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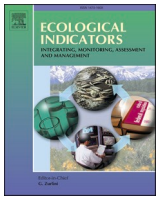

Environmental DNA reveals coastal fish biodiversity response across the Atlantic-Indian Ocean environmental transition gradient

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A B S T R A C T

The South African coast exhibits high spatial and environmental variability, which supports a diverse biodiversity of fish, and other marine species, providing valuable ecosystem services, including fisheries, nutrient cycling, habitat stabilization and tourism. However, ongoing anthropogenic pressure, including climate change and land-use alterations, are contributing to coastal biodiversity loss, necessitating the need for effective conservation strategies and a deeper understanding of the environmental factors driving marine biodiversity dynamics. Environmental DNA (eDNA) offers a non-invasive means to assess marine biodiversity and is becoming widely adopted as effective means to conduct ecological and biodiversity assessments . Here we looked to determine the spatial and environmental effects on coastal fish biodiversity across the entire extent of South Africa's coast using environmental DNA (eDNA). From 31 sites we detected 310 unique amplicon sequence variants (ASVs), representing 157 genera and 89 families. Genus, family and ASV richness all significantly increased with increasing temperature ($p < 0.01$), aligning with the west-to-east temperature gradient. Beta-diversity also significantly increased with difference in temperature ($p < 0.01$) and distance ($p = 0.01$), with turnover having a stronger influence compared to richness difference. We identified 22 key genera as indicators of spatial biodiversity change, and with hierarchical modeling provide insights into spatial and environmental influences on specific genera distributions along the coast This study underscores the utility of eDNA as a powerful, cost-effective, and non-invasive tool for capturing biodiversity dynamics across extensive spatial scales, offering a comprehensive understanding of the factors shaping coastal biodiversity and supporting global marine biodiversity monitoring and conservation efforts.

1. Introduction

The transition between the Atlantic and Indian Oceans zone, along the coast of South Africa, is characterized by strong environmental gradients, including temperature, salinity and productivity (Drake and Griffen, 2010; Griffiths et al., 2010). A combination of historical and contemporary processes along the South African coast have directly influenced evolutionary and biogeographic dynamics of many marine species (Dalongeville et al., 2022; Griffiths et al., 2010; Phair et al., 2019). As a result the coastline spanning South Africa is a known marine biodiversity hotspot, with levels of endemism for some taxonomic groups, including several invertebrate groups (Griffiths et al., 2010; Heemstra and Heemstra, 2004). However, coastal habitats along South Africa are also recognized as some of the fastest in decline due to effects of climate change and other anthropogenic factors (Hobday and Pecl, 2014; Mead et al., 2013).

Coastal fish communities are an integral part of the world's marine biodiversity, contributing significantly to the diversity of marine species and the ecosystem services they provide. Globally, many coastal communities are reliant on sustainable coastal fisheries for their livelihood, often as a primary food source (Tidd et al., 2023). Coastal fish biodiversity is vital for many African communities, directly supporting the livelihoods of over 2 million people, contributing ~ 20 % of the region's total fish production (FAO, 2020) and supporting ~ 28,000 small-scale fishers in South Africa (Macdonald, 2019). However, the sustainability of coastal fish biodiversity is increasingly threatened by overexploitation, habitat degradation, and climate change, underscoring the urgent need for effective management strategies to ensure their long-term viability and continued contribution to human welfare (Sowman, 2006; Virdin et al., 2023). Given the combination of environmental and anthropogenic pressures, including governance and law enforcement challenges (Taljaard et al., 2019), marine biodiversity management requires the development of effective strategies that can monitor and protect coastal fish biodiversity.

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South Africa marine fish biodiversity is exceptionally rich and diverse, with over 2000 species inhabiting a wide array of habitats, including rocky reefs, sandy shallows and estuarine systems (Heemstra and Heemstra, 2004; Whitfield, 2019), with 13–16 % of fishes endemic to southern Africa (von der Heyden, 2011). Fish species richness along the South African coast generally increases from west to east, with warmer tropical water hosting a larger number of species (Whitfield, 2019). There is also a noticeable change in biological community structure from east to west (Harrison and Whitfield, 2008), along with increased endemism, particularly along the south-eastern coastal area (Turpie et al., 2000). Temperature and salinity are suggested as important drivers of changes in the population structure of coastal species (Nielsen et al., 2020; Teske et al., 2019) and eukaryotic community dynamics (Dalongeville et al., 2022) along the South African coastline. While not extensively documented, species shifts in response to environmental variation have been recorded for sections of the coastline, including fishes (James et al., 2013; Potts et al., 2015) and invertebrate species (Whitfield, 2019), suggesting environmental clines may facilitate changes in biodiversity along the coast. However, effectively assessing fish biodiversity along the vast, ~2800 km coastline of South Africa is logistically and financially challenging with capture methods, such as trawling or line netting, potentially causing harm to targeted species during sampling and visual surveys likely to miss small or cryptic species.

Environmental DNA (eDNA) combined with high throughput sequencing provides an efficient and non-invasive means to assess biodiversity dynamics at large spatial and temporal scales (Gu et al., 2023; Hänfling et al., 2016; Seymour et al., 2021). Research has repeatedly shown that eDNA metabarcoding is an effective and reliable method to accurately assess biodiversity trends, and is a valuable tool to supplement biodiversity data (Seymour et al., 2020; Si et al., 2025; Stoeckle et al., 2020; Valentini et al., 2016). Recent efforts regarding applications of eDNA in South African coastal environments have shown eDNA based assessments are highly effective for detecting local and regional patterns of biological diversity across a number of taxonomic groups and marine environments (Czachur et al., 2022; Holman et al., 2021; Oosthuizen et al., 2023). South African eDNA based bioassessment is particularly important given South Africa's extensive and diverse coastal ecosystem, the heavy impact through anthropogenic activities in coastal areas (Mead et al., 2013), and the elevated effect of climate change on the area (Hobday and Pecl, 2014).

There is a limited body of research on the application of eDNA metabarcoding for aquatic biodiversity assessments across the African continent, with most studies focused primarily on regional or localized scales (Czachur et al., 2022; Oosthuizen et al., 2023). While eDNA methods have demonstrated global effectiveness, their application in Africa remains relatively limited (von der Heyden, 2023). Regional eDNA studies are essential for validating and refining these approaches within diverse environmental and ecological contexts, thereby enabling a comprehensive assessment of their effectiveness in detecting biodiversity and elucidating the environmental drivers shaping community composition and species assemblages across different scales (Keck et al., 2023; Seymour et al., 2020). Here we utilized eDNA metabarcoding to conduct a full biodiversity survey across the full extent of the South African coastline (~2800 km), covering the cool temperate to tropical biogeographic regions, across the full rangeof coastal environmental variation. In addition, we also assessed the extent to which environmental factors influence community dynamics and underlying species occurrences along the South African coast. Specifically, we addressed three key objectives: 1) utilize environmental DNA metabarcoding to assess coastal fish biodiversity across ~ 2800 km of the South African coastline; 2) investigate the influence of environmental variation, including pH, salinity, chlorophyll, substrate, and water temperature, on biological richness and changes in beta-diversity; and 3) determine which biological groups are most impacted by shifts in environmental conditions, which may indicate important contributors to overall regional biodiversity stability and indicators of localized environmental sensitivity.

2. Methods

Environmental DNA samples were collected from 33 coastal sites along the extent of the South African coast (Fig. 1). For each sampling site we conducted two sampling events, in October 2017 and May 2018. Samples consisted of surface water collected from nearshore using 2L plastic containers, which were cleaned using 10 % sodium hypochlorite prior to sampling. For each sampling event we collected triplicate 2L water samples, which were filtered using 0.22 μm Sterivex filter units (Merck Limited, Darmstadt, Germany), using 60 ml sterile plastic syringes to hand pump water through the filter. Hand pumping was conducted for each filter until the filters clogged whereby no more water was able to pass freely through the filter. Filters were pump dried, preserved with 2 ml buffer ATL (Qiagen, Hilden, Germany) and stored at room temperature prior to extraction. For each sampling site we also filtered 1L of distilled water, immediately after each set of samples, as a negative control. DNA extraction occurred in a designated eDNA clean room at Stellenbosch University, which included disinfecting all surfaces with 10 % sodium hypochlorite and using UV lights on all benches and pipettes for 30 min before and after DNA extractions. DNA extractions utilized a modified Qiagen DNeasy extraction kit & protocol (Qiagen, Hilden, Germany) following Czachur et al. (2022).

