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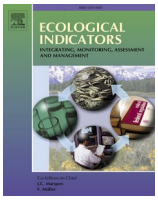

Addressing data-deficiency of threatened sharks and rays in a highly dynamic coastal ecosystem using environmental DNA

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ABSTRACT

Marine biodiversity loss is accelerating, leading to the elevated extinction risks of many species, including sharks and rays. To mitigate these losses, information on their distribution and community composition is needed. Monitoring these (often) mobile species is challenging, especially in remote, highly dynamic and turbid coastal areas. Here, we use an environmental DNA (eDNA) approach to: (1) establish the presence and distribution of elasmobranch species, (2) compare this to a conventional fisheries-dependent approach, and (3) determine the influence of season, area-based protection and habitat on elasmobranch community composition in the highly dynamic Bijagós Archipelago in Guinea-Bissau (West Africa). We collected 127 seawater samples and detected elasmobranch DNA in 58 (45.7%) of these samples, confirming the presence of 13 different elasmobranch species (2 sharks, 11 rays), including seven threatened species. Eight of the species detected by the eDNA-approach were also recorded in a fisheries observer program, which recorded another eight species not detected by the eDNA approach. The most commonly occurring species, based on the number of eDNA sampling locations were the pearl whipray (*Fontitrygon margaritella*), smalltooth stingray (*Hypanus rudis*), scalloped hammerhead shark (*Sphyrna lewini*), and the blackchin guitarfish (*Glaucostegus cemiculus*). Species composition and richness differed significantly before (January-March) and after the rainy season (November-December). Furthermore, we showed that community composition and species richness did not differ between protected (MPA) and non-protected areas of the archipelago. Thus, we confirm that eDNA approaches are a valuable and non-invasive tool to study threatened shark and ray species in data-deficient and dynamic coastal areas, especially when combined with conventional monitoring methods such as fisheries-dependent information.

1. Introduction

Globally, coastal ecosystems are threatened by anthropogenic stressors, such as pollution, coastal development and overexploitation, causing a collapse in richness and diversity of associated species (Worm et al., 2006, Cardinale et al., 2012, He and Silliman, 2019). The loss of species may hamper the functioning and health of ecosystems, and can lead to a loss of ecosystem services (Worm et al., 2006, Palumbi et al., 2009, Hammerschlag et al., 2019). Therefore, monitoring the status of biodiversity and individual species within ecosystems is essential to ensure future ecosystem health and the preservation of ecosystem services (Cardinale et al., 2012, Cooley et al., 2022).

In marine ecosystems, top- and *meso*-predators such as sharks and rays (i.e. elasmobranchs), can have important roles in coastal ecosystems (e.g. Heithaus, 2010, Heupel et al., 2014, Roff et al., 2016, Heithaus et al., 2022). However, recent findings suggest that currently approximately 33% of all shark and ray species are threatened with extinction due to overfishing and habitat degradation (Dulvy et al., 2021). Due to their ecological roles, the loss of these species may influence ecosystem services of marine ecosystems, such as productivity of

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fisheries, detoxification of marine waters, and carbon sequestration ('blue carbon', Heithaus et al., 2008, Atwood et al., 2015, Küpper and Kamenos, 2018).

Assessing the conservation status (e.g. IUCN Red List status) of species is an important step towards the implementation of management actions that enable species' protection. However, specific information for the appropriate assessment of conservation status is missing for many shark and ray species or local/regional populations (Dulvy et al., 2021). This includes information on the local presence, distribution and abundance of elasmobranch species. Monitoring biodiversity is costly and requires appropriate (research) capacity, causing data-deficiency to be more profound in developing regions. The resulting deficiency of essential information impairs species' status evaluation and hampers the implementation of (cost-)effective conservation strategies.

A relatively novel approach to monitor the occurrence of marine species is the use of environmental DNA (eDNA), which involves the metabarcoding of DNA traces of marine species in the water column or associated sediments (e.g., Thomsen et al., 2012). This approach simplifies species monitoring, increases species coverage (i.e., inclusion of cryptic, rare and highly mobile species and limiting misidentification), is non-invasive and cost-effective compared to other, traditional monitoring approaches (Thomsen et al., 2012, Miya, 2022). Over the past years, the application of environmental DNA has been increasingly used to confirm the presence of fish species in both freshwater and marine waters, and more recently to study the composition of elasmobranch communities (Bakker et al., 2017, Boussarie et al., 2018, Dunn et al., 2022). In addition, eDNA approaches have been successfully applied to determine seasonal abundance (Postaire et al., 2020), population sizes (Sigsgaard et al., 2016), and presence of highly cryptic species, for example the presence of sawfishes (*Pristis* spp.) in estuaries (Lafferty et al., 2018, Schweiss et al., 2019, Lehman et al., 2020). Although these relatively novel approaches are promising to address elasmobranch communities in highly data-deficient regions, less is known about the success of this technique to study elasmobranch communities in highly dynamic environments such as intertidal ecosystems, experiencing strong (tidal) currents.

To determine if environmental DNA can be used to tackle data-deficiency in highly dynamic, tropical coastal ecosystems, we aimed to study a highly data-deficient shark and ray community in the West African region. The coastal waters of the West African bioregion have a high occurrence of threatened endemic elasmobranch species (Derrick et al., 2020), and are a global hotspot for the most evolutionary distinct elasmobranch species (i.e., a measure of a species' evolutionary isolation) (Stein et al., 2015). However, the region currently also experiences one of the highest levels of fishing effort in the world (Kroodsma *et al.,* 2018, Leurs et al., 2021). Industrial fisheries surrounding protected coastal areas in West Africa (Leurs et al., 2021), and small-scale fisheries within these areas both threaten elasmobranch populations due to their high targeted and non-targeted catches (Kyne et al., 2020, Lemrabott *et al.,* in prep, Leurs *et al.,* in prep., Moore et al., 2019). Yet, the presence and community composition of elasmobranch fishes in coastal areas within the region remains poorly understood, hampering adequate conservation of this threatened species group. In addition, recent disappearance of species like largetooth sawfish (*Pristis pristis*) from the wider region and the occurrence of cryptic species, such as the African wedgefish (*Rhynchobatus luebberti*) asks for a more comprehensive approach to elasmobranch monitoring (Leeney and Poncelet, 2015, Moore, 2017).

Here, we determined if the environmental DNA approach can be used to successfully study elasmobranch communities in a tropical, data-deficient and highly dynamic intertidal environment. Specifically, we used an eDNA approach to: (1) establish the presence and distribution of elasmobranch species within a highly dynamic tropical intertidal ecosystem, (2) compare the eDNA-based species richness and composition of the archipelago to preliminary small-scale fisheries data, and (3) determine if eDNA-based species richness and community composition differed across seasons (i.e., before and after the rainy season), across tidal phases, between protected and non-protected areas and with distance to mangrove forest. Although the Bijagós Archipelago is one of the largest intertidal areas in the region, supporting local (artisanal) fisheries and likely functioning as a nursery area to both coastal and pelagic fish species (including commercial species captured in the industrial fisheries in the wider region) (Correia et al., 2021), information on the distribution of elasmobranch species in lacking. The only information on elasmobranch species within this area originates from inferred species distributions (IBAP, 2012), studies limited to a single species or island (Cross, 2015, Leeney and Poncelet, 2015), and recorded captures by industrial fishing fleets operating outside the archipelago (Diop and Dossa, 2011, Leurs et al., 2021). We aimed to provide information that is essential for the successful implementation of more efficient conservation measures for these threatened species, for future ecological studies focusing on the ecosystem functioning of the Bijagós, and to learn if and how this relatively novel approach can be used in remote, highly dynamic, and data-deficient environments to study sharks and rays.

2. Methods

2.1. Study area

The Bijagós Archipelago (11° 15′ 0″ N, 16° 5′ 0″ W) is located in Guinea-Bissau (Fig. 1), in the extended estuary of the Geba river. The archipelago consists of 88 islands and islets, which are lined by dense mangrove forests, intertidal mudflats and are connected through a complex system of gullies and channels. With over 350 km^2 of mangrove forests and 760 km^2 of intertidal flats, the archipelago is recognized as an important area for (migratory) shorebirds (Salvig et al., 1994, Meijer et al., 2021), teleosts (Correia et al., 2021), and sea turtles (Catry et al., 2002), and was designated as a UNESCO Biosphere Reserve in 1966 (IBAP, 2012, UNESCO, 2020). In 2014, the archipelago was also recognized as an important wetland under the RAMSAR Convention (IBAP, 2012, RAMSAR, 2014).

