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Mesophotic protected habitats as refugia for the most at-risk elasmobranch species

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ABSTRACT

Coastal areas experience among the highest cumulated human pressures across the world's oceans with severe consequences on some fish populations which become scarce and at risk of local extirpation. Marine protected areas (MPAs) are essential for conserving these threatened populations, but they often cover only the most superficial waters and are not three-dimensionally extensive enough, leaving most mobile species with a large home range like elasmobranchs vulnerable to human pressures. Here we explored the role of mesophotic and deep-water habitats as refuges for elasmobranchs and the most at-risk species in the Parc Naturel Marin du Cap Corse et de l'Agriate, a large MPA in the Mediterranean Sea. Using the metabarcoding of 200 environmental DNA samples of seawater filtered between 1 and 835 m depth we revealed that fish species diversity was the highest in the mesophotic habitat (30-149 m). We also found the highest number of endangered species, including Evolutionary Distinct and Globally Endangered (EDGE) species such as *Rostroraja alba* and *Squatina squatina*, in this key habitat. Species composition varied significantly with depth, revealing a stratification linked to human pressure and environmental conditions. Protecting both mesophotic and deeper habitats (below 150 m) thus appears crucial to preserving biodiversity, species with unique functional roles but also those with unique phylogenetic diversity, in order to ensure ecosystem resilience in the face of climate change and overexploitation. The full protection of deep-water refuges within the western Mediterranean basin is essential to ensure the preservation of the most at-risk species, with the possibility of rewilding by connectivity other depleted adjacents habitats.

1. Introduction

As a result of human activities causing major changes to ecosystems, we have entered a new geological era, recently coined as the Anthropocene (Latour, 2017). This era is characterized by a massive defaunation (McCauley et al., 2015; Finn et al., 2023) under the combined actions of habitat destruction, pollution, introduction of non-indigenous species, climate change and over-exploitation. Among all

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species affected by humans, elasmobranchs (sharks, skates and rays) are the second most threatened group just after marine mammalian (Sherman et al., 2023) mainly because of overfishing (Dulvy et al., 2014; Dulvy et al., 2021; Pacoureau et al., 2021; Pacoureau et al., 2023; Finucci et al., 2024). To counteract these pervasive impacts, Marine Protected Areas (MPAs) have been created as conservation tools designed to protect biodiversity (Grorud-Colvert et al., 2021; Knip et al., 2012). They also aim to promote resilient marine ecosystems and provide societal benefits (Marcos et al., 2021).

Yet even though MPAs can be useful to protect some fish species (Yates et al., 2016; Sanchez et al., 2024), they are not effective for all of them due to the limited extent of their territory in all three dimensions, both vertically and horizontally (Bonnin et al., 2021; Dwyer et al., 2020). The benefits of protection by large MPAs can also be insufficient when it comes to safeguarding mobile and widely distributed species such as elasmobranchs in a vast area which is also difficult to control globally (Di Lorenzo et al., 2022; White et al., 2017).

Recent studies highlight the role of remote areas and MPAs as ultimate refuges for large home-range species (Letessier et al., 2019; Conners et al., 2022; Zhang et al., 2024). Such refuges provide favorable conditions to host persistent and large populations which can then serve as sources of dispersal towards less favorable but connected habitats through a spillover effect (Roberts et al., 2005; Gaines et al., 2010; Di Lorenzo et al., 2020). Yet, such quasi-intact areas no longer exist on anthropized coasts (Williams et al., 2022). In these regions, conservation strategies have often focused on protecting two-dimensional shallow areas without considering deeper habitats, such as the mesophotic and rariphotic reefs (Mantas et al., 2024; Mathon et al., 2024), while impacts are three dimensional (Jacquemont et al., 2024). Currently, only the surface of the MPA over the oceans is considered as a measure of conservation effort and target (Maxwell et al., 2020; Jefferson et al., 2021). Indeed, MPAs are still mainly designed to protect shallow coastal habitats, ignoring deeper waters which can be highly exposed to threats like fisheries (Soares et al., 2019; Finucci et al., 2024; Jacquemont et al., 2024; Mathon et al., 2024) but can also be refuges for the most threatened species (Mantas et al., 2024) like apex predators (Brown et al., 2022). It is thus urgent to assess the potential refuge of mesophotic and deeper layer habitats (Velasco-Lozano et al., 2024). This assessment is essential to establish effective MPA networks made of stepping-stone climatic oases that could ensure long-term species persistence (Roberts et al., 2017), in line with current and future international marine policies (Podda and Porporato, 2023).

The Mediterranean Sea, which is the largest enclosed sea on Earth, has been exploited by humans since the beginning of civilizations (Bianchi and Morri, 2000; Coll et al., 2010). Today, major cumulated human impacts like pollution, habitat destruction, exploitation of resources, spread of non-indigenous species and intense maritime traffic occur across the Mediterranean Sea (Drius et al., 2019; Laviola et al., 2022). As a result, no intact coastline remains (Williams et al., 2022). In this context, Mediterranean elasmobranchs, representing 48 shark species from 18 families and 38 batoid species from 11 families (Serena et al., 2020), are particularly vulnerable (Maioli et al., 2023). In the north-western Mediterranean basin, the islands of Corsica and the Balearic archipelago are less impacted by human activities and can be considered as refuges for elasmobranchs (Pichot et al., 2024). However, these islands are experiencing overtourism and climate change which may limit their refuge capacity (Zittis et al., 2023; Monti et al., 2018). Therefore, their mesophotic and deeper protected habitats may represent ultimate and more secure refuges for threatened elasmobranchs.

In this study, we evaluate the potential role of mesophotic and deeper layers as refuges for fishes in the largest French Mediterranean MPA located in the Northern part of the Corsica Island. Indeed, even on this island, which is relatively untouched by human activities, the conservation of some fish species like elasmobranchs remains a major concern. This is due to the impact of tourism, habitat degradation, and pollution concentrated mainly on the coast and, above all, fisheries bycatch (Bousquet et al., 2022). Previous studies show that human pressure decreases with depth and distance from the coast, and that mesophotic habitats can therefore serve as refuges for species targeted by fishing (Lindfield, 2015; Brown et al., 2022). Yet, the assessment of biodiversity in these deep refuges remains challenging (Andrzejaczek et al., 2022; Das et al., 2022) but is essential if we expand the protection coverage to meet the new conservation target by 2030, i.e., 30 % of the ocean within MPAs (Jorgensen et al., 2022). Moreover, these habitats may host fishes with unique ecological roles and functional traits (Stefanoudis et al., 2023), including the most endangered elasmobranchs (Dulvy et al., 2024; Pimiento et al., 2023), such as EDGE (Evolutionarily Distinct and Globally Endangered) species. Thus, protecting mesophotic and rariphotic habitats can be essential to preserve unique evolutionary lineages that make a significant contribution to global biodiversity (Scherson and Faith, 2018).

To date, biodiversity inventories in shallow, mesophotic and deeper marine waters are largely based on classical techniques like fisheries catches or underwater cameras with well-known limitations and biases (Colton and Swearer, 2010; Jacquemont et al., 2024; Polanco Fernández et al., 2021; Marques et al., 2021a). Biodiversity monitoring of deep-sea fishes can be greatly enhanced using environmental DNA (eDNA) metabarcoding, which allows better detection than traditional techniques, particularly for rare and threatened species, with the advantage of being less costly and non-invasive (Boussarie et al., 2018; Mathon et al., 2022; Miya, 2022; Muff et al., 2023; Rozanski et al., 2024). eDNA corresponds to the genetic material obtained directly from an environmental sample and is made of a complex mixture of intracellular (from living cells) or extracellular DNA originating from fish shed skin, urine, feces, or carcasses (Taberlet et al., 2012). The extraction, amplification with universal primers and sequencing of eDNA from seawater produces genetic sequences that can be assigned to species or taxa using a genetic reference database (Casey et al., 2021).

The aim of this study is to compare fish, elasmobranch, and the most at-risk species diversity at different depths in the Cap Corse and Agriate Marine Park (North Corsica Island). The goal is to assess whether the mesophotic and deeper layers can act as refuges within this MPA to avoid or alleviate human pressure and climate change occurring in shallow waters (Collins et al., 2018). To address this question, we analyzed 200 environmental eDNA samples collected at different depths ranging from 1 to 835 m.

2. Material and methods

2.1. Study locations and depth ranges

Corsica Island is located 160 km away from the French mainland and <90 km from the Italian mainland. It has a relatively low mean population density of 39 inhabitants/km^2 (Insee, 2023), a quasi-absence of industrialization, and a weak industrial fishing pressure (Paolo et al., 2024). Only six trawlers operate almost exclusively in the Corsican Channel, at the east of Cap Corse, fishing at depths ranging from 200 to 400 m (SIH, 2017; https://parc-marin-cap-corse-agriate.fr/documentation/analyse-risque-peche-pnmcca).

