

## RESEARCH ARTICLE

# Benchmarking eleven biodiversity indicators based on environmental DNA surveys: More diverse functional traits and evolutionary lineages inside marine reserves

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**Handling Editor:** Rucha Karkarey**Abstract**

1. To mitigate the ongoing threats to coastal ecosystems, and the biodiversity erosion they are causing, marine-protected areas (MPAs) have emerged as powerful and widespread conservation tools. Strictly no-take MPAs, also called marine reserves, undeniably promote fish biomass and density, but it remains unclear how biodiversity responds to protection. Identifying which facets of biodiversity respond to protection is critical for the management of MPAs and the development of relevant conservation strategies towards the achievement of biodiversity targets.
2. We collected 99 environmental DNA (eDNA) samples inside and outside nine marine reserves in the Mediterranean Sea to assess the effect of protection on 11 biodiversity indicators based on fish traits, phylogeny and vulnerability to fishing. We controlled for the effect of environmental heterogeneity (habitat, bathymetry, productivity, temperature and accessibility) using a principal component analysis, and for spatial autocorrelation due to potential unmeasured factors.
3. We found a positive and significant effect of protection on only 3 out of 11 indicators: functional and phylogenetic diversity but also the ratio between demersal and benthic species richness. Rather, total fish richness responded significantly and negatively to protection. We did not detect any significant effect of protection on threatened and elasmobranch species richness, probably due to their large home range compared to the size of Mediterranean marine reserves.

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4. *Synthesis and applications*: Our findings highlight the importance of looking beyond the mere number of species to fully depict and understand the effect of marine reserves on biodiversity and evaluate the effectiveness of conservation measures. Rather, we propose a dashboard of three eDNA-based indicators that can provide an early signal of ecosystem deterioration or recovery. eDNA metabarcoding offers a powerful tool to supply site-specific and standardized taxonomic-, phylogenetic- and trait-based biodiversity assessments, in complement to other classical techniques, such as visual censuses or video surveys, able to estimate species abundance but also individual life-stage and size.

#### KEYWORDS

bioindicator, crypto-benthic fish, elasmobranch, environmental DNA metabarcoding, marine protected areas, Mediterranean Sea, monitoring

## 1 | INTRODUCTION

Marine biodiversity is under major threats worldwide due to increasing habitat degradation, overexploitation of resources, biological invasions and climate change (e.g. Yan et al., 2021). Marine-protected areas (MPAs) are the main conservation tools to curb the multiple threats on species and ecosystems, particularly no-take MPAs prohibiting fishing activities, also called marine reserves (Costello & Ballantine, 2015) or fully protected areas (Grorud-Colvert et al., 2021).

Generally, marine reserves host larger and more abundant fish than nearby fished areas (Grorud-Colvert et al., 2021; Marcos et al., 2021), and promote the demographic recovery of endangered or overexploited species (Giakoumi et al., 2017; Waterhouse et al., 2020). Comparatively, few studies have investigated or reported whether biodiversity—and which of its components—responds positively to protection. Sanabria-Fernandez et al. (2019) show that MPAs are more effective in protecting fish biomass than diversity, especially for threatened species, while Villamor and Becerro (2012) reveal a higher functional diversity of fishes and invertebrates inside than outside MPAs. Species that are particularly sensitive to human pressures—such as fisheries targets, apex predators and large piscivores, but also threatened species and those exhibiting slow life histories with long life spans, late sexual maturity and low fecundity rates (e.g. sharks and rays)—are expected to respond positively to protection (Cinner et al., 2018; Claudet et al., 2010). However, highly mobile taxa, such as sharks and pelagic fishes, are less likely to respond to protection since reserve areas are much smaller than their home range (Dwyer et al., 2020; Juhel et al., 2018). Besides, several studies have shown marginal or no difference in species richness between MPAs and nearby unprotected areas (Giakoumi et al., 2017; Loiseau et al., 2021; Soykan & Lewison, 2015), and a recent study even suggests that a higher species richness can be detected outside than inside MPAs (Boulanger et al., 2021). This rather counter-intuitive pattern might be explained by a higher species turnover in frequently disturbed areas (Dornelas

et al., 2019) or by trophic cascades limiting the diversity of low-trophic species (crypto-benthic fishes) under higher predation pressure inside marine reserves (Boulanger et al., 2021).

