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Of Biogeography, Fishes and Kelp: Environmental DNA Metabarcoding the Great African Seaforest

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ABSTRACT

Aim: The biodiversity associated with kelp forest ecosystems is understudied globally, including in South Africa, where kelp forests have expanded their range under climate change. This study used environmental DNA (eDNA) metabarcoding to assess fish biodiversity across an expansive range (~1000 km) of South Africa's kelp forests, contributing to a contemporary fish biodiversity baseline. We examined whether this baseline reflects existing biogeographical patterns, including the leading edge of an expanding kelp forest.

Location: South Africa.

Methods: We conducted eDNA metabarcoding, targeting the mitochondrial 12S rRNA gene for fishes, on 192 aquatic eDNA samples collected at eight sites spanning two ecoregions (Southern Benguela and Agulhas) and three kelp forest types (Namaqua, Cape and Agulhas). Intra-site spatiotemporal samples were collected to capture variability in eDNA signals at small spatial (< 600 m) and temporal (~24 h) scales.

Results: In total 140 operational taxonomic units (OTUs) from 39 marine fish families were detected, with 40 OTUs resolved to genus and 25 to species, encompassing a wide range of functional groups. OTU richness increased from west to east and differed significantly ($p < 0.05$) between ecoregions. Communities were significantly different ($p < 0.05$) across all biogeographical and intra-site predictors. While OTU accumulation curves did not fully saturate, spatiotemporal sampling at sites with lower species richness captured a larger proportion of extrapolated diversity. Notably, De Hoop, located at the kelp forest's leading edge, exhibited particularly distinct community patterns.

Main Conclusions: This research demonstrates that eDNA metabarcoding effectively detects diverse fish communities in a marine biodiversity and climate change hotspot, revealing biogeographic and local structuring patterns. However, gaps in the 12S reference database limit taxonomic resolution, highlighting the need to expand reference sequences. Continued development of eDNA metabarcoding across a range of spatiotemporal scales will enhance our understanding of biodiversity dynamics in South Africa's coastal ecosystems.

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1 | Introduction

In an era of unprecedented biodiversity loss and global climate change, ecosystem-scale conservation is vital to address shifting ecological baselines across broad spatial and temporal scales (Pörtner et al. 2023). This is particularly relevant for nearshore marine ecosystems, which pose unique management challenges given their proximity to direct anthropogenic disturbance from both marine and terrestrial environments, in addition to the impacts of climate change (Crain et al. 2009; He and Silliman 2019; Lu et al. 2018). Kelp forests, as the most widely distributed marine biome, provide economic, social and ecological benefits, including fisheries provisioning, carbon sequestration, and habitat for diverse marine species (Blamey and Bolton 2018; Eger et al. 2023; Jayathilake and Costello 2021). However, they also exhibit unstable and often declining global abundance trends, with rapid, short-term fluctuations driven by local stressors such as temperature anomalies and herbivory (Bell et al. 2020; Hamilton et al. 2022; Krumhansl et al. 2016; Wernberg et al. 2023). These fluctuations can lead to hysteresis, where ecosystem recovery from degradation is slow or requires intervention (Eger et al. 2022; Hamilton et al. 2022). The unique dynamics of kelp forest ecosystems, combined with the high biodiversity characteristic of their complex three-dimensional habitat, complicate efforts to establish ecosystem baselines and monitor shifts over time (Hamilton et al. 2022; Krumhansl et al. 2016; Smale et al. 2023; Steneck et al. 2002).

Amid escalating anthropogenic impacts, South Africa's kelp forests remain a regionally significant component of the broader kelp ecosystems along southern Africa's coast, recognised for providing substantial ecological and socio-economic benefits (Blamey et al. 2015; Blamey and Bolton 2018; Majiedt et al. 2019). The southern African kelp forests include the major canopy-forming stands of *Ecklonia maxima* and *Laminaria pallida* along the southwestern coastline (Field et al. 1980; Rothman et al. 2017), forming a large-scale marine ecosystem popularly known as the Great African Seaforest (GASF). Spanning approximately 1000 km, the South African range of the GASF (Figure 1; Dunga et al. 2024) exists along the unique intersection of the Atlantic and Indian Oceans, resulting in distinct oceanographic regimes driving environmental clines from west to east (Griffiths et al. 2010; Sink et al. 2019, Dalongeville et al. 2022). The west coast is characterised by cool-temperate conditions from the northward flowing Benguela Current, as well as intensive upwelling driving increased nutrient availability (Hutchings et al. 2009). Comparatively, the warm-temperate south coast is influenced more strongly by the warm south westerly flowing Agulhas Current, which is characterised by more nutrient-poor conditions (Lutjeharms 2007). The interplay of the two currents along the southwest coast results in a dynamic oceanographic regime within the easterly distribution range of the kelp forest (Kirkman et al. 2016; Rouault et al. 2010; Smit et al. 2017).

The unique environmental conditions drive a distinct gradient in marine biodiversity, with well understood biogeographic patterns for coastal fish and invertebrate species along the coastline (Awad et al. 2002; Dalongeville et al. 2022; Griffiths et al. 2010; Turpie et al. 2000). Further, the southwest coast has been recognised for its high species and genetic diversity and is considered a priority area for conservation (Dalongeville et al. 2022; Pfaff et al. 2019). In addition to broad biogeographical patterns, South Africa's kelp forests have been suggested to encompass three biogeographical subtypes of kelp forests: the Namaqua, Cape and Agulhas Kelp Forests (Figure 1; Dunga 2019; Sink et al. 2019). Notably, there has been an eastward expansion of *E. maxima* along the south coast into De Hoop, which is likely associated with increased cold-water upwelling events, although the mechanistic environmental drivers remain unclear (Bolton et al. 2012; Rouault et al. 2010). The impact of this range expansion on kelp-associated biodiversity assemblages in this region has not been investigated, highlighting the need for spatiotemporal biomonitoring to better elucidate potential shifts in community structure across the range of the GASF, as observed in kelp systems elsewhere (Beas-Luna et al. 2020).

South Africa's kelp forests were extensively studied in the 1970s and 1980s, building a foundational understanding of the functioning of these unique ecosystems (e.g., through investigating their nutrient cycling), but little research was carried out on kelp forest-associated biodiversity (Branch and Griffiths 1988; Field et al. 1980; Stephenson and Stephenson 1972; Velimirov et al. 1977). Additionally, research has since become fragmented, with limited focus on ecosystem-wide dynamics, even as pressures on these habitats have increased (Blamey et al. 2015; Griffiths et al. 2010; Majiedt et al. 2019; Pfaff et al. 2019). Consequently, a contemporary baseline understanding of GASF-associated biodiversity, including fishes, remains incomplete. Additionally, distributional data for many species is lacking (Turpie et al. 2000; von der Heyden 2011), limiting the scope of long-term monitoring (Atkinson 2022; Blamey et al. 2015; Kirkman et al. 2021; Pillay et al. 2018) and hindering our understanding of connectivity patterns, such as those between protected areas (Solano-Fernández et al. 2012; von der Heyden 2009). For example, even among the relatively well-studied marine fishes, an estimated ~25% of the total endemic fish species remain undocumented, and distributional data for most species is limited, often consisting of just a single reference point within the region (von der Heyden 2011). Research also disproportionately focuses on commercially valuable species, while cryptic, small-bodied and endangered species are not always well represented (Griffiths et al. 2010; Guy et al. 2021; von der Heyden 2011).

A complementary monitoring technique that rapidly provides large-scale biodiversity data is essential for overcoming the detection limitations of fragmented and labour-intensive conventional subtidal surveys in the GASF (Blamey et al. 2015; Dafforn et al. 2016; Deiner et al. 2017). Environmental DNA (eDNA) metabarcoding surveys offer a promising tool for biodiversity assessments but are also subject to some constraints, including taxonomic assignment challenges where reference databases are incomplete or suffer from marker resolution limitations (Bani et al. 2020; Gaither et al. 2022; Courtaillac et al. 2024). In marine systems, eDNA-based studies have revealed fine-scale spatiotemporal species distributions (i.e., < 1 km over a few hours; Courtaillac et al. 2024; Ely et al. 2021; Jeunen et al. 2019; Port et al. 2016), whilst covering a broad spectrum of functionally diverse biodiversity (Aglieri et al. 2021; Lamy et al. 2021; Stat et al. 2017), and have even detected community shifts across biogeographical boundaries (Fraija-Fernández et al. 2020;

