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ORIGINAL ARTICLE

Environmental DNA highlights fish biodiversity in mesophotic ecosystems

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Abstract

Mesophotic marine ecosystems are characterized by lower light penetration supporting specialized fish fauna. Due to their depths (−30−−150 m), accessibility is challenging, and the structure of mesophotic fish assemblages is generally less known than either shallow reefs or deep zones with soft bottoms which are generally trawled. Environmental DNA metabarcoding from seawater filtered in situ could improve our ability to monitor the diversity of mesophotic ecosystems. Here, we developed and tested a submersible standalone pumping device allowing targeted marine water filtering to explore the biodiversity of two mesophotic ecosystems, one temperate along the Provence coast in the North-Western Mediterranean Sea and one tropical at the seamount La Pérouse in the Western Indian Ocean. We filtered water samples from depths ranging between 0 and 200 m in the Mediterranean Sea and between 60 and 140 m in the Indian Ocean and applied a metabarcoding protocol using the teleo primer pair targeting the 12S mitochondrial rDNA (Actinopterygii and Chondrichthyes). For both study regions, our eDNA surveys were able to recover highly diverse fish assemblages, and the compositional analysis of eDNA samples showed both a marked signal of fish compositional turnover and overlapping taxa between depth zones. Further, we observed that a substantial number of species were found in samples collected in depths beyond their reported depth range suggesting an underestimation of species' depth tolerances. eDNA metabarcoding should thus complement existing knowledge of species' geographic distributions across space and depth. Overall, our results demonstrate the potential of eDNA metabarcoding for future mesophotic surveys as it allows fast and broad biodiversity assessment.

Florian Holon and Loïc Pellissier share senior authorship

KEYWORDS
biomonitoring, environmental DNA, marine biodiversity, mesophotic ecosystem, North-Western Mediterranean Sea, Western Indian Ocean

1 | INTRODUCTION

Although there has been substantial progress in marine biodiversity surveys to investigate shallow reef fishes (Antão et al., 2020; Edgar et al., 2020), large, remote, and deep areas, such as mesophotic marine ecosystems (hereafter MEs), remain under-sampled (Costello & Chaudhary, 2017). MEs include mesophotic coral ecosystems and temperate mesophotic ecosystems (Pyle & Copus, 2019; Loya, 2019), which are characterized by lower light penetration and by environmental factors generally more stable than their shallow-water counterparts, with classically colder waters (Cerrano et al., 2019; Kahng et al., 2019). They are found in subtropical, tropical, and temperate regions at depths ranging from approximately 30 to 150 m below the sea surface depending on water clarity (Loya et al., 2016; Loya, 2019). MEs support high biodiversity (Andradi-Brown et al., 2021), including species restricted and specialized to the mesophotic zone but also sharing species with their shallower counterparts (e.g., Lesser et al., 2019; Rocha et al., 2018; Soares et al., 2018). Sampling in these areas is difficult due to the presence of hard bottom at this depth range which limits the use of traditional methods such as scuba diving or grabs and trawls. Since our knowledge of ME biodiversity and ecology remains limited (Eyal et al., 2021; Kahng et al., 2017; Loya, 2019), we urgently need novel monitoring methods to document the organization of these ecosystems and their ongoing changes (Frade et al., 2018).

Mesophotic ecosystems are galvanizing research interest, both because of their unique biodiversity from invertebrates to fishes (Andradi-Brown et al., 2021; Lesser et al., 2019), but also because of their potential to act as refugia for shallow-water species from anthropogenic disturbances (Bongaerts et al., 2010). It was widely assumed that MEs have a reduced vulnerability to threats because of their remoteness and depth and could therefore sustain fish populations that are eroding on surface reefs (Eyal & Pinheiro, 2020; Laverick et al., 2016; Smith et al., 2019). However, there is increasing evidence showing that these disturbances go beyond the shallow MEs (Smith et al., 2019). Vertical connectivity and overlapping species composition between shallow and ME waters support the hypothesis of faunal exchange across these depth ranges and suggest that MEs could offer refugia for shallow species (Loya et al., 2016; Tenggardjaja et al., 2014). In contrast, other studies indicate that fish species assemblages in MEs are distinct from those of shallow waters as they host unique communities (Rocha et al., 2018). On coral reefs, species compositional turnover along a depth gradient suggests a transition between upper and lower MEs around 60 m, where assemblages, largely composed of shallow reef species, become distinct to MEs (Lesser et al., 2019; Loya et al., 2016). In temperate waters, such as in the Mediterranean Sea, the thermocline is closer to the surface (Bouzinac, 2003) with less marked species turnover within MEs. Hence, additional research on species turnover along depth gradients from the surface to mesophotic ecosystems is needed in the near future. Despite recent advances, MEs remain understudied across the world, and regional knowledge gaps are substantial (Pyle & Copus, 2019). Improved methodology to survey MEs would provide insights into the ecology of MEs and predict whether MEs can truly act as refugia from anthropogenic and natural disturbances.

Environmental DNA (eDNA) metabarcoding could be a powerful method to monitor MEs. Indeed, as compared to traditional sampling methods, eDNA metabarcoding is non-invasive and less cost- and time-intensive (Deiner et al., 2017). However, to date, it has primarily been used to survey (sub-)surface and coastal marine areas (e.g., Polanco Fernández, Marques, et al., 2021; West et al., 2020), while little effort was invested into eDNA metabarcoding studies focusing on deeper waters (but see Andruszkiewicz et al., 2017; Juhel et al., 2020; McClenaghan et al., 2020). To recover comprehensive biodiversity signals in an ecosystem, a large volume of water is required (Bessey et al., 2020; Cantera et al., 2019), but eDNA sampling at deeper depths remains challenging. The volume of filtered seawater and the number of replicates have been shown to influence the number of taxa (Stauffer et al., 2021) so only an appropriate protocol can provide accurate biodiversity assessments through eDNA metabarcoding within MEs. Therefore, the challenge is to sample a volume of water large enough as well as an adequate number of filtration replicates directly in situ, to avoid the possible risk of cross-contamination (Stauffer et al., 2021; Thomsen & Willerslev, 2015). The use of Niskin samplers to study deeper waters is a common method (e.g., Andruszkiewicz et al., 2017; Juhel et al., 2020; McClenaghan et al., 2020) as it allows the collection of eDNA at specific depths but this method has the drawback of filtering ex-situ. Therefore, the development of a submersible in situ pump for eDNA surveys in deep waters could enable the filtration of large volumes of seawater close to habitats or substrates and target micro-habitats while limiting the risk of contamination.

In this study, we developed and tested a standalone and in situ water filtration system toward application for eDNA metabarcoding in MEs and inventoried the fish composition of MEs in two marine regions. We sampled MEs along the Provence coast in the North-Western (NW) Mediterranean Sea and the La Pérouse seamount in the Western Indian Ocean (WIO). The eDNA metabarcoding sequences were analyzed at the species level and clustered into molecular operational taxonomic units (MOTUs). For both regions, we first investigated the ability of eDNA metabarcoding to detect taxa compared with known regional fish composition. Second, by sampling at different depths, we tested whether eDNA can recover differences in fish species composition with species turnover between the surface, shallow mesophotic, and deeper mesophotic

ecosystems. Since MEs support diverse but also unique species communities (Lesser et al., 2019; Rocha et al., 2018), we anticipate the detection of different species compositions depending on the sampling depth. Finally, we investigated the differences between documented fish depth ranges and the depth at which species were detected with our eDNA approach. We expect an underestimation of the species' depth range due to existing knowledge gaps in MEs (Kahng et al., 2014; Turner et al., 2019).

2 | MATERIALS AND METHODS

2.1 | Study areas

2.1.1 | Provence coast

The first study area is the Provence coast located in the South of France along the NW Mediterranean Sea (Figure 1a). The sampling stations are located between Marseille (43°17'N, 5°22′E) and Nice (43°42'N, 7°15'E) and are drop-offs from the surface to 200 m in the immediate proximity of deeper trenches exceeding 2000 m depth. The Mediterranean Sea is not only the world's largest and deepest enclosed sea but also a marine biodiversity hotspot (Coll et al., 2010).

2.1.2 | La Pérouse

The second study area is La Pérouse (19°43'S, 54°10'E), a shallow seamount located between Madagascar and Réunion Island in the WIO (Figure 1b; Marsac et al., 2020). La Pérouse is an extinct volcano with a summit depth at 60 m below the sea surface. The plateau of the summit has a maximum length of 12 km and a maximum width of 4 km. One side of the seamount might have collapsed in the past, leading to a less common crescent-shaped summit. The abyssal plains that surround this isolated pinnacle are at a depth of 5000 m (Durville et al., 2021; Marsac et al., 2020). Despite its proximity to Réunion Island (160 km northwest), La Pérouse and its surroundings are still poorly studied (Marsac et al., 2020; Roberts & Ternon, 2020).

