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ABSTRACT

Aim: The Mediterranean Sea is one of the most anthropized seas in the world but also a marine biodiversity hotspot with many fish species under threat. The main goal of the study is to test whether on the heavily fished and anthropized Mediterranean coast, the less impacted Corsica and Balearic Islands, can be considered as refugia for threatened and elasmobranch fishes independently of protection by marine reserves.

Location: The French Mediterranean coast and three north-western Mediterranean islands: Corsica and also Mallorca and Minorca from the Balearic archipelago.

Methods: We performed 187 fish surveys using environmental DNA metabarcoding on three islands and 109 along the continental coast. Of the 78 surveys on islands 22 correspond to no-take marine reserves and of the 109 continental surveys 26 were carried out within reserves. After eDNA filtration, extraction, amplification, and sequencing we estimated the number of fish species but also the number commercial, threatened and elasmobranch fish species on each sample. We then performed an ANOVA by permutation to test the effect of insularity and protection on these four biodiversity metrics. We also modelled these four biodiversity metrics as a function of protection and human pressure but also environmental, habitat and sampling conditions. We also built species accumulation curves to obtain asymptotes representing the potential regional pools for each species category on both island and continental coasts.

Results: We obtained a total of 175,982,610 reads over the 187 eDNA samples that were assigned to 153 fish species including 17 elasmobranch species among which 7 were only detected on islands. We observed a higher total fish richness on continental than island surveys regardless of protection but a higher threatened and elasmobranch fish richness on the island than on continental surveys. We obtained a significant, negative and predominant human gravity impact on the diversity of elasmobranch species. The modelled asymptote reached 148 teleostean fish species on islands and 196 on the continental coastline with a very similar

rate of diversity increase with sampling effort but the shape of the species accumulation curves differed markedly for elasmobranchs with a stronger increase in diversity with sampling effort on islands.

Main Conclusions: Our findings highlight that Mediterranean islands can be refugia for sharks and rays but also threatened fishes in this overexploited region. Our results also suggest that reducing or banning trawling activities may play a key role for conserving vulnerable fishes, beyond the benefits of no-take marine reserves, which appear limited on these large home-range species.

1 | Introduction

The Anthropocene is marked by a massive defaunation affecting both terrestrial and aquatic ecosystems (Jaureguiberry et al. 2022; Ripple et al. 2019). Yet, the lack of knowledge about the status and distribution of many species and the paucity of data make the assessment of this biodiversity erosion challenging and incomplete (Isbell et al. 2023; Luypaert et al. 2019). Indeed, taxonomic inventories provide the most elementary data of such assessment, but they are notoriously scarce and biased particularly in the vast ocean (Henderson et al. 2016; Mora et al. 2011; Serena et al. 2020). Among marine organisms, fishes are the main protagonists of the link between marine ecosystems and human societies since they provide essential contributions to people such as biomass production, food security, nutrient cycling, and cultural value (Villéger et al. 2017). However, fishes are severely threatened by overexploitation, degradation of their habitats, pollution and climate change which can lead to the local extirpation of some species (Mellin et al. 2016). Elasmobranchs (sharks, rays and skates) are particularly affected by anthropogenic activities due to their K-selected life history traits (Nuez, Gazo, and Cardona 2021; Pimiento et al. 2020). Of all vertebrates, elasmobranchs also represent the taxonomic group with the highest number of threatened species (76%) on the Red List of the International Union for Conservation of Nature (IUCN) and overexploitation has the greatest impact (Dulvy et al. 2021; Pacoureau et al. 2021). As mobile top predators these species play unique ecological roles in coastal ecosystems like top-down control of meso-predators, horizontal and vertical nutrient transports, and removal of weak or diseased individuals (Roff et al. 2016; Sherman et al. 2023). Yet, human threats tend to be the most pronounced in fish biodiversity hotspots like the Indo-Pacific Coral Triangle and the Mediterranean Sea (Dulvy et al. 2014; Walls and Dulvy 2020) even in partially or poorly protected areas (Di Lorenzo et al. 2022; MacNeil et al. 2020). So, in these regions, the extent to which the most isolated areas from human activities and no-take marine reserves can be considered as refugia for elasmobranch and threatened fishes is of primary importance in conservation but is still poorly investigated. In this study we assessed the level of fish biodiversity in different protected and unprotected areas of Mediterranean islands remote from the mainland as well as along the highly anthropized French coast using environmental DNA or eDNA metabarcoding.

The Mediterranean Sea is the largest enclosed sea on Earth (0.82% in surface area and 0.32% in volume of the world ocean), hosting around 17,000 species, equivalent to around 10% of the world's marine biodiversity, and has been exploited by mankind since the dawn of civilizations (Bianchi and Morri 2000; Coll et al. 2010). Nowadays, with a human density of approximately 480 million inhabitants along its 46,000 km of coastline, to which is added a third of world's tourism (500 million expected in 2030), the Mediterranean Sea experiences one of the highest cumulated human pressures across the world's oceans (Drius et al. 2019; Laviola et al. 2022). As a consequence, most elasmobranchs have almost disappeared from the Mediterranean coasts due to intensive fishing pressure while remaining populations are scarce (Dulvy et al. 2014; Ferretti et al. 2008; Giovos et al. 2021). For instance, the angel shark (*Squatina squatina*), which gave its name to the famous Bay of Angels in Nice, was common along the entire Mediterranean coastline in the past but has almost disappeared because of fishing pressure except in some last refugia (Faure et al. 2023; Gordon 2019). Islands and remote areas are known to host many species vulnerable to human activities and thus can act as biodiversity refugia in the Anthropocene (Letessier et al. 2019; Yesson et al. 2021). In the Mediterranean Sea, some islands are still relatively remote with a low human population density—except during the summer touristic season—and a limited exploitation of natural resources. Yet, their capacity to serve as refugia for threatened species is poorly known.

Here, we hypothesize that Corsica and the Balearic Islands, respectively belonging to France and Spain, can be the last refugia in the North-Western Mediterranean basin for threatened and elasmobranch fish species owing to their relative geographical isolation from the nearest continental coast and relatively weak human pressure. With reduced or non-existent trawling activities and limitations or adaptations concerning other types of fishing gears, these islands can therefore be considered as potential refugia for marine fishes in general and elasmobranch species in particular. However, robust, standardised and extensive biodiversity data are lacking to test this hypothesis.

Detecting and monitoring rare and elusive fishes, like most elasmobranchs, remain challenging in marine environments as some species bury themselves under sediments or mimic the colour of the substrate (*Squatina squatina*, *Torpedo marmorata*), some can also live in dense seagrass beds (*Dasyatis pastinaca*, *Dasyatis tortonosei*), others in the deep sea (*Hexanchus griseus*, *Dipturus batis*, *Dalatias licha*) or in the vast open sea (*Prionace glauca*, *Mobula mobular)* (Ebert and Dando 2021; Last et al. 2016; Louisy 2022). To date, the local census of coastal fishes is largely based on classical techniques of underwater visual observations or fisheries catches with well-known limitations and biases (Colton and Swearer 2010; Fernández et al. 2021; Marques et al. 2021a). The census of coastal fishes can be improved by using eDNA metabarcoding since it can detect more species compared to traditional surveys (Boussarie et al. 2018; Mathon et al. 2022). eDNA is the genetic material obtained directly from an environmental sample corresponding to the DNA that organisms release in their environment, through the shedding of cells from the skin, body fluids, metabolic waste, gametes or blood (Miya 2021; Taberlet et al. 2012). The extraction, amplification

with universal primers and sequencing of eDNA from seawater produces genetic sequences that can then be assigned to species or taxa using genetic reference databases (Casey et al. 2021) to provide ecological indicators (Dalongeville et al. 2022).

The objective of the study is to compare the sparsely anthropized Spanish and French islands of the northwestern Mediterranean basin with the highly urbanised and fished French continental coastline to test whether islands can be considered as refugia for threatened fishes and elasmobranchs. To test this objective, we analysed 187 eDNA samples, collected in no-take marine protected areas or marine reserves and mesophotic reefs, along the French North-Western Mediterranean coasts, and along Corsica and Balearic Islands. We then modelled fish taxonomic composition and diversity in terms of total species richness but also commercial, threatened and elasmobranch species richness with key factors like island, protection and human pressure but also factors summarising environmental, habitat and sampling conditions. We also tested several models of species accumulation curves to predict the level of regional fish diversity on continental and island coasts.