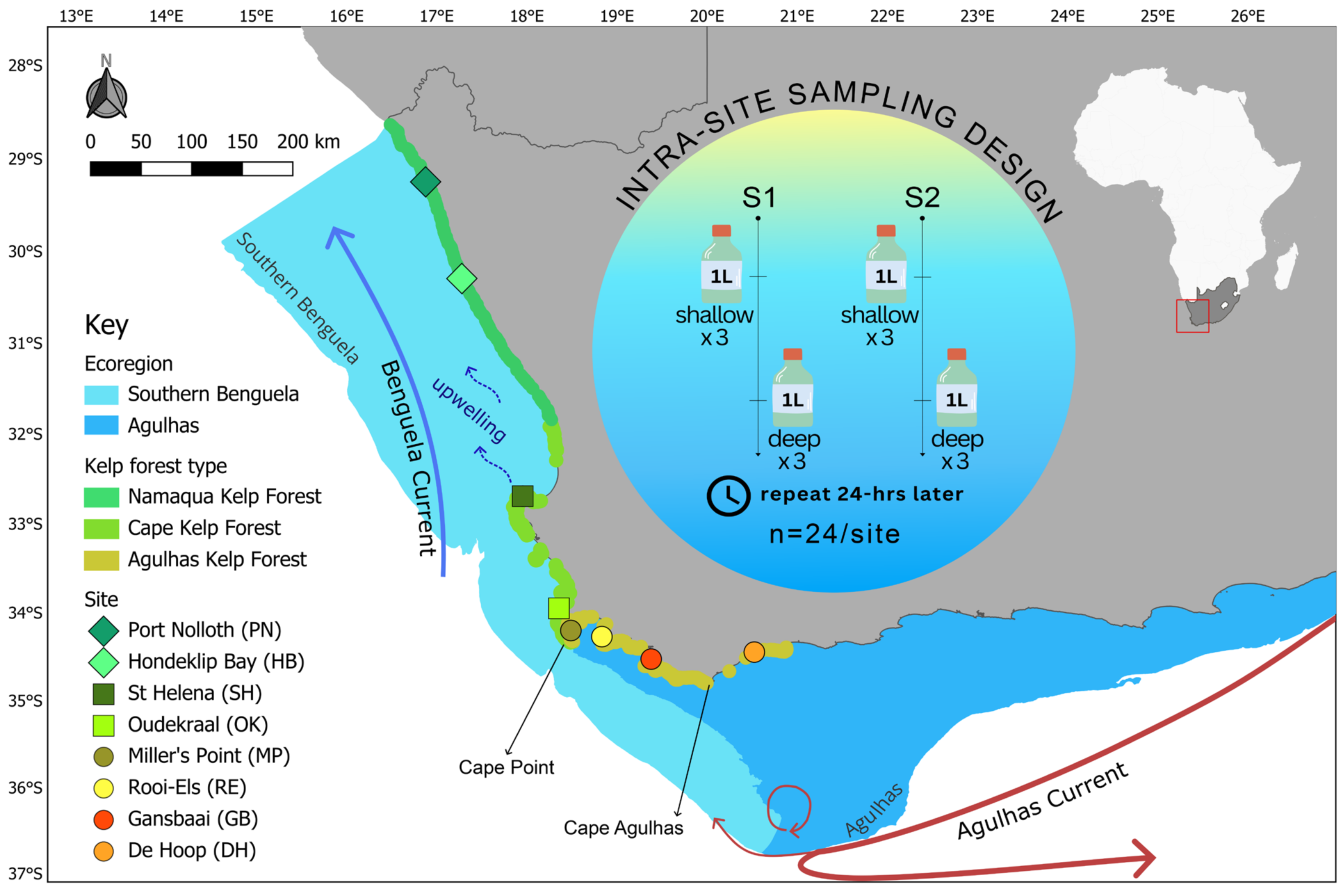

FIGURE 1 | Sampling locations for aquatic environmental DNA (eDNA) collection covered the entire range of the Great African Seaforest in South Africa. The sampling design captures biogeographical and intra-site variation in eDNA signals (Courtaillac et al. 2024). Sites were selected within both the Southern Benguela and Agulhas ecoregions, as well as the three kelp forest subtypes. Intra-site samples were collected at two stations along the shoreline (S1 and S2), at two depth ranges (shallow: ±1 m; deep: ±4 m) per station, repeated at two time points, ~24 h apart. The dominant currents of the region, the Benguela and Agulhas currents are indicated by arrows.

Naputo et al. 2024; West et al. 2021). Consequently, many regions globally are adopting eDNA-based surveys into national biomonitoring frameworks due to their scalable, non-intrusive and sensitive nature (De Brauwer et al. 2023; Kelly et al. 2023; Norros et al. 2022; Stepien et al. 2024).

However, eDNA surveys remain underutilised for ecological community monitoring within an African context (von der Heyden 2023), and only a few studies explore the spatiotemporal patterns of marine taxa, including fishes (Courtaillac et al. 2024; Czachur et al. 2022; Rossouw et al. 2024, 2025), as well as broader metazoan and bacterial diversity (Holman et al. 2021; Oosthuizen et al. 2023; Rossouw et al. 2024; Singh et al. 2021). This presents a clear opportunity to leverage eDNA surveys to extensively characterise community structure across the GASF, providing information which can support more effective management, for example, by potentially revealing the impacts of anthropogenic disturbances on coastal biodiversity (Bakker et al. 2017; DiBattista et al. 2020; Mathon et al. 2023).

As such, this study utilises eDNA metabarcoding to detect fish biodiversity across an expansive range (~1000 km) of South Africa's kelp forest ecosystems, contributing to a contemporary fish biodiversity baseline at the ecosystem-scale. Specifically, we (1) assessed the extent to which eDNA surveys could detect a broad array of fish diversity associated with the GASF and (2) differentiated fish community richness and composition between sites across distinct ecoregions and kelp forest types, thus providing a comprehensive review of fish community diversity across the GASF.

2 | Methods

2.1 | Sampling Design

Sampling was conducted over 1 month in the early austral winter (April–May 2023), covering eight sites spanning the South African distribution of the GASF. Sites were selected within the Southern Benguela and Agulhas ecoregions, as well as the three kelp forest types identified by Dunga (2019) (Figure 1). The sites Oudekraal (OK), Miller's Point (MP), Gansbaai (GB), as well as De Hoop (DH) at the eastern leading edge of the GASF range, are located within Marine Protected Areas (MPAs).

To account for variability in eDNA signals along the South African coastline (Courtaillac et al. 2024; Rossouw et al. 2024), intra-site sampling included triplicate 1 L seawater samples collected at two shoreline stations (S1 and S2; 200–600 m apart) and two depth ranges (±1 m and ±4 m) per station, which was repeated after 24 h (Figure 1). Sampling stations were selected primarily based on shoreline accessibility, and all samples were collected at low tide, approximately 1 m above the ocean floor. Permits were granted by the Department of Forestry, Fisheries and the Environment (XX), CapeNature (XX) and South African National Parks (XX).

2.2 | Processing Aquatic eDNA Samples

Each 1 L sample was filtered immediately after collection using a sterile 50 mL luer lock syringe to manually push the water through a Sterivex filter unit (Merck Millipore; pore size 0.22 μm). Post-filtering, each Sterivex unit was filled with 2 mL of ATL buffer (Qiagen) and stored at room temperature. Negative controls were prepared at each site (field blanks, $n = 8$) by processing 500 mL of sterile distilled water through a Sterivex filter unit and otherwise treated equally to the seawater samples for the remainder of the workflow.

DNA extraction was carried out in a PCR-free laboratory that was thoroughly cleaned using 10% sodium hypochlorite solution, with worktops and pipettes exposed to UV light for 30 min before and after setup. DNA was extracted directly from the filter units using the DNeasy Blood and Tissue kit (Qiagen) with a modified protocol (Czachur et al. 2022) that involved adding 70 μL proteinase K directly to the Sterivex filter unit and incubating at 56°C overnight. All DNA extracts were quantified using a Qubit dsDNA HS kit (Thermo Fisher Scientific) and stored at −20°C until PCR-based library preparations. Laboratory blank controls ($n = 2$) of sterile, UV-treated Milli-Q water were incorporated into the workflow to monitor potential contamination from the laboratory setup.

A total of 202 samples, including eDNA extracts ($n = 192$), field blanks ($n = 8$), and lab blanks ($n = 2$) were sent for library preparation and bioinformatic analyses at SimplexDNA, Switzerland. The hypervariable region of the 12S rRNA gene (163–185 bp) was targeted using the MiFish-U-F/R primers (Forward: 5′-GTCGGTAAAACTCGTGCCAGC-3′; Reverse: 5′-CATAGTGGGGTATCTAATCCCAGTTTG-3′; Miya et al. 2015). Full PCR-based library preparations and sequencing protocols are outlined in Appendix S1 in Supporting Information.