2.1.3 | eDNA sampling methods, filtration, and treatment

During the field and laboratory processes, a strict contamination control protocol was followed (Goldberg et al., 2016; Valentini et al., 2016). For each water sample, we used disposable gloves, as well as single-use filtration equipment. We applied three different eDNA sampling methods to collect seawater samples from various depths ranging from 0 to 200 m:

1. Athena method (subsurface transect sampling): surface eDNA was sampled in situ with the Athena® peristaltic pump (Proactive Environmental Products LLC, Bradenton, Florida, USA; nominal flow of ~1.0 L/min). The filtration process lasted 30 min to collect a water volume of ~30 L directly from the boat (Polanco Fernández, Marques, et al., 2021a). The entry of the tube was positioned at 20 centimeters below the water surface.
2. Niskin method (fixed-point sampling): we collected water samples using 10 L Niskin water samplers at different depths. A pressure sensor coupled to the Niskin water sampler was used to control the sampling depth. After bringing the Niskin water sampler to the surface, we poured the content into a sterile single-use plastic bag and placed the inlet of the pump in the bag for ex-situ filtering. The water (10 L) was then filtered for 10 min using the Athena® peristaltic pump (Proactive Environmental Products LLC, Bradenton, Florida, USA; nominal flow of ~1.0 L/min).
3. Submersible method (deep transect sampling; Figure 2): we used the novel submersible pump (Subspace, Geneva, Switzerland; nominal flow of ~1.0 L/min) to collect samples at various depths during close circuit rebreather dives. The pump has an internal battery and can be activated underwater. This allows in situ filtration directly at the targeted habitat and depth. The filtration lasted for 30 min to collect a total water volume of ~30 L.

We used these three filtration protocols to collect and filter water throughout a VigiDNA® 0.2 µM cross-flow filtration capsule (SPYGEN, le Bourget du Lac, France) using disposable sterile tubing for each filtration capsule. After the filtration process, the remaining water in the capsules was emptied. The capsules were then filled with 80 ml of lysis conservation buffer (CL1 buffer SPYGEN, le Bourget du Lac, France) and stored at room temperature in the dark.

2.2 | eDNA field sampling

2.2.1 | Provence coast

In the Provence coast, a total of 35 samples from 14 stations were collected from July 1st to July 19th, 2019 (Figure 1a; Supplementary material 1, Table S1). Using the Athena method, a total of nine samples of surface water were collected at three stations (Beach Rock Cassis 5, Cap Lardier 1, and Cap Negre 1: three replicates). With the Niskin method, we performed nine eDNA samples at five stations with depths ranging from 0 to 200 m (Cap Lardier 2: five replicates and Beach Rock Cassis 1–4: no replicates). The remaining 17 samples were obtained at six stations using the Submersible method (Banc de Magaud, Beach Rock Cassis 6, Cap Lardier 3, Cap Negre 2, and Tombant Americain: three replicates and Imperiaux 1: two replicates) on board *Cetravim*,a saturation diving barge during the GOMBESSA 5 expedition (https://gombessa-expeditions.com/gombessa-5/). The two replicates collected at the Imperiaux 1 site (MS1) were collected during the ascent from 65 to 10 m, while the remaining samples collected using the submersible pump were taken in depths between 65 and 130 m. All samples were collected in the daytime.

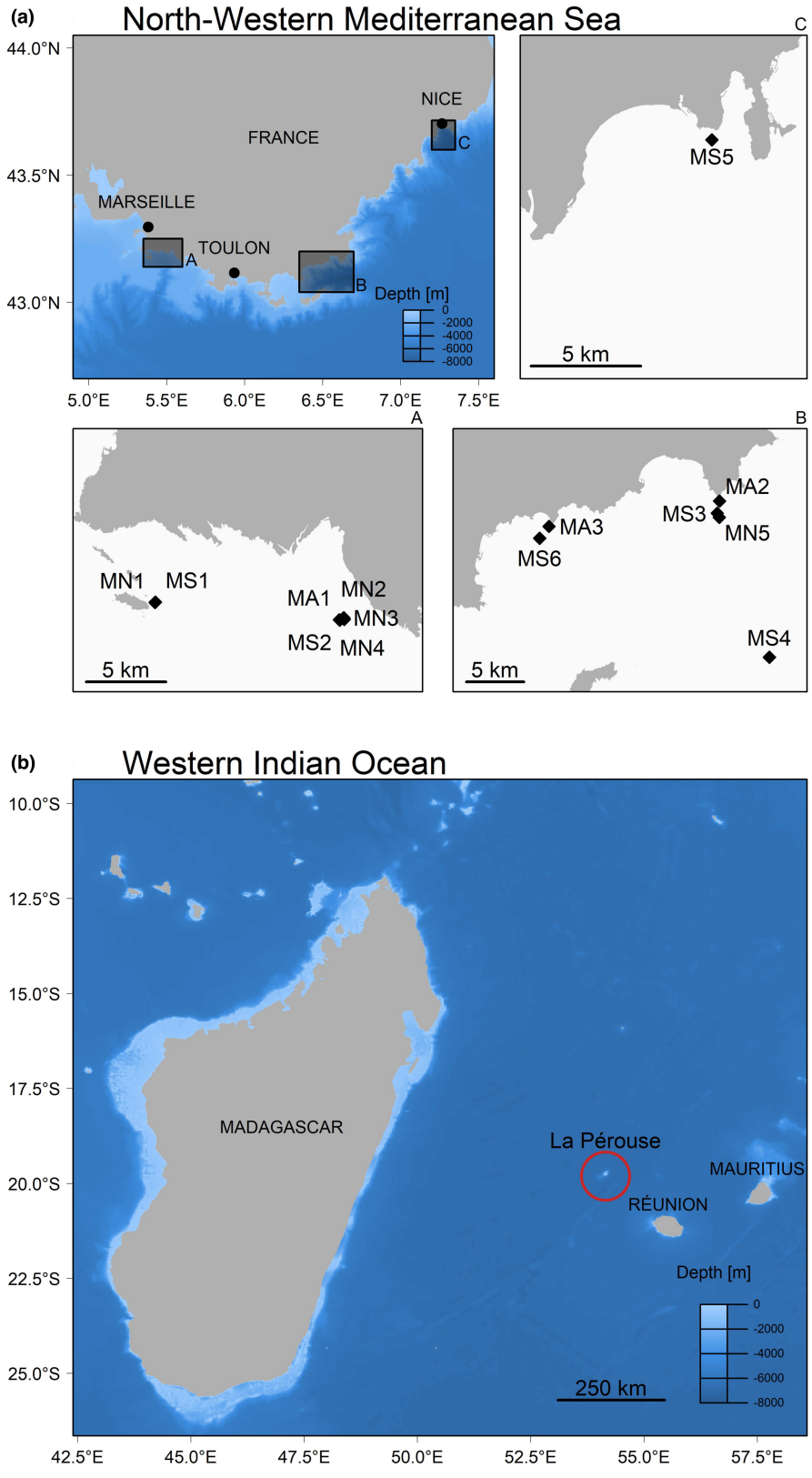

FIGURE 1 Area of eDNA sampling (a) in the coastal North-Western Mediterranean Sea and (b) at the seamount La Pérouse in the Western Indian Ocean. More information about the sampling protocol can be found in supplementary material 1 (Tables S1 and S2; GEBCO Bathymetric Compilation Group 2021, 2021)

2.2.2 | La Pérouse

At La Pérouse, a total of eight water samples were collected during the MONT LA PÉROUSE expedition (https://gombessa-expeditions.com/mont-la-perouse/) on board the ship *La Curieuse* to the seamount La Pérouse from October 27th to November 5th 2019 (Figure 1b; Supplementary material 1, Table S2). During close circuit rebreather dives, water samples from various depths, ranging from 60 to 140 m below the sea surface, were collected using only the Submersible method (eight stations: no replicates). Out of the eight