2.1. Library preparation and sequencing

Metabarcode sequence libraries were built using a two-step protocol (Bohmann et al., 2022; Seymour et al., 2020). Due to budget limitations, we were unable to sequence all replicate samples and decided to pool the replicate extracts (pooling within sampling site) prior to library creation. PCR1, including the 12S barcode amplification attachment of adapter ends, was carried out at Stellenbosch University. We utilized the Mifish U/E 12S primers 12S including MiFish-U forward (5′-GTCGGTAAAACTCGTGCCAGC-3′) and Mifish-U reverse (5′-CATAGTGGGGTATCTAATCCCAGTTTG-3′), Mifish-E forward (5′-GTTGGTAAATCTCGTGCCAGC-3′) and Mifish-E reverse (5′-GTTTGATCCTAATCTATGGGGTGATAC-3′) (Miya et al., 2015). MiFish primers are the most commonly used primers for eDNA fish biodiversity assessment, particularly for marine fish. The MiFish U primers target a wide range of fish species, with MiFish E primers being better suited for targeting elasmobranch species (Miya et al., 2015). PCR1 was carried out in triplicate 25 μl reaction volumes per sampled, containing 1 μl of template DNA, 0.75 μl of each primer (MiFish-U Forward and Reverse, Mifish-E Forward and Reverse), 12.5 μl of KAPA HiFi HotStart Ready Mix (Roche, USA), 1 μl of 5 mg/ml bovine serum album (BSA) solution, and 7.5 μl RNASE-free water. Replicate PCRs were then pooled. PCR1 included an initial denaturation at 95 °C for 3 min, followed by 35 cycles of 98 °C for 20 s, 65 °C for 15 s, and 72 °C for 10 s, followed by a final extension at 72 °C for 5 min. PCR1 products were cleaned using two rounds of MagBio High Prep PCR PB kit, using a 0.5x ratio for the first round and a 0.8x ratio for the second round. PCR2 was carried out to attach unique index tags to each of the sample library pools (Seymour et al., 2020), using 25 μl reaction volume with 7.5 μl of the cleaned PCR1 product, 5 μl of the index primers and 12.5 μl KAPA HiFi HotStart Ready Mix per reaction. PCR cycle conditions were the same as for PCR1, but with the number of cycles reduced to 15. PCR2 product was then cleaned using MagBio High Prep PCR PB kit using the same double cleaning protocol used to clean the PCR1 product. Cleaned PCR2 product was then checked using gel electrophoresis, quantified using Qubit (Life Technologies Ltd, USA), normalized to 4 nM, and pooled. Pooled amplicon libraries were then sequenced on a MiSeq (Illumina, USA) using 2 x 150 bp chemistry at the Institute for Microbial Biotechnology and Metagenomics (IMBM) at the University of the Western Cape.

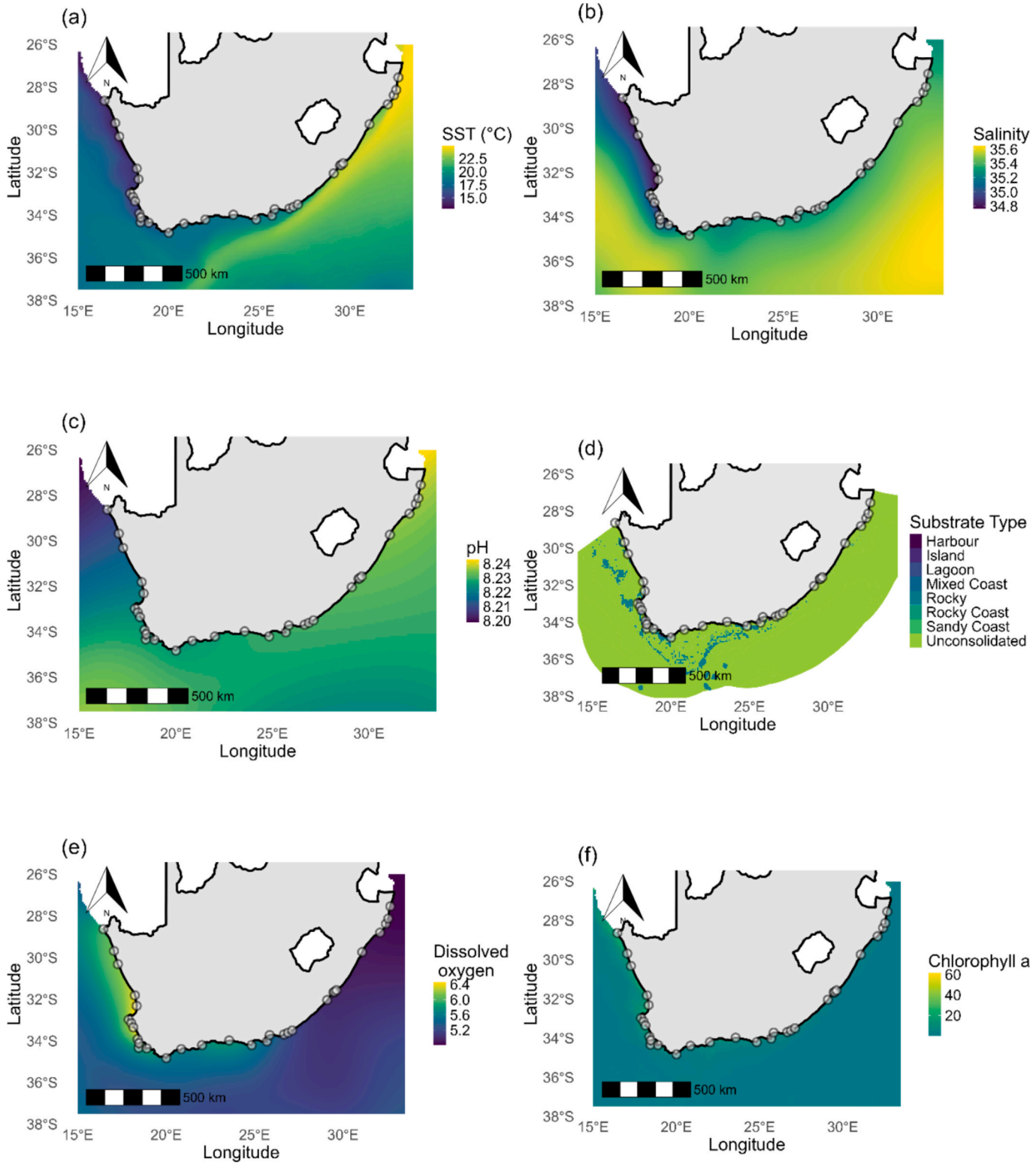

Fig. 1. Environmental gradients and substrate types along the South African coastline. Sampling sites are indicated by white circles in each subplot (a) mean annual sea surface temperature (SST, °C). (b) mean annual salinity (ppt). (c) Mean annual pH (d) predominant substrate, as classified in the National Biodiversity Assessment (e). Mean annual dissolved oxygen (mg/L). (f) Mean annual chlorophyll *a* concentration (μg/L).

2.2. Bioinformatic processing

Sequenced samples were demultiplexed using the Illumina MiSeq software. Subsequent bioinformatic processing was performed using the MiFish Pipeline version 2.40 (Miya et al., 2015; Zhu et al., 2023). MitoFish is part of the MitoFish Suite of web-based bioinformatics tools that automates MiFish-primer metabarcoding sequence data (Zhu et al., 2023). The MiFish Pipeline automates the processing of MiFish metabarcoding data by performing quality filtering, dereplication, chimera removal, and clustering to generate Amplicon Sequence Variants

(ASVs). MitoFish then assigns taxonomy to each ASV based on the MitoFish reference database. The output includes an ASV table detailing read counts per sample alongside taxonomic annotations for each ASV. From this table we removed all fish that were strictly associated with freshwater as non-target species. We also corrected the taxonomic assignment for each ASV based on percent match to the assigned reference sequence, including species at 98 %, genus at 95 % and family at 90 % (Miya et al., 2015). All other ASVs not assigned to at least the family level were removed from subsequent analysis. The full taxonomic annotation was then checked against a local database, Smith's Sea Fishes (Smith and Heemstra, 2012), and global databases, including FishBase (https://www.fishbase.org), and the World Register of Marine Species (WORMS), to ensure assigned taxonomy was consistent with known South African, and wider, coastal fish biodiversity. We utilized the full ASV annotation to describe the total biodiversity across the sampling sites. Due to the limited barcode data for South African marine fish we restricted our downstream biodiversity analysis to genus and family to avoid any potential miss-identifications arising from the incomplete reference database (von der Heyden, 2023). The dataset was then reduced to genus-level and family-level tables for assessing spatial and environmental effects on biodiversity.