This lack of consensus about protection effect on biodiversity is partly due to the inherent difficulty to exhaustively detect species in a vast water volume with diverse, and sometimes hidden, habitats (e.g. holes in shallow rocky reefs) and the cryptic or elusive behaviour of some fishes (Boussarie et al., 2018; Brandl et al., 2018). Environmental DNA (eDNA) metabarcoding has proven to offer more accurate and wider biodiversity assessments than classical census methods, particularly for reef fishes (Aglieri et al., 2021; Polanco Fernández et al., 2021; Sigsgaard et al., 2020), and to provide a very local signal (Gold et al., 2021; Jensen et al., 2022; Miya, 2022). This non-invasive method is based on retrieving DNA naturally released by organisms in their environment, then amplifying and sequencing a specific marker to identify corresponding species (Miya, 2022). eDNA metabarcoding then provides a list of molecular operational taxonomic units, whose sequences can then be assigned to species or taxa thanks to a genetic reference database (e.g. Marques et al., 2021), to ultimately produce a taxonomic composition for each sampled site. Yet, beyond the mere taxonomic richness, many other biodiversity indicators or metrics can be relevant to monitor ecosystems under degradation or restoration (D'agata et al., 2014; Smit et al., 2021; Soykan & Lewison, 2015), but remain to be tested using eDNA surveys.

The Mediterranean sea is a hotspot for both biodiversity and human impacts (Micheli et al., 2013), so offers an appropriate context to test the effect of MPA on fish biodiversity. Although 6% of its surface is covered by MPAs, only 0.06% is fully protected by marine reserves (Claudet et al., 2020). In this study, we took advantage of 99 eDNA samples within and outside nine Mediterranean marine reserves, partly published in Boulanger et al. (2021), and a well-completed genetic reference database (75% coverage of the regional fish species pool), to test 11 biodiversity indicators based on fish traits, phylogeny and vulnerability, while controlling for habitat and environmental heterogeneity.

## 2 | MATERIALS AND METHODS

### 2.1 | Study area and sampling

Our study focused on the strict no-take zone of nine no-take marine reserves located in the north-western Mediterranean Sea (Figure 1). These reserves were established at least 6 years before sampling and cover an area between 0.65 and 10.7 km<sup>2</sup> (see Table S2 in Supporting Information).

Sampling was conducted in June and July 2018, 2019 and 2020 (Table S2). For each marine reserve, we sampled two or three sites: one inside the reserve (i.e. within the strict boundaries of the no-take or fully protected area) and one or two in fished areas from 5 to 10 km away from the reserve boundaries (Figure 1 and Table S2). Some samples outside marine reserves were taken in a partially protected area with very little restrictions on fishing. Such weakly protected areas have been shown to provide little if no ecological benefit (Grorud-Colvert et al., 2021; Turnbull et al., 2021), in particular for Mediterranean fish (Giakoumi et al., 2017; Zupan et al., 2018). We thus considered all areas outside marine reserves as fished.

We sampled four replicates within each site in shallow waters (5–15 m) with standardized conditions in terms of habitat, depth and distance from the coastline. Each sample consists of 30 L of seawater filtered in 30 min along a 2-km transect from a boat navigating parallel to the coastline. When the reserve was smaller than 2 km wide the boat did shorter back-and-forth transects to strictly filter inside the boundaries. We collected seawater 1 m below the sea surface using a sterile tube and a peristaltic pump and filtered through a VigiDNA 0.2 µm cross-flow filtration capsule. Immediately after filtration, the capsule was emptied from the remaining water and filled with 80 ml of CL1 Conservation buffer and stored at room temperature until extraction.

### 2.2 | eDNA extraction, sequencing and analyses

eDNA extraction was performed in a dedicated room for water DNA sample extraction, equipped with positive air pressure, UV

treatment and frequent air renewal, with decontamination procedures conducted before and after each extraction (Polanco Fernández et al., 2021).

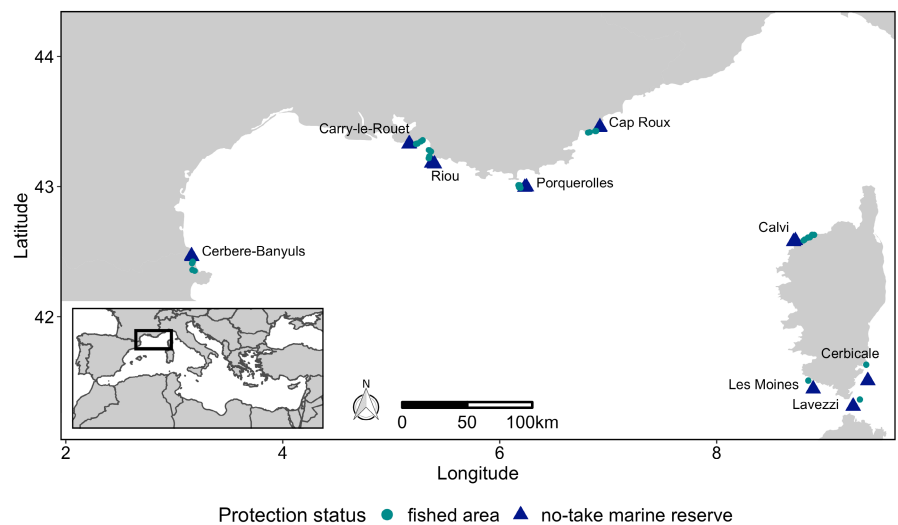
We carried out PCR amplification using the primer pair *teleo*, targeting a 64 bp fragment of the mitochondrial DNA 12S rRNA gene, specific to teleost fishes and elasmobranchs (Valentini et al., 2016). This *teleo* marker was shown efficient to detect fishes owing to its high interspecific variability and its short size so low degradation rate (Collins et al., 2019; Zhang et al., 2020).