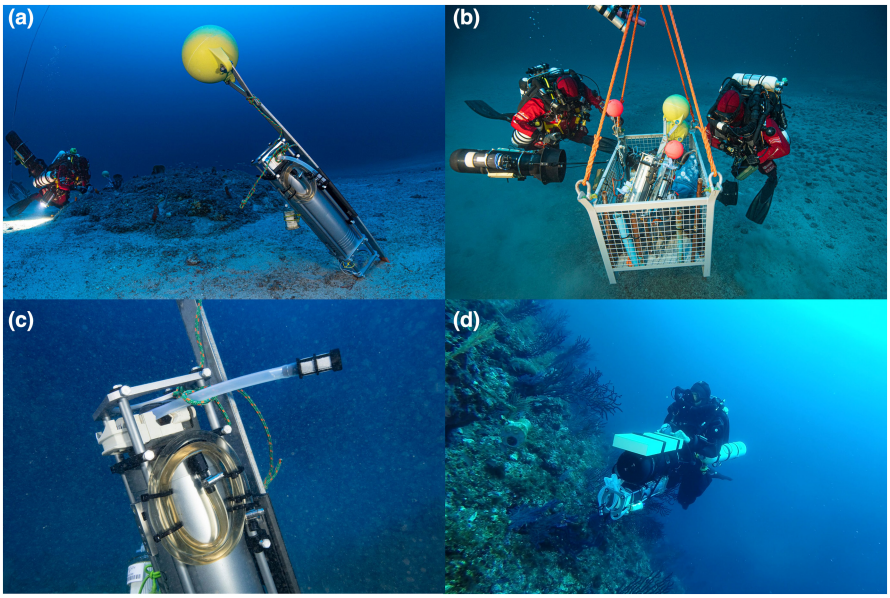

FIGURE 2 Montage of pictures showing the submersible pump used for sampling along the Provence coast (North-Western Mediterranean Sea) and at the seamount La Pérouse (Western Indian Ocean). Photo credits: (a) Laurent Ballesta, Gombessa expéditions, Andromède Océanologie (2019), (b & c) Laurent Ballesta, Gombessa 6: Mission cap Corse, Gombessa expéditions, Andromède Océanologie (2021) and (d) Régis Hocdé, location: Calanques of Marseille, Riou island

water samples, six were collected during the day, while two were collected at night (IS2_1 at 62 m and IS7_1 at 100–125 m).

2.3 | Laboratory procedures

eDNA extraction, amplification, and library preparation were performed at SPYGEN, a controlled eDNA laboratory (Le Bourget du Lac, France), and the sequencing steps at Fasteris (Geneva, Switzerland). Each of these steps was carried out in separate dedicated rooms. Suitable clean working and room conditions, as described in Pont et al. (2018), were ensured in each step to prevent contamination. For DNA extraction, each filtration capsule was prepared as described in the protocol of Pont et al. (2018). DNA extraction was carried out twice per filtration capsule using NucleoSpin® Soil (MACHEREY-NAGEL GmbH & Co., Düren Germany) and by following the manufacturer's instructions from the 6th step on. The samples were further tested for inhibition by qPCR (Biggs et al., 2015), and, in the case of inhibition, samples were diluted five-fold. In a final volume of 25 µl, amplifications were carried out by using 3 µl of DNA extract as the template (Pont et al., 2018). The amplification mixture contained 1 U of AmpliTaq Gold DNA Polymerase (Applied Biosystems, Foster City, CA), 10 mM Tris–HCl, 50 mM KCl, 2.5 mM $MgCl_2$, 0.2 mM each dNTP, 0.2 µM "teleo" primers (Valentini et al., 2016), 4 µM human blocking primer for the "teleo" primers (Civade et al., 2016) and 0.2 µg/µl bovine serum albumin (Roche Diagnostic, Basel, Switzerland). The primers were 5′-labeled to facilitate the matching of each sequence to their respective sampling station during the sequencing process. The PCR mixture was denatured at 95°C for 10 min, followed by 50 cycles of 30 s at 95°C, 30 s at 55°C, and finally 1 min at 72°C and a final elongation step at 72°C for 7 min. For each filtration, a total of 12 replicate PCRs were run. The PCR replicates were sequenced separately, and the results for each PCR replicate were summed up for each sample with the 12 individuallytagged PCR replicates after bioinformatic curation (Pont et al., 2018). To perform the amplification, we used the teleo primer pair (forward: -ACACCGCCCGTCACTCT, reverse: -CTTCCGGTACACTTACCATG), which has been shown to accurately detect our target taxa Actinopterygii and Chondrichthyes (Collins et al., 2019; Polanco Fernández, Richards, et al., 2021b).These primers target a specific circa 60 bp fragment within the mitochondrial 12S ribosomal DNA gene (Valentini et al., 2016). After the amplification, the quantification of the samples was performed using capillary electrophoresis (QIAxcel; Qiagen GmbH) and the purification with the MinElute PCR purification kit (Qiagen GmbH), respectively. The quantification step was repeated, and the resulting purified PCR products were then pooled in equivalent volumes (Pont et al., 2018). The three libraries were prepared according to the MetaFast protocol (Fasteris, 2020). Following the manufacturer's instructions, paired-end sequencing (2 × 125 bp) was performed on an Illumina HiSeq 2500 sequencer on a HiSeq Rapid Flow Cell v2 with the HiSeq Rapid SBS Kit v2 (Illumina) and a MiSeq (2 × 125 bp) with the MiSeq Flow Cell Kit v3 (Illumina). To monitor potential contaminants, a total of four negative extraction controls and two negativePCR controls (ultrapure water, 12 replicates) were amplified and sequenced in parallel to the samples. We found no fish sequence reads within our PCR controls.

2.4 | Species pipeline – OBITools filtering analyses

We used two bioinformatic pipelines: one to assign sequences to known species available in the reference database, and a second one into MOTUs since the reference database is incomplete (Marques et al., 2021). The first bioinformatics pipeline, the Species pipeline, was used following the protocol of Valentini et al. (2016) to process the raw sequence reads into taxa. For that, we applied programs implemented in the OBITools toolkit (https://git.metabarcoding.

org/obitools/obitools.git Boyer et al., 2016). During the first main step – pre-processing – reads were assembled using illuminapairedend (OBITOOLS). Reads were further demultiplexed and primers trimmed using ngsfilter. Sequences were cleaned using obiclean at default parameters. The taxonomic assignments were performed by the ecotag algorithm from the OBITools toolkit. This algorithm relies on the National Center for Biotechnology Information (NCBI) phylogeny tree as a reference base (Boyer et al., 2016). During the second main step, several quality thresholds were applied. We discarded occurrences below ten reads and sequences that were not assigned to the targeted taxa. To correct for potential tag-jumps, sequences with an abundance of less than 0.001% per taxon and per library were discarded (Polanco Fernández, Marques, et al., 2021a; Schnell et al., 2015).

2.5 | MOTU pipeline – SWARM Clustering analyses

The second bioinformatics pipeline, the MOTU pipeline, followed the protocol described in Marques et al. (2020) to process the raw reads into MOTUs, acting as proxies of species richness in the absence of a complete reference database. This is extremely valuable, as according to GAPeDNA v.1.0.1, only 36% and 30% of the species are sequenced for the teleo marker in the respective ecoregion: Western Mediterranean Sea and Mascarene Islands (https://shiny.cefe.cnrs.fr/GAPeDNA/; Marques et al., 2021). The clustering of sequences into MOTUs further lowers the risk of overestimating the species richness due to intraspecific variation (Brandt et al., 2021). During the pre-processing step, reads were assembled using VSEARCH (Rognes et al., 2016). Reads were further demultiplexed and primers trimmed using CUTADAPT (Martin, 2011). With the unsupervised SWARM algorithm, sequences were clustered into MOTUs based on sequences' proximity and abundance (parameter d = 1, -f option enabled). The taxonomic assignment relies also on the NCBI phylogeny tree as a reference base (Boyer et al., 2016). For the assignment, the most abundant sequence within each cluster was used as a representative sequence (Marques et al., 2020). During the second main step, the same cleaning steps as for the Species pipeline were applied, with the addition of removing MOTUs present in a single PCR replicate over the data set to avoid overestimation of richness due to spurious sequences arising in a single PCR event. In the post-clustering step, the post-clustering curation algorithm LULU was applied with default parameters to further clean erroneous MOTUs (Frøslev et al., 2017; Marques et al., 2020). A limitation of this pipeline is the clustering and the application of stringent thresholds, which might discard some rare but real species or group distinct related species together within the same MOTU. These precautionary steps are however needed to better approximate species richness in the absence of a complete reference database and to reduce the risk of numerous false positives (Marques et al., 2020).

We excluded four sample replicates from the analysis as both pipelines could only retrieve non-targeted taxa. These were three samples collected with the Niskin method in Cap Lardier 2 (MN5_1 at 0 m, MN5_4 at 150 m, MN5_5 at 200 m) and one sample collected with the Submersible method in Tombant Americain (MS5_1 at 120 m).

