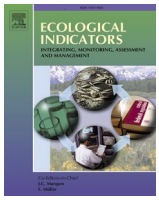
Ecological Indicators 166 (2024) 112290

Contents lists available at ScienceDirect

Ecological Indicators

journal homepage: www.elsevier.com/locate/ecolind



Original Articles

Seasonal dynamics of Mediterranean fish communities revealed by eDNA: Contrasting compositions across depths and Marine Fully Protected Area boundaries

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ARTICLE INFO

Keywords:
Depth
Fish
Fully Protected Area
Lightly Protected Area
Mediterranean Sea
MPA
Season
Teleo

ABSTRACT

Marine fish communities suffer from anthropogenic pressures and climate change, which influence their spatio-temporal dynamics. Marine Protected Areas (MPAs) have been established worldwide to preserve these communities, while mesophotic ecosystems could provide natural refugia. Assessing the extent to which MPAs and deeper ecosystems can mitigate human and climate change impacts requires regular monitoring of temporal community dynamics. Environmental DNA (eDNA) surveys – being time- and cost-effective – can provide valuable insights on biodiversity change. Here, we initiated a long-term study based on eDNA monitoring in an MPA in the north-western Mediterranean Sea that includes areas with various protection levels. Specifically, from June 2021 to January 2023, we collected eDNA samples during the summer, fall, and winter seasons from shallow water (20 m depth), at 40 m depth, and from the mesophotic zone (80 m depth) in a Fully Protected Area (FPA) and in a nearby Lightly Protected Area (LPA) in the Riou archipelago (France). In this short period and relatively small area, we detected a total of 113 actinopterygian and chondrichthyan taxa. Species with high fishing vulnerability had higher detection rates in the FPA than in the LPA, suggesting a positive impact of FPAs on the conservation of these threatened species. A marked seasonal signal in species detections, including significantly lower detections of several species in winter, indicated a combined effect of species biological changes and migration behavior. The seasonality trend was stronger in the FPA than in the LPA, indicating that such areas may modify sub-yearly patterns in communities and ecosystem processes. Fish composition was associated with water depth, with marked species dissimilarities between shallow waters and the mesophotic zone, implying that multiple depths should be considered in MPA monitoring to fully capture the response of biodiversity to management. Our results point to the importance of temporal information combined with extensive sampling across depths and protection levels to fully understand the ecological dynamics and structure of coastal fish communities.

1. Introduction

Marine ecosystems suffer from anthropogenic pressures and climate change, which alter the spatio-temporal dynamics of communities (Comte et al., 2021). To counteract these threats, Marine Protected Areas (MPAs) have been established worldwide (Grorud-Colvert et al., 2021). MPAs are dedicated to the conservation of nature and biodiversity, as well as the provision of ecosystem services and the protection of cultural values (IUCN, 2018). They vary in size, depth, age, and level of protection. MPAs vary in their management, from areas under protection according to the law but without concrete regulations (Pieraccini et al., 2017; Grorud-Colvert et al., 2021), to Partially Protected Areas

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https://doi.org/10.1016/j.ecolind.2024.112290
Received 21 March 2024; Received in revised form 19 June 2024; Accepted 21 June 2024
Available online 26 June 2024
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(PPAs) where low-impact human activities (e.g., tourism, fishing) are allowed but regulated (i.e., multiple-use MPAs; IUCN, 2018), to Fully Protected Areas (FPAs) where all extractive activities (e.g., mining, fishing) are prohibited (Claudet et al., 2020). These various management approaches result in contrasting ecological benefits (Edgar et al., 2014; Zupan et al., 2018; Sève et al., 2023). While well-designed and enforced MPAs (i.e., FPAs) have been recognized as an efficient conservation tool, especially for exploited species (Giakoumi et al., 2017; Sala and Giakoumi 2018), the ecological effectiveness of PPAs is more uncertain (Costello and Ballantine 2015; Turnbull et al., 2021). Likewise, the capacity of MPAs to counteract or buffer the impact of climate change or heat waves on ecological communities remains debated (Freedman et al., 2020; Jacquemont et al., 2022; Smith et al., 2023; Benedetti-Cecchi et al., 2024). Assessing MPA effectiveness over time requires regular biomonitoring to control for ecosystem health stability or improvement via changes in biodiversity (Nickols et al., 2019; Gold et al., 2021). Biodiversity assessments in MPAs are mainly performed through surveys which are time-consuming, labor-intensive and require taxonomic expertise (e.g., Underwater Visual Census, UVC; Usseglio 2015). Therefore, modern biodiversity assessments, such as environmental DNA metabarcoding (eDNA; Taberlet et al., 2012) and remote sensing (Zong et al., 2024) could complement these well-established methods.

The species composition within and outside an FPA might shift over time after its establishment, due to seasonal variations or the large-scale changes in assemblages under climate change (Smith et al., 2023). Additionally, the effects on species communities might take some time to manifest following the establishment of an FPA (Claudet et al., 2008; Ziegler et al., 2024). Moreover, marine fish communities undergo seasonal variations (e.g., Henriques et al., 2013; Jia et al., 2020). Some species regularly migrate to track the habitat conditions (e.g., food availability, thermoregulation) required for the different stages of their life cycle (e.g., reproduction, recruitment; Henriques et al., 2013), while others are present all year, ignoring the seasonal fluctuations in local conditions.

Community composition can also vary spatially, including along depth gradients. Changes in environmental conditions with increasing water depth, such as the reduced light penetration and lower temperatures (Cerrano et al., 2019; Kahng et al., 2019) that characterize mesophotic ecosystems (−30 to −150 m; Loya et al., 2016), require specific adaptations that are restricted to certain species (Bridge et al., 2016). Consequently, mesophotic fish assemblages are distinct from those in shallow zones (Rocha et al., 2018; Lesser et al., 2019). Deeper habitats might further serve as refugia for shallow-water fish species threatened by anthropogenic or climatic pressures (e.g., Lindfield et al., 2014, 2016; Muff et al., 2023). Additionally, spatial variations in community composition can result from climate changes combined with fishes' dispersal capacities that allow some species to colonize new habitats with favorable environmental conditions (Comte and Olden 2017, Pinsky et al., 2020). These shifts in fish geographical distribution occur worldwide (e.g., Campana et al., 2020), but species responses differ across regions and spatial scales (Eme et al., 2022; Chaikin and Belmaker 2023). These variations can translate to local species losses or gains, as well as species turnover over time (Blowes et al., 2019).

Biomonitoring time series offer considerable advantages for assessing fish community spatio-temporal dynamics, as the unidirectionality of time (i.e., changes always precede the visible impacts; Bálint et al., 2018) makes it possible to identify causality between environmental or anthropogenic changes and ecological dynamics (Dornelas et al., 2013; Anneville et al., 2019). However, the availability of temporally well-resolved and standardized time series on fish species composition is limited in marine ecology (e.g., McLean et al., 2019; Maureaud et al., 2024). The application of eDNA analyses could facilitate MPA monitoring (e.g., Aglieri et al., 2021). eDNA corresponds to DNA released by organisms in the environment, and metabarcoding enables the identification of multiple species based on their genetic markers (Taberlet et al., 2012). eDNA metabarcoding provides an efficient way to track fish community changes over time (see Sigsgaard et al., 2017; Jensen et al., 2022 for seminal works). Compared with other sources of temporal data (e.g., trawling, UVC), eDNA metabarcoding can provide temporal information for a broader range of species, including those often overlooked in standard studies, such as cryptic species (Boulanger et al., 2021; Juhel et al., 2022) or large pelagic species (Veron et al., 2023). In addition, eDNA sampling does not necessitate *in situ* taxonomic expertise (Yoccoz 2012), and it is independent of the type of habitat sampled (e.g., West et al., 2020; Muff et al., 2023), making it a time-effective method for regular monitoring. By identifying more pelagic, reef-associated, and cryptobenthic species, eDNA offers a fresh view on assembly rules across spatial scales (Mathon et al., 2022). Temporal eDNA metabarcoding has already proven to be efficient in detecting seasonal changes (e.g., Sigsgaard et al., 2017; Stoeckle et al., 2017). Given these advantages, eDNA metabarcoding time series may provide an understanding of temporal fish dynamics that complements that derived from traditional monitoring. In turn, it could provide stakeholders with new insights into important knowledge gaps regarding the sub-yearly temporal dynamics of fish communities, helping them to improve the long-term management of MPAs.