The PCR mixture was denatured at 95°C for 10 min, followed by 50 cycles of 30 s at 95°C, 30 s at 55°C and 1 min at 72°C, and a final elongation step at 72°C for 7 min. We ran 12 replicate PCRs per sample, and prepared nine libraries using the MetaFast protocol. We used the MiSeq Flow Cell Kit Version3 to perform paired-end sequencing (2 × 125 bp).

Paired-end sequencing outputs were handled using the ObiTools toolkit (Boyer et al., 2016). First, reads were merged using *illumina-paired-end*, trimmed and demultiplexed using *ngs-filter*, dereplicated using *obiuniq*, and reads identified as errors were discarded using *obiclean* with default settings. We discarded observations with less than 10 reads and accounted for tag-jumps and index-bleeding (Marques et al., 2021). Taxonomic assignments were performed using *ecotag* on a combination of publicly available sequences from ENA (downloaded in June 2021) and of our Mediterranean database, comprising 386 sequences from 156 species. See Appendix S1 in Supporting Information for more details.

### 2.3 | Reserve effect on fish biodiversity indicators

For each eDNA sample, we calculated 11 biodiversity indicators only based on species occurrences (i.e. non-quantitative) since eDNA metabarcoding does not provide reliable estimates of fish abundance in our Mediterranean system (Sanchez et al., 2022). The description, calculation and hypothesis behind each indicator are detailed in Table 1. For all analyses, the indicators based on species number



**FIGURE 1** North-Western Mediterranean map indicating sites where environmental DNA (eDNA) samples were collected outside (dots) and inside (triangles) nine marine reserves (i.e. no-takes MPAs).

**TABLE 1** Description and rationale for the 11 biodiversity indicators calculated inside and outside of the nine reserves. Mean value ( $\pm$  standard deviation) across all 99 samples are given for each indicator. Species traits and information used to calculate the indicators were extracted from the FishMed database (Albouy et al., 2015) and FishBase (Froese & Pauly, 2010)

Indicator	Data source	Calculation	R package version	Expected protection effect	Hypotheses	Mean ( $\pm$ SD)	Range	Reference
Species richness (R)		Total number of species		Negative	Higher R outside protected areas due to higher diversity of lower trophic level species	23.0 ( $\pm$ 13.4)	1–54	Boulanger et al. (2021) and Loiseau et al. (2021)
Functional diversity (FD)	FishMed	Number of functional groups. Fish functional groups were created using species traits and the application of 'clustering by fast search and find of density peaks' algorithm based on the Gower distance between species pairs (Moullot et al., 2021)		None or positive	Human impacts affect species sharing similar traits. Reserves could restore functional space	8.45 ( $\pm$ 3.3)	1–16	Cinner et al. (2020) and D'agata et al. (2014)
Phylogenetic diversity (PD)	Fish Tree of Life (Rabosky et al., 2018)	Sum of the total phylogenetic branch length for teleost species in a sample (Faith, 1992)	fishtree 0.3.4 (Chang et al., 2019) pd function in picante 1.8.2 (Kembel et al., 2010)	None or positive	PD can capture unmeasured functional diversity	11.1 ( $\pm$ 4.8)	1.5–21.8	D'agata et al. (2014)
Large fish index (LFI)	FishMed	Number of species with an average body length >20 cm ( <a href="http://www.reeflifesurvey.com/indicators/">www.reeflifesurvey.com/indicators/</a> )		None or positive	Exploitation targets large fishes. But reserve effect is expected on abundance and biomass rather than large species richness	16.9 ( $\pm$ 9.3)	0–40	Cinner et al. (2018) and Juhel et al. (2018)
Crypto-benthic richness (Crypto)	FishMed	Benthic families having >10% of species with average adult size <5 cm (listed in Brandl et al., 2018)		Negative	Higher diversity of small sized species due to reduced predation outside reserve	5.9 ( $\pm$ 3.6)	0–13	Boulanger et al. (2021) and Brandl et al. (2018)
Ratio dermo-pelagic/benthic species (DeBRa)	FishMed	Number of demersal and pelagic species/ (Number of benthic species+1)		Positive		0.84 ( $\pm$ 0.6)	0–6	
IUCN Red List species richness (RedList)	Red List of Threatened Species (IUCN, 2021)	Weighted sum of Red Listed species with weights VU = 1, EN = 2, CR = 3	rredlist 0.7.0 (Chamberlain & Salmon, 2020)	Positive	Reserves offer protection to vulnerable species	0.86 ( $\pm$ 1.4)	0–7	Loiseau et al. (2021)
Chondrichthyen species richness (Chondri)		Number of shark and ray species		None	Chondrichthyen species are highly mobile and unlikely to stay inside reserves	0.49 ( $\pm$ 0.9)	0–4	Dwyer et al. (2020)
Vulnerability (Vulner)	FishBase	Mean of all species sensitivity to exploitation calculated from 8 life-history traits	rfishbase 3.1.9 (Boettiger et al., 2012)	Positive	Sensitive species are expected to respond more strongly to protection	44.8 ( $\pm$ 6.1)	10.0–69.1	Cheung et al. (2005)
Commercial species richness (Commercial)	FishMed	Number of species with a commercial interest		None or positive	Commercial species are likely present inside and outside reserves, hence protection is expected to affect their biomass and abundance but not necessarily the number of commercial species	15.8 ( $\pm$ 8.7)	0–36	Giakoumi et al. (2017) and Yan et al. (2021)
Highly commercial species richness (HighL_commerc)	FishMed	Number of species of high commercial value (targeted by commercial fisheries)		None or positive		4.4 ( $\pm$ 2.6)	0–11	

