



'OCEANS AND LAKES'

INTERUNIVERSITY MASTER OF SCIENCE IN MARINE AND LACUSTRINE SCIENCE AND MANAGEMENT



Developing eDNA as a tool for assessing fish diversity on East African coral reefs

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Abstract

Coral reefs support some of the highest levels of biodiversity in the world but are increasingly threatened by a range of anthropogenic pressures, underlining the need for effective biodiversity monitoring. Environmental DNA (eDNA) metabarcoding has emerged over the last decade as a promising non-invasive technique for detecting aquatic organisms from water samples and has the potential to complement traditional underwater visual census (UVC) surveys. This study evaluated the performance of the 12S mitochondrial marker using Oxford Nanopore sequencing to characterise shallow coral reef fish communities along the southern Kenyan coast from filtered water samples. More specifically, it examined marker performance, the influence of protection status and geographic location on fish communities, and differences in species detection between eDNA and UVC surveys.

Sampling sites included both no-take and multiple-use areas in Diani, Mombasa, Shimoni, and Watamu. Amplification of fish DNA using the MiFish-U primers and subsequent sequencing across two independent runs successfully identified reef fish diversity at the family, genus, and species level. Overall, 41 families, 61 genera, and 71 species were identified across all reefs. We detected most expected reef-associated families, as well as the cryptic Gobiidae, which further illustrates one of the main advantages of eDNA metabarcoding, namely its ability to detect taxa that are commonly missed by UVC surveys. Geographic location had an influence on fish communities but not protection status. The Watamu Marine National Park showed greater taxonomic richness, while unprotected reefs in Shimoni showed comparable species compositions. Functional trophic composition was dominated by grazers and detritivores, while corallivores were absent, suggesting a shift towards ecological conditions commonly associated with coral reef degradation. While eDNA and UVC surveys recovered comparable taxonomic richness, overlap in detected species was limited.

Despite technical limitations associated with incomplete reference databases and sequencing performance, this study demonstrates that eDNA metabarcoding using Oxford Nanopore Technology can provide reliable ecological insights into Kenyan coral reef ecosystems.

Keywords: MPA, bioinformatics, Western Indian Ocean, next generation sequencing, high throughput sequencing, reef monitoring

Table of contents

Abstract	2
1. Introduction	5
1.1. Coral reefs and global warming	5
1.2. Conservation management in East Africa.....	5
1.3. Coral reef fish families	7
1.4. Diversity monitoring approaches	8
1.5. Scope of this study.....	9
2. Materials and methods	9
2.1. Sampling	9
2.2. DNA extraction	10
2.3. DNA amplification.....	11
2.4. Library preparation and sequencing.....	11
2.5. Bioinformatics	12
2.6. Video analysis.....	13
2.7. Statistical analyses	13
3. Results	14
3.1. DNA extractions and amplifications.....	14
3.2. Bioinformatic pipeline performance	14
3.3. Taxonomic resolution.....	15
3.4. Protection measures and fish communities	17
3.5. Methodology comparison.....	19
4. Discussion	22
4.1. Technical performance	22
4.2. Ecological interpretations	24
4.3. Comparing monitoring approaches	25
4.4. Optimising future research.....	26
5. Conclusion	27
Acknowledgements	29
References	30
Appendix A	45
Appendix B	46
Appendix C	47
Appendix D	49

Appendix E.....	51
Appendix F.....	54
Appendix G	55
Appendix H	59
Appendix I.....	62
Appendix J	63

1. Introduction

1.1. Coral reefs and global warming

Coral reefs, tropical shallow marine habitats, are among the most biodiverse ecosystems on Earth (Spalding et al., 2001). They support nearly a quarter of all marine life while providing essential ecosystem services to coastal communities, including sustaining fish stocks for fisheries, providing coastal protection from flooding and storms, and economic benefits through tourism (Moberg & Folke, 1999; Roberts et al., 2002; Buddemeier et al., 2004; Costanza et al., 2014; Ferrario et al., 2014; Hoegh-Guldberg et al., 2015).

Rising sea surface temperatures may trigger the expulsion of endosymbiotic algae from coral tissue, depriving species of their primary source of energy. This process, referred to as coral bleaching, can cause significant mortality among corals when heat stress is intense and/or prolonged. The ecological consequences include reduced coral cover, shifts in coral community structure, and alterations of associated reef fish communities (Loya et al., 2001; Stuart-Smith et al., 2018).

Human-driven climate change has increased heat stress in the oceans, resulting in more frequent, severe, and widespread mass coral bleaching events (Eakin et al., 2009; Heron et al., 2016; Oliver et al., 2018; Skirving et al., 2019; Eakin et al., 2026). These bleaching events rarely occurred before the 1980s (Glynn, 1993), but they have become progressively more common in recent decades and are now regarded as one of the dominant threats to coral reefs worldwide along with changes in ocean chemistry due to anthropogenic activities (Eakin et al., 2009; Eakin et al., 2010; Gattuso et al., 2014; Heron et al., 2016; Hoegh-Guldberg et al., 2017; Hughes et al., 2017; Hughes et al., 2018).

Between June 2014 and May 2017, reefs were impacted by the Third Global Coral Bleaching Event (Eakin et al., 2017; Eakin et al. 2018b; Sully et al., 2019). This was the first documented bleaching event that lasted significantly longer than one year (Eakin et al., 2018a; Eakin et al., 2018b; Skirving et al., 2019; Little et al., 2022). In some regions, bleaching continued even after the official end of this event (Burt et al., 2019; Raymundo et al., 2019). During this period, 80 % of reefs were exposed to moderate to severe bleaching, with a recorded mortality of 35 % (Eakin et al., 2026). Although exceptional at the time, similar or more severe impacts are now being observed during later events, including the ongoing Fourth Global Coral Bleaching Event (Reimer et al., 2024). Continued ocean warming is therefore expected to accelerate global coral loss and reef degradation, potentially resulting in irreversible ecosystem decline (Eakin et al., 2026).

1.2. Conservation management in East Africa

The Western Indian Ocean (WIO) includes several East African countries such as Comoros, Kenya, Madagascar, Mauritius, Mozambique, Seychelles, Somalia, South Africa, Tanzania, along with French territories such as Réunion and Mayotte. Its coral reefs support the livelihoods of approximately 60 million people, a number expected to grow strongly in the coming decades. As a result, proper management is crucial to sustain these resources (Spalding et al., 2007; Briggs

& Bowen, 2011; Barnes-Mauthe et al., 2013; Obura et al., 2017; Popova et al., 2019; Obura et al., 2021).

