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Integrating Environmental DNA Metabarcoding and Remote Sensing Reveals Known and Novel Fish Diversity Hotspots in a World Heritage Area

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ABSTRACT

Aim: Shark Bay, a UNESCO World Heritage site in Western Australia, is highly vulnerable to climate change, yet its fish biodiversity remains poorly understood at fine spatial scales. We integrated environmental DNA (eDNA) metabarcoding with high-resolution remote sensing to assess and extrapolate fish diversity patterns, providing a scalable framework for biodiversity monitoring in dynamic coastal ecosystems.

Location: Shark Bay, Western Australia.

Methods: We analysed 270 water samples across 560 km^2 using fish-specific 16S and 12S rRNA metabarcoding, comparing our results to earlier studies using conventional methods including seining, trawling, fisheries reports, and fish traps. We linked biodiversity patterns to key environmental variables, including depth, salinity, sea surface temperature, and habitat characteristics derived from high-resolution satellite imagery. To predict fish biodiversity across unsampled areas, we employed machine-learning models, enabling spatial extrapolation of eDNA data across the seascape.

Results: eDNA metabarcoding identified 106 fish species across 132 genera and 71 families, with substantial overlap with conventional monitoring but broader coverage at higher taxonomic levels. Fish richness increased with decreasing salinity, high channel habitat coverage, and moderate depths with high seagrass coverage. We delineated five distinct fish communities (A–E): two shallow seagrass communities—one in sparse seagrass (A) and another in dense seagrass (B), one in channel habitats (C) with the greatest fish diversity; one in deep sandy waters (D) and one in medium-depth, seagrass-free areas (E). Additionally, we detected several tropical species, suggesting poleward shifts due to rising water temperatures.

Main Conclusions: This study highlights the utility of combining marine eDNA metabarcoding with remote sensing to detect fine-scale biodiversity. The integration of machine learning enables spatial upscaling and timely responses to habitat changes,

enhancing marine conservation and management. By identifying key environmental drivers of fish diversity, this approach supports proactive conservation strategies, providing a scalable model for biodiversity monitoring under climate change.

1 | Introduction

Climate change and destructive fishing practices pose significant threats to marine ecosystems (Worm et al. 2009; Hoegh-Guldberg and Bruno 2010; Halpern et al. 2007), disrupting abiotic niches and the complex biotic interactions sustaining marine biodiversity (Carroll et al. 2024). Addressing these threats requires efficient monitoring of aquatic biodiversity across taxonomic levels and environmental conditions (García Molinos et al. 2016; Miya 2022). While large-scale studies are important, localised analyses are crucial for identifying fine-scale biodiversity patterns and habitat-specific conservation needs (Hill et al. 2020; Tittensor et al. 2019), offering insights for targeted management in vulnerable regions.

Environmental DNA (eDNA) has become an efficient tool for monitoring biodiversity in marine environments (Blackman et al. 2024). Analysis of eDNA involves extracting genetic material released by organisms into their surroundings through samples like water, soil, or air (Taberlet et al. 2012; Pawlowski et al. 2020). These analyses can target single species (barcoding) or multiple taxa simultaneously (metabarcoding) (Taberlet et al. 2012). Initially used to confirm species presence or absence (Thomsen et al. 2012; Keck et al. 2022), recent studies suggest that eDNA read counts may also provide an indication of relative abundance and biomass (for review: Rourke et al. 2022).

Environmental DNA metabarcoding offers marked advantages over traditional methods of monitoring fish biodiversity, which can be invasive, labour-intensive, or limited in scope (Gaither et al. 2022; Miya 2022; Thomsen and Willerslev 2015). Environmental DNA assays, when analysed alongside environmental factors such as salinity, sea surface temperature (SST), or water depth, provide unprecedented insights into the environmental drivers of marine species distribution (Budd et al. 2023; Bush et al. 2017; DiBattista et al. 2022).

At small scales, eDNA metabarcoding offers high-resolution information about local biodiversity across microhabitats, even in environments influenced by dynamic tides and currents (Jeunen et al. 2019; Yamamoto et al. 2017). For example, Port et al. (2016) identified distinct vertebrate communities separated by only 60 m. Such localised patterns likely result from the rapid decay, dilution, and dispersion of DNA as it moves away from its source (Foote et al. 2012; for review: Miya 2022). These approaches are invaluable for mapping biodiversity, understanding local environmental drivers, and identifying conservation priorities within smaller geographic areas.

At large spatial scales, eDNA can reveal broad biodiversity patterns reflecting environmental gradients. For instance, DiBattista et al. (2022) combined metabarcoding with remote sensing (RS) to study fish community shifts in the Omani and Arabian Sea. They compiled 10 years of RS data on SST and chlorophyll a (Chlo-a) concentrations at a resolution of 5×5 km and combined it with eDNA-derived communities of bony fishes and invertebrates from 15 locations along the entire coastline. They successfully detected community shifts predicted by local SST and Chlo-a values.

In freshwater systems, the combination of eDNA and RS has been used to identify spatial scales of environmental drivers, such as terrestrial land use, that influence aquatic biodiversity (Zhang et al. 2023; Zong et al. 2024). As such, eDNA metabarcoding holds promise for tracking large-scale biodiversity trends with minimal sampling effort. When combined with widely available RS data and predictive models, eDNA metabarcoding could further enhance our ability to monitor ecosystem changes at broader scales (Keck, Brantschen, and Altermatt 2023).

Shark Bay, Western Australia, provides a model study system for applying eDNA metabarcoding in combination with RS, given its diverse seascape, environmental gradients, and iconic ecological value. A World Heritage Area and Marine Park Area (Department of Conservation and Land Management 1996), it hosts one of the largest and most diverse seagrass assemblages globally, supporting a myriad of invertebrates, finfish, elasmobranchs, reptiles, the world's largest dugong (*Dugong dugon*) population, and the longest-studied Indo-Pacific bottlenose dolphin (*Tursiops aduncus*) population (for review: Sutton and Shaw 2020).

Taking into consideration Shark Bay's extensive and diverse seagrass meadows, shallow embayments, and the high faunal diversity, the ecosystem is particularly vulnerable to climate change. The shallow, sheltered nature of the bay intensifies the effects of marine heatwaves (Hetzel 2014), which have already led to large-scale seagrass die-offs (Strydom et al. 2020). These losses have triggered cascading effects across the food web, impacting fisheries, dugongs, dolphins, turtles, and seabirds (Caputi et al. 2014; Nowicki et al. 2019; Pearce et al. 2011; Wild et al. 2019). This vulnerability underscores the value of integrating eDNA metabarcoding with remote sensing for monitoring and managing such ecologically sensitive systems.

Shark Bay's unique environmental conditions create diverse microhabitats that support varied local fish communities. Studies using conventional methods showed differences in Shark Bay's fish diversity across depth profiles, salinity gradients, substrate characteristics, seasons, and time of day (for review: Sutton and Shaw 2020). Yet, most of these studies were restricted either in geographic scope (focusing on target areas within parts of the bay, e.g., Heithaus 2004), taxonomic breadth (focusing on a single genus or species, e.g., Jackson et al. 2003) or summarised fish catches across large parts of the bay without any specific habitat focus (e.g., Taylor et al. 2018, 2019).

Here, we combine eDNA metabarcoding with high-resolution RS data to rapidly and comprehensively assess how environmental factors influence fish biodiversity in two large areas of Shark Bay. This approach aims to capture key environmental drivers of fish biodiversity patterns across an ecologically significant area vulnerable to climate change and is applicable to shallow coastal bays and other sensitive marine ecosystems

worldwide, thereby informing future biodiversity monitoring efforts.

We studied fish diversity across two areas in inner Shark Bay—one in the eastern gulf and one in the western gulf—spanning a salinity gradient, various depths, and diverse habitats. Using two fish-specific metabarcoding assays, we first compared our eDNA-based findings with conventional fish studies in Shark Bay to assess the taxonomic breadth and validity of our results. Next, we integrated metabarcoding data with satellite-derived environmental variables to evaluate their influence on fish biodiversity metrics. Finally, we used a predictive modelling approach to map fish diversity patterns across our study sites. This method provided detailed local insights while allowing for broader spatial assessments, offering valuable information for conservation planning.

2 | Material and Methods

2.1 | Environmental DNA

2.1.1 | Sampling Design

Shark Bay, located in Western Australia, encompasses an area of approximately 22,000 km^2 across two major gulfs, with water depths ranging from 1 to about 30 m (Sutton and Shaw 2020). The Peron Peninsula bisects the bay. Water exchange is limited in southern reaches, thus creating hypersaline regions, which form a north–south salinity gradient that classifies the bay as an inverse estuary (Hetzel et al. 2013). Its seascape is a mosaic of mangroves, shallow sandbanks, seagrass meadows, and deeper channels, with seasonal temperature gradients governed by the Capes Current in summer and the Leeuwin Current in winter (Pattiaratchi and Hetzel 2018).

Our sampling areas (combined ca. 557 km^2) comprised two long-term dolphin research sites within the eastern (ca. 230 km^2) and western (ca. 327 km^2) gulfs of Shark Bay. These areas were selected because they span key environmental gradients and habitat types, while also aligning with ongoing ecological research on the region's resident bottlenose dolphin populations (Connor and Krützen 2015). As dolphin behavioural data is typically collected during the austral winter, we aimed to capture a representative snapshot of fish biodiversity during this season.