A total of 59 sequencing libraries were generated and sequenced across 33 sampling sites. After initial bioinformatics processing 2 libraries, encompassing 2 sites, were removed due to low sequencing output, resulting in a final raw sequence output of 57 libraries across 31 sites. Prior to within-sample (i.e. replicate) pooling for downstream analysis, we used a linear model to test for the effect of seasonality or within site replication on log ASV read count and another linear model to test the same effects on ASV richness. We found no significant effect of season or replicate on read count ($p = 0.796$, $p = 0.917$), genus richness ($p = 0.348$) or family richness ($p = 0.741$). Rarefaction curves were then assessed on each of the site-specific pools to check for ASV diversity saturation across each of the sites, with all curves showing saturation for each of the locations using function *rarefy* in the r-package vegan.

2.3. Environmental data

Environmental data for each site were extracted from the Bio-ORACLE database using the r-package *sdmpredictors*, including mean annual pH, mean annual temperature (C°), mean annual salinity (psu), mean annual Chlorophyll *a* (mg/m^3) and mean annual dissolved oxygen ($\mu mol/m^3$). Substrate were assigned based on the 2011 South Africa National Institute of Biodiversity Report (Driver et al., 2012).

2.4. Statistics

Data analyses were performed in R version 4.2.2 (R Core Team, 2024).

Biological fish diversity was analyzed at the genus, family and ASV level using the corresponding genus and family tables derived from the ASV table (also used). The lack of complete reference libraries means that many sequences could not be confidently assigned beyond the genus or family level, reducing the likelihood of false or unreliable taxonomic assignments at finer scales. Therefore, conducting analyses at these higher taxonomic levels helps ensure more accurate and robust community comparisons across sampling sites. The ASV level assessment, without taxonomic assignment, can serve as an approximate assessment of species and potential finer scale patterns in the data.

For each level of analysis, the data was normalized across sites by converting sequence reads per genus per site to relative frequencies of each species within each site. Genus richness was calculated as the number of unique genera occurring at each site, indicated by sequence read frequency being greater than zero. To determine whether environmental factors were attributed to changes in richness, we applied a generalized linear model (GLM), with a negative binomial distribution, to test the effect of annual temperature, annual salinity, chlorophyll *a*, pH and substrate on genus and family richness. We used a backwards model selection approach to select the most parsimonious model starting with a full model including all additive and associated pairwise interaction terms as explanatory variables (Borcard et al., 2018). Models were reduced by removing individual terms from the model and comparing the reduced model to the original model using Akaike's Information Criterion via the *stats* package in R (R Core Team, 2024). We utilized a GLM with a negative binomial distribution due to the noted over-dispersion of the data (Borcard et al., 2018).

Changes in biodiversity can be assessed using beta-diversity, which quantifies the difference in composition between communities (Podani et al., 2013; Seymour et al., 2024). Beta-diversity can be further partitioned into components of richness difference (i.e. nestedness) and replacement (i.e. turnover) to further assess the underlying mechanism facilitating changes between communities (Podani et al., 2013). Heightened levels of replacement are largely indicative of environmental forcing, such as filtering or dispersal limitations, whereas, high richness difference can highlight species-poor locations indicating dominance of specialist species, localized species extinction or establishment/immigration issues (Lazarina et al., 2023). As such, partitioning beta-diversity into components of richness difference and species replacement provides insights into the ecological processes shaping community assembly and the sensitivity of species to environmental change (Legendre, 2014; Seymour et al., 2021; Seymour et al., 2024).

Beta-diversity was calculated as Jaccard dissimilarly using the function beta.div.comp in the *vegan* package in R (Oksanen et al., 2024), following the Podani framework (Podani et al., 2013). We also calculated the partitioned beta-diversity components for the Jaccard dissimilarity into replacement (turnover) and richness difference (nestedness) to enable further disentanglement of the mechanistic dynamics associated with the observed difference between sampling sites. To assess the influence of environmental factors on beta-diversity, and the decomposed components of beta-diversity, we utilized a distance-based redundancy analysis (dbRDA) approach via the package *vegan*. Principal Coordinates Analysis (PCoA) using the *cmdscale* function in R was used to calculate the distance variables from the site distance matrix. For each component of beta-diversity, total, replacement and richness difference, we used the function *dbrda* to test the influence of temperature and the first distance variable against each of the response variable matrices.

We utilized the beta-diversity distance matrices to assess the genus and family-level contribution to beta-diversity (SCBD) (Borcard et al., 2018). SCBD is a decomposition of beta-diversity into individual contributions to assess specific (i.e. genus, family) influence on overall beta-diversity patterns. Individual SCBD values above the mean SCBD are consider significant and are attributed with greater influence on the among site differences(Borcard et al., 2018). Similarly, we also checked site contributions to species level beta diversity (LCBD).

To quantify the influence of environmental and distance on genus occurrence while accounting for community structure and site-level variation, we applied a hierarchical model of species communities (HMSC) using the R package Hmsc (Ovaskainen et al., 2017). The response matrix (Y) comprised the binary presence/absence of each genus at each site. The environmental data (X) included annual temperature, salinity, pH, chlorophyll, substrate, and the first distance variable. Site identity was included as a random effect to account for variation in community composition not explained by the measured predictors. The HMSC model used a probit link function appropriate for binary data. Markov chain Monte Carlo (MCMC) sampling was used to estimate model parameters, with four parallel chains run for 500 posterior samples each, a thinning interval of 20, and a burn-in period of 100 samples per chain. Convergence was assessed using effective sample size and the Gelman–Rubin diagnostic; all key parameters exhibited satisfactory mixing and convergence (effective sample size > 400, potential scale reduction factor < 1.1). Model fit was evaluated using both

in-sample predictions and five-fold cross-validation, to assess the conditional and unconditional explanatory power with Tjur's R^2. posterior means and 95 % credible intervals for each predictor's effect on each genus were extracted. Effects were considered significant if the credible interval did not overlap zero.

3. Results

Annual temperature averaged 18.4 °C across the sites, ranging from 13.6 to 24.5 °C (Fig. 1a). Annual salinity averaged 35.2 ppt across all sites and ranged from 34.8 to 35.4 ppt (Fig. 1b). pH averaged 8.23 and ranged from 8.20 to 8.24 (Fig. 1c). Substrate was predominately mixed coast (10 sites) or sandy coast (19 sites) with 1 site classified as a lagoon and 1 as rocky coastline (Fig. 1d). Dissolved oxygen averaged 5.6 mg/L and ranged from 4.8 to 6.4 mg/L (Fig. 1e). Chlorophyll *a* levels averaged 5.4 µg/l, ranging from 0.2 to 16.8 µg/l (Fig. 1f). Dissolved oxygen was highly correlated with temperature (0.95), so we only used temperature in subsequent analyses to avoid model over-fitting.

A total of 3,191,899 paired end reads were generated from the MiSeq run after trimming. After quality filtering and denoising, 2,856,367 clean sequence reads were utilized for subsequent taxonomic assignment. Reads per sample ranged from 408 to 193,706. After removing non-marine assignments, the final dataset encompassed 310 ASVs with 157 genus level assignments and 89 unique families (Fig. 2). Genera richness averaged 28 across the sites and ranged between 3 and 118 (Fig. 3a and Fig. 4). Endemic genera ranged from 0 to 24 (mean 1.9) across the sites, with 18 sites having 0 endemics. The sites with the highest endemism (6, 13 and 24) occurred along the eastern edge of the study area (Fig. 3c).