2 | Material and Methods

2.1 | Study Locations

Corsica island (160 km from French mainland, < 90 km from Italian mainland) is poorly urbanised with a relatively mean low population density of 39 inhabitants/km^2 versus 315 along the continental French Mediterranean coast (Insee 2023), a quasi-absence of industrialization and a weak industrial fishing pressure with six trawlers in Corsica (SIH 2017) compared to 44–49 along the continental French Mediterranean coastline for a similar length (Ifremer 2021). The Balearic archipelago is composed of four main islands, two of which were sampled during our study: Mallorca and Minorca. This archipelago is located in the middle of the western Ligurian basin at a distance of 75 km from the Spanish coast with an area of approximately 28,290 km^2, and a shoreline length of 1723 km. Balearic islands are more urbanised than Corsica with a density of 240 inhabitants/km^2 but the sampled areas of northeast of Mallorca and Menorca remain sparsely populated. Moreover, trawling and similar fishing methods have been banned in a great part of the Menorca channel since 2016 (Farriols, Ordines, and Massutí 2021).

The French Mediterranean coast hosts 77 marine protected areas (MPAs) among which some are highly protected (no-take zones) and are called marine reserves (Costello 2015). Corsica, with a shoreline of 1046 km, is comparatively less protected (0.28%) with 3265,5 ha of marine reserves than the French coastal mainland (0.44%) with 5396 ha along the 901 km shoreline (MedAMP 2017). The Balearic Archipelago is the region of Spain accounting for the most MPAs with 10 well-enforced MPAs spread over the four islands, plus a maritime-terrestrial national park located on Mallorca Island. The total protected surface adds up to 500,000 ha. Marine reserves represent 17.67% of the Balearic waters (Barrientos and Vaquer-Sunyer 2022).

The Corsica Island and islands of the Balearic archipelago share similar distances to the European continent (Figure 1).

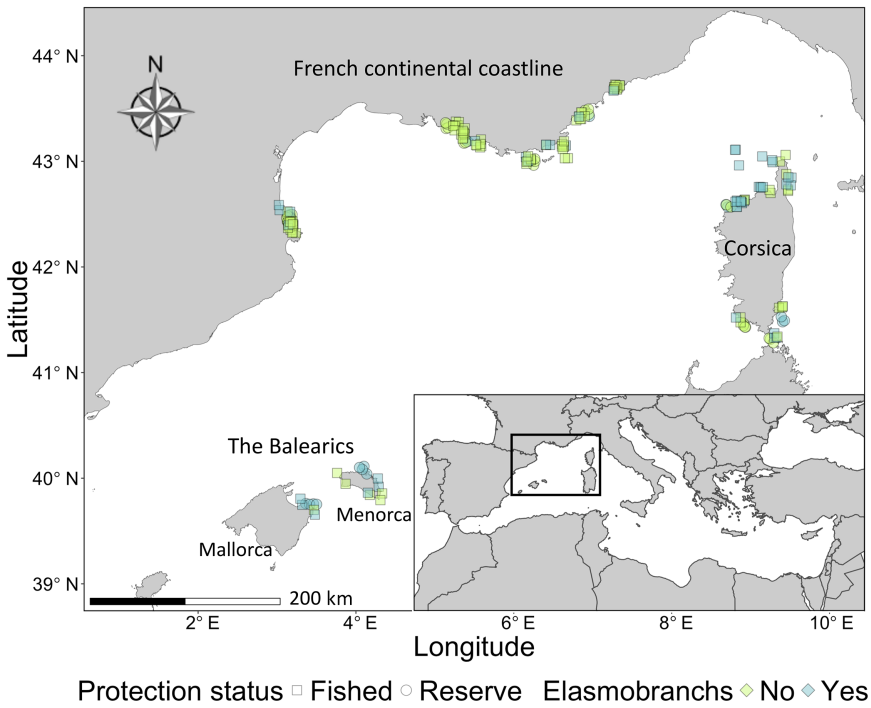

FIGURE 1 | Map of the 187 eDNA samples along the North-Western Mediterranean coast with the presence of elasmobranchs and protection by a marine reserve indicated by different colours and symbols, respectively. Each of the 53 sampling locations is symbolised by a dot (non-protected) or by a square (MPAs).

2.2 | eDNA Sampling

We filtered 204 samples of seawater in 53 locations (Figure 1) between 2018 and 2021 in the same type of habitats for island and continental coasts, inside and outside 11 marine reserves. These habitats consisted of a patchwork of *Posidonia oceanica* meadows associated with rocky shores and sandy patches. Among these locations, 50 were sampled at least twice (two or more replicates) and 3 were sampled only once since some samples were removed when less than five species were detected. All filters were sampled in spring and summer. The effects of seasonality were controlled in our analyses with sea surface temperature and chlorophyll-a as variables.

Finally, after retaining only filters containing at least five species, i.e. the threshold chosen to calculate biodiversity indicators on samples with a minimum realistic number of fish species detected (Dalongeville et al. 2022), we selected a total of 187 samples with 78 samples on islands (Corsica (58) and Balearic (20)) and 109 along the French continental coast (Figure 1). Of the 78 filters collected along the islands coast, 56 correspond to locations outside reserves (44 for Corsica and 12 for the Balearic) and 22 correspond to marine reserves (14 for Corsica and 8 for the Balearic). For the continental coast, of the 109 filters sampled, 83 were carried out outside reserves while 26 were carried out within.

Among the 187 eDNA samples, 166 were collected by filtering seawater along a 2-km transect at 1 m below the surface while 16 deep samples were performed by filtering seawater as close as possible to the bottom by scuba divers equipped with a submerged peristaltic pump (Muff et al. 2023) and five by filtering a volume of 30 L water samples collected by Niskin bottles at 0, 40, and 50 two times and at 100 m depth outside marine reserves along the French continental coastline. In total, we had three methods, but regardless of the method used, each sample consisted of a 30 L volume of seawater filtered using a device with a nominal flow of 1 L/min allowing to collection of eDNA with a sterile VigiDNA 0.2 μM cross-flow filtration capsule. We also used the same molecular (sequencing depth) and bioinformatic procedures (see Section 2.3) to make the analyses comparable. Due to constraints, we used different methods since we could not sample shallow, mesophotic and deep reefs with the same effort but we controlled for these methodological differences in the models (see Section 2.7).

At the end of each filtration, the capsules were filled with 80 mL of CL1 conservation buffer before being closed hermetically and then shaken for at least 1 min while rotating to resuspend eDNA in the conservation buffer. The labelled capsule was then stored at room temperature until extraction. We followed a strict contamination control protocol (Goldberg et al. 2016) using disposable gloves and single-use filtration equipment for each water sample.

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 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 at 15,000 *g* for 15 min 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 samples were pooled before the amplification step. After the DNA extraction, the samples were tested for inhibition following the protocol described (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 DNA extract as the template. The amplification mixture contained 1 U of AmpliTaq Gold DNA Polymerase (Applied Biosystems), 10 mM Tris–HCl, 50 mM KCl, 2.5 mM MgCl2, 0.2 mM of each dNTP, 0.2 μM of each primer, 4 μM human blocking primer for the "teleo" primers (Valentini et al. 2016), and 0.2 μg/μL bovine serum albumin (BSA, Roche Diagnostic).