2.3 | Bioinformatic Analyses

Raw FASTQ files were demultiplexed using Ultraplex (Wilkins et al. 2021) for the first batch to validate indices, after which the MiSeq platform software was used for demultiplexing the second batch. Paired-end reads of both sample batches were processed together with a modified version of Apscale v1.6.3 (Buchner et al. 2022). Primer-trimming was performed using *cutadapt* (Martin 2011), merging, quality filtering, and dereplication with VSEARCH (Rognes et al. 2016), and operational taxonomic unit (OTU) clustering with SWARM (Mahé et al. 2022). The modified version of Apscale was run with the following parameters: maxEE was set to 2 (discarded reads with >2 expected errors), the minimum and maximum read lengths were set to 153 bp and 220 bp respectively (discarded merged reads that were not within the target amplicon length), and default parameters were used for paired-end merging, dereplication, clustering, denoising, and LULU filtering (Froslev et al. 2017).

Taxonomies were assigned to OTUs using BLAST searches (Altschul et al. 1997) against the MIDORI2 srRNA database (Leray et al. 2022) v256 (MIDORI2_UNIQ_NUC_GB256_srRNA_BLAST), with percentage identity cut-offs of 98 (species), 95 (genus), 90 (family), 85 (order), 80 (class), and 75 (phylum). Taxonomic assignments were manually reviewed, and unassigned OTUs were queried against the Genbank non-redundant nucleotide database using the BLASTn algorithm with a 98% identity cutoff. OTUs not assigned to marine fishes were removed. Assignments at all taxonomic levels were validated using FishBase, the World Register of Marine Species (WoRMS) database, Smith's Sea Fishes (Smith and Heemstra 1986), Coastal Fishes of the Western Indian Ocean (Heemstra et al. 2022), and local taxonomic expertise.

2.4 | Data Processing, Statistical Analysis, and Data Visualisations

All analyses and plots were performed in R version 4.3.1 (R Core Team 2023). Rarefaction curves were developed using *rarecurve* of the 'vegan' v.2.6–4 package (Oksanen et al. 2022) to assess sequencing effort. Most samples ($n = 173$) exhibited sufficient sequencing depth (see Appendix S2 and Figure S2.1), but OTU richness was only partially captured in 19 samples, which were excluded from further analyses.

Fish OTU richness and community comparisons were analysed using a semi-quantitative approach performed on a normalised dataset, using the eDNA Index model (Courtaillac et al. 2024; Kelly et al. 2019), which reduces potential primer biases in PCR amplification by comparing each taxon's read proportion in a sample to its maximum proportion across all samples. This normalises variations in amplification efficiency and facilitates comparisons in the relative abundance of a taxa between samples, allowing for more robust comparisons between communities. Observed OTU richness and community composition variations were tested between biogeographical and intra-site predictors (Figure 1 and Table 1).

Observed OTU richness per sample was calculated using the *specnumber* function from the 'vegan' package (Oksanen et al. 2022). To test differences in OTU richness across predictor variables, a univariate permutational multivariate analysis of variance (PERMANOVA; *adonis2* function; permutations = 999) was conducted, following the conversion of the data into a Euclidean distance matrix with the *vegdist* function. The PERMANOVA model was refined through a stepwise selection process. Batch effects were controlled by including batch as the first predictor and constraining permutations within batches using the "strata" argument in *adonis2*. This approach ensured that batch-related variation was accounted for before evaluating the proportion variance explained by other predictors and prevented permutations from mixing samples across batches. For significant PERMANOVA results, differences in sample dispersion were computed using

TABLE 1 | Biogeographical and intra-site predictors used to analyse variation in observed kelp forest fish OTU richness and community composition.

Predictor	Levels	Sample size
Ecoregion	Southern Benguela	85
	Agulhas	88
Kelp forest type	Namaqua	44
	Cape	41
	Agulhas	88
Sites	Port Nolloth (PN)	23
	Hondeklip Bay (HB)	21
	St Helena (SH)	17
	Oudekraal (OK)	24
	Miller's Point (MP)	24
	Rooi-Els (RE)	16
	Gansbaai (GB)	24
	De Hoop (DH)	24
/Day	D1	~12/site
	D2	~12/site
/Station	S1	~12/site
	S2	~12/site
/Depth	Shallow	~12/site
	Deep	~12/site

Note: Sample size varies for intra-site predictors due to removal of 19 samples with poor sequencing depth.
Abbreviation: OTU, operational taxonomic unit.

Levene's test (*leveneTest* function of the 'car' v.3.1–2 package; Fox and Weisberg 2023). Pairwise comparisons of OTU richness between sites was performed per batch using the *pairwise.adonis2* function of the pairwiseAdonis v.0.4.1 package (Martinez Arbizu 2020). Observed OTU richness was visualised as boxplots of OTU richness per sample within each site using the packages of 'phyloseq' v.1.41.1 (McMurdie 2013) and 'ggplot2' v.3.4.4 (Wickham 2016).

Variability in community composition across predictors was visualised using a three-dimensional nonmetric multidimensional scaling (NMDS) ordination with *metaMDS* function in the 'vegan' package, where a three-dimensional solution was selected to reduce stress and improve data representation. The best solution (i.e., with the lowest stress value) was chosen from 20 separate runs of real data. The Bray–Curtis dissimilarity index was applied, leveraging the relative abundances within the eDNA index, to represent compositional dissimilarity and proportional species turnover between samples. Plots were developed using the 'plotly' v.4.10.4 package (Sievert et al. 2024). Variability in community composition across the predictors was tested using a multivariate PERMANOVA (permutations = 999; method = Bray-Curtis). As in the univariate analysis, the multivariate PERMANOVA model was determined through a stepwise selection process, with batch effects accounted for in the analysis. For significant results, sample dispersion was tested using a permutational analysis of multivariate dispersion (PERMDISP; *betadisper* function of the 'vegan' package). Pairwise comparisons of community composition were performed between the three kelp forest types and eight sites using *pairwise.adonis2* (Martinez Arbizu 2020). To eliminate the influence of batch effect, kelp forest pairwise comparisons were performed using samples from batch B exclusively, and site pairwise comparisons were performed per batch. Where pairwise comparisons were significant, indicator OTU analyses were performed to indicate taxa driving differences in community compositions among predictors (De Cáceres and Legendre 2009; Dufrene and Legendre 1997), using the *multipatt* function of the 'indicspecies' v.1.7.15 package (De Cáceres et al. 2024). This approach calculates the specificity and fidelity of OTUs within predictor groups, where the highest specificity value ($A = 1$) indicates OTUs exclusively associated with the group of interest, and the highest fidelity value ($B = 1$) indicates OTUs present in all samples in the group. As such, OTUs with $p < 0.05$ and $A > 0.8$ were highly associated with the respective predictor-level community.

The extent to which the eDNA sampling protocol captured localised fish biodiversity was assessed using OTU accumulation curves. All samples from across days, stations and depths within a site were pooled to form a site-wide accumulation curve. A multi-model approach was implemented to fit asymptotes onto each regional accumulation curve, using the *specaccum* function of the 'vegan' package. The "random" method was used to generate 1000 accumulation curves. This approach considered six different accumulation models (Lomolino, Michaelis–Menten, Gompertz, asymptotic regression, logistic curve and Weibull growth curve) and weighted them using the Akaike information criterion (AIC; Aho et al. 2014). A weighted mean asymptote was determined that represented the theoretical maximum number of OTUs that could be detected at each site. The best-fit model selected from the lowest AIC value was then extrapolated to determine the number of samples required to detect 90% of the theoretical maximum number of OTUs, using the *predict* and *specslope* functions.

3 | Results

3.1 | Sequencing Effort

The Illumina MiSeq run resulted in a cluster density of 678 cluster/mm^2 and 97.89% ≥Q30. A total of 30,152,466 raw reads were merged, trimmed and filtered, resulting in 28,259,078 sequence reads mapped to an OTU. Non-fish taxa were detected, along with freshwater fish OTUs ($n = 43$), which were manually removed from the dataset (see Appendix S3). All contaminants (OTUs present within a negative control) and their relative proportions are listed in Appendix S3. Any OTU for which more than 0.001% of its total reads originated from a negative control was removed from the dataset. Reads per sample ranged from 2452 to 2,296,686 (mean = 156,928.7 ± 189,905.8). One sample from De Hoop was an outlier with 2,296,686 reads, and when omitted, the average reads per sample decreased to 144,488.2 ± 96,665.7.

3.2 | Taxonomic Assignments

A total of 140 marine fish OTUs were detected across all samples, with 113 assigned to order, 83 to family, 40 to genus, and 25 to species (Figure 2). All OTU reads belonged to 24 different orders of bony fishes (Actinopterygii), with no reads assigned to Chondrichthyans. The Blenniiformes and Spariformes were the most speciose orders, containing 44 and 16 OTUs respectively (Figure 2).