GLM results showed a significant positive effect of temperature on genera richness ($p < 0.01$), with non-significant effects of pH ($p = 0.06$). All other model parameters (i.e. salinity, chlorophyll and substrate type) were nonsignificant and removed during model selection. Average family level richness was 24 across sites and ranged between 4 and 75 (Fig. 3b). GLM results showed a significant positive effect of temperature on family richness ($p = 0.01$), with non-significant effects of pH ($p = 0.06$). All other model parameters (i.e. salinity, chlorophyll, and substrate type.) were non-significant and removed during model selection. ASV richness per site ranged from 15 to 150, with a mean of 65 (Fig. 3c). GLM results found a significant effect of temperature on ASV richness ($p = 0.01$) and a non-significant effect of pH ($p = 0.08$), similar to genus and family-level results. Other variables (salinity, chlorophyll and substrate type) did not show significant effects and were removed during model selection.

Genus-level beta-diversity (similarity) averaged 0.21 between sites, ranging from 0 to 0.66, indicating moderate community turnover along

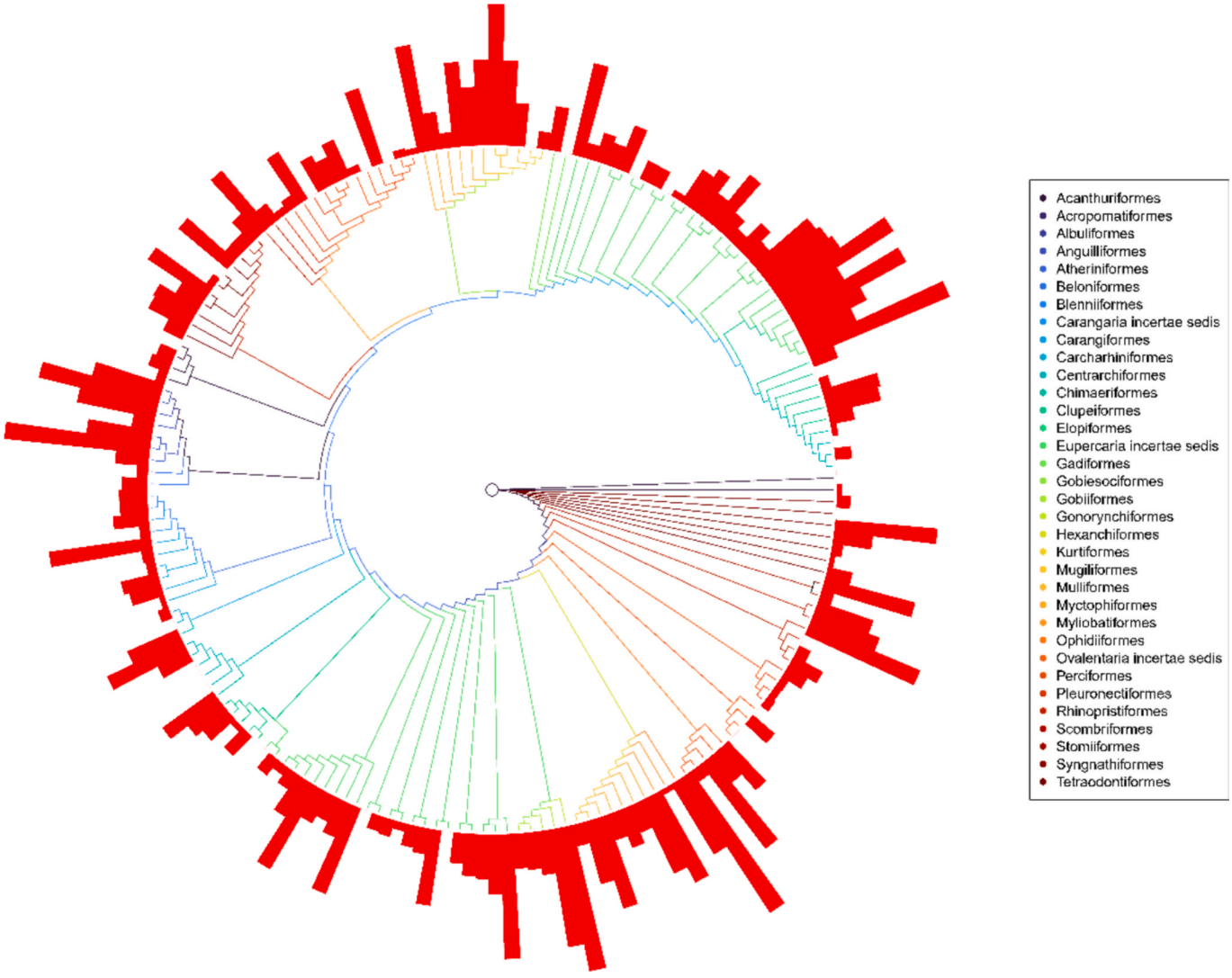

Fig. 2. Dendrogram showing taxonomic relationship of the 157 genera (unique tips) detected. Colors correspond to unique families with names provided in the legend. The red bars above each of the tips indicate the number of proportional reads for the corresponding genus.

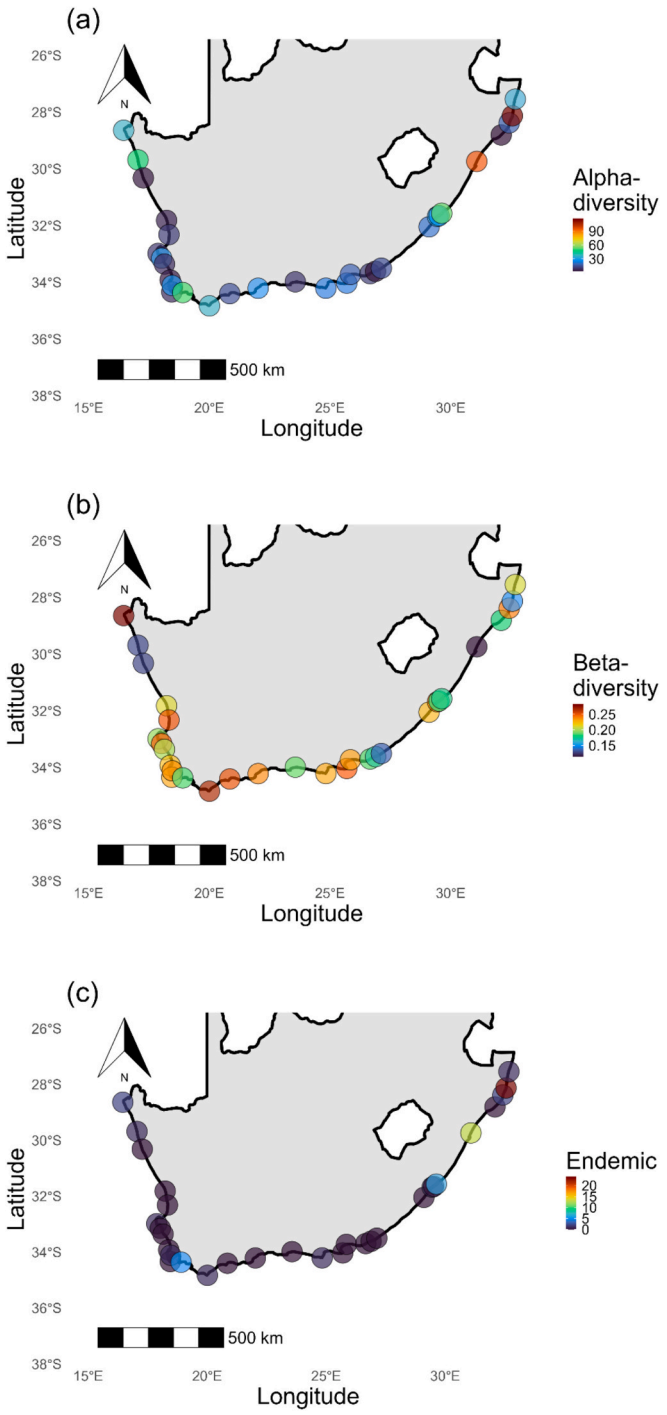

Fig. 3. Spatial patterns of fish diversity and endemism across South African coastal sites as detected through eDNA metabarcoding. (a) Genus-level alpha diversity (number of genera per site) along the coastline, with warmer colors indicating higher richness. (b) Map of mean pairwise beta-diversity (Jaccard dissimilarity), with higher values indicating greater community dissimilarity. (c) Map of the number of endemic genera detected at each site.