Its coastline stretches 1046 km, yet only 0.28 % (32,655 km^2 of MPAs) of its waters are protected (MedAMP, 2017). In its northern part, the Parc Naturel Marin du Cap Corse et de l'Agriate (hereafter PNMCCA), created in 2016, covers an area of 6830 km^2. This makes it the largest MPA in metropolitan France and one of the most important MPAs in the Mediterranean (https://medpan.org). This MPA encompasses 225 km of coastline from the eastern end of Cap Corse (town of Bastia) to the western part including the Gulf of Saint Florent and extending as far west as the commune of Belgudè in Balagne. This MPA is extending out to 40 nautical miles offshore (Fig. 1) with deep habitats (>2000 m). This MPA also hosts unique ecological features like four large submarine canyons close to the coast. The PNMCCA also encompasses continental shelves, slopes and seamounts, as well as the abyssal

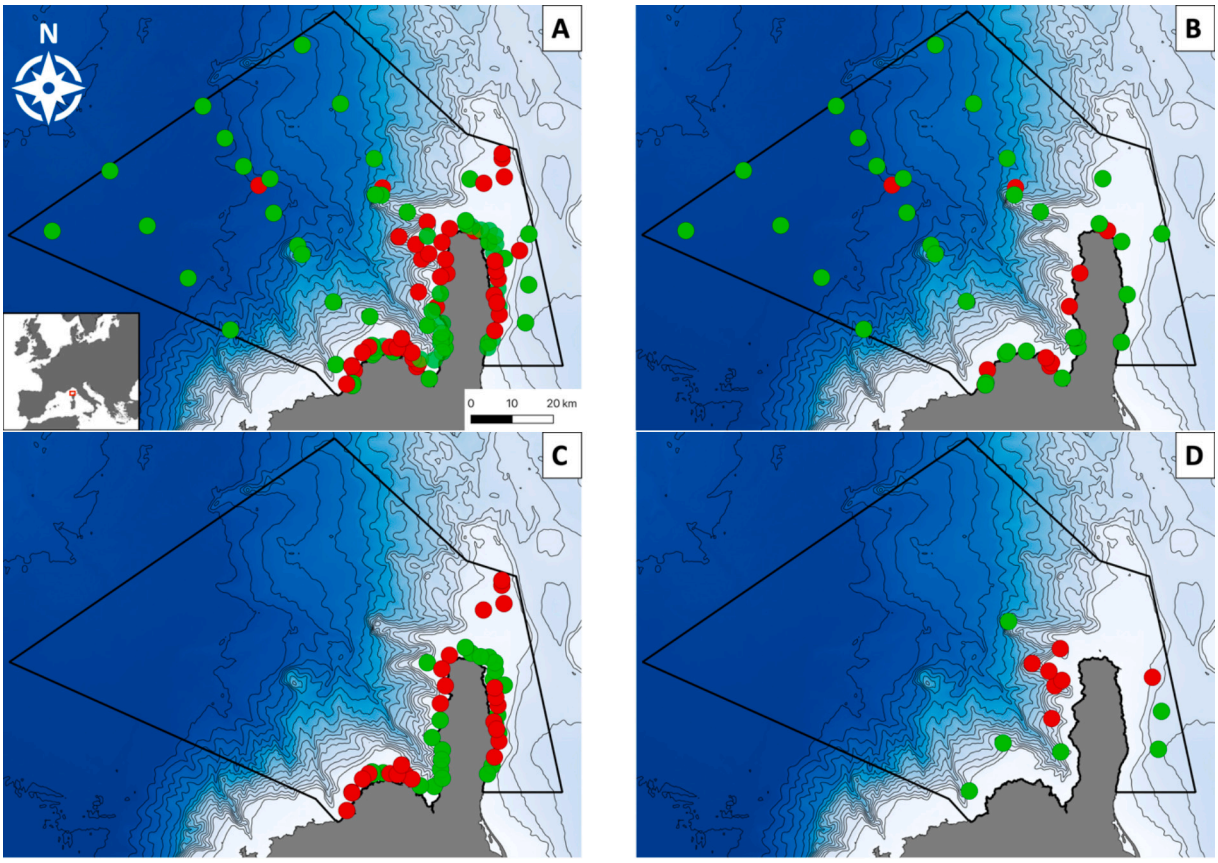


Fig. 1. Maps of the 200 eDNA samples (A) in the waters of the marine park (Cap Corse et Agriate) and at different depths (B: shallow; C: mesophotic; D: deeper layers). The presence of elasmobranchs (sharks and rays) detected in eDNA samples is indicated by green dots and absence by red ones. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

plain, making it an ideal area for the conservation of umbrella species such as elasmobranchs and all associated communities.

Fishing management within the MPA ensures an innovative approach to conservation. Indeed, the park includes two fishing exclusion zones created in 1977 and 1983 with local fishermen (https://www.medamp.org/), where fishing and diving are strictly prohibited. The park is also part of the Pelagos Sanctuary, a transnational protected area dedicated to the conservation of marine mammals in the north-western Mediterranean. Furthermore, regulation of fishing over most of its surface area is currently under discussion, to limit the harvesting associated with recreational fishing. Small-scale artisanal fishing is generally carried out at depths between 15 and 150 m. For trawlers, which operate almost exclusively in the Corsican Channel to the east part of Cap Corse, fishing depths range from 200 to 400 m (https://parc-marin-cap-corse-agriate.fr/documentation/analyse-risque-peche-pnmcca). With its current level of protection and the desire to extend it towards areas of interest, the PNMMCA currently benefits from very low fishing pressure and a relatively preserved habitat in the waters of the north-western Mediterranean basin (Pichot et al., 2024).

In this MPA, the diversity of habitats is important ranging from shallow *Posidonia* meadows to deep-water canyons with innovative management practices. So, this MPA is a cornerstone of biodiversity conservation in the Mediterranean. In addition to the 21.42 km^2 of fully protection, its unique combination of features underlines its role as an archetypal situation for the integration of coastal and deep-sea habitat protection, as highlighted by recent studies (Jacquemont et al., 2024). This park also plays an essential role in protecting coastal and shallow-water habitats, as it hosts 100 km^2 of *Posidonia oceanica* meadows, one of the largest surfaces in the Mediterranean. These meadows serve as nursery habitats for numerous marine species, including some vulnerable elasmobranchs (Faure et al., 2023).

In the PNMCCA, our study was conducted over three depth ranges with a shallow layer between 0 and 29 m, a mesophotic layer between 30 and 149 m and a deeper layer beyond 150 m (Duhamet et al., 2023) to check whether the different habitats could serve as refuges for fishes (Fig. 1).

2.2. eDNA sampling

We filtered 200 samples of seawater in different locations of the MPA (Fig. 1 and Supplement S1) between 2018 and 2023 in coastal and pelagic habitats at depths varying between 1 and 835 m. These different habitats consist of *Posidonia oceanica* meadows associated with rocky bottoms and sandbanks in shallow coastal areas. Beyond this depth, the substratum corresponds to either soft, rhodolith beds or hard bottoms.

With the exception of subsurface, samples were collected as closed as possible from the seafloor. In these locations, among one to five replicate of water samples were collected and all filters (i.e., a sample of 30 or 60 L of filtered water depending on depth) were sampled in spring and summer to avoid the effects of seasonal variability. Samples were performed during 30 or 60 min at a flow rate of 1 L/min. For each sample, we used a sterile igiDNA® 0.2 μM crossflow filtration capsule (Fig. 2).

We employed different methods to account for the varying requirements of sampling shallow, mesophotic, and deeper layers, as a single approach was not feasible for all depths (see Supplement S1).

We used the water column stratification defined by Duhamet et al. (2023). The shallow layer between the surface and 29 m is followed by the mesophotic layer between 30 and 149 m and deeper layers between 150 and 835 m. Out of the 200 filters collected, 93 correspond to the shallow layer, 83 were collected in the mesophotic layer, 24 in deeper