2.6 | Data cleaning and analysis

The taxonomic assignments, which were gained through two bioinformatics pipelines (NCBI taxonomy), were first corrected to fit the FishBase taxonomy by using the R package *rfishbase* (Boettiger et al., 2012). We then manually verified these retrieved taxa by using the online version of FishBase (Froese & Pauly, 2021). When the geographical occurrence of the taxa did not match the respective sampling area, we reassigned the taxa to the next possible taxonomic level known to occur in this area (Supplementary material 2, Tables S3 and S4). This verification procedure was used to avoid false positives, as taxonomic classification errors can occur due to the lack of taxonomic resolution on the metabarcode for some species coupled with gaps in the reference database for both regions, as well as the presence of some potentially erroneous sequences in NCBI (Marques et al., 2021).

2.7 | Comparison of eDNA taxa identification to synthesis data

Using the outputs of the Species pipeline, we compared the detection capabilities of eDNA to the known occurrences of taxa based on a synthesis of previous data sets. Before the comparison, we adapted the taxonomic information of all synthesis data according to the FishBase taxonomy by using the R package *rfishbase* (Boettiger et al., 2012). For all taxa at the species level, we further obtained their habitat category from FishBase (Froese & Pauly, 2021).

2.7.1 | Provence coast

For the Provence coast, we used data from the Marine Species Identification Portal as our synthesis data (Albouy et al., 2013; MSIP, n.d.). This data set contains 635 Mediterranean fish species, along with their geographic distribution on a 0.1 resolution grid system (Albouy et al., 2013). The list combines endemic, exotic, and ubiquitous species from existing atlases (Golani et al., 2002; Quignard & Tomasini, 2000; Whitehead et al., 1986) with the species bathymetric ranges adapted according to depth ranges from FishBase (Froese & Pauly, 2010) and Louisy (2015) using the bathymetry of the Mediterranean Sea obtained from ETOPO2 v2 (NOAA National Geophysical Data Center, 2006). For our synthesis data, we included only species from the list occurring within the same cells (0.1 × 0.1°) where eDNA samples were collected and species belonging to the two targeted taxa Actinopterygii and Chondrichthyes. This resulted in a total of 359 species, out of which 29% are sequenced for the 12S mitochondrial position and

the teleo primer (https://shiny.cefe.cnrs.fr/GAPeDNA/ Marques et al., 2021).

2.7.2 | La Pérouse

For La Pérouse, we compared the eDNA data to two different synthesis data. The first synthesis data were a species list based on the checklist of fish species of La Réunion from Fricke et al. (2009). This checklist includes a total of 986 taxa (Actinopterygii and Chondrichthyes) occurring in freshwater, transitional waters, and marine habitats. In our synthesis data, we only included the 969 taxa that occur in marine habitats. 34% of the 961 taxa that were assigned to the species level were sequenced for the 12S mitochondrial position and the teleo primer (https://shiny.cefe.cnrs.fr/GAPeDNA/ Marques et al., 2021). The second synthesis data consisted of data collected during the same expedition as the eDNA data (Durville et al., 2021). The species list was compiled using films and photographs taken during nine dives (one dive at night). It included 147 different taxa belonging to both classes Actinopterygii and Chondrichthyes. 133 species were identified up to the species level, with 36% being sequenced for the 12S mitochondrial position and the teleo primer (https://shiny.cefe.cnrs.fr/GAPeDNA/ Marques et al., 2021).

2.8 | Compositional analysis between eDNA samples

We investigated compositional differences in eDNA samples by using the outputs of the MOTU pipeline. For both marine regions, we constructed a MOTUs presence-absence matrix for each sample. Using these matrices, we calculated the Jaccard distance assessing the MOTU dissimilarity between samples (R package *vegan*; function *vegdist*, Oksanen et al., 2020). To ordinate the compositional differences between sampling stations for each region, we performed a principal coordinates analysis (PCoA) on the Jaccard distance matrix using the R package *ade4* (function *dudi.pco*, Dray & Dufour, 2007). We then plotted the ordination values in a geographic space and additionally reported the explained deviation of each axis. To test the effect of depth (categories: 0 m, 20–99 m, ≥100 m) on MOTU composition dissimilarity (Jaccard distance), we performed a Permutational Multivariate Analysis of Variance (PERMANOVA, 9999 permutations) by using the R package *vegan* (function *adonis*, Oksanen et al., 2020). We further analyzed for both marine regions the number of MOTUs shared by samples found in the depth categories described above. We finally computed the MOTU compositional dissimilarity as turnover (β_{jtu}) and nestedness (β_{jne})components of the β diversity between the surface, shallow mesophotic, and deeper mesophotic ecosystems using the function *beta.pair* of the R package *betapart* (Baselga et al., 2021).

2.9 | Comparison between species-reported depth ranges and sampled depths

We used the outputs of the Species pipeline to analyze the differences between the documented fish depth ranges and the depths at which species were detected with our eDNA approach. The former ecological trait was gathered from FishBase (Froese & Pauly, 2021). For the sampled depths, we used the term "species-depth occurrences" since most species were present in more than one eDNA sample. When the sampled depth had a range between two depths (e.g., 100–140 m), and then, we used the mean of the range for further analyses. In the first step, we computed the percentage of species-depth occurrences that were found within each species documented depth range. Species with a lower depth limit up to 300 m below sea surface were then plotted with their depth range and their respective sampled depth. The limit was chosen on one hand for reasons of clarity and on the other hand as the excluded species were found predominantly within their lower depth limit. The second step included a Welch two-sample *t*-test to investigate whether the signal of eDNA is as strong when found outside each species documented depth range as when it was found inside this documented depth range. The rationale behind this test was the "abundant-center" effect stipulating that species abundances are expected to be greatest at the center of their environmental niches and decline further away from these "optimal" environmental conditions (Brown et al., 1995). This pattern was shown for reef fishes (Waldock et al., 2019), so we can expect a lower number of sequence reads, if proportional to abundance (Carvalho et al., 2022), in samples outside a species-reported depth range. We therefore compared the mean number of reads of the two groups. The null hypothesis posits that the mean number of reads sampled within a species-reported depth range was equal to the mean number of reads sampled outside of a species-reported depth range. As a third step, we ran a Kendall rank correlation test for the species-depth occurrences sampled outside of the species' depth range to assess the relationship between the number of reads and the difference between the sampled depth and the species' depth range. We calculated this difference by subtracting the species' published lower depth limit from the sampled depth for cases where the species' sampled depth was below its lower depth limit. If the sampled depth was above the species' published upper depth limit, then we subtracted the sampled depth from the species' upper depth limit. The null hypothesis posits that there was no significant correlation between the number of reads and these calculated differences corresponding to a niche shift. For the statistical tests, a *p*-value of 0.05 was chosen as a statistical significance criterion.

We used the statistical programming environment R v.4.0.3 for the data cleaning, the downstream analyses, and for producing the graphics and maps (R Core Team, 2020).

3 | RESULTS

3.1 | Detection capabilities of eDNA metabarcoding

3.1.1 | Provence coast

Using the outputs of the Species pipeline, we estimated diversity in taxa composition and compared results to synthesis data. For the Provence coast, we obtained 10,817,609 reads from 35 water samples and found a total of 811 distinct fish sequences (Supplementary material 3; Table S5). Overall, eDNA metabarcoding identified 41 species, 57 genera, 43 families, and 33 orders. In comparison to the eDNA approach, the synthesis data of this region counted 359 species covering 249 genera and 128 families. For both approaches, eDNA and synthesis, about half of all taxa were classified as demersal (Table 1; synthesis 47.4% and eDNA 48.8%) and the remaining species were either bathypelagic, benthopelagic, pelagic-neritic, pelagic-oceanic, or reef-associated species. Bathydemersal species were only included in the synthesis data (6.7%). The species lists obtained from eDNA included 10.9% of species found in the synthesis data. In return, the eDNA approach recorded two species (*Buenia affinis* and *Corcyrogobius liechtensteini*) that were not present in the synthesis data. Further, the number of genera in the synthesis data was more than four times higher than the genus richness recovered by the eDNA method (synthesis $n = 249$ and eDNA $n = 57$). However, the family Gobiidae was for both, synthesis data ($n = 13$) and eDNA samples ($n = 11$), the family with the highest number of genera. While for the synthesis data the family Sparidae ($n = 12$) had the second-highest richness, only two genera were present for this family in eDNA samples. The families with the second-highest genus richness ($n = 5$) in the eDNA data were Labridae and Scombridae, which also both had a high genus richness in the synthesis data with each family having eight genera. The family Myctophidae (synthesis $n = 8$ and eDNA $n = 4$) was for both approaches among the five families with the highest genus richness.