We then used a primer set targeting teleostean that also works very well with elasmobranchs (Bylemans et al. 2018): "Teleo" (forward: ACACCGCCCGTCACTCT, reverse: CTTCCGGTACACTTACCATG) (Taberlet 2018; Valentini et al. 2016), amplifying a mean sequence length of 64 bp. This primer was 5′-labelled with an eight-nucleotide tag unique to each PCR replicate for "Teleo", allowing the assignment of each sequence to the corresponding sample during sequence analysis. The tags for the forward and reverse primers were identical. 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. Twelve PCR replicates were run per filtration. 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. Thirty-six libraries were prepared using the MetaFast protocol (Fasteris). For one library, the paired-end sequencing (2 × 125 bp) was carried out using an Illumina HiSeq 2500 sequencer on a HiSeq Rapid Flow Cell v2 using the HiSeq Rapid SBS Kit v2 (Illumina), for four libraries the sequencing was performed 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. Library preparation and sequencing were performed at Fasteris [https://www.fasteris.com/en-us/]. Fifty-four negative extraction controls and 14 negative PCR controls (ultrapure water, 12 replicates) were amplified and sequenced in parallel to monitor possible contaminants.

2.4 | Bioinformatic Analyses and Taxonomic Assignments

Following sequencing, reads were processed to remove errors and analysed using programs implemented in the OBITools toolkit (Boyer et al. 2016) based on a previous protocol (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 analysed 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.

Taxonomic assignment of the remaining sequences was performed using the ecotag program using a combination of publicly available sequences from ENA release 142 updated in 2022 and our Mediterranean genetic database, comprising already published sequences from 117 species (Boulanger et al. 2021) and 56 new species (39 teleostean and 17 elasmobranch species) all together representing 26% of the regional fishes species pool and 76% of all elasmobranch species of the regional North-Western Mediterranean pool. 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). Using the "Teleo" primer pair with all the sequences obtained in our filters we were capable of discriminate almost all elasmobranchs except three species of rays (*Raja clavata*, *Raja polystigma* and *Raja asterias*) which share the same barcode and two torpedo rays (*Torpedo torpedo* or *Tetronarce nobiliana*) because we have not yet obtained the corresponding barcodes. Moreover, reference databases are notoriously incomplete, limiting the identification of many species and thus the potential of eDNA metabarcoding to monitor a wide breadth of species (Marques et al. 2021b; Yoccoz 2012).

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 76% and for all fishes to 81% of the regional pool (Charbonnel, Coudre, and Francour 2017). Samples were collected from fisheries landings, with the assistance of veterinarian from the European Association of Zoos and Aquaria (EAZA) or from Museum collections.

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 for 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 a single one occurring in the Mediterranean, it was assigned to the Mediterranean species.

Species names were verified using the *rfishbase* R package (Boettiger, Lang, and Wainwright 2012).

After the taxonomic assignation steps, considering the incorrect assignment of a few sequences to the sample due to tag jumps (Schnell, Bohmann, and Gilbert 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.

2.5 | Biodiversity Metrics

Four metrics we considered to assess the effects of islands combined with human factors like protection or fishing pressure. The first metric encompasses all fish species present including all taxonomic fish groups, regardless of their ecological roles, economic importance, or conservation status. The second metric, commercial species richness, includes fishes that are of significant economic value for professional fisheries. The third metric corresponds to fish species that are classified as threatened, or red listed, according to the International Union for Conservation of Nature (IUCN). This metric includes both teleost fishes like the dusky grouper (*Epinephelus marginatus*) and elasmobranch fishes like the spinetail devil ray (*Mobula mobular*). The last metric comprises all Elasmobranch species, a subclass of cartilaginous fishes that includes sharks, rays, and skates. The value obtained per eDNA filter or sample corresponds to the cumulated number of fish species per category (all, commercial, threatened or elasmobranch species).

2.6 | Environmental, Habitat and Sampling Variables

A set of six explanatory variables were used to model each biodiversity metric. Beyond the protection level (inside marine reserve vs. outside) and the island factor (island vs. continental sampling location), we included the sampling depth (1–200 m), the diversity of habitats around the sampling location, the industrial fishing pressure and the human gravity as explanatory variables (Table 1). This latter variable is a good proxy of ecosystem accessibility by humans and is measured as the ratio between the population size of the nearest human settlements

TABLE 1 | list and data sources of human, environmental and habitat variables used in the models.

Category	Variable	Source
Habitat	Principal habitat: - Coralligenous bottoms - Posidonia meadows (*Posidonia oceanica*) - Soft bottoms - Bathyal zone	DONIA EXPERT (2023) Medtrix platform https://medtrix.fr/portfolio_page/donia-expert/
	Number of different habitat types	
Human	Industrial fishing pressure	Paolo Kroodsma et al. (2024)
	Human gravity	Cinner et al. (2018)
Climate	Mean sea surface temperature the week of the survey (7 days before sampling)	MARS3D (Model for Applications at Regional Scales; Lazure and Dumas 2008) Model F2-MARS3D-MENOR1200 Spatial Resolution of 1.2 km Time-step of 3 h
	Mean annual sea surface temperature (period 2000–2014)	bio-ORACLE v2.2 (Assis et al. 2018; Tyberghein et al. 2012) https://www.bio-oracle.org/ Spatial Resolution of 5 arcmin.
Bathymetry	Mean bathymetry Min bathymetry	Andromède Océanologie & SHOM (https://data.shom.fr/)
Productivity	Mean surface chlorophyll-a during the week of the survey (8 days including sampling day)	NASA Aqua MODIS (NASA Goddard Space Flight Center 2018) https://modis.gsfc.nasa.gov/data/dataprod/chlor_a.php Spatial resolution of 4 km
	Mean annual surface chlorophyll-a (period 2000–2014)	bio-ORACLE v2.2 https://www.bio-oracle.org/ Spatial resolution of 5 arcmin

divided by the squared travel time to reach each sampled location (Cinner et al. 2018). It includes several human pressures like recreational fishing, small-scale fishing, chemical and noise pollution, and human disturbance caused by tourism.

To control for other secondary variables related to environmental, habitat and sampling conditions, we considered the sampling method (surface, dive transect or Niskin drop), the mean and minimum bathymetry over the sampling transect, the sea surface temperature and the productivity (Chl a) of the sampling week and year, and the principal habitat at the sampling location. Then we performed a Principal Coordinate Analysis (PCoA) on these eight secondary variables to extract five synthetic and orthogonal axes, representing 81% of variation, to limit the effects of collinearity between explanatory variables and to take into account missing data in the comparison of sampling locations (Boulanger et al. 2021). All environmental and all habitat variables are detailed in Table 1.

2.7 | Statistical Analyses

We first assessed how the composition of fish and elasmobranch species varied across eDNA samples and in response to the protection status, insularity and other environmental or sampling factors using a distance-based redundancy analysis (dbRDA). We calculated the Jaccard distance between samples to quantify the species dissimilarity based on presence-absence data. We then conducted a partial dbRDA using the function *capscale* from the R package *vegan* (Oksanen 2016) to model the multispecies response to the protection level (reserve or outside), the insularity (island or continent) and their interaction (protection × island) as explanatory variables, while including the five PCoA axes as covariates to account for their potential confounding effects (Legendre and Legendre 2012). The global model as well as individual axes and variables were tested for significance using ANOVA-like tests with 999 permutations (function *anova.cca*). Species scores along the first and second dbRDA axes were further visualised to assess the species response to explanatory variables with a focus on commercial, elasmobranch, and threatened species.

We then employed an Analysis of Variance (ANOVA) with permutations to test the differences in the four biodiversity metrics among samples using protection and island, but also their interaction, as factors. The permutation approach was chosen due to its robustness in handling non-normal data distributions and its ability to provide exact *p*-values under the null hypothesis. To do this, we ran 999 permutations using the *aovp* function in the *lmPerm* package.

We then modelled each of the four fish biodiversity metrics using Generalised Linear Models (GLM) and 10 explanatory variables. More precisely, for each diversity metric, a full model was fitted using protection, industrial fishing pressure, human gravity, habitat diversity, depth and the first five axes of the PCoA as explanatory variables. Total species richness and commercial species richness were modelled with a gaussian distribution, whereas elasmobranch and threatened species richness were modelled using a quasi-poisson distribution to account for the high number of zeros.

To assess the importance of variables in each model, we used a multimodel inference approach providing the AIC weight for each variable corresponding to the proportion (0–1) of best models (Delta AIC < 2) selecting each variable (Bartoń 2022). The most parsimonious model was then selected based on the Akaike criterion using a descending and ascending stepwise selection with the *stepAIC* function of package MASS and partial effects of the explanatory variables selected in the parsimonious models were visualised using the *visreg* package in R (4.3.0).