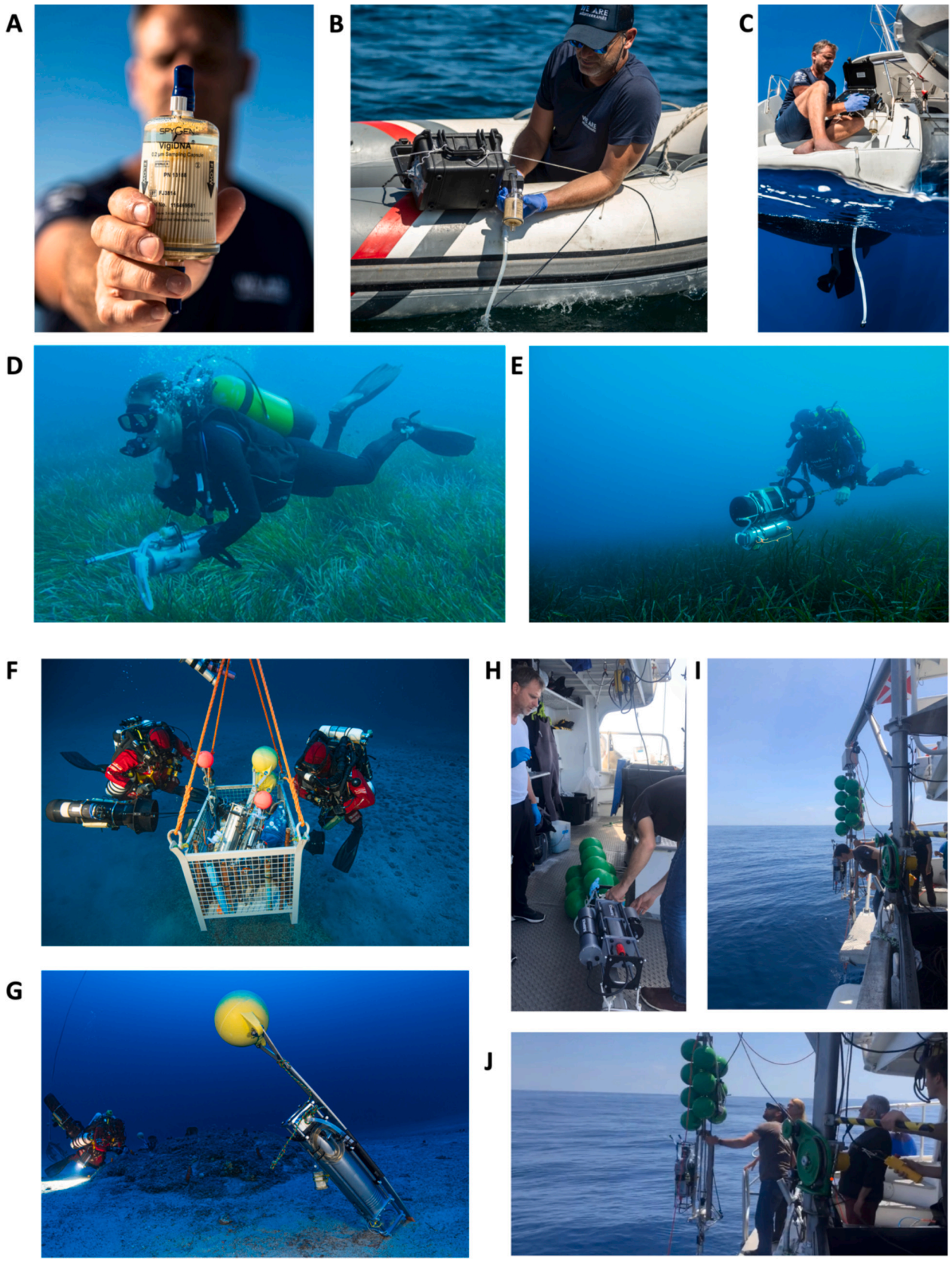


Fig. 2. Assemblage of pictures showing the capsule filter (A), the surface pump (B and C), the submersible pump used by scuba divers (D to G) and submersible deep pump (H to J) used for eDNA sampling in the waters of the Cap Corse and Agriate Marine Park. Photo credits: (A to C) Greg Lecoeur, BIODIVMED 2023, (D) Nacim Guellati (2022), Laurent Ballesta, Gombessa expéditions, Andromède Océanologie (2019), (E to G) Laurent Ballesta, Gombessa 6: Mission cap Corse, Gombessa expéditions, Andromède Océanologie (2021), (H) Adèle Barroil and (I and J) Franck Pichot.

layers. Among these filters, 120 were collected near to the coast (<2.96 km), 10 over the canyon heads, 28 in the open ocean, 4 above the seamounts and 38 offshore between approximately 20 and 60 km (Fig. 1).

2.3. eDNA processing

DNA extraction, amplification, and sequencing were performed in separate dedicated rooms equipped with positive air pressure, UV treatment, and frequent air renewal. Two extractions per filter were performed following a protocol described by Polanco Fernández et al. (2021). Each filtration capsule, containing the CL1 buffer, was agitated for 15 min on an S50 shaker (cat Ingenieurbüro™) at 800 rpm. The buffer was then emptied into two 50 mL tubes before being centrifuged for 15 min at 15,000*g*. The supernatant was removed with a sterile pipette, leaving 15 mL of liquid at the bottom of each tube. Subsequently, 33 mL of ethanol and 1.5 mL of 3 M sodium acetate were added to each 50 mL tube and stored for at least one night at −20 °C. The tubes were then centrifuged for 15 min at 15,000*g*, at 6 °C, and the supernatants were discarded. After this step, 720 μL of ATL buffer from the DNeasy Blood & Tissue Extraction Kit (Qiagen GmbH) were added to each tube. Each tube was then vortexed, and the supernatant was transferred to a 2 mL tube containing 20 μL of Proteinase K. The tubes were finally incubated at 56 °C for 2 h. Subsequently, DNA extraction was performed using NucleoSpin® Soil (Macherey-Nagel GmbH & Co) starting from step 6 and following the manufacturer's instructions, and two DNA extractions were carried out per filtration capsule. The elution was performed by adding 100 μL of SE buffer twice. The two DNA extractions form a single filtration capsule were pooled together before the amplification step. Extracted eDNA was tested for inhibition following the protocol described by Biggs et al. (2015). If a sample was considered inhibited, it was diluted fivefold before the amplification. DNA amplifications were performed in a final volume of 25 μL, using 3 μL of extracted DNA as the template using "teleo" primers following the protocol described in Valentini et al. (2016). This primer set ("teleo" forward: ACACCGCCCGTCACTCT, reverse: CTTCCGGTACACTTACCATG; Taberlet et al., 2018; Valentini et al., 2016), amplify a fragment of the 12S mtDNA gene with a mean sequence length of 64 bp. The primers were 5′-labeled with an eight-nucleotide tag unique to each PCR replicate, allowing the assignment of each sequence to the corresponding sample during bioinformatic analysis. The tags for the forward and reverse primers were identical. Twelve PCR replicates were run per sample. After amplification, the samples were quantified using capillary electrophoresis (QIAxcel; Qiagen GmbH) and purified using the MinElute PCR purification kit (Qiagen GmbH). Before sequencing, purified DNA was quantified again using capillary electrophoresis. The purified PCR products were pooled in equal volumes to achieve a theoretical sequencing depth of 1,000,000 reads per sample. In total, forty-three libraries were prepared and sequenced on Illumina sequencers. During the period this study (between 2018 and 2024), Illumina's sequencing technologies have evolved significantly and different platforms were used depending on the number of samples to be processed. Seventeen libraries were prepared and sequenced at Fasteris (https://www.fasteris.com/en-us/) using the MetaFast protocol (Fasteris), of these, three were sequenced on a NextSeq Mid (Illumina) using a NextSeq Mid kit (Illumina) and a MiSeq (2 × 125 bp) with the MiSeq Flow Cell Kit v3 (Illumina) was used for the sequencing of the remaining libraries following the manufacturer's instructions. Seven libraries were prepared using the TruSeq PCR-free kit (Illumina) and sequenced on a MiSeq (2 × 125 bp) with the MiSeq Flow Cell Kit v3 (Illumina), following the manufacturer's instructions, at DNA Gensee (https://www.dnagensee.com/en/). The remaining libraries were prepared using the TruSeq PCR-free protocol and sequenced NextSeq 1000 (Illumina) using a NextSeq P1 or P2 kit (Illumina). Before switching to a different sequencer or library preparation protocol, we conducted comparative studies to ensure that no significant differences were observed between the platforms or the library preparation methods. This was carried out to avoid introducing any bias into the eDNA results due to changes in sequencing technology. Negative extraction controls and negative PCR controls (ultrapure water, 12 replicates) were performed for each batch of DNA extraction and DNA amplification, and they were amplified and sequenced in parallel of the samples to monitor possible contaminants.

2.4. Genetic reference database

Genetic reference databases are the cornerstone of any eDNA studies, since without them, the identification of many species, and therefore DNA metabarcoding, remains limited (Keck et al., 2023; Marques et al., 2021b; Yoccoz, 2012). At present, the genetic reference databases for the TELEO genetic marker available at the global scales are notoriously incomplete.

At the onset of our study, only 57 % of North-Western Mediterranean elasmobranch species were referenced in the assembled database for the 12S mt rRNA fragment targeted by the "teleo" primer. To improve the taxonomic coverage for elasmobranchs, our study increased the reference database in elasmobranch to 88.4 % of the regional pool (Charbonnel et al., 2017). For the Mediterranean Sea we have collected fish tissues with the help of professional fishermen, museums and the European Association of Zoos and Aquariums (EAZA), to obtain more teleo 12 S barcodes in our eDNA metabarcoding analysis (Boulanger et al., 2021). Of the 86 elasmobranch species that can be observed in the Mediterranean Sea (Serena et al., 2020), 76 species (88.4 %) have a barcode available for the teleo 12S marker using a combination of publicly available sequences from GenBank and our Mediterranean genetic database, comprising already published sequences (Boulanger et al., 2021) and 17 new elasmobranch species of the regional North-Western Mediterranean pool. Some of these, such as the porbeagle (*Lamna nasus*), for example, are probably extinct in the western part of the basin, while still present in the eastern part (https://www.iucnredlist.org/). Others, originating from the Red Sea known as lessepsian migrants, such as the honeycomb stingray *(Himantura uarnak*), are not widely distributed in the Mediterranean to date (https://www.fishbase.se/). Here, we chose to consider all the species potentially present in the western Mediterranean basin.

Taxonomic assignment of the remaining sequences was performed using the *ecotag* program using a combination of publicly available sequences from GenBank [release 247] and our Mediterranean genetic database. The DNA was extracted from tissue samples and a 12S rRNA gene fragment of 675 bp encompassing the "teleo" marker fragment was targeted using the forward primer V05F_898 and the reverse "teleo" primer (Thomsen et al., 2016). Certain taxa could not be resolved at the species level due to limitations inherent to the genetic marker used (e.g., low interspecific variability) or the lack of their genetic sequences in the reference database (88.4 % completion). These are common limitations in environmental DNA (eDNA) metabarcoding, particularly for taxonomic groups such as elasmobranchs, where some species share highly similar gene sequences, making fine-scale taxonomic resolution challenging. In our case, only 3 of the 78 Mediterranean elasmobranch species (*Raja clavata*, *Raja polystigma* and *Raja asterias*) included in our genetic reference database share the same barcode (12S rRNA), meaning that species-level identification remains trustable for most elasmobranchs using metabarcoding.

To assess whether the observed taxonomic representation in our samples adequately reflects the potential diversity in the surveyed habitats, we evaluated species diversity saturation with sampling effort across depth layers. Species accumulation curves were constructed for each depth layer (Shallow, n = 93; Mesophotic, n = 83; Deeper layers, n = 24) using the specaccum function in the vegan R package (Supplementary material S2). A Lomolino model was subsequently fitted using the *fitspecaccum* function to estimate asymptotic species richness.

2.5. Bioinformatic analyses and taxonomic assignments

Following sequencing, reads were processed to remove errors and analyzed using programs implemented in the OBITools toolkit (Boyer et al., 2016; Valentini et al., 2016). The forward and reverse reads were assembled with *illuminapairedend*, using a minimum score of 40 and retrieving only joined sequences. The reads were then assigned to each sample using the *ngsfilter*. After this step, each sample was analyzed individually before merging the taxon list for the final ecological analysis. Sequences shorter than 20 bp, or with fewer than 10 occurrences were excluded using the *obigrep* program. The *obiclean* program was then run to remove sequences likely corresponding to errors with default settings.