(FD, PD, LFI, Crypto, RedList, Chondri, Commercial and High\_commerc) were expressed as a proportion of the total species richness in the sample to avoid redundant information.

We then used generalized linear models (GLMs) to test the effect of protection on each of the 11 indicators while accounting for habitat and environmental heterogeneity between sites. Distribution of the indicators was checked using histograms (Figure S1). Accordingly, we modelled the proportion of Red-Listed species and the proportion of chondrichthyans using a quasi-Poisson distribution, best suited for zero-inflated distributions, whereas all other indicators were modelled using a Gaussian distribution.

We used a total of nine variables to account for habitat and environmental variability between samples (see Appendix S1 and Table S1). We performed a principal component analysis (Figures S2–S4) on the eight habitat and environmental variables to reduce the dimensionality of the dataset and build models with independent variables. We used the first four principal components which explained 80% of the variance between samples (Figure S2) as explanatory variables in our GLMs. To account for spatial autocorrelation due to potentially overlooked factors, we estimated a spatial autocovariate on each initial GLM residuals, using the function *autocov\_dist* of package *SPDEP* (Bivand & Wong, 2018), and included it as an additional predictor in the final models.

We calculated the  $R^2$  for each model to determine their accuracy, and we estimated 'partial effects' of the *protection* variable (i.e. its contribution conditional to habitat and environmental variables)

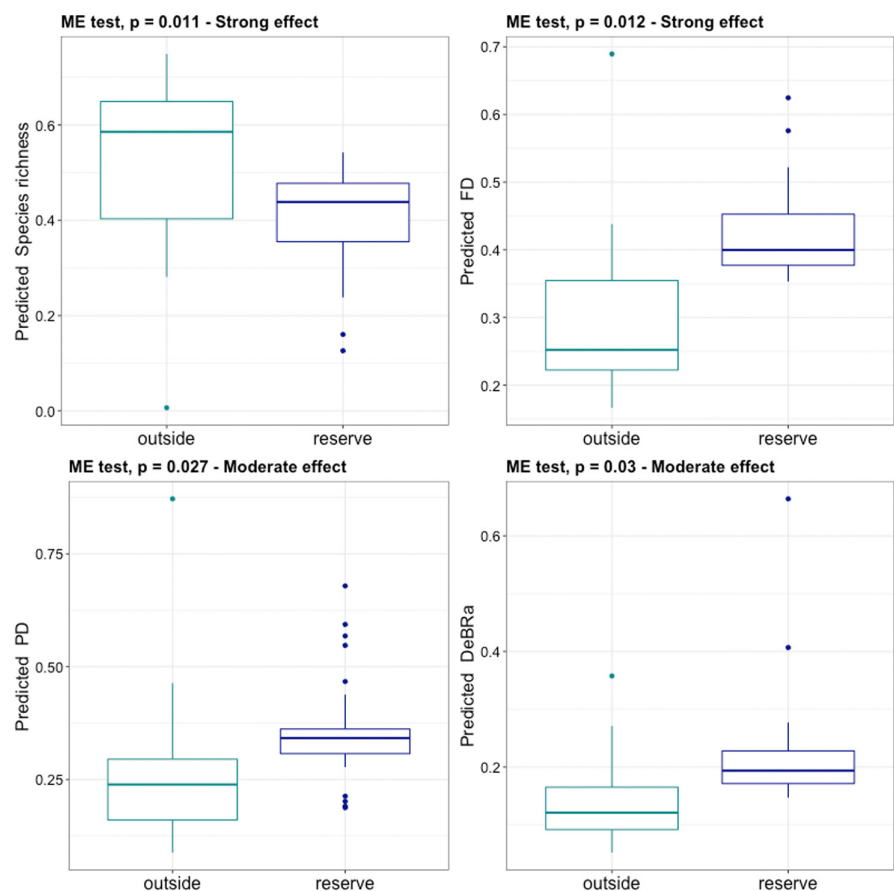
using the *margin* function of the R package *MARGINS* version 0.3.26 (Leeper, 2021). The conditional predicted values of the indicators inside vs. outside reserves and the test of marginal effects are given in Figure 2.