Since taxonomic diversity is likely underestimated given our sample size, we modelled species accumulation curves to obtain asymptotes representing the potential regional pools for each species category on both island and continental coasts (Juhel et al. 2020). This regional fish richness can be coined as γ-diversity by opposition to local or sample fish richness which is called α-diversity. We tested and compared five different accumulation models (Lomolino, Michaelis–menten, Gompertz, asymptotic regression and logistic curve) using the Akaike information criterion (AIC) for each species category (Aho, Derryberry, and Tery 2014).

3 | Results

3.1 | Metabarcoding and Taxonomic Diversity

A total of 175,982,610 reads assigned to fish were obtained over the 187 eDNA samples after bioinformatic curation and cleaning. From the 187 samples, after the assignment procedure, a total of 153 fish species were detected, including at least 17 elasmobranch species (6 sharks and 11 rays and skate species). Overall, 126 species were detected on continental coastlines and 117 on islands with 90 of these species found on both. Among the 17 detected elasmobranch species, seven were unique to islands while four were unique to the continental coast including one pelagic and critically endangered species in the Mediterranean Sea, the great Blue Shark (*Prionace glauca*), potentially present along all the North-Western Mediterranean coast and detected opportunistically (Figure 2). Six elasmobranch species were common to both continental and island coasts.

3.2 | Species Dissimilarity Across Locations

The partial dbRDA (adjusted $R^2 = 0.014$, overall F-test = 2.0, $p = 0.001$) revealed a significant effect of insularity (F-test = 2.4, $p = 0.001$), protection (F-test = 1.9, $p = 0.001$) and their interaction (F-test = 1.8, $p = 0.001$) while controlling for other factors. This result suggests that insularity has more effect than protection on species composition and that protection effect is different whether fish communities are from island or continental locations. The dbRDA showed a distinction between samples collected on island vs. continental coastline along the first axis and between those collected in protected vs. fished areas on the second axis, with the combination of island and protection hosting the most distinct fish communities (top right dark blue in Figure 3A). Species compositions in insular fished areas are more heterogeneous than their counterparts inside marine reserves.

On average, elasmobranch and threatened species tend to be more present on islands than on the continent regardless the level of protection (longer arrows towards the right side of the first axis than on the left side, Figure 3C,D). By contrast, commercial species show no particular trend in this insular vs. protection space (Figure 3B), even if for example the Atlantico bonito (*Sarda sarda*) are more present along continental coastline while the dusky grouper (*Epinephelus marginatus*) or the red mullet (*Mullus barbatus*) are more present along insular coastlines.

3.3 | Island and Protection Effects

We used an analysis of variance (ANOVA) with permutations to test the differences between samples collected within or outside marine reserves and on insular vs. continental coasts for the four biodiversity metrics (Table 2). We show that insularity had a significant effect on each of the four biodiversity metrics while protection had no effect. The interaction between both factors was neither significant suggesting that the insularity effect is consistent whatever the level of protection. When plotting the distribution of biodiversity metrics according to the insularity and protection effects we show that total and commercial fish richness per sample was higher on island than continental coasts but the opposite for threatened and elasmobranch species richness (Figure 4).

3.4 | Main Correlates of Fish Biodiversity Metrics

We used GLMs to explore the effect of five explanatory variables in association with five PCoA axes on total fish richness but also commercial, threatened and elasmobranch species richness. The coefficient of determination, denoted by R^2, ranged between 0.17 and 0.42. GLMs indicated a significant and predominant human gravity effect on elasmobranch biodiversity (Table 3), with eDNA samples showing, on average, higher elasmobranch richness under lower human pressure (Figures 5 and 6A). By contrast all potential explanatory variables had no significant influence on total and commercial fish richness which are mainly driven by environmental, habitat or sampling related variables (Table 3). Yet, industrial fishing pressure had a consistent negative effect on all biodiversity metrics, albeit non-significant. Habitat diversity had a positive effect on total and commercial fish richness but negative on threatened and elasmobranch richness, albeit non significantly. The protection effect was never significant nor retained in the most parsimonious model for any biodiversity metric (Table 3). Depth had a consistent negative

FIGURE 2 | Teleost and elasmobranch species detected using eDNA metabarcoding in 187 eDNA samples along North-Western Mediterranean coasts. The Venn diagrams show species richness overlap between continental and island coastline samples for all fish species (A) and for elasmobranch species specifically (B). Horizontal barplots display the relative frequencies of each elasmobranch species across samples in the two sets of locations (C).

effect on three biodiversity metrics, albeit non-significant, but was positive for threatened species suggesting they are more present in deep refugia (Table 3).

For elasmobranch species the most predominant effect is human gravity suggesting that, beyond fishing pressure, human presence induces disturbances responsible of elasmobranch biodiversity erosion along the Mediterranean coast. Depth had only a positive, albeit non-significant, for threatened species suggesting a potential refugia (Table 3).

3.5 | Species Accumulation Curves

The Lomolino model was selected after the evaluation of the five models using the Akaike information criterion (AIC) for all species accumulation curves. The modelled asymptote represent the estimated overall diversity or γ-diversity (measure of the overall fish diversity cumulated within a region) – and reached 148 teleostean fish species on islands and 196 on the continental coastline with a very similar rate of diversity increase with sampling effort (Figure 8A). For the