This region contains a large number of marine protected areas (MPAs; Figure 1), designated as no-take areas where tourism is permitted while protecting marine ecosystems from intensive small-scale fisheries (McClanahan et al., 2005). In Kenya specifically, these sites are often adjacent to multiple-use marine reserves, where fishing is allowed using hook-and-line gear, gill nets with mesh sizes larger than one inch, and basket traps. Seaweed harvesting, octopus fishing, and the harvesting of crabs and lobsters are also allowed. Banned practices include aquarium fishing, coral and mangrove harvesting, shell collection, speargun fishing, and the use of drag nets (McClanahan et al., 2005; Frontani, 2006; Tuda et al., 2014).

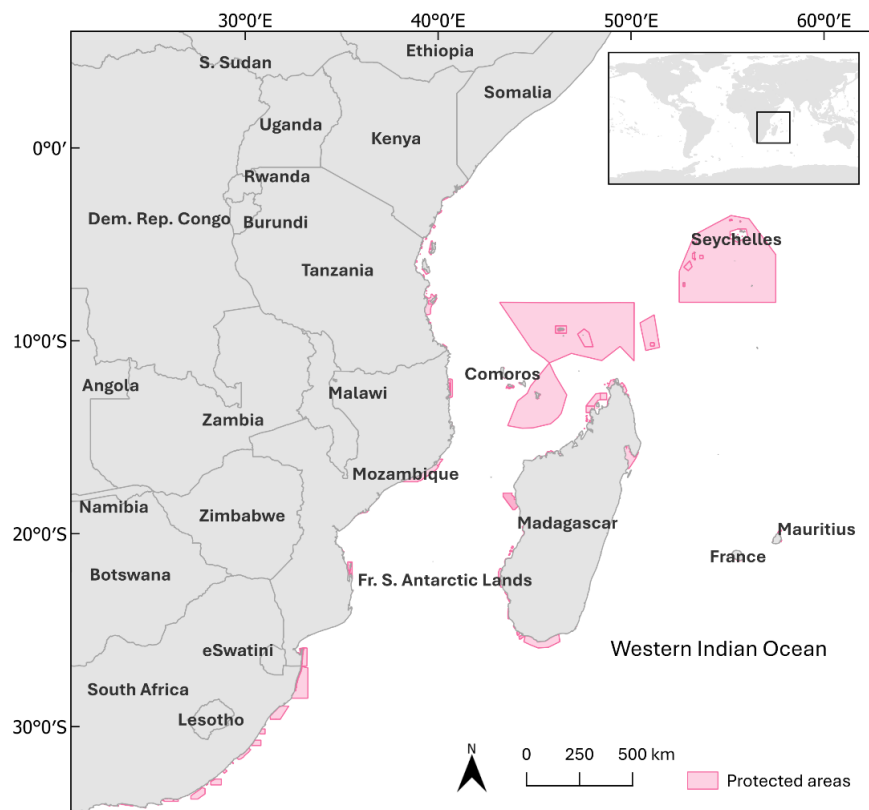


Figure 1. Overview of protected areas in the Western Indian Ocean, data derived from The World Database on Protected Areas (UNEP-WCMC and IUCN, 2026).

However, these management measures do not protect these areas from illegal fishing, the impact of mass tourism, pollution from beach users and nearby hotels, and climate change. Management effectiveness is often hindered by weak enforcement and increasing resource-use conflicts driven by a growing fishing community (McClanahan et al., 2005; Frontani, 2006; Tuda et al., 2014). Inefficient management remains one of the largest causes of the failure of successful conservation of global marine and terrestrial protected area systems. Estimates suggest that nearly two-thirds of MPAs worldwide are managed ineffectively, undermining their ability to deliver expected conservation outcomes and creating a false sense of protection (Kelleher, 1998; Mora et al., 2006; Hargreaves-Allen et al., 2011; Anthony et al., 2014). In the Kenyan Diani-Chale Marine Reserve for instance, fishing pressures remained persistent for

several years after official protection was declared due to weak enforcement capacities and limited management effectiveness (Alidina, 2005). Participatory governance represents a potential approach for increasing management effectiveness by promoting shared decision-making between governmental bodies and local resource users (Lockwood, 2009; Plummer & Armitage, 2010; Ayers & Kittinger, 2014). Social network analyses can provide valuable insights into these relationships and help identify opportunities for optimising management structures and governance strategies (O’Leary et al., 2020).

1.3. Coral reef fish families

The WIO is recognised as the second most important marine biodiversity hotspot in the Indo-Pacific, after the Coral Triangle in the East of Southeast Asia and the Western Pacific. Around 2,400 fish species are found in the region, with the highest richness concentrated along the East African continental coastline (Carpenter & Springer, 2005; Barber & Meyer, 2015; Obura, 2015). Several biodiversity hotspots along the Tanzanian coastline, Mayotte, southern Kenya, and parts of northern Mozambique have been identified (McClanahan et al., 2024).

Not unlike coral reefs in other parts of the world, this diversity is under significant threat, primarily due to fishing activities and bleaching events (Jacquet et al., 2010; Ateweberhan et al., 2011; Samoilys et al., 2017; Rehren et al., 2020). Large groupers are particularly vulnerable to overfishing and are widely used as indicators of both benthic condition and exploitation intensity (Hussey et al., 2014). In some regions, the absence of recent sightings suggests that certain grouper species might be locally extinct (Samoilys et al., 2022; Samoilys et al., 2025b). Significant reductions in biomass have been observed in several fish families, such as Acanthuridae, Balistidae, Epinephelidae, Lutjanidae, and Plectorhinchinae (sweetlips, a subfamily within Haemulidae), especially in areas within 100 km of impacting human activities. In contrast, fishing-restricted areas and deeper habitats showed a significantly higher fish biomass (McClanahan, 2019). Fish diversity is equally influenced by biological and environmental variables such as fish biomass, larval retention, and reef depth.

Fish communities are widely used to track changes in global marine biodiversity, with fish abundance and distribution recognised as one of the three Essential Ocean Variables (EOVs) (Miloslavich et al., 2018; Global Ocean Observing System, 2025). Following this approach, the Global Coral Reef Monitoring Network (GCRMN), under the International Coral Reef Initiative, uses underwater visual census (UVC) surveys to assess coral reef ecosystems (Obura et al., 2019). Within shallow coral reefs in the WIO, monitoring focuses on a set of 18 families. This includes Acanthuridae (surgeonfish), Balistidae (triggerfish), Caesionidae (fusiliers), Carangidae (trevallies), Chaetodontidae (butterflyfish), Epinephelidae (groupers), Haemulidae (grunts), Labridae (wrasses), wrasse subfamily Scarinae (parrotfish), Lethrinidae (emperors), Lutjanidae (snappers), Monacanthidae (filefish), Mullidae (goatfish), Nemipteridae (breams), Ostraciidae (boxfish), Pomacanthidae (angelfish), Pomacentridae (damselfish), Siganidae (rabbitfish), and Tetraodontidae (pufferfish) (Parravicini et al., 2013; Samoilys et al., 2022). Notably, Blenniidae (combtooth blennies) and Gobiidae (gobies), despite being valuable biodiversity indicators, were excluded since their cryptic behaviour makes them difficult to survey using UVC (Munday et al., 2008).