We only retained taxonomic assignments matching at the species level with a 98 % sequence match and full coverage over the sequence length. We validated species-level assignments only if the sequence matched a species known to occur in the Mediterranean Sea. In case of low sequence taxonomic resolution, with sequences matching to several species, species-level assignment was kept only if a single species was occurring in the study area. For example, if a sequence matches two or more species both occurring in the Mediterranean as it was the case for the three rays (*Raja clavata*, *Raja polystigma* and *Raja asterias*), this sequence was not considered further in the study. If a sequence matches two or more species with only one occurring in the Mediterranean, it was assigned to the Mediterranean species. Species names were verified using the *rfishbase* R package (Boettiger et al., 2012).

After the taxonomic assignment steps, considering the incorrect assignment of a few sequences to the sample due to tag jumps (Schnell et al., 2015), all the sequences with a frequency of occurrence <0.001 per sequence and per library were discarded. Then, the data were curated for Index-Hopping (MacConaill et al., 2018) with a threshold empirically determined per sequencing batch using experimental blanks (i.e., combinations of tags not present in the libraries) for a given sequencing batch between libraries. After the filtering pipeline, the extraction and PCR negative controls were completely clean, and no sequence reads remained in those samples.

2.6. Biodiversity metrics

Seven metrics were used to assess the effects of depth on biodiversity (Dalongeville et al., 2022). The first metric, species richness (α-diversity), encompasses all fish species present in a given eDNA sample, including all taxonomic groups, regardless of their ecological role, economic importance, or conservation status. The second metric, commercial species richness, includes only fishes that are of significant economic value to professional fishermen according to Fishbase (Froese and Pauly, 2010). The third metric corresponds to the number of fish species that are classified as threatened, or red listed, according to the International Union for Conservation of Nature (IUCN). This metric includes both teleosts like the dusky grouper (*Epinephelus marginatus*) and elasmobranchs like the angelshark (*Squatina squatina*). The fourth metric corresponds to the number of elasmobranch species present in a sample. The fifth and the sixth metrics describe respectively the phylogenetic and functional diversity which are crucial for understanding anthropogenic gradients (Dalongeville et al., 2022; Zhao et al., 2024) and ecosystem functioning (Lin et al., 2024; Dulvy et al., 2024). Their calculation was carried out using functional diversity indices based on Hill's numbers, without considering species abundance (Chiu and Chao, 2014).

Functional diversity was computed using ten fish functional traits common to both teleost and elasmobranch species (Villéger et al., 2017): body length, trophic level, the coefficient associated with growth (a), the size coefficient in the size-weight relationship (b), its growth factor (K), the preferred mean temperature, whether the species is demersal (lives near the bottom) or pelagic (lives in the water column), the ratio between body size and length as well as the ratio between width and length.

Phylogenetic diversity was computed only for teleost fishes detected in our samples since elasmobranchs cannot be branched on the same monophyletic tree. The length of the various branches of the phylogenetic tree connecting the species present in a sample was measured to represent the evolutionary relationships between species pairs. Greater cumulative branch length indicates greater evolutionary diversity. The hill index used ($q = 1$) allows each species to contribute proportionally to its presence in the sample, without favoring or ignoring rare or abundant species. Phylogenetic diversity values were then normalized to allow comparison between samples, regardless of size or total number of species.

The seventh metric corresponds to the number of species being both endangered and evolutionary unique so identified as Evolutionarily Distinct and Globally Endangered (EDGE) species. The EDGE value for a given species in a taxonomic group combines both the extent of unique evolutionary history it represents (evolutionary distinctiveness, or ED) and its conservation status (global endangerment, or GE) as described by Isaac et al. (2007). For sharks and rays EDGE values were calculated by the Zoological Society of London or ZSL (https://www.edgeofexistence.org/).

In order to assess species dissimilarity between the different depth ranges, we also calculated β-diversity with the Sorensen's indices based on the proportion of common and unique species encountered between pairs of layers (Koleff et al., 2003). We investigated the turnover and nestedness components of β-diversity (Baselga., 2010). The turnover component refers to the change in species composition from one depth to another, indicating how one species may be replaced by another. Nestedness, on the other hand, indicates a loss or gain of species without replacement; for instance, if certain species are present at one depth but not at another, this reflects a nested pattern. Our analyses included a total of 200 samples cumulating species within the meta-community, referred to as γ-diversity. These calculations were conducted for all fishes, including elasmobranchs, as well as for elasmobranchs alone.

2.7. Statistical tests

To compare the level of biodiversity metrics between the different depth ranges (shallow, mesophotic and deeper layers), we used Kruskal-Wallis statistical tests as most metrics were characterized by a non-normal distribution. The Bonferroni correction for multiple testing was then applied by dividing the significance level α by the total number of tests performed (Dinno, 2015). This is a conservative method that reduces the probability of false positives. When the Kruskal-Wallis test was significant (p-value < 0.05) we performed a Dunn's test for post hoc pairwise comparisons. To perform all the statistical calculations, we used version 4.4.2 of R software.

3. Results

3.1. Fish metabarcoding and detections across depths

A total of 106,486,135 reads assigned at the species-level were obtained over the 200 eDNA samples after bioinformatic processes. From the 200 samples, after the assignment procedure, a total of 174 fish species were detected, including 21 elasmobranchs (9 shark, 8 ray and 4 skate species), 109 commercial species, 13 threatened species and 2 EDGE species: the white skate *Rostroraja alba* (EDGE score: 5.96; ED score: 47.41; Endangered) and the angelshark *Squatina squatina* (EDGE score 6.68; ED score: 48.8; Critically Endangered).

Species accumulation curves of shallow ($n = 93$) and mesophotic ($n = 83$) layers both exhibited clear plateaus with narrow confidence intervals (Supplementary material S2), indicating that sampling effort was sufficient to capture local fish and elasmobranch species diversity. In contrast, the species accumulation curve for deeper layers ($n = 24$) did not reach an asymptote and showed wide confidence intervals, revealing

that additional sampling effort (estimated at ~40–50 sites) would be required to achieve comparable completeness.

Across all depth ranges, elasmobranchs were detected in 136 out of the 200 eDNA samples (Fig. 1, Table 1, Fig. 3). We found at least one elasmobranch species in 71 % of shallow samples (n = 66), in 63 % of mesophotic samples (n = 52) and in 67 % (n = 18) of deeper samples. When we looked at the percentage of eDNA samples containing at least one EDGE species, we found that the highest value was for the mesophotic (15.7 %). In shallow and deeper layers, EDGE species were not detected (0.0 %).

Concerning all fishes, 130 species including 12 elasmobranch species (8 ray, 1 skate, and 3 shark species) were detected in the shallow layer. In the mesophotic layer, 129 species were recorded, including 16 elasmobranch species (7 ray, 3 skate, and 6 shark species). Finally, in deeper layers, 67 species were detected, including 11 elasmobranch species (3 ray, 2 skate, and 6 shark species) (Fig. 3).

Among the 21 detected elasmobranch species, three were considered as a critically endangered species (Fig. 3) in the Mediterranean Sea, the angelshark (*Squatina squatina*), the common eagle ray (*Myliobatis aquila*) and the duckbill eagle ray (*Aetomylaeus bovinus*). All three are present in the mesophotic and two of them were detected in shallow waters (*Aetomylaeus bovinus*, *Myliobatis aquila*). Three elasmobranch species were considered as endangered and four as vulnerable (Fig. 3). Two critical endangered species were detected in the mesophotic (*Rostroraja alba*, *Squatina squatina*) and the third (*Mobula mobular*) was detected both above and below the mesophotic layer; the dotted lines in Fig. 3 symbolize the potential presence and non-detection of this species in this depth range. Only one endangered species (*Mobula mobular*) was detected in shallow and deeper layers (Fig. 3). Four vulnerable species were detected in the different depth ranges with three of them detected in the mesophotic (*Dasyatis pastinaca*, *Torpedo marmorata* and *Scyliorhinus stellaris*) as well as in the deeper layers (*Torpedo marmorata*, *Etmopterus spinax* and *Scyliorhinus stellaris*). In the shallow layer, only two vulnerable species were detected (*Dasyatis pastinaca* and *Torpedo marmorata*).

Among the 21 elasmobranch species detected in our eDNA samples, two are classified as strictly benthic and thus closely associated with the seafloor, according to FishBase (*Tetronarce nobiliana* and *Aetomylaeus bovinus*). These species were detected both in subsurface waters and in samples collected near the seafloor across shallow and mesophotic layers (Fig. 3, Table in Supplementary material S1). Regarding pelagic species such as the pelagic stingray (*Pteroplatytrygon violacea*) and the spinetail devil ray (*Mobula mobular*) and dermesal ones like the common torpedo (*Torpedo marmorata*) and the lesser spotted dogfish (*Scyliorhinus canicula*), we found the same patterns. All these species were detected across different depth ranges despite sampling in subsurface on near-seafloor (Fig. 3, Table in Supplementary material S1).

The mesophotic (water layer between 30 and 149 m) represents the depth range with the highest number of endangered species (10), including 8 threatened elasmobranch species (Table 2, Fig. 3). The relative frequency of endangered species per eDNA sample was respectively of 8.6 and 5.4 % for all fishes and elasmobranchs in the shallow layer, 12.0 % and 9.6 % in the mesophotic and 7.4 and 6.0 % in deeper layers. We therefore observed an increase in relative threatened species frequency with depth. The greatest specific richness (Table 2) with 52 species including 3 elasmobranchs (*Dasyatis pastinaca*, *Dasyatis tortonosei* and *Torpedo marmorata*) was found in the western part of the PNMCC corresponding to a *Posidonia oceanica* meadows in the mesophotic at a depth of 35 m.

The highest number of elasmobranch species detected in one sample comprised 7 species in two locations, one in the mesophotic (sandy bed in the western part of the PNMCCA in the Agriates at 47 m depth) and one in the deeper layers corresponding at the east side of the PNMCCA corresponding to the Corsica canal offshore of the town of Bastia at a depth of 400 m.

