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Is it worthy to use environmental DNA instead of scientific trawling or video survey to monitor taxa in soft-bottom habitats?

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ABSTRACT

Non-extractive techniques such as video analysis are increasingly used by scientists to study marine communities instead of extractive methods such as trawling. Currently, environmental DNA (eDNA) analysis is seen as a revolutionary tool to study taxonomic diversity. We aimed to determine which method is the most appropriate to describe fish and commercial invertebrate diversity comparing bottom trawl hauls, video transects and seawater eDNA. Our results reveal that video detected the lowest number of taxa and trawling the highest. eDNA analysis is powerful to describe marine bony fish communities, but some taxa of importance for the ecosystem such as elasmobranchs, crustaceans or molluscs are poorly detected. This may be due to several factors such as marker specificity, incomplete reference gene databases or low DNA release in the environment. For now, the various methods provide different information and none is exhaustive enough to be used alone for biodiversity characterisation.

1. Introduction

In light of the mounting anthropogenic pressures and the concomitant loss of biodiversity (Cardinale et al., 2012; Díaz and Malhi, 2022; Lawlor et al., 2024), it is more imperative than ever to characterize biodiversity, evaluate, manage and protect populations of species and communities. Standardised biodiversity monitoring is already underway (Costello et al., 2017; Gotelli and Colwell, 2001), particularly in areas of commercial and conservation importance, such as coastal environments. Due to their multifunctionality (e.g. fishing and recreational activities, marine energies, transport) and their ecological role (e.g. nurseries, spawning grounds), coastal environments are likely to change rapidly (O'Hara et al., 2021).

In soft-bottom habitats, sampling methods are usually extractive, negatively affecting organisms and their habitats. Scientific trawling has been traditionally used to collect data on bentho-demersal biodiversity. It allows an accurate identification of the sampled species and provides abundance estimates. Its major bias is that it samples a specific body size range based on the mesh size and net opening (Costello et al., 2017). The scale of seafloor destruction by scientific trawling is minor compared to commercial trawling, however it still raises ethical and conservational issues (Trenkel et al., 2019). These issues have led scientists to use new tools. In recent years, less invasive methods have been used. Underwater video, baited or not, towed or fixed (Mallet and Pelletier, 2014), and more recently environmental DNA (hereafter eDNA) which has the potential to improve marine biodiversity monitoring (Danovaro et al., 2016), are among these methods. Underwater video has been quite recently developed and used to monitor populations and communities, as well as to collect data on their surrounding environment. However, video analysis, even with the help of artificial intelligence technologies, is still time-consuming, as videos need to be analysed or annotated. Moreover, underwater video usually provides data at a low taxonomic resolution, with many taxa identified at higher levels than species, due to poor quality image or bad visibility because of the water turbidity. It is rather adapted to identify larger and slow or non-elusive taxa. As underwater videos are often deployed during day-time, nocturnal species may be missed. In that context, eDNA is seen as a revolutionary tool to study species richness in marine environments. eDNA analysis is non-invasive and very accurate in species determination (Danovaro et al., 2016), given that targeted genetic markers and furnished

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databases are used. This method consists in collecting sediment or seawater, from which targeted genetic material is amplified. Sampling is quick compared to the other two methods and requires filtering of a few litres of water or a few grams of sediment. However, its efficiency depends on the markers used (Freeland, 2017) and species identification requires prior sequencing and sharing in databases (Schenekar et al., 2020; Weigand et al., 2019).

The performance of different sampling methods has been previously compared two by two, e.g. Underwater visual census (UVC) or baited remote underwater videos (BRUV) *versus* eDNA analysis (Boussarie et al., 2018; Kopp et al., 2023; Mathon et al., 2022; Nguyen et al., 2020; Polanco Fernández et al., 2020; Stat et al., 2019) or eDNA analysis *vs.* trawling (Thomsen et al., 2016; Zhou et al., 2022; Zou et al., 2020) but to the best of our knowledge, very few studies has compared the performance of three methods to determine marine biodiversity on a large geographical scale (e.g. Aglieri et al. (2021) for fish community). The Grande Vasière is a wide area of soft-bottom habitats in the Bay of Biscay that has been monitored annually since 2004, using first scientific trawling and then underwater video campaigns (ICES, 2015; Mérillet et al., 2018). These long-term datasets make this area an ideal candidate to test the effectiveness of well-established sampling methods compared to eDNA analysis. Implementing effective monitoring tools that are cost- and time-effective, as well as non-invasive, is an important challenge for both fishing and conservational objectives. The aim of this study was to determine which sampling method is the most effective for identifying fish and commercial invertebrates in soft-bottom habitats. Within this overarching goal, we further tried to identify the advantages and drawbacks of each method and to determine whether eDNA analysis could potentially replace trawling or video in the near future.

2. Methods

2.1. Study area and data collection

The Grande Vasière is located in the north-eastern part of the Bay of Biscay in the north-east Atlantic (Fig. 1). It is an area of high commercial importance for the *Nephrops* fishing industry in Europe and covers 18,360 km^2 of soft and muddy substrate. Data were collected using three different sampling methods and covered the whole area. The three datasets were sampled at the same time of year, in late spring/beginning of summer, in order to be sure to collect the same type of communities. Overall, 37 water samples were collected for eDNA, 141 tows for scientific trawling and 225 underwater video transects were deployed (Figs. 1 and 2a).

2.2. Environmental DNA surveys

2.2.1. Sampling

In May 2021 and May 2022, water samples were collected with a Niskin bottle 1 m above the seafloor for eDNA analysis during the PELGAS campaign (Doray and Duhamel, 2022) at depth ranging from 53 to 139 m. The PELGAS campaign follows a sampling scheme consisting in systematic line transects perpendicular to the French coast (Doray et al., 2018). All sampling points located on these transects and located in the Grande Vasière were selected. For each sample, between 2 and 4 L of seawater were filtered on 0.45 µm Sterivex filters. For each sample, all the equipment was sterilised and single-use gloves and masks were used during filtration to avoid contamination. Control samples (filtration of distilled water on site) were also taken and analysed. The control samples were taken at the beginning and end of the cruise and at the first and second thirds of the samples taken for taxonomic richness assessment. All samples were stored at −20 °C onboard until shipment to the lab and DNA extraction.