Coastal marine ecosystems of the Mediterranean Sea have been greatly impacted by anthropogenic pressures, such as fishing (Micheli et al., 2013; Guidetti et al., 2014), habitat loss and degradation (Chefaoui et al., 2018), and maritime traffic and pollution (Coll et al., 2012; Sharma et al., 2021). Consequently, a 34 % reduction in the abundance of important forage fish species, including both non-commercial and commercial species (e.g., anchovy [*Engraulis encrasicolus*] and pilchard [*Sardina pilchardus*]) and a 41 % reduction in top predators (e.g., sharks, large pelagic fishes) have been observed (Piroddi et al., 2017). In addition, the Mediterranean Sea is impacted by climate change (Tuel and Eltahir 2020), with an increase in sea surface temperature (SST; 0.035 °C/year; Pastor et al., 2020) and sea level (Taibi and Haddad 2019) observed during the last decade, along with the arrival of non-indigenous species (Sala et al., 2011; Zenetos and Galanidi 2020) and the northward expansion of southern Mediterranean species (Azzuro et al., 2011). To limit anthropogenic pressures, 6 % of the Mediterranean Sea is currently designated with some level of protection, however, only 0.23 % are highly or fully protected (Claudet et al., 2020). The northwestern Mediterranean Sea notably harbors the Calanques National Park, a multiple-use MPA established to mitigate the anthropogenic pressures (e.g., overfishing) exerted on local communities. Regular monitoring of fish diversity in such MPA with eDNA (Aglieri et al., 2021) would improve our understanding of the extent to which the level of protection influences the presence of various types of species (e.g., large pelagic, cryptobenthic species), including those targeted by fishing.

In this study, we aimed to assess the temporal dynamics of marine fish communities from the Riou archipelago (France) in the northwestern Mediterranean Basin using a seasonal eDNA metabarcoding time series over 1.5 years. This area, located within the Calanques National Park, harbors both FPAs and PPAs. Our objective was to decipher how much the compositional variations in communities were influenced by: *(i)* the MPA protection level, *(ii)* seasonality, and *(iii)* habitat depth. We hypothesized that fishing prohibition in the FPA would have favored the arrival and settlement of target fish species (Blowes et al., 2020). We identified fish groups responsible for seasonal variations in community composition. Finally, we investigated whether communities at different depths exhibited different compositional changes in the MPA, hypothesizing that the varied environmental conditions in the Calanques National Park, including coves, steep cliffs, and clear waters (Blanc 2012), would have resulted in distinct fish communities within three depths zones: shallow water (20 m), 40 m depth, and the mesophotic zone (80 m).

2. Material and methods

2.1. Study area

In the north-western Mediterranean Sea, the area from Marseilles to Cassis (over 20 km, France) features numerous steep cliffs and massive rock formations resulting from erosion caused by waves and marine currents locally known as the Calanques (Blanc 2012). This area is characterized by clear and low-productivity oligotrophic waters (Poulain et al., 2012), due to the Ligurian-Provençal current between Corsica and the French mainland coast (Millot 1991), that drives nutrient transport westward, notably towards the Gulf of Lion. The seasonal conditions in the north-western Mediterranean Basin also influence productivity throughout the year: strong water stratification limits biological production in summer, while the combination of low surface temperatures and strong winds lead to the upwelling of nutrients to the surface from late winter to spring (Lambert et al., 2017).

The Calanques National Park, established in 2012 near Marseille (France), covers 435 km^2 (Parc National des Calanques, 2024). The park was established to reduce the local anthropogenic pressure on the terrestrial and marine local ecosystems, such as overfishing (e.g., Pollard et al., 2018; Panayiotou et al., 2020). This multiple-use MPA harbors both FPAs and PPAs. In the Riou archipelago (south of Marseille), Moyades island is located within a 10.5 km^2 FPA where fishing and access are strictly prohibited (Fig. 1). Located 2 km farther, the "Impérial du large" island is part of the PPA of the park, defined as a Lightly Protected Area (LPA; Grorud-Colvert et al., 2021), where marine traffic under a speed of 5 knots is allowed (Fig. 1). Specific fishing regulations also apply, including maximum quantities (7 kg/person/day), quotas (e.g., John Dory, *Zeus faber*), forbidden species (e.g., dusky grouper, *Epinephelus marginatus*; brown meager, *Sciaena umbra*), and seasonal bans (Parc National des Calanques, 2024). The two locations are characterized by similar rocky habitats with sandy sediments at the base of the drop-off, and they include coralligenous reefs housing a great diversity of species (Parc National des Calanques, 2024).

