomass (Gilbey et al., 2021; Lamb et al., 2019), and is impacted by the transport of DNA molecules away from their source, potentially leading to false positives or false negatives (Barnes and Turner, 2016; Hansen et al., 2018). While the incompleteness of reference databases appears to be the main current limitation of the method (Polanco Fernández et al., 2021), Condachou et al. (2023) highlighted that eDNA metabarcoding was an efficient approach to assess the FD of fish assemblages, even when some sequences cannot be assigned at the species level.

Beyond the advantages and limitations of survey methods, which have been extensively explored in past studies (French et al., 2021), there is a need to compare their abilities to assess the TD and FD of fish communities. Indeed, since different survey methods do not detect the same pool of species, it is essential to understand how the selection of a given method affects diversity estimates (Bosch et al., 2017). This is crucial, given that ecological conclusions derived from these estimates could be different from one method to another (Aglieri et al., 2021). In this context, several authors aimed to compare UVC and eDNA metabarcoding and showed that the eDNA approach had a higher species detection effectiveness (Aglieri et al., 2021; Rey et al., 2023; Valdivia-Carrillo et al., 2021). Additionally, Aglieri et al. (2021) found that eDNA metabarcoding outperformed UVC, BRUV and experimental fishing, in assessing functional metrics of fish diversity. However, in these previous studies several fish species observed with UVC remained undetected with eDNA (i.e., false negatives). For example, Valdivia-Carrillo et al. (2021) demonstrated that combining eDNA and UVC doubled the number of species identified, as each method detected a highly different set of species. Therefore, these authors suggested combining survey methods to obtain a more comprehensive description of fish communities. In a context where eDNA metabarcoding approach is continuously improving, more studies are needed to assess the complementarity between UVC and eDNA methods. Additionally, to our knowledge, only a few studies (i.e., Aglieri et al., 2021; Polanco Fernández et al., 2021; Rey et al., 2023) have considered functional traits in the comparison of UVC and eDNA and only Rey et al. (2023) discussed combining them into an integrated monitoring strategy.

Here, we compared UVC and eDNA metabarcoding fish community data collected in four Marine Protected Areas (MPAs) in the North-western part of the Mediterranean Sea (France). The eDNA approach was recently improved by optimizing both the sampling strategy and the primer set selection (Roblet et al., 2024), making it possible to detect many fish species. Presence-absence data were used to compare the

ability of the UVC and eDNA metabarcoding methods to assess i) the taxonomic and ii) the functional diversity of fish communities, as well as (iii) to evaluate the complementarity between these two methods. Finally, (iv) we addressed the challenge of integrating UVC and eDNA metabarcoding into a common monitoring approach.

2. Materials and methods

2.1. Study area

Field sampling was performed inside and outside four MPAs, during September and October 2022. These MPAs, namely, Cap Roux (CR), Pequerolle (PEQ), Cap d'Ail (CDA) and Roquebrune - Cap Martin (RQB) are situated in the Northwestern part of the Mediterranean Sea along the French Riviera coast between Saint-Raphaël and Menton (Fig. 1). Each MPA encompasses typical coastal habitats of the Mediterranean Sea such as *Posidonia oceanica* meadows, and rocky and coralligenous reefs. Additionally, RQB MPA features artificial reefs. To ensure a robust comparison between methods, eDNA and UVC fish surveys were conducted within 24 h for each sampling zone.

2.2. Underwater visual census

UVC strip transects were performed using the conventional method developed by Harmelin-Vivien et al. (1985) to monitor fishes in the Mediterranean Sea. Transects were 25 m long and 5 m wide, covering 125 m². Along each transect, a trained diver identified and recorded the abundance and the size of all fishes encountered while swimming at constant speed. However, only presence-absence data were used in this study for comparison with eDNA. After completing the transect, on the way back, the diver focused on counting cryptobenthic species that could have been missed because they are more difficult to spot (e.g., fishes belonging to Blenniidae, Gobiidae or Scorpaenidae families, often hiding in holes and faults).

Depending on the surveyed MPA, two to four habitats (e.g., *Posidonia oceanica* meadow, rocky reef, sandy patches and artificial reefs) were

sampled using UVC (Table S1). For artificial reefs, the strip transect technique is not adequate, so the UVC method was adapted to allow the observation of species hiding inside the reef structure (Appendix S1). For each of these habitats, four sites were sampled per MPA (Table S1). For each site, six transects were conducted. In the case of artificial reefs, two to four modules were surveyed per site. A total of 294 UVCs were performed for this study.

2.3. Metabarcoding: water sampling

We have followed the eDNA metabarcoding sampling method described in Roblet et al. (2024) which is highly effective in detecting fish species. This sampling strategy relies on the simultaneous filtration of two samples along the same transect, one from the surface (i.e., one meter below the surface) and the other from the bottom (i.e., one meter above the seafloor). For bottom samples, sea-water filtration was performed by divers with the underwater pump attached to a diver propulsion vehicle. For the surface sample, sea-water filtration was conducted from a boat following the divers to ensure that surface and bottom samples were collected along the same transect (Fig. S1). Four transects with two replicates for each (i.e., surface and bottom samples), were conducted per MPA, resulting in a total of 32 eDNA samples of 30 L (Table S2).

Immediately after sampling, 50 mL of Longmire buffer solution (Longmire et al., 1997) was injected into the eDNA capsules to allow the long-term conservation of eDNA before the laboratory procedure. eDNA samples were always manipulated with gloves to avoid contamination. Back at the lab, capsules were shaken vigorously and the eDNA solution was stored at room temperature in the dark until extraction.

During the field campaign, several negative field controls were performed to check for contamination that could have occurred during capsule handling on the boat (Table S2). These controls consisted of 1 L of ultrapure water filtered with a capsule connected to the surface pump. They were treated the same way as true field samples.