2.2. Sample collection and preservation

We sampled surface water in five different regions within the archipelago: Urok, Soga, Rubane, Bubaque, and Orango (Fig. 1). Samples were collected both before (January and February) and after (October to December) the rainy season of 2019. At each sampling location, we took a 2-liter water sample using a sterilized sampling bottle (i.e., using a 10% bleach solution) and submerging the bottle completely underwater, to prevent sampling the biofilm on the water surface. For each sample, we recorded the surrounding habitat (Table 1), geographic coordinates, and storage time (i.e. time between sample collection and filtration) for each sample. Retrospectively, we determined the distance of the sampling location to the entrance of the Geba river, distance to the nearest mangrove edge, and whether a sample was taken in or outside one of the two marine protected areas (Fig. 1). Sampling time was used to determine the tidal phase and amplitude based on the tide table for Bubaque (11.33° N/15.87° W). We estimated that compared to high tide in Bubaque, the high tide was one hour later in the Urok sampling region, and one hour earlier in the Orango region. To account for potential variability in these high and low tide estimates, we considered samples taken within 30 min to or from high tide as 'high-tide samples', and similarly for low tide. Samples taken in between low and high tide are referred to as 'receding tide' and 'incoming tides'. Straight after sample collection, samples were wrapped in aluminum foil and stored in an insulated cooling box until filtration. Upon return to the base camp or whenever the situation in the field permitted, samples were filtered as soon as possible using a portable, battery-operated vacuum pump (Makita 16V vacuum pump). The pump was connected to a Nalgene erlenmeyer flask with a sterilized filter holder and funnel on top. Samples were filtered using sterile mixed cellulose ester filters (MERCK and

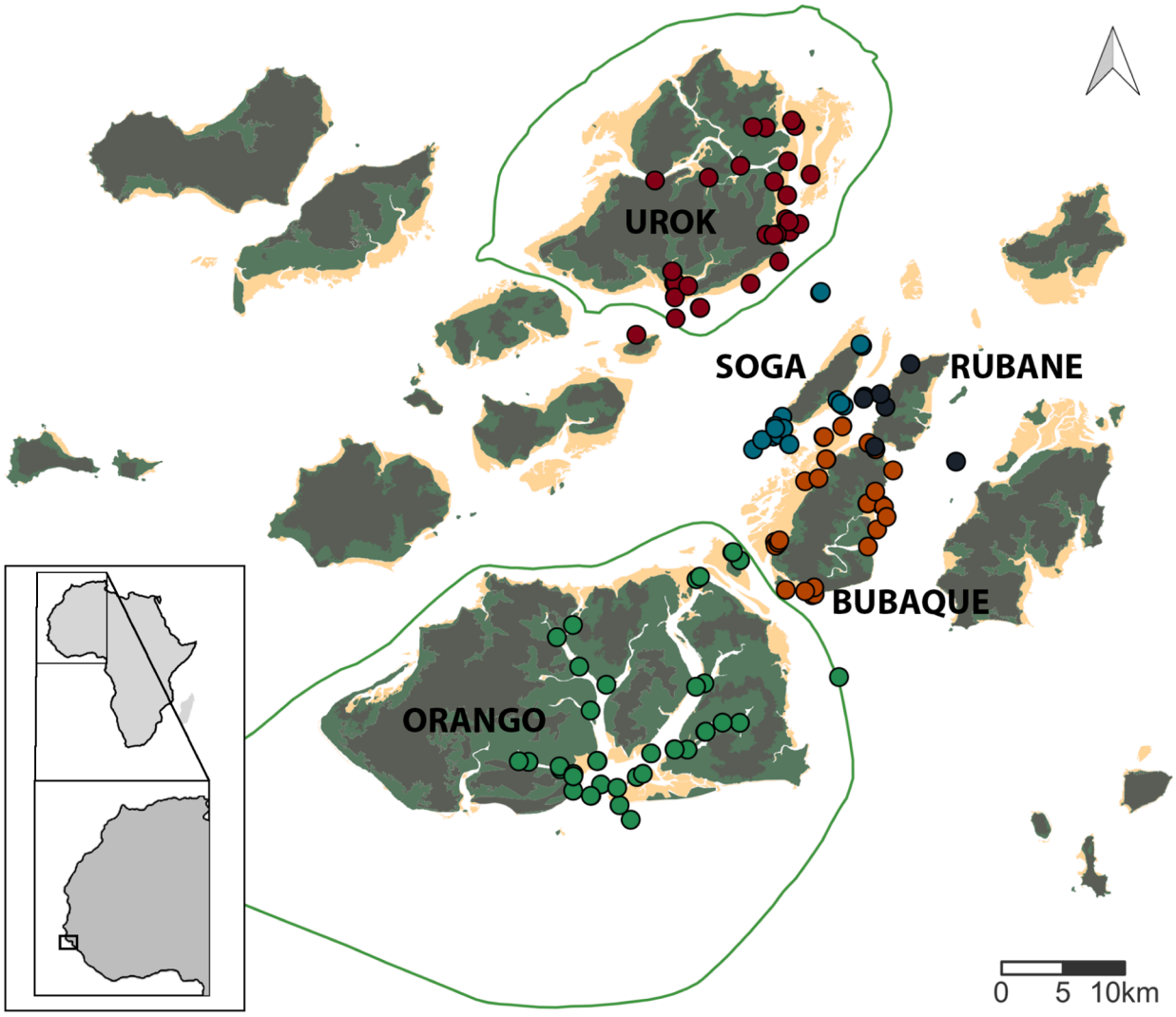

Fig. 1. Overview of the sampling locations in the Bijagós Archipelago in Guinea-Bissau. Sampling was conducted in five different regions: Urok (n = 35; red), Soga (n = 19; light blue), Rubane (dark blue), Bubaque (n = 28; orange), and Orango (n = 38; green). The island's upland (dark green), mangroves (green) and intertidal areas (yellow) are shown. The marine protected areas (MPAs) are outlined in green.

Table 1
The definitions of habitats assigned to each sample (see Leurs et al., 2023).

Habitat	Definition
Intertidal	Habitat that is submerged during high tide and emerges at low tide (generally shallower than 5 m in depth).
Gully	Water stream that is embedded in intertidal flats and/or mangrove forests (width of <250 m).
Minor channel	A water stream which is more than 250 m and <1 km wide, and has no direct connection to the ocean.
Main channels/ subtidal waters	Main water bodies with a width of over 1 km and with a direct connection to the ocean.

PALL filters, 47 mm Ø, 0.45 μm pore size). Depending on suspended material, we used multiple filters to filter a single 2-liter sample. As access to electricity during expeditions was not always guaranteed due to the remoteness of the field sites, each filter was subsequently stored in a Longmire's lysis buffer, which allows for sample storage without cooling (Williams *et al.,* 2016; Spens *et al.,* 2017; Taberlet et al., 2018). Sampling bottles, filter holders and funnels were sterilized using a 10% bleach solution in between sampling efforts by rinsing the materials for at least two minutes. To determine any possible causes of contamination during sampling and equipment sterilization, we took a negative control sample for each sterilized batch of equipment by filtering bottled mineral water (i.e. equipment blank). The filters of these controls were stored the same way as filters used for sample filtration.

2.3. *Metabarcoding of samples*

2.3.1. *DNA extraction*

Prior to DNA extraction, filters and buffer solution were merged for samples for which multiple filters were used to filter the 2L water samples (i.e. due to filter clogging). Filters and buffer solutions of these subsamples were merged in a 50 ml vial and were stored submerged in Longmire's lysis buffer. This process was conducted in an ultra-violet (UV) box with sterilized forceps at the genetics laboratory of the University of Groningen. Materials were sterilized using 50% bleach and subsequent rinsing with DNA-free water. Samples were then stored in the fridge (at about 2 °C) until DNA extraction. We applied a standard phase-separation and precipitation DNA extraction method based on phenol–chloroform (Minamoto et al., 2016). DNA quantities of every sample were determined using a spectrophotometer (Nanodrop 2000). Subsequently, DNA extracts were cleaned by gel extraction using the

Promega Wizard® SV Gel and PCR Clean-Up System. This clean-up step was necessary because of carry-over of PCR inhibitor originating from ingredients of the Longmire's lysis buffer. The obtained clean DNA was then used as the PCR template.

