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Environmental DNA (eDNA) metabarcoding is a method to detect taxa from environmental samples. It is increasingly used for marine biodiversity surveys. As it only requires water collection, eDNA metabarcoding is less invasive than scientific trawling and might be more cost effective. Here, we analysed data from both sampling methods applied in the same scientific survey targeting Northeast Atlantic fish in the Bay of Biscay. We compared the methods regarding the distribution of taxonomic, phylogenetic, and functional diversity. We found that eDNA captured more taxonomic and phylogenetic richness than bottom trawling and more functional richness at the local scale. eDNA was less selective than trawling and detected species in local communities spanning larger phylogenetic and functional breadths, especially as it detected large pelagic species that escaped the trawl, even though trawling detected more flat fish. eDNA indicated differences in fish community composition that were comparable to those based on trawling. However, consistency between abundance estimates provided by eDNA metabarcoding and trawl catches was low, even after accounting for allometric scaling in eDNA production. We conclude that eDNA metabarcoding is a promising method that can complement scientific trawling for multi-component biodiversity monitoring based on presence/absence, but not yet for abundance.

Keywords: Actinopterygii, Bay of Biscay, beta-diversity, Chondrichthyes, functional diversity, metabarcoding, phylogenetic diversity, taxonomic diversity .

Introduction

Human pressures on ecosystems can result in a rapid loss of species, genes, and ecosystem functions, representing a high risk for ecosystem integrity and human well-being (Díaz *et al.*, 2006; Cardinale *et al.*, 2012). Marine regions, especially highly productive coastal areas (Watanabe *et al.*, 2018), are threatened by human activities (e.g. fishing, nutrient pollution, human population growth, and ocean acidification; Halpern *et al.*, 2015), altering ecosystem composition, functioning, and services (Worm *et al.*, 2006). In particular, fishing activities can cause population collapse and local extirpation of species (Jackson *et al.*, 2001; Lotze and Worms, 2009). Scientific trawl surveys are conducted to inform catch management decisions and ensure the sustainability of fisheries (Trenkel *et al.*, 2019). However, they are costly and generally available only for the wealthiest countries (Trenkel *et al.*, 2019), which are more efficient in managing their marine resources (Hilborn *et al.*, 2020).

Scientific bottom trawling is the traditional method used to monitor marine bentho-demersal ecosystems and assess fish populations. By catching individuals, bottom trawling enables a quantitative estimate of fish abundance/biomass and provides information about population size structure, age at maturity, and physiological conditions, which help to determine fish quotas (Trenkel *et al.*, 2019). However, it is subject to sampling biases, such as variable catch probability according to fish size, fish behaviour (Benoít and Swain, 2003), and weather conditions during sampling (Poulard and Trenkel, 2007). Moreover, this method requires costly marine surveys with large research vessels, taxonomic expertise to identify fish, and is invasive, which raises ethical concerns (Trenkel *et al.*, 2019). In recent years, environmental DNA (eDNA) metabarcoding has emerged as a new tool applied in ecology (Deiner *et al.*, 2017), including in the marine realm (e.g. Gilbey *et al.*, 2021). eDNA is a genetic material obtained directly from environmental samples without isolating the individuals and is characterized by a complex mixture of intracellular and extracellular DNA (Taberlet *et al.*, 2012). In the eDNA metabarcoding method, species presence is detected through water filtration, polymerase chain reaction (PCR) amplification with one or several universal primers, sequencing using a high-throughput sequencer, and comparison of

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sequences with a genetic reference database (Fraija-Fernández *et al.*, 2020). eDNA metabarcoding is a non-invasive technique that capitalizes on the DNA persistence in water to detect taxa within a few hours or days after organisms have left the area (Collins *et al.*, 2018) and does not require any *in situ* taxonomic expertise (Yoccoz, 2012). Despite the openness and the dynamism of the marine system, which presents significant potential for DNA dilution and transport, several studies have demonstrated that eDNA metabarcoding enables to detect the local signature of distinct communities over short spatial distances (e.g. Port *et al.*, 2016; Jeunen *et al.*, 2019; Muff *et al.*, 2023). This technique is attractive in ecological research with various objectives, including species detection and mapping (Nester *et al.*, 2020), understanding species behaviour (Takeuchi *et al.*, 2019), deep-water monitoring (Everett and Park, 2018), and characterizing of fish diversity and habitat preference (Stoeckle *et al.*, 2017). Moreover, eDNA metabarcoding could complement and even reduce the number of trawls performed by surveys (Trenkel *et al.*, 2019). Despite these attractive aspects, eDNA metabarcoding requires laboratory facilities and equipments as well as expertise in both molecular ecology and bioinformatic to analyse the data effectively. The first comparative study between eDNA and trawling for fish (Thomsen *et al.*, 2016) indicated that eDNA holds promise but detects lower richness than trawling. In contrast, as the availability and quality of databases improve and eDNA techniques become more refined, recent studies comparing scientific trawling and eDNA monitoring methods showed that eDNA detects a higher species richness than trawling, with especially good performance for both rare (low abundance) and pelagic species (Weltz *et al.*, 2017; Afzali *et al.*, 2020).

So far, studies comparing the performances of eDNA metabarcoding and trawling methods mostly focused on the taxonomic biodiversity component by comparing the number of taxa captured by both methods, as well as the taxa preferentially captured by only one method (Fraija-Fernández *et al.*, 2020; Stoeckel *et al.*, 2020; Jiang *et al.*, 2023). However, providing a more holistic view of biodiversity also requires considering the diverse ecological functions performed by an organism within an ecosystem (Villéger *et al.*, 2017), measured as functional diversity. Studying functional diversity is crucial to identify shared biological functions and assess functional redundancy in ecosystems. The degree of redundancy is positively linked to resilience against disturbance (Borrvall *et al.*, 2000; Elmqvist *et al.*, 2003), as species with similar functional niches may replace each other if one faces extinction or collapse. Exploring functional diversity allows us to understand the ability of communities to maintain ecosystem functioning despite disturbances and to continue providing ecosystem services (Diaz *et al.*, 2006). Finally, biodiversity represents millions of years of evolution, and phylogenetic diversity (PD) acknowledges this as a key component of biological heritage (Winter *et al.*, 2013). As PD captures the successful evolutionary material filtered by millions of years of selection, it is often used as an integrative proxy to assess functional diversity, accounting for unmeasured and cryptic—yet important—functional traits (Winter *et al.*, 2013; Tucker *et al.*, 2017). Beyond the differences in taxonomic composition captured by eDNA metabarcoding and bottom trawling, it remains unclear whether both sampling methods capture taxa exhibiting similar or distinct functions and PD.

To represent the complexity of the taxa distribution across functional space or within a phylogenetic tree, it is common to use a multi-faceted approach, decomposing diversity into independent facets called richness, divergence, and regularity (e.g. Mason *et al.*, 2005; Scheiner *et al.*, 2017). Richness relates to how much of the observed/sampled phylogenetic tree or functional space is filled by the taxa, while divergence and regularity represent how the tree is structured or how the space is filled (Schleuter *et al.*, 2010). Divergence and regularity require characterizing the distances among taxa estimated from a phylogenetic tree or a functional space built from multiple functional traits (Tucker *et al.*, 2017). Divergence offers a broad indicator of the distances among species, while regularity indicates whether the species are evenly distributed in the functional space/phylogenetic tree or located at heterogenous distances (Tucker *et al.*, 2017). Combining functional divergence (FDiv) and regularity indices informs about the degree of functional redundancy and functional originality of a community (Mouillot *et al.*, 2013). So far, we lack a clear understanding of whether eDNA metabarcoding and trawling capture similar or distinct signals for the different facets of the functional and phylogenetic components of biodiversity.

Providing quantitative assessment of species abundance or biomass within ecosystems is crucial for scientific marine surveys aiming to define fish stock status and propose fishing quotas (Trenkel *et al.*, 2019). In large open marine systems, understanding whether eDNA concentration, specifically the number of eDNA reads provided by eDNA metabarcoding can serve as a reliable source of information for fish abundance or biomass represents an important challenge (Fraija-Fernández *et al.*, 2020; Stoeckle *et al.*, 2020). In open marine systems, several studies using species-specific approaches to quantify eDNA concentration [e.g. quantitative PCR (qPCR)] have revealed very strong relationships (e.g. Shelton *et al.*, 2019, 2022; Fukaya *et al.*, 2021). On the contrary, studies comparing traditional sampling methods, including trawling with eDNA metabarcoding reveal an overall positive relationship between relative quantitative estimates. However, such relationships remain weak with considerable variability among species (Lamb *et al.*, 2018; Fraija-Fernández *et al.*, 2020; Liu *et al.*, 2022; Rourke *et al.*, 2022). Moreover, allometric scaling of physiological rates associated with eDNA production and allometric relationship between body mass and body surface area indicate that larger individuals tend to have a lower eDNA production rate per mass unit (Yates *et al.*, 2021a, 2022). Several studies have shown that accounting for such allometric scaling in eDNA production improved the relationships between organism abundance and eDNA reads count both within (Maruyama *et al.*, 2014) and among species (Yates *et al.*, 2022).