Recently, coral reef monitoring has shifted from only family-level indicators towards also including functional trophic groups, which better reflect the ecological roles of species. Four trophic groups essential for reef resilience have been proposed, represented by six fish families (Warmuth et al., 2024; Samoilys et al., 2025a). Corallivores, represented by Chaetodontidae, are dependent on live coral and are widely used as indicators of benthic condition (Hourigan et al., 1988; Pratchett et al., 2006; Russ & Leahy, 2017). Grazers and detritivores, including Acanthuridae and Siganidae, feed on algal turf and detritus and provide an indication of macroalgal abundance (Marshall & Mumby, 2012; Heenan & Williams, 2013). Excavators, comprising Scarinae, contribute to reef resilience by removing pieces of coral during feeding, promoting bioerosion, and creating settlement space for corals while reducing fleshy and turf algae (Heenan & Williams, 2013; Hussey et al., 2014). Primary piscivores, including Lutjanidae and Epinephelidae, occupy high trophic levels and are associated with fishing pressure (Jennings & Polunin, 1997; Hussey et al., 2014; Nash & Graham, 2016; Samoilys et al., 2025a).

Using trophic groups has its advantages but stays rather coarse when relying only on family-level identifications. Accurate species-level identification would greatly enhance it but is often challenging during UVC surveys. As a result, the GCRMN in the WIO region continues to rely on family-level indicators (Samoilys et al., 2025b).

1.4. Diversity monitoring approaches

Fish abundance has been traditionally assessed through methods such as catch analysis and UVC surveys. As a more recent alternative, environmental DNA (eDNA) analysis, utilises genetic material collected from environmental samples, including faeces, sediment, and water (Taberlet et al., 2018). In addition to being cost-effective and non-invasive, this approach also enables the detection of rare and elusive species (Coble et al., 2019; Tsuji et al., 2019). Its origins can be traced back to microbiological studies examining microbial communities in marine sediments (Ogram et al., 1987). This field later expanded through the first DNA metabarcoding study (Handelsman et al., 1998) and the reconstruction of ancient permafrost ecosystems (Willerslev et al., 2003). Further studies expanded its applications to the development of a method for the detection of invasive American bullfrog populations in freshwater systems (Ficetola et al., 2008) and the identification of spawning and migration patterns of potamodromous fish (Thalinger et al., 2019). Combined with advances in sequencing technologies, these developments have supported the increasing use of eDNA in conservation programmes (Taberlet et al., 2018).

Selecting an appropriate sampling and analysis approach is crucial as it directly affects species detection quality and potential biases, particularly through the choice of primer pair and targeted DNA region (Beng & Corlett, 2020; Van Der Loos & Nijland, 2020). Genetic markers targeting mitochondrial and nuclear genes are commonly used, with mitochondrial DNA often favoured due to its rapid evolution and the presence of multiple copies in each cell. Additionally, it has proven reliable for analysing degraded DNA, distinguishing between vertebrate species, and monitoring fish communities (Rees et al., 2014; Freeland, 2016).

Several universal fish primer pairs that target short regions of the mitochondrial 12S rRNA (Riaz et al. 2011; Taberlet et al. 2012; Miya et al. 2015), 16S rRNA (Evans et al. 2015; DiBattista et al. 2017), cytochrome *b* (Thomsen et al. 2012), and cytochrome *c* oxidase I (Balasingham et al. 2017)

genes, as well as the control region (Lee et al., 1995) are available. Among these, primer pairs targeting short 12S regions are generally considered the standard for eDNA metabarcoding of fish, with the MiFish-U universal primers recommended for fish biodiversity studies (Miya et al., 2015; Miya et al., 2020; Shu et al., 2020). By amplifying a hypervariable region of the 12S rRNA gene that is shorter than 200 base pairs, these primers enable taxonomic identification at family, genus, and species level using high-throughput platforms such as Illumina or Oxford Nanopore (Miya et al., 2015). However, successful identification ultimately depends on the quality and coverage of the reference database used (Taberlet et al., 2018).

1.5. Scope of this study

This study explores the performance of the 12S marker targeted by the MiFish-U primers for the analysis of fish diversity derived from coral reef water samples by asking the following research questions. First, can 12S be used as a genetic marker to detect fish diversity in coral reefs using eDNA samples? Second, can this information be used to assess ecological patterns, such as the impact of MPAs and geographic location on fish diversity? And lastly, does this technique detect similar fish diversity as UVC surveys?

Together, these questions help determining the potential of eDNA for ecological studies and marine conservation efforts.

2. Materials and methods

Analysing environmental DNA (eDNA) generally follows a structured workflow of sampling, DNA extraction, PCR amplification, and sequencing (Figure 2). Resulting sequencing data are processed and interpreted using bioinformatics and statistical methods.

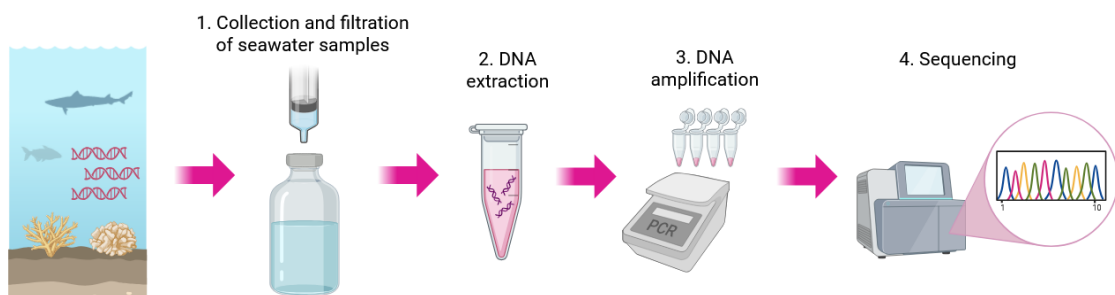


Figure 2. Schematic overview of environmental DNA analysis (created using Biorender).

2.1. Sampling

Seawater samples were collected from seven sites along the central and southern coast of Kenya from September to October in 2025: two at Watamu, Mombasa and Shimoni, and one at Diani (Figure 3; Appendix B). These locations were selected to compare reefs within no-take marine protected areas (MPAs) with nearby reefs that were either unprotected or designated as marine reserves, where certain fishing practices are permitted. Protected and non-protected reefs were separated by less than 9 km. At Diani, there is currently no no-take zone, only Diani-Chale Marine National Reserve, assuring partial protection.