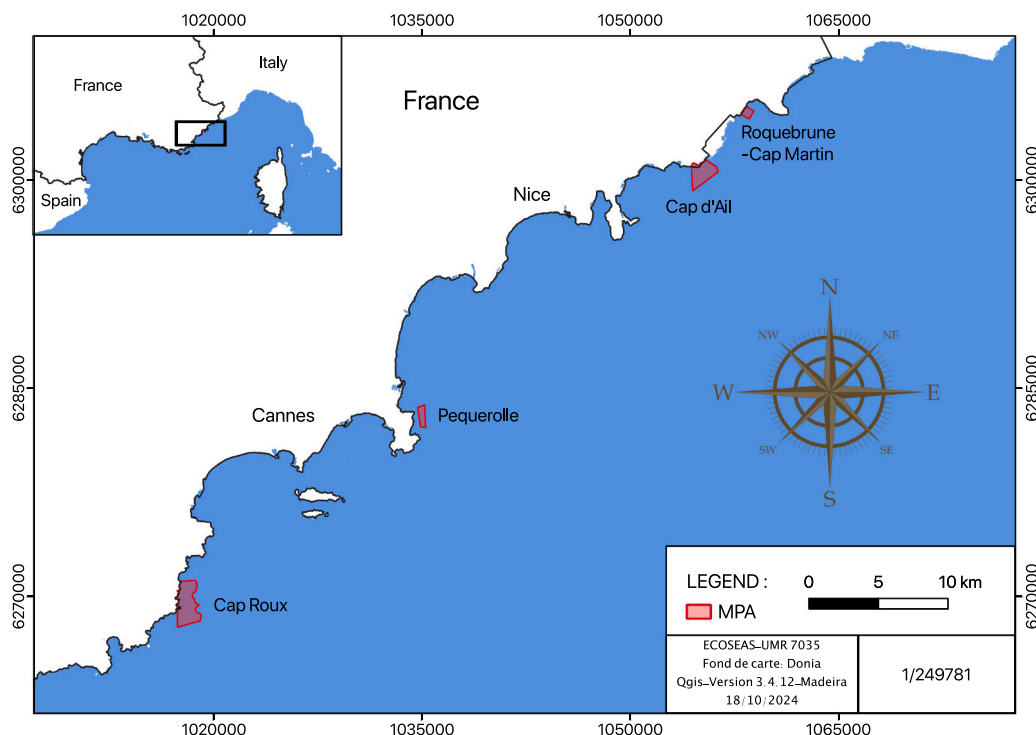


Fig. 1. Location of the four MPAs surveyed.

2.4. Metabarcoding: lab processing

The metabarcoding experiments, from DNA extraction to bioinformatic sequence analysis, were performed by Argaly (Sainte-Hélène-du-Lac, France; <https://www.argaly.com/en>), a private company specialising in biodiversity surveys based on eDNA. DNA extraction and PCR amplification were performed in dedicated laboratories for handling eDNA samples. Extraction was conducted following the NucleoSpin Soil kit protocol (Macherey Nagel) with the following modifications: the 50 mL falcon tubes were centrifuged for 1 h at 12,000g. The pellets were then resuspended in ATL buffer and proteinase K for 2 h at 56 °C to lyse cells and cell debris. The extraction procedure was continued according to the manufacturer's protocol and the resulting DNA extracts were eluted in a final volume of 100 µL of elution buffer.

In this study, we used three primer sets selected from the recent pilot study (Roblet et al., 2024): Fish16S, an Actinopterygii specific primer set that targets the 16S rRNA locus; AcMDB07, which is also Actinopterygii specific and targets the 12S rRNA gene; and Vert16S, which targets the 16S locus but is Vertebrate specific (Table 1). Therefore, these primer sets may detect both Actinopterygian and Chondrichthyan fish species. These primer sets each showed promising results, allowing the detection of many fish species, and were complementary, which justifies combining them (Roblet et al., 2024).

For each of these three primer sets, the extracted DNA from each sample was amplified in 12 replicates. Each PCR replicate was uniquely identified by a combination of two eight-base tags appended to the PCR primer at the 5' end. These tags were used during bioinformatics analysis to assign sequences to the corresponding replicate. Following amplification, all replicates were pooled per marker and purified with the MinElute purification kit (Qiagen). Library constructions and sequencing were then performed by Fasteris (Geneva, Switzerland). One (AcMDB07 and Vert16S) or two (Fish16S) libraries were prepared using the corresponding pool of amplicons with the Metafast protocol (<https://www.fasteris.com/en-us/NGS/DNA-sequencing/Amplicons-sequencing/Short-Amplicon-sequencing>), designed to minimize sequencing artefacts (Table S3). The libraries were then sequenced in several Illumina MiSeq runs with paired-end reads of 2 × 250 bp (for AcMDB07 and Vert16S) or 2 × 150 bp (for Fish16S).

Various quality controls were conducted at each step of the protocol to identify potential contamination, ensuring an accurate interpretation of the results. For each primer set, the following controls were performed: four negative extraction controls, three negative PCR controls, two positive controls (i.e., seawater eDNA samples from other projects that were collected in the Atlantic Ocean and processed by Argaly) and eight bioinformatic controls. The success of the amplifications and purifications was confirmed on a 2 % agarose gel (*E-Gel Power Snap*, Invitrogen).

2.5. Metabarcoding: bioinformatics

Argaly conducted the bioinformatic steps, using the following procedure: the raw sequence data for each primer were analysed using the suite of OBITools programs (<https://pythonhosted.org/OBITools/welcome.html>; Boyer et al., 2016), which is designed specifically for

metabarcoding data analysis. For each primer set, paired sequences were assembled (“illumina-paired-end” command). Then, sequences with an alignment score ≥ 40 (i.e., corresponding to an overlap of at least 10 bases) were assigned to the corresponding amplification replicate, thanks to the tags inserted in the 5' end of the primers (“ngsfilter” command). The resulting dataset was dereplicated (“obiuniq” command), then filtered (“obigrep” command) to remove low-quality sequences (i.e., containing at least one N), sequences whose length did not belong to the length range observed in silico for the target group, and singletons (i.e., sequences observed only once in the dataset). Sequences sharing 97 % identity were grouped into clusters using SumaClust (Mercier et al., 2013). The abundances of sequences within each cluster were summed for each PCR replicate. The cluster head, representing the most abundant sequence in the cluster, was chosen as the representative sequence. Clusters appearing <10 times in a sample were deleted. A taxonomic assignment of the cluster heads was performed using the “ecotag” command to obtain a list of MOTUs (Molecular Operational Taxonomic Units). The reference sequences used for this taxonomic assignment were obtained by performing an in-silico PCR on the public sequence database GenBank (v.249) using the ecoPCR program (Ficetola et al., 2010). This in silico PCR was conducted using the PCR primers associated with each marker, allowing a maximum of three mismatches per primer and retaining only sequences assigned at least at the family level.

The R package “metabar” (Zinger et al., 2021) was then used to remove artefactual sequences that are present in low abundance in the metabarcoding data, but which may influence the ecological conclusions that can be drawn from them (Calderón-Sanou et al., 2020). This process included removing i) MOTUs with sequence similarity to any sequence in the reference database below 0.95, as they are potential chimeras; ii) MOTUs whose frequency over the entire dataset is maximum in at least one negative control (“max” method of the “contaslayer” function), because they are potential contaminants; and iii) MOTUs with a relative frequency < 0.03 % within a PCR replicate (“tagjumpslayer” function), because they are potentially artefacts generated during library construction for sequencing (i.e., “tag jumps”; Schnell et al., 2015). PCR replicates with a sequencing coverage <100 sequences were also removed and then the remaining PCR replicates were aggregated by sample using the “aggregate_pcrs” function. Finally, MOTUs represented by <10 reads in a sample were recoded as absent in that sample.