2.3.2. Primer details

For species identification in elasmobranchs, the fast-evolving, mitochondrial protein-coding gene NADH dehydrogenase subunit 2 (NADH2) has been successfully applied (Naylor et al., 2005, 2012). The universal elasmobranch primers of Naylor et al. (2005), binding to the ASN and ILE tRNA regions, target a 1,044 bp fragment of NADH2. To amplify a shorter fragment from eDNA samples with potentially degraded DNA, we used the ASN primer variant called 'ChimeraF' ('5-AAGGACTACTTTGATAGAGT-'3) (Naylor et al., 2005) in combination with two newly designed reverse primers yielding an amplicon of ca. 320 bp. The first reverse primer NADH2 'miniSharkR2′ ('5-GGAATRATGGCTAATGTGTT-'3) targets both sharks and rays, and the second reverse primer 'miniSharkR5′ ('5-CCTATTCAAACTAGGAGTC-'3) was specifically designed to target shark species. For subsequent sequencing the following tails were attached to the primer: 5′-GATGTGTATAAGAGACAG_Forward-primer-3′ and 5′-CGTGTGCTCTTCCGATCT_Reverse-primers-3′. We then used in-silico testing of the newly designed primers using the Geneious v8.0. software package. To do this we tested the primers on species from different shark (Hemigaleidae, Leptochariidae, Carcharhinidae, Sphyrnidae, Scyliorhinidae, Squatinidae, Triakidae, Hexanchidae, Alopiidae, Lamnidae, Odontaspidae, Ginglymostomatidae) and ray (Rhinidae, Glaugostegidae, Myliobatidae, Rhinobatidae, Dasyatidae, Torpedinidae, Pristidae, Rhinopteridae, Gymnuridae, Rajidae) families and optimized primers further by minimizing mismatches with reference sequences. No bias towards any shark and ray family was detected using this approach. In addition, we performed tests with the primers in the laboratory on common shark and ray species from the West African region (i.e. *Fontitrygon margaritella* and *Rhizoprionodon acutus*).

2.3.3. PCR and sequencing

Polymerase Chain Reaction (PCR) was set up in a DNA-free room. Each sample was amplified in triplicate to avoid PCR bias. AccuStart II PCR ToughMix© was used, as DNA in the collected samples may have been degraded due to biological processes or degradation caused by exposure to UV-light. The reaction volume was 10 μl including 5 μl AccuStart, 1 μl of each primer (10 μM), 1 μl ddH2O and 2 μl DNA template. The PCR profile was 3 min at 94 °C, followed by 35 cycles of 1 min at 94 °C, 30 sec at 48 °C, and 1 min at 72 °C, and a final extension at 72 °C for 10 min. Annealing temperature was set to 48 °C to minimize taxonomic bias (Ishii and Fukui, 2001). PCR products were sequenced on a MiSeq© (Illumina) Sequencer at the Department of Human Genetics, Leiden University Medical Center, with the aim for read-depth set at 50,000 reads per sample. Libraries were prepared with the MiSeq© V3 kit, generating 300-bp paired-end reads. Since the V3-kit does not normalize, i.e. leaves the relative presence of the initial PCR product intact, this library preparation method allows assessing the relative contribution of taxa to read abundance of each PCR product.

2.3.4. Lab controls

For each sampling period (before and after the rainy season) two negative extraction controls were included to test the Longmire's lysis buffer stock solution as a source of contamination: one with the first and one with the last batch of extractions. Additionally, negative control samples were taken from each PCR master mix to track possible contamination of PCR reagents.

2.3.5. Creation of OTU table

We extracted unique, high-quality barcode reads (molecular operational taxonomic units, abbreviated as OTU) using the software USearch 9.2 (Edgar, 2010). First, paired-end reads were merged to a consensus sequence, removing the sequencing adaptors. Primer sequences were removed by truncating each end by 25 bp, the length of the longest PCR primer. For the full dataset quality filtering was set at default E-value of 0.4 and read-truncation to 220 bp. To simplify clustering, truncated reads were de-replicated by assigning a count to unique reads, this way merging identical reads in both orientations. Subsequently, the singletons were removed (see e.g. Frøslev et al., 2017). Using the UPARSE-OTU algorithm (Edgar, 2010) reads that were minimally 97% identical were clustered. This was replicated using a threshold of 100% similarity for clustering and yielded no differences in detection of species. The consensus sequence of each cluster was assigned an OTU ID, resulting in a OTU sequence table. This algorithm also filters chimeras. For each sample the number of reads (paired and with primers truncated) that matched with each OTU was determined, resulting in an OTU frequency table. The default identity match of 97% was used. The final OTU frequency table was adjusted for the negative extraction and PCR controls, by deducting the number of reads found for an OTU in the pooled negative extraction controls from each cell in the OTU table. The final OTU table was blasted against the mitochondrial genome database of Chondrichthyes constructed and curated by the Florida Program for Shark Research (FPSR) at the Florida Museum of Natural History of the University of Florida (see Naylor et al., 2012). At the time of this analysis, the database contained 94% of known genera and 72% of known chondrichthyan species, plus potential new species and population-level variants. The database has been curated by taxonomic experts to exclude any wrongly identified haplotypes. During blasting only the match with the lowest E-value was retained for each OTU.

Of the 127 samples that we collected and sequenced, 58 (45.7%) contained elasmobranch DNA. A total of 886,097 reads, 88.1% (780,581) could be taxonomically assigned to 110 unique OTUs; 40 OTUs were assigned with high taxonomic certainty using percentage identity of $\geq$ 95% and query coverage of $\geq$ 85%. Of these, 25 OTUs were assigned to 13 elasmobranch species, accounting for 218,047 (24.6%) reads in total. The remaining 15 OTUs were assigned to teleosts (7.16% of reads; primarily *Sarotherodon melanotheron*), humans (0.04% of reads) and plant/bacteria (<0.01% of reads). Of these elasmobranch species, 11 ray species were identified accounting for 180,227 reads (82.7%) of the total number of target reads. Two shark species were detected, accounting for the rest of the target reads (17.3%). Elasmobranch reads per sample ranged from 0 to 6,521 (399.4 ± 976.2, mean ± standard deviation) after corrections for contamination.

2.4. Fisheries observer program

Data from a pilot fisheries observer program was used to compare to the number of species detected in the eDNA survey. From February to September 2021, a total of 122 small-scale fishing boats operating within the Archipelago were sampled in the main fishing port of Bissau. Of each boat, the elasmobranch catches were identified to species level and information on the fishing trip (e.g. fishing location, duration) was documented. To compare the fisheries observer data to the eDNA results only boats fishing within the Urok, Soga, Rubane, Bubaque, and Orango regions were included in the analyses (n = 44). Due to the limited sample size, species richness and composition between fisheries observer and eDNA data could only be compared on an archipelago level.

2.5. Statistical analyses

To minimize the influence of species presence due to cross-contamination, a species was considered to be present when the number of reads exceeded ten. To study the species composition across different variables (e.g. season, tidal phase, MPAs), we determined the frequency of occurrence for each species by dividing the total number of locations that a species was detected by the total number of sample locations. We used non-metric dimensional scaling to visualize species

composition of sampling locations at which at least one species was detected, and determined significant differences in relative species composition across the different seasons, protected areas and tidal phases using a permutational analysis of variance (PERMANOVA). We determined the species richness for each sampling location as the number of species that was detected (S). We used a generalized linear model with a negative binomial error distribution to determine the relation between species richness and predictor variables. We conducted a Tukey's range test to test for differences among sampling season and tidal phases. Since the number of reads for a specific species can be influenced by PCR conditions (Taberlet et al., 2018) or ecological events (e.g a deceased individual or reproduction/spawning, Barnes and Turner, 2016), we limited species-specific analyses to presence-absence. We used a general linear model with a binomial error distribution to determine the significance of independent variables in predicting the presence of a species. We included season, region, distance to nearest mangrove, distance to the Geba river entrance, habitat, and tidal phase as independent variables. The presence of a species was only modeled for species that were detected at 10 or more sampling locations, resulting in an exclusion of rare species from this analysis. Model selection was performed based both on the Akaike's Information Criterion (AIC) and Bayesian Information Criterion (BIC).