Sparidae (seabreams and porgies) was the most abundant family (Figure 2; 16 OTUs), representing 15.5% of the total reads across samples, including reads not assigned to a family (Figure 3). Other notable families included Mugilidae (mullets; 2 OTUs; 8.44% reads) and Clinidae (klipfishes; 17 OTUs; 8.12% reads). Families such as Syngnathidae (seahorses and pipefishes), Triglidae (gurnards), and Soleidae (soles) had lower abundances, each contributing less than 0.3% to the total reads (Figure 3). The indicator OTUs did not cluster clearly by site, and most indicator OTUs were from sites within the Agulhas ecoregion, particularly De Hoop ($n = 19$; Figure 2). Most of the indicator OTUs assigned to species-level have commercial importance, and some, such as *Trachurus* sp. (Horse mackerel) and *Epinephelus marginatus* (Yellowbelly rockcod),

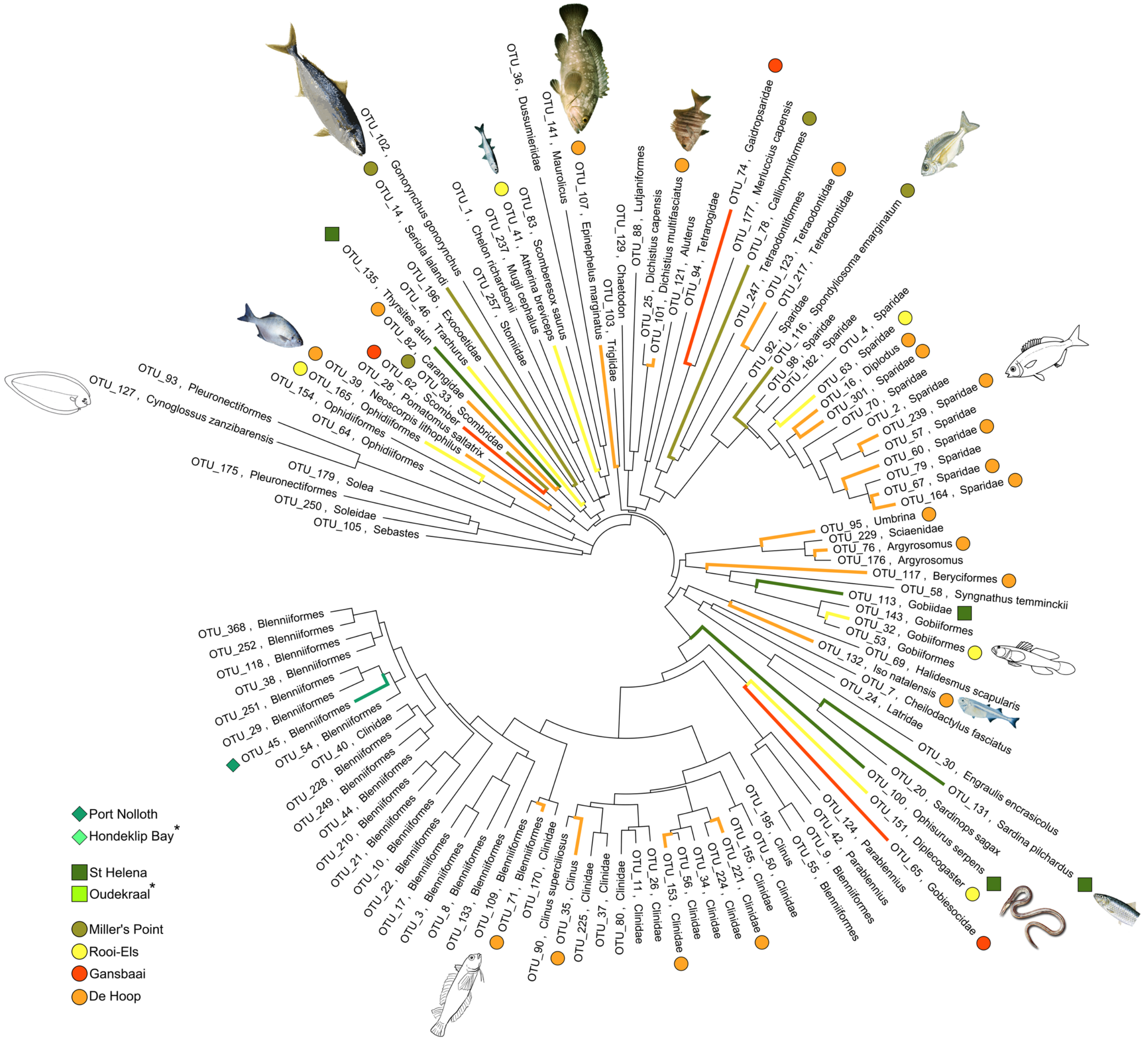

FIGURE 2 | Environmental DNA (eDNA) metabarcoding at eight sites across the South African distribution of the Great African Seaforest detected 140 distinct marine fish operational taxonomic units (OTUs). The OTUs belonged to 39 families, with 40 OTUs resolved to genus and 25 to species, encompassing a wide range of functional groups. The OTUs depicted in this neighbour-joining dendrogram ($n = 118$) are those assigned a taxonomic rank more specific than class. Branches of significant ($p < 0.05$) and highly specific ($A > 0.8$) indicator OTUs are colour-coded by sampling site, indicating their strong association with the respective site-level community. Coloured sketches of species-level indicator OTUs are included, non-coloured sketches correspond to the important taxonomic groups of Pleuronectiformes, Spariformes, Gobiiformes, and Blenniiformes. OTUs are labelled by their lowest taxonomic rank.*No indicator OTUs were detected at Hondeklip Bay and Oudekraal.

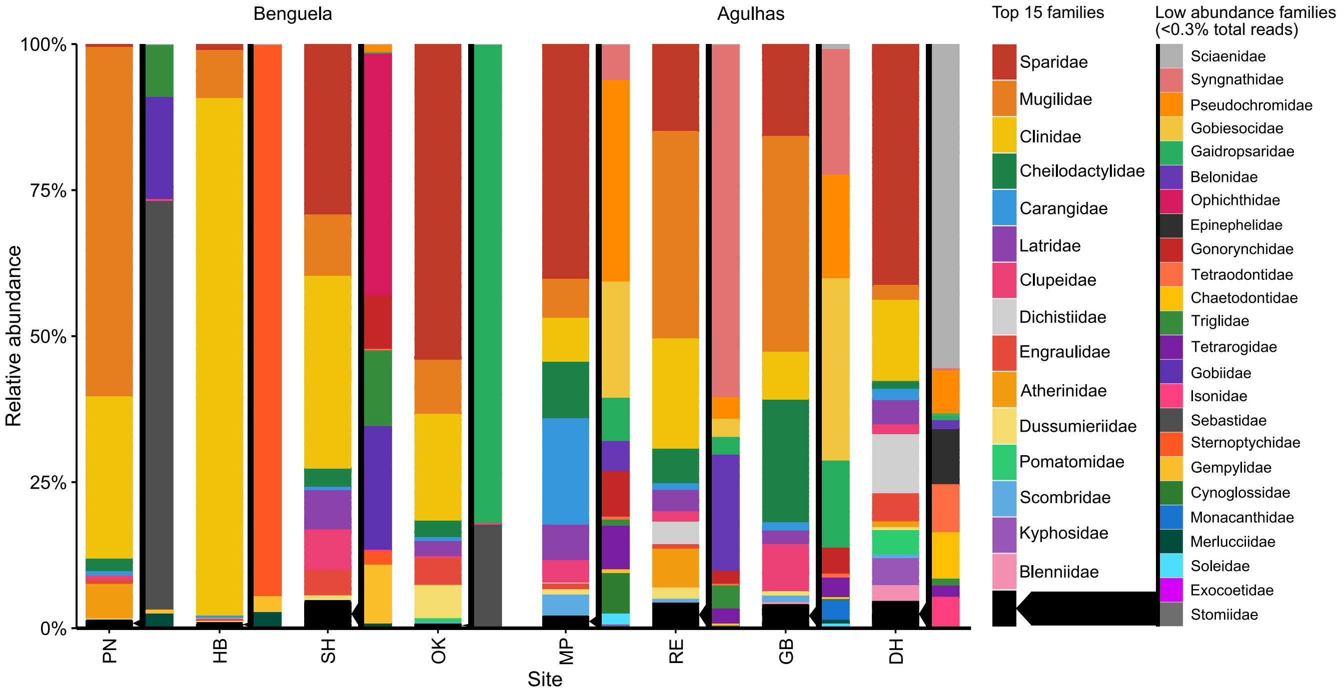

FIGURE 3 | Relative abundance of marine fish families across eight study sites of the Great African Seaforest. Each pair of bars represents a site (labelled from west to east), with sites grouped by ecoregion. The left bar in each pair displays the relative abundances of the top 15 most abundant families, with low-abundance families (< 0.3% total read counts) coloured in black. The right bar in each pair expands the low-abundance families for greater resolution. Abundances are calculated proportionally to total read counts per sample, including reads not assigned to a family, so as to not inflate family abundances in samples that possessed many unassigned operational taxonomic units (OTUs). Site codes for the Benguela ecoregion: Port Nolloth (PN), Hondeklip Bay (HB), St Helena (SH), Oudekraal (OK); and for the Agulhas ecoregion: Miller's Point (MP), Rooi-Els (RE), Gansbaai (GB), De Hoop (DH).

are listed as Vulnerable on the IUCN Red List. Approximately two thirds of these indicator species are resident species, while *Sardina pilchardus* (European pilchard, but likely a misidentification due to a lack of a reginal barcode), *Seriola lalandi* (Giant yellowtail), *Thyrsites atun* (Snoek) and *Trachurus* sp. are oceanodromous.