To maximise the biological signal while minimising sampling effort, we employed a stratified random sampling design, thus enhancing sample representativeness and efficient capture of underlying biological patterns (Altermatt et al. 2023; Carvalho et al. 2016). This approach requires the definition of 'sampling units' which we derived from the 2016 "Shark Bay Marine Habitat Classification" a by-product of the 2016 seagrass map from Strydom et al. (2020) published in Sutton and Shaw (2020).

To account for the diffuse nature of eDNA signals, we divided both gulf study sites into 500 × 500 m grid cells (hereafter sampling grid), and samples were collected at the geographical centre of each selected grid cell. This ensured a minimum sampling distance of 500 m. We considered this distance adequate, as other eDNA studies in nearshore marine environments report effective sampling ranges from < 100 (O'Donnell et al. 2017; Port et al. 2016) to 800 m (Yamamoto et al. 2017). Each grid cell was assigned to a 'sampling unit', and grid cells were randomly selected from each unit. A detailed description of the study area and sampling design is given in the supporting information (SI Section S1: Table S1; Figure S1).

2.1.2 | Sampling and Extraction

All eDNA samples were collected between August 30 and September 27, 2021, by filtering seawater through 0.45 μm CN (Cellulose-Nitrate) filters using a peristaltic pump (GeoPump, Geotech Environmental Equipment Inc., Denver, Colorado) directly from the boat on site. At each location, we collected six samples of 3 L each, filtering a total of 18 L of seawater per location. We immediately stored the filter papers in Longmire's solution (Longmire et al. 1997) at room temperature until eDNA extraction, following the procedure described by Bizzozzero et al. (2024). In total, we sampled 45 locations (Figure 1; Figure S1) and collected 274 samples, including four field negative controls.

Samples targeted the mixed layer of the water column, specifically the upper to mid-water depths. At sites shallower than 7 m ($n = 20$), sampling was done at half the total depth; at deeper sites ($n = 25$), a fixed depth of 3.5 m was used due to hose length and pump power limitations. Overall, location depths ranged from 0.5 to 15.7 m. Given the well-mixed nature of Shark Bay, with minimal stratification and strong vertical mixing from tides or wind (Hetzel et al. 2015) the sampling depths are expected to capture representative eDNA from the entire mixed layer (Dukan et al. 2024).

Field blanks were collected by filtering 250 mL of molecular-grade water (AppliChem) directly on the boat using the same equipment and protocols as for eDNA sampling. The controls were taken both before and after each of the two sampling campaigns (in the eastern and western gulfs of Shark Bay) to account for potential contamination introduced during field handling.

2.1.3 | PCR, Library Preparation, and Sequencing

To cover a broad range of fish species, we amplified the samples targeting two fish-specific metabarcodes in different genomic regions as recommended by Kumar et al. (2022): a 16S rRNA gene fragment, hereafter Fish16S, and a 12S rRNA gene fragment, hereafter MiFish12S (Table 2). For each metabarcode, we generated and sequenced separate libraries following a published protocol (Bizzozzero et al. 2024). In short, we followed the Illumina MiSeq dual-index two-step PCR amplicon sequencing protocol, which prevents the generation of between-sample chimeras due to tag jumps, as reported in Schnell et al. (2015). In the first PCR (PCR1), the target sequence was amplified using the fish-specific primers with an overhang specific to the Illumina indexing primers and a heterogeneity spacer at the 5′-end (Blackman et al. 2021). PCR1 was done in triplicates and subsequently pooled for the second PCR (PCR2), where Nextera XT indices were incorporated using a reduced number of amplification cycles.

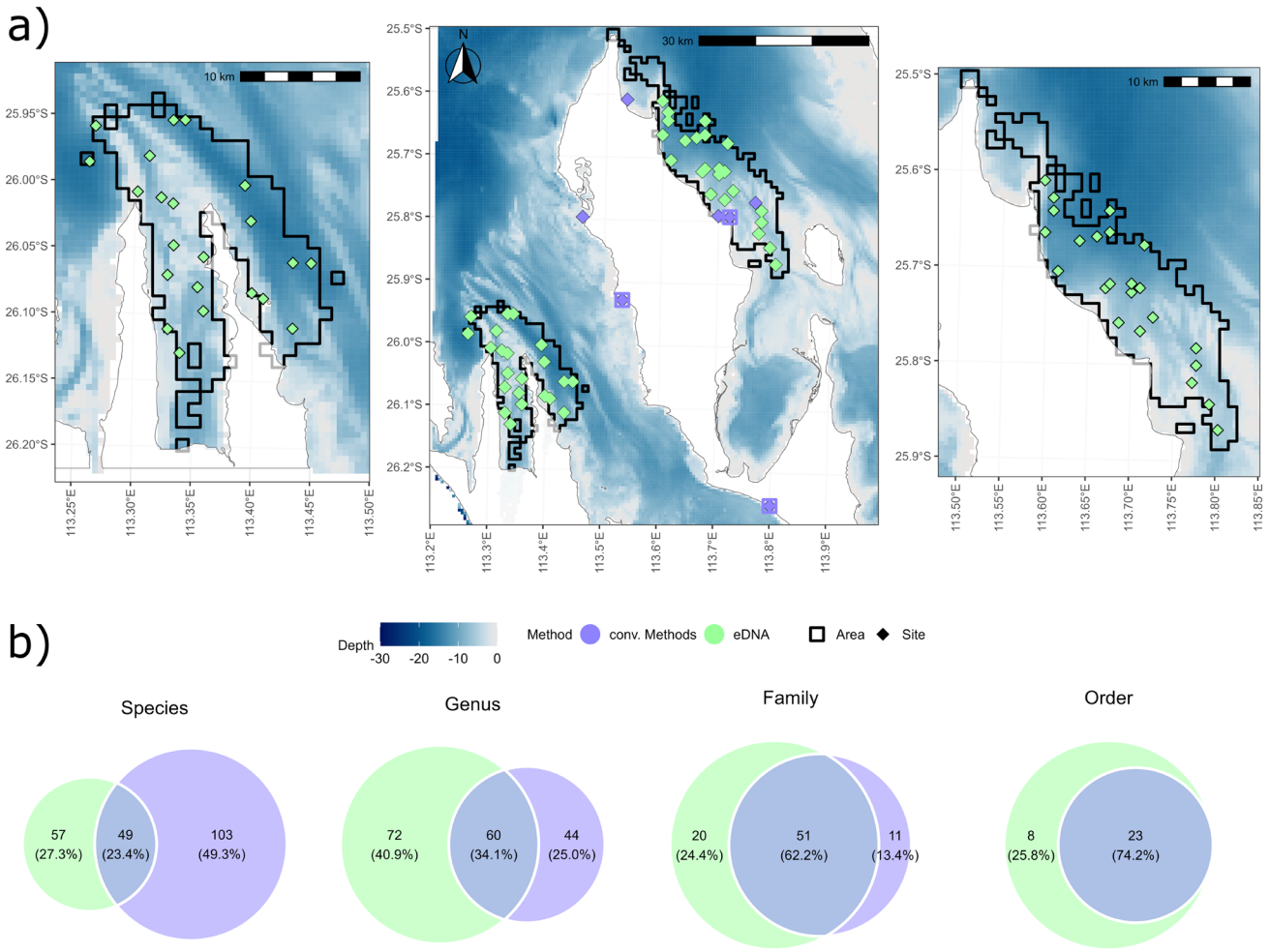

FIGURE 1 | (a) Overview of the eDNA sampling locations (green) and the areas or sites where fish biodiversity was assessed using conventional methods (periwinkle) in Shark Bay, Western Australia. In each eDNA sampling location, we collected six samples of 3 L of water. Landmasses are shown in white. The term "site" refers to sampling at the exact location, while "area" refers to the broader regions assessed by conventional methods, which involved collecting information from boats departing from these locations and surveying the adjacent areas. The study sites are delineated according to the survey effort and outlined in black. The background map depicts the water depth derived from bathymetry data (Beaman 2023). The maps are projected to the universal transverse mercator zone 49 south, using the GRS80 ellipsoid (b) Comparison of fish taxa detected with eDNA and conventional methods. Comparisons were made on different taxonomic levels: Species, genus, family, and order all within the class Actinopterygii.

We checked for possible contaminants, including several negative controls and two positive controls: a mock community (MC: Table S5) added to PCR1; and a positive index control, DNA from *Salmo trutta*, an alien species absent in Shark Bay, added to PCR2 (PC_{Index}). The PC_{Index} allowed us to detect cluster assignment errors during sequencing (Galan et al. 2018 and SI Section S2).