3. Results

3.1. Species presence and distribution

A total of 13 species were detected as part of our eDNA survey, with 7 (53.8%) of these species currently being listed as threatened on the IUCN Red List. The four most common species in the study area based on the total number of sample points that a species was detected are the pearl whipray (18.9%; N = 24 locations), smalltooth stingray (*Hypanus rudis*, 14.2%; N = 18 locations), scalloped hammerhead shark (*Sphyrna lewini*, 12.6%; N = 16 locations), and blackchin guitarfish (*Glaucostegus*

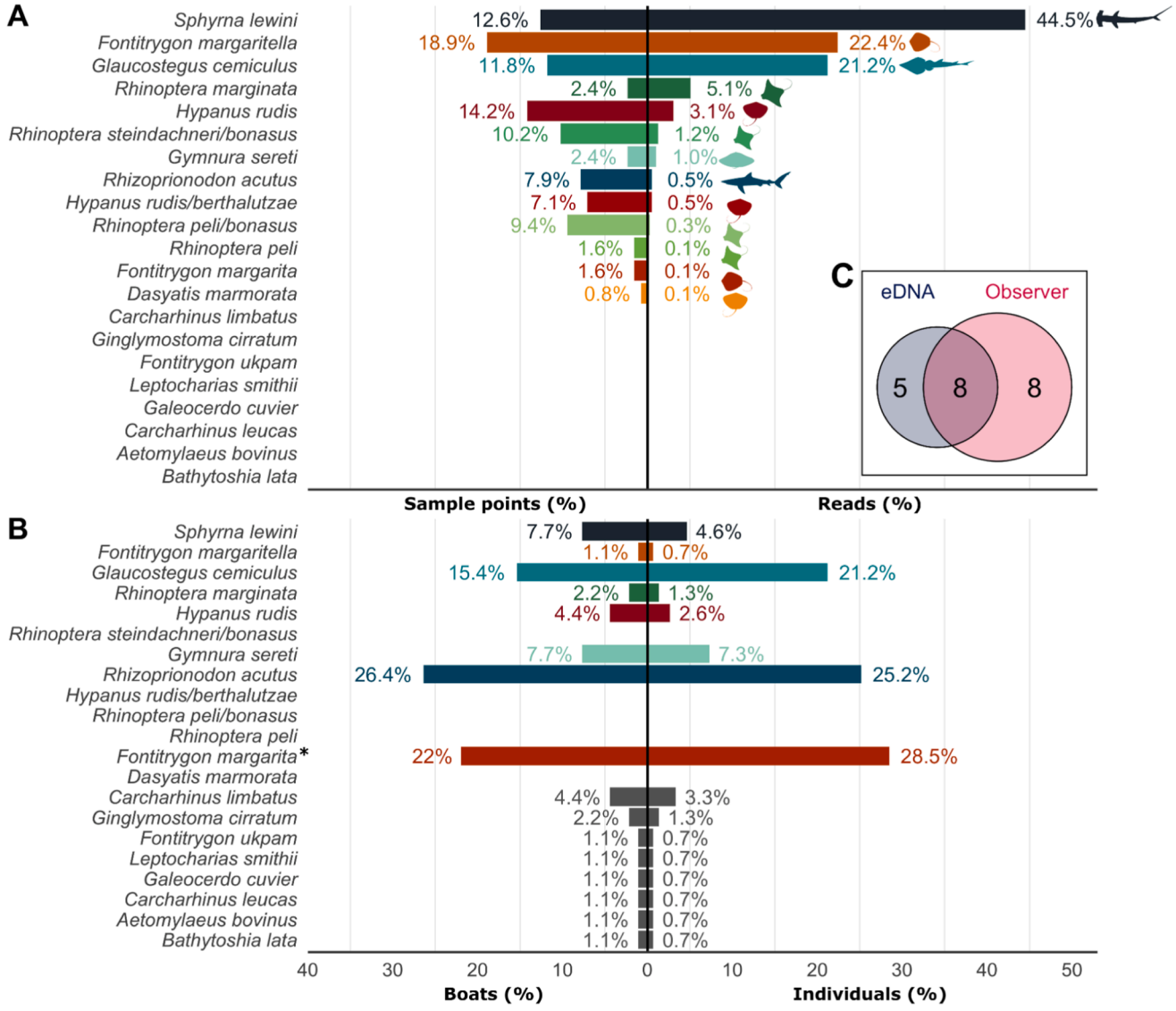

Fig. 2. The species that were detected using environmental DNA in 2019 as a proportion of sampling points (n = 127) that a species was detected and as a proportion of the total number of reads (A). The species observed during the fisheries observer program in 2021 are shown as a proportion of the boats that captured the species, and the proportion of the total number of individuals of elasmobranchs that were captured (B). Species were detected either by both methods or only by the eDNA survey or by the observer (C). Different colors indicate different species detected as part of the eDNA survey, with color tint indicating species group (sharks = blue, benthic rays = orange/red, benthopelagic rays = green, guitarfishes = light blue). Species only detected as part of the observer program are shown in gray. *Possibly includes observations of *Fontitrygon margaritella* due to misidentification.

cemiculus, 11.8%; N = 15 locations) (Fig. 2A). These four species were detected in the majority of study regions, except for the blackchin guitarfish and smalltooth stingray, which were not detected in the Rubane and Soga regions respectively (Fig. 3). Relatively rare species, such as the African cownose ray (*Rhinoptera peli*) and the marbled stingray (*Dasyatis marmorata*) were only detected in Urok and southern Orango (Fig. S1). When considering the total number of reads per species, the most common species were the scalloped hammerhead shark (44.5%), pearl whipray (*Fontitrygon margaritella*, 22.4%), blackchin guitarfish (21.2%), and the Lusitanian cownose ray (*Rhinoptera marginata*, 5.1%). This differed from fisheries-dependent information, as the most caught species were the milk shark (*Rhizoprionodon acutus*, 26.4%), daisy whipray (*Fontitrygon margarita* 22.0%, but likely includes *F. margaritella* due to frequent misidentification), blackchin guitarfish (15.4%), scalloped hammerhead shark (7.7%), and Seret's butterfly ray (*Gymnura sereti*, 7.7%) (Fig. 2B). The eDNA approach and observer program overlapped in documenting the presence of eight species, whereas five additional species were only detected with the eDNA approach and eight other species were recorded only in the catches of local fishers (Fig. 2C).

We determined that eDNA-based species richness within the study area ranged from 0 to 7 species per location, with a mean of 1.0 spp. (95% CI: 0.75–1.27 spp.) (Fig. 4). Seven of the species detected using the eDNA approach are classified as threatened based on the IUCN Red List: The milk shark (*Rhizoprionodon acutus*) and pearl whipray (*Fontitrygon margarita*) are listed as Vulnerable, the Seret's butterfly ray (*Gymnura sereti*) as Endangered, and the scalloped hammerhead shark, blackchin guitarfish, Lusitanian cownose ray (*Rhinoptera marginata*), and the smalltooth stingray are listed as Critically Endangered. The fisheries observer recorded an additional seven threatened species that were not detected using the eDNA approach. Of these, the blacktip shark (*Carcharhinus limbatus*), nurse shark (*Ginglymostoma cirratum*), barbeled houndshark (*Leptocharias smithii*), bull shark (*Carcharhinus leucas*), and brown stingray (*Bathytoshia lata*) are listed as Vulnerable, and the thorny whipray (*Fontitrygon ukpam*) and duckbill eagle ray (*Aetomylaeus bovinus*) as Critically Endangered.

3.2. Effects of season, protective status and habitat

We determined that both species richness and species composition differed significantly before and after the rainy season (Fig. 5A-C; richness: d.f. 1, $F = 4.46$, $p = 0.04$, composition: d.f. = 1, $F = 7.79$, $p < 0.01$), and that species composition differed between non-protected and protected areas when seasonality is taken into account. These seasonal differences are caused by a higher occurrence of the pearl whipray and the cownose ray *Rhinoptera steindachneri* cf. *bonasus* after the rainy season, and higher occurrence of the scalloped hammerhead shark and the blackchin guitarfish before the rainy season (Fig. 5B). This was supported by a higher detection probability of the scalloped hammerhead (d.f. = 1, $X^2 = 10.4$, $p < 0.01$) and blackchin guitarfish (d.f. = 1, $X^2 = 11.1$, $p < 0.01$) before the rainy season (Table S4).