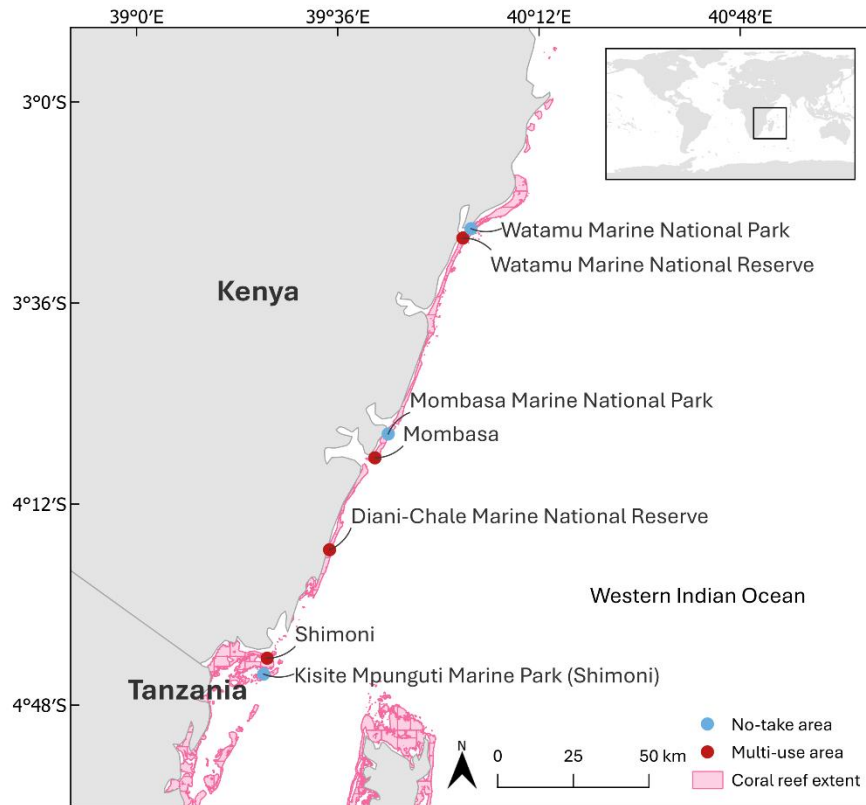


Figure 3. Sampling sites along the southern Kenyan coast, with coral reef data derived from the Allen Coral Atlas (2020).

Transect lines were deployed across shallow coral reefs for video surveys. At each sampling location, three 50 m transects parallel to the coastline were filmed for underwater video analysis and three seawater replicates were collected near the reef benthos using plastic bags, filtered using a 60 mL screw-on syringe and 0.45 μm eDNA Dual Filter Capsules (Sylphium molecular ecology), and preserved in 3.5 mL cetyltrimethylammonium bromide (CTAB) buffer. Unless stated otherwise, 4.5 L of water was filtered during low tides in shallow coral reefs. For four samples, field conditions did not allow for the required volume to be filtered completely (Appendix B).

2.2. DNA extraction

DNA was extracted using the Sylphium eDNA isolation kit SYL002 (Sylphium Inc, Groningen, The Netherlands) following the manufacturer's protocol. Extractions were performed in a DNA-free workspace equipped with DNA-free equipment and filter pipette tips, using gloves. Surfaces were decontaminated before the start of the extractions using a 10 % bleach solution and ultraviolet irradiation for a minimum of 1 hour. Internal negative controls (INCs) were included, consisting of preservation buffer from the sampling kit. All extractions were stored at -20°C until amplification.

Extraction quality and yield were evaluated using a NanoDrop™ 2000 spectrophotometer (Thermo Fisher Scientific Inc., Waltham, Massachusetts, USA). Concentration was measured and purity was assessed using the 260/280 and 260/230 ratios (Thermo Scientific, 2012).

2.3. DNA amplification

Two polymerase chain reaction (PCR) protocols, a one-step and a two-step approach, were applied to the same samples to compare sequence yield. First, a two-step protocol suggested by Collard et al. (2025) was applied, performing two consecutive PCR reactions using the forward (5'-TTTCTGTTGGTGCTGATATTGCGTCGGTAAACTCGTGCCAGC-3') and reverse (5'-ACTTGCCTGTCGCTCTATCTTCCATAGTGGGGTATCTAATCCCAGT-3') MiFish-U primers targeting the 12S marker.

The first PCR was performed in a total volume of 13 µL using 0.78 µL magnesium chloride (MgCl₂, 25mM), 3.5 µL nuclease-free water (VWR Chemicals, Water Molecular Biology Grade), 6.5 µL 2X Phire Plant Direct PCR Master Mix (Thermo Scientific, F-160), 0.61 µL of the forward primer (0.5 µM), 0.61 µL of the reverse primer (0.5 µM), and 1 µL of DNA extract. An internal negative control was included, using 1 µL nuclease-free water instead of DNA extract.

The PCR was run with the following temperature profile: an initial denaturation at 98 °C for 300 seconds, followed by 35 cycles of denaturation at 98 °C for 8 seconds, annealing at 50 °C for 8 seconds, and extension at 72 °C for 20 seconds, and finally a cool down to 12 °C.

Quality control of the two-step PCR products was performed using gel electrophoresis on a 1 % agarose gel, prepared by dissolving 1 g of agarose in 100 mL of 1X Tris/Borate/EDTA (TBE) buffer. The solution was heated until fully dissolved, then supplemented with 10 µL of GelRed® Nucleic Acid Stain (BIOTIUM; 0.5 mL; 10,000X in Water). Each well was loaded with 2 µL of PCR product, with 2 µL of O'GeneRuler Express DNA fixed size fragments mix (Thermo Scientific, SM1563, ready-to-use, 50 µg, 0.1 µg/µL) in the first lane. Electrophoresis was run at 85 V for 37 minutes. To successfully generated PCR-products, 1 µL of barcode (Appendix C) was added and a second PCR reaction was performed with the same temperature profile as the first PCR reaction but ran for 30 cycles.

Since barcode retention in the two-step protocol was minimal, causing the loss of more than 90% of sequenced reads, a one-step PCR protocol was attempted, using primers with incorporated barcodes (Appendix C). The one step PCR was run with the following temperature profile: an initial denaturation at 98 °C for 300 seconds, followed by 35 cycles of denaturation at 98 °C for 30 seconds, annealing at 50 °C for 30 seconds, and extension at 72 °C for 30 seconds, and finally a cool down to 12 °C.