In this study, we compared the results of eDNA metabarcoding and scientific bottom trawling for marine fish biodiversity monitoring in the Bay of Biscay (BoB), a Northeast Atlantic Shelf region known to be highly productive for fisheries (Moullec *et al.*, 2017). We expected eDNA metabarcoding to detect more taxa than bottom trawling, as it has been shown to perform better in this respect (Afzali *et al.*, 2020; Stoeckle *et al.*, 2020; Liu *et al.*, 2022), especially by detecting more rare species (Nester *et al.*, 2020). Furthermore, we predicted that eDNA metabarcoding would cover a larger spectrum of functional space than bottom trawling. This is because while bottom trawling is designed to target demersal species, eDNA metabarcoding can detect demersal species but also species occurring in the water column, such as pelagic fish, as well as

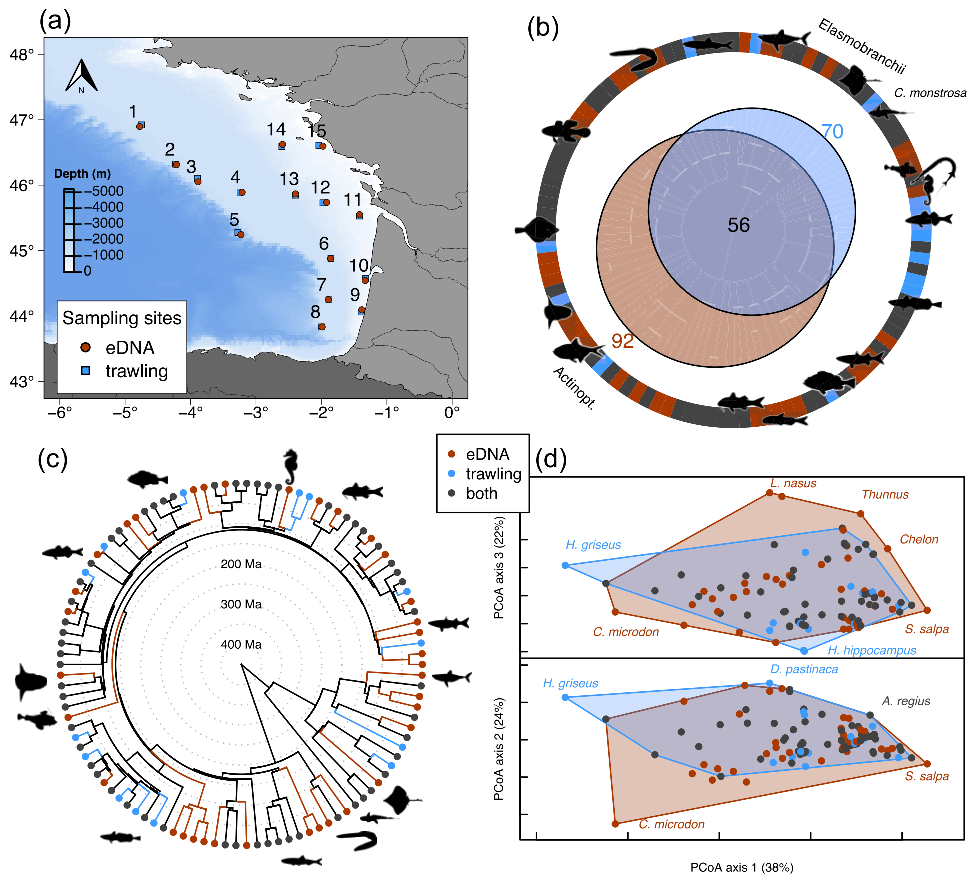

Figure 1. (a) Map of sampling sites in the Bay of Biscay in 2019. (b) Venn diagram showing the number of taxa detected by each method and their overlap after aggregation. Colours on the outer ring correspond to taxa detected by eDNA metabarcoding (red), trawling (blue), or both methods (black) at the regional scale. (c) Phylogenetic tree at the regional scale calibrated in absolute time. Each tip corresponds to a detected taxon, with colour indicating the detection method(s). Branch lengths are proportional to the evolutionary history expressed in million years (Ma). (d) Functional space at the regional scale, determined by principal coordinate analysis (PCoA; axes 1–2 and 1–3). Each point indicates one taxon, and the distance between taxa reflects the Gower distance between species based on their functional traits. Point colour indicates the sampling method(s) that detected the species across all sites. The coloured polygons are the convex hulls of all taxa detected by eDNA metabarcoding (red) and trawling (blue) .

species that can escape the trawl that have distinct functional traits. In contrast, we expected PD to be similar for eDNA and bottom trawling. To test these hypotheses, we considered several occurrence-based biodiversity indicators, including taxonomic, functional, and PD indices. Moreover, as the understanding of the relationships between the number of DNA reads and fish abundance is currently a major obstacle to the use of eDNA approaches in biodiversity monitoring (Fraija-Fernandez *et al.*, 2020; Yates *et al.*, 2019), we investigated the distributions and correlations between fish catches (number of individuals) and the number of eDNA reads while accounting for allometric scaling in eDNA production among taxa (Yates *et al.*, 2022).

Material and methods

Study area

The BoB, stretching between the northern coast of Spain and Brittany in western France, is an intracontinental sea largely open to the Atlantic Ocean. The BoB continental shelf (80000 km^2) is mostly a flat sedimentary area, with a triangle shape, narrow in the south and broader in the north. The continental shelf breaks at around 200 m depth, and a steep slope extends down to the Atlantic abyssal plain. The region is influenced by the Gulf Stream (Palter, 2015) and by freshwater inputs from the Loire and Garonne rivers (Lazure *et al.*, 2009). Consequently, the BoB is a heterogenous and highly productive area that has been identified as a major area of fish spawning and a key migration path (Borja *et al.*, 2019), supporting a high level of fishing activities (Guénette and Gascuel, 2012). As the BoB represents a transition zone between the northern and southern temperate provinces of the Northern Atlantic, its ecosystem is influenced by both provinces and therefore has higher biodiversity than adjacent areas (Punzón *et al.*, 2016). The French international EVHOE bottom trawl survey is carried out annually during autumn in the BoB to monitor demersal fish resources (Laffargue *et al.*, 2021). We chose 15 sites from the 2019 EVHOE survey for eDNA sampling. All sites were located on the continental shelf (26–170 m depth), except one on the upper slope with a depth of 1045 m (Figure 1a; Table 1).

Data acquisition by eDNA and trawling

To perform eDNA sampling, we collected water samples at 15 sites (Figure 1a). At each site, we sampled seawater using Niskin bottles deployed with a circular rosette. There were

Table 1. Summary of the biodiversity indicators, taxonomic richness, phylogenetic diversity, and functional diversity, measured by eDNA and trawling at each sampling site and considering all sites (γ diversity).

Site	Depth	Taxonomic richness		Phylogenetic diversity		Functional diversity	
		eDNA	Trawling	eDNA	Trawling	eDNA	Trawling
1	148	35	15	4 490	2 440	5.33^{-}	4.77
2	170	41	19	$5\,240^{+}$	2 790	6.61	4.63
3	156	38	19	4 920	2 690	6.36	4.02^{-}
4	129	37	16	4 860	2 580	6.57	4.21
5	1045	36	19	4 510	3 300	5.61	5.44
6	113	44	20	4 940	2 860	5.96	3.55^{--}
7	131	37	22	4 560	2 880	6.08	4.13^{-}
8	144	48	10	$5\,920^{+}$	1 710	5.75^{-}	3.23^{-}
9	34	50	13	5 520	1 860	6.29	3.26^{-}
10	36	33	20	3 770	2 490	4.80^{--}	4.46
11	38	43	19	4 990	$1\,810^{--}$	7.52	2.44^{---}
12	71	51	23	5 650	2 910	6.40	3.57^{--}
13	101	55	26	5 910	3 220	6.37	3.43^{---}
14	65	41	18	4 490	2 520	4.90^{---}	3.04^{--}
15	26	44	18	4 990	$1\,910^{-}$	6.03	3.75^{-}
All		92	70	$9\,180^{+++}$	7 060	8.79	9.36^{++}

For phylogenetic and functional diversity, significant standardized effect sizes (SES) showing over-dispersion (+) or clustering (−) are indicated.

nine bottles on the rosette, each of them able to hold ~5 l of water. At each site, we first cleaned the circular rosette and bottles with freshwater, then lowered the rosette (with bottles open) to 5 m above the sea bottom, and finally closed the bottles remotely from the boat. The 45 l of sampled water was transferred to four disposable and sterilized plastic bags of 11.25 l each to perform the filtration on-board in a laboratory dedicated to the processing of eDNA samples. To speed up the filtration process, we used two identical filtration devices, each composed of an Athena® peristaltic pump (Proactive Environmental Products LLC, Bradenton, Florida, USA; nominal flow of 1.0 l min^{-1}), a VigiDNA 0.20 μm filtration capsule (SPYGEN, le Bourget du Lac, France), and disposable sterile tubing. Each filtration device filtered the water contained in two plastic bags (22.5 l), which represent two replicates per sampling site. We followed a rigorous protocol to avoid contamination during fieldwork, using disposable gloves and single-use filtration equipment and plastic bags to process each water sample. At the end of each filtration, we emptied the water inside the capsule that we replaced by 80 ml of CL1 conservation buffer and stored the samples at room temperature following the specifications of the manufacturer (SPYGEN, Le Bourget du Lac, France).