Although we determined that both species richness and composition across protected and non-protected areas did not differ significantly (Fig. 5D-F), species composition differed significantly between protected and non-protected waters if seasonality is taken into consideration (d.f. = 1, $F = 2.29$, $p = 0.04$) (Fig. 5G). After the rainy season, species composition within the MPAs significantly differed from

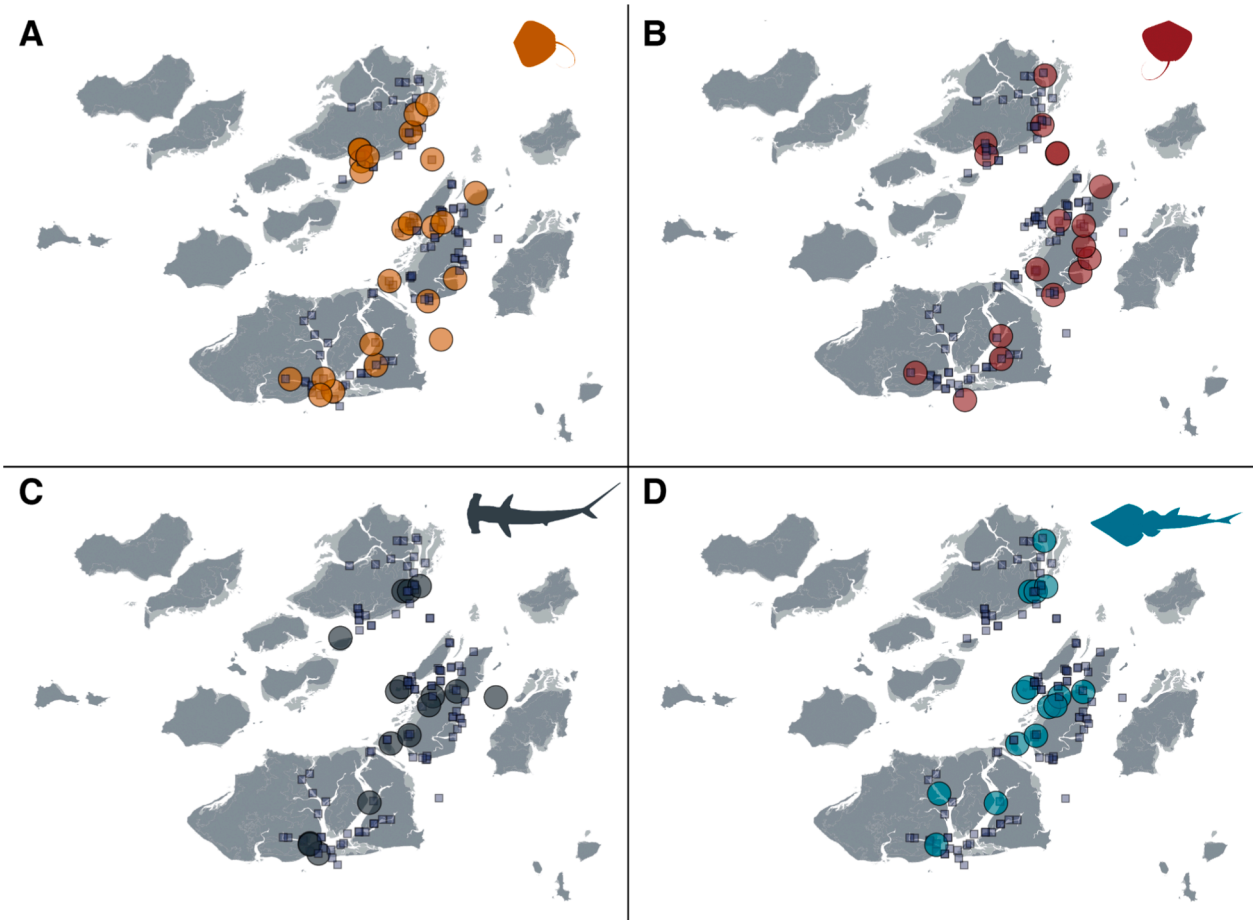

Fig. 3. The sample points where the four most common species were detected using eDNA: (A) pearl whipray (*Fontitrygon margaritella*), (B) smalltooth stingray (*Hypanus rudis*), (C) scalloped hammerhead shark (*Sphyrna lewini*), and (D) blackchin guitarfish (*Glaucostegus cemiculus*). Grey squares indicate locations where the species was not detected. The distribution maps of the remaining species detected in this study are shown in Fig. S1.

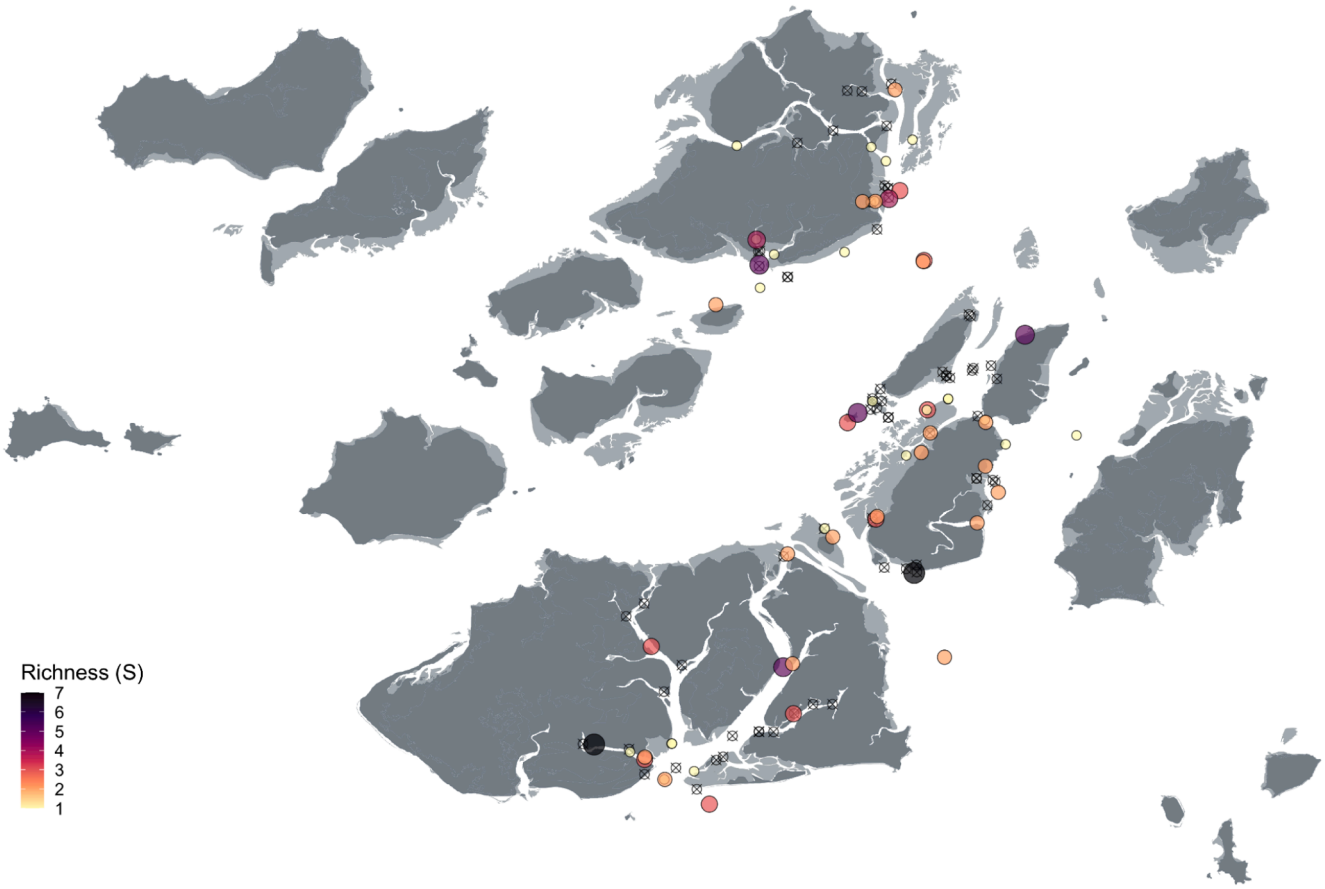

Fig. 4. Species richness - the number of detected species - for every sampling point within the study area. Sampling points with a low species richness are indicated by a small, yellow/orange point, and sampling points with a high species richness are indicated by dark purple colors and a larger point. Sampling points with no elasmobranch species detected are indicated with crossed dots.

locations outside the MPAs (d.f. = 1, F = 3.67, p = 0.03), but also from locations both in- and outside MPAs before the rainy season (d.f. = 1, F = 6.40, p = 0.001; d.f. = 1, F = 6.51, p = 0.002). These differences are caused by a higher occurrence of the pearl whipray within the MPAs after the rainy season, and the higher occurrence of the scalloped hammerhead shark and guitarfish before the rainy season in both protected and non-protected areas (Fig. 5H).