the coast (Fig. 3b). The partitioned components of beta-diversity included replacement (turnover) and richness difference (nestedness), where replacement averaged 0.43 and ranged from 0.07 to 0.95 and richness difference averaged 0.24 and ranged from 0 to 0.73. Notably, approximately 52 % of the site pairs exhibited high replacement values (>0.5), indicating that community differences were primarily driven by the replacement of genera rather than simple differences in the number of genera. Conversely, high richness differences (>0.5) were observed between 12 % of the site pairs, suggesting these may be suspect to local environmental filtering. Three sites had high average richness difference, including HB (0.49) UM (0.46) and CV (0.49), which included both eastern (HB) and western (UM and CV) sites, indicating filtering effects are likely highly localized. The Local Contributions to Beta Diversity (LCBD) analysis indicated no individual sites with significant influence on overall beta-diversity, reflecting a relatively even distribution of community variation across the sampled locations.

The family-level beta-diversity analysis indicated an average similarity of approximately 0.25 between sites, with a range from 0 to 0.70, reflecting moderate community variation across the coastline. Partitioning the beta-diversity revealed that community differences were primarily driven by replacement (turnover), which averaged 0.50 and ranged from 0.13 to 0.88, whereas nestedness contributed less, with an average of 0.25 and a range from 0 to 0.69. About 49 % of site pairs exhibited high turnover values (>0.5), indicating that shifts in dominant families, were the main factor shaping community dissimilarity. High richness difference (<0.05) was observed between 9 % of the sites pairs, with the main contributors being UM (0.46), HB (0.49) and CV (0.49), mirroring the results from the genus-level results. The LCBD analysis showed no individual sites with significant influence on overall beta-diversity, suggesting that community variation was evenly distributed across locations.

The ASV-level beta-diversity analysis demonstrated an average similarity of 0.20 between sites, with a range from 0 to 0.60. Partitioning the beta diversity revealed that community differences were predominantly driven by replacement, which averaged 0.53 and ranged from 0.14 to 0.97, while nestedness contributed an average of 0.27, ranging from 0 to 0.79. About 57 % of site pairs exhibited high turnover values (>0.5), emphasizing that community dissimilarity was mainly due to the replacement of ASVs rather than differences in richness. High richness difference (>0.5) was observed between 14 % of the site pairs, with the main contributors again being UM (0.46), HB (0.49) and CV (0.49). The Local Contributions to Beta Diversity (LCBD) analysis indicated UM as having a significant influence ($p = 0.001$) on overall beta-diversity.

Genus contributions to beta diversity analyses indicated 22 of the 157 genera contributed above the mean stressor value (Fig. 5), indicating greater than expected influence on beta-diversity, including *Chelon*, *Clinus*, *Halidesmus*, *Engraulis*, *Neoscorpis*, *Diplodus*, *Dentex*, *Rhabdosargus*, *Chorisochismus*, *Takifugu*, *Sardinops*, *Parablennius*, *Caffrogobius*, *Cheilodactylus*, *Decapterus*, *Mugil*, *Argyrops*, *Monodactylus*, *Dichistius*, *Scomber*, *Kyphosus*, and *Sardinella*. The family contributions to beta-diversity analysis found 15 of the 89 families contributing above the stressor value, including Mugilidae, Clinidae, Sparidae, Pseudochromidae, Engraulidae, Kyphosidae, Gobiidae, Blenniidae, Carangidae, Tetraodontidae, Clupeidae, Haemulidae, Gobiesocidae, Cheilodactylidae, Scombropidae.

Distance-based redundancy analysis of beta-diversity, and its partitioned components were assessed for temperature following the explanatory variables selected from the alpha-diversity and site contribution to beta-diversity analyses. For the genus-level assessment total beta-diversity showed a significant effect with temperature ($p = 0.041$) and a non-significant effect of distance ($p = 0.093$). Replacement (i.e. turnover) showed no significant effect of temperature ($p = 0.715$) or distance ($p = 0.972$). Richness difference (e.g. nestedness) showed a significant effect of temperature ($p = 0.016$) and distance ($p = 0.0221$). Similarly, for the family level analysis temperature was significant ($p = 0.049$) and distance non-significant ($p = 0.077$) vs total beta-diversity. Family richness difference showed a significant effect of temperature ($p = 0.049$) and distance ($p = 0.012$) with replacement having non-significant associations with temperature ($p = 0.604$) and distance ($p = 0.986$). For the ASV analysis there was a non-significant effect of temperature ($p = 0.057$) and distance ($p = 0.115$) for total beta-diversity. Richness difference showed a significant effect of temperature ($p = 0.030$) and distance ($p = 0.026$) and replacement showed a non-significant effect of temperature ($p = 0.739$) and distance ($p =$

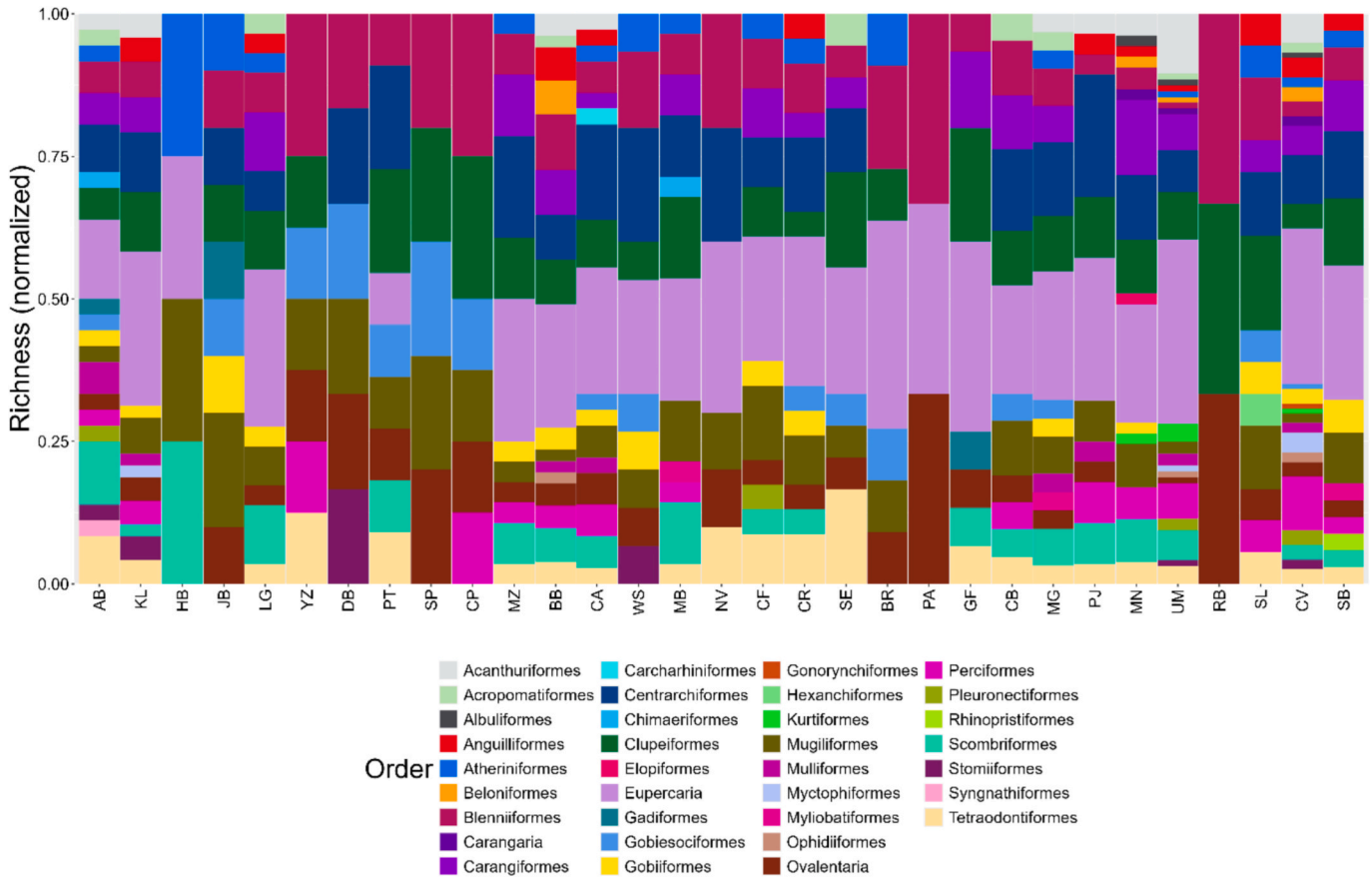

Fig. 4. Bar chart of the genera richness (within site relative frequency) across sites (ordered from west to east). To highlight the taxonomic variation within and among sites order level groups are indicated by color as depicted in the figure legend.