Due to the incompleteness of the 12S sequence database for the region (Courtaillac et al. 2024; Czachur 2021; Rossouw et al. 2024, 2025), manually reviewing OTU assignments was crucial to validate taxonomic detections and refine assignments to ensure consistency with regional biogeography. For example, four OTUs were assigned to *Sparus* spp. due to a ~95% sequence similarity, although the *Sparus* genus is monotypic and is not distributed in southern Africa. As such, these assignments were corrected to family-level (Sparidae). Similarly, OTU 116 was assigned to *Spondyliosoma cantharus*, a northern Atlantic species not found in southern Africa. However, the only other species in the genus, *Spondyliosoma emarginatum*, is endemic to the region but lacks a 12S reference barcode. Given this, we confidently reassigned OTU 116 to *S. emarginatum* rather than treating it as a false positive. Further, two OTUs were assigned to *Engraulis encrasicolus*: one with 100% sequence similarity and another with 98%. However, because *E. capensis*, a species endemic to southern Africa, lacks a 12S barcode, it is unclear whether the OTU with 98% similarity represents *E. capensis* or reflects population structure within *E. encrasicolus*. Given this ambiguity, the reads from both OTUs were combined and treated as a single OTU.

3.3 | Biogeographical and Intra-Site Patterns in the eDNA Signal

3.3.1 | OTU Richness

The batch effect was minimal but significant, explaining $<0.003\%$ of the variation in observed OTU richness ($R^2=0.00003$, $p=0.001$). Most of the variation in OTU richness was explained by ecoregion, where richness differed significantly between the Southern Benguela and Agulhas ($R^2=0.27431$, $p=0.001$; Figure 4a). Site as a main effect explained negligible variation in OTU richness across samples, and sites nested within each ecoregion did not significantly differ in OTU richness ($R^2=0.01542$, $p=0.290$). Richness increased across sites from west to east, with the exception of De Hoop (Figure 4a), where 90 OTUs were detected as compared to the 102 OTUs in Gansbaai. Within each site, OTU richness differed significantly across all three intra-site hierarchical sampling factors (Table 2), with day accounting for the greatest variation in richness ($R^2=0.12562$, $p=0.001$; Figure 4b). Additionally, significant interactions were observed between day and depth, and station and depth, indicating that variation in OTU richness between depths was influenced by both sampling day and station. The dispersion of fish OTU richness was homogeneous for all predictors except ecoregion, suggesting that, for most predictors, richness differences are primarily due to differences in centroid locations rather than variations in heterogeneity among samples (Levene's test; $p>0.05$, Table 2).

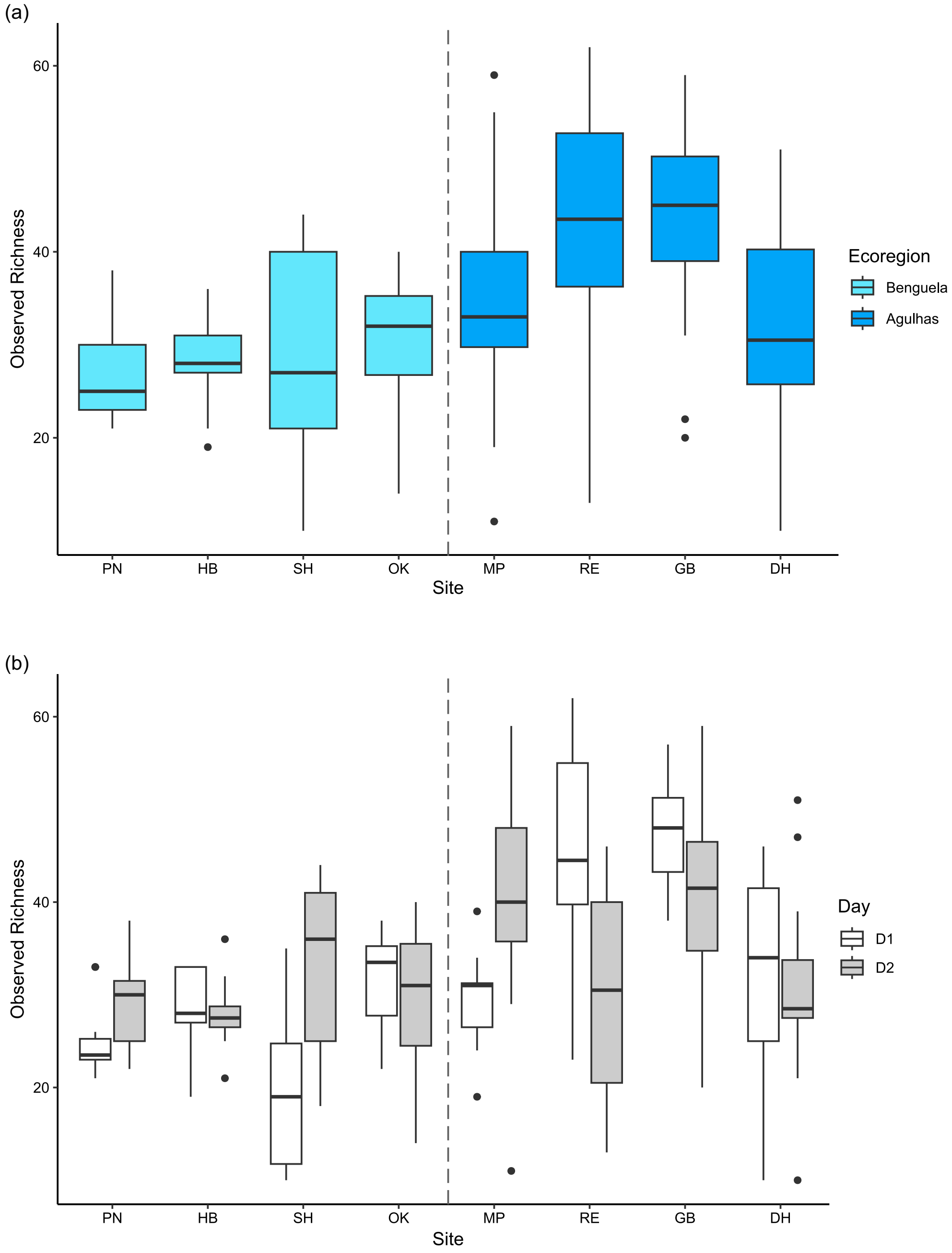

FIGURE 4 | Boxplots illustrating the observed fish operational taxonomic unit (OTU) richness among samples for each kelp forest site, ordered from west to east. (a) Most of the variation in OTU richness was explained by ecoregion, and richness differed significantly between the Southern Benguela and Agulhas. Richness increased across sites from west to east, with the exception of De Hoop. (b) Intra-site variation in OTU richness among spatiotemporal sampling points revealed that day accounted for the greatest significant variation in intra-site richness. Site codes for the Benguela ecoregion: Port Nolloth (PN), Hondeklip Bay (HB), St Helena (SH), Oudekraal (OK); and for the Agulhas ecoregion: Miller's Point (MP), Rooi-Els (RE), Gansbaai (GB), De Hoop (DH).

3.3.2 | Community Composition

The batch effect was significant and explained ~9% of the variation in observed OTU richness ($R^2 = 0.09369$, $p = 0.001$). Communities differed significantly between ecoregions ($R^2 = 0.11846$, $p = 0.001$; Figure 5) and between kelp forest types ($R^2 = 0.08688$, $p = 0.001$; Figure 5). Although site as a main effect explained minimal variation in community composition, significant differences were observed between sites nested within each ecoregion and their respective kelp forest types, accounting for the largest proportion of variation between samples ($R^2 = 0.19614$, $p = 0.001$; Figure 5). Pairwise analyses indicated that all sites and kelp forest types within respective batches exhibited significantly different community compositions ($p < 0.001$). Within each site, communities differed significantly across all three intra-site hierarchical sampling factors (Table 3), with day accounting for the greatest variation ($R^2 = 0.09909$, $p = 0.001$). Additionally, significant interactions were observed between day and depth, day and station as well as depth and station (Table 3), indicating

TABLE 2 | Summary table of the univariate PERMANOVA comparing variation in kelp forest fish OTU richness among key biogeographical and intra-site predictors.