TABLE 1 The number of species per habitat category along the Provence coast (North-Western Mediterranean Sea) for the samples collected by eDNA and for the synthesis data. The table is presented in decreasing order of importance according to eDNA data

Provence coast		
	eDNA data	Synthesis data
Demersal	20 (48.8%)	170 (47.4%)
Reef-associated	6 (14.6%)	36 (10.0%)
Benthopelagic	5 (12.2%)	46 (12.8%)
Pelagic-neritic	4 (9.8%)	27 (7.5%)
Pelagic-oceanic	4 (9.8%)	25 (7.0%)
Bathypelagic	2 (4.9%)	31 (8.6%)
Bathydemersal	—	24 (6.7%)

3.1.2 | La Pérouse

For la Pérouse, out of 5,653,393 reads from eight eDNA samples, we found a total of 721 distinct sequences (Supplementary material 3, Table S5). We detected 57 fish species, as well as the presence of 102, 56, and 33 different genera, families, and orders, respectively. The first synthesis data included a total of 969 distinct taxa, 961 of which were identified at the species level. Overall, 494 and 165 genera and families were present respectively. 47 species were included in the first synthesis data and also detected by the eDNA approach. While 914 species listed in the first synthesis data were not detected by eDNA, eDNA identified ten additional species (*Acanthurus bariene*, *Cirrhitichthys oxycephalus*, *Crossorhombus valderostratus*, *Diaphus splendidus*,*Hirundichthys oxycephalus*, *Lachneratus phasmaticus*, *Pseudocheilinus evanidus*, *Scombrolabrax heterolepis*, *Thalassoma lutescens*,and *Zebrasoma desjardinii*). The second synthesis data, which was gathered during the same expedition as the eDNA data, discovered a total of 147 distinct taxa. 133 distinct species were identified, and overall, 90 and 35 genera and families, respectively, were present. 34 species were detected by both eDNA and the camera approach of the second synthesis data. While with eDNA we found 23 additional species, the camera approach could identify 99 additional species. Most species detected by the eDNA approach and the two synthesis data were categorized as reef-associated (Table 2; eDNA: 75.4%, synthesis 1: 71.7%, and synthesis 2: 94.0%). In all three data sets, species classified as bathypelagic, benthopelagic, and pelagic-oceanic species were included. The eDNA data set and the first synthesis data both contained demersal and pelagic-neritic species, whereas pelagic species were only present in the first synthesis data ($n = 1$). In terms of taxonomic richness and composition, the first synthesis data ($n = 494$) had the highest genus richness per family followed by the eDNA approach ($n = 102$) and the second synthesis data ($n = 90$). The families with the highest genus richness in the eDNA samples were Labridae ($n = 11$), Balistidae ($n = 7$), Myctophidae ($n = 7$), Apogonidae ($n = 5$), Muraenidae ($n = 5$). For the first synthesis data, the family Gobiidae ($n = 29$) was the family with the highest genus richness, followed by the following four families Labridae ($n = 25$), Blenniidae ($n = 18$), Carangidae ($n = 16$), and Serranidae ($n = 16$). The families with the highest genus richness in the second synthesis data were Labridae ($n = 11$), Serranidae($n = 8$), Balistidae ($n = 5$), and Lutjanidae($n = 5$).

3.2 | Fish community composition from different depths

3.2.1 | Provence coast

We used the MOTU pipeline to investigate the compositional dissimilarity of fish communities among eDNA samples depending on their sampling depth. For the Provence coast, we identified a total of 7,265,928 reads from 35 eDNA samples, resulting in a total of 113 distinct MOTUs (Supplementary material 3, Table S5). Within the

31 samples containing MOTUs, we detected on average 77.0 ±80.4 MOTUs per sample.Overall, we detected 30 different species, as well as the presence of 46, 31, and 21 different genera, families, and orders, respectively. For the PCoA of the eDNA samples, the percentage of variance explained by the first two axes was 27.0% (axis 1: 14.54% and axis 2: 12.46%; Figure 3a). The surface water samples filtered with the Athena method had on average 129.3±112.9 MOTUs per sample and formed two clusters according to their filtration replicates (MA replicates, gray diamonds in Figure 3a). The Athena surface samples collected at Cap Negre 1 (MA3 replicates) were more heterogeneous, especially the MA3_1 replicate which only contained ten MOTUS. The samples collected with the Niskin method had on average 19.2 ±21.4 MOTUs per sample and did not show a strong pattern along the depth gradient (MN replicates, triangles in Figure 3a). The Submersible method (MS replicates, circles in Figure 3a) had on average 69.2 ±54.3 MOTUs per sample. The mid-depth samples collected at Imperiaux 1 (MS1 replicates, orange circles in Figure 3a), which were collected by continuously sampling from 65 to 10 m, are close to the Athena surface samples in the upper right corner. All Submersible samples between 60–120 m, with the exception of one mid-depth Banc de Magaud replicate (MS4_3, 6 MOTUs), formed a cluster in the upper left corner. Overall, there was a significant effect of depth (categories: 0 m, 20–99 m, ≥ 100 m) on MOTU composition (PERMANOVA: $p = 2.51$, $R^2 = 0.15$, $p = 0.001$). Approximately the same numbers of distinct MOTUs were found at the surface (0 m, $n = 80$) and mid-depth (20–99 m, $n = 83$) but fewer MOTUs were detected in the deep waters (≥ 100 m, $n = 34$). The deep-water samples share 30 and 23 MOTUs with the mid-water and surface water samples, respectively. The mid-water and surface waters on the other hand share 53 MOTUs (Figure 4a). The pairwise Jaccard's dissimilarity index calculated between the depth categories showed that the surface and the deep-water samples differed the most ($\beta_{jac} = 0.747$). The difference in MOTU composition was mainly explained by turnover ($\beta_{jtu} = 0.489$) and less by nestedness

TABLE 2 The number of species per habitat category at La Pérouse (Western Indian Ocean) for the samples collected by eDNA and for both synthesis data. The table is presented in decreasing order of importance according to eDNA data

La Pérouse			
	eDNA data	Synthesis data 1	Synthesis data 2
Reef-associated	43 (75.4%)	689 (71.7%)	125 (94.0%)
Benthopelagic	5 (8.8%)	52 (5.4%)	3 (2.3%)
Pelagic-oceanic	5 (8.8%)	38 (4%)	4 (3.0%)
Demersal	2 (3.5%)	102 (10.6%)	–
Bathypelagic	1 (1.8%)	26 (2.7%)	1 (0.8%)
Pelagic-neritic	1 (1.8%)	22 (2.3%)	–
Bathydemersal	–	31 (3.2%)	–
Pelagic	–	1 (0.1%)	–