Manual verification of the data output from the bioinformatic pipeline and modification of the automatic taxonomic assignments were performed for each primer set dataset as follows:

- non-fish taxa and freshwater fish MOTUs were deleted;
- MOTUs assigned to marine fish were reviewed by blasting the sequences on Genbank and were modified if needed following these criteria:
 - Based on biogeographic data, if a sequence was assigned to a non-Mediterranean species, the assignment was changed to the next lowest possible taxonomic rank known to occur in the Mediterranean Sea. If there was only one known species of this particular genus or family occurring in the Mediterranean Sea, the assignment was changed to that species.

Table 1
Information on the three primer sets used in the study.

	AcMDB07	Fish16S	Vert16S
Reference	Bylemans et al., 2018	Shaw et al., 2016	Vences et al., 2016
Target gene	12S	16S	16S
Forward primer	GCCTATATACCGCCGTCG	CGAGAAGACCCTWTGGAGCTTIAG	AGACGAGAAGACCCYdTGGAGCTT
Reverse primer	GTACACTTACCATGTTACGACTT	GGTCGCCCCAACRAAG	GATCCAACATCGAGGTCGTAA
Annealing T °C	55	55	65
Amplicon length (without primers)	276–289 bp	31–94 bp	161–298 bp
Illumina MiSeq Run	2 × 250 bp	2 × 150 bp	2 × 250 bp

- Based on biogeographic data, when a sequence was assigned to a taxonomic rank higher than the species level and there was only known one species with this genus or family occurring in the Mediterranean Sea, we changed the assignment to that species.

Datasets of the three primer sets were then transformed into presence-absence data and pooled into a single common dataset. As we were not interested in intraspecific diversity, MOTUs assigned to the same taxa were pooled.

2.6. Statistical analyses

2.6.1. Pre-treatment of the data

Since eDNA metabarcoding does not yet provide reliable information on fish abundance or biomass (Hansen et al., 2018; Sanchez et al., 2022), the comparison of UVC and eDNA was only based on presence-absence data. It should also be noted that eDNA and UVC do not have the same taxonomic resolution for some taxa (e.g., eDNA allowed us to identify several species of Gobiidae whereas for UVC these taxa were registered at the family level). Thus, to allow a fair comparison between methods, when there was an uneven taxonomic resolution for a given taxon, the taxa recorded by the method with higher resolution were condensed together and assigned to the lowest taxonomic rank identified by the other method (e.g., every species of Gobiidae identified with eDNA were pooled and assigned at the family level) (Table S4). As some fish taxa were not identified at the species level, hereafter we will use the term “taxa” instead of “species”. In addition, the two methods were not comparable at the replicate scale. Thus, to allow a robust comparison between UVC and eDNA datasets, we compared both methods at the sampling zone scale (Appendix S2). In total, four sampling zones were defined per MPA (Table S5). Out of these 16 sampling zones, three were removed because of incomplete sampling effort with eDNA (i.e., the pump stopped prematurely) and thus these zones were also discarded from the UVC dataset (Table S5).

2.6.2. Statistical analyses used to assess taxonomic diversity

All statistical analyses were conducted in R v4.3.1 (<http://www.R-project.org>; R Core Team, 2024). The differences between the UVC and eDNA datasets were represented using a Venn diagram. The frequency of occurrence (i.e., percentage of the number of sampling zones where a given taxa was detected out of the total number of sampling zones) for each fish taxa found through both methods was computed to further investigate these differences.

Species rarefaction curves were computed at the replicate scale for each survey method to evaluate the relative sampling effort needed to have comparable estimates of fish richness (function “ggiNEXT” of “iNEXT” package; Hsieh et al., 2016). After checking for normality and homoscedasticity (Shapiro and Bartlett tests), we compared the mean number of fish taxa detected per replicate (Welch’s *t*-test) and per sampling zone (Student’s *t*-test) between the UVC and eDNA methods. A Student’s *t*-test was also conducted to assess the differences between the methods in terms of average taxonomic distinctiveness (AvTD₂; Clarke and Warwick, 1998). The AvTD index corresponds to the mean taxonomic distance between pairs of taxa comprising a given sampling zone and provides information on the taxonomic breadth of fish communities. It was calculated using “taxa2dist” and “taxondive” functions of the “vegan” package (Dixon, 2003), excluding three taxa identified at the family level.

For taxonomic beta diversity, a nonmetric multidimensional scaling (nMDS) based on the Jaccard distance matrix was first computed to visualize the ordination of the data grouped by sampling method and MPA. A permutational multivariate analysis of variance (PERMANOVA) was then run on this distance matrix to evaluate the effects of these two main factors and their interaction on community composition (“adonis2” function of the “vegan” package), followed by post hoc pairwise comparisons (“pairwise.adonis2” function of the “pairwiseAdonis” package;

Martinez Arbizu, 2020).

2.6.3. Statistical analyses used to assess functional diversity

In addition to the Taxonomic Diversity (TD), we also compared the ability of UVC and eDNA to assess the Functional Diversity (FD) of fish assemblages. Seven traits, linked to key ecosystemic functions performed by fishes (Villéger et al., 2017), were selected: maximum size, mobility, period of activity, schooling behaviour, vertical position in the water column, depth-range, and diet (Table S6). The first six traits were coded as ordinal variables whereas diet was coded as a factor variable. Trait values were assigned to each species using available information from Fishbase (Froese and Pauly, 2002) and from three functional databases, namely, FishMed (Albouy et al., 2015), Gaspar (Kulbicki et al., 2013) and the database compiled by Aglieri et al. (2021). When information on a given species was missing, trait values from sibling species (i.e., same genus or family) occurring within the same region were used. In addition, for taxa registered at a taxonomic rank higher than species level, we used trait values that were the most common in species belonging to these taxa. Although there may be some inaccuracies in the functional traits for these taxa, several papers have shown that FD estimates have good reliability when species belonging to the same genus or family as the target taxa are used to attribute traits (Condachou et al., 2023; Ford and Roberts, 2020).

The “mFD” package (Magneville et al., 2022) was used to compute several FD indices. First, taxa sharing the same trait values were grouped into functional entities (FEs) and the mean number of FEs per replicate (Wilcoxon’s rank sum test) and per sampling zone (Student’s *t*-test) was compared between UVC and eDNA. Functional redundancy (FRed), an index corresponding to the number of fish taxa divided by the number of FEs (Mouillot et al., 2014), was calculated and we compared the mean value of FRed estimates per sampling zone for each survey method (Wilcoxon’s rank sum test). FEs were also used to build rarefaction curves at the replicate scale (“iNEXT” package). For functional beta diversity, a community matrix based on FEs was compiled. An nMDS and a PERMANOVA were then conducted on this matrix to assess the effects of the sampling method, the MPA and the interaction between these two factors on functional composition.