In the mesophotic layer, this sample encompassed 1 skate (*Raja brachuyra*), 4 rays (*Dasyatis pastinaca*, *Dasyatis tortonosei*, *Myliobatis aquila* and *Torpedo marmorata*) and 2 sharks (*Scyliorhinus canicula* and *Prionace glauca*). In deeper layer, the sample encompassed 2 skates (*Leucoraja naevus* and *Dipturus oxyrinchus*), 1 ray (*Pteroplatytrygon violacea*) and 4 sharks (*Scyliorhinus canicula*, *Squalus blainville*, *Hexanchus griseus* and *Galeus melastomus*). In these two samples the seven elasmobranch species were respectively associated to a specific richness of 49 (42 teleost fishes) and 35 (28 teleost fishes) species. Concerning shallow layers, we detected a maximum of 4 elasmobranch species in 6 samples.

Several commercially important species were detected across the entire depth range. The surmullet (*Mullus surmuletus*), a demersal species of high economic value, was found at all depths, highlighting its wide bathymetric range. Similarly, pelagic species such as the Atlantic chub mackerel (*Scomber colias*), the Atlantic bluefin tuna (*Thunnus thynnus*), the saddled seabream (*Oblada melanurus*), the black seabream (*Spondyliosoma cantharus*), and the swordfish (*Xiphias gladius*) were also detected throughout the water column from the surface to deeper layers.

Regarding threatened species, two species were identified across all sampled depths. The ocean sunfish (*Mola mola*), a pelagic species listed as vulnerable, was consistently detected throughout the column at all depths, illustrating its broad vertical distribution. Likewise, the marbled electric ray (*Torpedo marmorata*), a demersal species, was also observed at all depths.

EDGE species (*Squatina squatina* and *Rostroraja alba*) were only detected in the mesophotic layer. We detected *Rostroraja alba* in 12 eDNA samples while *Squatina squatina* was only detected in one sample (Table 2, Fig. 3).

3.2. Comparing biodiversity metrics between depths

We observed that the shallow layer hosted 130 species (Table 2), with a minimum of 1 and a maximum of 50 different species per sample (mean: 17.8 ± 14.3). In the mesophotic, 129 species were detected, with a minimum of 2 and a maximum of 52 species per sample (mean: 22.9 ± 11.1). On the other hand, and due to a smaller number of samples (n = 24), deeper layers had the lowest cumulated number of species (67 species), including 11 elasmobranch species, with between 2 and 35 species per sample (mean: 10.3 ±7.2).

Concerning elasmobranch diversity, we observed that the shallow layer contained a total of 12 species, with a minimum of 0 and a maximum of 4 species per sample (mean: 1.24 ± 1.2). In the mesophotic layer, 16 elasmobranch species were detected, with a minimum of 0 and a maximum of 7 species per sample (mean: 2.8 ± 3.0). Due to a smaller number of samples (n = 24), deeper layers had the lowest cumulated number of species (11) with between 0 and 7 species per sample (mean: 1.21 ±1.5 for elasmobranch species).

We observed significantly different species richness values between depth categories for all fishes (Fig. 4A) and commercial fishes (Fig. 4B) with more species in the mesophotic. There was no significant difference between depths for threatened and elasmobranch species richness (Fig. 4C and D). We observed a significant difference in phylogenetic richness between depth ranges with shallow and mesophotic layers having a greater diversity than the deeper layer (Fig. 4E). There was a significant difference in functional diversity between the mesophotic, having the highest level, and both shallow and deeper layers (Fig. 4F).

3.3. β-Diversity across the different depth layers

A Venn diagram was used to illustrate the overlap of all fish (Fig. 5A) and elasmobranch species (Fig. 5B) between depth layers. For all fishes including elasmobranchs (Fig. 5A), 32 species (18.4 % of the 174 species) are shared by all depth ranges while a maximum of 23 species were unique to one depth range (23 for shallow, 19 for mesophotic and only 12 for deeper layers). Shallow and mesophotic were the layers sharing the most important number of species (n = 65) in comparison to mesophotic and deeper layers (n = 13) and shallow and deeper layers (n =

Table 1
Number of samples and the percentage of samples containing at least one or more Elasmobranch species and one or more EDGE species by depth range.

Depth range	Number of samples	Percentage of filters with elasmobranchs	Percentage of filters with EDGE species
Shallow (0–29 m)	93	71.0 %	0.0 %
Mesophotic (30–149 m)	83	62.6 %	15.7 %
Deeper layers (>150 m)	24	75.0 %	0.0 %

10). Concerning elasmobranchs (Fig. 5B), there was no species present only in the shallow layer. In the mesophotic and in deeper layers we found 3 species that were not present in other depth ranges. Mesophotic is also the depth range with the most species in common with shallow (n = 7) and deeper layers (n = 3), encompassing 16 out of the 21 elasmobranch species detected over the 200 samples.

The dissimilarity between depth ranges was calculated for all fishes (Fig. 5C) and for elasmobranchs only (Fig. 5D). The greatest dissimilarity was observed between the mesophotic and deeper layers for all fishes (47 %) and between shallow and deeper layers (57 %) for elasmobranchs. The dissimilarity between shallow and mesophotic layers was the lowest for all fishes with 34 % and between mesophotic and deeper layers (38 %) for elasmobranchs.

The nestedness (Fig. 5E to F) and turnover (Fig. 5G to H) components of Sorensen's β-diversity provided insights into the structure of fish communities across depth ranges. For all fishes (Fig. 5E), we observed low nestedness values ranging from 13 % to 16 % between depth layers, with shallow and mesophotic layers showing the highest nestedness (16 %) while shallow and deeper layers showed the lowest nestedness (13 %). A similar pattern was observed for elasmobranch species (Fig. 5F), with nestedness values ranging from 14 % between deeper layers and the other depth ranges to 17 % between shallow and mesophotic layers. It means that the different depth layers tend to host different species assemblages and are not sub selections of the richest assemblage.

Concerning the turnover (Fig. 5G and H), which measures the rate of species replacement between different depth layers (proportion of species change), we observed a larger number of species being replaced between shallow and deeper layers for elasmobranch species (27 %). We also observed that species replacement was very low between shallow and mesophotic layers for both all fish and elasmobranch species only (8 %). Concerning elasmobranchs, we calculated a rate of 24 % species replacement between the mesophotic and deeper layers and of 27 % between shallow and deeper layers. The lowest value for the turnover was observed between shallow and mesophotic layers (8 %). It means that the deep fish assemblage tends to be different from the mesophotic and shallow fish assemblages.

4. Discussion

Our study sheds new lights about the role of deep habitats, including coastal to offshore mesophotic areas, as biodiversity refuges in a large MPA already identified as a sanctuary for skates, rays, sharks and other threatened species (Pichot et al., 2024). We reveal that α-diversity was significantly higher in the mesophotic layer than in other depth layers for all fishes, including commercial species. However, this difference disappears when considering only elasmobranchs or threatened species. β-diversity is high between depth layers for all fishes, but neither turnover nor nestedness dominates the pattern.

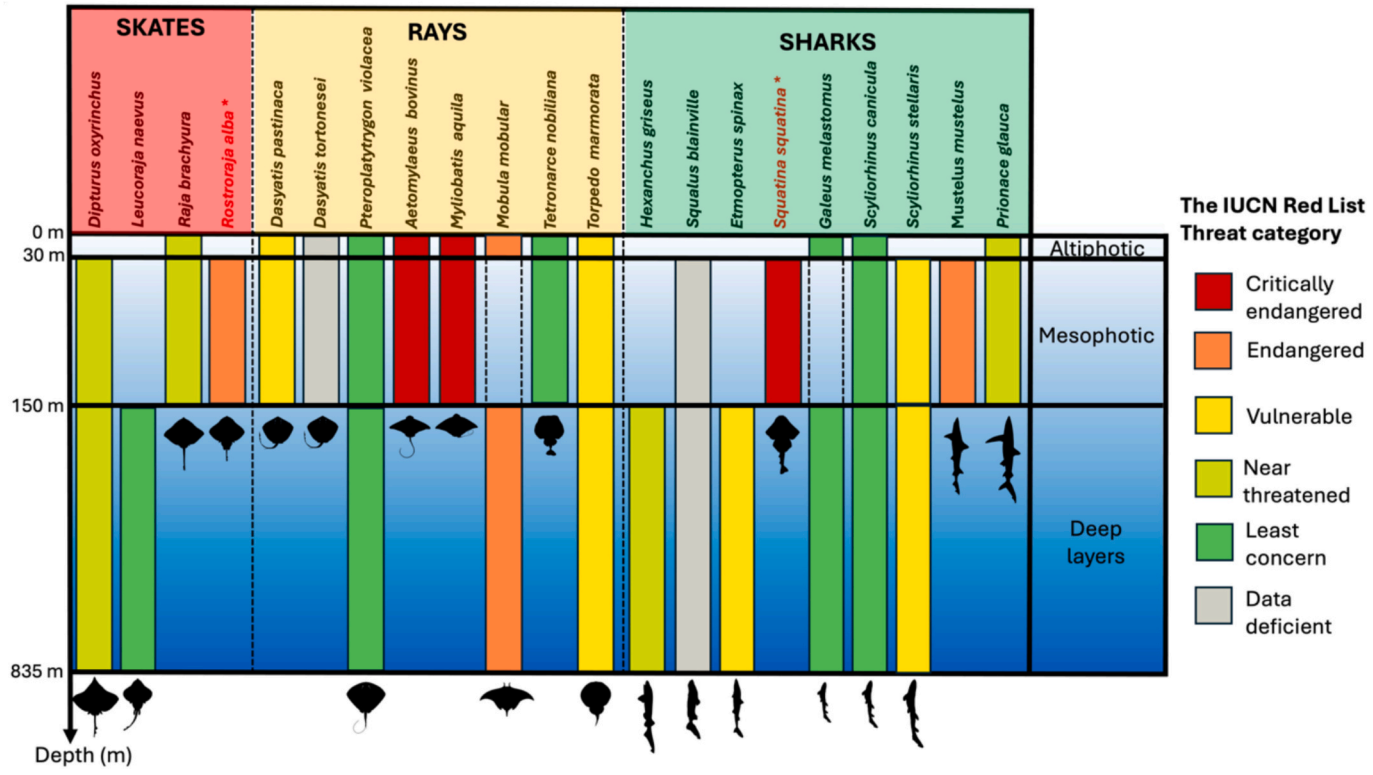


Fig. 3. Distribution of elasmobranch species according to depth. The name, IUCN status, and depth range of species where their eDNA was detected is indicated with a rectangle colored differently according to the IUCN status. The two species in red with an asterisk are Evolutionarily Distinct and Globally Endangered (EDGE). Dotted rectangles represent the assumption of species presence at some different depths where they were not detected during our sampling campaign. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Table 2
Cumulative totals of various species categories by depth range: total fish species, commercial species, threatened species, elasmobranch species, threatened elasmobranch species and EDGE species richness by depth range.