2.4. Library preparation and sequencing

Prior to sequencing, 1 µL of each barcoded amplicon was pooled into a 1.5 mL Eppendorf™ DNA LoBind™ tube (Thermo Fisher Scientific Inc.). These were then size-selected using the SPRIselect Double Size Selection Protocol (Beckman Coulter, Inc.; 2026) with a left-right bead ratio of 1:1 to remove fragments shorter than 200 base pairs (bp).

Following this, library preparation was performed using the Ligation Sequencing Kit V14 (Oxford Nanopore Technologies, SQK-LSK114) according to the manufacturer's protocol (Oxford Nanopore Technologies, 2026a). This protocol was also used to prime and load the MinION Mk1D portable nanopore sequencing device (Oxford Nanopore Technologies, MD-102418) equipped

with a FLO-MIN114 (R10) flow cell (Oxford Nanopore Technologies, MinION or GridION Flow Cell R10 Version).

Sequencing was initiated using the MinKNOW protocol (Oxford Nanopore Technologies, 2026b) and ran for 24 hours.

2.5. Bioinformatics

Bioinformatic analysis was performed using the VUB HPC-cluster Hydra on the Vlaams Supercomputer Centrum (VSC; <https://hpc.vub.be>). A detailed overview of the code used can be found [here](#).

Reads were first demultiplexed according to their barcode using the software SeqKit2 (v2.12.0; Shen et al., 2024) prior to quality control, allowing for a maximum mismatch of three. Read statistics, including count and quality metrics, were extracted with the tool NanoStat (v1.6.0-gfbf-2025a; De Coster et al., 2018). Further quality filtering was performed using the module chopper (v0.12.0b-GCCcore-14.2.0; De Coster & Rademakers, 2023), selecting reads with a minimum quality score of Q = 10 and lengths between 150 and 400 bp.

Consensus sequences, which represent clusters of highly similar reads that likely belong to the same species (Bhargava & Sharma, 2013) were built first using the software VSEARCH (v2.30.6-GCC-14.2.0; Rognes et al., 2016), stacking identical sequences and dereplicating them. Sequences were ranked by abundance to help identify rare reads that might reflect contaminants or sequencing errors. Then, still using VSEARCH, molecular operational taxonomic units (MOTUs) were identified. A cluster of consensus sequences were accepted as a MOTU with a minimum similarity of 97 %, a minimum query coverage of 80 %, a minimum target coverage of 50 %, and using the full identity of the shortest sequence alignment algorithm.

The quality filtered sequences were then aligned to the reference sequences (MOTUs) using the tool minimap2 (v2.29-GCCcore-13.3.0; Li, 2018). The resulting alignments were subsequently used to build initial consensus sequences for the MOTUs using the module Racon (v1.5.0-GCCcore-12.3.0; Vaser et al., 2017), with filtering criteria maximum length 500 bp, maximum error rate 0.3, maximum number of mismatches 3, and maximum gaps length 4 bp. The selected sequences were then aligned again using minimap2, converted into BAM-files using the software SAMtools (v1.22.1-GCC-14.2.0; Danecek et al., 2021), and filtered for long repeats, a typical Oxford Nanopore artefact, using the module Medaka (v2.2.1-foss-2025a-CUDA-12.8.0; Lee et al., 2021). This resulted in a final consensus sequences file containing all MOTUs. A new minimap2 alignment was then conducted to assign each read to its respective MOTU and quantify the number of reads per MOTU, producing a read count table. To account for an inflated amount of MOTUs caused by sequencing errors, the post-clustering LULU algorithm was applied to identify and remove these artificial clusters using minimum co-occurrence 0.95 and minimum match of 84 % to consider a MOTU as an error as filtering criteria (Frøslev et al., 2017).

A reference database was developed containing 12S fish sequences, retrieved from GenBank at the National Center for Biotechnology Information (NCBI). Finally, MOTUs were assigned to sequences in the reference database using the tool BLAST+ (v2.17.0-gompi-2025a; Benson et al., 2016) which ran in custom-made python scripts using the SciPy-bundle (v2025.06-gfbf-2025a;

Virtanen et al., 2020). Assignments were classified in descending identity and accepted when they corresponded to the following three criteria: 80 % identity, 70 % query cover, and minimum alignment length of 80 bp.

Taxonomic assignments were accepted at minimum identity thresholds of 85 % for family, 90 % for genus, and 95 % for species. Identifications that reached the minimum threshold for species-level but did not occur in Kenyan waters were demoted to genus level.

We accept different MOTUs as a proxy for different species when they are identified as belonging to the same genus, but we are aware of the fact that we are not able to demonstrate that they actually are different species. Nevertheless, they will be referred to as species throughout the manuscript.

2.6. Video analysis

Video analysis data was obtained from footage collected simultaneously with eDNA sampling by Acevedo Ramírez (2026).

Data collection consisted of two survey methods: transect and free-search exploratory surveys. During the transect surveys, a 50 m transect tape was deployed along the reef and filmed using two approaches. The first involved a snorkeller equipped with a GoPro Hero 12, oriented at 90 °C to the substrate to record fish in the water column. The second method used a remotely operated vehicle (ROV) equipped with a forward-facing camera angled slightly downward. A five-minute interval was observed after transect deployment and before each filming session to allow fish to resume normal behaviour. Each transect was recorded for approximately 2-3 minutes.

Additional exploratory surveys were conducted using the ROV to capture fish outside the transect area. For analysis, only video segments containing at least two minutes of stable footage were retained.

2.7. Statistical analyses

All analyses were conducted in R (v4.4.1; R Core Team, 2024), using the packages ggven (v0.1.19; Yan, 2025), stats (v4.4.1; R Core Team, 2024), tidyverse (v2.0.0; Wickham et al., 2019), and vegan (v2.6-8; Oksanen et al., 2024).

The eDNA dataset was filtered to exclude non-fish taxa, species not occurring in the WIO (Heemstra et al., 2022), and all taxa detected in the negative controls. All non-MPA sites were classified as unprotected.

Relative read proportions of the top 10 taxa between the two sequencing runs were compared and visualised using horizontal bar plots. A Wilcoxon signed-rank test was applied to test for differences between sequencing runs. Effects of protection status and sampling location on fish community composition were analysed using Permutational Multivariate Analysis of Variance (PERMANOVA) based on Jaccard dissimilarities and visualised with non-metric multidimensional scaling (NMDS) ordinations. For each PERMANOVA, a corresponding betadisper analysis and permutation test were conducted to assess the assumption of homogeneity of multivariate dispersion. Functional trophic composition was summarised as

relative proportions of trophic groups and visualised using stacked bar plots. Methodological comparisons between eDNA and video surveys included taxa richness comparisons using boxplots and Wilcoxon signed-rank tests. It also included Venn diagrams of family- and genus-level detections, with differences assessed using Fisher's exact tests. Differences in species-level community composition between methods, protection status, sampling location, and their interactions were further analysed using PERMANOVAs, betadisper analyses, permutation tests, and NMDS ordinations.