Predictor	Df	SS	R^2	F	p	Dispersion
Batch	1	0.7	0.00003	0.013	**0.001**	0.6911
Ecoregion	1	5602.2	0.27431	108.561	**0.001**	**< 0.0001**
Ecoregion:Site	5	315.0	0.01542	1.221	0.290	
Ecoregion:Site:Day	8	2565.5	0.12562	6.214	**0.001**	0.5403
Ecoregion:Site:Station	8	1311.6	0.06422	3.177	**0.002**	0.6764
Ecoregion:Site:Depth	8	1834.7	0.08983	4.444	**0.001**	0.0535
Ecoregion:Site:Day:Station	8	766.0	0.03751	1.856	0.064	
Ecoregion:Site:Day:Depth	8	875.6	0.04287	2.121	**0.026**	
Ecoregion:Site:Station:Depth	8	1113.8	0.05454	2.698	**0.010**	
Residual	117	6037.8	0.29564			

Note: Final model after stepwise selection: Euclidean distance matrix ~ Batch + Ecoregion/Site/(Day + Station + Depth)^2; permutations, 999; strata, SampleMetadata$Batch. Significant values are indicated in bold ($p < 0.05$). Levene's test probabilities for homogeneity of dispersion are also shown for significant PERMANOVA results.

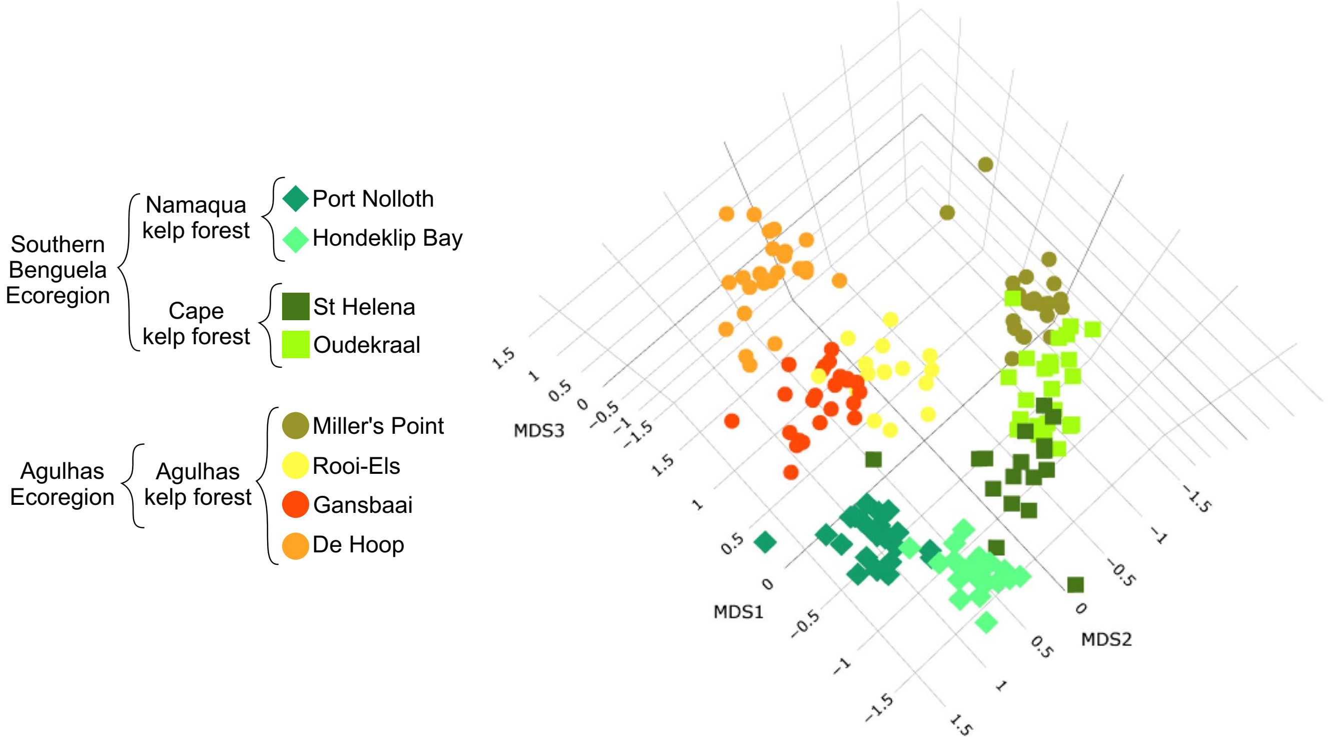

FIGURE 5 | Distinct communities are observed among predictor variables. Ecoregions, kelp forest types and sites exhibit clear community clusters with some overlap in this three-dimensional nonmetric multidimensional scaling (NMDS) ordination.

that community differences between depths and stations were influenced by sampling day, and that community differences detected at each station were influenced by depth sampled. Most predictors have variable dispersion among samples, which could be driving the differences in communities (PERMDISP; $p > 0.05$, Table 3). However, NMDS ordination suggested that both centroid and dispersion differences were likely driving the significant community differences among predictors (Figure 5, Figure S2.2 in Appendix S1).

3.4 | Regional-Scale OTU Accumulation

Accumulation curves varied across the eight sites, reflecting differences in both OTU diversity and sampling completeness. The weighted mean asymptotes, representing expected richness (R_E), ranged from 57 to 140 OTUs (horizontal solid lines; Figure 6). The site-specific accumulation curves, representing observed richness (R_O), capture various proportions of RE: PN = 95.26%, HB = 90.85%, SH = 84.35%, OK = 81.84%,

TABLE 3 | Summary table of the multivariate PERMANOVA comparing variation in kelp forest fish community composition among biogeographical and intra-site predictors.

Predictor	Df	SS	R^2	F	p	Dispersion
Batch	1	6.514	0.09369	50.585	**0.001**	**0.0016**
Ecoregion	1	8.237	0.11846	63.960	**0.001**	**< 0.0001**
Ecoregion:KelpForestType	1	6.041	0.08688	46.909	**0.001**	**< 0.0001**
Ecoregion:KelpForestType:Site	4	13.638	0.19614	26.475	**0.001**	**0.0375**
Ecoregion:KelpForestType:Site:Day	8	6.890	0.09909	6.688	**0.001**	**0.0136**
Ecoregion:KelpForestType:Site:Depth	8	2.073	0.02981	2.012	**0.001**	0.1050
Ecoregion:KelpForestType:Site:Station	8	4.041	0.05812	3.923	**0.001**	0.9774
Ecoregion:KelpForestType:Site:Day:Depth	8	2.315	0.03329	2.247	**0.001**	
Ecoregion:KelpForestType:Site:Day:Station	8	3.361	0.04834	3.263	**0.001**	
Ecoregion:KelpForestType:Site:Depth:Station	8	1.356	0.01949	1.316	**0.043**	
Residual	117	15.068	0.21670			

Note: Final model after stepwise selection: eDNA_index ~ Batch + Ecoregion/KelpForestType/Site/(Day + Depth + Station)^2; permutations, 999; strata, SampleMetadata$Batch. Significant values are indicated in bold ($p < 0.05$). PERMDISP probabilities for homogeneity of dispersion among samples are also shown for significant PERMANOVA results.