Depth range	Fish species richness			Number of commercial species	Number of threatened species	Elasmobranch species richness			Number of threatened elasmobranch species	Number of EDGE elasmobranch species
	Species detected	Min - Max	Mean ± SD			Species detected	Min - Max	Mean ± SD		
Shallow (0–29 m)	130	1–50	17.8 ±14.3	84	8	12	0–4	1.24 ±1.2	5	0
Mesophotic (30–149 m)	129	2–52	22.9 ±11.1	85	10	16	0–7	2.8 ±3.0	8	2
Deeper layers (>150 m)	67	2–35	10.3 ±7.2	45	5	11	0–7	1.21 ±1.5	4	0

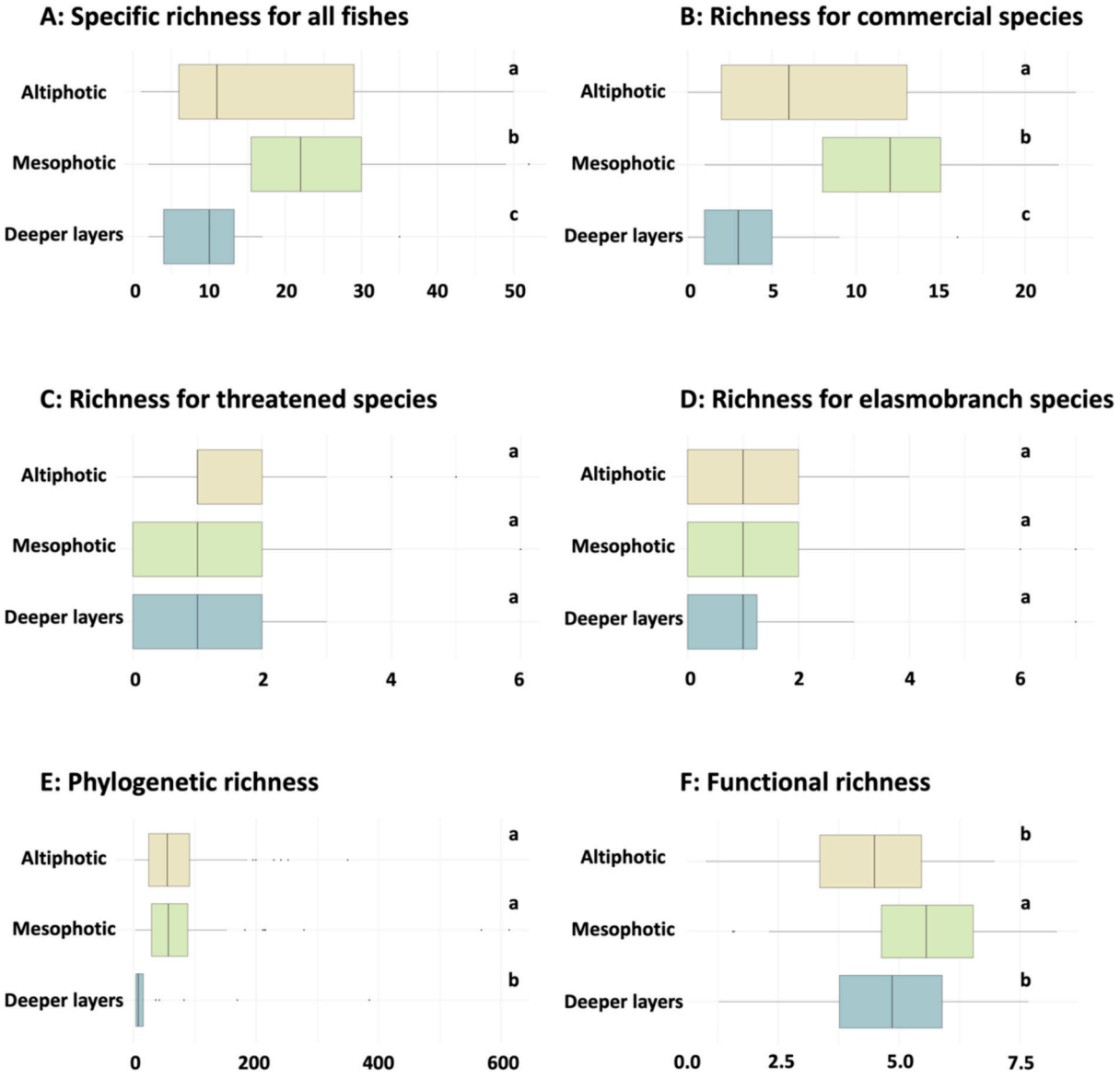
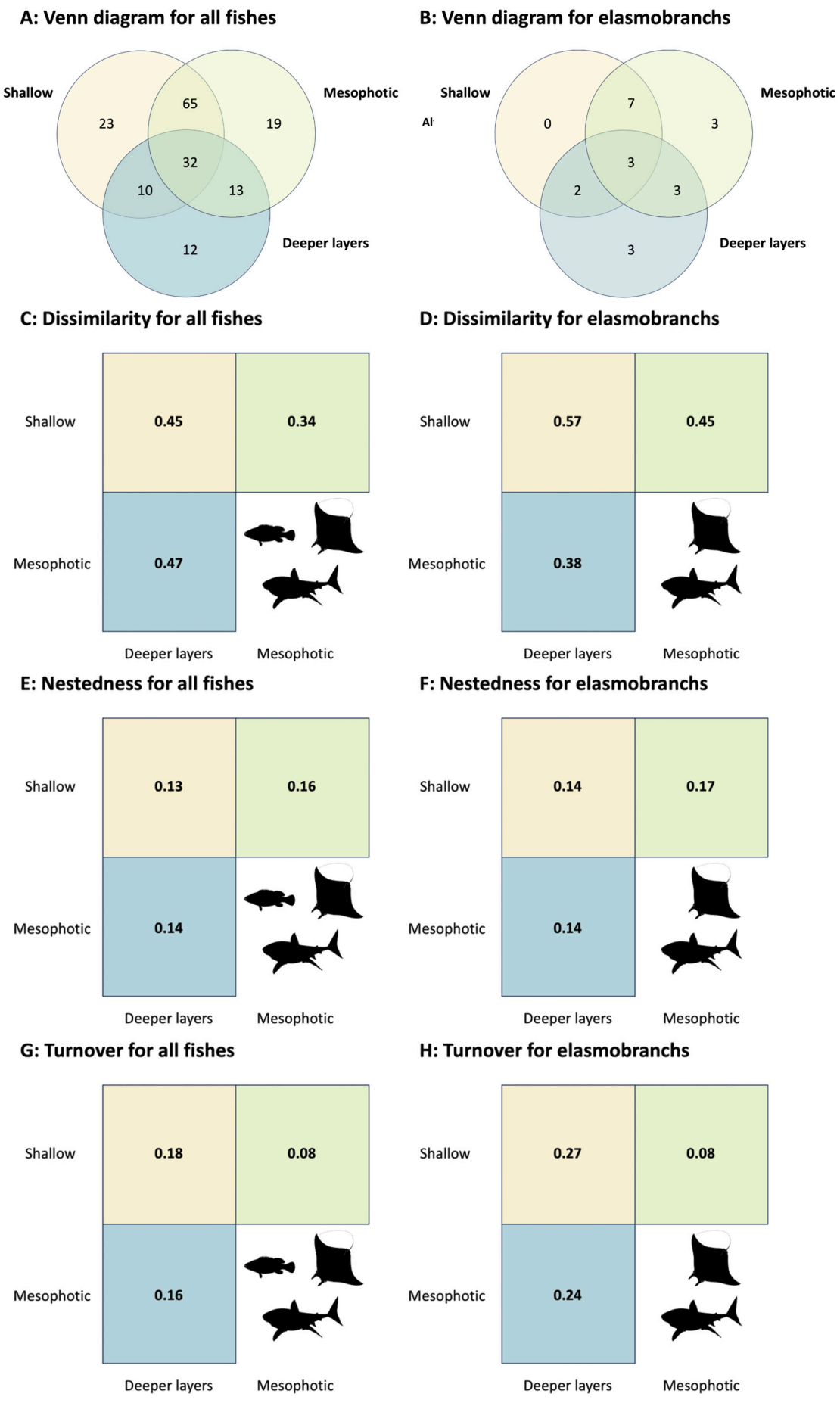


Fig. 4. Boxplots showing the distribution of six biodiversity metrics for eDNA samples as a function of depth range (A: specific richness, B: commercial richness, C: threatened richness, D: threatened species, E: phylogenetic richness and F: functional richness). Grey line represents the 25 and 75 % confidence intervals. We represent the significance between all depth ranges compared two by two with a different letter. Adjusted p-values (p-adj) correspond to the correction of the initial p-value to account for multiple comparisons. The Bonferroni correction was applied to assess significance.