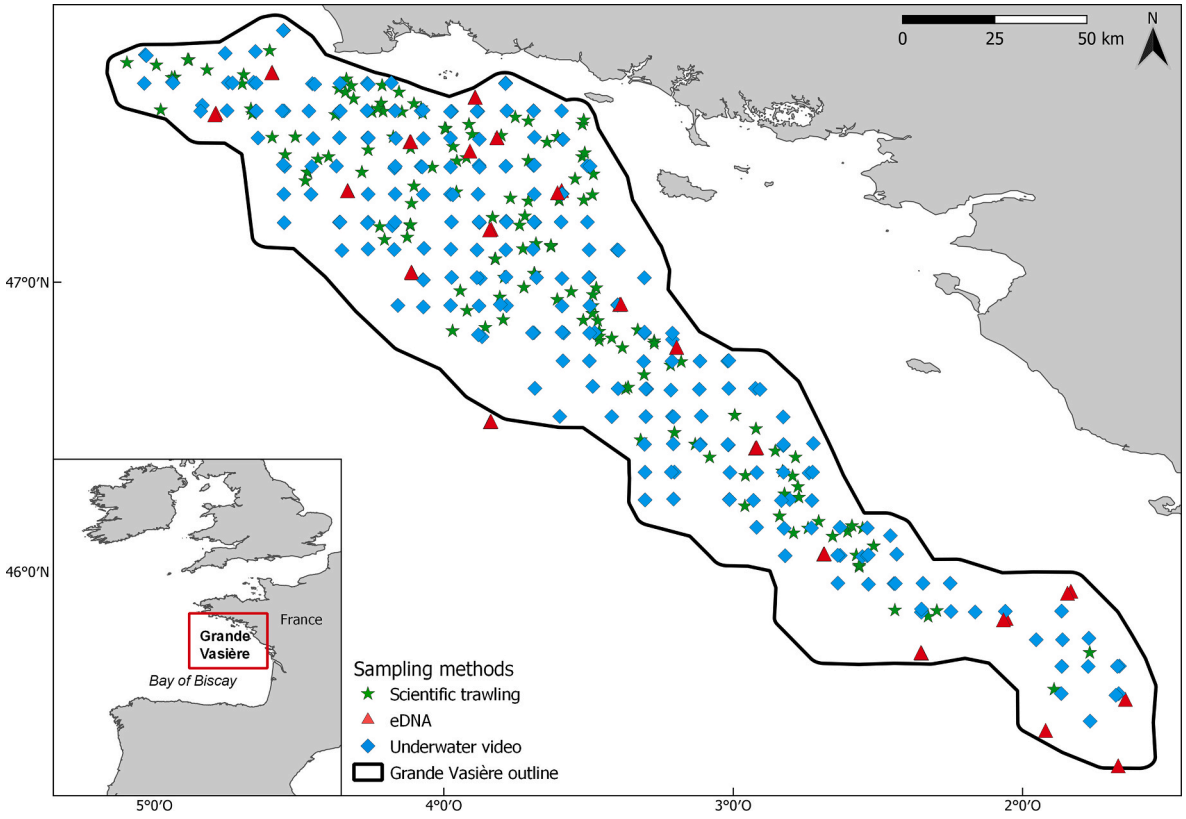

Fig. 1. Sampling of bentho-demersal species of the Grande Vasière (Bay of Biscay) by three sampling methods; scientific trawling (green stars), eDNA analysis (red triangles) and underwater video (blue diamonds).

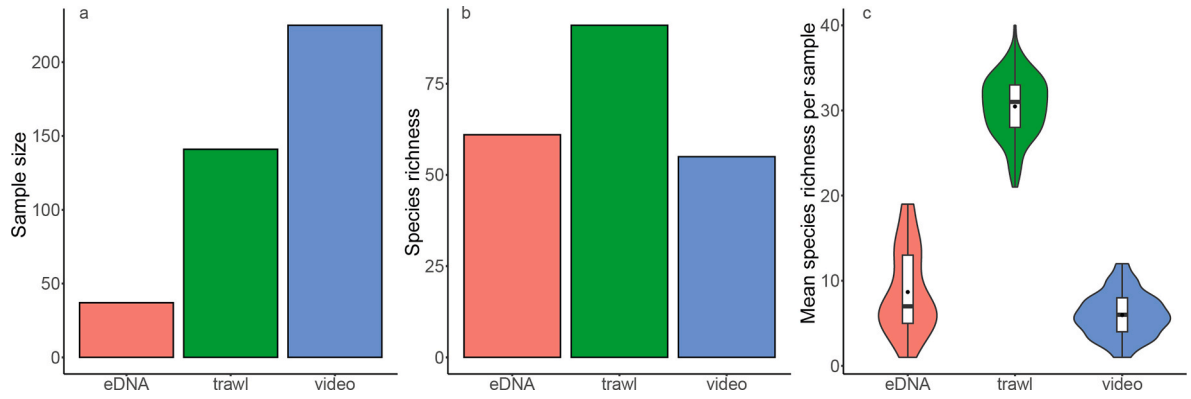

Fig. 2. a) Sampling size per method. b) Total taxonomic richness per method. c) Mean taxonomic richness per sample for each method. The violin plots show the distribution of taxonomic richness and the boxplots show the mean, median and interquartile range of the taxonomic richness. Pink = eDNA analysis, green = scientific trawling, blue = underwater video.