0.984). Plotting the dbRDA (Fig. 5) showed a transitioning pattern from the eastern Benguela coastal ecoregion though the central Agulhas coastal ecoregion to the eastern Natal-Delagoa coastal ecoregion (Fig. 6).

Explanatory power (mean TjurR^2) of the HMSC model was 0.182. Unconditional predictive power was 0.042. Conditional predictive power was 0.179. Hierarchical modelling of species communities revealed consistent and strong environmental effects on the occurrence of fish genera across the sampled sites. Annual temperature was a key predictor, with all 181 genera exhibiting a significant positive association between occurrence probability and temperature (95 % CI: 0.125 to 1.239, not overlapping zero; Table S1). This pattern indicates that higher temperatures markedly increase the likelihood of genus presence throughout the region, which coincided with increased alpha diversity at higher temperature sites. Conversely, distance did not show a significant effect for any genera with the mean ranging close to zero. Chlorophyll was a moderate key predictor with 3 genera showing a positive association (95 % CI: 0.001–0.154, not overlapping zero) and 66 showing a negative association (95 % CI: −0.242–0, not overlapping zero) with occurrence probability. Salinity was a not a key predictor with 1 genus showing a positive association with occurrence probability (95 % CI: 0.002–7.883, not overlapping zero). pH was a not a key predictor with 2 genera showing a positive association with occurrence probability (95 % CI: 6.127–278.236, not overlapping zero). Substrate was a not a key predictor with no genera showing an association with occurrence probability. The variance partitioning analysis revealed that annual temperature was the dominant driver of species occurrence, explaining an average of 52 % of variance across genera (23–58 %). Distance contributed an average of 30 % across genera (18–57 %). Other environmental variables such as pH, habitat, salinity, and chlorophyll explained minimal variance (less than 10 % on average), suggesting limited influence on the distribution patterns (Fig. 7).

4. Discussion

The South African coastline, at the transition of the Indian and Atlantic Oceans, represents a marine biodiversity hotspot that includes ~ 2000 species of fishes distributed from cool-temperate to tropical environments, including a number of endemic species (Griffiths et al., 2010; Heemstra and Heemstra, 2004). In this study we successfully employed an eDNA metabarcoding approach to comprehensively assess coastal fish biodiversity along ~ 2800 km of South African coastline and detected 157 genera across 89 families noted from the region. We also found strong associations with temperature on genus, family and ASV richness with specific indications of temperature potentially impacting beta-diversity and specifically the richness difference component between sites. We further established which genera are primarily driving the notable changes in community differentiation along the coast, thereby highlighting key biological entities linked to regional biodiversity stability and environmental sensitivity.

4.1. *Detecting a diverse fish assemblage using eDNA metabarcoding*

We accounted for 89 of the 270 (~32 %) known fish families; (Heemstra and Heemstra, 2004), highlighting the remarkable biological diversity of South African coastal fishes using a rapid and cost effective eDNA surveillance method. Three aspects are worth highlighting here. One, that our findings support other recent studies in biodiverse hotspots with complex ecosystems in providing an effective means to capture high biodiversity survey data while also effectively capturing key

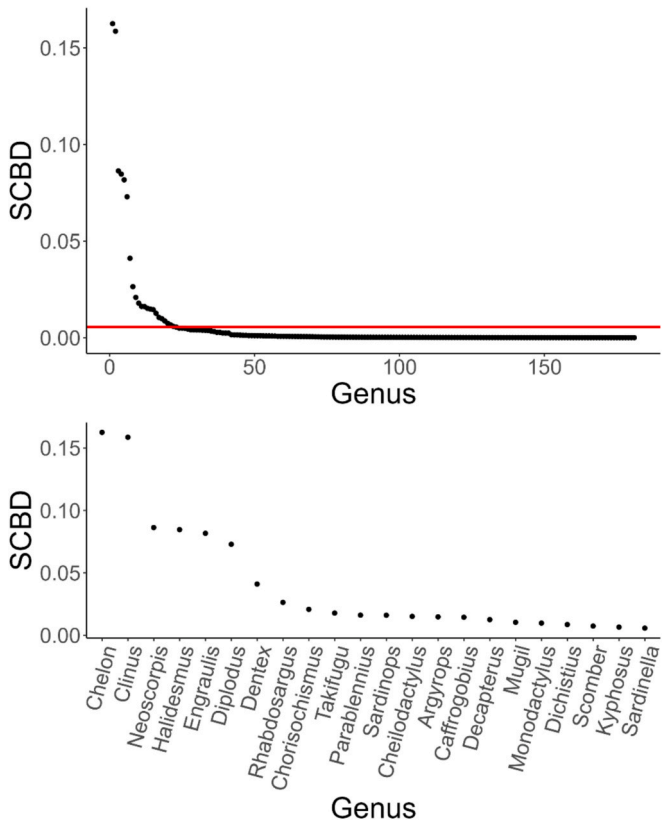

Fig. 5. Top panel shows the genus level species contribution to beta-diversity (SCBD) (y-axis) with all genera as single points ordered from most contributing (left) to least (right). Bottom panel shows the genera that are above the mean contributing scores (i.e. are having the greatest influence in describing the differences between communities).

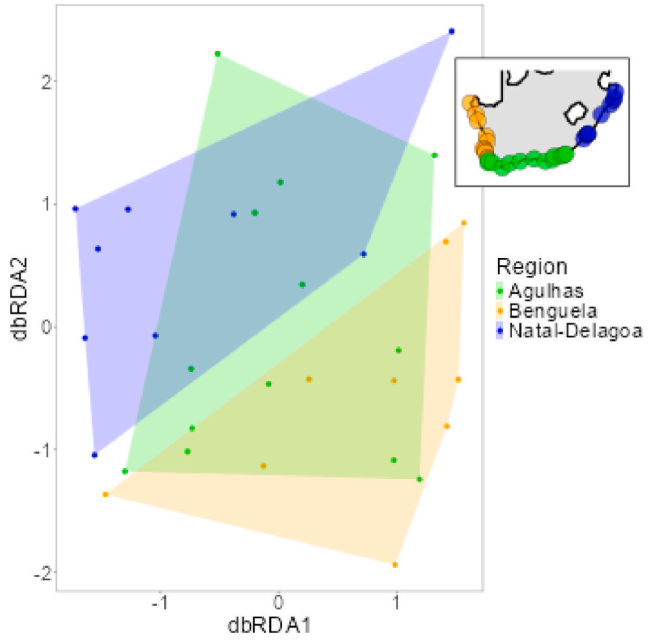

Fig. 6. Distance-based redundancy analysis (dbRDA) of genus-level community composition. dbRDA ordination plot showing the relationship between fish community composition and key environmental variables. Sites are colored by major coastal ecoregion (Agulhas, Benguela, Natal-Delagoa). The subplot illustrates the region transition across the sampling sites along the coast of South Africa.

trends in biodiversity dynamics across sites associated with abiotic and physical factors (Marwayana et al., 2022; Mathon et al., 2022; Zainal Abidin et al., 2022). Secondly, it is important to note that increased survey efforts at finer spatial or temporal scales may also be needed to fully capture total biodiversity, particularly in tropical and subtropical regions (Collen et al., 2008). As noted elsewhere, this study found elevated coastal fish richness, in a South African context, which is facilitated by the strong environmental gradient along the transition from the Eastern Atlantic to the Western Indian Ocean (Spalding et al., 2007). Thirdly, of the 310 AVS, we were able to resolve 157 to genus using the existing reference database, thus underreporting the true diversity of fishes detected in this study. This highlights the continuing need for increasing the regional reference barcode database (Courtaillac et al., 2024; Rossouw et al., 2024; von der Heyden, 2023).