3. Results

3.1. DNA extractions and amplifications

The extracted samples yielded an average nucleic acid concentration of 80.6 ± 54.3 ng/ μ L, whereas the internal negative controls (INCs) reached only 5.3 ± 2.8 ng/ μ L. They exhibited mean purity ratios of 1.8 ± 0.2 for the 260/280 and 2.5 ± 4.4 for 260/230 ratio, respectively (Appendix D). Both polymerase chain reaction (PCR) protocols successfully amplified the target region in all samples, producing distinct bands of approximately 300 base pairs. Based on these successful amplifications, the resulting PCR products were selected for sequencing.

3.2. Bioinformatic pipeline performance

Two independent sequencing runs generated the sequence data. The first analysed samples amplified with the two-step PCR protocol and the corresponding INC, whereas the second processed PCR products generated using both the one- and two-step protocols as well as the INC of the one-step amplification. The first run produced 4.55 million reads, which resulted in 8,068 identified molecular taxonomic units (MOTUs). Surprisingly, only 32 of these were retained after quality control and taxonomic assignment. Conversely, the second run generated fewer reads. However, a larger proportion was recovered after and substantially more identified MOTUs were recovered. In the end, 128 MOTUs were assigned from reads of the second run (Table 1).

Table 1. Read counts and sequence retention at each processing step for both sequencing runs, ending with the amount of identified molecular operational taxonomic units (MOTUs). The first run processed only two-step polymerase chain reaction (PCR) products, while the second run analysed both one-step and two-step PCR products. Data was derived from seawater samples collected in southern Kenya, amplified using a one-step and two-step polymerase chain protocol, and sequenced using Oxford Nanopore sequencing across two separate runs.

Step	Run 1	Retention (%)	Run 2	Retention (%)
Reads generated	4,550,000	100.00	166,280	100.00
Reads retained by MinKNOW	3,950,000	86.81	127,480	76.67
Demultiplexed reads	103,328	2.27	53,761	32.33
Quality and length filtered	8,068	0.18	31,749	19.09
Curated MOTUs	8,068	0.18	26,719	16.07
Identified MOTUs	32	0.00070	128	0.077

Protocol performance was directly compared by amplifying PCR product of samples SH_001, SH_002, SH_003, SH_007, SH_008, SH_009, MO_001, and MO_006 following both PCR protocols and sequencing them together in the second run. Since this run clearly did not perform optimally,

samples SH_007, MO_001, and MO_006 did not generate any reads for both protocols. For the five remaining samples, 18,073 demultiplexed reads were recovered, of which 95.30% originated from one-step PCR products, compared to 4.70% from two-step PCR products. This clearly demonstrated that in the two-step protocol, barcode ligation did not perform optimal and caused the loss of a substantial part of the data.

3.3. Taxonomic resolution

The first sequencing run produced 8,068 curated MOTUs, but following taxonomic assignment coupled with quality filtering, only 32 were retained, representing 800 reads. Additional removal of negative controls, non-fish taxa, freshwater species, and marine fish species not occurring in the Western Indian Ocean (WIO) further reduced the dataset to 24 unique MOTUs and 34 reads. Taxonomic assignment identified these sequences as 16 families, 15 genera, and nine species. In comparison, the second sequencing run produced substantially more assignments and more reads, retaining 128 taxonomically assigned MOTUs from a total of 23,719 and representing 13,054 reads. Following the same ecological and contamination filtering steps as for the first run, 100 unique MOTUs and 334 reads remained, representing 38 families, 56 genera, and 67 species (Table 2).

Table 2. Genetic marker success comparison between the two sequencing runs. Unique molecular operational taxonomic units (MOTUs), and total reads were compared on several levels, such as all taxa found in the eDNA data, fish taxa occurring in the Western Indian Ocean (WIO), family, genus, and species. Data was derived from seawater samples collected in southern Kenya, amplified using a one-step and two-step polymerase chain reaction protocol, and sequenced using Oxford Nanopore sequencing across two separate runs.

Level	Run 1 MOTUs	Run 1 total reads	Run 2 MOTUs	Run 2 total reads
All taxa	32	800	128	13,054
WIO fish taxa	24	34	100	334
Family	16	31	38	333
Genus	15	27	56	322
Species	9	12	67	273

Both in the first and the second sequencing run, more than half of all MOTUs were represented by a single read (Appendix E).

Pomacentridae represented the largest proportion of family-level assignments in both sequencing runs. The first run was further characterised by an equal representation of Acanthuridae, Holocentridae, Pempheridae, and Siganidae, while Labridae and Holocentridae were the next most abundant families in the second run (Figure 4).

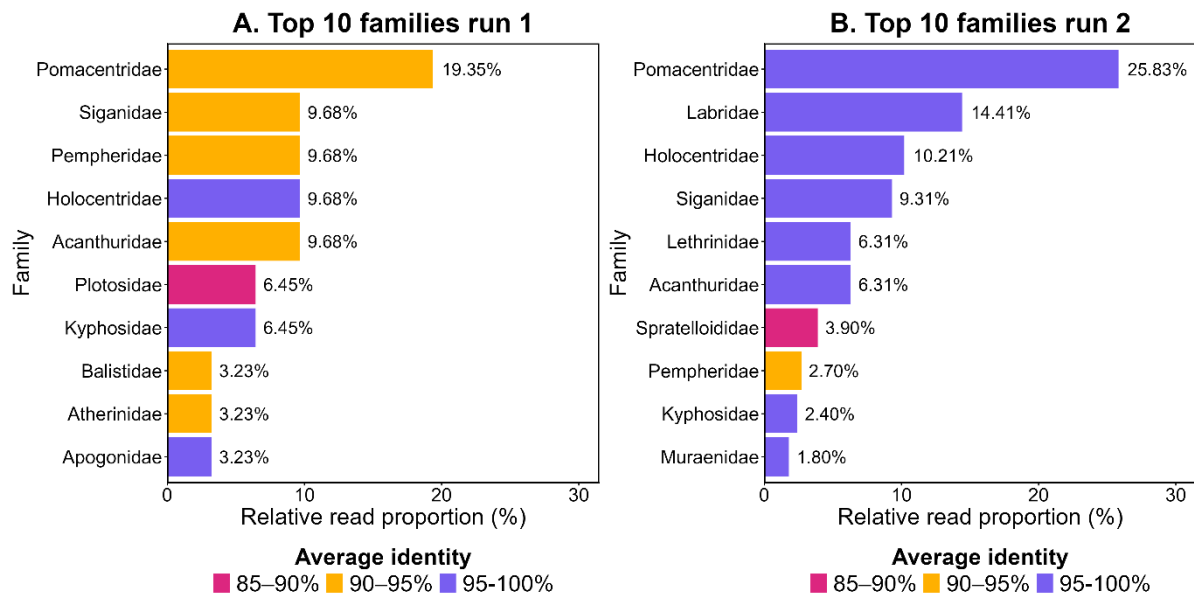


Figure 4. Relative read proportions of the top 10 detected fish families between (A) the first sequencing run and (B) the second. Taxa were grouped into three distinct categories depending on their average identity. Data was derived from seawater samples collected in southern Kenya, amplified using a one-step and two-step polymerase chain protocol, and sequenced using Oxford Nanopore across two separate runs. The first run contained two-step products, while the second run contained products from both protocols.