2.1.4 | Data Processing and Taxonomic Assignments

To facilitate data processing, the UNOISE3 workflow, as part of the USEARCH framework (v11.0.667_i86linux64), was applied (Edgar 2016). After removing PhiX-related reads (usearch:filter_phix) and those with low complexity (i.e., simple repeats or regions highly enriched with just one base; usearch:filter_lowc, TH = 25), the paired-end reads were merged (usearch:fastq_mergepairs, min. overlap = 30, min. %identity = 60, min merged length = 100). To improve the merging process, low-quality read ends were trimmed (usearch:fastx_truncate, R1/R2: 75/150). The primer sites were then removed (usearch:search_pcr), and the amplicon reads were filtered based on standard quality criteria (minimum mean quality = 20; length range: MiFish12 = 100–300; Fish16S = 150–350, and GC-content range = 30–70). The cleaned amplicon reads were processed into operational taxonomic units (OTUs) using the zero-radius clustering approach (ZOTUs). Finally, the cleaned amplicon reads were mapped to the ZOTUs to generate count tables.

Taxonomic classification was performed using SINTAX, a k-mer-based method (Edgar 2016). ZOTUs from the MiFish12S dataset were annotated with the MIDORI2 srRNA database (GB248), whereas the Fish16S dataset was enriched through annotations from multiple sources, including MIDORI2 (GB259), MitoFish (v397), and NCBI RefSeq (Fish-16S-v240202). To address the lower number of references available for the Fish16S metabarcode compared to the MiFish12S metabarcode, we supplemented the MIDORI2 reference database with additional reference sequences.

We processed and analysed our data in Rstudio V2022.07.2 (RStudio Team 2022), using R 4.3.0 (R Core Team 2023). To improve data quality, we used read counts from positive and negative controls (detailed in Section S6) to remove non-target taxa and external contaminants. We examined all samples, negative controls, and mock communities for external contamination originating from laboratory work and reagents by identifying non-target taxa (*Homo sapiens*, Hominidae, Primates, *Sus scrofa*, *Bos frontalis*, *Bos taurus*, Cypriniformes) and excluded them. We further checked for external contaminants; ZOTUs that had their maximum abundance in negative controls instead of samples, but did not find any (Taberlet et al. 2018). ZOTUs were filtered using a false assignment threshold (MiFish12S: 0.155%, Fish16S: 0.048%) based on PC_{Index} reads to correct for sequencing errors (Galan et al. 2018). Finally, samples with dysfunctional PCRs (MiFish12S and Fish16S: M2046, M2117; MiFish12S only: M1027) were identified and removed by plotting log-transformed read counts against taxonomic richness, where failed amplifications clustered with negative controls (Taberlet et al. 2018).

We evaluated the correctness of taxonomic assignments by checking whether the identified taxa were documented in the tropical Indo-West Pacific marine bioregion (Briggs and Bowen 2012). This was based on data from the Global Biodiversity Information Facility (GBIF 2001; accessed: 24.04.2024), the Australian Faunal Directory (ABRS 2020; accessed: 24.04.2024), and FishBase (FishBase 2021; accessed 24.04.2024). If a taxon was not recorded in the region in any of these databases, we reassigned it to a lower taxonomic level that more plausibly occurs in Shark Bay (SI Section S3: Table S2).

2.1.5 | Comparison With Studies Using Conventional Methods From Historical Records

To complement the broader plausibility assessment across the tropical Indo-West Pacific marine bioregion, we conducted a more targeted analysis specific to Shark Bay. We compared taxa identified in all eDNA samples and both metabarcodes (MiFish12S: $n = 267$, Fish16S: $n = 268$) with historical records from the eastern and western inner gulfs, focusing on areas close to our sampling sites (Figure 1).

This refined comparison was based exclusively on peer-reviewed studies or official reports ($n = 6$) using conventional methods including beach seining, otter trawls, surveys of recreational fisheries, and fish traps (Black et al. 1990; Heithaus 2004; Serrano et al. 2017; Taylor et al. 2018, 2019; Travers and Potter 2002). Although only some of these studies were designed to assess fish biodiversity, we included all because they documented fish species not reported elsewhere (SI Section S4: Table S3). We further assessed these results with regard to IUCN redlist entries (IUCN 2023; SI Section S8).

2.2 | Environmental Data Acquisition and Processing

We extracted marine habitat types, that is, channel, sand, sand/silt, seagrass, and turf algae, from the 2016 "Shark Bay Marine Habitat Classification" created by Strydom et al. (2020) as a by-product of their seagrass map and published as map in Sutton and Shaw (2020), which also informed our sampling design. Given the potential variability in seagrass extent in Shark Bay across different years (Strydom et al. 2020), we adjusted the seagrass extent in the habitat map using 2021 Sentinel-2 (level 2A) satellite imagery (Copernicus 2023a), applying a random forest algorithm (Table S4) in the Google Earth Engine (Gorelick et al. 2017) with a custom JavaScript (SI Section S5). We acquired the highest available resolution (10–1000 m) of satellite-derived data describing SST, Chlo-a, and total suspended matter (TSM) of our region of interest (Table 1; Figure S3). Furthermore, we constructed bathymetry derived values (depth, slope, complexity) based on publicly available bathymetries (Beaman 2023; Lebrec et al. 2021) (Table 1; Figure S3). Environmental variables were aggregated to the 500 × 500 m eDNA sampling grid. Continuous variables with higher resolution, such as bathymetry, Chlo-a, and TSM, were processed using 'bilinear' reprojection ('raster' package; Hijmans 2023) for bathymetry, while median values were calculated for Chlo-a and TSM. Percentage coverage of categorical habitat types (10 × 10 m grid) was calculated within the sampling grid (Figure S2; Figure S3). For further details on data acquisition and processing, see SI Section S5.

2.3 | Fish Diversity Analysis

2.3.1 | Fish Richness

We performed analyses of fish richness and structure at the genus level, given most reads were assignable at this level (Figure 1b, Table 2). For a more comprehensive assessment, these analyses were also replicated at the species and family levels (SI Sections S12–S14: Tables S8–S12; Figures S9–S25).

For the richness analysis, all samples from the same sampling location were combined, thus representing the full 18 L of water sampled per location. To ensure equal sampling effort, we only included locations where all six samples had been successfully sequenced ($n = 42$). We evaluated the correlation between read numbers per location and observed richness but found no significant relationship (SI Section S9: Figure S5,S6; Table S7). Having equal sampling efforts and with read depth not affecting richness counts, we used observed richness, without rarefaction, as our richness measure. To obtain a more comprehensive assessment, we also combined the data from both metabarcoding assays, that is, adding genera from the Fish16S assay that had been missing from the MiFish12S assay. We assessed our sampling effort's effectiveness in capturing fish richness at each location by calculating the mean percentage coverage of observed versus expected richness based on genus accumulation curves.

To evaluate the environmental factors influencing the genus richness, we used a negative binomial generalised linear model with a log link function as implemented in the 'MASS' package (Venables and Ripley 2002). To avoid multicollinearity, we excluded highly correlated environmental predictors (Pearson's correlation > 0.7; Zong et al. 2024). Abiding by this criterion, we excluded the layers associated with chlorophyll a concentration (Chlo-a median) and total suspended matter (TSM median, TSM sd) (SI Section S11: Figure S8). Furthermore, we excluded one of the habitat type

TABLE 1 | Summary of acquired environmental information used in this study.

Environmental variable	Data source	Spatial resolution	Original projection	Temporal resolution	Original value/units	Derived value/units	Modifications from original	Citation
Marine habitat types	Publication	10 m	Proj: UTM Zone: 49S Ellps: GRS80	2021	One layer with 24 habitat types	Individual layers of each marine habitat type (channel, sand, sand/silt, seagrass, turf algae) coverage in % of 500 m cell	See detailed modifications in SI	Strydom et al. (2020)
Bathymetry (depth)	Australian Government—Geoscience Australia	250 m	Proj: longlat Datum: WGS84	2023	Depth [m]	Depth [m], slope [°]	Projected onto sampling grid "proj: UTM zone: 49S ellps: GRS80" and to a resolution of 500 m	Beaman (2023)
Complexity	Publication	10 m	Proj: UTM Zone: 49S Datum: WGS84	2017–2020	Depth [m]	Sd within 500 m grid cell	Projected onto sampling grid "proj: UTM zone: 49S ellps: GRS80" and to a resolution of 500 m	Lebrec et al. (2021)
Sea surface temperature (SST)	European Space Agency (ESA) Sentinel-3A: SLSTR Level 2 Sea Surface Temperature (SST) non-time-critical data	1000 m	Proj: longlat Datum: WGS84	2021-08-29–2021-09-28	Bi-daily SST [Kelvin]	Median SST, and monthly median daily difference in SST; converted to °C	Projected onto sampling grid "proj: UTM zone: 49S ellps: GRS80" and to a resolution of 500 m	Copernicus (2023b), Copernicus Sentinel-3A data, 2021
Chlorophyll a (Chlo-a)	European Space Agency (ESA) Sentinel-3A: OLCI Level 2 Ocean Colour Full Resolution	300 m	Proj: longlat Datum: WGS84	2021-08-29–2021-09-28	Daily CHL_NN [mg/m^3] log10 scaled	Median and sd Chlo-a concentration	Projected onto sampling grid "proj: UTM zone: 49S ellps: GRS80" and to a resolution of 500 m	Copernicus (2023c), Copernicus Sentinel-3A data, 2021

(Continues)

TABLE 1 | (Continued)

Environmental variable	Data source	Spatial resolution	Original projection	Temporal resolution	Original value/units	Derived value/units	Modifications from original	Citation
Total Suspended Matter (TSM)	European Space Agency (ESA) Sentinel-3A: OLCI Level 2 Ocean Colour Full Resolution	300 m	Proj: longlat Datum: WGS84	2021-08-29–2021-09-28	Daily TSM_NN [g/m^3] log10 scaled	Median and sd TSM	Projected onto sampling grid "proj: UTM zone: 49S ellps: GRS80" and to a resolution of 500 m	Copernicus (2023c), Copernicus Sentinel-3A data, 2021
Salinity	Global Ocean Physics Analysis and Forecast level 4, Monthly mean fields for product	0.083°	Proj: longlat Datum: WGS84	01/09/2021	Monthly mean practical salinity unit	Monthly mean practical salinity unit	Projected onto sampling grid "proj: UTM zone: 49S ellps: GRS80" and to a resolution of 500 m	E.U. Copernicus Marine Service Information (2023)
Distance to shore	Calculated using the R package 'geosphere' (dist2Line) and a shapefile of Australia's current coastline	500 m	Proj: UTM Zone: 49S Ellps: GRS80	NA	Distance to shore [m]	Distance to shore [m]		Hijmans (2022)

TABLE 2 | A summary of sequencing metrics, ZOTUs, and taxonomic assignment resolution for MiFish12S and Fish16S metabarcodes.