Species richness was influenced by the tidal phase (d.f. = 3, F = 3.75, p = 0.01), with the highest number of species detected in samples taken during incoming tide (1.59 ± 0.28 spp.) (Fig. S2). This coincides with the higher probability of detecting the most commonly detected species, the pearl whipray, during incoming tide (d.f. = 1, z = 2.18, p = 0.03) (Table S4).

Although the distance to the Geba River and habitat type had no significant influence on the species richness and detection probability of a species, the distance to the nearest mangrove forest had a significant influence on the probability to detect three ray species, the pearl whipray, blackchin guitarfish, and the cownose ray *Rhinoptera steindachneri* cf. *bonasus* (Fig. 6). Samples taken further away from the mangrove edge had a higher probability to detect the pearl whipray (d.f. = 1, X^2 = 4.5, p = 0.03) and *Rhinoptera steindachneri* cf. *bonasus* (d.f. = 1, X^2 = 5.9, p = 0.02), whereas the probability of detecting a blackchin guitarfish decreased when moving further from the mangrove edge (d.f. = 1, X^2 = 4.0, p = 0.05).

3.3. Sampling effort and storage

Lastly, increased sampling effort correlated with an increase in the number of species detected in our study. The maximum species richness (S = 13) was reached at 124 samples taken, which constitutes 96% of the total sampling effort of this study (Fig. S3). We also determined that extended storage times (0.03–7.2 h) due to remoteness of the study sites did not negatively impact the number of species detected (*Spearman* r = 0.09), but storage time had a slight negative correlation with the read abundance of a sample (*Spearman r* = -0.19).

4. Discussion

For effective marine conservation, information on species presence, richness and community composition is essential, especially in regions where resources for conservation are limited. In remote, highly dynamic and often highly turbid ecosystems like intertidal areas, resolving data-deficiency of a species group can be challenging as many other observational methods are either unsuitable or require high research and financial capacity. In this study, we aimed to solve data-deficiency of elasmobranch species in the remote and dynamic Bijagós Archipelago in Guinea-Bissau, comparing an eDNA approach with fisheries observer data. We confirmed the presence of 13 elasmobranch species (2 sharks and 11 rays, including 7 threatened species) in the Bijagós Archipelago using an eDNA approach, including the spatial distribution of these threatened species throughout the archipelago. An additional 8 species, including 7 IUCN threatened species were solely detected by the fisheries observer program. In addition, our study confirms that species composition and richness of the elasmobranch community is mostly influenced by seasonal changes related to changes before and after the rainy season, and less by differences between habitats (e.g. proximity to

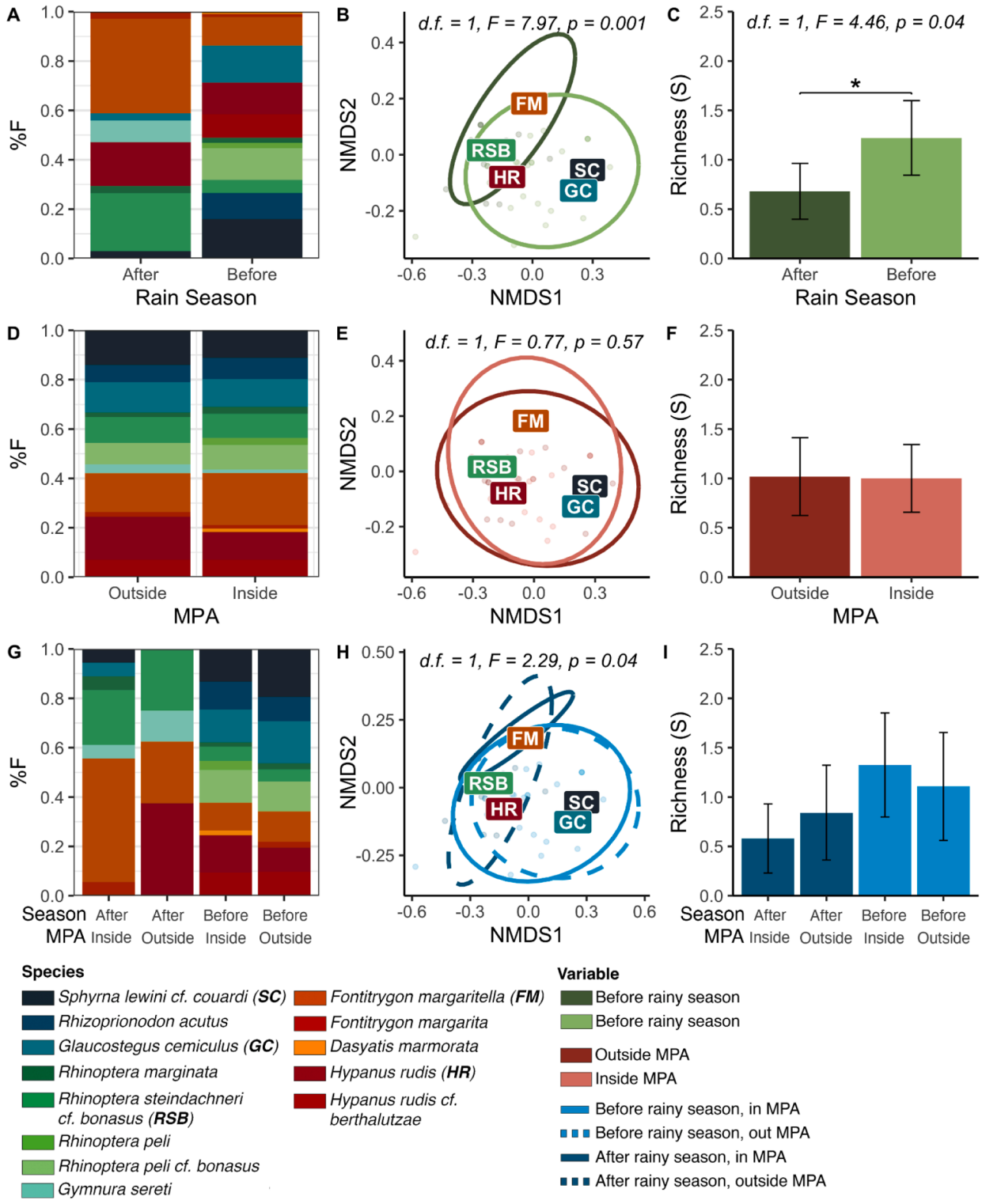

Fig. 5. The influence of season (A-C), marine protected areas (D-F) and their interaction between (G-I) on the frequency of occurrence of a species (%F; left column), species composition (NMDS; center column), and the species richness (S; right column). Species are indicated by their different colors, with the five most common species indicated in the NMDS (FM = *Fontitrygon margaritella*, RSB = *Rhinoptera steindachneri* cf. *bonasus*, HR = *Hypanus rudis*, SC = *Sphyrna lewini* cf. *couardi*, GC = *Glaucostegus cemiculus*).

mangroves and estuary) or protective status of the sampling area. Our results show that an eDNA approach can successfully be used to tackle data deficiency of the presence of threatened shark and ray species on a local scale in highly dynamic coastal areas, including the indication of priority areas for the conservation of critically endangered species.