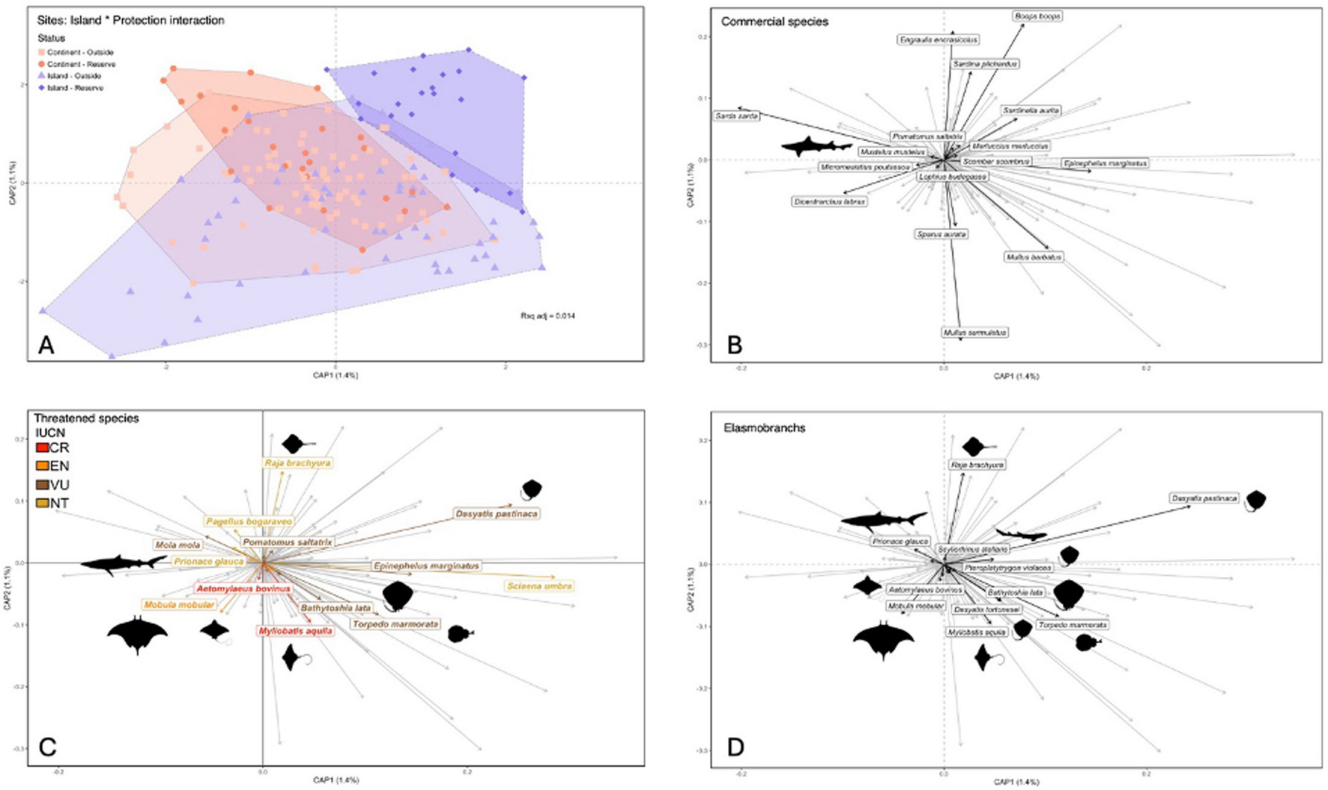

FIGURE 3 | Fish and elasmobranch species composition in response to the island vs. continental location, protection level and their interaction using a distance-based redundancy discriminant analysis (dbRDA). Each dot represents an eDNA sample (i.e., fish community) while their shapes and colours represent the island vs. continental location and protection level, respectively (A). Species scores highlight the position of commercial species (B), threatened species according to their IUCN status (C) and elasmobranch species (D). Only the names of species with a score above 0.05 on each axis are depicted.

TABLE 2 | Results of the ANOVA with permutations testing the effects of insularity and protection on the four taxonomic biodiversity metrics: All, commercial, threatened and elasmobranch fish richness. *p*-values, obtained by comparing the observed statistic with the distribution of statistics obtained by permuting the group labels, show whether the differences observed between groups are statistically significant. Only low *p*-values indicate that the differences observed between groups for the four metrics are statistically significant (*$p < 0.05$, **$p < 0.01$, ***$p < 0.01$).

Biodiversity metric	Explanatory variable	*R* Sum of square	*p*
Total species richness	Island	554.8	**0.02985***
	Protection	1.9	0.80392
	Island × Protection	388.3	0.19332
Commercial species richness	Island	522.8	**< 2e-16****
	Protection	10.6	1.0000
	Island × Protection	71.5	0.2606
Threatened species richness	Island	25.571	**< 2e-16****
	Protection	2.369	0.06360
	Island × Protection	2.679	0.07815
Elasmobranchs richness	Island	22.957	**< 2e-16****
	Protection	0.109	1.0000
	Island × Protection	0.099	0.5733

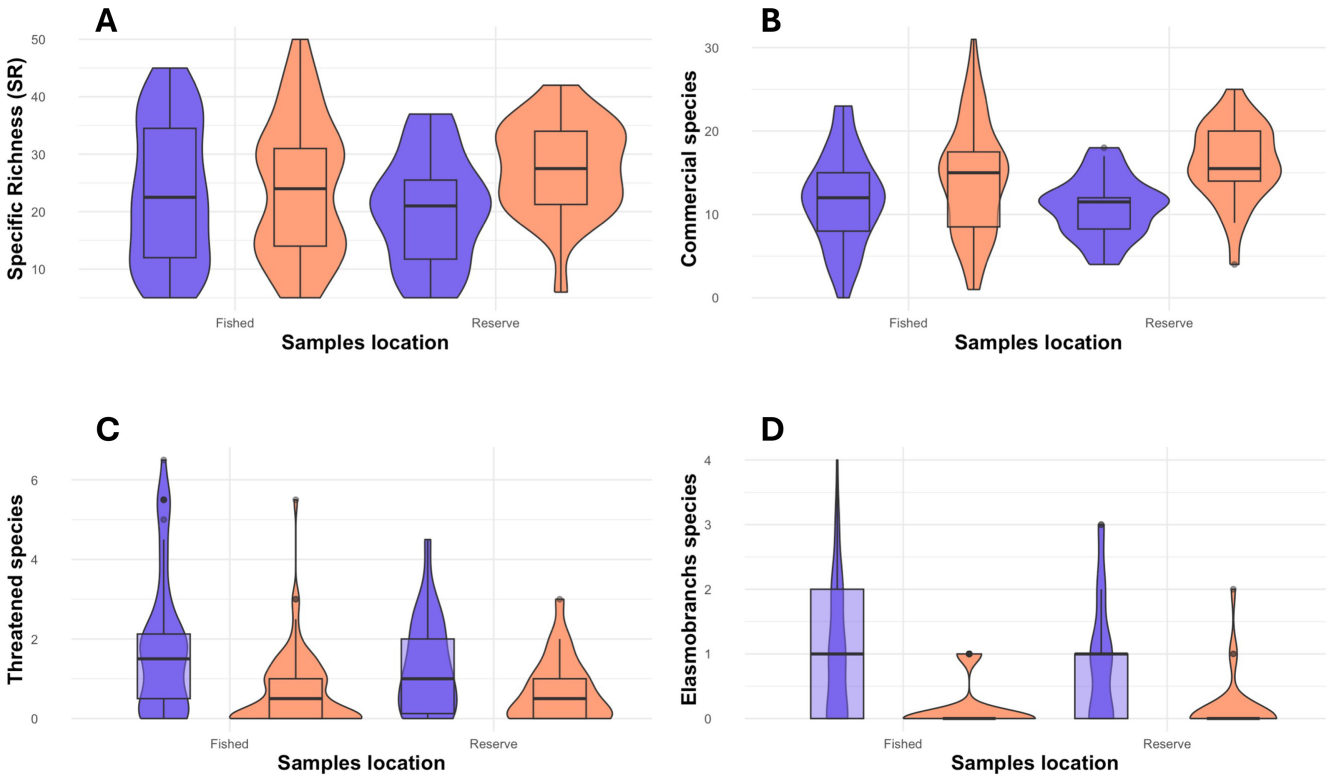

FIGURE 4 | Plots from the ANOVA with permutation using the *aovp* function (package "lmPerm") showing the distribution of the four biodiversity indices for continental and island eDNA samples from inside or outside marine reserves (Table 2). Shaded area represents the 95% confidence interval.

TABLE 3 | Results of GLMs testing the effects of five explanatory variables and five PCoA axes on the four biodiversity metrics in terms of species richness for all fish species, only commercial, threatened and elasmobranch species. AIC weight represents the importance of each variable in the best models and the *t*-value or *z*-value their influence on the predicted variables along with significance (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$). Only factors retained in the most parsimonious models (according to the selection procedure based on AIC) are in bold for AIC weight values and used to draw partial plots of the main effects in Figures 4 and 5.

	Species richness		Commercial species		Threatened species		Elasmobranch species	
	AIC weight	*t*	AIC weight	*t*	AIC weight	*z*	AIC weight	*z*
Human gravity	**0.27**	0.155	**0.41**	0.882	**0.28**	−0.462	**0.98**	**−2.156***
Industrial fishing density	**0.62**	−1.648	**0.56**	−1.280	**0.50**	−1.371	**0.28**	−0.441
Protection	0.26	−0.245	0.26	0.241	0.32	0.732	0.29	0.666
Depth	0.30	−1.175	0.26	−0.293	**0.54**	1.564	0.28	−0.028
Habitat diversity	**0.88**	1.754	**0.55**	0.989	0.33	−0.860	**0.60**	−1.908
Axis 1	**0.65**	**2.215***	**0.96**	**2.711****	**0.83**	**−2.212***	**1.00**	**−3.239****
Axis 2	0.49	1.596	0.26	0.202	**0.97**	**2.689****	**0.98**	**3.148****
Axis 3	**1.00**	**−3.426*****	**1.00**	**−3.845*****	**0.77**	**−2.494***	0.28	−0.387
Axis 4	**0.73**	**−2.310***	**0.90**	−2.470	0.27	−0.072	**0.96**	**−2.664****
Axis 5	0.31	0.772	0.28	0.280	**0.72**	1.786	**0.69**	1.734
R^2	0.31		0.30		0.17		0.42	