Metric	MiFish12S	Fish16S
Original name	MiFish-U-F/R	Fish16SF/D-2R
Forward primer seq.	5′-GTCGGTAAAACTCGTGCCAGC-3′	5′-GACCCTATGGAGCTTTAGAC-3′
Reverse primer seq.	5′-CATAGTGGGGTATCTAATCCCAGTTTG-3′	5′-CGCTGTTATCCCTADRGTAACT-3′
References	Forward and reverse: (Miya et al. 2015)	Forward: (Berry et al. 2017), Reverse: (DiBattista et al. 2017)
Average length (bp)	170.0	203.6
Raw reads	19,961,920	16,934,796
Post-QC reads	17,057,831	16,289,206
ZOTUs	487	430
Median reads per sample	42,211	56,046
Min reads per sample	6744	10,990
Max reads per sample	191,623	99,987
Species-level assignment	33%	67%
Genus-level assignment	70%	90%
Family-level assignment	82%	96%

Note: The metrics include metabarcode name; the sequence of the targeted primers; references for the primers; the average read length; number of raw and processed (post-QC) reads used for creating ZOTU tables; the sequencing depth statistics (median, minimum, and maximum read counts per sample) after processing, applying the false assignment threshold, excluding contaminations, removing dysfunctional PCRs, and excluding controls. The taxonomic resolution is detailed at the species, genus, and family levels after taxonomic reassignment based on geographic context.

layers since all these layers originated from a single map and are therefore correlated. We chose to exclude 'turf algae' as it was associated with the highest level of uncertainty (Strydom et al. 2020).

After fitting the model, we observed overdispersion indicative of overfitting. Thus, we excluded Chlo-a standard deviation, slope, and distance to shore due to limited variability in our sampling design. To avoid further over-parameterisation, we included only interaction terms previously shown to be relevant for fish diversity in Shark Bay, that is, interactions between depth and seagrass and depth and sand (Heithaus 2004). After fitting the model, we checked for multicollinearity using the *vif* function from the 'car' package (Fox and Weisberg 2019) while omitting interaction terms and considering vif > 10 as a value of concern (Field 2012). We then used a minimal adequate model including only the significant predictors and those with a trend to predict genus richness across our study sites. In case of significant interactions, we kept the associated main term and its interaction.

2.3.2 | Fish Structure and Definition of Fish Communities

To analyse differences in genus structure in relation to environmental characteristics, we used a semi-quantitative approach by applying a Wisconsin double standardisation to our community matrix as per Kelly et al. (2019), using the *wisconsin* function of the 'vegan' package (Oksanen et al. 2022), resulting in standardised relative read counts. We then pooled the relative read counts of all samples from each sampling location ($n = 45$). Given that three locations had only five samples (due to dysfunctional PCRs), we randomly selected five samples from each location to ensure equal effort; thus, sample locations were represented by 15 L of filtered water. The same samples were chosen for both metabarcodes. We then agglomerated the data to the genus level and combined the two metabarcoding assays as previously described.

To determine which environmental predictors significantly influenced genus structure at different locations, we conducted a Canonical Correspondence Analysis (CCA) using the *cca* function in the 'vegan' package (Oksanen et al. 2022). A CCA was chosen due to its robustness in dealing with non-normally distributed data and is appropriate for longer and shorter environmental gradients. After accounting for multicollinearity, we used depth, channel percentage, Chlo-a sd, complexity, distance to shore, sand percentage, sand/silt percentage, seagrass percentage, slope, salinity, SST median, SST daily difference, and the interaction between depth and seagrass, and depth and sand, as predictors. We employed a forward stepwise model selection process via 'vegan's *ordiR2step* function (Oksanen et al. 2022) to select the best subset of environmental variables. On the final model, we performed a significance test using permutated ANOVAs ($n_{\text{permutations}} = 9999$). After fitting the model, we checked for multicollinearity using the *vif.cca* function of the 'vegan' package (Oksanen et al. 2022) while omitting interaction terms (vif > 10; Field 2012).

To extrapolate our findings across the study area, we firstly grouped our sampling locations into distinct clusters of similar relative fish structure, hereafter fish communities, using *k*-means clustering, as implemented in the package 'stats' (R Core Team 2023). We used Bray–Curtis dissimilarity to calculate the distances between samples using the vegdist function of the 'vegan' package (Oksanen et al. 2022). To obtain initial centres

for subsequent *k*-means clustering, we performed hierarchical clustering using various linkage methods: single, complete, average, Ward's minimum variance, and weighted average, applied via 'vegan's hclust (Oksanen et al. 2022) and 'cluster's agnes (Maechler et al. 2019) functions. We calculated the cophenetic correlation coefficients (CCC) to assess the fit of each linkage method and selected the best-fitting model based on its CCC value. To determine the optimal number of clusters, we applied the 'elbow' method (Holmes and Huber 2019), plotting within-cluster sum of squares (WSS) for *k* values ranging from 1 to 20 and choosing the number of clusters after the last sharp decline, that is, the 'elbow'.

Once the fish communities were established, we used the environmental characteristics of the sampling locations of each fish community to train a machine-learning random forest algorithm (Kuhn 2008). A random forest approach was chosen for its robustness in handling non-linear relationships. For this, we selected only the environmental variables that significantly contributed to fish distribution, as identified in the CCA. We weighted the selected environmental variable layers by multiplying the standardised *F*-values of the previously performed permutated ANOVA on the CCA. We extracted the values of each weighed layer at each sampling location using *extract* of the 'raster' package (Hijmans 2023) and trained random forest model (method = "rf") using the 'caret' package (Kuhn 2008), with the fish communities as the response variable and the weighed environmental values as predictors.

Due to the small sample size ($n = 45$), we used 'Leave-One-Out Cross-Validation' (LOOCV) to maximise the use of available data. To address unequal sample size among fish communities, we applied up-sampling during cross-validation. This technique reduces bias towards majority classes and improves the model's ability to accurately predict minority fish communities. We optimised the model by tuning the "mtry" parameter, which controls the number of variables randomly sampled at each split. We began by setting mtry $= \sqrt{p}$, where p is the number of predictors, and tested additional values (mtry = 2, 3, and 4) to select the value that maximised model accuracy. We then used this model to predict fish community affiliation of all raster cells within the study area based on their weighed environmental properties using the function *predict* via the 'stats' package (R Core Team 2023).

To identify areas where the random forest algorithm struggled to assign fish community affiliations, we extracted and plotted maximum assignment probabilities using *predict* with type set to "prob" and calculating a maximum raster layer from the resulting raster stack using *calc* of the 'raster' package (Hijmans 2023). Low maximum probabilities indicated assignment difficulties, as no clear signal was identified favouring a particular fish community. The lowest probability possible was 25% indicating no clear signal for any fish community.

2.3.3 | Fish Community—Genus Associations

We identified genera that were significantly associated with fish communities. To investigate genus-habitat associations with relative abundance data, we calculated group-equalised point-biserial coefficients using the *multipatt* function of the 'Indicspecies' package (Cáceres and Legendre 2009) and tested for significance using permutation tests ($n_{permutations} = 9999$). We also allowed associations to fish community pairings to allow a genus to be associated with two fish communities.

3 | Results

3.1 | Library and Taxonomic Assignments

Using the MiFish12S and Fish16S libraries, we generated 487 and 430 ZOTUs, respectively, with taxonomic assignments detailed in Table 2 and Table S6. The Fish16S library showed higher assignment rates at the species, genus, and family levels (Table 2). We identified one freshwater fish species, the fortescue grunter (*Leiopotherapon aheneus*), that occurs in a tributary. While this detection is plausible, we excluded it from our analyses since our focus was on marine species. See SI Sections S6 for sequencing results of positive and negative controls, and SI Section S7 (Figure S4) for a comparison of the MiFish12S and Fish16S metabarcodes.