Then, additional FD indices were computed based on a multidimensional trait-space. Pairwise functional trait-based distances were calculated using Gower’s distance, an index that uses mixed variable types (Legendre and Legendre, 1998). A principal coordinate analysis (PCoA) was performed on this distance matrix to generate the multidimensional space in which FD indices are calculated. Mean absolute deviation (mad) and root of mean square deviation (rmsd), two indices used to assess the quality of the PCoA based multidimensional space (“mFD” package), showed that the first four axes provided the best representation of the trait-based distances between taxa. This 4D multidimensional space was thus used to compute three FD indices: i) Functional richness (FRic; Villéger et al., 2008), an index corresponding to the proportion of the functional space filled by the community (i.e., convex hull volume); ii) the average functional distinctiveness (AvFD; Weiher et al., 1998), which represents the mean functional distance between all pairs of taxa comprising a sampling zone, and iii) the functional dissimilarity, an index referring to beta diversity and corresponding to the proportion of nonoverlap in the functional space of the convex hull volumes containing the taxa detected by each method (Villéger et al., 2013). Differences between FRic estimates for UVC and eDNA were visualised with the “mFD” package on the global pool of taxa. Then, the Jaccard coefficient was used to compute the functional dissimilarity between methods. Finally, FRic and AvFD indices were averaged at the sampling zone scale and compared statistically to assess differences between the methods (Welch’s *t*-test for FRic and Student’s *t*-test for AvFD).

3. Results

3.1. Taxonomic diversity

A total of 2,292,818 paired-end reads were recovered from the Illumina MiSeq runs after applying the filtration steps (351,427 for AcMDB07; 468,089 for Fish16S and 1,473,302 for Vert16S; Table S3). The MOTU clustering process resulted in 46 MOTUs for AcMDB07, 99 for Fish16S and 124 for Vert16S but 27 were assigned to non-marine or non-fish taxa and were therefore removed.

Modification of assignments to adjust for taxonomic resolution resulted in the loss of 39 species identified by eDNA, condensed into six genera and three families and the loss of 13 species identified by UVC, condensed into three genera (Table S4).

Overall, combining taxa detected with eDNA (three primer sets combined) and UVC, we identified a total of 60 fish taxa (48 at the species level, nine at the genus level and three at the family level). Out of these 60 taxa, eDNA performed better with the detection of 57 taxa (95 % of the global pool) whereas UVC recorded 35 taxa (58.3 % of the global pool) (Table S7). 25 were unique to eDNA, three were unique to UVC and 32 were detected by both methods (Fig. 2).

The frequency of occurrences for the 32 taxa concomitantly detected by UVC and eDNA, showed differences between the two methods (Fig. S2). For example, Blenniidae, Gobiidae and Mugilidae families were more often detected with eDNA whereas almost all species of Sparidae were spotted in more sampling zones using UVC (Fig. S2).

At the replicate scale, we detected significantly more fish taxa with eDNA (15.7 ± 7.06) than with UVC (6.74 ± 3.57) (Welch's *t*-test, $p < 0.001$) (Fig. S3). This result was further illustrated by the rarefaction curve which showed that only two eDNA replicates are sufficient to identify as many fish taxa as ~14 UVC replicates (Fig. S4). At the sampling zone scale (i.e., the spatial scale chosen to allow a fair comparison between methods), the average number of fish taxa detected was quite similar for eDNA (23.2 ± 6.11) and UVC (21.7 ± 5.34) (Student's *t*-test, $p > 0.05$) (Fig. 3-a).

Nevertheless, the AvTD index was significantly higher for eDNA (82.4 ± 5.76) than for UVC (71.7 ± 3.48) (Student's *t*-test, $p < 0.001$) (Fig. 3-b). This result indicates that sampling zones present a wider taxonomic breadth when surveyed with eDNA than with UVC.

The nMDS plot showed a clear separation between sampling zones surveyed with eDNA and with UVC (Fig. 4). This result was statistically supported by a PERMANOVA performed on the same distance matrix, showing a significant difference in fish composition depending on the survey method ($p < 0.001$, Table S8). Taxonomic composition was also significantly influenced by the factor "MPA" ($p < 0.001$). Post hoc pairwise analyses revealed that the fish composition of the PEQ MPA

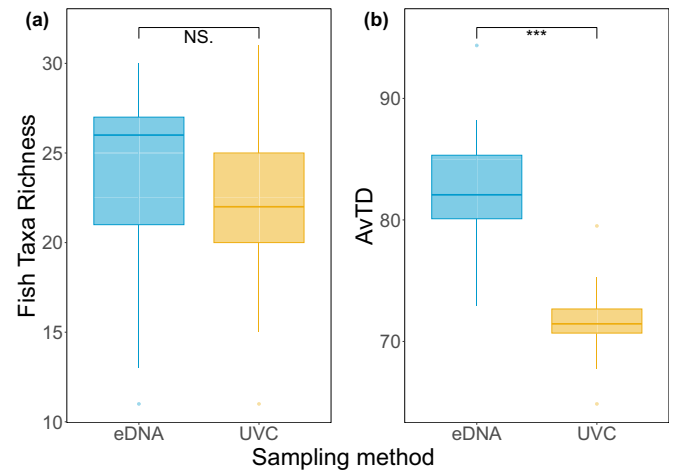


Fig. 3. Boxplots showing (a) the average taxa richness, and (b) the mean AvTD index per sampling zone depending on the survey method ($n = 13$ for eDNA; $n = 13$ for UVC; NS.: $p > 0.05$; ***: $p < 0.001$).

was significantly different from the compositions of the three other MPAs (Table S8). This was highlighted on the nMDS plot with PEQ sampling zones being spaced away from the two clusters (i.e., one for each survey method) in which the three other MPAs are merged (Fig. 4). Finally, although the interaction between "Sampling method" and "MPA" was significant ($p < 0.05$), none of the post hoc pairwise tests were significant (Table S8).

3.2. Functional diversity

A total of 45 FEs were generated from the association of the 60 fish taxa with the seven functional traits. eDNA was able to recover 43 of these FEs (17 of them were unique to eDNA and 26 were shared with UVC) while UVC recovered 28 of them (two of them were unique to UVC) (Fig. S5). At the replicate scale, we detected significantly more FEs with eDNA (14.2 ± 6.13) than with UVC (6.01 ± 2.90) (Wilcoxon rank test: $p < 0.001$) (Fig. S6). The rarefaction curve based on FEs showed that ~20 UVC replicates were needed to recover as many FEs as were recovered with two eDNA samples (Fig. S7). The mean number of FEs per sampling zone was not significantly different between eDNA (20.5 ± 4.84) and UVC (17.7 ± 4.40) (Student's *t*-test, $p > 0.05$) (Fig. 5-a).