2.2.2. DNA extraction, PCR amplification and high-throughput sequencing

The following steps were performed by ID-Gene Ecodiagnostics, a laboratory specialised in environmental genomics. In the laboratory, bottom water eDNA (100 µl) was extracted using the DNeasy Powerwater Sterivex kit (Quiagen), following manufacturer instructions. All DNA extracts were stored at −20 °C. All samples were then amplified using three mitochondrial markers: a 280bp long fragment of the 16S rRNA gene specific to vertebrates and molluscs [16Smix F and R, degenerated version of primers 16S from Kitano et al. (2007), reference herein], a 380bp long fragment of the COI gene commonly used as barcode for animals [forward mlCOIintF from Leray et al. (2013)), reverse dgHCO-2198 from (Meyer, 2003)], and a 220bp long fragment of the 16S gene specific to Decapods (MiDeca-F from (Komai et al., 2019), MiDecaMod-R modified from Komai et al. (2019), reference herein) (see Online resource 1 for detailed information about the primers and PCR conditions). For each sample and marker, seven PCR reactions and one negative control were performed. In the case of MiDeca marker, a second round of amplifications (reamplification) with the same set of primers using the first PCR as template was performed in order to concentrate decapod DNA and facilitate detection. For 16S and COI markers, 1 µl of DNA extract was used as template, and 2 µl for MiDeca primers. Tagged primers bearing 8 or 9 nucleotides attached at each primer's 5′-extremity were used to enable multiplexing of all PCR products in a unique sequencing library (Esling et al., 2015). The results of the 7 PCR reactions were pooled and quantified. These pools were then quantified with capillary electrophoresis using QIAxcel instrument (Qiagen). Equimolar concentrations of PCR products were pooled for each library and purified using High Pure PCR Product Purification kits (Roche Applied Science). Libraries preparation was performed using Illumina TruSeq® DNA PCR-Free Library Preparation Kit. The libraries were then quantified with qPCR using KAPA Library Quantification Kit and sequenced on a MiSeq instrument using paired-end sequencing for 500 cycles (2 X 251bp) with Standard kit v2 for each marker.

2.2.3. High-throughput sequencing (HTS) data analysis

The data analysis was performed using SLIM pipeline (Dufresne et al., 2019).The raw sequences were demultiplexed using the DTD software (https://github.com/yoann-dufresne/DoubleTagDemultiplexer) to retrieve unique tag-encoded primers combinations associated to each sample (allowing no mismatches). The further steps including the quality filtering, were performed using DADA2 v1.12.1 R package (Callahan et al., 2016). These include the quality filtering with the *filterAndTrim* function (maxN = 0, truncQ = 2, rm.phix = TRUE and maxEE = 2), the trimming of primers using the cutadapt v2.4 software (Martin, 2011), the filtering of any read that still contain traces of primers, the filtering of any read below 20 bp, the training of errors models using the *learnErrors* function of DADA2 with default settings and the inference of Amplicon Sequence Variants (ASVs) using the *dada* function with default settings. Finally, the overlapping paired-end reads were merged using the *mergePairs* function with the option 'trimOverhang' set to true.

The total reads/sample in original MiSeq output, reads/sample after bioinformatic processing as well as number of reads/sample for each taxa by markers are given in Online resource 2. A substantial proportion of reads were unassigned in some samples with all three markers. A description of unassigned reads in control samples is available in Online Resource 3. Taxonomic assignment was then performed using the function IdTaxa in the *decipher* R package (Wright, 2016), with the default parameters and a threshold of 60% following Murali et al. (2018). We used a curated database (NCBI) comprising all metazoan species present in the studied area for which sequences were available in GenBank and for which assignment was verified by phylogenetic trees (See Online resource 4 for detailed information about the coverage of reference database).

2.3. Scientific trawl surveys

The bottom trawl surveys occurred in May 2012 and May 2013. Sampling is defined each year within the Grande Vasière based on a randomly stratified strategy from a sedimentary map of the area established by Bouysse et al. (1986): sampling points are allocated and randomly placed within each sedimentary strata based on its total surface and the relative activity of fishing boats within them. The fishing gear used was a bottom twin trawl 15m/21.20m (1.80m height, 8m x 2 width, 80 mm mesh size). Each tow lasted 30 min at 3.5 knots. Depth ranged between 60 and 133 m. The totality of the catch was sorted and as the first aim of the survey was species stock evaluation, all the fish, commercial crustaceans and cephalopods were identified onboard by the scientific crew. Species identification was done at the lowest possible taxonomical level based on (Garren et al., 2020) guide, mainly at species level. Whenever needed, a binocular was used for examination of morphological features (e.g. cephalopod's tentacular clubs with suckers).

2.4. Underwater video surveys

The underwater video surveys occurred in May 2019 and July 2020. 225 stations for video observation were selected randomly using a fixed isometric grid with a 4.5–4.7 nautical mile interval (Vacherot et al., 2019). An underwater sledge equipped with lights, a High-Definition Camera with an oblique angle to the seabed and data loggers (turbidity, depth, salinity) was deployed at each station. Once stable on the seabed, the sledge was towed behind the vessel at a low average speed of 0.85 knots for 10 min, to allow for a detailed examination of the seabed. Depth ranged between 47 and 173 m. Each video was then read

twice by a qualified scientist and the bentho-demersal species were identified up to the lowest taxonomic level possible. When it was not possible to go to species level, higher taxonomic levels were used such as genus, family or class.

2.5. Data analysis

As the bottom trawl survey identifies and counts only commercial species within invertebrates captured by bottom trawling, we had to exclude non-commercial invertebrate species identified with eDNA and underwater video from the analyses. Conversely, all fish captured during these campaigns were identified, regardless of their commercial status, therefore we chose to keep all fish species for a more exhaustive comparison of the three. A Kruskal-Wallis test followed by a Dunn's test was performed on the list of identified taxa, to test the differences in taxonomic richness per sample between the three methods. A non-parametric test has been preferred since normality assumptions were not met for eDNA data (Shapiro tests, $p < 0.05$).