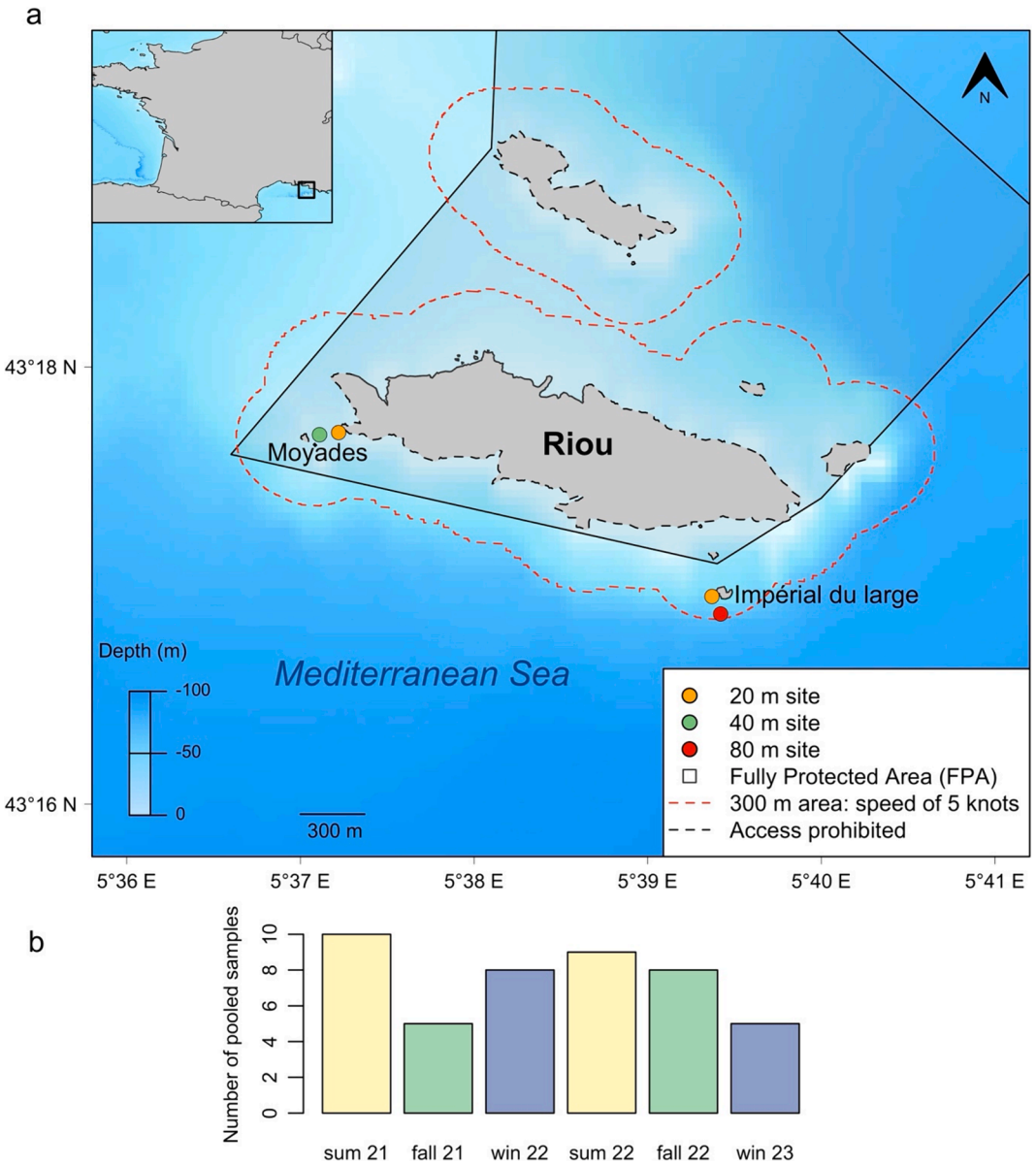

Fig. 1. (a) Map of the study area in the Riou archipelago (Calanques National Park, France) with the eDNA sampling sites and their respective depths, near Moyades island and "Impérial du large" island. (b) Number of pooled samples collected during each season over the full sampling period (sum = summer, win = winter, 21 = 2021, 22 = 2022, 23 = 2023).

2.2. Data acquisition by eDNA

The eDNA samples were collected between June 2021 and January 2023 in two locations (Moyades, M−FPA and "Impérial du large", I-LPA), during the summer (June to August), fall (mid-September to November) and winter (January to early March) seasons, with the exact sampling dates depending on the weather conditions. Unfortunately, no samples were collected during the spring due to logistical issues. For the M−FPA, samples were collected between Moyades island and Riou island, while for the I-LPA, they were collected on the south side of the "Impérial du large" island (Fig. 1). To take into account the existing bathymetry, in the M−FPA the samples were taken at two sampling sites with depths of 20 and 40 m, while in the I-LPA they were taken at two sites with depths of 20 and 80 m.

At each site, in-situ filtration of seawater was performed using a double-head submersible pump (Subspace, Geneva, Switzerland; nominal flow of ca. 1 L/min) strapped to an underwater scooter with 2 Vig-iDNA 0.20 μm filtration capsules (SPYGEN, le Bourget du Lac, France), along with disposable sterile tubing (Muff et al., 2023). The samples were collected along two horizontal transects (up to 400 m in length) during each closed-circuit rebreather dive, enabling the filtration of a water volume of 15 L/filter per depth, as close as possible to the substrate (Hocdé et al., 2023). Two filter replicates were collected by two divers at each sampling site, except in two cases where bad weather conditions or logistical issues meant that only one replicate was sampled.

After the filtration, the remaining seawater was emptied from the capsule back on the boat and replaced by a 80 mL CL1 conservation buffer (SPYGEN, le Bourget du Lac, France). To prevent any contamination, a strict protocol was followed during the entire process, requiring disposable gloves and single-use filtration equipment. Finally, the samples were stored at room temperature (ca. 20 °C). Over the whole sampling period, 45 filter replicates were sampled in the M−FPA and 44 in the I-LPA, for a total of 89 filter replicates.

2.3. eDNA extraction, amplification, sequencing, and data processing

The two filter replicates were pooled prior to the amplification step (except for the two cases without replication), leading to 22 pooled samples (30 L) and one of 15 L for the M−FPA and 21 pooled samples plus one of 15 L for the I-LPA (Supp. Mat. 1). DNA extraction, amplification, and high-throughput sequencing were performed at SPYGEN, following the protocol from Polanco-Fernández et al. (2021a), in dedicated rooms set up with positive air pressure, ultraviolet (UV) treatment, and frequent air renewal. During DNA extraction, samples containing the CL1 buffer were agitated for 15 min, then centrifuged (15,000 × g). Subsequently, 33 mL of ethanol and 1.5 mL of 3 M sodium acetate were added to the tubes. After one night of storage at −20 °C, a second centrifugation was carried out (15,000 × g for 15 min at 6 °C), followed by the addition of an ATL buffer (720 μL) and proteinase K (20 μL). The mixture was incubated at 56 °C for 2 h. DNA extraction was completed using the NucleoSpin® Soil kit (MACHEREY-NAGEL GmbH & Co., Düren, Germany), following the manufacturer's instructions. The elution was performed by adding 100 μL of SE buffer twice (see Polanco-Fernández et al., 2021a for the detailed protocol).

After DNA extraction, the samples were tested for inhibition following the protocol described in Biggs et al. (2015). If a sample was inhibited, it was diluted five-fold before amplification. DNA amplifications were conducted 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, Foster City, CA, USA), 10 mM Tris–HCl, 50 mM KCl, 2.5 mM $MgCl_2$, 0.2 mM of each dNTP, 0.2 μM of each primer listed below, 4 μM human blocking primer (Valentini et al., 2016), and 0.2 μg μL^{-1} bovine serum albumin (BSA; Roche Diagnostics, Basel, Switzerland). The teleo primers (forward: ACACCGCCCGTCACTCT, reverse: CTTCCGGTACACTTACCATG; Valentini et al., 2016) were used, amplifying a region of roughly 60 bp base pairs on average (range 29–96 bp) of the mitochondrial 12S region, designed to capture both teleost (Valentini et al., 2016) and Elasmobranchii taxa (Polanco-Fernández et al., 2021). The primers were 5′-labeled with an eight-nucleotide tag unique to each polymerase chain reaction (PCR) replicate, enabling the assignment of each sequence to the corresponding sample. The tags for the forward and reverse primers were identical for each PCR replicate. 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 was performed at 72 °C for 7 min. Twelve PCR replicates were run per sample to increase the probability of detecting rare species (Ficetola et al., 2015) and to filter the chimeras by comparing the results of the 12 parallel PCRs during the bioinformatics step. After amplification, capillary electrophoresis (QIAxcel; Qiagen GmbH, Hilden, Germany) was used to titrate the samples, and the samples were purified using the MinElute PCR purification kit (Qiagen GmbH). Before sequencing, purified DNA was titrated again using capillary electrophoresis. The purified PCR products were then pooled into equal volumes to achieve a theoretical sequencing depth of 1,000,000 reads per sample.

Library preparation and sequencing were performed at Fasteris (Geneva, Switzerland). The MetaFast protocol (a ligation-based method) was applied to prepare four libraries, which were sequenced separately. Paired-end sequencing was carried out using a MiSeq sequencer (2 × 125 bp, Illumina, San Diego, CA, USA) on two MiSeq Flow Cell Kits (v3; Illumina), following the manufacturer's instructions. To control for potential contamination, two negative extraction controls and one negative PCR control of ultrapure water (12 replicates) were also amplified and sequenced in parallel to the samples.

The sequence reads were analyzed using the OBITools package (http://metabarcoding.org/obitools; Boyer et al., 2016), following the protocol described by Valentini et al. (2016). Forward and reverse reads were assembled using the *illuminapairedend* program, with a minimum score of 40 and retrieving only the joined sequences. The reads were then assigned to each sample using the *ngsfilter* program, and a separate dataset was created for each sample by splitting the original dataset into several files using *obisplit*. After this step, each replicate was analyzed individually before the taxon lists were merged. Strictly identical sequences were clustered together using *obiuniq*. Sequences shorter than 20 bp or with an occurrence lower than 10, as well as those labeled "internal", were removed using the *obiclean* program, as they most likely corresponded to PCR substitutions and indel errors.