For functional beta diversity, we found similar results to those reported for taxonomic beta diversity, with significant effects of the factors "Sampling method" ($p < 0.001$) and "MPA" ($p < 0.001$) and of their

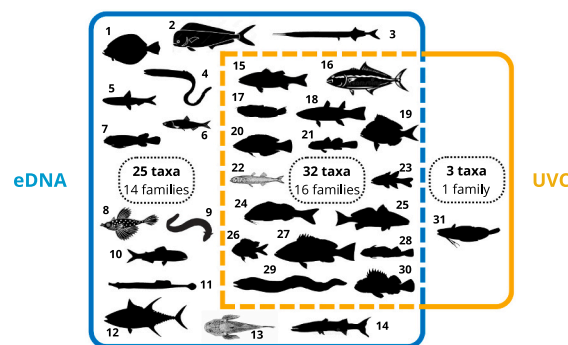


Fig. 2. Venn diagram showing the overall number of fish taxa and families detected for a given method ($n = 26$ for eDNA; $n = 246$ for UVC). Fish silhouettes represent the families detected for each survey method (1: Bothidae; 2: Coryphaenidae; 3: Belonidae; 4: Congridae; 5: Clupeidae; 6: Engraulidae; 7: Gobiessocidae; 8: Dactylopteridae; 9: Ophichthidae; 10: Myctophidae; 11: Syngnathidae; 12: Scombridae; 13: Uranoscopidae; 14: Sphyrnaeidae; 15: Moronidae; 16: Carangidae; 17: Blenniidae; 18: Mugilidae; 19: Sparidae; 20: Labridae; 21: Gobiidae; 22: Atherinidae; 23: Apogonidae; 24: Mullidae; 25: Sciaenidae; 26: Pomacentridae; 27: Seranidae; 28: Tripterygiidae; 29: Muraenidae; 30: Scorpaenidae; 31: Phycidae). (Image' sources: R package "fishualize"; dreamstime.com; shutterstock.com; stock.adobe.com; pngtree.com; fishesofaustralia.net).

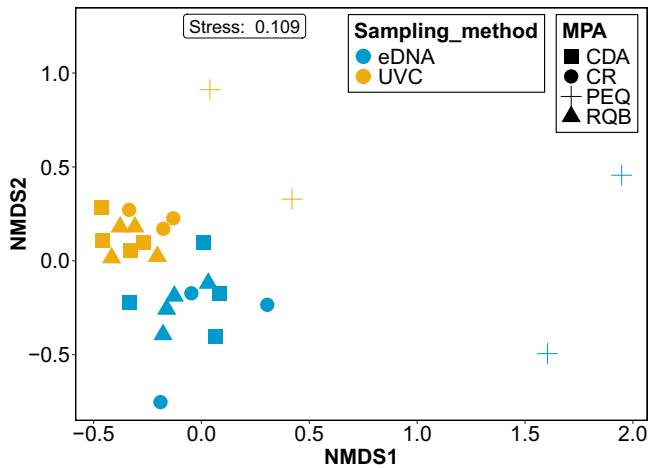


Fig. 4. Nonmetric multidimensional scaling (nMDS) plot based on the Jaccard index, showing differences in the taxonomic composition of fish assemblages depending on the survey method (indicated by colours) and on the MPA (indicated by shapes). Each point corresponds to a given sampling zone ($n = 13$ for eDNA; $n = 13$ for UVC).

interaction ($p < 0.05$) (Table S9). Post hoc pairwise analyses revealed that the functional composition of the PEQ MPA was significantly different from the compositions of CDA and RQB, but none of the post hoc tests conducted on the interaction were significant (Table S9). The separation between PEQ sampling zones and the zones of the other MPAs was clearly highlighted on the nMDS plot (Fig. S8).

At the sampling zone scale, the AvFD index was significantly higher for eDNA (eDNA: 38.4 ± 2.38 , UVC: 30.1 ± 3.57 , Student t-test: $p < 0.001$) (Fig. 5-c) while the FRed estimate was statistically lower (eDNA: 1.13 ± 0.0584 , UVC: 1.23 ± 0.0584 , Wilcoxon rank test: $p < 0.001$) (Fig. 5-b). Our results also showed that the mean FRic value per sampling zone was significantly higher for eDNA (0.342 ± 0.153) than for UVC (0.132 ± 0.0778) (Welch's t-test, $p < 0.001$) (Fig. 5-d). These results indicate that eDNA can detect fish assemblages that are less functionally redundant and that display a broader range of functional traits

than UVC.

At the global scale of the study, the FRic value was much higher for eDNA. Taxa detected with eDNA filled a functional volume representing 99.4 % of the global functional space whereas UVC filled only 31.1 % of this volume (Fig. 6).

The functional dissimilarity between eDNA and UVC volumes was 0.69 and 97.7 % of this value was due to the nestedness component of beta diversity. This highlights that the taxa detected by UVC filled a subset of the eDNA functional space rather than displaying functional traits unique to UVC (Fig. 6).

eDNA was better at detecting very small and cryptobenthic taxa such as Gobiidae, the flat fish *Arnoglossus thori* or *Conger conger* (Fig. S9-a and S9-d). Using this method, we also identified more pelagic taxa, either very large and very mobile piscivorous taxa (e.g., *Thunnus* or *Belone belone*) or small schooling taxa (e.g., *Sardina pilchardus* or *Sardinella aurita*) (Fig. S9-a, S9-b, S9-c, S9-d and S9-e). These taxa, only detected with eDNA, were vertices of the convex hull volume of the entire fish community (Fig. 6). On the other hand, UVC performed well at recording mobile and demersal fishes such as *Coris julis* or *Diplodus sargus* (Fig. S9-a and S9-c).

4. Discussion

Having effective and non-destructive methods for the monitoring of coastal fish assemblages is essential. In this context, we compared the abilities of eDNA metabarcoding and UVC to assess the Taxonomic Diversity (TD) and the Functional Diversity (FD) of fish communities.

4.1. Comparing eDNA metabarcoding and UVC on the regional species pool (gamma diversity)

Overall, 95 % of the 60 fish taxa identified in this study were detected with eDNA, while 58.3 % were observed with UVC. Notably, 25 fish taxa were only detected with eDNA. Our finding aligns with previous studies which showed that eDNA metabarcoding has a higher species detection efficiency than traditional survey methods (Aglieri et al., 2021; Alexander et al., 2022; Boussarie et al., 2018; Gold et al., 2023; Marques et al., 2021a; Port et al., 2016; Rey et al., 2023; Sard