For the bottom trawl sampling method, we counted the number of individuals per species for the trawl haul closest to the eDNA sampling site (mean distance 2.85 ± 1.5 km, minimum distance 0.25 km, maximum distance 5.8 km). Trawling was carried out during daylight for 30 min at a speed of around 4 knots. The catch of each haul thus integrated 3.5 km of distance and around 20 m in the horizontal direction (trawl opening between wings). The trawl was a standard GOV 36/47 ("Grande Ouverture Vertical") with a 4-m vertical opening and a 20-mm mesh size in the codend. Taxonomic experts identified, counted, and weighed the sampled fish during the survey (Laffargue *et al.*, 2021). We performed the fish identification at the species level, however, taxa that could not be unambiguously identified were grouped at the genus level. For example, *Trachurus mediterraneus* and *T. trachurus* were lumped in *Trachurus* sp. Additional information about sampling is available on the GitHub page indicated in the section "Data availability".

eDNA extraction, amplification, sequencing, and data processing

We processed the eDNA capsules at SPYGEN, following the protocol proposed by Polanco-Fernández *et al.*, (2020). The extracted DNA was tested for inhibition by qPCR (Biggs *et al.*, 2015). If the sample was identified as inhibited, it was diluted five-fold before amplification. We performed the DNA amplifications in a final volume of 25 μl, using 3 μl of DNA extract as the template. The amplification mixture contained 1 U of AmpliTaq Gold DNA Polymerase (Applied Biosystems, Foster City, CA, USA), 10 mM Tris–HCl, 50 mM KCl, 2.5 mM $MgCl_2$, 0.2 mM of each dNTP, 0.2 μM of each primer listed below, 4 μM human blocking primer (Valentini *et al.*, 2016), and 0.2 $\mu g\,\mu l^{-1}$ bovine serum albumin (BSA; Roche Diagnostic, Basel, Switzerland). To perform the amplification, we used the teleo primers (forward: ACACCGCCCGTCACTCT, reverse: CTTCCGGTACACTTACCATG; Valentini *et al.*, 2016) that amplify a region of 64 base pairs on average (range 29–96 bp) of the mitochondrial 12S region, designed to capture both teleost and Elasmobranchii taxa (Polanco-Fernández *et al.*, 2021). We 5′-labelled the primers with an eight-nucleotide tag unique to each PCR replicate, assigning each sequence to the corresponding sample. The tags for the forward and reverse primers were identical for each PCR replicate. We ran 12 PCR replicates per sample to increase the probability of detecting rare species (Ficetola *et al.*, 2014). We denatured the PCR mixture at 95°C for 10 min, followed by 50 cycles of 30 s at 95°C, 30 s at 55°C, and 1 min at 72°C, and we completed a final elongation step at 72°C for 7 min. After amplification, we quantified the samples using capillary electrophoresis (QIAxcel; QIAGEN GmbH, Hilden, Germany), and we purified them using the MinElute PCR Purification Kit (QIAGEN GmbH). Before sequencing, we quantified the purified DNA again using capillary electrophoresis. We pooled the purified PCR products into equal volumes to achieve a theoretical sequencing depth of 1000000 reads per sample. During all these laboratory steps, we applied a meticulous contamination control protocol (Valentini *et al.*, 2016). Specifically, we performed DNA extraction, amplification, and high-throughput sequencing in distinct dedicated rooms set up with positive or negative air pressure, UV treatment, and frequent air renewal, and we dressed in full protective clothing before entering a room. We amplified two negative extraction controls and one negative PCR control of ultrapure water (12 replicates) and sequenced them in parallel to the samples. We did not detect any contamination.

We performed library preparation and sequencing at Fasteris (Geneva, Switzerland). Specifically, we prepared four libraries using the MetaFast protocol (a ligation-based method) and sequenced them separately. We carried out paired-end sequencing using a MiSeq sequencer (2 × 125 bp, Illumina, San Diego, CA, USA) on two MiSeq Flow Cell Kits (v3; Illumina), following the manufacturer's instructions. We analysed the sequence reads using the OBITools package (http://metabarcoding.org/obitools; Boyer *et al.*, 2016), following the protocol described by Valentini *et al.* (2016). We assembled forward and reverse reads using the *illuminapairedend* program, with a minimum score of 40 and retrieving only the joined sequences. We then assigned the reads to each sample using the *ngsfilter* program and created a separate dataset for each sample by splitting the original dataset into several files using *obisplit*. After this step, we analysed each replicate sample individually before merging the taxon list. We dereplicated strictly identical sequences using *obiuniq*. We removed sequences shorter than 20 bp, those with an occurrence <10, and those labelled "internal" by the *obiclean* program due to PCR substitutions and indel errors. We performed taxonomic assignment of the sequences using the *ecotag* program with a genetic reference database formed by combining two sources: (i) the EMBL genetic reference database including 16128 sequences from 10546 species across all organisms (European Molecular Biology Laboratory, <www.ebi.ac.uk>, v141, downloaded in January 2020; Baker *et al.*, 2000) and (ii) a custom-built 12S reference database from sequenced samples taken from individual fish during previous EVHOE trawl surveys, currently containing 84 sequences belonging to 68 species of Atlantic fish. We confirmed taxonomic assignment at different taxonomic levels only when the following conditions were met: species (match > 98%), genus (96% < match ≤ 98%), family (90% < match ≤ 96%) (Marques *et al.*, 2020). We discarded all sequences with a frequency of occurrence <0.001 per sequence and per library to account for tag jumps (Schnell *et al.*, 2015). We further corrected for index-hopping (MacConaill *et al.*, 2018) with a threshold empirically determined using experimental blanks between libraries. We only kept species and genera from the identified sequences for diversity analyses. To ensure that our biodiversity estimates were conservative, we removed taxa identified in only one PCR replicate and that had fewer reads than the 10% quantile threshold of all reads.

eDNA filter replicability

We quantified the dissimilarity between sets of taxa sampling units (between filter replicates within sites or between sites) by calculating the Jaccard dissimilarity index (β_{jac}; Baselga, 2012), which ranges from 0, when taxonomic compositions are identical between sampling units, to 1, when they are completely distinct. To disentangle whether the taxonomic dissimilarities between sampling units were driven by taxonomic turnover or by a difference in richness, we decomposed the β_{jac} index into the taxonomic turnover (β_{jtu}) and the nestedness-resultant components (β_{jne}; Baselga, 2012). Following Rozanski *et al.* (2022), we assumed that good replication between replicates within a site would result in low overall dissimilarity (β_{jac}) dominated by the nestedness component, indicating that most of the species' composition was detected in the two replicates (i.e. from the two filters). In addition, we expected good replication if the average dissimilarities between replicates within sites were smaller than the average dissimilarity among sites. To compute the average dissimilarity among sites, we averaged the pairwise dissimilarities between eDNA sample replicates belonging to different sites. We then pooled the species list of the two sample replicates per site for the subsequent biodiversity analyses.

Data aggregation

The lowest taxonomic level at which we assigned the sequences was the species level, but in some cases, we could only identify the sequences at the genus level. For ambiguous assignments, we aggregated all species belonging to the same genus to the genus level when one observation was restricted to a genus identification. For example, we merged the detected taxa *Notoscopelus*, *N. elongatus*, and *N. kroyeri* into the genus *Notoscopelus*. When the genus had only one known species in the region, we replaced it by the species. For example, the genus *Sardina* and the species *S. pilchardus*, and we combined them into *S. pilchardus*. As several taxa could not be unambiguously identified at the species level for the bottom trawl dataset (as previously indicated in the section "Data acquisition by eDNA and trawling"), we used the taxonomic level (species or genus) for taxa detected by trawling and by eDNA sampling (Supplementary Table S1). To perform taxa aggregation and analyses based on fish clades, we retrieved the taxonomic classification from the Barcode of Life Data System (BOLD; Ratnasingham and Herbert, 2007) and the World Register of Marine Species (WoRMS; Horton *et al.*, 2022), querying them online through the *taxize* package v0.9.99 (Chamberlain and Szöcs, 2013) in R v4.1.2 (R Core Team, 2023).

Fish traits and phylogeny

We measured functional diversity using nine traits associated with several ecosystem functions (habitat, feeding, reproduction, and mobility; Villéger *et al.*, 2017): maximum length, average depth and its range, trophic level, position in the water column, body shape, reproduction mode, fertilization mode, and parental care type (Supplementary Table S2). We retrieved 89% of the traits from the online Fishbase database (Froese and Pauly, 2022) and complemented missing values with information from experts and from a reference guide (Quéro *et al.*, 2003) to fill a trait table to 95%. We centred and normalized all quantitative traits. For taxa detected or merged at the genus level, we randomly selected one species of that genus occurring in the Eastern Atlantic from the reference guide (Supplementary Table S2). We repeated this random selection 100 times. We then computed 100 distance matrices between all pairs of species, based on the 100 trait tables, using the Gower distance, which accounts for different types of traits and missing data (de Bello *et al.*, 2021). To calculate PD indices, we used a distribution of 100 phylogenetic trees delineated at the species level to account for phylogenetic uncertainty. We used a similar phylogenetic approach as in Rozanski *et al.* (2022). The method used to create the trees is explained in Supplementary Materials Method S1.