Taxonomic assignment of the molecular operational taxonomic units (MOTUs) was performed by applying the *ecotag* program with a combination of two genetic reference databases: (*i*) the EMBL genetic reference database comprising 16,128 sequences from 10,546 species across all organisms (European Molecular Biology Laboratory, https://www.ebi.ac.uk, v141, downloaded in 2023; Baker et al., 2000) and (*ii*) a custom-built 12S reference database from sequenced samples collected during previous surveys, containing 2,678 sequences corresponding to 937 species (in June 2023) from both the Mediterranean Sea and the eastern Atlantic Ocean. The latter database is estimated to contain 81 % of the fish species present in the north-western Mediterranean Sea (Duhamet, 2023). The sequences were assigned at different taxonomic levels: species (match = 100 %), genus (90 % ≤ match < 100 %), and family (85 % ≤ match < 90 %). These thresholds were chosen considering the high coverage of the custom-built database and in accordance with previous studies (e.g., Polanco-Fernandez et al., 2021b; Mathon et al., 2022, 2023). All sequences with a frequency of occurrence lower than 0.001 per sequence and per library were discarded, as these result from tag jumps (Schnell et al., 2015). Further, a correction was made to account for index-hopping (MacConaill et al., 2018), with a threshold empirically determined using experimental blanks between libraries. This correction removed all reads present in plates where the combination of tags was not present in the library and was later applied for each plate position.

2.4. Taxonomy cleaning

The 45 pooled eDNA samples yielded a total of 32,785,799 reads (average reads/sample = 642,858 ± 460,819. Of the 196 taxa detected with the pipeline, 97 were assigned at the species level, 64 at the genus level, and 35 at the family level. Next, taxa that were assigned at the family level and only detected in one pooled sample were removed, as were some genera/families for which some representative species were detected at the species level, as done in Rozanski et al. (2022). Fish taxa that do not occur in the study area were also removed, such as taxa from the Pacific Ocean (e.g., *Oncorhynchus*; Froese and Pauly 2024) that may correspond to species genetically related to local ones that are currently missing from the reference database, as well as freshwater and brackish-water taxa whose detection may come from fluvial discharge from the Rhone river (e.g., *Cyprinidae*, *Percidae*; Froese and Pauly 2024; see Supp. Mat. 2 for the final list of taxa).

2.5. Modeling of compositional dissimilarity

To identify and visualize the degree of compositional dissimilarity between the 45 pooled samples, β−diversity was first computed based on the presence–absence Jaccard's dissimilarity index (Baselga 2012; *betapart* R package; Supp. Mat. 3), followed by a principal coordinates analysis (PCoA; Fig. 2).

A generalized dissimilarity model (GDM) was implemented on the Jaccard dissimilarity matrix to explore the main drivers of the dissimilarity between pooled samples (Mokany et al., 2022). Five non-correlated environmental variables (Supp. Mat. 4) were considered as predictors of taxa composition. Four of these variables were directly derived from the sampling sites: (1) the coordinates (longitude, latitude) of the sites, converted directly in the model to the Euclidean geographic distance between two samples in a pair (Mokany et al., 2022); (2) the sampling depth (m); (3) the day of year of the sampling, as a seasonal indicator; and (4) the day of sampling over the full 1.5-year sampling period, as a time indicator. The local sea surface temperature (SST) anomaly, retrieved from Copernicus Marine Service ("MED-NRT-L4" dataset; 2022) was the fifth predictor considered, computed as the daily optimally interpolated (L4) maps of foundation SST over the Mediterranean Sea at high (HR = 0.0625°) and ultra-high (UHR = 0.01°) spatial resolution from 2008 to 2022.

The GDM was implemented using the *gdm* package (Fitzpatrick et al., 2021) in R v.4.2.0 (R Core Team 2022), with default settings for the number of splines (3) and knots (3). The GDM was run for all pairs of samples in the whole study area (Fig. 3; Supp. Mat. 5–7) and within each site separately (M−FPA and I-LPA), removing the geographic distance predictor in the latter case (Supp. Mat. 5, 6, 8, 9). The statistical significance of the models and the predictors' importance were assessed with a permutation test ("gdm.varImp" R function; gdm package; Fitzpatrick et al., 2021) across 1000 permuted site–pair tables. This statistical test allows a comparison of the deviance explained from the fitted model and that derived when all predictor variables are randomly permuted between sites (Fitzpatrick et al., 2013; Mokany et al. 2014, 2022). Predictor variables with a p-value < 0.05 were considered to have a significant influence.

2.6. Hierarchical modeling of communities

Using the taxa composition matrix for each sample taxa occurrences were compared across the three sampled seasons (summer, fall, winter). Hierarchical modeling of species communities (HMSC; Ovaskainen et al., 2017, Ovaskainen and Abrego 2020) was combined with eDNA-derived presence–absence data for this analysis, following Polanco-Fernández et al. (2022). HMSC operates as joint species distribution models (JSDM) that incorporate a hierarchical component, which helps

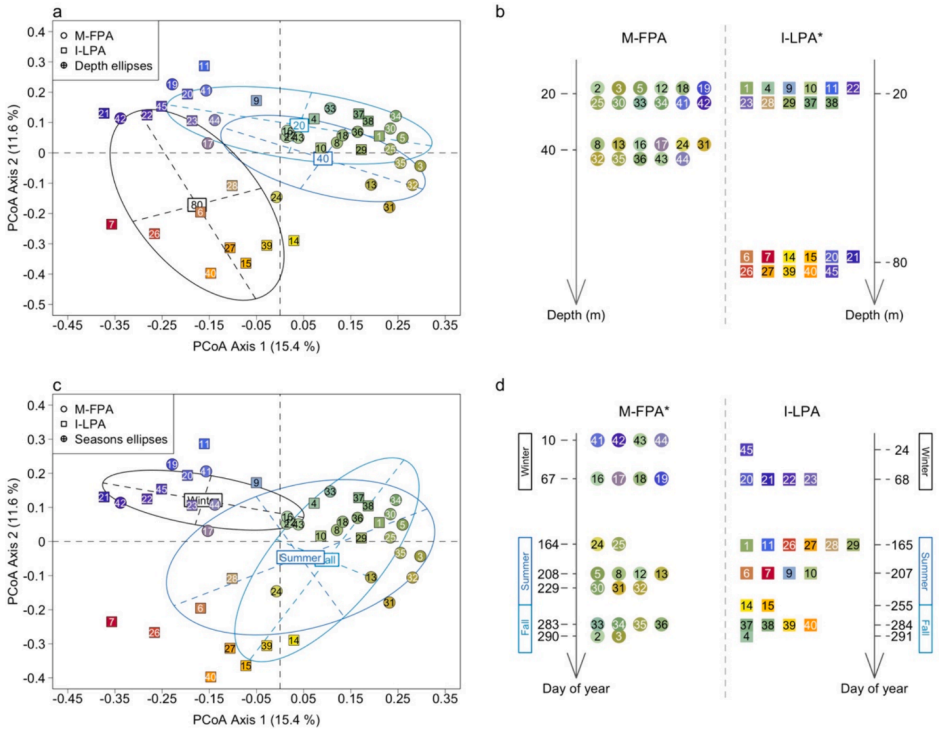

Fig. 2. (a)–(c) First two axes of the principal components analysis (PCoA) based on Jaccard dissimilarity, showing differences in taxa composition between all pairs of samples. M−FPA corresponds to the Fully Protected Area (Moyades), while I-LPA corresponds to the Lightly Protected Area ("Impérial du large"). Ellipses display the dispersion of the filters according to (a) sampling depth (20, 40, or 80 m) and (c) season (summer, fall, or winter). (b) Sampling depth and (d) day of year of sampling of the corresponding sample according to their location in the M−FPA or I-LPA. Point colors correspond to the position in the PCoA space: points with similar colors have similar taxa compositions. * indicates a significant influence of (b) depth or (d) day of year of sampling according to the generalized dissimilarity model (GDM) results.