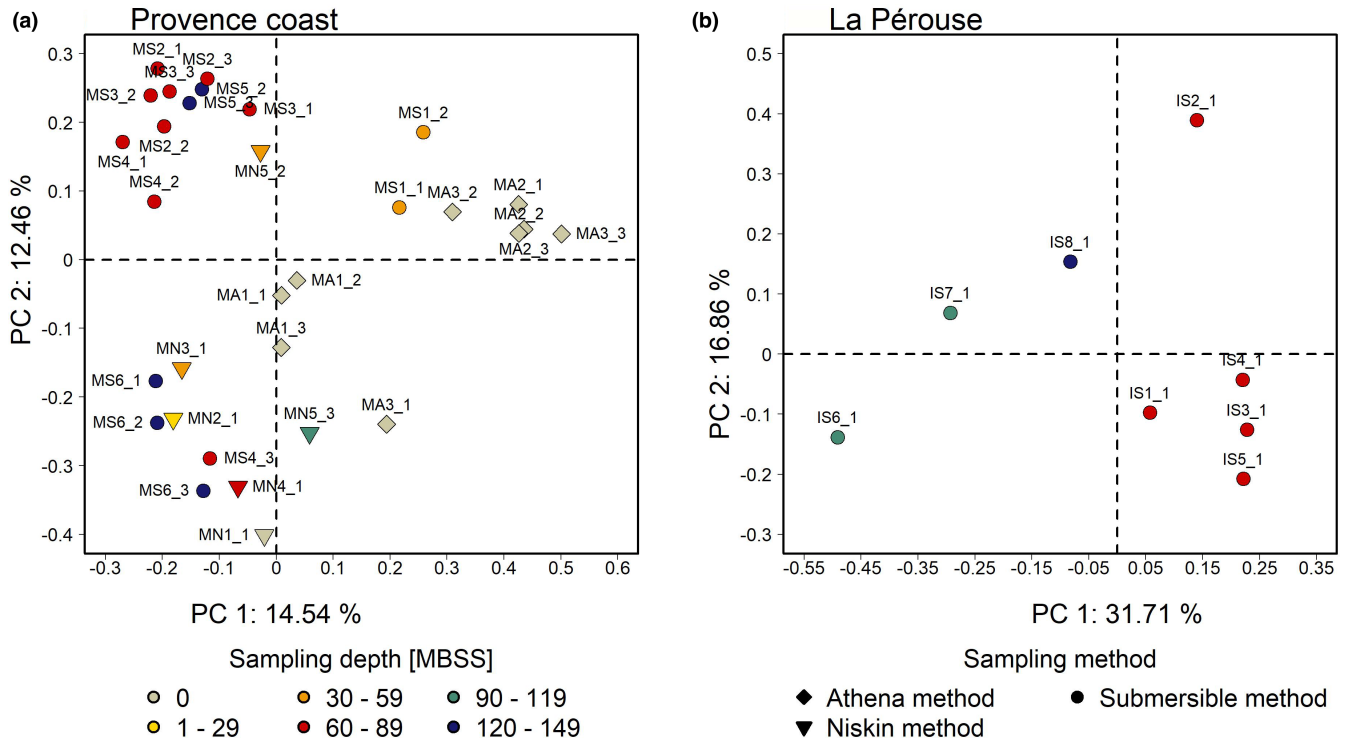

FIGURE 3 Compositional differences (PCoA) were calculated using the Jaccard distance matrix, which is based on the MOTUs presence-absence matrix between eDNA samples collected (a) along the Provence coast in the North-Western Mediterranean Sea and (b) at La Pérouse in the Western Indian Ocean. The color indicates the depth of each sample, and the shape represents the used sampling method. More information about the sampling protocol can be found in supplementary material 1 (Tables S1 and S2)

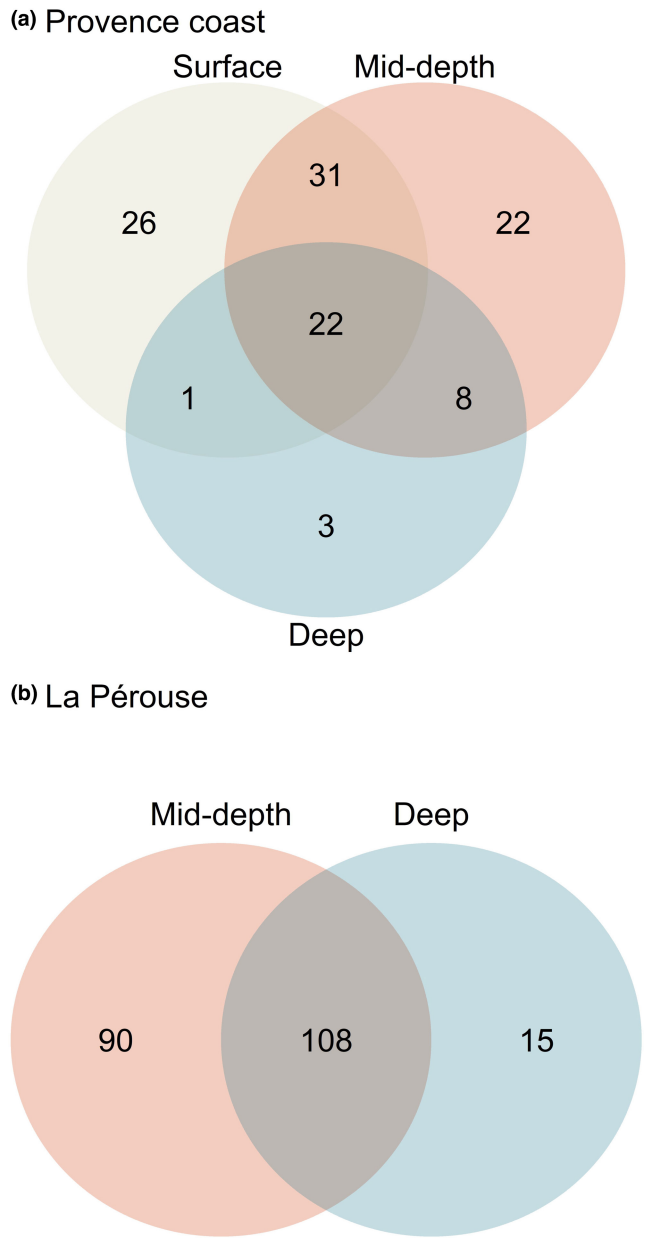

FIGURE 4 Venn diagrams showing the number of MOTUs found (a) in the three depth zones of the coastal North-Western Mediterranean Sea and (b) in the two depth zones of the seamount La Pérouse in the Western Indian Ocean

(β_{jne} = 0.258). The pairwise Jaccard's dissimilarity index between the mid-depth and the deep-water samples reached a value of β_{jac} = 0.655 and was mostly explained by nestedness (β_{jne} = 0.445) and less by turnover (β_{jtu} = 0.211). In return, the difference between the surface and the mid-depth water samples (β_{jac} = 0.518) was almost exclusively explained by turnover (β_{jtu} = 0.505, β_{jne} = 0.013).

3.2.2 | La Pérouse

For La Pérouse, 3,157,242 reads were produced from eight eDNA samples and a total of 213 MOTUs could be distinguished (Supplementary material 3, Table S5). On average, we detected 326.5 ± 134.6 MOTUs per sample and out of these MOTUs, we identified a total of 40, 76, and 46 species, genera, and families, respectively. For the PCoA of the La Pérouse eDNA samples, the first two axes explained 48.57% of the total variance (axis 1: 31.71% and axis 2: 16.86%; Figure 3b). On the first axis, there is a distinction visible between the samples collected around 60 m (red circles in Figure 3b) and the deep-water samples (≥ 100 m; green and blue circles in Figure 3b). The mid-depth samples (IS1_1 – IS5_1, around 60 m) were all clustered in the lower right corner, except one sample (IS2_1) which was collected during the night. From the more dispersed deep-water samples, the IS7_1 sample (green circle in Figure 3b) was collected at night, while the samples IS6_1 (green circle in Figure 3b) and IS_8 (blue circle in Figure 3b) were taken during the daytime. In total, depth (categories: 60–99 m, ≥ 100 m) had a significant effect on MOTU composition (PERMANOVA: F = 2.28, R^2 = 0.28, p = 0.02). The comparison between the MOTUs found in the mid-depth water (n = 198) and the MOTUs found in depths below 100 m (n = 123) showed that they share 108 MOTUs, including 21 taxa assigned to the species level. 90 and 15 MOTUs were found only in mid-depths and deep-water samples, respectively (Figure 4b). The pairwise Jaccard's dissimilarity index between the two depth categories reached a value of β_{jac} = 0.493, with a similar amount explained by both turnover (β_{jtu} = 0.217) and nestedness (β_{jne} = 0.276).

3.3 | Differences between species-reported depth ranges and detected depths

3.3.1 | Provence coast

Due to incomplete depth range available in FishBase for four out of the 41 detected species in the Provence coast, the following species were excluded from this analysis: *Chelon labrosus*, *Corcyrogobius liechtensteini*, *Lesueurigobius suerii*, and *Oedalechilus labeo*. The published depth ranges of the remaining 37 species extend from 0 to 2878 m (mean upper depth limit: 14.1 ± 26.7 m; mean lower depth limit: 364.8 ± 556.5 m) (Froese & Pauly, 2021). Most species were present in more than one eDNA sample resulting in a total of 155 species-depth occurrences. 54.2% (n = 84) of all occurrences were found within species-reported depth ranges. Divided into the depth categories, there were 98 species-depth occurrences above 60 m, out of which 42.9% match the published depth ranges. Between 60 and 99 m and below 100 m, a total of 39 and 18 species-depth occurrences were found, respectively. The percentage matching the species-reported depth ranges was 74.4% and 72.2%, respectively. We observed species both below their published lower depth limit and above their published upper depth limit (Figure 5). For example, *Symphodus ocellatus* was sampled at 0, 38, and 70 m (documented depth range of 1–30 m) while *Spicara maena* was detected at 0, 38, and 130 m (documented depth range of 30–130 m) (Froese & Pauly, 2021). No statistically