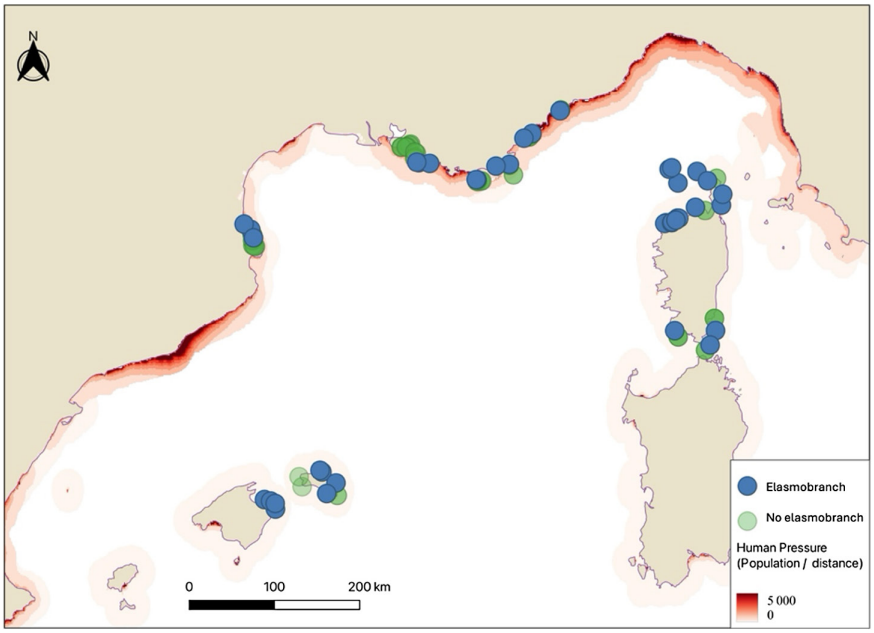

FIGURE 5 | Map showing human pressure (gravity) along the continental and insular coastline. The 187 samples are represented by dots showing those for which elasmobranchs are detected in the filters (blue dots) and those for which only teleost are present (green dots).

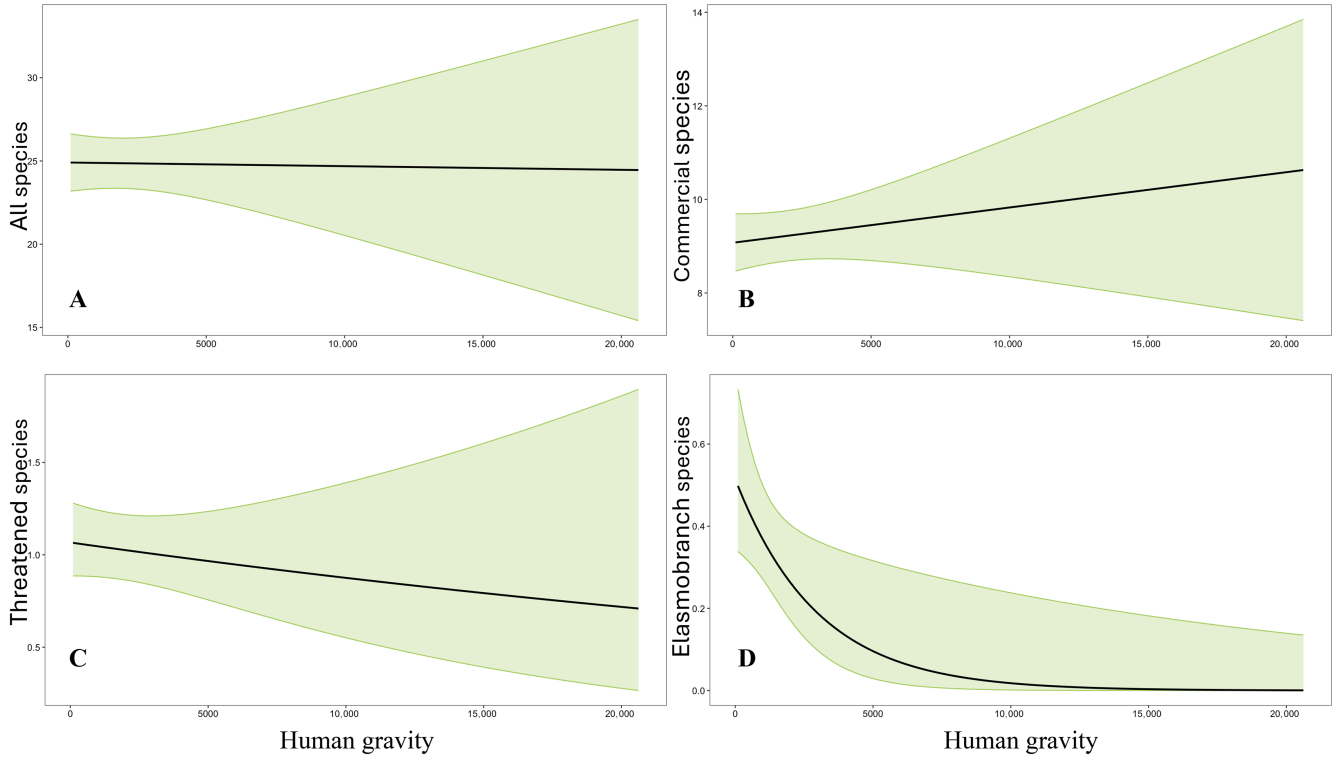

FIGURE 6 | Partial regression plots from the GLMs showing the modelled taxonomic diversity indices (A: Species richness, B: Commercial species, C: Threatened species and D: Elasmobranchs species) as a function of human gravity (population density per square distance to the sampled location) while controlling for all other variables selected in the most parsimonious models (Table 3). Shaded areas represents the 95% confidence intervals.

continent, the accumulation curve was well below the asymptote, indicating the need for a more substantial eDNA sampling effort to detect all species assumed to be present along the continental coastline. For elasmobranchs, the asymptote was still higher on the continent than on islands (24 vs. 21) but the shape of the species accumulation curves differed markedly with a stronger increase in diversity with sampling effort on islands (Figure 8D). We obtained similar patterns

for threatened species (Figure 8C). Concerning commercial species (Figure 8B), if the number of expected species corresponds to the number of detected species for the islands, i.e. we almost reached the asymptote of 19 species with our sampling effort, this is not the case for the continent where we should identify up to 25 species.

4 | Discussion

This study is based on an extensive eDNA sampling along the French mainland coastline and the coastline of Corsica and Balearic Islands in the north-western Mediterranean Sea coupled to a quasi-exhaustive genetic reference database for all fishes (81% of known species of the regional pool including teleostean and elasmobranch fish species). In this survey, we assess the role of Mediterranean islands as a potential refugia for fish species beyond the effect of protection with a focus on elasmobranchs and threatened species on the IUCN Red List.

We reveal that, on average, local fish richness or α-diversity for threatened and elasmobranch species, is higher along the insular than the continental coasts (Figure 4). We are thus able to confirm our initial hypothesis that islands in this highly impacted region constitute a refugia for sharks and rays but also threatened teleost fishes. Low anthropogenic pressure along island coasts appears to be the main correlate of the greater diversity of elasmobranchs (Table 3, Figures 5 and 6). Their life-history traits prevent a high degree of resilience to human activities such as noise pollution, artisanal fishing or pleasure boating (Boussarie et al. 2018; Dwyer et al. 2020), which are closely related to the size of human populations on coast and the time needed to access potential living areas known as human gravity defined by Cinner et al. (2018). In contrast, industrial fishing pressure (Figure 7) has no major impact on most biodiversity metrics, except that of threatened species which can be more dependent on habitat quality and trawling activities while fixed nets also impact elasmobranchs (Di Lorenzo et al. 2022). This pattern confirms that anthropization and fishing pressure are the primary causes of elasmobranch defaunation (Dulvy et al. 2021).