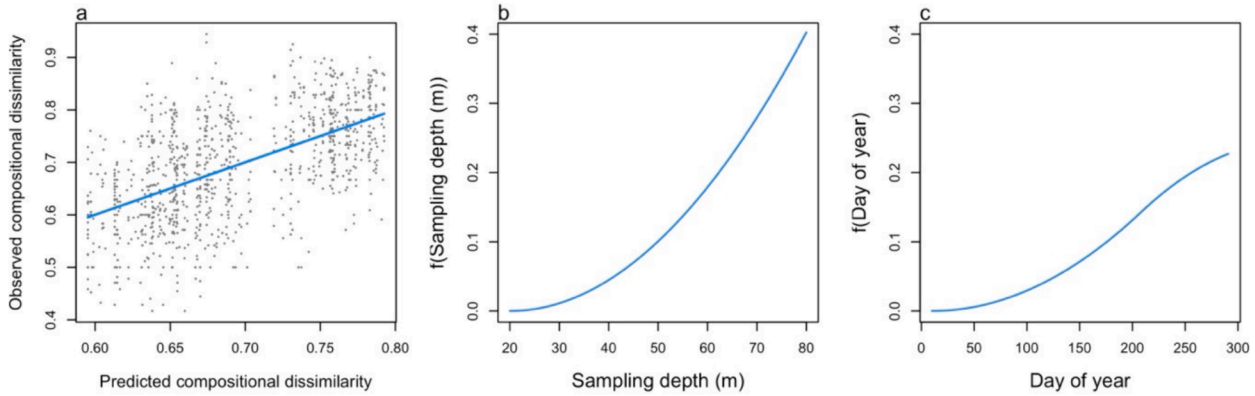

Fig. 3. Results from the generalized dissimilarity model (GDM). (a) Observed versus model-predicted dissimilarities. Each gray point represents a sample pair, while the blue line corresponds to equality, i.e., perfect model prediction. (b) and (c) Spline functions of the predictor variables sampling depth and day of year, both of which had a significant influence ($p < 0.05$; Supp. Mat. 6) on the compositional dissimilarity.

us to understand taxa responses to environmental covariates (Warton et al., 2015). To avoid biased and false environmental responses for taxa with only a few occurrences (Ovaskainen and Abrego 2020), taxa that occurred in fewer than three filters and in more than n-2 filters were first removed (n = 45). This preliminary step led to the exclusion of 50 out of 112 taxa (44.6 %). Therefore, the model was applied to the 62 remaining taxa (n_s) from 29 different fish families. HMSC was also applied to both the M−FPA and the I-LPA separately, considering 50 and 34 taxa, respectively.

The taxa presence–absence matrix (size $n \times n_s$) was used as the response variable in the HMSC (Ovaskainen et al., 2017). Season (summer, fall, winter) was included as a fixed effect. A taxa-specific regression parameter was estimated to compare their occurrences across the three seasons. A "probit" regression was applied in all analyses. Due to the irregular sampling design, the sampling depth (20 m, 40 m, 80 m), the location (M−FPA, I-LPA), and the sample itself (n = 45) were included as random effects to control for unexplained variations at the sample level.

The HMSC model was fitted using the *Hmsc* R package (Tikhonov et al., 2020), assuming default prior distributions (Ovaskainen and Abrego 2020). For parameter estimation, four Markov Chain Monte Carlo (MCMC) chains were used, each run for 37,500 interactions, of which the first 12,500 were discarded as burn-in. The chains were thinned by a factor of 100, resulting in 1000 posterior samples per chain. MCMC model convergence was assessed using the potential scale reduction factors (e.g., Gelman and Rubin 1992). To assess the explanatory power of the probit model for each species, the observed and predicted occurrences were compared using the area under receiver-operator curve (AUC; Pearce and Ferrier 2000) and Tjur R^2 (Tjur 2009) statistics. The three pairs of seasons (summer–fall, summer–-winter, and fall–winter) were then compared; for each pair, the proportion of species displaying positive or negative responses to the two considered seasons, with 95 % credible intervals of coefficients that did not overlap zero, was calculated. The same procedure was performed to compare species occurrences in both FPA and LPA, considering the location (M−FPA or I-LPA; X matrix) as a fixed effect and including the sampling depth, the day of year of the sampling (seasonality effect), and the sample itself as random effects.

3. Results

3.1. Taxonomic assignment

After the taxonomy cleaning step, the total number of reads was 28,062,260 (average reads/filter = 623,605 ± 429,75) across the 45 pooled eDNA samples. We identified 113 taxa, including 110 Actinopterygians from 52 different families (e.g., Sparidae, Scombridae, Gobiidae) and 3 Chondrichthyans (*Scyliorhinus canicula*, *Raja* sp., and *Torpedo marmorata*; Supp. Mat. 2). Among the 113 taxa detected, 92 were assigned at the species level, 17 at the genus level, and 4 at the family level (Callionymidae, Gaidropsaridae, Istiophoridae, Sternoptychidae; Supp. Mat. 2).

3.2. Modeling compositional dissimilarities

When comparing the taxa compositions between samples, we found that the average dissimilarity between all samples was $\beta jac_{mean} = 0.69 \pm 0.10$ and was mainly due to taxa turnover ($\beta jtu_{mean} = 0.50 \pm 0.18$, representing 72.5 %; Supp. Mat. 3). We detected 98 of the total 113 taxa in the M−FPA and 73 in the I-LPA. The average dissimilarity between the M−FPA and the I-LPA was $\beta jac_{mean} = 0.70$, with turnover accounting for 71 % of the dissimilarity (Supp. Mat. 3), indicating that some taxa were unique to a specific location. Notably, of the 39 unique taxa occurring in the M−FPA, we detected 46 % (6/13) of the Gobiidae, 66 % of the Tripterygiidae (2/3) and 75 % (3/4) of the Mugilidae (Supp. Mat. 10). In the I-LPA, the 15 unique taxa were more spread out along the phylogeny, but 60 % (9/15) of them were only detected at 80 m, including some mesopelagic taxa (e.g., *Chauliodus sloani*, *Lophius Budegassa*, *Merluccius merluccius*; Supp. Mat. 10).

The first two axes of the PCoA explained 27 % of the total inertia of species compositional variations (Fig. 2a, c). We observed an ordination of samples according to depth (Fig. 2a, b): while samples had a more similar taxa composition between 20 and 40 m in the M−FPA, they displayed stronger dissimilarities between 20 and 80 m in the I-LPA. For both locations and depths, taxa dissimilarities were driven by the turnover component, accounting for 69 % of the dissimilarities in the M−FPA between 20 and 40 m and 82 % in the I-LPA between 20 and 80 m (Supp. Mat. 3). We also detected a seasonal differentiation between communities in the M−FPA: taxa compositions were more similar in summer and fall than in winter (Fig. 2c, d). Although turnover was the main contributor, the nestedness component still accounted for 41 % of the dissimilarity between fall and winter and 36 % between summer and winter (Supp. Mat. 3). However, in the I-LPA, although we detected a compositional similarity within samples collected in winter, those sampled in summer or fall did not show an intraseasonal similarity pattern (Fig. 2c, d).