Finally, we tested spatial autocorrelation in the residuals of our models using a Moran's  $I$  test. To test the potential effect of the unbalanced sampling (more samples outside than inside reserves), we randomly selected four samples outside reserve for each region and ran the same analyses. We tested the effect of reserve age and size by replicating the analyses while replacing the 'protection' explanatory variable with age and size, and only considering the samples taken inside the reserves (31 samples).

### 3 | RESULTS

#### 3.1 | Detected sequences and fish species

We recovered a total of 53,765,575 sequences after bioinformatic filtering, with an average of 548,628 sequences per eDNA sample (min = 1178; max = 1178,732;  $SD = 274,660$ ). After taxonomic assignment, a total of 113 fish species covering 84 genera and 46 families were identified (Table S4), with an average taxonomic richness of 23 ( $\pm 13$ ) species per sample (Table 1). The most represented families were Gobiidae (15 species) and Labridae (14 species), whereas 28 families were represented by a single species.



**FIGURE 2** Results of the generalized linear models (GLMs) testing the effect of protection on the biodiversity indicators while accounting for habitat and environmental heterogeneity but also spatial autocorrelation. Boxplots represent the conditional predicted values of the indicators inside vs. outside reserves, with the test of marginal effects (ME  $p$ -values). Only the indicators showing a significant strong to moderate response (Muff et al., 2021) to protection are represented (four indicators). The other seven indicators are presented in Figure S6.

The most frequent species were the damselfish (*Chromis chromis*; detected in 95% of samples), the saddled seabream (*Oblada melanura*; 89% of samples) and the bogue (*Boops boops*; 86% of samples), whereas some rare species such as the grey triggerfish (*Balistes capricus*), the velvet belly lanternshark (*Etmopterus spinax*) or the short-snouted seahorse (*Hippocampus hippocampus*) were detected in only one sample.

Nine species were exclusively detected inside a reserve while 27 species were exclusively recorded outside (Figure S5, Table S4). Among the 27 species exclusively found in fished sites, 26% (7 species) were cryptobenthic fishes. In contrast, pelagic species represented 44% (4 species) of the species identified only inside reserves.

Two elusive shark species were detected outside reserves: the smooth-hound shark *Mustelus mustelus* and the velvet belly lanternshark *Etmopterus spinax*, classified, respectively, 'Endangered' and 'Vulnerable' on the IUCN Red List of Threatened Species. The 'Critically Endangered' eagle ray *Myliobatis aquila* and the 'Vulnerable' brown stingray *Bathytoshia lata* were also only detected outside reserves (Figure S5, Table S4).