The relationship between taxa diversity and sampling effort was assessed through the analysis of rarefaction curves. These curves represent the number of taxa detected depending on the sampling effort. The sample-based taxa rarefaction curves were calculated for each sampling method using the function *specaccum* from the *vegan* R package (Oksanen et al., 2020). In order to predict taxonomic richness detectability and evaluate the number of samples necessary to identify the maximum number of taxa for each method, five models were fitted on the rarefaction curves using the *nls* function in the *stats* R package; negative exponential models with 2 and 3 parameters, an exponential model, a Monod model and a rational function model. The AICc (corrected Akaike Information Criterion) was then calculated to select the best model for each method (*AICcmodavg* R package, Mazerolle (2020)).

Data were then pooled at the genus level to keep as much data as possible, while ensuring a biologically relevant resolution. Especially for underwater video, restricting the data at species level would have meant losing nearly 40% of data (Fig. 4). To this end, any taxa identified above the genus level was excluded and those identified at species level were grouped into genus. We then drew a Venn diagram on this restricted dataset (*ggvenn* R package, Yan (2021)) and a taxonomic tree (*ape* R package, Paradis and Schliep (2019)) to represent the number and identity of taxa detected by each method.

All graphs and statistical analyses were performed in R 4.0.3 and the map was created in QGIS 3.16.3-Hannover.

3. Results

3.1. Taxonomic resolution varies between methods

Both scientific trawling and eDNA analysis are the most accurate in terms of taxonomic resolution (Fig. 3). Around 92% of the taxa were identified to the species level with both methods. On the contrary, it was more difficult to identify taxa at the species level with underwater video. Nearly half of the taxa (42%) were identified above species level by this method, with 4% only being assigned at class level (and corresponding to Actinopterygii and Cephalopoda).

3.2. Differences in taxonomic richness

A total of 134 taxa were detected across the three methods. Scientific trawling detected the most taxa (91), while underwater video and eDNA detected 55 and 61 taxa respectively (Fig. 2b). The mean taxonomic richness per sample (Fig. 2c) was significantly different between methods (Kruskal-Wallis test, chi-squared = 279, $P < 0.001$). In particular, scientific trawling detected over four times more taxa ($SR \pm SD = 30.5 \pm 3.6$; Dunn's test, $P < 0.001$) than both underwater video ($SR \pm SD = 6 \pm 2.5$) and eDNA analysis ($SR \pm SD = 8.7 \pm 4.9$) (Fig. 2c). There was also a significant difference of taxonomic richness between eDNA analysis and underwater video ($P = 0.03$).

Half the identified genera (45 out of 91) were only detected by a single method, mostly by scientific trawling with the detection of 27 different genera, and 17 by eDNA analysis only (Figs. 4a–5). Underwater video identified just one genus (*Echiodon*) that has not been detected by either eDNA analysis or scientific trawling (Online resource 4). 23 different genera were detected by the three methods. The taxonomic tree highlights that the taxa identified by the three methods are among the most occurrent on the Grande Vasière, such as *Nephrops, Microchirus, Merluccius, Trisopterus, Micromesistius, Callionymus* (Fig. 5 and Online Resource 4). eDNA analysis detected taxa from Malacostraca, Elasmobranchii and Teleosteii classes (Fig. 4b). It did not detect any molluscs while scientific trawling and underwater video both detected taxa from Malacostraca, Teleosteii, Elasmobranchii, Bivalvia and Cephalopoda classes. Furthermore, scientific trawling was the only method to detect Petromyzonti (Fig. 4b and Online Resource 6).

3.3. Taxa detectability revealed for each method

The rarefaction curves show that scientific trawling detects the most taxa for the same number of samples, followed by eDNA analysis, and then underwater video which detects the lowest number of taxa (Fig. 6a and b). The asymptotes are different for each method, indicating that the three methods have different detectability rates. Scientific trawling and underwater video have both almost reached asymptotes, meaning that they have detected nearly the maximum number of taxa (corresponding to their detectability). eDNA analysis though, with 37 samples, does not reach the asymptote for the rarefaction curve. Out of the five models, the rational function, an asymptotic model with three parameters, had the best AICc for all three sampling methods (Table 1). The fitted curves for this model show that doubling the sampling effort for scientific trawling and underwater video, would increase taxonomic richness and allow the detection of only 8 and 5 new taxa respectively, as they have already nearly reached asymptote (Fig. 6c). Doubling the effort for eDNA would

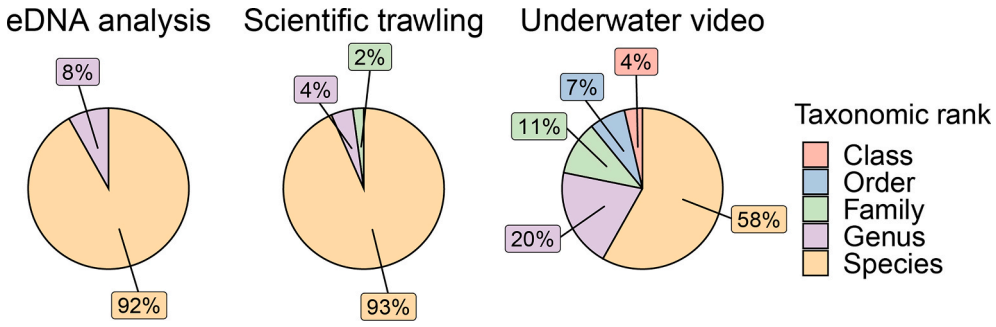

Fig. 3. Taxonomic resolution identified by eDNA analysis, scientific trawling and underwater video. Proportions show the number of taxa per taxonomic rank for each method.