In our study, we also compare marine reserves (i.e., no-take areas with enforcement) and fished areas along the islands and the French Mediterranean coastline to test whether these areas present different levels of fish biodiversity. Indeed, island or continental reserves, if located in comparable ecosystems, should in theory ensure the maintenance of species richness since the main driver of anthropogenic biodiversity loss is the overexploitation of resources (Jaureguiberry et al. 2022). Assuming that other parameters such as coastal urbanisation, habitat degradation, and pollution associated with human activities also play an important role (Carreño and Lloret 2021; Di Franco et al. 2020), we should observe a significant difference between the islands and the continent, the latter having a higher human population density and thus a more marked anthropogenic pressure embedded in the metric of human gravity (Figure 5). Contrary to such expectations, probability due to their size not being suitable for species with large home range, we found no clear reserve effect on the four species richness metrics and there is no interaction between the reserve and the island effects (Table 2). These results reinforce previous findings showing no protection effects on species

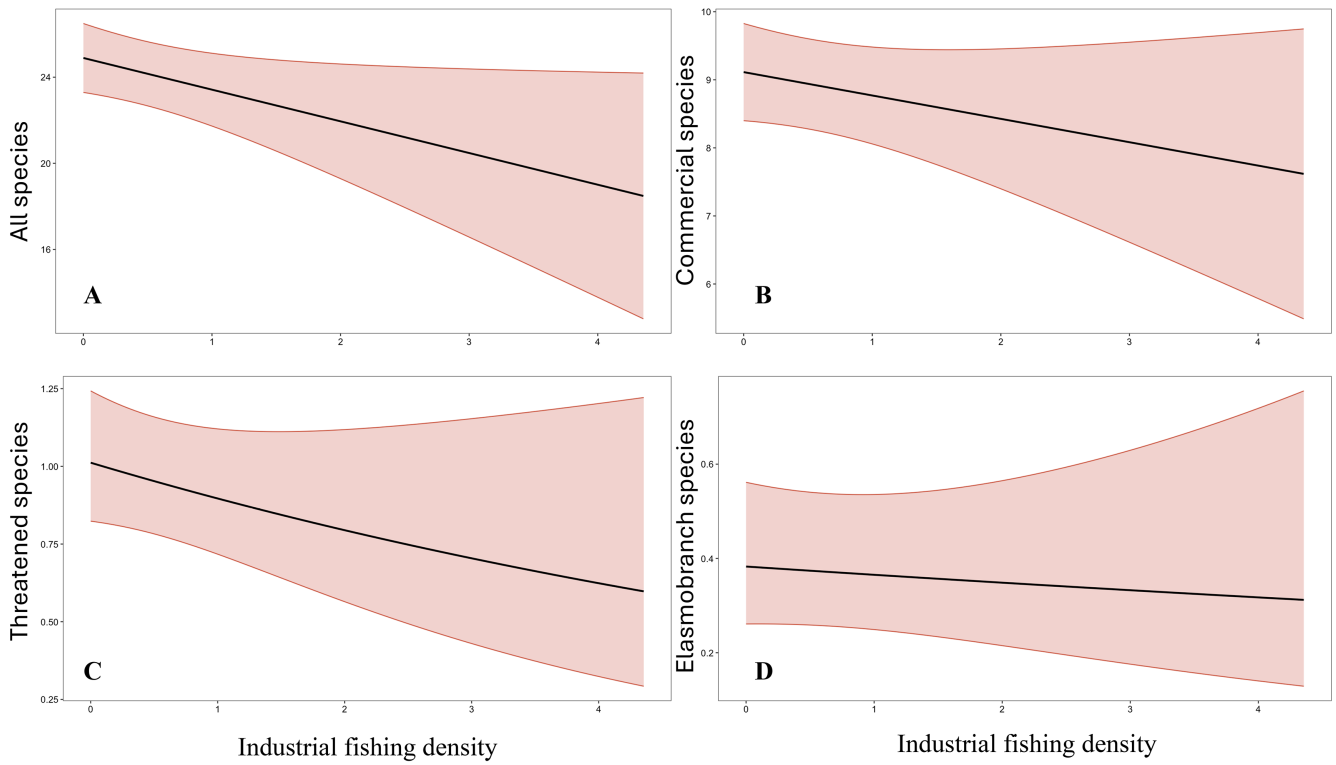

FIGURE 7 | Partial regression plots from the GLMs showing the modelled biodiversity metrics (A: Species richness, B: Commercial species, C: Threatened species and D: Elasmobranchs species) as a function of industrial fishing density while controlling for all other variables selected in the most parsimonious models (Table 3). Shaded areas represent the 95% confidence intervals.

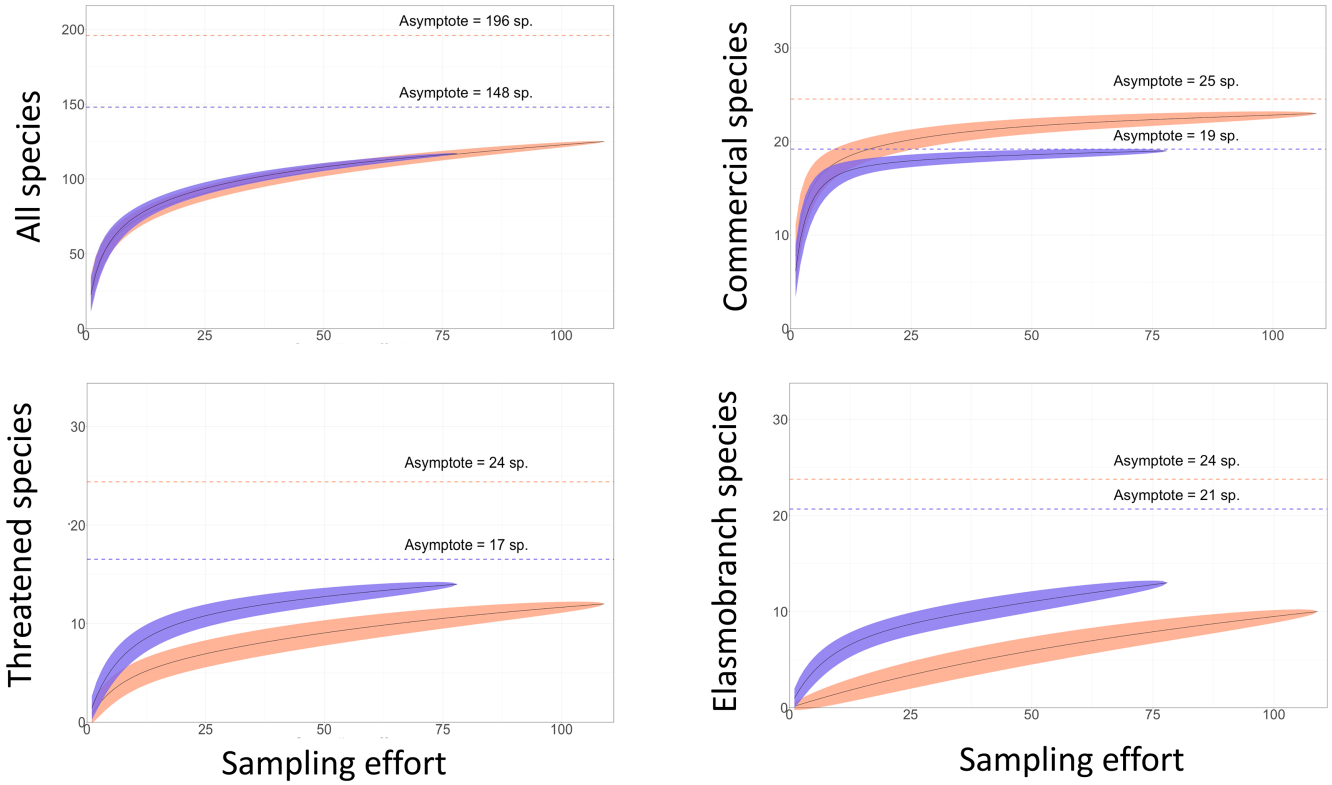

FIGURE 8 | Species accumulation curves of species richness on continental (orange) and island (purple) coasts using eDNA samples for all fish (A), commercial (B), threatened (C) and elasmobranch species (D). Asymptotes were estimated using the Lomolino model.

richness since different species react differently to human vs. protection effects (Sanchez et al. 2024) so, on the balance, species richness is either similar inside and outside protected areas (Loiseau et al. 2021) or sometimes even more important outside than inside (Boulanger et al. 2021). As shown by Sanabria-Fernandez et al. (2019), MPAs efficiency may vary within regions meaning that some of them can be ineffective so compliance measures are necessary. The Global Fish Watch initiative provides an estimate of industrial fishing pressure (Paolo Kroodsma et al. 2024) but in the Mediterranean more precise satellite images would be helpful to quantify artisanal and recreational fishing effort.