### 3.2 | Effect of protection on biodiversity indicators

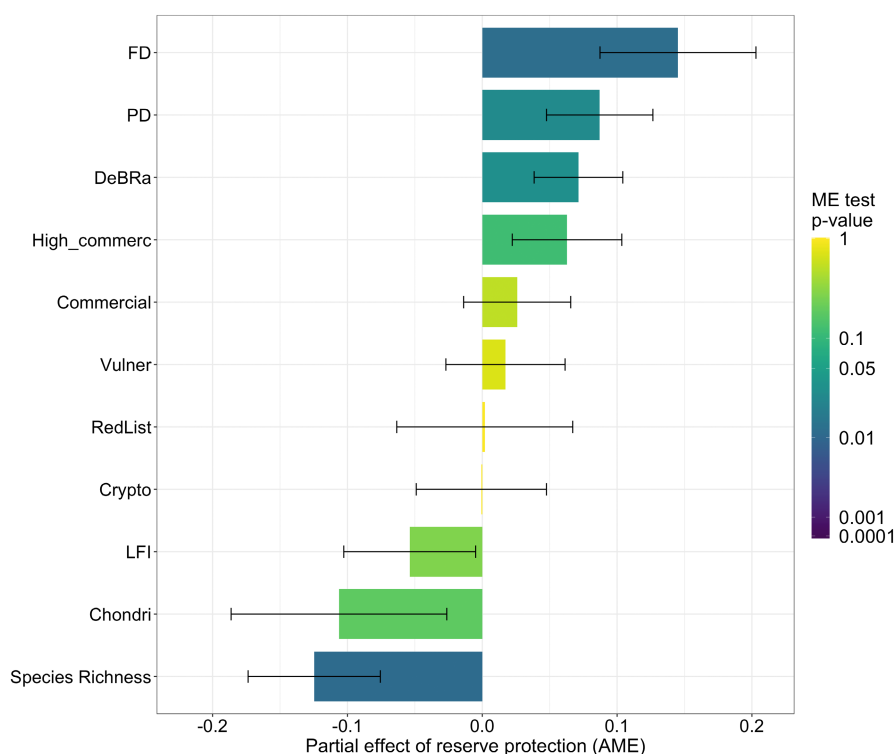
The mean, minimal and maximal values of each biodiversity indicator across all samples are provided in Table 1. The adjusted  $R^2$  of the final models ranged from 0.07 (LFI) to 0.40 (PD) with an average of 0.25. Our results showed a significant positive effect of protection on only three out of 11 biodiversity indicators after accounting for habitat and environmental variables but also

spatial autocorrelation (Figure 2): functional diversity *FD* (i.e. the proportion of different functional groups within a sample), phylogenetic diversity *PD* and the ratio of demersal-pelagic on benthic species richness. Our models revealed a significant 15% increase in *FD* and 9% increase in *PD* inside reserves compared to outside (Figure 3, Table S5). The *DeBRa* ratio increased by 7% with protection (Figure 3, Table S5), hence indicating a larger proportion of demersal and pelagic species inside reserves, and a relatively larger proportion of benthic species in fished areas. On the other hand, total species richness significantly decreased from an average of 30 ( $\pm 11$ ) species per sample in fished areas to 23 ( $\pm 11$ ) species inside marine reserves (Figure 2). We did not detect any significant effect of protection on the other seven biodiversity indicators (Figures S3–S6).

Across all final models, we did not detect any significant spatial autocorrelation in the residuals with Moran's *I* ranging from 0.00 to 0.27 and *p*-value ranging from 0.05 to 0.88 (Figure S7). The results obtained with the balanced dataset (i.e. with the same number of samples inside and outside reserves) were similar to the results found using the full dataset. We did not find any significant effect of reserve size or age on any of the indicator (Figures S8 and S9), except on the Crypto indicator for which reserve age had a slightly positive effect (average marginal effect *ME* = 0.004; *p*-value = 0.018).

## 4 | DISCUSSION

Here, we used eDNA surveys in nine marine reserves of the north-western Mediterranean Sea to assess the effect of protection on



**FIGURE 3** Partial effect of protection on biodiversity indicators from generalized linear models (GLMs). The coefficient ( $\pm$  confidence intervals at 95% level) is the average marginal effect (AME) of protection on each indicator, indicating its partial effect while accounting for the effect of other covariates (i.e. principal component analysis [PCA] axes and spatial autocorrelation). The colour gradient represents the strength of the evidence (*p*-value from the marginal effect test), following Muff et al. (2021).

various components of fish biodiversity including their traits, evolutionary history, commercial importance and vulnerability to exploitation, while controlling for habitat and environmental heterogeneity but also spatial autocorrelation. We show that only 3 out of 11 biodiversity indicators respond positively and significantly to protection. We also confirm our initial hypothesis and recent findings (Boulanger et al., 2021; Loiseau et al., 2021) that fish species richness can be higher outside than inside marine reserves, this pattern being driven by the diversity of cryptobenthic fishes notoriously missed by classical assessments (Brandl et al., 2018). Because of their high diversity, high fecundity and rapid turnover, we can assume that cryptobenthic communities can re-shuffle more rapidly than their demersal and pelagic counterparts in response to predation and human pressure. In other words, cryptobenthic fishes could be considered as 'pioneer-species' in coastal ecosystems, that is, hardy species establishing themselves in a disturbed ecosystem and triggering ecological succession (Swaine & Whitmore, 1988).