We found unique genera in 13 of the 31 sites sampled, with 6 sites having at least 2 unique genera, which is consistent with previous estimates of regional endemism in South Africa (Heemstra and Heemstra, 2004). While these findings are not entirely reflective of the wider biodiversity dynamics, some genera may be constrained by habitat type and be locally range limited given the high level of endemism in the region (Turpie et al., 2000; Whitfield, 2019). Given the difficulty in standardizing sampling protocols across diverse habitats and differing extent of anthropogenic pressure, the use of eDNA to rapidly assess biodiversity here provides a unique look at the potential for standardized and large-scale utilization. Thereby the findings here, while likely understating the total diversity, will nevertheless enable managers and researchers with a fast and reliable approach to expand or focus survey efforts.

4.2. *Environmental drivers of community structure*

The transition between the Atlantic and Indian Oceans at the southern tip of Africa and associated environmental clines shape a rich coastal biodiversity (Griffiths et al., 2010) with strong bioregionalization. It is broadly recognized that temperature is an important predictor of marine biogeographic patterns for marine fauna along shallow coastal margins (Belanger et al., 2012; Stuart-Smith et al., 2017) and that for South African fishes, including those in estuaries, temperature, as well as salinity are important drivers of bioregionalization (Harrison and Whitfield, 2006b; Maree et al., 2000). Here we found that coastal fish biodiversity is likely less influenced by salinity, but is still heavily influenced by temperature shifts across taxonomic levels. These findings highlight the importance of habitat differentiation in assessing changes in community composition.

We utilized the beta-diversity framework (Legendre, 2014; Seymour et al., 2024) to provide important insights into the variability of species composition between sites. The average similarity of 0.32 indicated moderate differences in community structure between sites, suggesting that each site has a unique community composition. This finding further supports the likely persistence of endemism across the South African coast (Heemstra and Heemstra, 2004). By partitioning beta-diversity into replacement and richness difference, we further characterized the underlying patterns of community variation across the South African coast. Sites with lower average richness differences were predominately observed in the southern portion of the coastline indicating a greater sharing of genera across sites with compositional differences primarily driven by species replacement. In contrast, sites with high average richness difference, more commonly observed at the extreme ends of the coastline, indicated sites that differed significantly in the number of species they hosted, reflecting variations in habitat suitability, environmental conditions, or ecological processes in shaping local community composition (Podani et al., 2013). Sites with high average richness greater than 0.4 (compared to the overall mean of 0.12) included Hondeklip Bay (HB), Cape Vidal (CV) and Umhlanga (UM), suggesting that these locations play a significant role in shaping the overall fish biodiversity patterns along the South African coast. Hondeklip Bay is a

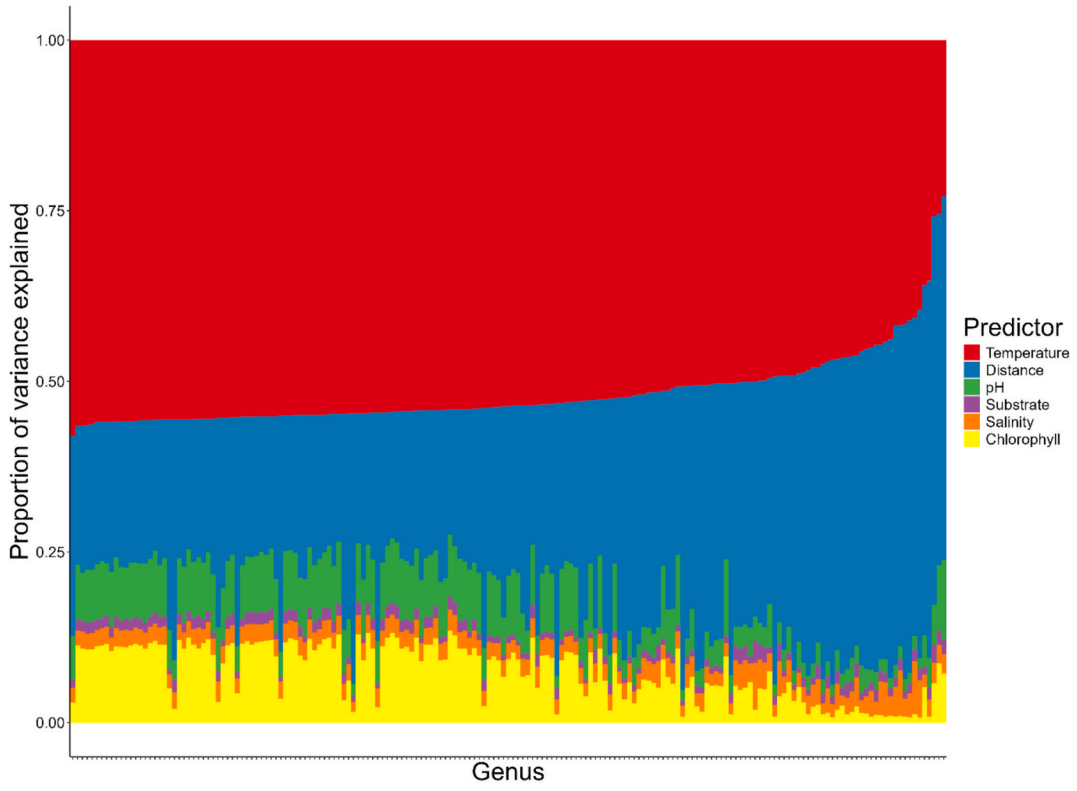

Fig. 7. Variance partitioning of environmental predictors on genus occurrence. Barplot showing the proportion of variance in genus presence/absence explained by each environmental variable (temperature, distance, pH, substrate, salinity, chlorophyll). Each bar represents a genus, and colors denote the predictor. Temperature is the dominant explanatory variable for most genera.

cold Atlantic Ocean site along the west coast with lower salinity that harbors low overall diversity (only four genera detected), whereas Cape Vidal is a sub-tropical site at the eastern end of the southern coast with four unique species, with a high level of richness (113 genera) and 24 genera unique to the site. Umhlanga is a site located on the tropical east coast harboring high richness (97 genera) of which 13 were unique and not detected elsewhere in our survey. These patterns, extremes at the ends of our sampling range, highlight the importance of biogeographic and environmental gradients in shaping community composition. The marked differences in diversity and endemism suggest that these regions may serve as distinct biogeographic units, with implications for regional conservation prioritization.

4.3. Contributions towards beta diversity

The species-specific contributions to beta-diversity analysis highlighted the importance of 22 genera in driving community dynamics. Genera *Chelon* and *Clinus* had the strongest influence on beta-diversity compared to the other 21 (~400 % greater average influence) followed by *Halidesmus*, *Engraulis* and *Neoscorpis* which had roughly 200 %, 197 % and 187 % greater average influence respectively. The causes for each genus contributing to beta-diversity are quite varied but appear to be associated with temperature and local endemism for some genera. In South Africa, the genus *Chelon* (a group of mullets) is typically found in a range of coastal habitats, including estuaries, lagoons, and shallow marine waters. These mullets prefer cooler waters, particularly along the western and southern coastlines, where they inhabit environments rich in detritus and algae (McGregor and Strydom, 2020). The genus *Clinus*, belonging to the family Clinidae, are commonly known as klipfish. Klipfish are typically found in rocky intertidal and subtidal zones and prefer cooler waters, particularly around the south-west coastline (Branch et al., 2022). The genus *Halidesmus*, belonging to the family Pseudochromidae, is a lesser-studied group of marine fish found in rocky reefs and subtidal zones along the South African coastline. *Halidesmus* exhibit niche environmental tolerances, often favoring localized and stable habitats with specific conditions, which reflects the limited observations from our survey efforts. Their presence in these specialized environments suggests a reliance on undisturbed rocky ecosystems, making them potentially sensitive to habitat alterations (Heemstra and Heemstra, 2004). The genus *Engraulis*, commonly known as anchovies, includes small, schooling fish that are widely distributed in both temperate and tropical coastal waters around the world. In South Africa, the species *Engraulis capensis* (the Southern African anchovy) is particularly significant and forms large shoals in the nutrient-rich waters of the Benguela Current along the western and southern coastlines. Our observations noted wide variations in *Engraulis* frequency and occurrence which might reflect this rapid migration and schooling behavior of this group (Smith and Heemstra, 2012). The genus *Neoscorpis*, represented by *Neoscorpis lithophilus* (commonly known as the stonebream), is a distinctive fish found along the rocky coastal areas of South Africa (Heemstra and Heemstra, 2004).