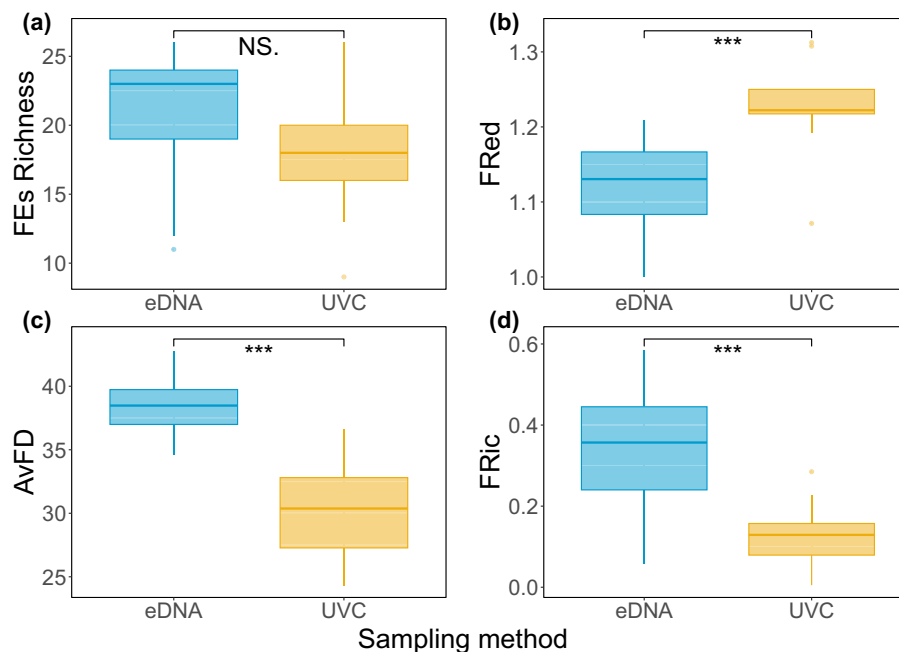


Fig. 5. Boxplots showing the average (a) FE richness, (b) FRed index, (c) AvFD index and (d) FRic index per sampling zone depending on the survey method ($n = 13$ for eDNA; $n = 13$ for UVC; NS.: $p > 0.05$; ***: $p < 0.001$).

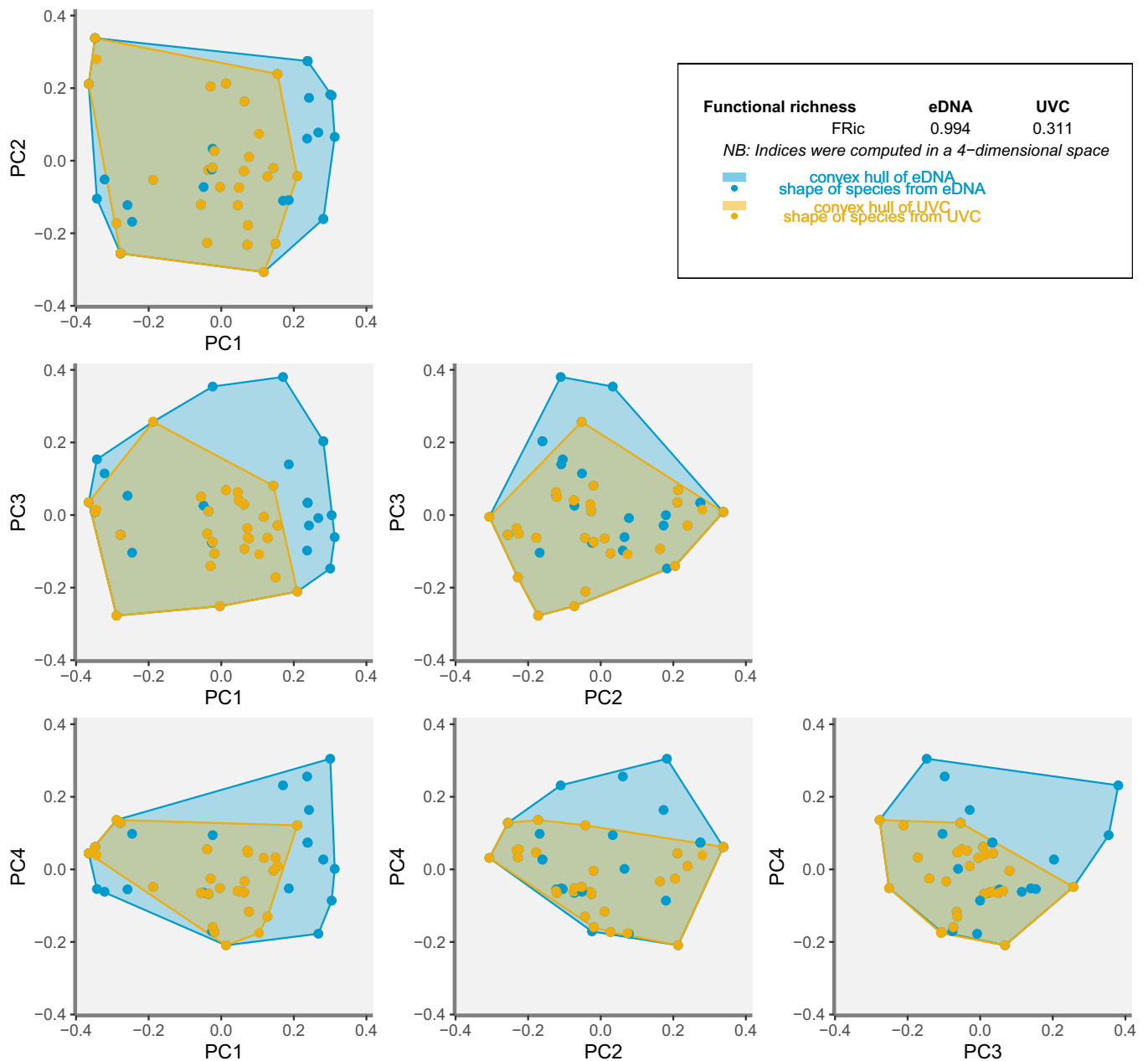


Fig. 6. Comparison between FRic estimates at the global scale for eDNA and UVC, computed in a PCoA-based 4D functional space ($n = 26$ for eDNA; $n = 246$ for UVC). Each subplot represents the functional space for a given pair of axes. Blue dots correspond to the fish taxa identified with eDNA and orange dots to those detected with UVC. The blue polygon represents the trait space covered by fish taxa detected with eDNA, whereas the orange polygon represents the trait space covered by taxa identified with UVC.

et al., 2019; Valdivia-Carrillo et al., 2021). This result could be linked to the broader spatiotemporal detection scale associated with eDNA (Valdivia-Carrillo et al., 2021). For example, local oceanographic conditions such as current can transport DNA molecules away from their source, potentially leading to the detection of species inhabiting adjacent habitats (Yamamoto et al., 2017). Our results support this idea as most of open water pelagic taxa identified in this study were only detected with eDNA. Additionally, eDNA might also capture genetic signal over a longer period due to the persistence of DNA molecules in the water column for several hours after their release depending on environmental factors controlling eDNA degradation (Collins et al., 2018). Therefore, species that were present in the area hours before seawater sampling could be detected (Aglieri et al., 2021). In our case, this might explain the detection of nocturnal species usually missed by

UVC, such as *Ophisurus serpens* or *Conger conger*, as well as species that rise in the water column at night (i.e., nychthemeral migrations) such as *Myctophum punctatum* or *Ceratoscopelus maderensis*. It should also be noted that while UVC mostly targets adult individuals, eDNA can detect fish species at any stage of their life cycle, potentially increasing the number of taxa detected (Valdivia-Carrillo et al., 2021).