3.2 | Comparison With Conventional Monitoring Methods From Historical Records

Collectively, the Mifish12S and Fish16S metabarcoding assays detected 106 species, 132 genera, 71 families, and 31 orders, all within the class ray-finned fish (Actinopterygii). Our review of historical records identified 152 different fish species, 104 genera, 62 families, and 23 orders, showing a high degree of consistency and overlap with our metabarcoding results at higher taxonomic levels: 32% at the species level, 58% at the genus level, 82% at the family level, and 100% at the order level. Taxonomic assignments did not consistently increase with lower taxonomic levels, as some could only be assigned to higher levels. While traditional methods have identified more fish species overall, metabarcoding revealed a broader diversity across genera, families, and orders (Figure 1).

3.3 | Fish Richness

Based on estimates from genus accumulation curves, our sampling effort per location covered 87% of the expected total genus diversity on average across locations (SI Section S10: Figure S7). Overall, the western gulf exhibited higher genus richness than the eastern gulf (Figure 3a). Genus richness increased with a higher percentage of channel habitat and decreasing salinity. Genus richness decreased with depth and increased with seagrass coverage (Table 3; Figure 3d; Figure S13). There was also a trend of higher richness correlating with sand/silt percentage. An assessment of model residuals revealed no remaining overdispersion or evident pattern, even when plotted against sampling date, longitude, or latitude, indicating no issues with temporal or spatial autocorrelation (Figure S10).

The minimal model used to predict genus richness across the study sites included depth, channel percentage, salinity, sand/

TABLE 3 | Fish (Actinopterygii) genus richness in Shark Bay, Western Australia modelled with environmental predictors using a negative binomial generalised linear model with log link function.

	Full model	Minimal model
Intercept	7.456 (1.835)***	**8.181 (1.122)*** **
Depth	−0.056 (0.013)***	**−0.06 (0.009)*** **
Channel percentage	0.591 (0.154)***	**0.565 (0.088)*** **
Complexity	−0.057 (0.108)	
Sand percentage	0.131 (0.185)	
Sand/Silt percentage	0.254 (0.149)°	**0.211 (0.092)***
Seagrass percentage	−0.39 (0.25)	**−0.533 (0.21)***
Salinity	−0.118 (0.034)***	**−0.112 (0.03)*** **
Sea surface temp. (daily diff.)	0.03 (0.04)	
Sea surface temp.	0.046 (0.064)	
Depth: Sand percentage	−0.012 (0.02)	
Depth: Seagrass percentage	0.125 (0.045)**	**0.141 (0.043)** **
Observations	42	42
Null deviance (df)	131.917 (41)	128.661 (41)
Residual deviance (df)	44.388 (30)	44.706 (35)

Note: The full model explained 66% of the variation and the minimal model used for extrapolation 65%. *p*-values < 0.05 are given in bold print.
***$p < 0.001$.
**$p < 0.01$.
*$p < 0.05$.
°$p < 0.1$.

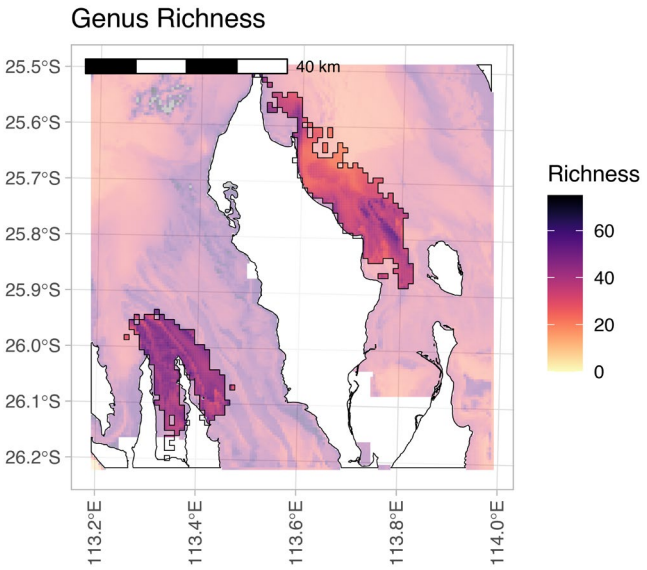

FIGURE 2 | Predicted fish genus richness (Actinopterygii) using the minimal model throughout the regions of interest (in full colour) that were representatively sampled, and throughout the rest of Shark Bay, Western Australia (faded). The map is projected to the universal transverse mercator zone 49 south, using the GRS80 ellipsoid.

silt percentage, and a depth and seagrass interaction (Table 3). The genus richness predictions indicated a gradient of richness in the eastern gulf, highest in the southern regions and areas closer to shore. In contrast, no such gradient was discernible in the western gulf. Here, the highest genus richness was found in shallow waters and channel habitats (Figure 2). The associated standard errors to the prediction plot illustrated challenges for the model to predict areas with moderate depth but high seagrass coverage (Figure S14), but those areas were not within our focus areas.

3.4 | Fish Structure and Definition of Fish Communities

For the genus structure, the forward stepwise selection resulted in a model that included four significant environmental variables accounting for 21% of the total variance: depth, channel percentage, seagrass percentage, and salinity (Table 4, SI Section S13). The variation in fish structure between the eastern and western gulfs was primarily influenced by salinity, as indicated by the clustering of sampling locations along axes 2 (Figure 4) and 3 (Figure S17).

To identify communities with similar relative fish structures, we applied k-means clustering. Among several hierarchical methods tested, average linkage clustering yielded the highest CCC (CCC = 0.79) and was therefore used to initialise cluster centres. Using the elbow method, we determined the optimal number of clusters—that is, distinct fish communities—to be five (Figure S15). In the CCA plots (Figure 4; Figure S17), colouring the sampling locations according to their assigned fish community revealed distinct patterns. Fish communities A and B were associated with shallow waters and increased seagrass coverage; however, community B occurred in the highest seagrass coverage and increased salinity (Figure S19). Fish communities C and D were found in deeper waters, with community C located in channel habitat. Fish community E occurred in areas with medium depth and salinity (Figure S19).

The random forest algorithm was trained on the environmental layers significantly shaping fish structure (depth, salinity, seagrass, and channel percentage) and used to identify regions in our study sites with similar environmental characteristics as the sampling locations of each fish community (Figure 4). The final model, using two randomly selected variables at each split (mtry = 2), achieved an accuracy of 76% and a Kappa coefficient of 0.69 based on leave-one-out cross-validation. This level of agreement between predicted and observed fish communities indicates a good reliability of the model's classification. The maximum assignment probability raster revealed that the algorithm had difficulties assigning environmental characteristics to a specific fish community in the southern regions of the eastern gulf (Figure S24).

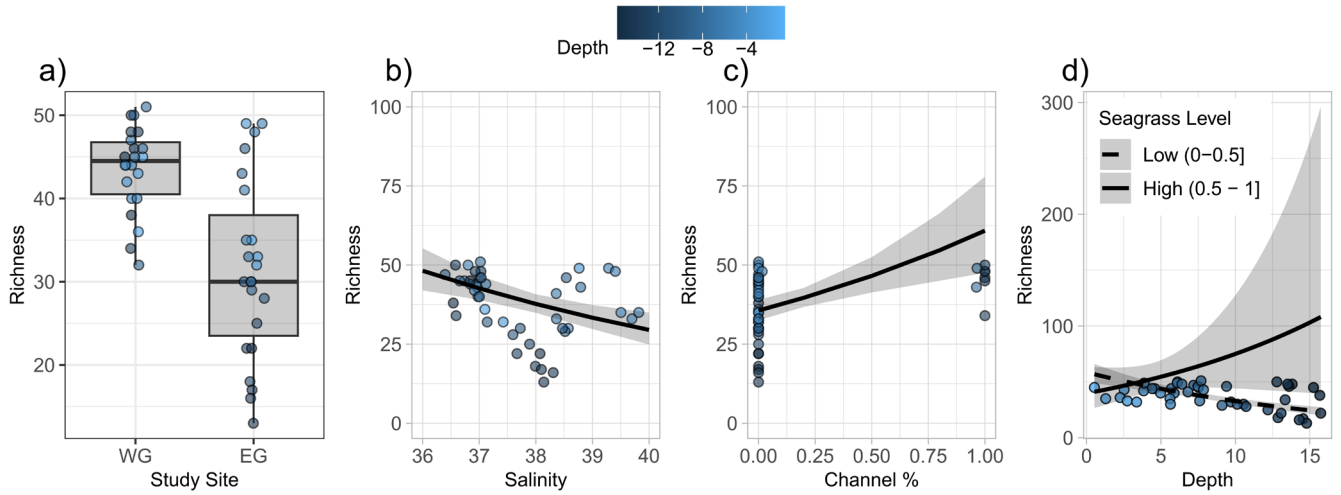

FIGURE 3 | (a) Observed fish genus richness (Actinopterygii) in the western and eastern gulf of Shark Bay, Western Australia. (b–d) Conditional effect plots of the significant predictors of genus richness from the full model. Each panel shows modelled effects as lines and observed data points coloured according to depth at the sampling location. (d) Shows the only significant interaction term in the model: Depth × seagrass cover. For visual clarity, we display conditional effects for two representative levels of seagrass (0 and 1), and plot the observed data grouped into low (0–0.5) and high (0.5–1) seagrass categories.