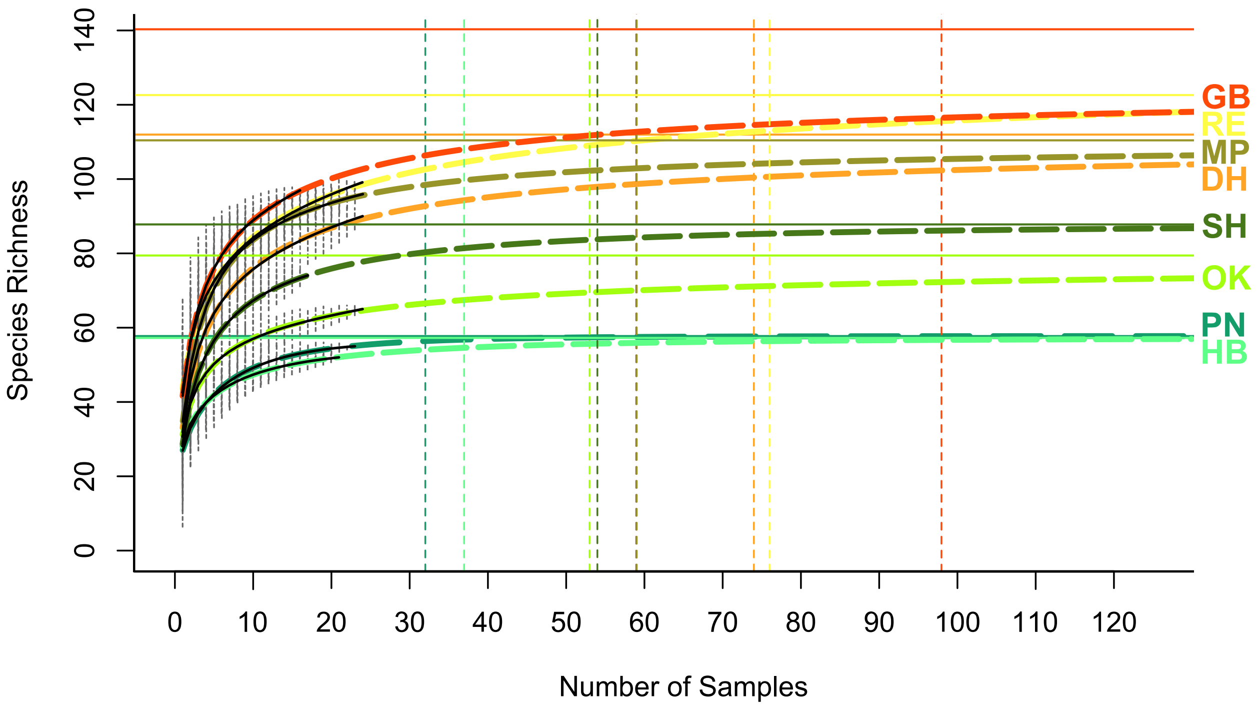

FIGURE 6 | Regional operational taxonomic unit (OTU) richness accumulation curves from environmental DNA (eDNA) samples collected within each of the eight kelp forest study sites. The black curves represent the observed accumulation of OTUs within each site (observed richness: R_O), while the coloured dashed curves show extrapolations to the expected richness (R_E) based on weighted mean asymptotes (solid horizontal lines), derived from multi-model fits. Vertical dashed lines indicate the estimated number of samples required to capture approximately 90% of the R_E. The colour of each curve and its associated lines corresponds to the site, with site codes labelled at the tail of each curve. Site codes according to location from west to east: Port Nolloth (PN, $n = 23$), Hondeklip Bay (HB, $n = 21$), St Helena (SH, $n = 17$), Oudekraal (OK, $n = 24$), Miller's Point (MP, $n = 24$), Rooi-Els (RE, $n = 16$), Gansbaai (GB, $n = 24$), De Hoop (DH, $n = 24$).

MP = 86.89%, R_E = 79.08%, GB = 72.62%, DH = 80.38%. None of the intra-site sampling efforts fully captured OTU richness, and the proportion of R_E captured decreased from west to east, except at De Hoop, which had the lowest OTU richness among the Agulhas sites. Extrapolating the accumulation curves to their respective asymptotes suggests that between 32 and 98 samples would be needed to capture approximately 90% of the R_E, depending on the OTU richness of each site (vertical dashed lines; Figure 6).

4 | Discussion

Kelp forest ecosystems in South Africa span approximately one third of the coastline, providing an extensive three-dimensional habitat of ecological significance (Blamey and Bolton 2018; Dunga et al. 2024). Due to the many benefits that kelp forests provide, such as supporting high levels of biodiversity (Blamey and Bolton 2018; Smale et al. 2023; Steneck et al. 2002), research and conservation interest in the GASF is increasing, although existing studies on faunal diversity within the system are spatiotemporally limited or primarily focus on individual taxa (Blamey et al. 2015; Katharoyan et al. 2024; Morris and Blamey 2018; Rossouw et al. 2024). To address this biodiversity knowledge gap, this study assessed the effectiveness of aquatic eDNA-based surveys in capturing fish community dynamics across the GASF. By leveraging eDNA, this approach detected functionally diverse fish communities, including species unlikely to be recorded together through other survey methods, at both broad ecoregion and localised site scales.

4.1 | Building a Fish Biodiversity Baseline for the GASF

An updated census of the biodiversity associated with the highly valuable and expanding kelp forest ecosystems of South Africa needs to be developed (Blamey et al. 2015; Katharoyan et al. 2024; Pfaff et al. 2019; Rossouw et al. 2024), along with ongoing monitoring of shifting baselines to understand ecosystem responses to escalating anthropogenic pressures and climate change impacts (Mihoub et al. 2017). To contribute to a contemporary fish biodiversity baseline in the GASF, this study conducted eDNA sampling across eight littoral sites encompassing the full biogeographic extent of the kelp forests.

A broad range of marine fish OTUs were identified across 24 orders, including common kelp forest-associated taxa such as *Dichistius capensis* (Galjoen) and *Cheilodactylus fasciatus* (Redfingers). The eDNA sampling approach also identified species that may have been overlooked in traditional surveys, such as the elusive *Ophisurus serpens* (Serpent snake-eel) and the small-bodied *Syngnathus temminckii* (African longnose pipefish). Additionally, this study captured both benthic (Gobiesocidae, Soleidae) and more mobile pelagic species (Sparidae, Carangidae and Clupeidae). These findings underscore the capacity of eDNA-based surveys to capture a wide array of diversity possessing a multitude of functional groups, including taxa that are typically underrepresented in biodiversity assessments, such as cryptic or small-bodied species (Aglieri et al. 2021). Moreover, beyond documenting species richness, this approach contributed valuable updates to OTU distribution data. For instance, the last recorded sighting of *Epinephelus marginatus* in De Hoop on GBIF was in 2012 (event ID: 3cc0860b-fc21-11e2-ac3f-081965b14959); this study now provides a more recent record of this species at the same location. While these findings enhance our understanding of biodiversity in the region, a significant limitation of the eDNA approach lies in the need for improvements to the regional 12S reference database, which is essential for more accurately resolving taxonomic diversity and distributions (Czachur et al. 2022; Rossouw et al. 2024, 2025; Singh et al. 2021). The identification of the majority of OTUs only at the order or family level within the Blenniiformes and Sparidae highlights the necessity for expanded 12S barcoding efforts targeting these important groups.

The intra-site spatiotemporal sampling design of this study was important for accounting for the ecology of eDNA, particularly its transport and persistence (Barnes and Turner 2016), enabling a more comprehensive representation of fish communities at each site. The consistent finding of significant differences in species richness and beta-diversity across intra-site predictors underscores how hierarchical spatiotemporal sampling can effectively detect fine-scale fluctuations in eDNA signals (Courtaillac et al. 2024). Further, increasing the number of spatiotemporal sampling points within each site also enhanced observed richness, allowing for the accumulation of larger proportions of expected richness. Overall, our sampling effort, of 192 L of water, identified 140 marine fish OTUs, representing ~17% of the marine fish recorded in FishBase for the upper 250 m of the Southeast Atlantic Ocean in South Africa (FishBase 2024). The limited temporal scope of this study means that seasonal variation in fish presence and uncertainties around the ecology of eDNA may have influenced detection rates, contributing to an incomplete sampling record. Additionally, spatial sampling restricted to littoral sites may potentially under-represent species associated with deeper reefs, offshore habitats, or highly transient species. However, upscaling of eDNA applications and species' barcoding regionally will contribute to a more comprehensive biodiversity baseline over time. This highlights the importance of increasing eDNA survey frequency and replication for improved biodiversity records going forward (Courtaillac et al. 2024; Czachur et al. 2022; Rossouw et al. 2024; West et al. 2024).

While our increased natural sampling effort improved detection reliability, it is recommended to address potential detection errors (false negatives and false positives) using robust statistical approaches and increased technical (PCR) replication (Ficetola et al. 2015, 2016). As eDNA biodiversity data for the system accumulates over time, Bayesian frameworks can help evaluate detection reliability by integrating prior information and cumulative data (Lahoz-Monfort et al. 2016). For instance, interpreting detections of offshore, pelagic species in a coastal nearshore ecosystem in the context of the local ecological community, along with combining local expertise and occupancy modelling, could help clarify whether these taxa represent transient or ecologically relevant presences within the target ecosystem (Gold et al. 2022). For example, in the case of the exclusive detection of *Sardina pilchardus* at St Helena, this may be a result of nearby fishing port activity, where occupancy modelling could provide a more robust understanding of whether this is a case of a false positive, or reflects the true presence of the signal through human-induced import into the system, or indeed is just a misidentification due to the lack of the 12S barcode for the regional species. This contextual approach can enhance our understanding of how stochastic events influence detected communities, such as the presence of larvae from adjacent marine habitats (e.g., deep-sea or offshore) (Andruszkiewicz et al. 2019;

Collins et al. 2022; Valdivia-Carrillo et al. 2021). Further, although the 98% species-level cut-off we utilised in this work is frequently reported in other studies (e.g., Boulanger et al. 2021; Van Nynatten et al. 2023), it will be necessary to review cut-offs used for taxonomic assignments for southern African marine species as more barcode and phylogenetic information becomes available. Future studies, alongside improvements in genetic reference data, are likely to revise the number of species reported from eDNA surveys and provide a more accurate assessment of marine biodiversity and its evolutionary dynamics in the region.