The GDM, implemented to decipher which environmental variables influenced the observed compositional dissimilarities, explained 31.5 % of the deviance (Supp. Mat. 5). Among the five predictors selected, the day of sampling over the 1.5-year sampling period and the geographic distance had no influence on the model construction and were discarded. The sampling depth and the day of year of sampling both had a

significant influence ($p < 0.05$; Fig. 3, Supp. Mat. 6), with depth being the major contributor to the observed dissimilarities (importance = 86 %; Supp. Mat. 6). According to the spline function, the greater the sampling depth, the more the dissimilarities sharpened compared with the fish communities present at 20 m (Fig. 3b), as displayed by the PCoA (Fig. 2a, b). However, the SST contributed very little and not significantly (Supp. Mat. 6, 7). When each location was considered separately, only sampling depth had a significant influence on the compositional dissimilarities in the I-LPA (dev. explained = 16.4 %; $p < 0.05$; Supp. Mat. 5, 6, 8), although only between 20 and 80 m, while it was non-significant between 20 and 40 m in the M−FPA. On the other hand, the day of year of the sampling (proxy for seasonality) significantly influenced the taxa composition in the M−FPA (dev. explained = 15.9 %; $p < 0.05$; Supp. Mat. 5, 6, 9), as shown in the PCoA (Fig. 2c, d).

3.3. Species-specific responses to seasonality effect and site

The HMSC showed a good fit to the data, with an average AUC = 0.90 and Tjur $R^2 = 0.21$ for explanatory power. Its application to the 62 taxa revealed a clear seasonal occurrence response for most taxa (Fig. 4). Specifically, 93.5 % (58/62) had a higher probability of occurrence in fall (Fig. 4a) and 87 % (54/62) in summer (Supp. Mat. 11), compared with the winter baseline. Considering fall vs. winter, with 90 % posterior estimate support (i.e., confidence level), 80 % (50/62) displayed a higher probability of occurrence in fall and none in winter. For summer vs. winter, 63 % (39/62) of species had a higher probability of occurrence in summer at this confidence level, while only 2 % (1/62) had a higher probability in winter (Supp. Mat. 11). Regarding fall vs. summer, 88 % (55/62) of the taxa showed a lower occurrence probability in summer compared with the fall baseline, but when 90 % posterior estimate support was considered, only 30 % (19/62) had a significantly higher probability in fall and 2 % in summer (Fig. 4b). Of the seven families with at least three taxa, five had a higher probability of occurrence in fall for more than 90 % of their taxa when comparing fall vs. winter, with the probability reaching 100 % for Mugilidae and Blenniidae (Fig. 4a; Supp. Mat. 12). When considering summer vs. fall, two families of pelagic species – Scombridae and Mugilidae – had a significantly lower occurrence probability in summer for more than 90 % of their taxa (Fig. 4b; Supp. Mat. 12). Moreover, season explained 11.3 % of the model's total variance, while the random effects explained 4.7 % (sample), 2.7 % (depth) and 2.2 % (site) (Supp. Mat. 13a). When only the explained variance was considered, season accounted for 57.6 %, sample for 20.2 %, depth for 11.7 %, and site for 10.5 % (Supp. Mat. 13b).

The application of the HMSC to the M−FPA displayed a good explanatory power ($AUC_{mean} = 0.89$; Tjur $R^2 = 0.16$). Most taxa showed a clear seasonal occurrence, with 80 % (40/50) displaying a higher probability of occurrence in fall and 70 % (35/50) in summer (Supp. Mat. 14) compared with the winter baseline, when considering 90 % posterior estimate support. Regarding summer vs. the fall baseline, at this confidence level only 20 % of the species/taxa had a higher probability of occurrence in fall, while none did in summer (Supp. Mat. 14). Considering the I-LPA ($AUC_{mean} = 0.90$; Tjur $R^2 = 0.21$), the seasonal signal was less marked, with 62 % (21/34) and 35 % (12/34) of the taxa having a higher probability of occurrence in fall and summer, respectively, compared with the winter baseline (Supp. Mat. 15) with 90 % posterior estimate support. When considering summer vs. fall, we observed a negative response to summer for 53 % (18/34) of the taxa (Supp. Mat. 15).

The HMSC applied to the two locations M−FPA and I-LPA also showed a good explanatory power ($AUC_{mean} = 0.90$; Tjur $R^2 = 0.20$). When considering 90 % posterior estimate support, we observed a positive response for 66 % (41/62) of the taxa to the M−FPA and no negative responses associated with the I-LPA (Supp. Mat. 16). The top five species responding positively to the M−FPA were the East Atlantic peacock wrasse (*Symphodus tinca*), the garfish (*Belone belone*), the dusky grouper (*Epinephelus marginatus*), the red scorpionfish (*Scorpaena scrofa*), and the *Spicara* genus.

4. Discussion

We investigated the temporal dynamics of marine fish communities in the Riou archipelago using seasonal eDNA metabarcoding time series. Our study revealed a higher detection of vulnerable species within the M−FPA than in the I-LPA, suggesting a positive impact of this FPA on the conservation of threatened species (Giakoumi et al., 2017; Loiseau et al., 2021). A marked seasonal signal in species detections, including significantly lower detections of several species in winter, indicated a combined effect of species biological changes and migration behavior (Stoeckle et al., 2017; Thalinger et al., 2021). Composition also shifted along the depth gradient, with strong compositional dissimilarities between fish communities detected in shallow waters and in the mesophotic zone. Hence, monitoring of MPAs should consider multiple depths to fully capture the response of biodiversity to management. Together, our findings demonstrate the spatio-temporal complexity involved in monitoring MPAs.

4.1. Spatial variations in fish composition between the FPA and the LPA

MPAs are expected to benefit individual species differently, depending on the species-specific sensitivity to anthropogenic pressure (Claudet et al., 2010; Giakoumi et al., 2017; Boulanger et al., 2021). The species-specific HMSC approach applied here, incorporating a hierarchical structure combined with eDNA occurrences, indicated a positive occurrence response of most taxa inside the M−FPA (Polanco-Fernández et al., 2022). This FPA provides a suitable environment with potentially less disturbance compared with the I-LPA (Sarker et al., 2023). Marine FPAs have been demonstrated to be effective in protecting exploited species (Giakoumi et al., 2017) and act as refuges from fishing for species characterized by large body size and high trophic level (Claudet et al., 2010; Edgar et al., 2014). In our study, several species of high commercial interest that are particularly vulnerable to fishing pressures had a higher detection probability in the M−FPA than in the I-LPA, in line with previous findings from the north-western Mediterranean Sea (Giakoumi et al., 2017; Blowes et al., 2020; Boulanger et al., 2021). For example, the dusky grouper (*Epinephelus marginatus*), the zebra seabream (*Diplodus cervinus*) and the red scorpionfish (*Scorpaena scrofa*), which are sensitive to fishing (Lloret et al., 2012; Pollard et al., 2018; Froese and Pauly 2024), were preferentially, if not exclusively, detected within the M−FPA. In addition to protecting species, FPAs also support the restoration and preservation of marine habitats (Marcos et al., 2021). Therefore, they may indirectly benefit non-exploited taxa, such as the damselfish (*Chromis chromis*) and the black-faced blenny (*Tripterygion delaisi*), by providing better habitat conditions crucial for various life stages compared with nearby fished areas (e.g., Hopf et al., 2022). The use of eDNA metabarcoding enabled the detection of rare species, such as the short-snouted seahorse (*Hippocampus hippocampus*) in the I-LPA. This species is considered near threatened (NT) in the Mediterranean Sea, due to bycatch and habitat damage and loss (IUCN 2016). Due to its small size, cryptic behavior, and ability to camouflage in its environment (e.g., *Posidonia oceanica* meadows), this species is also difficult to observe (Goffredo et al., 2004; Gristina et al., 2015), and eDNA metabarcoding monitoring likely improves its detectability. Together, these examples illustrate that eDNA can help document the distribution and population occurrence trends of species with low detectability and high conservation priority.