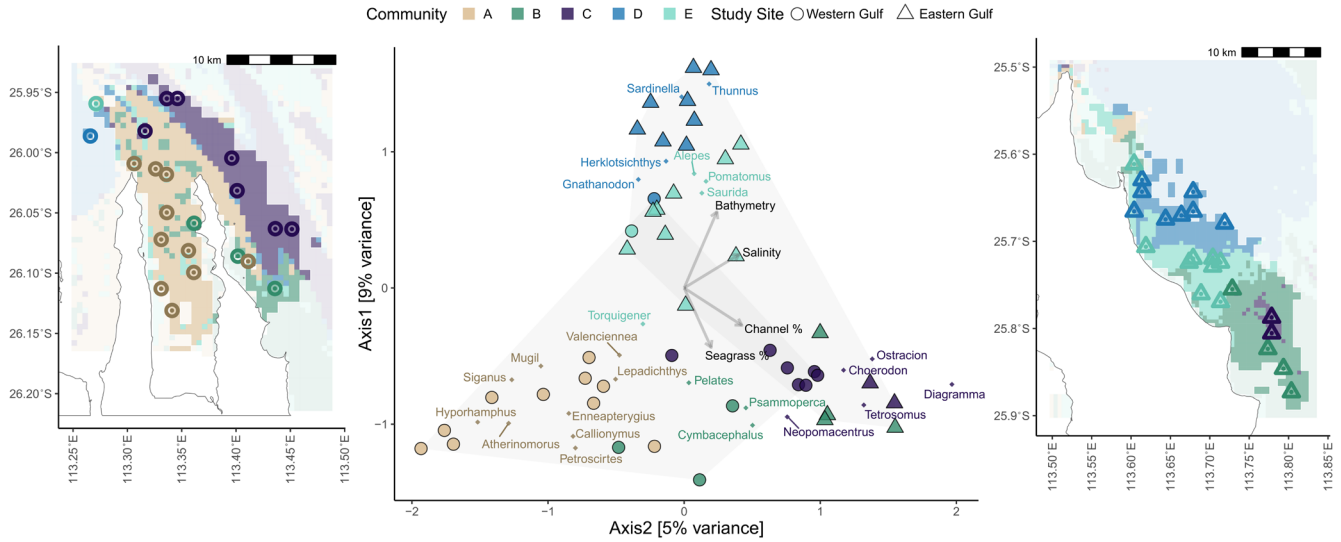

FIGURE 4 | Canonical correspondence analysis (CCA) biplot (centre) illustrating the relative fish genus structure (Actinopterygii) across sampling locations, along with maps of the western (left) and eastern (right) gulf sampling sites of Shark Bay, Western Australia. Sampling locations are represented by circles (west) or triangles (east) and colour-coded according to their respective fish community assignment (A–E). The maps are projected to the universal transverse mercator zone 49 south, using the GRS80 ellipsoid. In the CCA biplot, light grey convex hulls encompass all samples from the same gulf. Genera are displayed as small dots. Only genera significantly correlated with one fish community are shown and colour-coded accordingly. Arrows indicate the gradients of key environmental variables, including salinity, depth (bathymetry), channel, and seagrass percentage. The background tiles of the maps represent predicted fish community affiliations, with darker shaded areas marking the main study sites. Landmasses are shown in white.

The extrapolation (Figure 4) revealed distinct spatial patterns across the western and eastern gulfs. Areas dominated by the environmental conditions associated with fish community A were predominantly located in the western gulf, especially in the northern and central regions. In contrast, fish communities D and E, both characterised by different environmental profiles, were more prevalent in the eastern gulf. The northern and southern extremes of the eastern gulf exhibited fish community assignments similar to those found in the western gulf, suggesting some environmental overlap between certain areas of the two gulfs.

3.5 | Fish Community—Genus Associations

Our analyses revealed 25 genera that were significantly associated with only one fish community and eleven that were

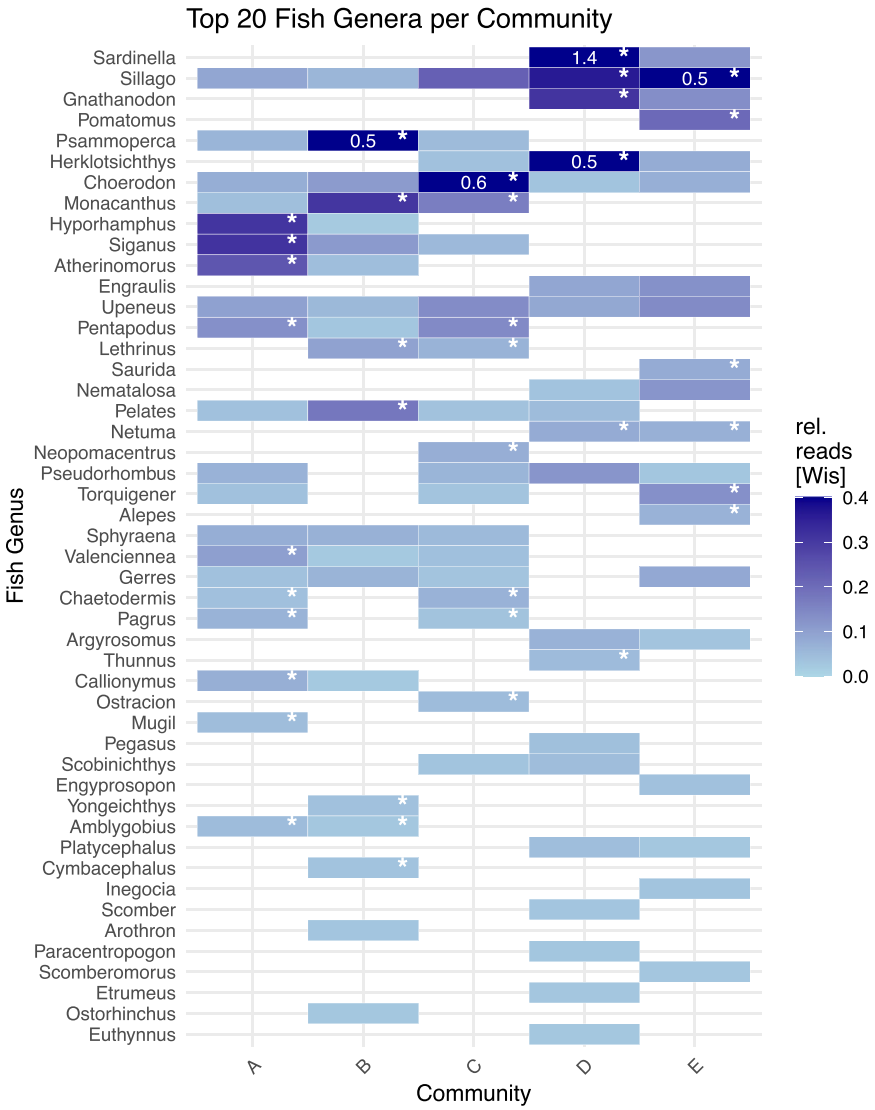

FIGURE 5 | Heatmap showing the top 20 fish genera (class Actinopterygii) by mean relative read counts (Wisconsin transformed) across each of the five fish communities within our study sites in Shark Bay, Western Australia. Each column represents a different fish community (A–E), and the fill gradient indicates the mean relative read counts of all samples within that fish community, with darker colours representing higher relative read counts. Values above 0.4 are capped at the maximum colour gradient to improve visualisation and avoid skewing, with actual values displayed on the plot. Genera with an asterisk (*) indicate significant associations with a specific fish community. Empty tiles indicate that the respective genus was not among the top 20 documented for the given fish community.

TABLE 4 | Permutation ANOVA of the CCA model on fish (Actinopterygii) genus structure in Shark Bay, Western Australia: $N = 45$, $X^2 = 1.08$, $F = 2.61$, $df = 4$, $p < 0.001$, $R^2_{adj} = 0.13$.

	df	χ^2	F	Pr (> F)
Depth	1	0.30	2.95	**0.001**
Channel percentage	1	0.28	2.70	**0.001**
Salinity	1	0.26	2.52	**0.001**
Seagrass percentage	1	0.21	1.99	**<0.01**
Residual	40	4.13		

Note: Significance of bold values indicates p-values < 0.05 are given in bold print.

associated with two different fish communities (Table 5, Figure 5).