γ and α diversity indices

We measured taxonomic richness using the species richness (SR) index, i.e. the number of taxa. To evaluate the impact of sampling effort on the number of detected taxa at the regional scale (i.e. γ diversity) for eDNA and trawling, we built a taxonomic accumulation curve fitted with an asymptotic model using the R package *vegan* v2.5–7 (Oksanen *et al.*, 2020). We estimated the accumulation rate and asymptotic richness for each sampling method and tested the average taxonomic richness difference per order between eDNA and trawling with a Pearson's -test. Since we considered all species equivalent and only accounted for taxa presence or absence, we did not consider the regularity and divergence facets for the taxonomic component. For the phylogenetic component, we measured phylogenetic richness by calculating the PD index (Faith, 1992), corresponding to the sum of all branch lengths in the phylogenetic tree associated with the sampled community. We measured phylogenetic divergence using the mean pairwise distance (MPD) index, defined as the average phylogenetic distance between all pairs of species (Tucker *et al.*, 2017). We estimated phylogenetic regularity by computing the variance in pairwise distances (VPD), calculated using the R package *PhyloMeasures* v2.1 (Tsirogiannis and Sandel, 2015).

For functional diversity, we assessed the functional richness facet using the 0-order ($q = 0$) functional Hill number (FD hereafter), denoting the number of equivalent functional entities at a site or in an assemblage (Chao *et al.*, 2019). FD is computed directly from the Gower distance matrix and not based on the reconstructed functional space. For comparison with FD, we also computed the FRic index (Villéger *et al.*, 2008), denoting the volume of the convex hull formed by species in the functional space (Mouillot *et al.*, 2013). The functional space was reconstructed using a principal coordinates analysis (PCoA) using the first 5 axes. We estimated functional regularity as functional evenness (FEve), which corresponds to the size of the minimum spanning tree linking all species in the functional space. We assessed the functional divergence (FDiv) by computing the mean distance of detected taxa from the centre of gravity of the functional space (Villéger *et al.*, 2008). We calculated these indices for each of the 100 generated functional spaces with the R package *mFD* v1.0.1 (Magneville *et al.*, 2021), and we retained the average value. For each functional trait, we also used a Pearson's -test to compare the ability of the two sampling methods to detect fish with different trait modalities. We computed all the taxonomic, phylogenetic, and functional diversity indices presented above at both the regional and local (site) scales to document the γ and α diversity, respectively.

Species richness influences both phylogenetic and functional richness (Tucker and Cadotte, 2013). However, the independence of these indices is crucial for comparing the three biodiversity components. Therefore, we decoupled phylogenetic and functional measures of diversity (PD, MPD, VPD, FD, FEve, FDiv) from their relationships with taxonomic richness for each sampling site by calculating the standardized effect sizes (SES). SES quantifies the difference between an observed phylogenetic/functional index of diversity and an expected distribution of the same diversity index under a null model of random association of taxa with their phylogenetic relationships or biological traits. The random association is performed by shuffling the species identity 99 times for each of the 100 phylogenetic trees and each of the 100 trait tables to get a null distribution of the diversity indices. SES are computed by subtracting the observed value of the diversity index of interest by the average diversity value obtained from the null distribution and divided by the standard deviation of the null distribution (Leprieur *et al.*, 2012). SES values <0 and conversely SES values >0 indicate that given the taxonomic richness, the observed diversity index of interest is lower and higher, respectively, than expected under a null model of random selection of taxa from the total pool of taxa. Considering that the null distribution follows a standard normal distribution, we used the 95% percentile interval (i.e. 0.025 and 0.975) to detect significant clustering and overdispersion for SES <-1.96 and SES >1.96, respectively, while values within this interval are considered not different from the null model. We computed SES values at both the regional and local scales to document γ and α diversity for the two sampling methods, using a regional pool of taxa combining the taxonomic lists provided by eDNA and trawling.

β diversity indices

First, we assessed how much the taxonomic dissimilarities between the eDNA and trawling methods within a site were driven by taxa turnover (β_{jtu}) or nestedness (β_{jnes}) by decomposing the Jaccard dissimilarity index (β_{jac}). Then, to assess how much the taxonomic dissimilarities among sites and among depth strata differed between the two sampling methods, we computed the Jaccard dissimilarity index (β_{jac}) among all pairwise site and method comparisons, using the R package *betapart* v1.5.4 (Baselga and Orme, 2012). Then, to visualize the similarities among sites and methods, we performed a PCoA on this Jaccard dissimilarity matrix, using the R package *ade4* v1.7.18 (Dray and Dufour, 2007). To further assess a potential depth effect on the species composition identified with the two sampling methods, we formed four equal-sized groups of sites based on a 100-m depth threshold, which corresponds to the median depth of sampling sites and separates shallow and deep sites (sites < 100 m, sites > 100 m). We drew ellipses of dispersion with a size equal to 1.5 times the standard deviation in principal directions of variance.

Qualitative comparison of abundance estimates

To assess if eDNA metabarcoding and bottom trawling could offer similar quantification of the relative taxa abundance, we fitted a linear model (LM) between the logarithm (base 10) of the number of individuals from trawling and the relative number of reads from eDNA metabarcoding whenever a species was detected by both methods within a site. First, we estimated a general relationship between abundance measures, considering all sites and species. We tested whether accounting for the model residuals' heterogeneity by performing a generalized least square (GLS) model using an exponential variance structure improved the model fit and affected the relationship. In addition, we tested the robustness of the GLS model by accounting for spatial autocorrelation in the model residuals using an exponential correlation structure (Zuur *et al.*, 2009). We tested the improvement in the model fit between LM and GLS using the Akaike information criterion (AIC) while fitting the GLS model using a maximum likelihood optimization approach implemented in the R package *nlme* (Pinheiro *et al.*, 2021). We assessed the explained GLS model variation using the Cox and Snell pseudo-R^2 (Cox and Snell, 1989).

Second, we fitted the same linear regressions among taxa for each eDNA filter independently within each site because the purified PCR products of each filter were pooled in equal volume before sequencing, which prevented a robust comparison of the number of eDNA reads between filters. Finally, we tested whether accounting for allometric scaling in eDNA production improved the relationships between organism abundance and eDNA reads count, and we explored which allometric scaling coefficient maximized such relationships (Yates *et al.*, 2022). To do so, we first computed the allometric scaled abundance of individuals per species through the following formula proposed by Yates *et al.* (2022):

$$\mathrm{APT_i} = (x_i^b) * \mathrm{N_i},$$

where APT is the allometric scaled abundance per trawl for the *i*th species, x_i is the individual mean weight of the *i*th species, N_i is the number of individuals of the *i*th species per trawl, and b is the interspecific allometric scaling coefficient. For values of 0 and 1, b corresponds exactly to the count of individuals per species per trawl and the total biomass per species per trawl, respectively. For each eDNA sample and its closest associated trawl, we determined the optimal b interspecific scaling coefficient. We did this by iteratively running all generalized linear models (GLM) between the number of eDNA copies and the allometric scaled abundance (b) for all values of b between 0 and 1, with an increment of 0.01. We implemented GLMs with a negative binomial distribution error and a log link function to account for the overdispersion of the number of eDNA reads, using the R package *MASS* (Venables and Ripley, 2002). We retained the model with the lowest AIC as the best model. We also estimated the explained model variation using a pseudo- R^2 based on deviance (Zuur *et al.*, 2009). To assess whether accounting for allometry provided a better model fit than the linear relationships between the relative number of eDNA reads and the log number of individuals per trawl, for each filter we compared the R^2 of the LM model in step 2 with the pseudo-R^2 of the GLM fitted with the best b coefficient in step 3. We also tested the best allometric coefficient for the relationships with the relative number of eDNA reads per taxa per filter using an LM to assess if accounting for allometry provided a better model fit than the linear relationships between the relative number of eDNA reads and the log number of individuals per trawl. The R scripts, along with the corresponding data, used to calculate the diversity indices and the abundance estimates and those used to create the figures in this manuscript are provided in the repository https://github.com/pierre-veron/eDNA-trawl.

Results

eDNA replicability

The average dissimilarity in taxa composition between the eDNA sample replicates of each site was $\beta_{\mathrm{jac}} = 0.372$ (*SD* ± 0.095) and was mainly explained by taxonomic turnover ($\beta_{\mathrm{jtu}} = 0.262 \pm 0.108$) rather than by the nestedness component ($\beta_{\mathrm{jne}} = 0.110 \pm 0.099$). When comparing eDNA sample replicates from different sites, we found $\beta_{\mathrm{jac}} = 0.536$ (*SD* ± 0.098), which is mostly driven by taxonomic turnover ($\beta_{\mathrm{jtu}} = 0.435 \pm 0.133$) rather than by the nestedness ($\beta_{\mathrm{jne}} = 0.101 \pm 0.085$). Thus, eDNA sample replicates from the same site were more similar than sample replicates from different sites.