Genus-level assignments differed more notably, where the first run was characterised by equal representation of *Abudefduf*, *Acanthurus*, *Myripristis*, *Siganus*, and *Stegastes*, while *Abudefduf* dominated the second run, followed by *Myripristis*, and *Siganus* (Figure 5).

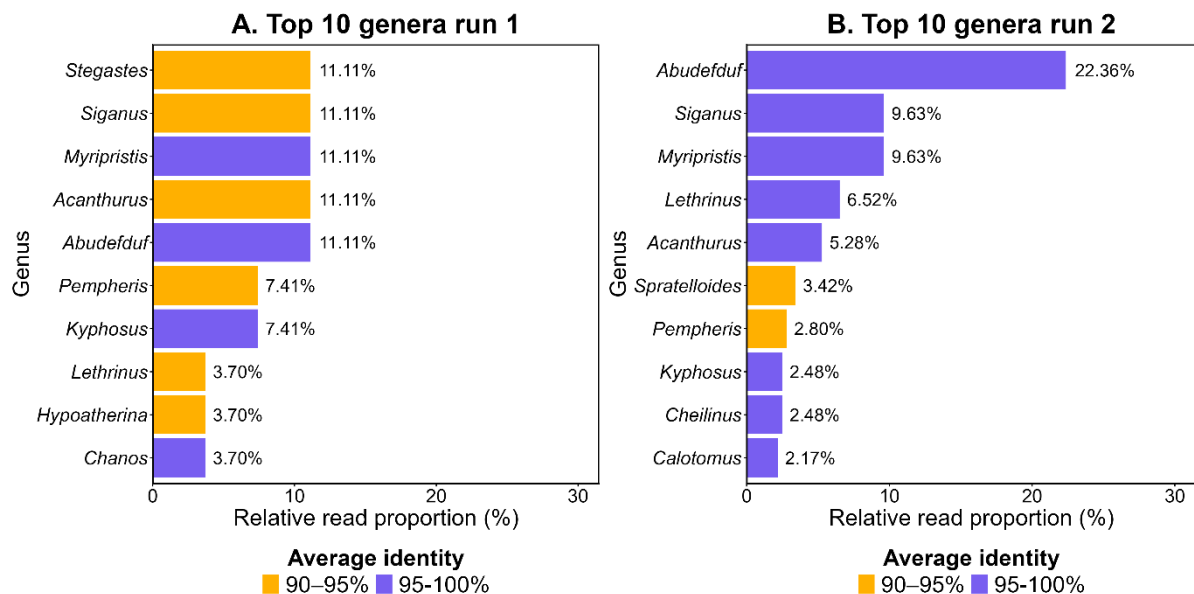


Figure 5. Relative read proportions of the top 10 detected fish genera between the (A) the first sequencing run and (B) the second. Taxa were grouped into two distinct categories depending on their average identities. Data was derived from seawater samples collected in southern Kenya, amplified using a one-step and two-step polymerase chain protocol, and sequenced using Oxford Nanopore sequencing across two separate runs. The first run contained two-step products, while the second run contained products from both protocols.

A similar pattern occurred at the species level, where *Abudefduf vaigiensis* and unidentified *Kyphosus* and *Myripristis* MOTUs equally represented the top three assignments in the first run.

In the second run *Abudefduf vaigiensis* was followed by an unidentified *Myripristis* MOTU and *Siganus sutor* (Appendix F).

Despite these differences, a Wilcoxon test did not detect any statistically significant difference between both runs. Nevertheless, due to the noteworthy imbalance in identified MOTUs, only data of the one-step PCR products of the second run was retained for further ecological analyses. Comparison between video data and assessing overall performance of the eDNA method included the entire dataset of both runs.

3.4. Protection measures and fish communities

Taxonomic assignments and richness differed among locations. The Watamu Marine National Park, no-take zone, produced the highest number of assignments at every taxonomic level, with the unprotected reefs in Shimoni ranking second. Across all three taxonomic levels, these two locations showed similar community compositions. In contrast, reefs in the Watamu Marine National Reserve, multi-use zone with fishing permitted, consistently showed the fewest assignments and the lowest MOTU richness across all taxonomic levels (Table 3; Appendix G), forming distinct communities (Appendix H).

Table 3. Taxonomic assignments and richness observed across locations at the family, genus, and species levels. Sampling locations include Diani-Chale Marine Reserve (DI_UNP), Shimoni (SH_UNP), Watamu Marine National Park (WA_MPA), and Watamu Marine National Reserve (WA_UNP). Data was derived from seawater samples collected in southern Kenya, amplified using a one-step polymerase chain protocol, and sequenced using Oxford Nanopore.

Sample	Family assignments	Family richness	Genus assignments	Genus richness	Species assignments	Species richness
DI_UNP	17	10	15	12	11	10
SH_UNP	43	22	39	27	29	23
WA_MPA	51	22	48	30	34	31
WA_UNP	9	4	9	6	6	5

Species-level community composition showed no significant effect of protection status ($R^2 = 0.11$, $F = 1.028$, $p = 0.46$; Figure 6A), while location had a significant effect ($R^2 = 0.26$, $F = 1.25$, $p = 0.03$; Figure 6B). Homogeneity of dispersion was satisfied for location ($F = 2.80$, $p = 0.14$), but not for protection status ($F = 15.53$, $p = 0.001$).