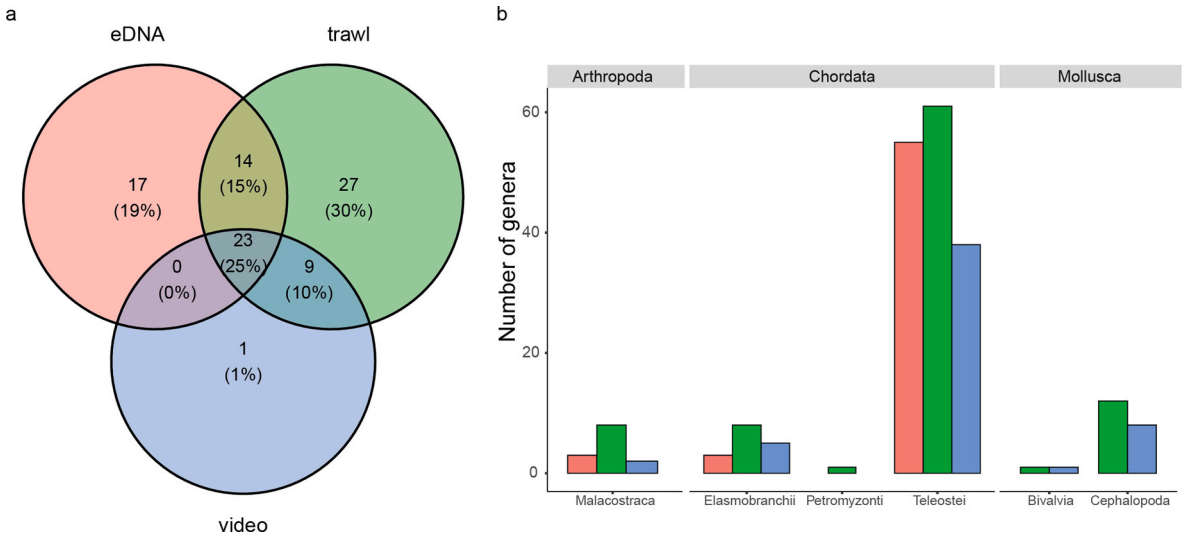

Fig. 4. a) Venn diagram showing the taxa identified at genus level detected by the three sampling methods. A detailed list of taxa detected by a single, two or three methods is available in Online Resource 5. b) Number of genera per phylum identified by each method. Pink = eDNA analysis, green = scientific trawling, blue = underwater video.

increase richness to 69 taxa (8 more). Asymptotes reached respectively 79, 105 and 62 taxa for eDNA analysis, scientific trawling and underwater video. More importantly, these models show that even if the number of samples for eDNA analysis and underwater video increased, these two methods would never reach the rate of detection of scientific trawling, which detects more than 25 taxa more than both other methods.

4. Discussion

A total of 134 fish and commercial invertebrates were detected by at least one of the three methods, among which scientific trawling identified 91 taxa, eDNA analysis 61 taxa and underwater video 55 taxa. When considering the number of taxa per sample, trawling remains the best performing method, but eDNA analysis revealed to be better than underwater video (Table 2).

Our study differs from other studies that identified more or an equal number of fish species with eDNA than with other sampling methods [e.g. Afzali et al. (2020) who found 71 species with eDNA vs 64 with trawling or Boussarie et al. (2018) who detected 44% more shark species with eDNA than with UVC or BRUV] but is consistent with other studies finding more species with trawling or video than with eDNA [see for example Stoeckle et al. (2021) for which 70–87% of the species detected with trawling were detected with eDNA or Nguyen et al. (2020) that identified 97 fish species with visual census vs. 79 by eDNA]. Similar to our study, Stoeckle et al. (2021) found that both eDNA analysis and trawling identified almost all the dominant species. Compared to trawling, eDNA enables identification of streamlined and small-sized species that probably escape the trawl through the mesh due to their body shape and size. For instance, 4 species of Gobiidae were identified by eDNA as well as 2 species of sand eel. The same pattern was noticed by Afzali et al. (2020) for several species of eelpout and sand lances that were much more detected in eDNA samples than in trawls. Nevers et al. (2018) also used eDNA for studying round goby presence in US lakes and found this method well-suited for this species, even for fish count as the eDNA concentration was positively correlated to the number of gobies. These differences may also be due to the fact that the study considered only vertebrates and mainly bony fish [see (Ruppert et al., 2019) for a review] whereas we considered a broader range of taxa, including cartilaginous fish, crustaceans, and molluscs. Furthermore, the primers employed as well as their ability to detect species is highly variable (Zhang et al., 2020). In the present study, even among Chordata, eDNA failed to detect petromyzontids and elasmobranchs that are usually rare species. No species of molluscs was detected by eDNA whereas both trawling and videos identified bivalves and cephalopods. eDNA studies in marine ecosystems have traditionally focused on fish taxa. However, an increasing number of studies are now considering benthos and other taxa than fish (Antich et al., 2021; Garlapati et al., 2019; Merten et al., 2021; Nguyen et al., 2020). Several factors could be examined to try to explain why eDNA analysis found less taxa than trawling. The number of replicates per site may have influenced species detection. For instance, when comparing fyke net and eDNA for freshwater fish determination, Shaw et al. (2016) found that two 1L water samples per site were insufficient for detecting rare taxa but that five 1L samples per site enabled a 100% detection rate. In highly diverse tropical ecosystems such as coral reefs, Stauffer et al. (2021) revealed that a number of sites comprised between 18 and 52 was required to describe regional diversity. For less diverse ecosystems, the number of replicates to qualify species biodiversity using eDNA is probably lower. Because the concentration of DNA may be low in environmental samples, the volume of seawater needed might be an important factor of variability. Most of the studies in marine sciences focused on eDNA for biodiversity monitoring, used around 1–2L per site (e.g. Stat et al. 2017; Boussarie et al. 2018; Afzali et al. 2020; Stoeckle et al. 2021) or even less (0.5L for (Thomsen et al., 2012), 0.25L for (Grey et al., 2018). As we used between 2 and 4L per site for 19 sites and as we compared the species richness at a regional scale pooling all the samples together, we are confident that our sampling was sufficient. Moreover, rarefaction curves modelling showed that doubling the number of eDNA samples would only add the detection of 6 more taxa. The discrete sampling that we have used could have led to miss some taxa in the eDNA samples. However, several authors agreed that eDNA can be displaced over long distance (Andruszkiewicz et al., 2019; Thomsen et al., 2012). Yet even with one single point sampling compared to the long distance of trawling or video transect, eDNA spatial resolution can be large in marine environment. Other approaches undertake continuous sampling (Maiello et al., 2022) or discrete samples combined (e.g. benthic, at 1m above sediment and in the water column and process them all together) to prevent a sampling bias. Sediment samples could also be considered for future researches as Turner et al. (2015) evidenced that sedimentary eDNA for carp was 8–1800 times more concentrated in sediment than in water, and remain detectable up to 132 days. Nevertheless, Shaw et al. (2016) have found that eDNA from water samples is a better match for taxa that are physically present at the time of sampling.