γ diversity

The taxonomic assignment of eDNA sequences resulted in the identification of 1039 unique sequences at the family or lower taxonomic level (33% species level, 34% genus level, and 33% family level), corresponding to 202 different taxa (128 genera or species). After data aggregation and harmonization between the two methods, we retained 92 taxa, among which 79 were Actinopterygii (27 orders; 74 genera; and 55 species), 12 Elasmobranchii (7 orders; 13 genera; and 9 species), and 1 Holocephali (*Chimaera monstrosa*). At each site, we detected 33 to 55 taxa (42.2 on average) based on 31300 reads on average.

Scientific trawl catches included 250000 fish individuals, with an average of 17000 individuals per sampling site. We identified 84 taxa, and after taxonomic harmonization we retained 70 taxa: 60 Actinopterygii (belonging to 22 orders; 57 genera; and 44 species), 9 Elasmobranchii (5 orders; 8 genera; and 5 species), and 1 Holocephali (*C. monstrosa*). We detected 56 common taxa between eDNA and trawling (Figure 1b). On average, 4.2 trawl hauls caught the same taxonomic richness (SR) as a single eDNA site (when pooling both replicates per site; Figure 2). The estimated asymptotic taxonomic richness $\mathrm{SR_{max}}$ was 93 for eDNA, 75 for trawling, and 108 for the combined dataset. The number of taxa detected per order differed significantly between eDNA and trawling ($\chi^2 = 71$, df $= 35$, $p < 0.01$; Supplementary Figure S1). eDNA detected fewer Pleuronectiformes, Gadiformes, Carangiformes, and Callionymiformes but more Spariformes and Beloniformes compared with trawling (Supplementary Figure S2).

Considering PD, the detected species had an estimated common ancestor dating back to 446 ± 44 Ma (Figure 1c). The total PD was 10040 ± 250 Ma (9180 ± 220 Ma for eDNA and 7060 ± 150 Ma for trawling). The SES showed that eDNA phylogenetic richness was significantly higher than expected under the null model, indicating phylogenetic overdispersion ($\mathrm{SES_{PD}} = 3.56$, $p < 0.01$), while for trawling it was not different from the null model ($\mathrm{SES_{PD}} = 1.49$, $p = 0.14$). By contrast, SES values of phylogenetic divergence (MPD) and regularity (VPD) were similar for both sampling methods, showing no significant deviation from a random selection of taxa ($\mathrm{SES_{MPD}} = 1.46$ for eDNA and 0.83 for trawling; $\mathrm{SES_{VPD}} = 0.95$ for eDNA and 0.90 for trawling).

Regarding functional diversity, the first three axes of the PCoA based on nine functional traits explained 84% of the traits' variation (axis 1: 38%, axis 2: 24%, and axis 3: 22%). The total measured functional richness (FD) was 9.46 ± 0.12 functional entities, and the functional richness was slightly higher for trawling ($\mathrm{FD_{trawling}} = 9.36$) than for eDNA ($\mathrm{FD_{eDNA}} = 8.78$). Both methods had a higher FD than expected under a random selection of taxa; however, only trawling showed significant functional overdispersion ($\mathrm{SES_{FD\_trawling}} = 2.69, p < 0.017; \mathrm{SES_{FD\_eDNA}} = 0.85, p = 0.4$). These results were confirmed by the FRic index based on the volume of the convex hull, with trawling and eDNA capturing 81% and 78% of the total functional space defined by the first five axes of the PCoA, respectively (Figure 1d). In terms of FDiv, both sampling methods (especially trawling) identified taxa that tended to be less functionally divergent than the

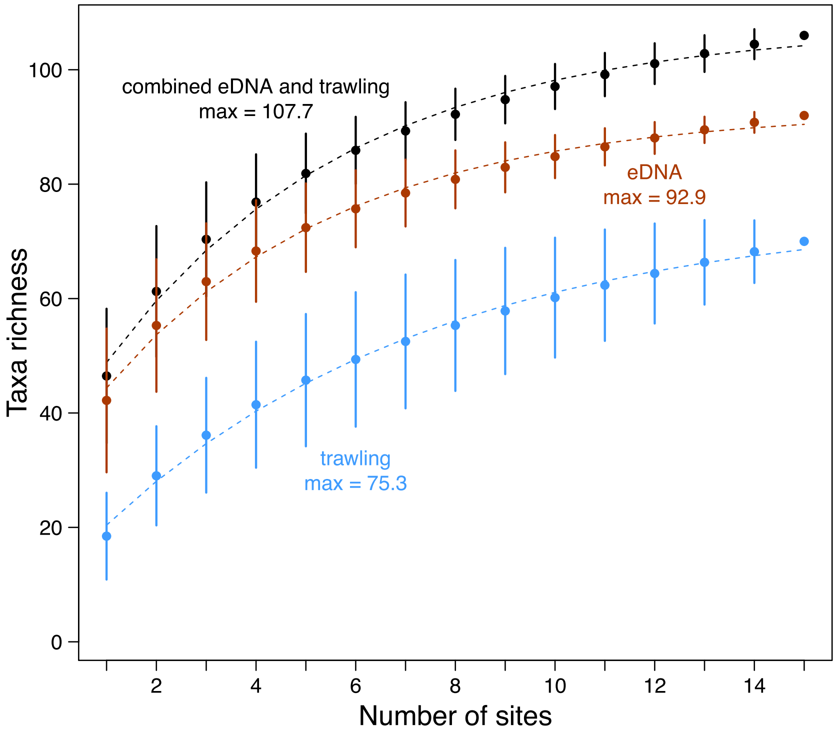

Figure 2. Taxonomic accumulation curves for taxa collected by eDNA metabarcoding (the two sample replicates from each site were pooled together), trawling or combined. The dotted lines correspond to a fitted asymptotic model. eDNA metabarcoding: rate of accumulation = 0.213, initial SR0 = 32.9; trawling: rate of accumulation = 0.150, SR0 = 11.5; combined dataset: rate of accumulation = 0.203, SR0 = 35.6. The asymptotic richness (max) is indicated for each method. Circles represent the average taxonomic richness for a given class of the sites sampled, and bars represent the standard deviation.

null model ($SES_{FDiv\_trawling} = -1.65$, $p = 0.099$; $SES_{FDiv\_eDNA} = -0.43$, $p = 0.67$). Finally, the SES values for functional regularity (FEve) showed no deviation from the null model, regardless of the sampling method ($SES_{FEve\_trawling} = -0.28$, $p = 0.78$; $SES_{FEve\_eDNA} = -0.015$, $p = 0.99$).

Fish detected by eDNA significantly differed in their habitat use compared with those caught by trawling: eDNA captured more pelagic and bathypelagic species but fewer demersal/benthic species than trawling ($\chi^2 = 14$, df = 4, $p = 0.006$; Supplementary Figure S2a). In terms of body shape, eDNA captured more fusiform and elongated fish and fewer flat fish than trawling ($\chi^2 = 18$, df = 6, $p < 0.001$; Supplementary Figure S2c). Moreover, the eDNA method detected more fish of low trophic level (<3) and fewer fish of intermediate trophic level (3–4) than trawling ($\chi^2 = 32$, df = 8, $p < 0.01$; Supplementary Figure S2a). Other traits (depth range, reproduction, and length) showed no differences between sampling methods.

α diversity

For all sites, eDNA systematically captured more taxonomic (details of taxa detected at each site and with each method are provided in Supplementary Figures S3 and S4 and S5), phylogenetic richness than trawling (Figure 3, Table 1; Supplementary Figures S3 and S6). eDNA sample capture in average 42.2 taxa (*SD* = 6.5), while trawling 18.5 (*SD* = 3.9; Supplementary Table S3). The SES of the PD index were significantly higher for eDNA (paired Student's *t*-test = 113, $p < 0.001$) and showed a trend for overdispersion ($SES_{PD} = 0.96 \pm 0.8$) and clustering ($SES_{PD} = -0.33 \pm 1.09$) for communities sampled with eDNA and trawling, respectively (Supplementary Table S3, Figure 3a). For eDNA, 2 sites out of 15 showed a significantly higher PD than the null model (sites 2 and 8, Figure 3b). For trawling, two sites (11 and 15) had a lower PD than the null model, and none had a higher value. Regarding functional richness at the local scale on average communities sampled by both methods tended to be all clustered (SES < 0; Figure 3a) but contrary to the results at the regional scale (γ diversity), functional richness of the communities sampled by eDNA ($SES_{FD} = -1.70 \pm 0.9$) tended to be less clustered than those sampled by trawling ($SES_{FD} = -2.3 \pm 0.91$) even though those difference remained non-significant (paired Student's *t*-test = 89, $p = 0.11$) despite that more sites (67%) showed significant clustering for trawling than for eDNA (27%). Only one site (8) showed a significantly low SES_{FD} for both methods. Both SES of the phylogenetic (MPD) and functional divergence (FDiv) indices were positive with eDNA and negative with trawling; however, differences were not significant (Supplementary Table S3), and none of the sites but one sampled by eDNA (site 4, $FDiv_{SES} = 2.37$) showed deviation from the null model (Figure 3c). For the regularity facet, the SES of the functional index (FEve) was >0 on average for eDNA and negative for trawling, while SES of the phylogenetic index (VPD) were negative on average for both sampling methods, however, none of the differences were significant (Supplementary Table S3). At local scale, SES of the regularity facet for the functional index were significantly clustered and overdispersed for four sites with trawling and eDNA, respectively, while for the phylogenetic regularity in-