4.1. α-Diversity of elasmobranch by depth range

We show that elasmobranch detections are not evenly distributed across depth (Fig. 3). The mesophotic and deeper layers host the greatest number of species per sample, albeit not significantly, compared to shallow layer where a maximum of 4 species is detected per sample.



(caption on next page)

Fig. 5. Dissimilarity in species composition between different depth layers (shallow, mesophotic, deeper layers) for all fishes including elasmobranchs (A, C, E, G) and elasmobranchs only (B, D, F, H). The Venn diagrams (A, B) show the distribution of species by depth, indicating the number of species shared and not shared between the different depth layers. Heatmaps are used for β-diversity computed with the Sorensen index of dissimilarity (C, D), including the nestedness (E, F) and turnover (G, H) components between depth layers.

Indeed, mesophotic samples recorded up to 7 species per sample, including 5 batoids and 2 sharks, reflecting a high density of elasmobranch species in this depth range. Moreover, the mesophotic is the only layer hosting EDGE species (*Rostroraja alba* and *Squatina squatina*), emphasizing its conservation importance. Among these two EDGE species, angelshark (*Squatina squatina*) holds an exceptionally high Evolutionary Distinctiveness (ED) score of 48.8, meaning it represents nearly 49 million years of unique evolutionary history. Classified as Critically Endangered (CR) on the IUCN Red List, this species is at an extremely high risk of extinction. Its combined EDGE score of 6.68 reflects both its evolutionary uniqueness and severe conservation status, making it a top global priority for conservation. The loss of this species would mean the disappearance of an ancient, irreplaceable lineage within the elasmobranch clade. *Rostroraja alba* (white skate) also exhibits a high ED score of 47.41, indicating its strong evolutionary distinctiveness. It is listed as Endangered (EN) on the IUCN Red List, signifying a high, though not critical, risk of extinction. With an EDGE score of 5.96, it is recognized as both a vulnerable and evolutionarily rare species. While slightly lower in priority than *S. squatina*, its conservation remains essential to safeguard a unique lineage within the Mediterranean's threatened elasmobranch diversity. Of the 16 EDGE species described in the Mediterranean Sea, all are represented in our genetic reference database. The non-detection of other EDGE species in our samples cannot therefore be attributed to the gaps of our reference base, which covers 100 % of EDGE species in the Mediterranean, but rather to the rare occurrence of these species in relation to their IUCN status.

A deep eDNA sample detects 7 different species of elasmobranchs, although their composition differs from that of the mesophotic, with 2 skates, 1 ray and 4 sharks. According to FishBase (Froese and Pauly, 2010), the only three mesophotic elasmobranch species missing from our samples (*Hexanchus griseus*, *Leucoraja naevus*, and *Etmopterus spinax*) are typically found in deeper waters. Although these species may be present in the mesophotic depth layer, Corsican fishermen report that they are usually encountered at greater depths, beyond the range covered by our sampling. The presence of unique elasmobranch species in the deep layer stresses the importance of not only protecting the mesophotic habitats, but where possible also protecting deeper areas. On the contrary, the absence of unique elasmobranch species in the shallow layer confirms that mesophotic habitats can play a crucial role in the conservation of species found at other depth ranges. So, protecting this layer but also shallow habitats are of major importance due to the necessity for some species to reproduce in shallow waters (Enajjar et al., 2015). Our results echo the urgent need to protect all depth layers and maintain their connectivity (O'Leary and Roberts, 2018; Goetze et al., 2021).

We also observe that the most remarkable mesophotic and deep eDNA samples in terms of elasmobranch diversity were filtered in opposite locations of the PNMCCA (on the extreme western and eastern parts) meaning that elasmobranchs are well represented in numerous locations as well as at different depth ranges and different environmental conditions (respectively above the *Posidonia oceanica* meadows at 47 m and in Corsica canal at 400 m above an area undescribed).

The higher percentage of eDNA samples with elasmobranch detections in shallow and deep layers (71 % and 75 %) respectively, compared with the mesophotic (62.6 %), masks an important fact: this latter depth range is the one with the highest proportion of eDNA samples with more than one elasmobranch species by sample (37 %) compared with shallow (30.1 %) or deeper layers (25.0 %). The mesophotic is also the layer with the highest number of threatened elasmobranch species being detected (8 of the 10) with 3 critical endangered species, 2 endangered species and 3 vulnerable species.

4.2. Fish β-diversity between depth ranges

The species composition dissimilarity between shallow and deeper layers (respectively 45 % and 57 % for all fishes and elasmobranchs only) indicates that nearly half of species differ between these habitats. Between the mesophotic and deep layers, species dissimilarity is lower but still important (respectively 47 % and 38 % for all fishes and elasmobranchs) suggesting a marked biodiversity stratification even for mobile species like fish (Rocha et al., 2018). These dissimilarities reflect unique ecological adaptations to extreme deep-sea conditions, such as low light availability, high pressure and specific prey types with almost half of the species found beyond a depth of 150 m being deep-sea species.

The lowest species dissimilarity is observed between shallow and mesophotic layers (34 %) for all fishes while the dissimilarity is higher for elasmobranchs (45 %). This low value observed for all fish could partly result from the presence of species adapted to different living conditions, but also from fishing pressure exerted more superficially for certain populations (Frank et al., 2018). With less restrictive reproductive strategies than elasmobranchs, the greater resilience of teleost fishes could indeed explain the differences observed in species dissimilarity between depth layers. In fact, late sexual maturity combined with low reproduction make elasmobranchs more sensitive to greater anthropogenic pressure in shallow waters (Simpfendorfer et al., 2023) so deep refuges are even more critical for these vulnerable species.

The presence of a greater number of commercial (n = 85) and endangered species (n = 10) in the mesophotic layer could be the consequence of a lower human impact preserving biodiversity. Mesophotic habitats host 79.8 % (67 of the 84) of commercial species and 75 % (6 of the 8) of threatened species present in shallow waters.

Indeed, the low values of both species turnover (0.08 for both groups) and nestedness (0.16 for all fish, 0.17 for elasmobranchs) between shallow and mesophotic layers indicate that no one process dominates between these two depth layers. This lack of a predominant process to explain β-diversity pattern suggests that the mesophotic layer, with the highest number of elasmobranch species, provides a unique assemblage of species but can also act as a refuge for taxa adapted to a wide range of depth and light conditions.

The β-diversity patterns detected here reinforce and complement the α-diversity results by showing that, while many elasmobranch species found in the shallow layer are also found in the mesophotic layer, the latter is not merely a subset of the former. Specifically, nestedness accounts for only 16 % of dissimilarity for all fishes and 17 % for elasmobranchs, suggesting limited species loss or gain along a richness gradient. This low level of nestedness indicates that most mesophotic communities are not simply subsets of shallow assemblages. Moreover, turnover values are also low (0.08 for both groups), suggesting that species replacement between these two depth layers is limited. Taken together, these low values imply no dominant structuring process, neither nestedness nor turnover, between the shallow and mesophotic layers, reinforcing the idea that each depth layer harbors distinct, though partially overlapping, communities. Mesophotic habitats can then act as a refuge for taxa adapted to a wide range of depth and light conditions.

The exceptional water transparency of Corsican oligotrophic waters challenges a simple interpretation based solely on light availability. Indeed, coralligenous habitats have been detected down to 112 m in Corsica, the deepest known occurrence in the western Mediterranean

(Bonacorsi et al., 2013). Therefore, we hypothesize that anthropogenic pressures such as fishing intensity and coastal disturbances in shallow waters may contribute to a depth-related stratification of elasmobranch biodiversity, possibly through a deepening process where vulnerable species are gradually pushed towards deeper, less disturbed layers (Frank et al., 2018).

4.3. Functional and phylogenetic diversity patterns

Taxonomic α and β-diversity pattern highlight the role played by the mesophotic habitat as a potential refuge for elasmobranchs and the most-at-risk species, but also for commercial and endangered species. The significantly greater functional richness in the mesophotic layer (Fig. 4F) amplifies the ecological importance of this depth range with critical and unique functions performed by species with particular traits (Laureto et al., 2015). By hosting communities with a greater functional richness, the mesophotic layer should thus be more resilient to disturbances (Auber et al., 2022), enabling it to better withstand potential human impacts such as fisheries but also global warming.

Within the mesophotic, the greater functional richness associated with the heterogeneity of coastal habitats observed within the PNMCCA should enhance ecosystem resilience (Bernhardt and Leslie in 2013). Protecting coastal zones with a strong bathymetric gradient would therefore be highly relevant to preserving ecological memory (Nyström and Folke, 2001). It would also enable species to recolonize habitats after disturbance, provided that connectivity is maintained (Goetze et al., 2021).

In terms of phylogenetic richness, we observe significantly higher values for the shallow layer (Fig. 4E). Since 74.6 % of species present in the shallow layer are also found in the mesophotic layer (Fig. 5A), we can hypothesize that the latter depth range limits biodiversity erosion and therefore the loss of evolutionary history in the shallow layer. The mesophotic layer preserves phylogenetic diversity (Winter et al., 2013) and evolutionary distinctiveness among species within a community (Scherson and Faith, 2018). This suggests that this depth layer can serve as a refuge beyond just maintaining taxonomic diversity. As for deeper layers, the harsher and more uniform environmental conditions such as low light, low productivity, prey scarcity, lower temperature, and higher pressure may exert stronger selective pressure on species. This is due to the need for specialized adaptations to survive in such extreme environments (Kyne and Simpfendorfer, 2010). These constraints could lead to species being grouped closer together on the phylogenetic tree, thereby reducing phylogenetic diversity (Kyne and Simpfendorfer, 2010).