In relation to the broader spatiotemporal detection scale associated with eDNA discussed above, we found that this molecular approach was better able to investigate functional metrics of fish diversity, supporting the results of Aglieri et al. (2021). Indeed, while UVC detected 28 FEs, 43 FEs were detected using eDNA, for a total of 45 FEs identified in this study. Additionally, FEs exclusively associated with eDNA presented more extreme and distinct trait combinations than the FEs detected with UVC. This was highlighted by our result on functional richness as this

index was much higher for eDNA (Marques et al., 2021a). The performance of eDNA to detect FEs could also be linked to the lack of selectivity of this method towards any functional traits (Aglieri et al., 2021). Indeed, while UVC is mostly biased towards common Mediterranean species, that are diurnal, mobile and demersal, eDNA was able to capture a more diverse range of functional traits. For example, we detected more piscivorous and highly mobile pelagic species with eDNA, probably because these species usually avoid divers (Langlois et al., 2010), so the width of UVC transects (i.e., 5 m) might be too small to spot them (Prato et al., 2017). eDNA was also better at detecting very small and cryptobenthic species that are less easily seen by UVC due to their size and because they are often hidden within the substrate (Ackerman and Bellwood, 2000; Baker et al., 2016). In addition, schooling taxa were preferentially detected with this molecular method, probably because they release DNA in large quantities (Kelly et al., 2014; Maruyama et al., 2014).

Another important finding is that eDNA required less field time than UVC to capture the same number of taxa and FEs. Indeed, rarefaction curves showed that two eDNA samples collected as many taxa as ~14 UVC replicates. Furthermore, two eDNA replicates were sampled simultaneously along the same transect over a period of 30 min, while 14 UVC replicates required >80 min. From a functional perspective, the difference was even higher with two eDNA samples yielding the same number of FEs as ~20 UVC replicates. These results suggest that eDNA allows a fast inventory of TD and FD (Marques et al., 2021a).

While previous studies showed that eDNA and conventional methods detected largely different sets of species (Stat et al., 2019; Valdivia-Carrillo et al., 2021), our results did not show the same pattern. In our case only three species and two FEs observed by UVC were missed by eDNA. Several explanations have been proposed in these studies to explain the high number of false negatives associated with eDNA, including under sampling, insufficient number of PCR replicates, incompleteness of reference databases and primer bias (Doi et al., 2019; Griffin et al., 2020; Rey et al., 2023; Valdivia-Carrillo et al., 2021). For example, Polanco Fernández et al. (2021) found that 60 % of the fish genera identified only with UVC did not have a reference sequence in the genetic database and Marques et al. (2021a) stated that the complementarity between eDNA and video censuses was mostly due to the incompleteness of current databases. Even if databases are continuously expanding, their current poor exhaustiveness is a major concern preventing effective eDNA-based monitoring (Marques et al., 2021b). In the medium term, this issue could be addressed by using a combination of primer sets that target different marker genes (Evans et al., 2017; Rey et al., 2023; Stauffer et al., 2021), allowing more sequences to be assigned thanks to the complementarity between databases (Kumar et al., 2022; Shaw et al., 2016). Conducting multiprimer surveys could also overcome the limitations of primer resolution and coverage associated with single-set approaches (Marques et al., 2021a). Unlike previous studies, we used a combination of three primer sets targeting different marker genes (i.e., 12S and 16S rRNA) that were amplified using 12 PCR replicates for each sample. Additionally, our sampling strategy relied on large volumes of seawater filtered simultaneously from the surface and the bottom (Roblet et al., 2024). This is expected to further improve the robustness of our approach as maximizing water volumes increases detection efficiency (Bessey et al., 2020; Stauffer et al., 2021) and sampling close to the substrate identifies additional species (Andruszkiewicz et al., 2017; Jeunen et al., 2020; Sigsgaard et al., 2020; Yamamoto et al., 2017). We thus attribute the low number of eDNA false negatives in this study to the robustness of our approach. However, it should also be noted that our study was conducted in the Mediterranean Sea, a region that benefits from a wider coverage of genetic databases than tropical areas (Marques et al., 2021b).

The three species uniquely detected with UVC in our study were *Pagrus pagrus*, *Phycis phycis* and *Serranus cabrilla*. Each of them has reference sequences in the database, so other reasons must explain their non-detection with eDNA. For example, *Pagrus pagrus* is morphologically

highly similar to the species belonging to *Pagellus* genus and these latter were detected within several eDNA samples but not with UVC. Thus, this false negative could be either due to a wrong identification by the divers performing UVC or by an incorrect assignation of the sequence in the database. Another reason could be linked to the differences in sampling locations between the two methods. Although eDNA and UVC censuses were performed within the same sampling zone, the eDNA transect was not exactly overlapping UVC sites in some cases, potentially preventing the detection of some species observed with UVC. For example, *Phycis phycis* is known to inhabit small and localised habitats during the day such as rocky faults. Thus, the microhabitat preferences of this species could explain its non-detection with eDNA (Troth et al., 2021), unless the seawater is filtered very close to an inhabited fault. Finally, although *Serranus cabrilla* was highlighted as uniquely associated with UVC, we did detect this species in our study, in one of the eDNA samples removed because of incomplete filtration volume. However, this species was only detected within one sampling zone with eDNA but in more than half of sampling zones with UVC. The lack of detection of this species is therefore likely due to several other reasons, including a low DNA release rate or PCR amplification biases (Valdivia-Carrillo et al., 2021).

4.2. Comparing eDNA metabarcoding and UVC at local scale (alpha diversity)

At the sampling zone scale, our results showed that the mean number of fish taxa identified was similar for each survey method. This finding was unexpected as eDNA detected many more taxa than UVC when all replicates were considered together. We found that this result was due to certain taxa being missed by eDNA at the sampling zone scale. Indeed, results of the frequency of occurrences for the 32 taxa jointly detected by the two methods, showed that some taxa were detected in more sampling zones for UVC than for eDNA. Although the hypotheses developed above to explain false negatives cannot be excluded, we believe that this finding is mostly due to an insufficient number of eDNA samples at the sampling zone scale. In fact, the sampling zone scale corresponded to only two replicates for eDNA while it consisted of between 12 and 24 replicates for UVC. In this context, Stauffer et al. (2021) have suggested that eDNA distribution is patchy in seawater and varies at a fine scale. Consequently, these authors among many others (Cantera et al., 2019; Guillerá-Arroita et al., 2017; Stat et al., 2019; Valdivia-Carrillo et al., 2021), highlighted the need to collect more samples to prevent false negatives. Even though we filtered 30 L per sample, which is much more than for many studies (reviewed in Shu et al., 2020), we still would have needed a greater sampling effort to be exhaustive with eDNA at the sampling zone scale. On the other hand, combining 26 eDNA samples overall was sufficient to detect these taxa. This result could also be attributed to the adjustment of the taxonomic resolution which led to the loss of 39 eDNA-detected species (merged into nine taxa) whereas only 13 UVC-spotted species were collapsed (merged into three taxa).