Our results reiterate that species richness cannot provide a relevant conservation target, as community composition differs among levels of protection. The diversity of large mobile species, such as sharks and rays, is unaffected by protection. This rather concerning result may be explained by the small size and shallow depth of no-take Mediterranean reserves, in our case between 0.65 km<sup>2</sup> (Cerbère-Banyuls) and 10.74 km<sup>2</sup> (Calvi), compared to the home range of most predator or elasmobranch species (Dwyer et al., 2020; Juhel et al., 2018). This relatively limited area covered by our marine reserves may also explain why reserve size has no effect on most our biodiversity indicators.

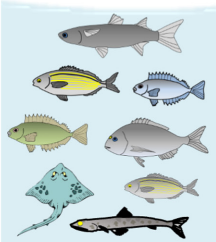



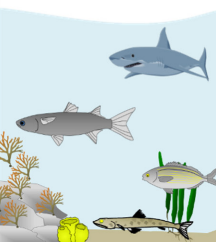










We observed a higher functional and phylogenetic diversity within reserves (Figures 2 and 3), which corroborates the findings of other studies using other assessment methods (Sanabria-Fernandez et al., 2019; Villamor & Becerro, 2012). Our findings confirm that eDNA offers a powerful tool to capture the breadth of functional and phylogenetic composition, since the method can detect rare, elusive and highly mobile taxa likely to exhibit distinct traits and lineages (Aglieri et al., 2021; Marques et al., 2021). The higher functional and phylogenetic diversity may, in turn, translate into wider functional roles played by fishes under protection, likely offering benefits for ecosystem functioning such as resilience or productivity (Tredennick et al., 2017).

We also found a higher, albeit not significantly, diversity of exploited fishes and species of high commercial value (i.e. targeted by industrial and small-scale fisheries) within reserves (Figures S3–S6). The biomass and abundance of commercial taxa are known to be higher inside than outside MPAs since they are particularly sensitive to fishing pressure and logically respond stronger and quicker to protection (Blowes et al., 2020; Giakoumi et al., 2017; Loiseau et al., 2021). However, since our eDNA-based commercial indicators are non-quantitative (i.e. based on species occurrences), they are not expected to display much variation between protection statuses. The *Commercial* indicator would be higher inside MPAs if some commercial species were completely extirpated from fished

areas, which is extremely unlikely, or if their higher abundance within MPAs increased their detectability due to increased eDNA shedding, which is more likely. Last, and contrary to expectations, we did not detect any reserve effect on the 'sensitivity to fishing' (i.e. vulnerability) indicator or on the Large Fish Indicator. This rather puzzling result may indicate that protection acts on the biomass and density of sensitive species (Cinner et al., 2018; Juhel et al., 2018), but that residual populations or early life stages, often undetectable by classical surveys, may still occur in fished areas. As an alternative hypothesis, species sensitive to fishing may take refugia in deeper areas (Frank et al., 2018), like mesophotic reefs, hence could not be detected by our coastal surface sampling in both marine reserves and fished areas.

We highlight the importance of looking beyond the number of species to fully depict and better understand reserve effect on biodiversity. From a monitoring perspective, assessing taxonomic or even vulnerable species richness is not enough and can be irrelevant to evaluate the status of ecosystems or the effectiveness of conservation measures. Rather, we propose a set of three eDNA-based indicators, independent of species richness, that can provide early signals of ecosystem deterioration or recovery (Figure 4). Soykan and Lewison (2015) proposed a set of community-level metrics to monitor the effect of MPAs on biodiversity and found that biomass-based metrics responded more consistently to protection than abundance-based metrics. Since eDNA does not yet allow accurate biomass quantification in the Mediterranean system (Sanchez et al., 2022), other occurrence-based indicators were to be found. We suggest that the monitoring of MPAs and fisheries may rely on complementary monitoring techniques, including eDNA, like underwater visual censuses (UVCs), baited remote underwater videos (BRUVs) or bioacoustics (Marques et al., 2021; Sigsgaard et al., 2020; Valdivia-Carrillo et al., 2021) able to provide complementary indicators (size-based, abundance-based or occurrence-based). Each of these methods has its pros and cons, hence developing ecological indicators incorporating various sources of information might be the best way to fully depict the complexity of ecological communities. For example, Aglieri et al. (2021) compared four monitoring methods (visual census, underwater video, fishery catches and eDNA) and showed that eDNA is the best suited to capture the functional diversity of coastal fish communities, due to its lack of selectivity towards a specific trait. More generally, eDNA offers reliable and broad species inventories detecting small, cryptic, nocturnal, rare and elusive species that are often missed or not-targeted by conventional methods (Gold et al., 2021; McElroy et al., 2020; Miya, 2022). On the other hand, UVCs or BRUVs are compulsory to assess the abundance and size distribution of focal taxa, such as commercial species or conservation targets. Hence, we advocate the use eDNA metabarcoding to complement other survey techniques, to expand monitoring activities across space (e.g. deep sea or areas where diving is unsafe) and time (e.g. increased monitoring frequency) to improve the overall biodiversity assessment.