HMSC provides a powerful extension of traditional community analyses by allowing for explicit partitioning of variance in species occurrence among environmental and spatial factors. With regards to South African coastal fish biodiversity dynamics, our results echo a growing body of literature that highlights the pre-eminent role of temperature as a determinant of species distributions and community structure (Harrison and Whitfield, 2006b; Stuart-Smith et al., 2017). The strong and consistent temperature signal observed across all genera in the HMSC analysis provides empirical support for the notion that the biogeographic boundaries and high turnover observed along the South African coast are primarily thermally mediated. This is particularly salient for taxa with restricted ranges or specialized habitat requirements, as these are the genera most likely to be both sensitive indicators of environmental change and key contributors to regional biodiversity (Maree et al., 2000; Teske et al., 2019). By showing that

temperature consistently explains the largest proportion of variance in genus-level presence, even after accounting for distance and other environmental gradients, our study supports current research that suggest changes in ocean temperature are likely to have pronounced effects on the composition and stability of local fish assemblages (Potts et al., 2015).

4.4. eDNA biodiversity assessments beyond species level assignments

Environmental DNA (eDNA) metabarcoding has greatly improved our ability to assess fine scale spatio-temporal dynamics, offering a non-invasive, high-resolution, and cost-effective alternative to traditional monitoring methods (Miya, 2022; Seymour et al., 2021; Seymour and Smith, 2023; Si et al., 2025). While the number of families detected is incomplete the survey effort still exceeds traditional efforts, while also providing a non-invasive and cost-effective approach. Importantly, our findings align with broader eDNA research, which has consistently demonstrated its capacity to reveal fine-scale biodiversity patterns driven by environmental gradients, (Erős et al., 2024; Seymour et al., 2021; Seymour et al., 2024; Shi et al., 2023). For instance, we observed a significant increase in richness with rising temperatures ($p = 0.01$), consistent with the west-to-east temperature cline along the South African coast. This highlights the utility of eDNA in capturing the responses of fish communities to localized environmental drivers, a critical advantage for understanding and managing biodiversity in dynamic coastal ecosystems. By providing a more complete and accurate assessment of fish biodiversity, eDNA-based approaches not only enhance our ecological understanding but also inform conservation strategies and sustainable resource management (Gehri et al., 2021; Li et al., 2024).

Genus- and family-level assessments, facilitated by the comprehensive environmental DNA (eDNA) dataset generated in this study, proved highly effective for large-scale biodiversity monitoring along the South African coast. Our results demonstrate that genus- and family-level analyses yielded congruent patterns in alpha and beta diversity, with both taxonomic resolutions capturing the pronounced influence of temperature on richness and community composition (Nguyen et al., 2020; Seymour et al., 2021). Notably, genus-level data provided greater sensitivity in detecting fine-scale replacement and identifying key taxa that disproportionately contributed to beta-diversity, consistent with findings that higher-resolution taxonomic approaches reveal more nuanced ecological dynamics (Blackman et al., 2023; Li et al., 2024). This enhanced sensitivity was particularly evident in the partitioning of beta-diversity, where genus-level analyses captured spatial variation in both turnover and nestedness, aligning with recent work demonstrating the value of intermediate taxonomic resolution in eDNA studies, especially in regions with incomplete barcode reference libraries (Gold et al., 2021; von der Heyden, 2023). While family-level analyses effectively summarized broad biodiversity patterns, they tended to mask subtle community shifts, underscoring the advantages of genus-level assessments for detecting localized effects of environmental filtering and turnover, especially in complex, heterogeneous marine environments (Si et al., 2025; Stat et al., 2017). Our results further suggest that genus-level resolution strikes a practical balance between the efficiency of coarser taxonomic assessments and the ecological insight afforded by species- or ASV-level approaches. The inclusion of ASV-based, taxonomic-free metrics corroborated these patterns, revealing hidden diversity and cryptic turnover that may be overlooked by traditional taxonomic assignment (Seymour et al., 2020), a finding echoed by recent *meta*-analyses of eDNA applications in marine systems (Keck et al., 2022; Stat et al., 2017).

While our study demonstrates the utility of eDNA metabarcoding for assessing marine fish biodiversity across large spatial scales, several methodological limitations must be acknowledged. First, the sampling protocol employed 6 L of surface water per site, with sites sometimes separated by up to 100 km. Although previous studies have shown that eDNA can effectively capture community diversity within relatively small water volumes (Mächler et al., 2016; Port et al., 2016; Stat et al., 2017), the limited volume and spatial coverage may not fully represent the local fish assemblage or detect rare, transient, or low-abundance species (Sigsgaard et al., 2020). This is especially relevant in dynamic coastal environments, where eDNA distribution can be patchy due to hydrodynamics, tides, and species movement (Andruszkiewicz et al., 2017). Consequently, our estimates of alpha and beta diversity may underestimate true local diversity, and the detection of spatial turnover may be influenced by the resolution of sampling. To improve species detection, future studies could increase the number of replicate samples, include vertical and temporal sampling, and employ a denser network of sampling sites across environmental gradients (Goldberg et al., 2016; Lacoursière-Roussel et al., 2016). Another important consideration is the choice of genetic marker. We utilized the MiFish 12S primers, which are widely used for marine fish eDNA studies and offer broad taxonomic coverage (Miya et al., 2015). However, marker choice inherently influences taxonomic resolution, detection sensitivity, and the ability to discriminate closely related taxa (Collins et al., 2019). The MiFish primers, while effective for bony fishes, may have reduced sensitivity for certain elasmobranchs or deep-sea taxa, and are subject to biases arising from primer mismatches and incomplete reference databases (von der Heyden, 2023). This can result in false negatives or ambiguous assignments, particularly at the species level. Employing multiple primer sets targeting different gene regions, or using shotgun metagenomic approaches, could enhance taxonomic coverage and improve detection of cryptic or poorly represented taxa, when logistically feasible (Deiner et al., 2017).

Overall, the results from this study highlight the dominant role of thermal gradients in driving biodiversity patterns along the South African coast and the Atlantic-Indian Ocean transition zone. We specifically show that the variability in environmental conditions, particularly temperature, contributes to the unique marine coastal fish composition and community dynamics observed across different sites (Harrison and Whitfield, 2006a). Understanding these environmental influences is crucial for effective management and conservation strategies in the face of ongoing challenges, including climate change, landuse altercation and human resource demands. Our eDNA based approached combined with beta-diversity partitioning provided important insights into the biodiversity and genus-specific patterns shaped by environmental influences along the South African coast. By leveraging the rapid and exponential power or eDNA for modern biodiversity assessment we can build effective management and monitoring practices to ensure long-term sustainability and the preservation of marine biodiversity for the benefit of current and future generations.

CRediT authorship contribution statement

Mathew Seymour: Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Project administration, Methodology, Investigation, Formal analysis, Conceptualization. **Molly V. Clavey:** Writing – review & editing, Project administration, Methodology, Investigation, Funding acquisition, Data curation, Conceptualization. **Masaki Miya:** Writing – review & editing, Methodology, Formal analysis. **Simon Creer:** Writing – review & editing, Supervision, Methodology, Funding acquisition, Conceptualization. **Gary Carvalho:** Writing – review & editing, Supervision, Methodology, Conceptualization. **Sophie von der Heyden:** Writing – review & editing, Supervision, Conceptualization.

Declaration of competing interest

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

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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.2025.114186.

Data availability

Data will be made available on request.

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