While the average number of taxa per sampling zone was similar between methods, the AvTD index was significantly higher for eDNA than for UVC, meaning that fishes detected with eDNA exhibit a wider diversity of lineages. At the sampling zone scale, our results also showed a better assessment of functional metrics with eDNA. As FD is complex, no single index can summarise all the aspects of this dimension of diversity (Mason et al., 2005). Therefore, a combination of several functional indices is advised (Villéger et al., 2008). In this study, we demonstrated that three out of the four functional indices calculated revealed significant differences between UVC and eDNA at the sampling zone scale. Since the average taxa richness was similar between methods at this scale, these results suggest that eDNA metabarcoding exhibits an even greater ability to detect functional entities than taxonomic entities, in agreement with findings from Aglieri et al. (2021). Therefore, even though the sampling zone scale was characterised by relatively sparse sampling for eDNA, this method could still provide reliable information on ecosystem functioning (Marques et al., 2021a).

4.3. Fish assemblages' composition (beta diversity)

The taxonomic and functional compositions of fish communities were significantly different between sampling zones monitored with eDNA and zones surveyed with UVC. This was not surprising as we already showed that 25 taxa and 17 FEs were uniquely detected with eDNA. However, while these two survey methods give a different vision of the fish community, they both successfully highlighted differences in fish composition between MPAs. Indeed, PEQ sampling zones were spaced away from the zones of the other MPAs on the two nMDS plots (i.e., one based on taxonomic data and the other on functional data) for the two methods. Our results suggest that these differences could be associated with the presence or absence of a given habitat required for specialist taxa. While the three other MPAs present the same kind of habitats (i.e., *Posidonia oceanica* meadows and natural or artificial rocky reefs), there is no *P. oceanica* meadows within the PEQ MPA. As a consequence, the meadows specialist species identified in this study (e.g., *Diplodus annularis* or *Sarpa salpa*) were not detected in PEQ sampling zones. Additionally, stretches of sand were sampled in PEQ but not for the three other MPAs. This led to the detection of sand associated species such as *Arnoglossus thori* with eDNA. Nevertheless, these species were missed with UVC, probably due to the low ability of this method to detect cryptic species exhibiting mimetic behaviour (Pais and Cabral, 2017). This was illustrated on the two nMDS plots, as PEQ sampling zones were more widely separated for eDNA than for UVC. Therefore, eDNA appears to be even more efficient than UVC for highlighting differences in taxonomic and functional compositions between sampling areas. These results align with a growing body of literature which showed that eDNA was able to reveal marked spatial structure in fish composition across habitats (Nguyen et al., 2020; Polanco Fernández et al., 2021; West et al., 2020).

4.4. Integration of these two methods in a combined monitoring strategy

While we found that eDNA alone detected almost all the taxonomic and functional variants identified in this study, combining this method with UVC into an integrated monitoring program could still be of interest. It would depend on the underlying research question and should combine the respective benefits of each survey method (Thanopoulou et al., 2018). First, even if our results showed that eDNA outperformed UVC, these findings were based on presence-absence data as eDNA does not yet allow a reliable estimation of fish density and biomass (Hansen et al., 2018; Sanchez et al., 2022). Therefore, if quantitative data are required to address a given question, combining eDNA with other methods providing quantitative estimates might be necessary (Aglieri et al., 2021; Rey et al., 2023). In this sense, UVC has the advantage of providing reliable information on these metrics (Murphy and Jenkins, 2010). This is particularly important for an effective assessment of FD given that species' contributions to ecosystem functioning depend on their abundance (Díaz and Cabido, 2001). Additionally, size classes and therefore information on fish demography and life stages can be accessed with UVC, which is not possible with eDNA (Rey et al., 2023). Any combination of these two methods in an integrated monitoring program to study ecological processes will also depend on the spatial scale considered. For example, while the use of eDNA metabarcoding could provide an integrated view on fish composition across different habitats, UVC offers a higher spatial fidelity on a target habitat (Aglieri et al., 2021). Additionally, we found that eDNA required a robust sampling effort to be taxonomically exhaustive. Therefore, if the area studied is large, it might be costly to reach species richness saturation on localised sites of this area using eDNA. In this case, a meaningful way to integrate both methods could be to conduct long eDNA transects across the whole area, allowing the detection of many species inhabiting a wide range of habitats, while performing UVC at small spatial scale to provide information on localised habitats. To further improve the sampling coverage of the studied area, eDNA samples could be collected in deeper

areas, as this method is not restricted by depth, unlike UVC (i.e., usually ~30 m maximum depth, Andradi-Brown et al., 2016). Therefore, a combined approach using eDNA and UVC in the same monitoring program may be of value, depending on the research objectives.

4.5. Conclusion

In conclusion, we showed that eDNA outperformed UVC for the assessment of taxonomic and, to a greater extent, functional metrics of fish diversity. In contrast with previous studies, we found using presence-absence data that eDNA alone was able to make an outstanding characterisation of fish composition. We attribute this finding to the effectiveness of our eDNA metabarcoding approach. Together these results suggest that eDNA offers a great potential to survey complex marine systems encouraging its implementation in monitoring programs. Depending on the research question, combining eDNA with UVC in an integrated sampling strategy could be justified, especially when quantitative metrics are needed. Despite the current challenges associated with eDNA metabarcoding, mainly due to limitations in database comprehensiveness, future advances in this field promise to enhance our understanding of fish assemblages and help supporting their sustainable management.

CRedit authorship contribution statement

Sylvain Roblet: Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Fabrice Priouzeau:** Writing – review & editing, Validation, Supervision, Resources, Methodology, Investigation, Conceptualization. **Gilles Gambini:** Writing – review & editing, Methodology, Investigation. **Jean-Michel Cottalorda:** Writing – review & editing, Investigation. **Julie Marty Gastaldi:** Writing – review & editing, Investigation. **Alexis Pey:** Writing – review & editing, Investigation, Data curation. **Virginie Raybaud:** Writing – review & editing, Investigation. **Gilbers Romero Suarez:** Writing – review & editing, Investigation. **Christophe Serre:** Writing – review & editing, Resources, Investigation. **Cécile Sabourault:** Writing – review & editing, Validation, Supervision, Project administration, Methodology, Investigation, Funding acquisition, Conceptualization. **Benoit Dérillard:** Writing – review & editing, Validation, Supervision, Project administration, Methodology, Investigation, Funding acquisition, Conceptualization.

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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.scitotenv.2024.177250>.

Data availability

AcMDB07, Fish16S and Vert16S raw sequence reads as well as UVC data and the functional database are available at the public repository: <https://doi.org/10.5061/dryad.9cnp5hqts>

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