4 | Discussion

This study proved the utility of eDNA metabarcoding as a powerful, non-invasive tool for fish biodiversity monitoring in Shark Bay, a UNESCO-listed World Heritage site and Marine Protected Area vulnerable to the effects of climate change (Heron et al. 2020). The validated metabarcodes, targeting 16S and 12S rRNA genes, are recommended for ongoing biodiversity assessments in Shark Bay and similar ecosystems globally. The integration of eDNA sampling with high-resolution

TABLE 5 | Significant associations of fish genera (Actinopterygii) and fish communities based on group-equalised point-biserial coefficients (r_{pb}) and permutation tests in Shark Bay, Western Australia.

	r_{pb}	p	Fish community
Clingfishes (*Lepadichthys* sp.)	0.459	0.024	A
Dragonets (*Callionymus* sp.)	0.447	0.029	A
Mullets (*Mugil* sp.)	0.588	0.002	A
Silversides (*Atherinomorus* sp.)	0.710	0.000	A
Rabbitfishes (*Siganus* sp.)	0.669	0.001	A
Triplefins (*Enneapterygius* sp.)	0.514	0.002	A
Combtooth blennies (*Petroscirtes* sp.)	0.596	0.000	A
Garfishes (*Hyporhamphus* sp.)	0.625	0.000	A
Glider gobies (*Valenciennea* sp.)	0.519	0.006	A
Grunters (*Pelates* sp.)	0.545	0.004	B
Seaperches (*Psammoperca* sp.)	0.793	0.000	B
Flatheads (*Cymbacephalus* sp.)	0.460	0.025	B
Boxfishes (*Tetrosomus* sp.)	0.437	0.021	C
Boxfishes (*Ostracion* sp.)	0.511	0.002	C
Tuskfishes (*Choerodon* sp.)	0.861	0.000	C
Lyretail damselfishes (*Neopomacentrus* sp.)	0.581	0.001	C
Dragonets (*Diagramma* sp.)	0.664	0.000	C
Herrings (*Herklotsichthys* sp.)	0.561	0.001	D
Sardines (*Sardinella* sp.)	0.947	0.000	D
Trevallies (*Gnathanodon* sp.)	0.456	0.022	D
Tunas (*Thunnus* sp.)	0.420	0.024	D
Pufferfishes (*Torquigener* sp.)	0.479	0.018	E
Lizardfishes (*Saurida* sp.)	0.648	0.001	E
Scads (*Alepes* sp.)	0.613	0.001	E
Tailors (*Pomatomus* sp.)	0.397	0.010	E
Gobies (*Amblygobius* sp.)	0.441	0.026	A+B
Seabreams and porgies (*Pagrus* sp.)	0.434	0.042	A+C
Leatherjackets (*Chaetodermis* sp.)	0.476	0.015	A+C
Threadfin breams (*Pentapodus* sp.)	0.706	0.000	A+C
Gobies (*Callogobius* sp.)	0.412	0.038	A+C
Filefishes (*Monacanthus* sp.)	0.599	0.001	B+C
Emperors (*Lethrinus* sp.)	0.414	0.048	B+C
Gobies (*Yongeichthys* sp.)	0.422	0.045	B+E
Filefishes (*Paramonacanthus* sp.)	0.458	0.026	C+E
Sea catfishes (*Netuma* sp.)	0.501	0.011	D+E
Sillagos (*Sillago* sp.)	0.556	0.001	D+E

Note: Scientific names are given in brackets.

satellite-derived environmental variables allowed us to identify salinity, depth, seagrass, and channel habitats as key environmental drivers shaping fish genus richness and community structure. The extrapolation of our results using machine-learning models delineated actionable targets for conservation efforts. Our findings underscore the ecological importance of, and need for directed conservation efforts toward, not only the renowned seagrass meadows (Strydom et al. 2020) but the channel systems, which support distinct and highly diverse fish communities (this study), as well as a globally unique community of tool-using dolphins (Bizzozzero et al. 2019; Wild et al. 2019).

4.1 | Taxonomic Assignments

When comparing ZOTU assignments to taxa documented in the tropical Indo-West Pacific, we identified some unlikely taxonomic assignments for Shark Bay, primarily linked to the MiFish12S metabarcode. This likely reflects unequal coverage in databases, as MiFish12S studies have focused predominantly on the northern hemisphere (Miya et al. 2020), with limited cataloguing of southern hemisphere taxa. Inadequate reference sequences can result in erroneous assignments, where ZOTUs are matched to closely related species from other regions (Couton et al. 2022; Keck, Couton, and Altermatt 2023), emphasising the need for careful metabarcode selection and consideration of their respective limitations. Fewer misassignments were observed with the Fish16S metabarcode, which has been more extensively applied in Western Australian studies (Nester et al. 2020; Stat et al. 2019; West et al. 2021).

Notably, compared to the distributions in the Australian Faunal Directory (ABRS 2020; accessed: 24.04.2024), we identified species such as Dunker's pipefishes (*Halicampus dunckeri*), dwarf flatheads (*Onigocia oligolepis*), barred garfishes (*Hemiramphus far*), blacktip sardinellas (*Sardinella albella*), and black rock-cods (*Epinephelus fuscoguttatus*), with adjacent but more tropical distributions, suggesting potential climate-driven range shifts—a globally documented consequence of climate change, with several species moving poleward along the Australian coastline (Gervais et al. 2021; Wernberg et al. 2016). Verification through conventional monitoring methods will be essential (Darling et al. 2020; Keck, Couton, and Altermatt 2023), but these findings highlight the need for further investigation, particularly given the knowledge gaps on tropical species' range shifts and the lack of baseline monitoring along much of Western Australia's coast (Gervais et al. 2021).

4.2 | Comparison With Conventional Monitoring Methods From Historical Records

When compared to conventional monitoring methods from historical records, the eDNA approach captured a broader range of taxa at higher taxonomic levels, including genera, families, and orders, but fewer species overall. At higher taxonomic levels, despite being applied in a single season, our approach captured over half of the fish biodiversity documented in previous studies. Given the lack of temporal and limited spatial overlap with the conventional studies, we did not anticipate such extensive overlap. For instance, at the family level, our approach identified all but eleven families previously recorded. Ten of these families were represented in one or both eDNA reference databases, suggesting their absence may be genuine, unless there was signal dropout due to primer mismatch. The only family missing from the databases was the halibuts (Psettodidae), which thus could not be detected even if present. Overall, our approach uncovered a wider diversity of taxa compared to conventional methods, a pattern that has been observed in multiple prior studies (reviewed in: Miya 2022).

The broader taxonomic scope at higher levels detected with metabarcoding suggests many ZOTUs were amplified but could not be confidently assigned to species level. This is most likely due to the lack of a regional reference database, which led to misassignments at the species level and thus necessitated reassignment to the genus level. Overall, our findings highlight the efficiency of eDNA as a biodiversity monitoring tool, particularly at higher taxonomic levels in Shark Bay. At the species level, establishing a custom database is recommended (Dugal et al. 2022). The broader taxonomic assignments still enable robust ecological inference, particularly when assessing community structure and environmental drivers.

4.3 | Key Environmental Drivers of Fish Richness and Community Structure

Genus richness increased as salinity decreased, and salinity significantly influenced fish community structure. Similar patterns were observed previously in Shark Bay, where hypersaline regions lacked herbivorous species (Campbell 2017) and exhibited reduced top-down herbivory on seagrass (Bell et al. 2019). A negative relationship between salinity and fish richness has been observed in other tropical coastal lagoons (Sosa-López et al. 2007) and is expected in increasingly saline conditions (Smyth and Elliott 2016; Whitfield et al. 2012). As a key environmental variable, salinity can profoundly affect vital processes in aquatic organisms (Smyth and Elliott 2016). Fish inhabiting hypersaline environments require physiological adaptations to counter osmotic challenges (Laverty and Skadhauge 2012), explaining salinity's profound impact on fish structure and richness (Smyth and Elliott 2016). Applying this knowledge to the entire bay suggests a turnover in fish structure along its salinity gradient, highlighting the need to incorporate salinity variation into conservation strategies, as proposed for estuaries (Elliott and Whitfield 2011). These strategies include protecting habitats across salinity gradients, preserving transitional zones, and addressing salinity impacts caused by climate change and other human activities to maintain biodiversity and ecosystem function (Barletta et al. 2010; Elliott and Whitfield 2011).

The percentage coverage of channel habitat positively influenced genus richness and significantly shaped fish structure. These habitats were characterised by canyon-like deep-water areas with mixed substrates that were difficult to identify via multispectral satellite imagery (Strydom et al. 2020). In our study area, previous research showed that channel substrates were primarily rocky or sandy and hosted conical sponges (e.g., *Echinodictyum mesenterinum*) (Tyne et al. 2012). The

mixed substrates and underwater valleys likely provide greater structural complexity than bare sand flats. Habitat complexity enhances species diversity by providing more crevices, offering shelter, nesting sites, and water flow that brings nutrients to substrate-attached organisms, which in turn serve as food sources for fish and other higher trophic levels (Gratwicke and Speight 2005; for review: Lazarus and Belmaker 2021). This study is the first to identify Shark Bay's channel habitats as local fish diversity hotspots.

Depth and seagrass coverage both affected the fish community structure, an effect that has already been shown in Shark Bay (reviewed in Sutton and Shaw 2020) and elsewhere (Hutchinson et al. 2014; Jones et al. 2021). Depth and seagrass coverage also jointly influenced fish richness. In areas with low seagrass coverage, depth negatively affected genus richness, a pattern not previously reported in Shark Bay. While Heithaus (2004) found no difference in species richness between deep and shallow 'unvegetated areas' (sensu no seagrass coverage), Travers and Potter (2002) observed higher richness in deeper unvegetated areas. Unlike these studies, using RS data, we were able to distinguish the unvegetated areas (sand, sand/silt, and channel habitats) and detected higher richness only in channels (15–30 m depth), which reflects the complexity of Shark Bay's microhabitats and aligns with Travers and Potter's (2002) findings.