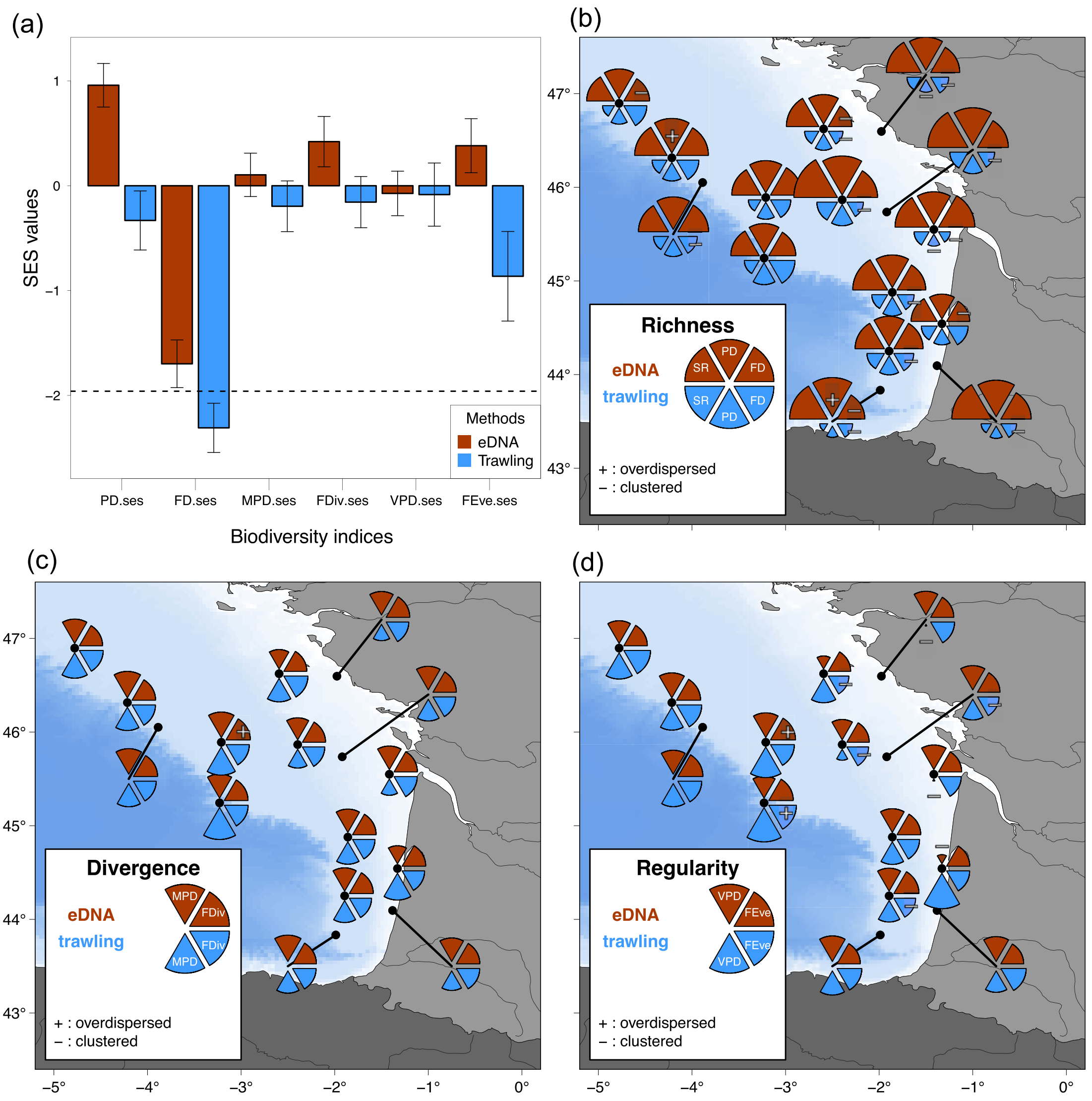

Figure 3. Comparison of the average standard effect size (SES) of the different phylogenetic and functional indices, including the richness, divergence, and regularity facets (a) associated with the two sampling methods (eDNA vs. trawling). Colour bars showed the average value over the 15 sites and error bars represent the standard error. The dashed horizontal line indicates a threshold of significant clustering (−1.96) for the SES of the indices. Spatial distribution of the α-diversity indices of (b) richness, (c) divergence, and (d) regularity separated by site and by sampling method for the three biodiversity components: taxonomic, phylogenetic, and functional. The radius of each slice is proportional to the observed value of the index. For functional and phylogenetic diversity, indices significantly different from the null model (based on the SES) are indicated with − for clustering and + for overdispersion.

dex, one site (10) and two (11 and 15) were significantly clustered for eDNA and trawling, respectively (Figure 3d), none of the other sites deviated from the null model regardless of the sampling method and the phylogenetic and functional components considered (Figure 3d).

β diversity

Within a site, the average dissimilarity in taxa composition between eDNA and trawling was high ($\beta_{jac} = 0.692 \pm 0.073$), equally driven by turnover ($\beta_{jtu} = 0.337 \pm 0.201$) and nestedness ($\beta_{jne} = 0.356 \pm 0.191$). The first three axes of the PCoA explained 51% of the total inertia of spatial species compositional variations (axis 1: 21.5%; axis 2: 17.8%; axis 3: 11.3%; Figure 4a ; Supplementary Figure S7). They showed a marked difference in species composition between trawling and eDNA, with the two sampling methods forming disjoint sets in the PCoA space (Figure 4a). Beyond sampling methods, sites were also clearly separated by depth, with the deep (>100 m depth) and the shallow sites (<100 m depth) forming almost disjoint ellipses (Figure 4a). Interestingly, the species composition was more stable among sites sampled by eDNA than by trawling, for both shallow and deep sites, as shown by the smaller ellipses for eDNA than for trawling. However, the spatial ordination of sampling sites from coastal (shallow) to offshore (deep) was similar for the two sampling methods (Figure 4b).

Abundance

Overall, across species and sites, we found a positive relationship between the average relative number of eDNA reads and the log number of individuals within a trawl (LM: $p < 0.001$, $R^2 = 0.14$; GLS: $p = 0.002$, Cox and Snell *pseudo-R* $^2 = 0.23$; Supplementary Figure S8). However, even though the relationship was unaffected by spa-

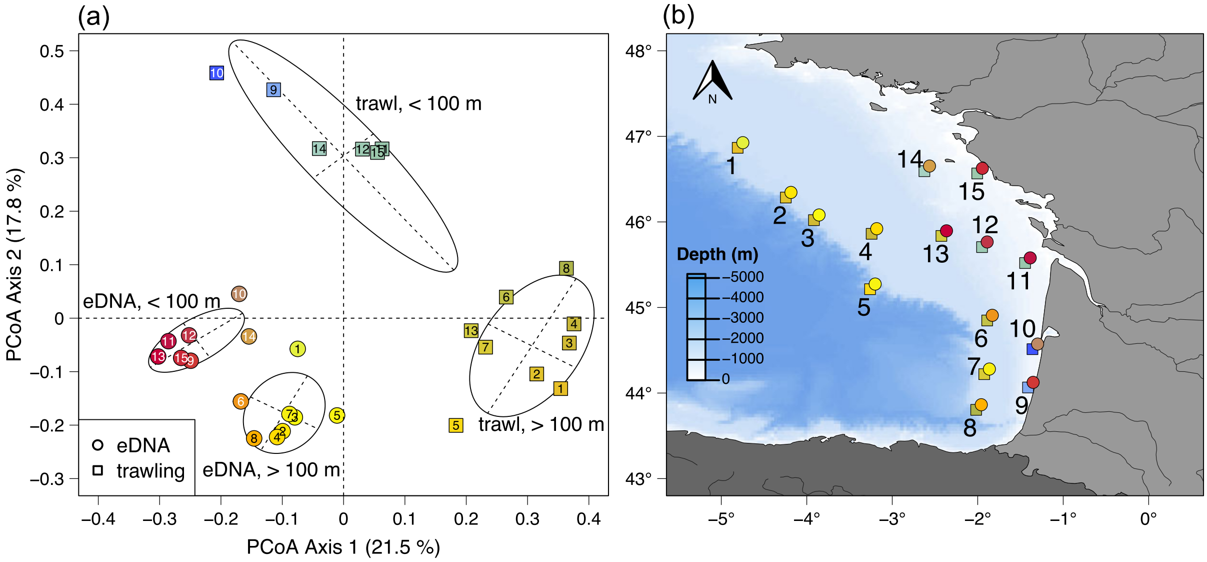

Figure 4. (a) First two axes of a PCoA showing the compositional differences between the species composition sampled by eDNA metabarcoding (circles) and by bottom trawl hauls (squares), based on the Jaccard dissimilarity distance. Ellipses display the dispersion of the sampling sites according to depth and sampling method. (b) Geographical positions of the corresponding sites, with seven sites are shallow sites (<100 m), while eight are deep sites (>100 m). The colour of each point corresponds to its position in the PCoA space: points with similar colours share a similar species composition.