As any monitoring tool, eDNA-based inventories necessarily involve limitations including false positives and false negatives,

	Indicator	Protection effect
<b>Fish biodiversity</b> 	<b>Species richness</b>	
	<b>Functional diversity</b>	
	<b>Phylogenetic diversity</b>	
<b>Fish ecology</b> 	<b>Large fish index</b>	
	<b>Crypto-benthic richness</b>	
	Ratio $\frac{\text{demerso-pelagic}}{\text{benthic}}$ Species	
<b>Fishery targets</b> 	<b>Vulnerability</b>	
	<b>Commercial species richness</b>	
	<b>Highly commercial species richness</b>	
<b>Rare species</b> 	<b>IUCN Red List species richness</b>	
	<b>Chondrichthyan species richness</b>	

**FIGURE 4** Summary of the response of biodiversity indicators to reserve effect based on generalized linear models (GLMs) while accounting for habitat and environmental heterogeneity but also spatial autocorrelation. Green arrows pointing up indicate a significant positive effect (e.g. more functional fish diversity inside reserves than outside). The red transparent arrow pointing down indicates a significant negative effect. The blue, horizontal arrows indicate equal indicator values inside and outside reserves. Icons were drawn by Arthur Escalas or downloaded from [clipart-library.com](https://clipart-library.com).

resulting from differences in eDNA shedding and PCR amplification among taxa (Kelly et al., 2019), and heterogeneous decay among systems and taxa that may affect species detectability. These limitations need to be recognized when interpreting eDNA metabarcoding results, and should be overcome by future research and technical developments (Miya, 2022). For instance, knowing the hydro-geomorphological features of a river network allowed the reconstruction of upstream distribution and abundance of freshwater species using eDNA (Carraro et al., 2018). More recently, Cantera et al. (2022) used eDNA and measurements of anthropic disturbance to show the spatial extent of deforestation impact on vertebrate biodiversity in Amazonia. Such assessments remain challenging in the marine ecosystem, but accounting for seascape connectivity may contribute to identify source-sink dynamics in eDNA passive drift, and better predict biodiversity patterns including species abundances.

Associated with relevant biodiversity indicators, eDNA metabarcoding offers a powerful non-invasive and cost-effective tool

for long-term biodiversity monitoring by providing standardized assessments in space and time that do not rely on taxonomic expertise. As we enter the United Nations Decade of Ocean Science for Sustainable Development and the Post-2020 Biodiversity Framework, collecting such knowledge is crucial to fully depict biological communities and to evaluate the progress made towards international conservation targets.

#### AUTHOR CONTRIBUTIONS

Alicia Dalongeville and David Mouillot designed the study. Emilie Boulanger, Julie Deter, Philippe Lenfant, Franck Pichot, Loic Sanchez, Florian Holon, Eric Charbonnel, Virginie Hartmann and Lola Romant conducted the fieldwork. Alice Valentini, Tristan Milhau, Stéphanie Manel, Veronique Arnal and Tony Dejean supervised the biomolecular analyses and Virginie Marques performed the bioinformatic analyses. Julie Deter, Florian Holon, Laure Velez, Thomas Bockel and Gwenaëlle Delaruelle collected the environmental data. Alicia Dalongeville, Emilie Boulanger and Lola Romant performed the



statistical analyses. Alicia Dalongeville wrote the first draft of the paper. Emilie Boulanger, Virginie Marques, Eric Charbonnel, Virginie Hartmann, Marie Catherine Santoni, Julie Deter, Alice Valentini, Philippe Lenfant, Pierre Boissery, Tony Dejean, Laure Velez, Stéphanie Manel and David Mouillot interpreted the results and corrected the paper. All authors approved the final version of the manuscript.

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## CONFLICT OF INTEREST

The authors declare no conflicts of interest.

## DATA AVAILABILITY STATEMENT

The bioinformatic pipeline is freely available in Gitlab at the following link: [https://gitlab.mbb.univ-montp2.fr/edna/snakeyaml\\_rapid\\_run\\_obitools](https://gitlab.mbb.univ-montp2.fr/edna/snakeyaml_rapid_run_obitools). All data and code are available in a GitHub repository at the following link: [https://github.com/AliciaDalongeville/eDNA\\_indicators\\_med.git](https://github.com/AliciaDalongeville/eDNA_indicators_med.git).

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## SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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