In areas with higher seagrass coverage, greater depth increased genus richness. Similar results were reported in the eastern gulf, where shallow seagrass areas had lower richness than deeper ones (Heithaus 2004). Conversely, Travers and Potter (2002) observed higher fish density and diversity in nearshore (shallow) seagrass beds than in offshore (deeper) areas in the western gulf, outside our sampling area and during the austral summer, suggesting perhaps seasonal effects.

Differences in the relationship between seagrass and depth across studies may reflect variations in seagrass species composition and structural attributes, like fragmentation and growth patterns (Jackson et al. 2006; Smith et al. 2012). Although we could not distinguish seagrass species composition, our findings likely reflect general trends across Shark Bay's predominant seagrass species (*Amphibolis antarctica, Posidonia australis*, and *Halodule uninervis*), described by Walker et al. (1988). Future research might explore species-specific effects, growth patterns, and seasonal dynamics to reconcile discrepancies with studies such as Travers and Potter (2002).

4.4 | Fish Communities and Associated Genera

We identified five fish communities associated with distinct environmental conditions. Communities A and B predominantly occurred in seagrass habitats. Several genera associated with these communities, such as grunters (*Pelates*), filefishes (*Monacanthus*), gobies (*Amblygobius* and *Youngeichthys*), and seaperches (*Psammoperca*) have been previously linked to seagrass in Shark Bay (Black et al. 1990; Linke et al. 2001). Community A was distinguished by its occurrence in deeper waters, lower salinity, and reduced seagrass coverage. Low seagrass coverage may increase edge effects, which are known to influence fish assemblages (Smith et al. 2012). As Shark Bay's seagrass meadows face degradation from heatwaves (Arias-Ortiz et al. 2018; Strydom et al. 2020), reduced seagrass coverage may drive shifts in fish communities—from dense seagrass habitats to those with fragmented seagrass patches, or sand-dominated habitats.

Fish community C had the highest genus richness of all communities and was predominant in channel habitats. The genus tuskfishes (*Choreodon*), of which *C. schoenleinii* is near threatened (IUCN 2023), was strongly associated with this community though it occurred at lower relative abundances in all communities. Fairclough et al. (2008) found that three tuskfish species in Shark Bay (*C. schoenleinii, C. cyanodus, C. cauteroma*) were primarily associated with inner gulf reefs and rocky shorelines, consistent with the channel habitats in this study. However, their study was limited to depths down to 12 m, whereas much of fish community C's habitat was deeper. This underscores the value of eDNA sampling for monitoring regions less accessible by conventional methods.

Fish community D was found in open, sandy, or sand/silty deep-water habitats. The associated genera—herrings (*Herklotsichthys*), sardines (*Sardinella*), trevallies (*Gnathanodon*), and tunas (*Thunnus*), potentially including the endangered Southern bluefin tunas (*Thunnus maccoyii*) (IUCN 2023)—are known pelagic schooling fish along Western Australia's coast (Hobday et al. 2009). This community is of interest under climate change, as small pelagic fish, such as sardines and herring, are highly sensitive to environmental shifts due to their small size and intermediate trophic level (Hobday et al. 2009). Research on small pelagic fish in Shark Bay is limited, likely due to their aggregative behaviour, short life span, and sensitivity to change (Barangé et al. 2009). Environmental DNA monitoring could provide valuable insights into this community's habitat use and potential seasonal shifts.

Fish community E was found in medium-depth, medium-salinity habitats with no seagrass. Associated genera included pufferfishes (*Torquigener*), previously linked to sandy habitats in Shark Bay (Travers and Potter 2002), as well as the red-listed, vulnerable tailors (*Pomatomus*) (IUCN 2023), scads (*Alepes*), and lizardfishes (*Saurida*), known pelagic or benthic species associated with sand or mud bottoms (FishBase 2021). While these genera are known in Shark Bay, this study is the first to identify the environmental conditions where they occur in relatively higher abundance.

4.5 | Challenges and Recommendations

Although our non-invasive approach captured a winter snapshot of fish biodiversity that overlapped broadly with prior research using conventional methods, this design had inherent limitations. While our sampling was restricted to upper and mid-water depths due to logistical constraints, this may limit detection of taxa with strong vertical habitat preferences or during rare stratification events. However, given the typically well-mixed nature of Shark Bay and the relatively large volume of water filtered per sample, we consider our approach sufficient to broadly capture community structure in this system. Nonetheless, future work incorporating multi-depth sampling could further validate

these assumptions and improve resolution of fine-scale vertical patterns.

We did not detect effects of environmental factors such as SST and habitat complexity on fish diversity, despite their known influence in Shark Bay (Heithaus 2004) and elsewhere (Lazarus and Belmaker 2021). Limited SST variation during winter likely contributed to this, although SST indirectly influences biodiversity via its effects on seagrass coverage and salinity. It must be noted that satellite-derived SST represents a snapshot of water temperature at the surface and its relation to water temperature in deeper layers depends on temporally varying mixing rates.

Our habitat complexity metric—depth standard deviation at 10 m resolution—may have been insufficient to detect effects, though some complexity was likely captured in the channel habitat category. Furthermore, wide confidence intervals in the depth–seagrass interaction at depths > 10 m were due to limited sampling, as seagrass in Shark Bay grows only down to 12 m (Tyne et al. 2012), and deeper vegetated areas fell outside the focal regions (Strydom et al. 2020). Narrower confidence intervals within core regions support the reliability of our findings. Future studies should sample vegetated areas across broader depths, seasons, and seagrass growth patterns to enhance model applicability beyond the primary study area.

5 | Conclusion and Conservation Implications

By integrating eDNA metabarcoding with high-resolution RS data, we provide a powerful and scalable blueprint for monitoring biodiversity in dynamic marine ecosystems. While species-level identification remains constrained by reference database coverage and often benefits from the complementary use of conventional methods, the strength of the eDNA approach lies in its scalability and efficiency. It enables rapid biodiversity assessments across large spatial scales with comparatively minimal field effort, offering valuable first insights into broad ecological patterns where traditional monitoring would be logistically or financially prohibitive.

Our study in Shark Bay not only corroborates previous findings from conventional methods but also unveils novel insights, including potential early signals of climate-driven shifts in fish communities. The detection of tropical species expanding southward into this temperate region aligns with broader patterns of poleward migrations driven by ocean warming (Gervais et al. 2021; Wernberg et al. 2016), underscoring the urgency of adaptive conservation strategies (Elmqvist et al. 2003). Given Shark Bay's vulnerability to environmental and anthropogenic pressures (Arias-Ortiz et al. 2018; Heron et al. 2020; Strydom et al. 2020), integrating eDNA metabarcoding with remote sensing into long-term monitoring programmes offers an efficient, data-driven approach for proactive ecosystem management.

More broadly, our findings demonstrate the potential of combining eDNA, remote sensing, and machine learning to advance biodiversity monitoring, providing a scalable framework that can be applied to marine systems worldwide. By integrating eDNA, remote sensing, and predictive modelling, this approach is transferable to other shallow coastal bays, estuarine systems, and marine protected areas worldwide. Future applications could refine these models by incorporating additional environmental predictors such as hydrodynamic modelling to account for eDNA dispersal dynamics. Similar integrative approaches have shown promise in freshwater and terrestrial systems (Zhang et al. 2025; Zong et al. 2024), reinforcing the utility of coupling eDNA with remote sensing-derived environmental predictors and machine learning to map biodiversity patterns to understand broader ecological patterns and even cross-ecosystem linkages. As climate change accelerates habitat shifts and biodiversity loss, such interdisciplinary approaches are not just valuable but essential for preserving ecological resilience and informing targeted conservation efforts at a global scale.

Author Contributions

M.R.B., M.K., and F.A. conceived the study. M.R.B., M.K., and F.A. designed the sampling. M.R.B. and S.M.M. acquired and processed the eDNA data in the laboratory. M.R.B., S.M.M., J.-C.W., and E.P.W. analysed and interpreted the data bioinformatically and statistically. M.R.B. and A.D.-R. acquired and processed the remote sensing data. M.R.B. and M.K. wrote the manuscript. M.K. and S.J.A. coordinate the long-term research project. M.R.B., S.M.M., S.J.A., E.P.W., A.D.-R., F.A., J.-C.W., E.P.W., and M.K. edited the manuscript.

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Ethics Statement

The study was conducted in accordance with the relevant national and international guidelines. As the experiments were performed with water samples only and did not involve any endangered/protected fish species or experiments on live specimens, an ethics statement is not required for this study. Permits for Shark Bay Dolphin Research's activities were obtained from the Department of Biodiversity, Conservation and Attractions, Western Australia (FO25000142-3).

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The raw sequence data supporting the findings of this study is openly available in the European Nucleotide Archive (ENA) under project number PRJEB84923 (https://www.ebi.ac.uk/ena/browser/view/PRJEB84923) and on Dryad (https://doi.org/10.5061/dryad.kh18932jk). A summary of the bioinformatic workflow is available on

GitHub: https://github.com/mbizzo/eDNA-unveils-fine-scale-fish-biodiversity.

Peer Review

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Supporting Information

Additional supporting information can be found online in the Supporting Information section. **Data S1. Data S2.**