We further showed the potential of eDNA to detect new species invasions in the case of the *Acanthopagrus* genus, whose species are native to the Pacific and Indian Oceans (Iwatsuki and Heemstra 2022; Froese and Pauly 2024). A few individuals of twobar seabream (*Acanthopagrus bifasciatus*) have recently been detected in the Mediterranean Sea (García-de-Vinuesa et al., 2020; Al Mabruk et al., 2021). The present

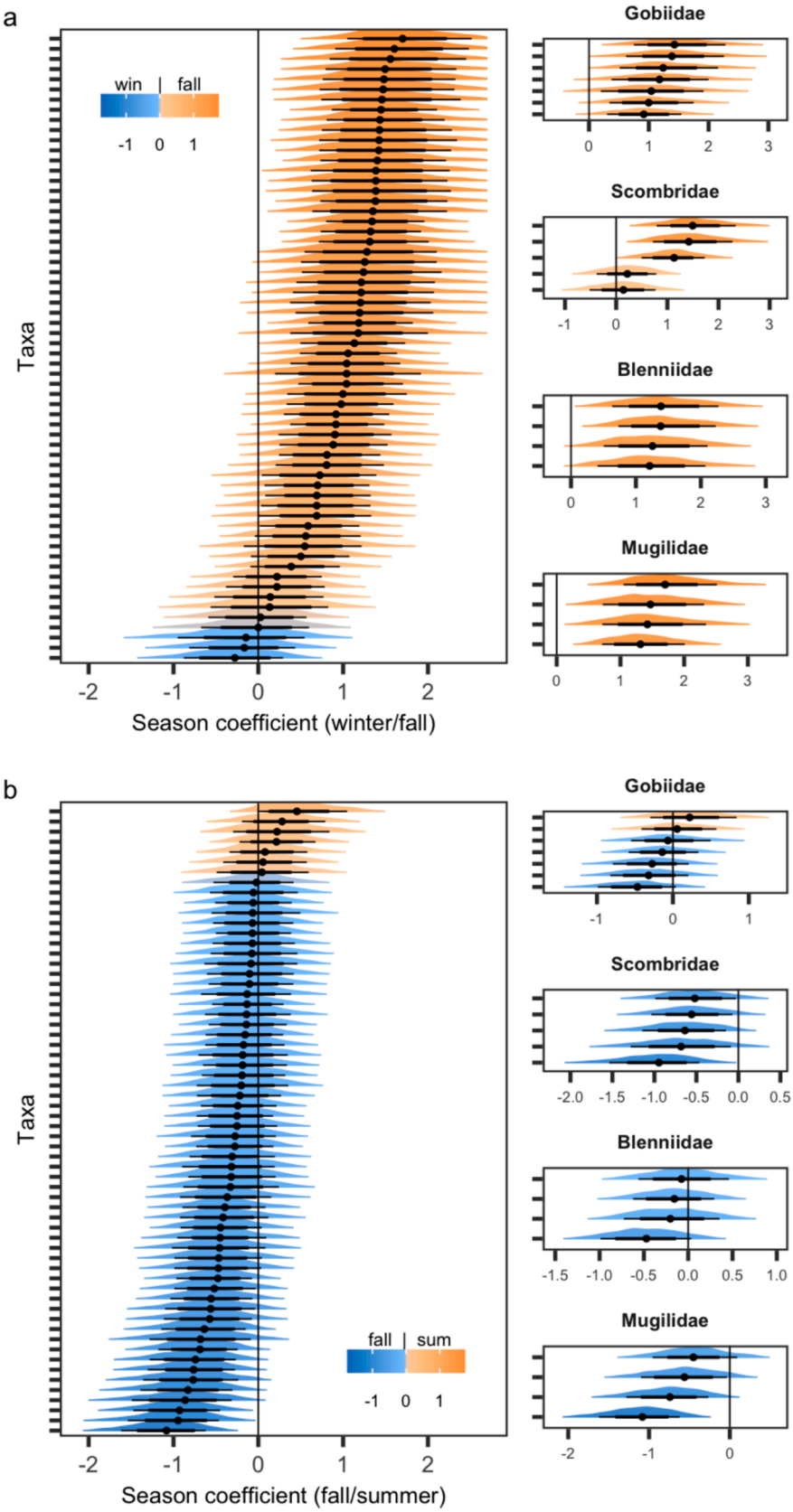

Fig. 4. Effect of season (orange = positive effect, blue = negative effect) on the occurrence probability of 62 taxa detected by environmental DNA (eDNA) and details for four fish families. Each horizontal line represents a taxon, with the colored histograms showing the 95 % credible intervals of parameter posterior estimates. Points represent the median of the posterior estimates, while thick and thin solid lines represent the 60 % and 80 % credible intervals, respectively. (a) Taxa responses to fall vs. winter, where a positive coefficient indicates a greater signal of occurrence in fall than in winter. (b) Taxa responses to summer vs. fall, where a positive coefficient indicates a greater signal of occurrence in summer than in fall (see Supp. Mat. 11 for winter vs. summer).

detection with eDNA may therefore correspond to this species, although the taxonomic assignment did not enable us to confirm its identity.

eDNA transportation in seawater may have influenced species detections in the M−FPA and I-LPA, which are located only 2 km apart. The study area may present surface and underwater − mostly westward − currents, stronger in winter than summer due to the wind intensity (Pairaud et al., 2012) that may have influenced eDNA circulation. While transport of eDNA material between the locations may have occurred, previous studies have demonstrated that eDNA becomes undetectable 1 h after being introduced into coastal seawater (Murakami et al., 2019). Moreover, spatial fidelity of eDNA detections has already been reported for sites located only a few hundred meters apart, despite water movements (e.g., Jeunen et al., 2019; West et al., 2020). Therefore, it is likely that eDNA degraded too rapidly to be detected away from its source. Together, these results demonstrate the importance of FPAs for the preservation of the most vulnerable species.

4.2. Seasonal changes in community composition

Seasonal changes in abiotic conditions driving shifts in biological activity might shape species detectability and occupancy recovered from eDNA (Barnes et al., 2014; Klymus et al., 2015). Our results highlight significant seasonal changes in species detections that were possibly related to either species behavior (Lacoursière-roussel et al., 2016) or abiotic conditions (e.g., temperature, UV, salinity; Barnes et al., 2014). The lower detections in winter might be related to changes in species biological and physiology rates, such as metabolism, movement, and excretion (Thalinger et al., 2021). In particular, feeding activity has been demonstrated to increase the amount of eDNA shed, due to increased metabolism and excretion (Klymus et al., 2015), while a decrease in food resources and feeding activity in winter may cause a decrease in the rate at which fish shed eDNA (Thalinger et al., 2021). The absence of detections of all the cryptobenthic species from the Blenniidae, Tripterygiidae, and Gobiidae families in winter could be explained by slowed activity when food is more limited, due to the higher energy needs per unit of mass of these species compared with those of larger fishes (Brandl et al., 2018).

Further, changes in taxa detectability might be attributable to the migratory behavior of species leaving the area during part of their life cycle (Henriques et al., 2013). We found lower eDNA detections of the Scombridae and Mugilidae families in summer, which include species whose life cycle involves seasonal migrations (e.g., Mediterranean *Thunnus thynnus* emigrating to the West and North Atlantic in July and August; Block et al., 2005). Migration patterns have already been highlighted with eDNA by Stoeckle et al. (2017) and Jia et al. (2020), who reported that seasonal detections aligned with the movements of fish between inshore waters and estuaries.