Our results also reveal that some species are detected more frequently inside marine reserves. This applies to commercially exploited species such as foraging species with low trophic level (*Sardina pilchardus*, *Boops boops*, *Sardinella aurita*). The presence of these prey species may favour the presence of species with higher trophic levels but also threatened mesopredators like the Ocean sunfish (*Mola mola*) along the continental coast or top predators like the bluefish (*Pomatomus saltatrix*) along the island coastline. The dbRDA shows an island-continent gradient where some species like the dusky grouper (*Ephinephelus marginatus*) are more present in insular locations without any protection (Figure 3C). Regarding the IUCN red list species (Figure 3D), some critically endangered species like the common eagle ray (*Myliobatis aquila*) are more present along the island coastline. At the opposite, certain species are more represented in continental (*Mustelus mustelus*, *Prionace glauca*) and island marine reserves (*Raja brachyura* and *Dasyatis pastinaca*). Other elasmobranchs (*Mobula mobular*, *Myliobatis aquila*) do not take advantage of protection due to their large home range usually greater than the one offered by most Mediterranean no-take reserves. A minimum of 50 km long protected areas is indeed required to significantly reduce annual fishing mortality for reef sharks (Dwyer et al. 2020). To achieve this, we need to provide appropriate protection for top predators such as elasmobranchs, which have an important home range, by avoiding trawling and fishing gears responsible for by-catches over large areas (Di Lorenzo et al. 2022). The Pelagos Sanctuary, an 87,500 km^2 maritime area covered by an agreement between Italy, Monaco and France for the protection of marine mammals (Notarbartolo-di-Sciara et al. 2008), could thus be partially extended to include vulnerable species, species with high commercial value and elasmobranchs. Because of its size, it would allow these species with large home range, to benefit from protection enabling them to be preserved and stocks to be replenished in this heavily depleted region of the world.

Total fish richness, but not elasmobranch and threatened fish richness, is significatively higher in shallow waters (Table 2). A similar result was found for tropical regions where mesophotic reefs can be refugia for fishery-targeted species only (Lindfield et al. 2016). It suggests that these species better persist at greater depth due to lower fishing effort. Even if islands seem to be a refugia for those species, we observe that mesophotic waters could be critical habitats for threatened species avoiding fishing pressure. Protecting such mesophotic waters acting as refugia is even more important for predators since shallow and small marine reserves may have little if no effect on these species (Dwyer et al. 2020; Carvalho et al. 2022).

Regarding γ-diversity, i.e. regional fish diversity, species accumulation curves show an asymptotic richer pool of fishes on the continent than along the island coasts (196 vs. 148 species) even if local species richness is lower on the continent. These opposite patterns can be explained by β-diversity or species turnover which is higher on the continental than island coasts. In other words, heavily anthropized areas host a locally lower species diversity of fishes but a higher species turnover in such disturbed areas. Yet, for elasmobranchs and threatened species, we observe a higher species richness for a lower sampling effort on islands (78 samples) than on the continental coast (109 samples). So, the asymptote could be reached with a lower sampling effort on island than on continental coast. This result could be explained by the higher diversity and area of habitats along the continental coastline than on islands regardless of the species category owing to the island theory of biogeography (MacArthur and Wilson 1967). The difference between the number of detectable species and the number of theoretical species present (asymptote) is much greater along the continental coast than along the insular coasts so a much higher sampling effort is needed on the continent coast to achieve a full species inventory. By analogy to the conservation paradox highlighted by Boulanger et al.,(2021) with more species outside marine reserves than inside, our results suggest a conservation island paradox where islands seem to be a refugia for vulnerable species with a high trophic level while hosting less species than their continental counterparts.

Yet, eDNA metabarcoding presents some limitations. Some detections were assigned at the genus level because they share the same sequence of the 12S mt rRNA fragment targeted by the "Teleo" primers probably due to their recent evolutionary radiation with low genetic divergence like species of the *Raja* genus (Ramírez-Amaro et al. 2018). As our study was carried out at the species level, we therefore discarded three potential species from our results (*Raja clavata*, *Raja asterias*, and *Raja montagui*). The high levels of biodiversity reported in this study along the coasts are therefore underestimated compared to the true levels if all species could be detected. Yet, some fish species are still missing in our genetic reference database (i.e., *Tetronarce nobiliana*) but its increasing coverage should in the near future allow us to refine our biodiversity inventory and its monitoring. To obtain a reliable estimate of biodiversity, we would need to increase the sampling effort to maximise species detection. Since some species with recent evolutionary radiation cannot be detected using this single marker, we may need to use a multi-marker approach to alleviate the limitations of individual markers in terms of resolution (Polanco et al. 2021). Moreover, the possibility of having a mesophotic refugia for fish biodiversity urges to collection of more eDNA samples and species tissues in deeper waters to stimulate the protection of such overlooked and unprotected habitats (Duhamet et al. 2023). Since some studies report the deepening of fish specimens with increasing size or age, including commercially harvested species (Frank et al. 2018), mesophotic habitats and deeper waters must also be protected to ensure the role of potential reservoirs for mature individuals allowing stock replenishment in shallower waters. The most commonly used metric for estimating biodiversity trends is species richness. However, simply measuring the presence of a species in a given location does not provide all the information needed to predict and monitor a biodiversity crisis. To measure biodiversity trends and the collapse of some species, eDNA studies should provide abundance estimations. To do this, we can use read numbers, but they cannot be considered as reliable proxies of species abundance in the sea (Rourke et al. 2022; Sanchez et al. 2022) but rather a proxy for the abundance of certain species (Liu et al. 2022; Mariani et al. 2021). As an alternative, we could also use longer sequences of mtDNA to obtain eDNA haplotypes that can provide information about intraspecific diversity and population size (Dugal et al. 2022). With these two measures (specific diversity and abundance), eDNA could then be a very powerful tool for estimating and monitoring biodiversity in many of its dimensions (Marques et al. 2021a), particularly in no-take MPAs where restrictions may only allow the use of non-invasive and non-destructive methods like eDNA metabarcoding (McGeady et al. 2023), potentially coupled with visual or video surveys.

In terms of conservation, the protection, extension and reinforcement of marine reserves on islands would be recommended so that these last refuges of the western Mediterranean Sea can play their role of seeding for the other areas with more depleted populations. A less restrictive alternative to extending large areas of protection would be to require longline fishermen to use hooks that limit by-catches. Indeed, we already know that hooks equipped with different types of magnets (neodymium, iron, boron or barium-ferrite) have the property to catch fewer elasmobranchs compared to control fishing hooks (O'Connell et al. 2011). The presence of these higher trophic level species enables them to play their important role in trophic cascades and ecosystem functioning (Natsukawa and Sergio 2022; Hammerschlag et al. 2019). In a global context where mankind's impact on ecosystems is increasingly marked, maintaining such functional diversity along highly anthropized coasts is essential to face up to present and future threats in terms of overexploitation, habitat degradation and global warming.

Author Contributions

David Mouillot was responsible for coordinating the field logistics and data collection. MARBEC was responsible for digitising the data and Franck Pichot performs data analysis and statistics. All authors drafted the manuscript. Franck Pichot prepared all figures for publication. All authors revised and edited the manuscript. David Mouillot, Julie Deter and Franck Pichot obtained the funding for this study.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The datasets *generated during* and/or *analysed during* the current *study* are *available in* the dryad *repository* [https://doi.org/10.5061/dryad.qbzkh18qh]. https://datadryad.org/stash/share/NZjwbAD_4yd_pVsBoPExpTUcO9AC4FtWZ2CQq196XGk

Peer Review

The peer review history for this article is available at https://www.webofscience.com/api/gateway/wos/peer-review/10.1111/ddi.13937.

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