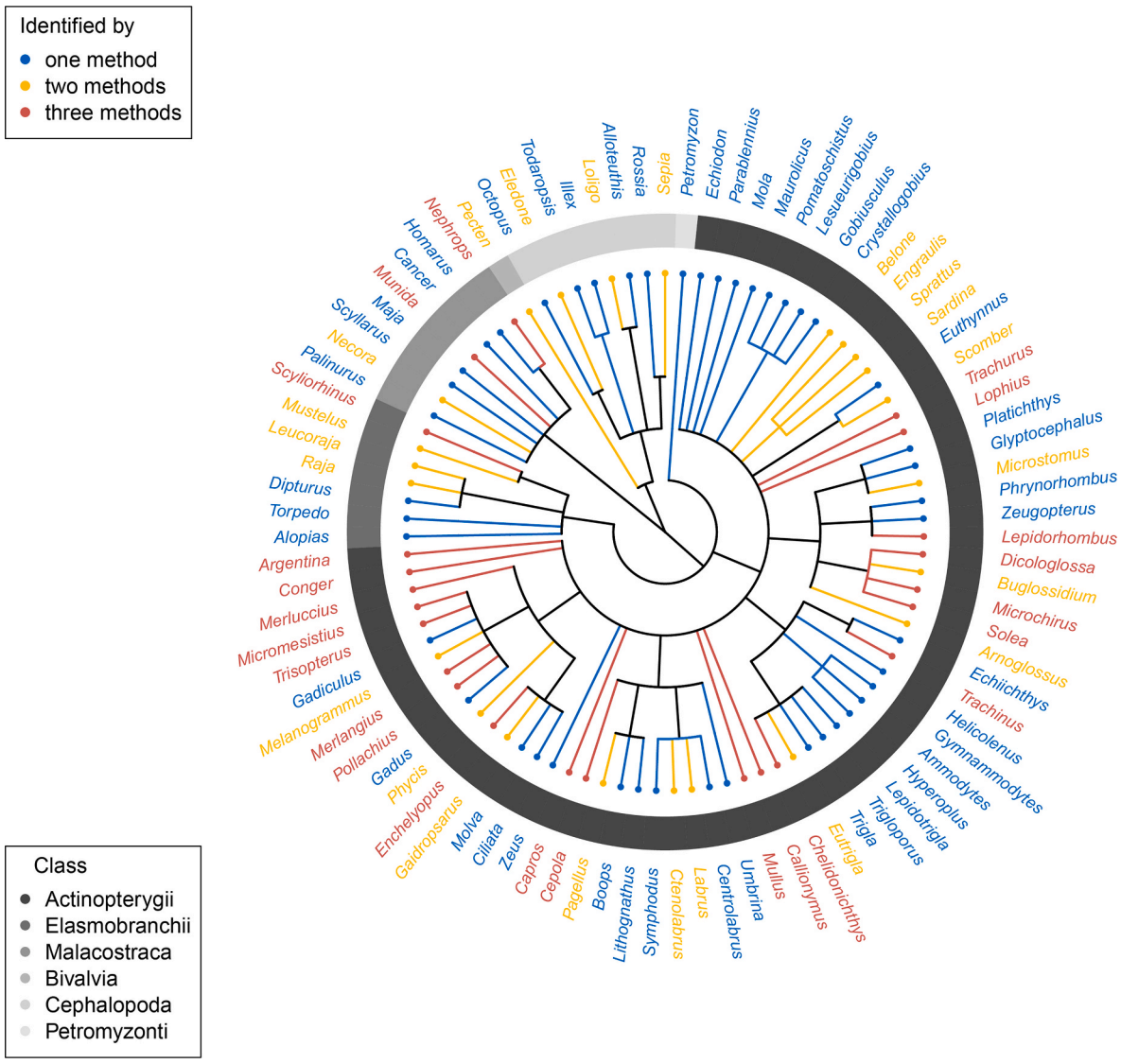

Fig. 5. Taxonomic tree of the genera identified in this study by eDNA analysis, scientific trawling and underwater video. Colours indicate the genera identified by either a single method (blue), two methods (yellow) or three methods (red). The grey shaded ring represents the class to which belongs each genus.