Seasonal trends may vary between FPAs and PPAs because anthropogenic activities such as tourism (e.g., recreational fishing, boat tours, diving) are more prevalent during the warm seasons in the Mediterranean region (Duro and Turrión-Prats 2019), which could impact fish communities (e.g., anthropogenic noise, visual disturbance, habitat degradation; Buscaino et al., 2016; Giglio et al., 2020). Anthropogenic disturbances can alter species behavior, potentially causing changes in feeding patterns, movement, or hiding. Therefore, FPAs mitigating human activities might serve as seasonal refuges, which may influence eDNA detections. This result aligns with the stronger seasonal eDNA signal found inside the M−FPA than in the I-LPA, with species being detected significantly more often in the M−FPA during summer and fall compared with winter. Therefore, FPAs may initiate modifications of sub-yearly patterns in communities and ecosystem processes.

eDNA detection relies on the persistence and degradation of DNA in seawater, which is related to biotic (e.g., extracellular enzymes, microorganisms) and abiotic factors (e.g., temperature, UV; Collins et al., 2018; Wang et al., 2021) that also fluctuate seasonally. Both eDNA degradation (e.g., Tsuji et al., 2017; Kasai et al., 2020) and eDNA shedding rate (Jo et al., 2019) increase with higher temperatures. Therefore, our strong eDNA signal in summer could suggest that the eDNA production rate is higher than the decay rate during that season. Moreover, Collins et al. (2018) reported no significant difference in eDNA decay rate between summer and winter in the western English Channel. These findings suggest that variations in eDNA persistence in the water, although present, may not be the primary factor contributing to the observed seasonal patterns. The compositional changes over time observed in our study stress the need to integrate seasonal monitoring in MPAs to assess seasonal variations in fish community composition and the underlying reasons.

4.3. Compositional dissimilarities across three depth zones

The composition of shallow and mesophotic assemblages may differ considerably (Rocha et al., 2018; Lesser et al., 2019), requiring the monitoring of fish compositional responses to MPAs across depths. We observed marked compositional dissimilarities between fish communities detected at 20, 40, and 80 m depth, with the most pronounced distinctions occurring between 20 and 80 m in the I-LPA. Our sampling of the lower mesophotic zone (80 m, I-LPA) led to the detection of more deep-water-specialized species used to low temperatures and low light penetration (Cerrano et al., 2019; Kahng et al., 2019), for instance the Madeira lantern fish (*Ceratoscopelus maderensis*) and Sloane's viperfish (*Chauliodus sloani*). Such compositional differences along a depth gradient in reefs have already been reported, with a significant break around 60 m depth, potentially attributable to shifts in light penetration and trophic resource availability (e.g., Lesser et al., 2019; Saenz-Agudelo et al., 2024). These distinct assemblages may induce different responses to the protection level. While our design did not allow us to compare the deep mesophotic zone inside and outside the FPA, we found a large number of species whose detections were restricted to the deeper I-LPA and that only occurred at 80 m. These taxa included deep species vulnerable to fishing, such as the European hake (*Merluccius merluccius*, usual depth range 70–370 m; Froese and Pauly 2024) or the blackbellied angler (*Lophius budegassa*; 70–1013 m; Preciado et al., 2006). Our results suggest that deeper reefs might serve as refuges for some species threatened by anthropogenic pressures (Claudet et al., 2011; Lindfield et al., 2014). Incorporating a broad depth range within FPA marine reserve boundaries has been reported to have a positive effect on fish biomass and abundance (Goetze et al., 2021). Therefore, FPAs should be designed in a way that includes a large depth gradient, to provide more opportunities for species to escape disturbances, which will increasingly include those related to climate change (e.g., Keppel et al., 2012; Brito-Morales et al., 2022; Giraldo Ospina et al., 2023).

The depth-related FPA signal may also be biased due to eDNA vertical transportation. According to previous studies, however, such transportation would be limited (Canals et al., 2021; Monuki et al., 2021) to 10–20 m from the original place of shedding (Allan et al., 2021). Considering the three depths of 20, 40, and 80 m sampled in this study, it is possible that some detections in deeper water correspond to species that normally occur shallower. The comparison between species' known depth ranges (retrieved from Duhamet 2022; Froese and Pauly 2024) and our eDNA detections resulted in a match for 66 % of the species, while 21 % were detected at deeper and 13 % at shallower depths than the known range. These findings suggest that while vertical eDNA transportation occurs, most detections accurately reflect species' typical depth ranges.

4.4. Sampling limitations

Our study underlines the ability of eDNA to recover local fish community composition over time across various depths and protection levels, but our analyses were associated with some sampling limitations. The sampling design could have been more balanced, with the addition of spring samples and comparable depth samples inside and outside the

FPA to better compare local community structure and seasonal dynamics. Further, including an unprotected area would have enhanced the study by enabling comparative analyses of fish biodiversity between protected and unprotected zones. Previous eDNA studies conducted in the north-western Mediterranean Sea have pointed to compositional fish dissimilarities between various FPAs and unprotected zones (e.g., Boulanger et al., 2021; Sanchez et al., 2022). With the present sampling design, we considered only two protected areas located only 2 km apart, so it was not possible to detect a strong contrast. The sampling effort allowed us to recover 71 % of the estimated local taxa (Supp. Mat. 17). A greater effort may have provided a more precise assessment of fish composition (Stauffer et al., 2021). Moreover, although eDNA metabarcoding may overcome some limitations inherent to traditional sampling (e.g., detection of rare/elusive species; Boulanger et al., 2021; time-effectiveness; Stoeckle et al., 2017), this method cannot provide accurate quantitative (e.g., abundance; Veron et al., 2023) and qualitative (e.g., development stage: larvae, juveniles, adults) data as classical methods can. These data are of great importance for assessing MPA effectiveness (e.g., fish stock recovery; Giakoumi et al., 2017). Therefore, integrating eDNA and standardized traditional monitoring together could give complementary insights into seasonal dynamics.

5. Conclusion

The use of eDNA metabarcoding time series revealed how fish communities are structured, in terms of taxonomic diversity, seasonally and by depth within and outside an FPA. While a “snapshot” of biodiversity at a single time point allows us to obtain a general picture of a local community, a time series enables the assessment of compositional variations over time and thus the investigation of reasons or processes behind the presence or absence of species in different periods (e.g., seasonal migrations, arrival of new species). Integrating regular and extensive sampling across depths and protection levels might more accurately guide conservation policies and MPA management. Further, the utility of time series extends beyond retrospective studies, also enabling forecasting of future changes (e.g., spreading of invasive species in the Mediterranean Sea; Azzuro et al., 2022) and a better understanding of such changes. We showed that eDNA metabarcoding can be a powerful tool to complement less frequent sampling using traditional methods, which still provide critical information on fish community biomass, body size structure, and intraspecific genetic variation.

CRediT authorship contribution statement

Romane Rozanski: Writing – original draft, Visualization, Investigation, Formal analysis, Data curation. **Laure Velez:** Writing – review & editing, Data curation. **Régis Hocdé:** Writing – review & editing, Resources, Methodology. **Agnès Duhamet:** Writing – review & editing, Resources. **Conor Waldock:** Writing – review & editing, Funding acquisition. **David Mouillot:** Writing – review & editing, Resources. **Loïc Pellissier:** Writing – review & editing, Supervision, Project administration, Methodology, Funding acquisition, Conceptualization. **Camille Albouy:** Writing – review & editing, Supervision, Methodology, Conceptualization.

Declaration of competing interest

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

Data availability

Data will be made available upon request and on Envidat (https://envidat.ch) in the "eDNA Data View" subsection: "Environmental DNA Marine Calanques 2021", "Environmental DNA Marine Calanques 2022", and "Environmental DNA Marine Calanques 2023".

Acknowledgments

We thank the OSU Pytheas, Septentrion Environnement and their staff for technical support and for providing their vessels. We thank D. Guillemain, F. Zuberer, Q. Schull, O. Bianchimani, and J. Leblond for contributing to the underwater filtering. We thank M. Dawes for the English language editing of this manuscript. This study was supported by the SNSF project “ShifteDNA” (grant no. 205556 to LP).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ecolind.2024.112290.

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