tial autocorrelation in the model residuals, the relationship was weak and the variability in eDNA read numbers increased with increasing fish abundance, as suggested by the better fit of the GLS model, which accounted for the heterogeneity in model residuals (exponential residual parameter > 0; Supplementary Figure S8). To avoid an influence of the standardization of the amount of PCR product among filter replicates, which could have affected the global relationship, we also ran the same analysis among species but per filter per site. Here, we found a significant positive relationship with trawl log-transformed abundance for 8 of the 30 filter replicates over the 15 sites (Supplementary Figure S9). Accounting for the optimal interspecific allometric scaling coefficient in the relationships with the number of eDNA reads did not improved the previous relationships between the relative number of eDNA reads and the trawl log-transformed number of fishes, as only six filters retained a positive and significant relationship (Supplementary Figure S10), and the explained variation of the models were lower on average by almost 6%. These relationships were mostly driven by one or two taxa, especially *Tachurus* sp., as only three filters kept a significant positive relationship after its removal. However, accounting for the optimal interspecific allometric scaling coefficient in the relationship with the relative number of eDNA reads per taxa (Supplementary Table S4), showed greater consistency with 11 filters having a significant positive relationship (Supplementary Table S4 and Figure S11) and the proportion of explained variation increased by 12% on average in comparison to the models accounting for the log of the number of fish. After removing *Trachurus* sp., the relationships remained for six filters. For the latter relationships, the optimization of the interspecific scaling coefficient revealed that for 15 filters the relationship was better fitted when the raw abundance was accounted for ($b = 0$), while for 11 filters the relationship was better when the raw fish biomass was accounted for ($b = 1$). For the four remaining replicates, the best interspecific scaling coefficient was distributed between 0.08 and 0.86 (Supplementary Table S4).

Discussion

In this study, we compared the detection and selectivity of eDNA metabarcoding with classical bottom trawling in the BoB in terms of taxonomic, functional, and phylogenetic diversity for fish communities. We showed that this method was able to detect higher taxonomic diversity than trawling, with less sampling effort, and that fish communities detected by eDNA metabarcoding also reflected differences in species composition with water depth and coastal proximity. In addition, this method captured communities with broader phylogenetic diversity than trawling. This trend was also true for functional diversity especially at the local scale but to a lesser extent than for the phylogenetic diversity as it is not independent from the taxonomic richness. The relationships between eDNA read numbers and trawl fish abundance or biomass were variable and unclear, preventing the use of eDNA metabarcoding for reliable quantitative abundance estimation, despite our efforts to account for the optimal interspecific allometric scaling abundance coefficient. Despite that we cannot totally rule out some potential contamination of eDNA samples among sites, we have shown through a sensitivity analysis that our results are robust to the removal of taxa, the most susceptible to contamination (e.g. the most abundant taxa showing multiple successive occurrences, see Supplementary Figure S12).

At the local scale, the eDNA method detected more taxonomic diversity than trawling, and on average 4.2 trawls were necessary to sample the same taxonomic richness as two eDNA filter replicates. Such a difference in sampling effort is already impressive and is even more so if we consider the difference in the volume of water sampled by the two methods, which is about 45 l per site for eDNA in comparison to the 280 million litres of water (3500 m distance × 20 m horizontally × 4 m vertically) sampled with a single trawl. Moreover, trawling requires an oceanographic vessel and a crew of around ten people just for sorting and identifying the catch (excluding the crew required to run the vessel), while eDNA demands less effort during sampling (a single person), though sampling sites still need to be reached. On the other hand, eDNA analysis does require a clean lab, time, and staff for post-sampling treatment of the collected samples. Most previous studies have concluded that eDNA metabarcoding can detect more taxa than classical methods with less sampling effort (e.g. Polanco-Fernández *et al.*, 2020), even compared with trawling (Afzali *et al.*, 2020). eDNA can detect certain species that are generally not detected by visual census (e.g. pelagic, mobile fish, or crypto-benthic species; Aglieri *et al.*, 2020; Boulanger *et al.*, 2021). In our case, species belonging to the genus *Thunnus* were only detected with eDNA metabarcoding, probably because they are large and fast pelagic fish able to escape the trawl and mainly found in the upper part of the water column. eDNA metabarcoding was also able to detect rare and vulnerable species (Polanco-Fernández *et al.*, 2021; Liu *et al.*, 2022), such as the marbled electric ray (*Torpedo marmorata*), the shark spiny dogfish (*Squalus acanthias*), and the ocean sunfish (*Mola mola*), which were not detected by trawling but are known to occur in the BoB.

Communities of fish detected by eDNA showed a similar spatial pattern in species composition as observed with trawling, mostly structured along the water depth gradient and the proximity to the coast (Figure 4a). Taxonomic dissimilarities among sites were stronger with trawling than with eDNA. Two mutually non-exclusive hypotheses could drive this result. First, although most studies have indicated a strong localization of eDNA signals (Polanco-Fernández *et al.*, 2020; Miya, 2022; Rozanski *et al.*, 2022), previous research has demonstrated the persistence of eDNA in temperate marine waters (Collins *et al.*, 2018; Andruszkiewicz *et al.*, 2019), permitting long-range transport. Such an effect may have homogenized the species composition sampled by eDNA, decreasing the taxonomic dissimilarities among sites. Second, the ability of eDNA metabarcoding to capture ubiquitous and abundant small and medium size pelagic species (e.g. *Trachurus* sp., *Engraulis* sp., *Sardina pilchardus*, *Scomber scombrus*, *Scomber colias*, *Sprattus sprattus*, see also Fraija-Fernandez *et al.*, 2020), occurring in most sites yet not always detected by trawling, have contributed to decrease the compositional dissimilarities among communities detected with eDNA (eDNA β_{jac} increases from 0.47 to 0.51 without those ubiquitous pelagic taxa). However, both sampling methods revealed gradients in species composition in the BoB that match those previously documented (Persohn *et al.*, 2009; Eme *et al.*, 2022). Community discrimination was mostly based on rare taxa in our survey, e.g. *Notoscopelus* sp., *Myctophum punctatum*, *Lampanyctus* sp., *Beryx splendens*, and *Xenodermichthys* sp. detected in offshore sites and *Argyrosomus regius*, *Umbrina* sp., *Alosa fallax*, and *Boops boops* mainly detected in coastal sites. Hence, the detectability by eDNA of rare species or species escaping the trawl (e.g. several pelagic species such as *Thunnus* sp., *Sarda sarda*, and *Squalus acanthias*) made it possible to detect a higher species richness and helped to refine species composition differences among environmental gradients.

In our study, eDNA metabarcoding sampled a diversity of taxa covering a broader part of the phylogenetic tree than trawling. Our results revealed that the richness facet was the most influenced by the sampling methods, while the divergence and regularity facets were rarely different from a null model of functional or phylogenetic association for both methods. Overall PD was higher when considering taxa detected by eDNA, and SES analyses showed that these taxa tended to be overdispersed on the phylogenetic tree, contrary to taxa caught by trawling that were more phylogenetically clustered. These results are in line with those reported by Rozanski *et al.* (2022), where communities detected with eDNA were phylogenetically overdispersed. The detection of rare taxa, or taxa rarely capture by the trawl spans in general more distinct phylogenetic lineages by including taxa belonging to Spariforms, Beloniforms, Myctophiformes, Alepocephaliformes, Squaliformes, while trawling is more prone to detect Gadiforms, Carangiformes and flat fish, which all belong to the order Pleuronectiformes. In terms of functional diversity, when comparing eDNA detection and trawling at the regional level, trawling captured more functional richness, due to the detection in one site of one peculiar species, the bluntnose sixgill shark (*Hexanchus griseus*). By contrast, at the site level the functional space occupied by taxa detected with eDNA was more extensive and diverse than that detected with trawling. However, this trend was mostly driven by the increase in taxonomic richness detected by eDNA and the strong link between taxonomic and functional richness. After correcting for differences in taxonomic richness, SES of the functional richness were on average less clustered for eDNA than for trawling but differences were not statistically significant (Figure 4a, Supplementary Table S3). Therefore, our results are consistent with those of Aglieri *et al.* (2020), who found that eDNA metabarcoding recovered communities with a wider spectrum of functions than fishery observations and visual/video censuses but are reported for the first time in comparison with trawling. Regardless the link with taxonomic richness, these results suggest that eDNA metabarcoding is less selective than trawling in detecting fish with a broader range of functions; it recovered more pelagic, bathypelagic, and fusiform taxa but fewer demersal and flat fish taxa than trawling. Indeed, the GOV bottom trawl targets fish living in or close to the seabed (up to around 4 m), explaining why trawling captures more flat fish (e.g. Pleuronectiformes) than eDNA metabarcoding, where samples were collected from around 5 m above the seabed. The bottom trawl, scraping the seabed, also favoured the detection of species buried in the sediments, which may be less prone to releasing eDNA in the water column. By contrast, pelagic taxa, such as *Mola mola*, *Thunnus* sp., and *Pagellus* sp., were almost exclusively detected by eDNA, even though they are known to be locally abundant in the BoB. The development of alternative eDNA sampling strategies where water is sampled from the seabed to higher up in the water column or close to the seabed after minimal sediment resuspension (i.e. in the trail of a towed underwater camera) offers great potential to improve the detection of benthic and flat fish in addition to pelagic species.