Beyond fish species, our study also focusses on commercial invertebrates. Considering multiple genetic markers increased the detection success, we decided to combine three markers among which MiDeca which is specific to Decapod detection. However, no bivalves nor cephalopods could be detected with the markers used. Several explanations could be formulated. First, the absence of detection of some taxonomic group may be linked to the selected markers' specificity. For cephalopods, de Jonge et al. (2021) recently identified a primer for eDNA analysis of Cephalopoda that we could have used to target this taxonomic group. Even for fish, the choice of markers could lead to differences in diversity. In Xiong et al. (2022) review on studies using eDNA to monitor fish diversity, it appears that the two primers we have chosen are commonly used for this purpose, in particular COI that is in fourth position in terms of frequency of selection. However, considering a list of 22 primer sets for eDNA metabarcoding of teleost fish, Zhang et al. (2020) underline considerable differences depending on the primer selection, mainly in terms of fish taxa richness, with a variation from 0 to 66 taxa for a given sample. The 16S and COI markers perform quite well, but our results would have been different using a 12S rRNA marker that has been shown to outperform other primers in terms of amplified fish diversity. Diversifying the primers used would change the shape of the rarefaction curve associated with eDNA to either approach the one associated with trawling or even go over it (Fig. 6). However, as it may be expensive to use multiple markers to increase detection probability, a compromise has to be found when studying several phyla (Collins et al., 2019; Freeland, 2017). Another explanation could be related to DNA release, dispersal, and degradation in seawater. According to Antich et al. (2021), eDNA in water samples is a poor surrogate for the analysis of benthic communities as only a few detections of benthic organisms could be made in close-to-bottom seawater. The authors argued that the rate of DNA shedding from benthic organisms is generally low. They recommend to sample by scraping the seafloor to capture the benthic diversity using eDNA, especially for Mollusca, Anthozoa, Phyllodocida and Decapoda. Thus, DNA shedding rates of benthic invertebrates is probably lower than those of fish, notably for organisms such as bivalves that are protected by a shell. For these animals, the main activities driving the shedding rate are filtering linked to feeding and burrowing (Sansom and Sassoubre, 2017). Considering three pelagic fish species, (Sassoubre et al., 2016) found that the shedding rate is highly variable depending on fish size. The same author as well as other authors (Thomsen et al., 2012) revealed that DNA degradation is faster in marine environments than in freshwater, with DNA concentration rapidly dropping below the detection threshold (after 3–4 days for *Sardinops sagax* and *Engraulis mordax*, 0.9 days for *Gasterosteus aculeatus* and 6.7

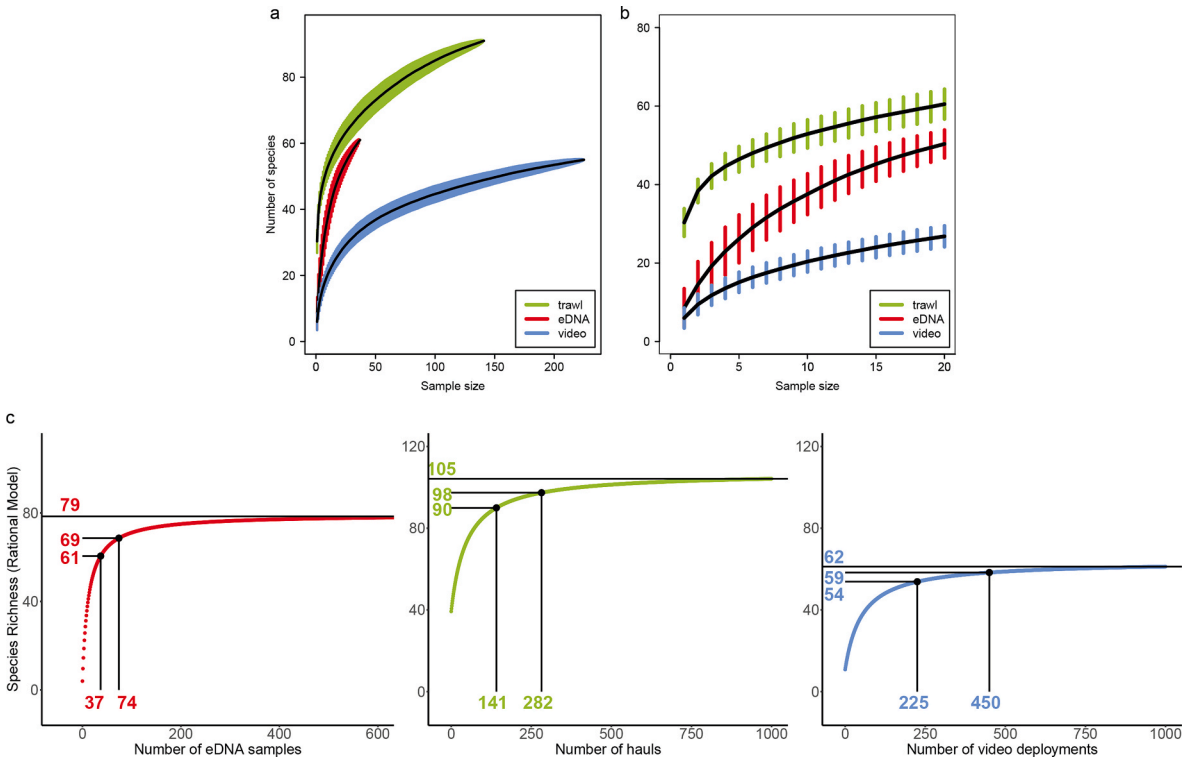

Fig. 6. Sample-based rarefaction curves (red = eDNA analysis; green = scientific trawling; blue = underwater video). a) Rarefaction curves of the accumulated taxonomic richness sampled by the three sampling methods (eDNA, scientific trawling and underwater video) in the Grande Vasière. The shaded areas indicate the confidence interval. b) Rarefaction curves zoomed on the first 20 samples of each method. The error bars indicate standard deviation. c) Estimated rarefaction curves fitted with the rational function model (Table 1) for each of the three sampling methods. The first coloured number in the left bottom corner corresponds to the present number of samples, the following number correspond to a doubling of the number of samples. The coloured numbers on the left top of the graphs correspond to the richness associated to the present number of samples, a doubling of the samples and asymptote.

Table 1
Models fitted for taxa rarefaction curves obtained from the three sampling methods used in this study, eDNA analysis, scientific trawling and underwater video. The best-fitting model is highlighted in bold and underlined.