4.1. Species presence and distribution

The four most commonly detected species, the pearl whipray, scalloped hammerhead shark, smalltooth stingray and the blackchin guitarfish, were detected on sampling locations throughout the

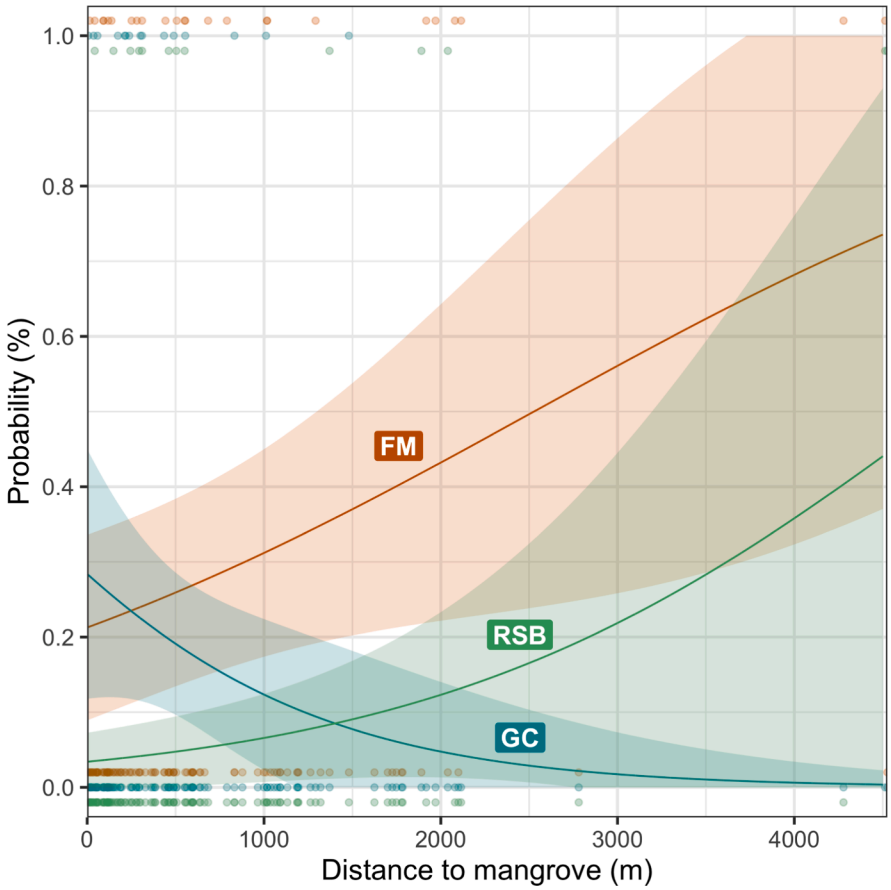

Fig. 6. The probability of detecting the pearl whipray (*Fontitrygon margaritella*, **FM**), the cownose ray *Rhinoptera steindachneri* cf. *bonasus* (**RSB**), and the blackchin guitarfish (*Glaucostegus cemiculus*, **GC**) with increasing distance from the mangrove edge.

archipelago. These results suggest that the Bijagós Archipelago is an important area for these elasmobranch species. Coastal areas are known to be important nurseries or feeding areas for many elasmobranch species (Knip et al., 2010). Intertidal areas such as the Bijagós Archipelago and the habitats it provides can play an important role as (seasonal) feeding refugia for (early life stages of) sharks and rays (Leurs et al., 2023). For example, the scalloped hammerhead shark is known to use shallow coastal areas during early life stages, before moving to a more pelagic habitat in deeper waters (Simpfendorfer and Milward, 1993, Zanella et al., 2019). This is confirmed by our preliminary results of the observer program that shows that the majority of scalloped hammerheads captured within the archipelago are immature (Leurs, unpublished data).

Like many elasmobranch species (Nagelkerken et al., 2008, White and Potter, 2004), the blackchin guitarfish likely rely on the extensive mangrove forests of the Bijagós Archipelago, and this benefit is potentially greatest for early life stages of this species. Our results show a higher probability to detect this species close to the mangroves, which coincides with the catches of newborns and young-of-the-year individuals close to the mangrove edge, suggesting this species uses the mangrove edge as a nursery habitat (Leurs, unpublished data). Alternatively, for the pearl whipray, all life stages are likely to use coastal areas, including intertidal habitats for feeding (Clements et al., 2022, Nauta *et al.,* submitted). The relative abundance of the species is potentially site-specific, as the pearl whipray is one of the most captured species in other coastal areas in The Gambia and Senegal (Moore et al., 2019, Jabado et al., 2021), but catches in the Banc d'Arguin in Mauritania are low (Lemrabott, 2023).

Rare species like the largetooth sawfish (*Pristis pristis*) and African wedgefish (*Rhynchobatos luebberti*) were not detected in this study. Sawfishes are considered to be extinct from the West African region, with the last documented sawfish record originating from 2004 from the Bijagós Archipelago (Robillard and Seret, 2006, Diop and Dossa, 2011, Leeney and Poncelet, 2015). Observations of the African wedgefish are increasingly rare within the region (Moore, 2017), however recent in-depth interviews and photographic evidence with fishers confirmed the capture of one specimen in March 2021 in the Bijagós (Leurs *et al.,* unpublished). Novel eDNA approaches have a higher sensitivity for species-specific detection of rare and cryptic species compared to the approaches used in our study (e.g. Droplet Digital PCR with species-specific primers, see Lehman et al., 2020). However, the discovery of rare and cryptic species in aquatic environments can also require an increase in sampling effort, for example increasing the size of the sampling area or a larger volume of water filtered (Bessey et al., 2020).

4.2. Season, habitat and protective status

We showed that species composition and richness of elasmobranch communities in the Bijagós Archipelago are mainly influenced by seasonality, with a higher species richness before the rainy season resulting in a different species composition across the two seasons. The rainy season in the Bijagós Archipelago causes an influx of freshwater between June and October, significantly lowering salinity levels (Cross, 2014, Campredon and Catry, 2016). As salinity can be one of the most important drivers of elasmobranch species composition in estuarine areas (Plumlee et al., 2018), it is likely that the observer changes are caused by changes in the abundance of species. In the Bijagós Archipelago the differences between the two seasons are likely caused by the

blackchin guitarfish and scalloped hammerhead shark, of which the presence was significantly lower after the rainy season. That the movements of hammerhead sharks and guitarfishes can be influenced by changes in precipitation has been confirmed for other coastal areas (Hensley et al., 1998, Corgos and Rosende-Pereiro, 2022). However, an increase in precipitation has also been linked to an increase in availability of crustaceans, the main prey of many guitarfishes (Lara-Mendoza et al., 2015). A decrease can also cause elasmobranch species to move away from coastal areas due to higher metabolic rates associated with maintaining osmoregulation (Meloni *et al.,* 2002). Our results suggest that the blackchin guitarfish and scalloped hammerhead shark possibly move to waters with a relatively higher salinity during or right after the rainy season.

Tidal phase influenced the number of species detected in a sample, with samples taken during incoming tide resulting in a higher species richness. This was likely partly due to the increased detection of the most common species, the pearl whipray, in samples taken during high tide. Other studies including the influence of tidal phase show contrasting results showing no to little effect of tides on eDNA and presence of species (Kelly et al., 2018, Jeunen et al., 2019, Lafferty et al., 2021). These studies suggest that eDNA-approaches can therefore be used as an appropriate method to determine relatively small-scale habitat use by species. However, our results show that tidal regime should be taken into account for sampling design and allow for comparison of study results between tidal phases. The most likely explanation for the observed differences in eDNA results between tidal phases are methodological (e.g., water mixing across adjacent habitats like mangroves or the pelagic), instead of ecological (e.g., differences in species presence).

The distance to the Geba river entrance was found not to have a significant influence on the species richness or presence of any species, which could also be caused by further tidal mixing throughout the archipelago. However, the distance to mangroves had a significant effect on the detection of three species. Both the pearl whipray and cownose ray *Rhinoptera steindachneri* cf. *bonasus* were negatively correlated with an increase in distance from the mangrove forest edge, whereas the detection of blackchin guitarfish was more likely closer to the mangrove edge. This is supported by earlier findings of newborn and young-of-the-year blackchin guitarfishes in shallow waters in close proximity to the mangroves (Leurs *et al.,* unpublished data), and other studies that confirm the use of mangrove edges by early life stages of elasmobranchs (e.g., Guttridge et al., 2012).