So far, the eDNA sampling approach presented here, with Niskin bottles deployed on a circular rosette 5 m above the seabed, cannot fully replace trawling because some species are only detected with this latter method. This result is confirmed by other studies showing that eDNA metabarcoding and traditional methods are complementary (Polanco-Fernandez *et al.*, 2020; Keck *et al.*, 2022). Such results can be partly explained by the lack of completeness of the genetic reference databases, which prevents a full taxonomic detection by eDNA metabarcoding (Miya, 2022), even though databases covering European areas are more complete than some other regions (Marques et *al*., 2021). In our study, we added to the 12S EMBL reference database a regional custom database, which have improved taxonomic assignment (Mugnai *et al*., 2023). Among the 14 taxa detected by trawling and not by eDNA (Figure 3), 5 were missing from the genetic reference database (*Ammodytes marinus*, *Gaidropsarus macrophthalmus*, *Microchirus variegatus*, *Mora moro*, and *Phycis blennoides*; the last four did not even have a representative of the same genus), and 6 were easy to discriminate with the teleo primer, preventing misassignment at a higher taxonomic level *(Hippocampus hippocampus*, *Lithognathus mormyrus*, *Phycis blennoides*, *Atherina presbyter*, *Hexanchus griseus*, and *Dasyatis pastinaca*). However, for three other species detected only by trawling, *Merlangius merlangus*, *Nezumia aequalis*, and *Lepidotrigla dieuzeide*, their detection was not possible with the teleo primer because they shared a similar barcode DNA sequence with other species known to be present in the region of interest. We did not exclude the species without a DNA barcode from the trawl data set to account for current eDNA metabarcoding weaknesses. However, the nine taxa detected by trawling and not by eDNA metabarcoding but present in the genetic reference database were rarely detected in our study (i.e. once or twice).

Biodiversity analyses also rely on quantitative data, i.e. estimates of fish abundance. Even though our results indicated a positive and significant relationship between the number of individuals caught by trawling and the number of eDNA metabarcoding reads for several sites, this relationship was weak and very uncertain . This result is consistent with the literature on classic metabarcoding approaches (Lamb *et al*., 2018; Rourke *et al*., 2022), even though some studies reported better relationships between relative biomass and number of log-transformed metabarcoding reads (e.g. Stoeckle *et al*., 2020). In the natural environment, the strength of the relationship between DNA copies and individual abundance decreases (R^2 = 0.51–0.57; Yates *et al*., 2019) in comparison to studies performed under control conditions or in naturae in lake ecosystems (R^2 = 0.8–0.91, Yates *et al*., 2019; Spear *et al*., 2021; Karlsson *et al*., 2022) involving species-specific qPCR methods (Pont *et al*., 2022). Two major non-exclusive considerations must be acknowledged. First, traditional quantitative sampling methods also have an inherent bias and only provide estimates of the fish abundance/biomass; such uncertainties may weaken the comparative signal with eDNA if the biases of the two sampling methods act in different directions (Rourke *et al*., 2022). Second, in the marine environment, the quantity of eDNA can be subject to fluctuations caused by many abiotic factors affecting the dispersal and the degradation rates of eDNA, such as currents, and temperature (Andruszkiewicz *et al*., 2019; Allan *et al*., 2021; Fukaya *et al*., 2021). Biotic factors such as the ontogenetic stage or fish behaviour, including metabolic activity, reproduction, and mating behaviour, can also strongly affect the eDNA emission rate (Danziger *et al*., 2022; Rourke *et al*., 2022). In addition, while several studies have shown that accounting for allometric scaling of eDNA production tends to improve the relationship between eDNA copies and fish abundance/biomass (Yates *et al*., 2021a, 2021b, 2022), our results were more mitigated. Indeed, even after accounting for the best interspecific allometric scaling coefficient, the relationships only slightly improved when modelling the relative number of eDNA reads rather than the raw number of eDNA reads; however, the relationships remained too variable among filters to be considered reliable (Supplementary Table S3 and Figures S10 and S11). The significant positive relationships were driven in 50% of the filters by the *Trachurus* sp. taxon showing extreme allometric scaled abundance values. These results confirm that the dominant taxa disproportionately drive this relationship between quantitative measures involving eDNA and fish quantities (Skelton *et al*., 2022). In addition, we did not find consistent interspecific allometric coefficients among the filter replicates and sites. This might be at least partially explained by the geographic distance between the trawling site and the eDNA sampling location. This distance is important and likely distorts the eDNA concentration due to hydrosystem dynamics (Fukaya *et al*., 2021). Without abundance and biomass estimates from external sampling methods, standalone quantitative eDNA approaches will remain unlikely to reliably disentangle abundance from biomass effects on eDNA copies numbers. For future research, we suggest that more points be sampled closer to the trawling sites and that novel quantitative metabarcoding methods such as high-throughput qPCR (HT-qPCR, Wilcox *et al*., 2020), metabarcoding and qPCR coupling (Pont *et al*., 2022), or qMiSeq approaches (Tsuji *et al*., 2022) be applied and associated with local hydrodynamic modelling (Andruszkiewicz *et al*., 2019; Fukaya *et al*., 2021). Currently, reliable use of eDNA metabarcoding for estimating fish abundance in a comparable manner to trawl catches remains elusive .

Despite our efforts to follow a strict sampling protocol, we cannot definitively rule out the possibility of contamination in the eDNA samples, as we did not perform negative controls at sea between the different samples. The Niskin bottles were the only none-single-use material employed for the eDNA sampling, so we took measures to thoroughly rinse the bottles three times before (including by the site's water) and one time after each use to avoid contamination. The negative samples for DNA extraction and PCR steps did not reveal any contamination. Several lines of evidence suggest that the biological signal remains strong. We observed a greater similarity among replicate eDNA samples from the same site (β_{jac} = 0.372 ± 0.095) compared to different sites (β_{jac} = 0.536 ± 0.098), and the presence of rare species at specific sites such as *Squalus acanthias*, which was detected at sites 1 and 3 but not at site 2 and reappeared at sites 11 to 13 (Supplementary Figure S5). We also observed distinct species compositions among different sites, in agreement with trawling methods (Figure 4). In addition, our results, including the quasi-systematic detection of small and abundant pelagic species (such as *Engraulis* sp., *Sardina Pilchardus*, and *Scomber scombrus*) or the frequent detection of *Pagellus bogaravero* by eDNA and not by trawling, are consistent with another eDNA study conducted in the same area (Fraija-Fernandez *et al*., 2020). Finally, we showed that after deleting 10 ubiquitous and/or abundant taxa showing multiple successive occurrences that were the most susceptible to drive the contamination, our conclusions remained similar (see Supplementary Figure S12).

In conclusion, in this study we compared the effectiveness of eDNA metabarcoding and classical bottom trawling in detecting similar spatial patterns of taxonomic, functional, and phylogenetic diversity of the marine fish communities in the BoB. We found that eDNA was able to detect higher taxonomic and phylogenetic diversity than trawling with less sampling effort. At the local scale, eDNA tended to detect functionally more divergent species than trawling, however, this trend was not totally independent from the increase in taxonomic richness. These findings confirm that eDNA metabarcoding is less selective than trawling and detects species spanning larger phylogenetic and potentially functional breadths, especially due to the identification of rare taxa and taxa that can escape the trawl, even though trawling detected more flat fish. However, because flat fish are functionally and phylogenetically clustered as they all belong to the order Pleuronectiformes, their greater detection by trawling cannot compensate for the wider phylogenetic and functional spectra of the additional taxa detected by eDNA. Finally, the correlations between the number of individuals per trawl and the absolute or relative number of eDNA reads were too variable and weak to support the use of eDNA metabarcoding as a reliable method of quantitative abundance estimate. This was true despite our attempts to account for allometric scaling in eDNA production, as done successfully in other studies (Yates *et al*., 2021a, 2021b, 2022). Overall, our results support the finding from a corpus of recent studies (Afzali *et al*., 2020; Fraija-Fernández *et al*., 2020; Stoeckle *et al*., 2020; Rozanski *et al*., 2022) that eDNA metabarcoding will gradually take its place within the scientific tools box to reliably investigate species occurrences and infer multi-component biodiversity patterns using presence–absence metrics. The constant improvement in the completeness of the DNA sequence reference database will help researchers to detect the remaining species currently missed. Further, with other sampling devices targeting the bottom substrate, we could enhance the detectability of benthic species buried in the sediment with eDNA metabarcoding. Finally, further developments should be guided towards a better understanding of eDNA ecology (production, fate, and degradation) to help build hydrodynamic models of eDNA concentrations in the environment to improve both species occurrence detection and quantitative estimates (Fukaya *et al*., 2021).

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Supplementary data

Supplementary material is available at the *ICESJMS* online version of the manuscript.

Author contributions

CA and DE jointly designed this study. DE and CA participated in the fieldwork. PV, MED, DE, CA, and RR analysed and interpreted the data. PV, DE, and CA jointly wrote the manuscript, and all the authors contributed to its improvement.

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Data availability

Data and code used to perform this research are available at https://github.com/pierre-veron/eDNA-trawl

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