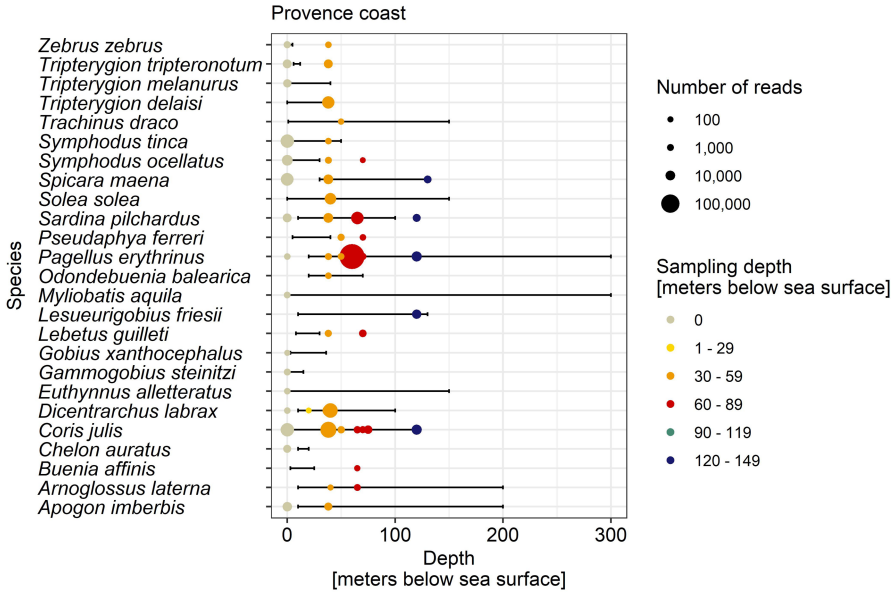

FIGURE 5 Fish species detected with eDNA metabarcoding along the Provence coast (North-Western Mediterranean Sea). The black lines show the species-reported depth ranges retrieved from Froese & Pauly (2021). Only species with a lower depth limit of up to 300 m are displayed for clarity reasons. The points represent species detections by eDNA. The size of the point is proportional to the total number of reads for a single species within one filter. Note that in samples taken at 150 and 200 m, no species were retrieved through the eDNA approach

significant difference was found between the mean number of reads of species-depth occurrences taken within a species documented depth range (M = 12,359) and the mean number of reads per sample outside a species documented depth range (M = 6289; Welch two-sample t-test, p = 0.10; Supplementary material 4, Table S6). For the species-depth occurrences found outside of the species-reported depth ranges, the mean difference between the sampled depths and the species-reported depths was 21.8 m (*Mdn* = 10.0 m, *SD* = 27.6 m, *min* = 1.0 m, *max* = 150.0 m). No significant correlation was found between the number of reads and the depth difference (Kendall rank correlation test, $\tau = -0.07$, $p = 0.43$; Supplementary material 4, Table S7).

3.3.2 | La Pérouse

For La Pérouse, one species (*Macropharyngodon bipartitus*) out of 57 detected species was excluded as the upper depth range was unknown (lower depth range: 30 m). This species was found in two filters at 65 m, which means that, regardless of its lower depth range, it is outside of the reported depth range from FishBase. The published depth ranges of the remaining species were between 0 and 8000 m (mean upper depth limit: 10.1 ±29.6 m; mean lower depth limit: 380.9 ±1142.1 m) (Froese & Pauly, 2021). Most species were present in more than one eDNA sample resulting in a total of 162 species-depth occurrences. Out of these occurrences, 48.1% (n = 78) were collected at a sampling depth within the expected depth range. Divided into the depth categories, there were 122 species-depth occurrences in mid-depth waters (60–99 m) and 40 species-depth occurrences in deeper waters (≥ 100 m). The percentage matching the species-reported depth ranges was 47.5% (n = 58) and 50.0% (n = 20), respectively. Three species-depth occurrences of two species (*Scombrolabrax heterolepis*: documented depth range of 100–900 m and *Lepidocybium flavobrunneum*: documented depth range of 200–1100 m) were found around 60 m, which is above their reported upper depth limit. However, more occurrences were detected below their lower depth range as for example *Hirundichthys oxycephalus* or *Sufflamen chrysopterum*, which have reported maximal depth ranges of 20 m and 30 m, respectively, but were both found below 60 m (Figure 6). The mean number of reads of the 84 species-depth occurrences collected outside the species documented depth ranges (M = 5628) and the mean of the 78 occurrences taken within the species documented depth ranges (M = 6108) did not vary significantly (Welch two-sample t-test, p = 0.84; Supplementary material 4, Table S6). For the species-depth occurrences collected outside of the species-reported depth range, no significant correlation was found between the number of reads and the difference between the observation depths and species-reported depths (Kendall rank correlation test, $\tau = -0.09$; p = 0.24; Supplementary material 4, Table S7). The mean difference between the two depths was 30.1 m (*Mdn* = 21.5 m, *SD* = 28.9 m, *min* = 1.0 m, *max* = 140.0 m).

4 | DISCUSSION

Beyond the well-established capacity of eDNA metabarcoding through the 12S marker to provide a comprehensive picture of fish biodiversity in shallow MEs (e.g., Boulanger et al., 2021; Polanco Fernández, Marques, et al., 2021a), our study further shows that this technique was able to assess highly diverse fish assemblages made of Actinopterygii and Chondrichthyes in two different oceanic regions: the shallow-to-mesophotic ecosystem along the Provence coast (NW Mediterranean Sea) and the mesophotic ecosystem at the seamount La Pérouse (WIO). By analyzing the MOTU dissimilarity along the shallow-mesophotic sampling depth, we show marked variations in species composition as well as the presence of unique and shared MOTUs in the different depth zones. Moreover, the high percentage of species-depth occurrences recorded outside

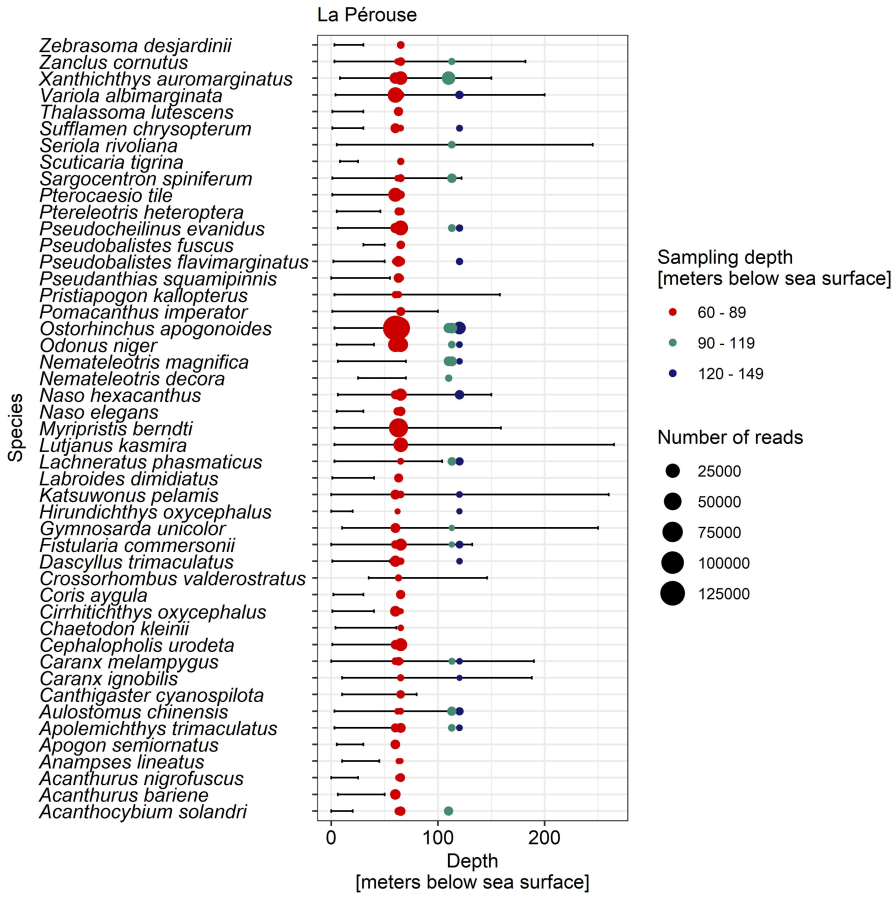

FIGURE 6 Fish species detected with eDNA metabarcoding at La Pérouse (Western Indian Ocean). The black lines show the species-reported depth ranges retrieved from Froese & Pauly (2021). Only species with a lower depth limit of up to 300 m are displayed for clarity reasons. The points represent species detections by eDNA. The size of the point is proportional to the total number of reads for a single species within one filter

of species-reported depth ranges is intriguing and promising since these ranges are potentially underestimated (Kahng et al., 2014), and eDNA surveys might be used to improve their accuracy. These insights are crucial since a substantial amount of marine biodiversity, particularly in deeper oceans, remains unknown (Turner et al., 2019).