Several highly commercial species were detected across all depth ranges, including both demersal species such as *Mullus surmuletus* and pelagic species such as *Scomber colias*, *Thunnus thynnus* or *Xiphias gladius*. These findings underscore the ecological connectivity and potential vulnerability of economically valuable species across different marine depth ranges.

With a higher functional diversity and a similar phylogenetic diversity than the shallow layer, the mesophotic appears therefore key for conserving the most at-risk species, functions and evolutionary lineages.

4.4. The multiple benefits of protecting deep marine waters

Our results underscore the importance of protecting mesophotic habitats, not only for the highly vulnerable elasmobranchs in the Mediterranean but also for teleost fishes, which could benefit from the effect of conserving elasmobranch populations. The ecological role of elasmobranchs as keystone (Fernández-Corredor et al., 2024) and umbrella species is particularly relevant in areas like the Pelagos Sanctuary. In such ecosystems, they contribute to the food web that supports marine mammals. Thus, safeguarding elasmobranchs would have cascading benefits (Ferretti et al., 2010) allowing a better ecosystem health and functioning.

As highlighted recently (Velasco-Lozano et al., 2024), the conservation effectiveness of deep refuges is linked to the diminishing effect of disturbance as depth increases. The mesophotic could therefore play a potential role as a refuge for communities threatened in shallower waters. Protecting the mesophotic alone, however, would not encompass all elasmobranchs, as some species (e.g., *Leucoraja naevus*, *Hexanchus griseus* and *Etmopterus spinax* which is a vulnerable species) are only detected in deeper layers. While the mesophotic layer may serve as a refuge for many shallow-water species, comprehensive conservation efforts must include deeper zones to ensure effective protection across elasmobranch habitats (Mathon et al., 2024).

The high functional diversity of the mesophotic highlights its ecological richness and demonstrates the need for targeted conservation measures. The mesophotic depth range harbors species with varied ecological roles and adaptations, enhancing ecosystem resilience. This greater functional diversity may indicate a wider range of ecological strategies and adaptations among the species present. The presence of EDGE species only in the mesophotic also stresses its need of protection if we consider that it is essential to ensure the conservation of species that are both evolutionarily distinct (i.e. phylogenetically valuable) and globally threatened and then at high risk of extinction (Scherson and Faith, 2018).

The disparity in sample size between superficial layers (176) and the deepest one (24) is critical, as demonstrated by our species accumulation curves. Future studies should aim to increase sampling effort both in the different layers of the mesophotic and beyond the mesophotic depth layer despite the stronger logistical and technical challenges. A more balanced sampling strategy across all depth layers is essential to better capture the full extent of elasmobranch diversity in deeper habitats.

4.5. The limits of eDNA metabarcoding and our sampling design

Despite its high potential to detect a wide array of species (Veron et al., 2023), including rare and elusive species (Boussarie et al., 2018), eDNA metabarcoding presents several methodological and interpretative limitations that must be carefully considered in future biodiversity assessments. Although our genetic reference database covers 88.4 % of potentially occurring elasmobranch species, taxonomic assignment remains incomplete for certain closely related lineages, particularly when reference sequences are missing or ambiguous (Boulanger et al., 2021).

Furthermore, spatial patterns of species distribution may be distorted by the variable persistence of eDNA in the environment (https://doi.org/10.1098/rspb.2019.140900) which can detect, for instance, the presence of the blackmouth catshark (*Galeus melastomus*) in the shallowest depth range, bearing in mind that this species is bathyal in the western Mediterranean basin but that juveniles can occur in shallower waters. Similarly, the dispersal capacity of eDNA can affect the accurate detection of mobile species such as the blue shark (*Prionace glauca*) or the spinetail devil ray (*Mobula mobular*), both identified in subsurface and near-bottom samples across different depths. The distribution of eDNA within the water column is often heterogeneous and influenced by a biotic factor such as currents, temperature, depth (McCartin et al., 2022; Sanchez et al., 2022). We detected 21 distinct elasmobranch species across multiple depth layers, including subsurface and near-seafloor sampling. However, 52 of the 73 species expected in the western Mediterranean Sea (Serena et al., 2020) were not detected, a pattern that may partly reflect the patchy nature of eDNA dispersal and detectability.

Although eDNA metabarcoding studies using a well-established database provide a valuable snapshot of biodiversity at a given time, the absence of temporal replicates limits the ability to detect compositional shifts within communities over time (Rozanski et al., 2024). This represents a significant limitation, particularly for widely dispersing species such as elasmobranchs with large home range, which may undergo seasonal migrations linked to prey availability or coincide with detection of rare species at the moment of sampling. However, this

constraint could be effectively addressed through the implementation of regular sampling campaigns and the development of eDNA-based time series. Moreover, this approach would also enable the early detection of newly arriving non-indigenous species (Sepulveda et al., 2020). In addition, PCR amplification biases can occur, favoring certain taxa depending on primer specificity or interspecific differences in DNA shedding rates (Sanchez et al., 2022).

Building upon these methodological considerations, further efforts should also focus on refining the spatial resolution of eDNA sampling, particularly across depth gradients, to better capture the full extent of fish biodiversity and validate the observed turnover between depth layers.

The high turnover of elasmobranch species observed between the two shallowest depth ranges and the deep layer also needs to be confirmed by a more exhaustive sampling at deeper layers. We also suggest sampling the mesophotic at three levels as described by Rocha et al., 2018 (upper between 30 and 59 m, middle between 60 and 89 m and lower mesophotic between 90 and 149 m) to investigate if depth ranges within the mesophotic are of major interest in terms of reservoir for elasmobranch biodiversity.

This limitation nevertheless highlights the need to continue improving reference databases, as well as considering alternative markers or multi-marker approaches to improve identification at the species level whose evolutionary radiation is too recent (Fontes et al., 2024). The use of complementary markers on the 12S increased the number of fish species detected (Ip et al., 2024). When the authors combined the previous markers with the Berry-Fish marker, the number of fish detected was again greater. By multiplying the number of markers, we could potentially limit the constraints associated with sharing the same barcode. It would also reduce barcode affinity problems during PCR.

4.6. *Implications for future monitoring and conservation strategy*

Even if access to these deeper areas remains challenging, human exploitation of the mesophotic remains important (Jacquemont et al., 2024; Maiorano et al., 2022) and human activities such as deep-sea fishing represent significant threats to the mesophotic (Enrichetti et al., 2019). Due to their remoteness from the coast, the accessibility and surveillance of these deep-water areas is difficult, often rendering their protection limited and ineffective. In the waters of PNMCCA, deep layers are located close to the coast (around 3.7 km offshore) where canyon heads are present. These canyons generally enhance productivity by creating biomass hotspots in otherwise nutrient-poor environments (Bianchelli and Danovaro, 2019), urging their effective protection. Protecting the mesophotic layer will also help conserve associated species across interconnected depth ranges. Elasmobranchs can be seen as umbrella species, providing benefits to other organisms, including marine mammals, which are already a focus of particular attention within the Pelagos Sanctuary. With the PNMCCA we have this great opportunity due to its own design to effectively protect species like elasmobranchs which require large areas to be adequately protected due to their extensive home range (Dwyer et al., 2020). In this marine park, even if some species live below shallow layers, some life stages impose spatial segregation during calving, and the nurseries of many species are identified in shallow coastal areas close to estuaries (Schlaff et al., 2020). The PNMCCA offers the opportunity to protect such corridors within its boundaries, by ensuring a bathymetric gradient from the surface to the deeper layers associated with nearby estuaries and canyons. Implementing these protective measures would not only ensure the conservation of species in deep-water refuges but also seed neighboring and depopulated areas. It therefore seems important to establish protected areas that span a continuum of depths from the coast to the open sea. This would help ensure better conservation of species across all bathymetric layers. Particular emphasis should be placed on the mesophotic layer, which contains nearly all elasmobranch species found in shallow layers, a zone that is more accessible to human activities. Within this refuge in the northern Mediterranean basin that Corsica represents (Pichot et al., 2024), the mesophotic zone can be seen as a "refuge within the island refuge". This depth range should be preserved through the implementation of protection measures targeting habitats that are not usually considered when establishing fully protected areas (Giménez et al., 2020).

In conclusion, our findings support the urgent need to preserve mesophotic and deeper layers in addition to shallow and coastal habitats. The new conservation agenda targeting 30 % protection coverage in the ocean by 2030 offers the unique opportunity to adapt the design of most MPAs towards the inclusion of deeper habitats. Protecting only shallow coastal areas leaves deeper habitats vulnerable to threats such as overfishing, which is particularly harmful to elasmobranchs due to their slow growth, late maturity, and low reproductive rates. The establishment of a coherent network of fully protected or no-take areas within the PNMCCA is essential to address the challenges of climate change and overfishing. Such a network would help mitigate the multiple human pressures and ensure the conservation of the most vulnerable species, ecological functions, and evolutionary lineages. The PNMCCA would then fully play its role as a refuge on this Mediterranean island and beyond.

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CRediT authorship contribution statement

Franck Pichot: Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Stéphanie Manel:** Writing – review & editing, Validation, Supervision, Formal analysis. **Laure Velez:** Writing – review & editing, Investigation, Data curation. **Jean-Baptiste Juhel:** Writing – review & editing, Data curation. **Laurent Ballesta:** Writing – review & editing. **Pierre Boissery:** Writing – original draft. **Morgane Bruno:** Writing – review & editing. **Maddy Cancemi:** Writing – review & editing. **Florian Holon:** Writing – review & editing, Investigation. **Jean-Jacques Riutort:** Writing – review & editing, Investigation. **Marieke Schultz:** Writing – review & editing, Visualization. **Nicolas Tomasi:** Writing – review & editing. **Alice Valentini:** Writing – review & editing. **Olivier Adam:** Writing – review & editing. **Julie Deter:** Writing – review & editing, Methodology, Funding acquisition. **David Mouillot:** Writing – review & editing, Visualization, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, 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.

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Data availability

Data will be made available on request.

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