Model	Model details			AICc		
	Formula	Asymptotic	Number of parameters	eDNA	Scientific trawling	Underwater video
Negative exponential (1)	$S = a + (b - a)e^{-cX}$	Yes	3	98.09	535.58	778.43
Negative exponential (2)	$S = a(1 - e^{-bX})$	Yes	2	150.25	979.14	1161.96
Exponential	$S = a + b\log(X)$	No	2	137.03	590.57	700.89
Monod	$S = a/(1 + bX^{-1})$	Yes	2	85.90	858.38	915.79
Rational function	$\boldsymbol{S = (a + bX)/(1 + cX)}$	**Yes**	**3**	**29.50**	**466.17**	**578.71**

Table 2
Pros and cons of the different techniques.

Method	Taxonomic resolution	Estimating taxonomic richness	Taxa detected	Detectability and number of samples required	Other benefits	Limitations
eDNA	Excellent	Better estimation of richness than underwater video but far behind bottom trawling	Most occurrent taxa from Malacostraca, Elasmobranchii and Teleosteii. No Mollucs detected	Rate of detection slightly below bottom trawling (asymptote not reached with 37 samples)	Potential detection of cryptic species	Completeness of reference databases, markers selection, number of reads. No reliable assessment of biomass currently
Bottom trawling	Excellent	Highest richness detected (~four times more taxa detected than with the other two sampling methods)	Most occurrent taxa from Malacostraca, Teleosteii, Elasmobranchii, Bivalvia and Cephalopoda.	Best rate of detection (asymptote almost reached with 141 samples)	Biomass estimation	Extractive and impacting to the environment. Identification can be time consuming.
Underwater video	Less accurate	Lowest richness detected	Most occurrent taxa from Malacostraca, Teleosteii, Elasmobranchii, Bivalvia and Cephalopoda.	Lowest rate of detection (asymptote almost reached with 225 samples)	Visualisation of the habitat	Identification of taxa is time-consuming if not automated. The estimation of biomass may be limited.

days for *Platichthys flesus*). For bivalves, (Sansom and Sassoubre, 2017) revealed that after 4 days, the concentration of eDNA was below the level of detection. To increase the detection for some taxonomical groups, methodological improvements simple to address may be considered such as adding markers, but intrinsic factors such as the DNA shedding rates or decay are difficult to overcome. However, as DNA decays quite fast (e.g. a persistence of eDNA of 48h in marine systems, Collins et al. 2018) we can be confident that the diversity reflected in our samples corresponds to the recent diversity.

When considering the number of samples needed to characterize the biodiversity in our area, we highlight that video sampling is probably the less adapted method to reveal the biodiversity of muddy habitats. Indeed, with 225 samples, 55 taxa were detected whereas 91 taxa were detected with 141 trawl hauls. Several explanations could explain this result. First video transects covered a smaller surface than trawl hauls (0.85 knots for 10 min with a camera width of 0.7m for video compared to 3.5 knots for 30 min with a 8m x 2 width for trawling). With increased numbers of videos sampling, the number of taxa detected reached a plateau (Fig. 3) but with a total number of taxa lower than other techniques. Videos were deployed only during day-time and so nocturnal fauna is missed. This is also the case for trawling that is operated only during daytime but not for eDNA sampling that capture the whole diversity. As the sledge is equipped with artificial lights, this issue could be easily overcome by deploying night-time videos. The noise and lights of the sledge may also have frightened mobile species causing them to flee from the field of view (Lorance and Trenkel, 2006; Stoner et al., 2008; Sward et al., 2019).

When compared to other non-invasive methods, it also seems that video is less efficient than eDNA as reported in other studies (Boussarie et al., 2018; Polanco Fernández et al., 2020). Indeed, eDNA analysis identified on average 9 taxa per sample against 6 taxa per sample for video. Compared to trawling or eDNA sampling, video analysis is time-consuming (up to tens of hours of work for a single transect depending on the diversity), with a low taxonomical resolution. A precise species identification needs trained observers as the degree of taxonomic resolution is dependent on scientists expertise (Ji et al., 2013). Even with trained observers, video analysis strongly depends on environmental conditions (especially in muddy habitats) and the determination often stop at the genus level or even higher due to water turbidity. It is worth mentioning that in a near future, deep learning would probably become efficient enough to detect automatically species on video and forgo time-consuming manual validation (Ditria et al., 2020; Marrable et al., 2022). Moreover, even if the sledge supporting the camera has a lower impact on the seafloor than towed fishing gear, eDNA sampling is far less invasive as the Niskin bottle used for seawater samples does not touch the seafloor at all. However as for eDNA, this method has the advantage of being non-extractive and can detect the most frequent taxa like the two other methods.

To conclude, scientific trawling allows to better describe the benthodemersal communities from the Grande Vasière in terms of number of taxa. However, with one order of magnitude less sampling effort and diminished impact on species and the seafloor, eDNA analysis allows to capture the presence of dominant taxa in the area. Moreover, a few samples rapidly lead to an asymptote in rarefaction curves. Video analysis is probably the less suitable method, with poor taxonomic resolution and much bias and time for video processing. Even if eDNA analysis does not allow a thorough determination of size, sex or abundance yet [but see for instance (Salter et al., 2019)], this method seems really promising in the future notably in areas inaccessible to other sampling techniques such as marine protected areas or offshore windfarms. For now, the best strategy for describing a whole marine community in soft bottom environments is probably a combination of monitoring methods.

CRediT authorship contribution statement

Anna Le Joncour: Writing – original draft, Methodology, Formal analysis, Data curation. **Maud Mouchet:** Writing – review & editing, Methodology, Data curation, Conceptualization. **Germain Boussarie:** Writing – review & editing, Visualization, Methodology, Data curation. **Gaël Lavialle:** Methodology, Data curation. **Laurence Pennors:** Methodology, Data curation. **Ludovic Bouche:** Methodology, Data curation. **Pierre Le Bourdonnec:** Methodology, Data curation. **Fabien Morandeau:** Methodology, Data curation. **Dorothée Kopp:** Writing – review & editing, Writing – original draft, Validation, Supervision, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

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

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.marenvres.2024.106667.

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