The species compositions detected by eDNA show that with a relatively small sampling effort of 35 samples for the Provence coast and eight samples at the seamount La Pérouse a broad snapshot of fish diversity can be obtained. For the well-studied Provence coast, the eDNA approach detected a subset of the potentially present species from the synthesis data, but this information combines multiple atlases based on data collected over many years (Golani et al., 2002; Quignard & Tomasini, 2000; Whitehead et al., 1986). A major difference with the synthesis data is that we have not detected any bathydemersal fish species, which could require filtering closer to the substrate to capture those species that live and feed on the bottom at depths beyond our present maximum eDNA sampling depth (Froese & Pauly, 2021). For the less studied seamount ecosystem of La Pérouse, the eDNA approach retrieved a more diverse fish community composition than the second synthesis data (camera approach), which was gathered during the same expedition. The camera approach, in return, led to more taxa being found that were assigned up to the species level (Durville et al., 2021). For both study regions, we found taxa that were not reported in the respective synthesis data. On the one hand, this can be due to incomplete synthesis data as for example the species *Diaphus splendidus* was also found by Cherel et al. (2020) at the seamount La Pérouse or the species *Corcyrogobius liechtensteinii*s known to occur in the Mediterranean Sea (Boulanger et al., 2021). On the other hand, such putative new records might only be due to closely related species having no barcode available. This could for example be the case for *Thalassoma lutescens*, as the closely related species *Thalassoma genivittatum* was detected by both synthesis data but is not sequenced for the 12S mitochondrial position and the teleo primer (Durville et al., 2021; Fricke et al., 2009; Marques et al., 2021). Overall, our results further demonstrate the applicability of eDNA metabarcoding in a variety of environments from the surface to mesophotic depths. Especially, the use of in situfiltering method enables the collection of large-volume water samples at the desired sampling depth and targeted habitat, which allows dynamic, more accurate, and comprehensive eDNA surveys.

As eDNA studies rely on reference database completeness (Marques et al., 2020) and MEs, or below, remain largely understudied (Pyle & Copus, 2019), we clustered sequences into MOTUs, which enables an accurate assessment of biodiversity gradients in the absence of a complete database (Marques et al., 2020; Mathon et al., 2022). The high numbers of MOTUs obtained for both study regions, especially with the Athena and the Submersible method, further highlight the potential of eDNA to reveal comprehensive species assemblages. For both study regions, more MOTUs are found in surface and mid-depth water samples than in deep-water samples, which matches

the findings of previous eDNA studies (Andruszkiewicz et al., 2017; Govindarajan et al., 2022). Moreover, we reveal that while a large fraction of MOTUs is shared between depth zones, each depth also has its own set of MOTUs (Figure 4). The presence of differences in fish community composition depending on sampling depth in both our study regions further shows the feasibility of applying eDNA metabarcoding on smaller scales and along depth gradients. However, the effect of depth on MOTU composition was only minor for both study regions. In regard to this, it is important to note that the three methods differ in the amounts of water sampled, resulting in a different number of MOTUs per sample. To improve results within and across methods, increasing the sampled water volume and number of sampling replicates is important to maximize the number of taxa obtained per sample, especially as DNA molecules in seawater samples can be patchy (Bessey et al., 2020; Stauffer et al., 2021). Nevertheless, with appropriate sampling protocols, eDNA metabarcoding is a promising tool to reveal community compositions at small spatial scales and along depth gradients.

In our study, we use eDNA detections as a proxy of a source species being present at the respective sampled location. This assumption must be treated with caution, as eDNA molecules might get transported away from the place where they are shed from an organism. Such vertical and horizontal transport mechanisms are very complex in marine systems and influenced by hydrological factors (e.g., wind, currents, site characteristics) as well as the abiotic (e.g., temperature, salinity), and biotic environment (e.g., biofilms, vegetation) (Harrison et al., 2019). The vertical eDNA movement in MEs has received little attention (but see Allan et al., 2021), but there is evidence that the vertical dispersal is limited (e.g., Govindarajan et al., 2022; Jeunen et al., 2020; Monuki et al., 2021; Murakami et al., 2019). Since much is still unknown about DNA persistence and transport in marine environments, more proof-of-concept research is needed, especially in terms of how eDNA is transported across marine environments and how these processes vary along depth gradients when environmental conditions change (Miya, 2022).

Beyond documenting species geographic range (e.g., Polanco Fernández, Marques, et al., 2021a; West et al., 2020), eDNA, combined with in situ filtration, could inform on marine fish species' depth ranges. Growing evidence suggests that species' lower depth range limits are underestimated (Kahng et al., 2014), partly due to the lack of sampling efforts in mesophotic and deeper regions (Pyle & Copus, 2019). In agreement, our comparison of the depth at which a species was detected with eDNA metabarcoding to the species' published depth range from FishBase shows for both study regions that only circa half of all eDNA species-depth occurrences fit the species' documented depth range (Froese & Pauly, 2021). As discussed in the previous section, such detections of species outside their reported depth range might also be explained by DNA being transported away from its source. However, other evidence suggests that the vertical dispersal of eDNA molecules is limited (e.g., Allan et al., 2021; Minamoto et al., 2017; Murakami et al., 2019) so that detection can indicate real depth range use. For example, the detection of *Sufflamen chrysopterum*, a species of Balistidae, in La Pérouse deeper than its published depth range within 1–30 m (Froese & Pauly, 2021) matches knowledge from other Balistidae species that inhabit deeper environments of the continental shelf mesophotic environments (Chasqui Velasco & González Corredor, 2019; Thresher & Colin, 1986). The absence of correlation between the number of reads and the depth difference also suggests that we poorly know the real depth boundaries of most species or that the "abundant-center" effect does not apply along the depth gradient. Therefore, our findings highlight the importance of integrating sampling across depth zones to gain a more complete understanding of local biodiversity patterns, especially community structure and turnover including the vertical dimension.

5 | CONCLUSION

Mesophotic ecosystems are widely understudied (e.g., Costello & Chaudhary, 2017; Kahng et al., 2014; Turner et al., 2019), and we show the potential of eDNA metabarcoding to reveal fish community compositions across the shallow-to-mesophotic depth gradient. An even more comprehensive taxonomic picture of local biodiversity can be revealed, by using the same water samples for subsequent analysis and applying primer sets specific to other taxa (McClenaghan et al., 2020). This represents a valuable methodological step forward since mesophotic ecosystems are harder to study than their shallow counterparts but are also potentially threatened (Pyle & Copus, 2019; Smith et al., 2019). Our eDNA surveys did not only detect variations in community composition depending on the sampling depth but also found shared MOTUs between samples collected at the surface, in mid-depth, and below 100 m. Based on the emerging evidence of the applicability and accuracy of eDNA surveys at small spatial scales (e.g., Andruszkiewicz et al., 2017; Jeunen et al., 2020; Minamoto et al., 2017), our findings highlight the potential of applying eDNA surveys along depth gradients and thereby gain relevant insights in vertically structured community assemblages. Further, eDNA might enhance species' reported depth ranges which currently lack precision and are biased (Kahng et al., 2014). These insights are crucial, especially because MEs face increasing threats such as climate change, overexploitation, or invasive species (Díaz et al., 2019; Smith et al., 2019). We thus advocate the use of eDNA for the investigation in mesophotic ecosystems to better understand, monitor, and predict changes in community composition. Future eDNA surveys should incorporate region-specific factors, such as thermocline, or hydrologic and hydrodynamic processes, to enhance accuracy and further validate our findings.

AUTHOR CONTRIBUTIONS

DM, FH, and LP jointly designed this study. LB, JB, TB, RH, JBJ, EB, NG, JD, and PD participated in the field data acquisition, MM, MJ, VM, AV, TD, SM, APF, and CA analyzed and interpreted the data. MM with the help of MJ and LP wrote the first draft, and all the

co-authors contributed to the writing and the improvement of the manuscript.

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CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest.

DATA AVAILABILITY STATEMENT

The R-scripts (https://doi.org/10.6084/m9.figshare.20713405.v1) and the raw sequencing data (Provence coast: https://doi.org/10.6084/m9.figshare.20713345 and La Pérouse: https://doi.org/10.6084/m9.figshare.20711686) are archived online and are publicly available.

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