4.2 | Environmental DNA Surveys Detect Biogeographical Patterns of Marine Communities

The kelp forests of South Africa represent a broad ecosystem type that spans two oceanographically distinct ecoregions, each characterised by unique species assemblages (Griffiths et al. 2010; Sink et al. 2019; Spalding et al. 2007). At Cape Point, the Southern Benguela ecoregion transitions into the Agulhas ecoregion along the south coast, where species richness exhibits an eastward increase (Awad et al. 2002; Stephenson and Stephenson 1972; Turpie et al. 2000). This transition results in markedly different communities on either side of the peninsula for seaweeds (Smit et al. 2017), invertebrates (Awad et al. 2002), and fishes (Solano-Fernández et al. 2012), although the biogeographic breaks for various taxonomic groups shift along a “transition zone” between Cape Point and Cape Agulhas (Dalongeville et al. 2022; Sink et al. 2019; Teske et al. 2011; Turpie et al. 2000). This distinct biogeographical context offers a valuable opportunity to assess how effectively eDNA based surveys can capture biogeographical influences on fish community dynamics across the GASF.

In our study, species richness and community composition were driven by an ecoregional effect across the Southern Benguela and Agulhas ecoregions. Specifically, we observed a west-to-east richness gradient alongside distinct fish communities that align with well-established biogeographical patterns in the region (Bustamante and Branch 1996; Dalongeville et al. 2022; Field et al. 1980; Turpie et al. 2000). For instance, the increased relative abundance of Sparidae and Cheilodactylidae in the south coast sites reflects the presence of reef-associated endemic fishes in the warm-temperate Agulhas ecoregion (Field et al. 1980; Turpie et al. 2000), further reinforced by the multiple Sparidae OTUs detected as indicator OTUs at the south coast sites. Additionally, commonly subtropical species, such as *Dichistius multifasciatus* and *E. marginatus*, were highly indicative of the easternmost site, De Hoop. While the lack of indicator OTUs within the Southern Benguela sites may reflect an increased similarity in fish communities across the west coast, likely due to lower species richness (Bustamante and Branch 1996), the high relative abundances of Clinidae and Gobiesocidae observed within these sites align closely with established biogeographical patterns. For example, Prochazka and Griffiths (1992) documented that intertidal fish communities along the west coast were dominated exclusively by these families, indicating a characteristic composition for the region.

Notably, fish communities from Miller's Point, which lies within the Agulhas Ecoregion, clustered more closely with those from Oudekraal within the Benguela Ecoregion, with this cross-ecoregional clustering potentially influenced by multiple factors. One possibility is that eDNA transport across Cape Point has introduced a signal from Benguela-associated taxa into Miller's Point samples. Given the hydrodynamic complexity of Cape Point, where tidal exchange and localised currents can facilitate cross-peninsula transport (De Vos et al. 2021), some degree of eDNA mixing between adjacent ecoregions is plausible. This clustering pattern may also indicate that the biogeographical break for fish occurs further east, within False Bay, rather than at Cape Point. If so, this could support a more gradual transition in fish community structure across this region rather than a discrete ecoregional boundary (Dalongeville et al. 2022; Sink et al. 2019; Teske et al. 2011; Turpie et al. 2000). This presents an interesting avenue for future research, particularly in the context of potential shifts in ecoregional boundaries under changing environmental conditions. However, despite some uncertainties around bioregional clustering, our results reinforce the conclusions by Czachur et al. (2022) and Holman et al. (2021) that eDNA is effective in detecting biogeographical patterns in South African coastal ecosystems.

Emerging broad-scale community patterns were identified in this study, including the distinctive community structure at De Hoop, which exhibited lower species richness compared to its neighbouring site to the west and featured a unique suite of indicator OTUs. This disparity may be related to its position at the leading edge of the *E. maxima* population (Bolton et al. 2012; Dahms and Killen 2023; Pinsky et al. 2020). For example, Beas-Luna et al. (2020) documented kelp forest community shifts in response to climate change, noting that kelp and associated detritivores migrated into cooler regions, while higher-level consumer groups shifted equatorward. Should spatial mismatches arise among functional groups as the GASF expands its range, ecosystem resilience and long-term dynamics could be affected. Moreover, the west–east orientation of the South African coastline further complicates range-shift dynamics, as it restricts more straightforward north–south migration pathways observed elsewhere globally (Pinsky et al. 2020). As such, ongoing monitoring is essential to assess the drivers and impacts of community changes at the leading edge of the GASF.

Also contributing to the biogeographical knowledge of the region are the distinct fish communities detected between kelp forest subtypes, supporting their proposed delineation (Dunga 2019; Sink et al. 2019). Importantly, protection levels across these subtypes vary markedly (Adams and Kowalski 2021; Kirkman et al. 2021). For instance, parts of the Namaqua Kelp Forest shoreline remain inaccessible due to diamond mining operations (Blair 2011; Griffiths et al. 2004) and only one ~500 km^2 coastal MPA, Namaqua National Park, offers protection of this ecosystem subtype. In contrast, the Cape Kelp Forest benefits from protection by three MPAs that cover a combined area of ~1300 km^2 (Sixteen Mile, Robben Island, and Table Mountain National Park (TMNP)), while the Agulhas Kelp Forest is protected by four MPAs that cover a combined area of ~730 km^2 (TMNP, Betty's Bay, Walker Bay, and De Hoop). Further, although all our sampling sites fall into MPAs, these provide different levels of protection, with strict no-take MPAs (such as De Hoop) to those allowing controlled exploitation of fishes (e.g., Betty's Bay MPA and Table Mountain National Park), in addition to illegal exploitation within MPAs. Given these disparities in protection and exploitation, and the distinct communities observed between the subtypes in this study, there is a need for more evenly distributed biomonitoring efforts across the Namaqua, Cape, and Agulhas subregions, including investigating the dynamics between protection level and community diversity (Boulanger et al. 2021).

Expanding biodiversity baselines within the kelp forest subtypes is important to track nuanced shifts in communities in line with relevant environmental pressures (Bakker et al. 2017; DiBattista et al. 2020), such as overexploitation on the west coast (Griffiths et al. 2004), or regime shifts due to shifting species distributions along the south coasts (Blamey and Branch 2012; Roy et al. 2007). Future research examining the differences in biodiversity between protected and unprotected areas within each kelp forest subtype would prove valuable in guiding more representative protection across this ecosystem (Capurso et al. 2023; Gold et al. 2021; Kirkman et al. 2021).

5 | Conclusion

While there is a need for increased eDNA metabarcoding capacity throughout Africa (von der Heyden 2023), the eDNA survey approach proves highly valuable in this study. The breadth of data obtained underscores the power of eDNA surveys as complementary ecosystem-scale monitoring tools, as has been demonstrated in other regions globally (De Brauwer et al. 2023; Kelly et al. 2023; Norros et al. 2022). However, reference databases urgently need to be populated to enhance taxonomic resolution, allowing archived eDNA data to be more effectively utilised over the long term, to enable the compilation of extensive distributional data and support baseline maps for tracking changes in species and community distributions over time (Bani et al. 2020; West et al. 2021). Additionally, there remains a critical need to develop an eDNA monitoring network in South Africa, alongside regulations governing the development and sharing of eDNA-derived data, to enhance its application within management frameworks (Hirsch et al. 2024; Shea et al. 2023; von der Heyden 2023).

Author Contributions

Conceptualization: Svdh, JL, KL; Data Curation: KL; Formal Analysis: KL; Funding Acquisition: Svdh, JL; Methodology; Investigation: Svdh, JL, KL; Project Administration: SvdH; Resources: SvdH; Supervision: Svdh, JL; Writing – Original Draft Preparation: KL, JL; Writing – Review; Editing: Svdh, JL, KL.

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Disclosure

Field study permissions: All sampling was conducted under permit granted by the Department of Forestry, Fisheries and the Environment (RES2023-49), CapeNature (CN32-87-24746) and South African National Parks (CRC/2023-2024/014—2023/V1).

Ethics Statement

The authors have nothing to report.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The original sequence data have been submitted to the NCBI SRA with accession number PRJNA1116967 and the full OTU table can be found in Supporting Information Table S3.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section.