Lastly, our results show that elasmobranch species richness and composition was similar between samples taken from protected and non-protected areas. This can be explained by the influences of horizontal water mixing due to tidal currents (Miya, 2022), or by the mobility of shark and ray species, which likely move between protected and non-protected areas within the archipelago. Another explanation that no differences were found between protected and non-protected waters is the continued (targeted) fishing of elasmobranchs within both areas (Moranghajogo, 2012**)**. These results suggest that the eDNA approach can be used successfully to determine changes in species composition of elasmobranch communities across seasons and habitats in dynamic coastal areas.

4.3. *eDNA-based monitoring of elasmobranch communities*

The eDNA approach and fishery observer program differed in the number of species that were recorded, suggesting that a combination of monitoring methods is recommended for a complete overview of the elasmobranch community in highly dynamic (coastal) environments (Polanco Fernandez et al., 2021). eDNA-approaches have been described to resolve the phantom diversity of sharks and rays (Ip et al., 2021). However, in our study no shark species of the genus *Carcharhinus* was detected using the eDNA approach, despite species from the genus being recorded amongst catches of the small-scale fisheries. All species that were recorded in the fisheries observer program and that were not detected using the eDNA-approach were present in the reference database of the Florida Museum for Natural History. Moreover, the large majority of DNA-reads assigned to elasmobranchs were assigned to ray species (82.7%). Possible explanations for differences in relative read abundance in eDNA-approaches are differences in mobility or site fidelity of species, physiological differences, or the use of the eDNA methodology itself. Sharks are generally thought to move over larger distances compared to benthic ray species (Braccini et al., 2016), this may cause DNA concentrations of more mobile species to be low compared to species with a high fidelity to the sample site. However, the differences in DNA shedding rates between species can also cause a bias in relative read abundance within marine communities (Tréguier et al., 2014, Stewart, 2019). Benthopelagic myliobatid rays (i.e. eagle rays) excrete considerably more mucus compared to other elasmobranch species (e.g. guitarfish, sharks) (Meyer and Seegers, 2012), possibly causing an imbalance in the detection of rays and shark species when the whole elasmobranch community is studied. However, another likely explanation for the differences could be caused by a lower occurrence of sharks within the archipelago due to their continued exploitation in and outside the archipelago. Differences in relative read abundances may also have a methodological origin. For example, PCR conditions might favor the amplification of specific species DNA (Miya, 2022). For this reason we used two different primers to amplify ray and shark DNA in each sample separately and pooled the PCR products prior to sequencing. Furthermore, the sampling design (i.e. sampled area and filtered sample volume) can influence the completeness of a species richness assessment, as an increase in water filtered increases the number of successfully detected species (Bessey et al., 2020). The selection of which genetic marker (i.e. reference library) and the length of PCR product used for the detection of elasmobranchs can also influence detection rates. Due to the degradation of eDNA, relatively short genetic markers have been used in combination with publicly accessible reference libraries for 18S and CO1 (e.g. Bakker et al., 2017). However, due to the continued taxonomical changes of elasmobranchs (e.g. Naylor et al., 2012), and misidentification of closely related species in public reference libraries, we used a short fragment of the mitochondrial NADH2 gene with sufficient resolution to distinguish expected species. The NADH2-gene was used previously to successfully target sharks using an eDNA-approach (Postaire et al., 2020). We used this approach as this allowed us to match eDNA results with the reference library curated by the Florida Museum of Natural History. This database was used to reassess the taxonomy of all known batoid species (see Last et al., 2016) and is updated continuously by experts in the field of elasmobranch taxonomy.

The storage time of samples (i.e. time between sample collection and fixation) can influence the read abundance (Eichmiller et al., 2016). Storage time was negatively correlated with read abundance in our study, whereas there was no correlation between storage time and the number of species that were detected in a species. This small negative correlation highlights the importance of adequate sample preservation and limiting storage time prior to filtration and sample fixation.

We emphasize that the translation of relative read abundance to relative species abundance should be done with caution, and recommend that - if likely factors influencing relative read abundance are not addressed - eDNA data should be translated into the presence/absence of species (Tréguier et al., 2014, Barnes and Turner, 2016, Stewart, 2019, Miya, 2022).

4.4. *Implications for elasmobranch monitoring and conservation*

The eDNA-approach used in our study successfully detected elasmobranch species throughout the study area, but failed to detect some species that were detected in the fisheries observer program. This highlights that different complementary methods can be used to determine and monitor elasmobranch community structure. We show that

eDNA approaches can be used to determine species presence in highly dynamic and remote study site in relation to seasonal and other environmental factors. Other studies have concluded that the combination of eDNA-approaches with conventional monitoring methods such as the collection of fisheries data, underwater visual census and (baited) video monitoring can improve the quality of collected data (Boussarie et al., 2018, Budd et al., 2021, Ip et al., 2021). Conventional methods often underestimate the presence of cryptic and rare elasmobranch species, these methods may be selective to specific species (e.g. due to elusiveness or selection bait used), or are less suitable to be used in specific areas (e.g. due to limited underwater visibility or a lack of fisheries to monitor). Hence the locality of the study area and elasmobranch community at hand determines which combination of monitoring methods is most appropriate, also taking the study objectives into account.

Our results suggest that the large majority (54%) of shark and ray species detected in this study are threatened with extinction on a global scale. In addition, in more than half of the samples collected no shark or ray DNA was detected, and only two shark species were identified based on the eDNA-approach, the milk shark and scalloped hammerhead shark. Elasmobranchs within the wider West African region are at risk from being caught within coastal areas like the Bijagós Archipelago by artisanal small-scale fisheries (Lemrabott, 2023, Moore et al., 2019), or by industrial fisheries on the outer edges of these areas once certain species leave their coastal habitats (e.g. ontogenetic habitat shifts/migrations) (Zeeberg et al., 2006, Leurs et al., 2021). The fishing effort of both types of fisheries has increased over the past decade and is a threat to the conservation status of sharks and rays within the wider West African region (Campredon and Cuq, 2001, Diop and Dossa, 2011, Kroodsma et al., 2018). We show that the eDNA-approach can be used to successfully determine the spatial distribution of threatened elasmobranch species in highly dynamic intertidal ecosystems. These results can support area-based conservation strategies for elasmobranchs or can be incorporated in the management of existing MPAs. Furthermore, our results show that the presence of critically endangered species, such as the blackchin guitarfish, is linked to mangrove forests. It is results like these that highlight the importance of mangrove conservation for coastal elasmobranch species (Nagelkerken et al., 2008), and can be used to revise fishing practices taking place in close proximity to the mangrove edge to minimize accidental bycatch of elasmobranch species.

Our results show that an eDNA-approach to elasmobranch monitoring can successfully be used in highly dynamic coastal areas with continued high exploitation of elasmobranchs to address the data-deficiency on elasmobranch presence, distribution and community composition. Especially, when this method is combined with conventional monitoring methods such as the collection of fisheries-dependent data. The information of this novel combination of techniques provides solid evidence on the distribution and status of threatened shark and ray species that can inform the management of elasmobranch species in remote and highly dynamic coastal ecosystems.

CRediT authorship contribution statement

Guido Leurs: Conceptualization, Methodology, Software, Validation, Formal analysis, Writing – original draft, Writing – review & editing, Visualization, Funding acquisition. **Yvonne I. Verkuil:** Methodology, Software, Validation, Data curation, Writing – review & editing. **Nadia Hijner:** Methodology, Writing – review & editing. **Franziska Saalmann:** Methodology, Writing – review & editing. **Lilísio Dos Santos:** Methodology, Writing – review & editing. **Aissa Regalla:** Methodology. **Samuel Ledo Pontes:** Methodology. **Lei Yang:** Software, Validation, Resources, Data curation, Writing – review & editing. **Gavin J.P. Naylor:** Software, Validation, Resources, Data curation, Writing – review & editing. **Han Olff:** Supervision, Writing – review & editing, Funding acquisition. **Laura L. Govers:** Conceptualization, Supervision, Writing – review & editing, Funding acquisition.

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 on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ecolind.2023.110795.

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