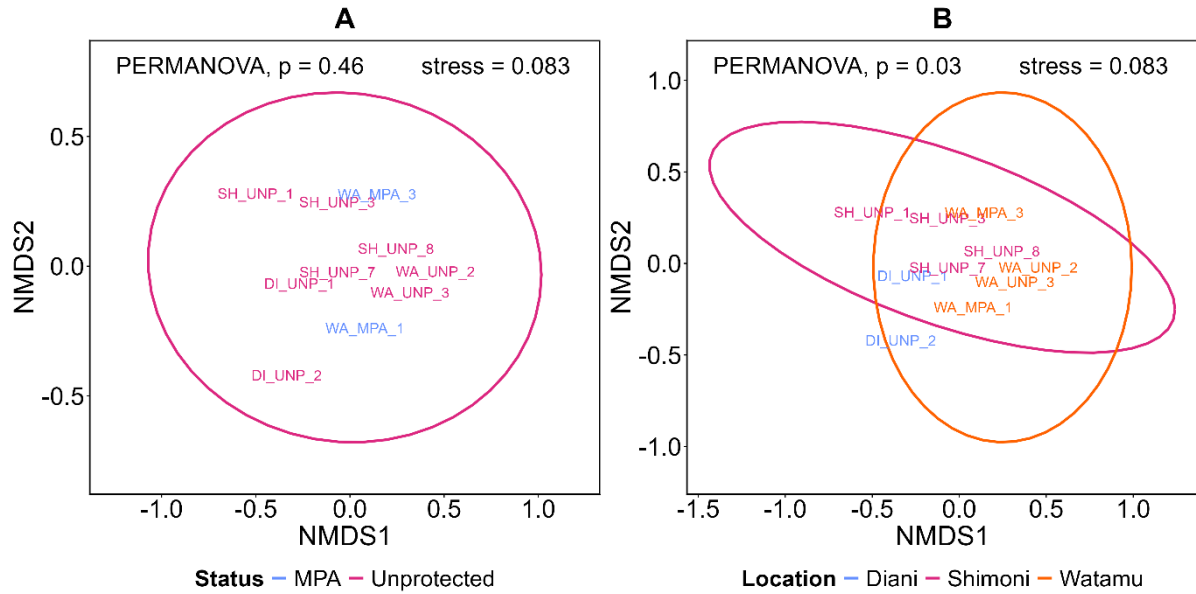


Figure 6. Non-metric multidimensional scaling (NMDS) ordination of fish communities. (A) Species communities structured by no-take zones in marine protected areas (MPAs) or unprotected areas with fishing permitted. (B) Species composition driven by geographic location. Permutational Multivariate Analysis of Variances (PERMANOVA)'s p-values and stress scores are presented within each panel. Data was derived from seawater samples collected in southern Kenya, amplified using a one-step polymerase chain reaction protocol, and sequenced using Oxford Nanopore. Sample SH_UNP_9 was excluded in order to generate a reasonable stress score.

Grazers and detritivores, represented by Acanthuridae and Siganidae, were the dominant functional groups across both protection statuses and all sampling locations, while corallivores, Chaetodontidae, were not detected. In contrast to unprotected reefs, piscivores including Lutjanidae and Epinephelidae were absent from MPAs. However, protection status did not significantly affect functional trophic composition ($R^2 = 0.060$, $F = 0.45$, $p = 0.62$; Figure 7A) and dispersion remained homogenous ($F = 1.98$, $p = 0.18$). Geographic location showed a significant association with functional trophic composition ($R^2 = 0.71$, $F = 7.38$, $p = 0.019$), with Diani characterised exclusively by grazers and detritivores, Watamu additionally supporting excavators such as Scarinae, and Shimoni supporting piscivores as well (Figure 7B). The assumption of homogeneity of dispersion was met ($F = 2.20$, $p = 0.24$).

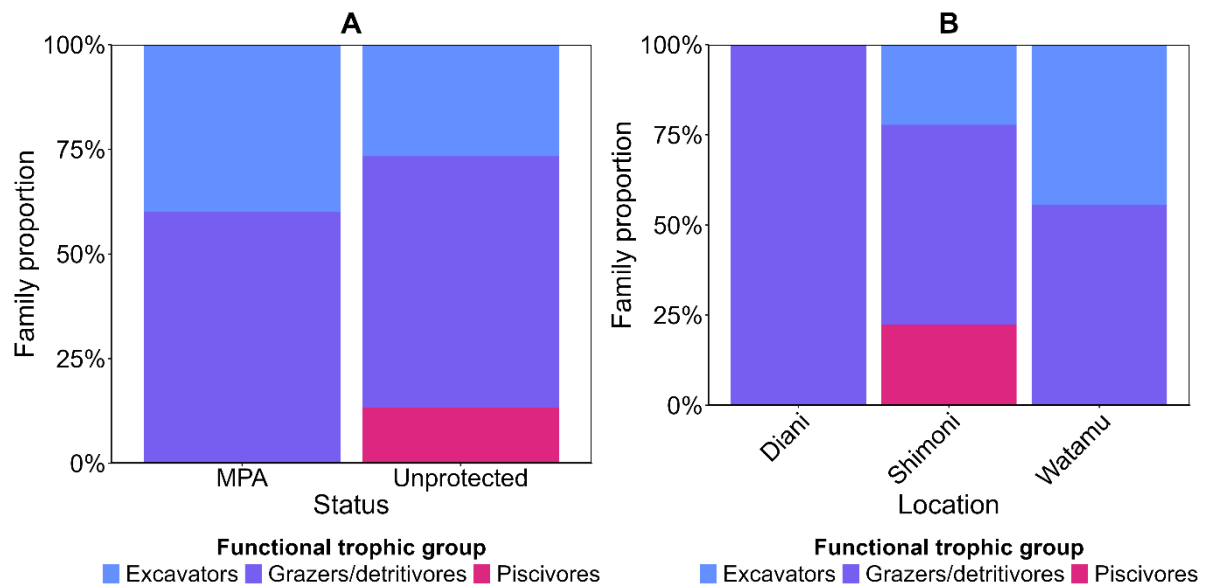


Figure 7. Comparisons of functional trophic structure among fish communities. (A) Relative proportions in marine protected areas (MPAs) and unprotected reefs. (B) Relative proportions across locations. Data was derived from seawater samples collected in southern Kenya, amplified using a one-step polymerase chain reaction protocol, and sequenced using Oxford Nanopore.

3.5. Methodology comparison

The highest taxonomic richness across all taxonomic levels occurred in the Watamu Marine National Park, no-take zone, according to eDNA surveys, whereas underwater visual census (UVC) surveys ranked another no-take zone, Kisite Mpunguti Marine Park, highest (Appendix I). Despite this difference, both methods detected comparable numbers of families, genera, and species across all sampling sites (family: $V=13$, $p=0.18$; genus: $V=14$, $p=1$; species: $V=5$, $p=0.29$; Figure 8A-C). Within MPAs, UVC surveys detected slightly more genera and species on average, although these differences were not significant (family: $V=2$, $p=1$; genus: $V=3$, $p=1$; species: $V=2$, $p=0.75$; Figure 8D-F). In unprotected reefs, eDNA detected slightly more families and genera, but these were, once again, not significant (family: $V=6$, $p=0.18$; genus: $V=7$, $p=0.62$; species: $V=0$, $p=0.18$; Figure 8G-I).

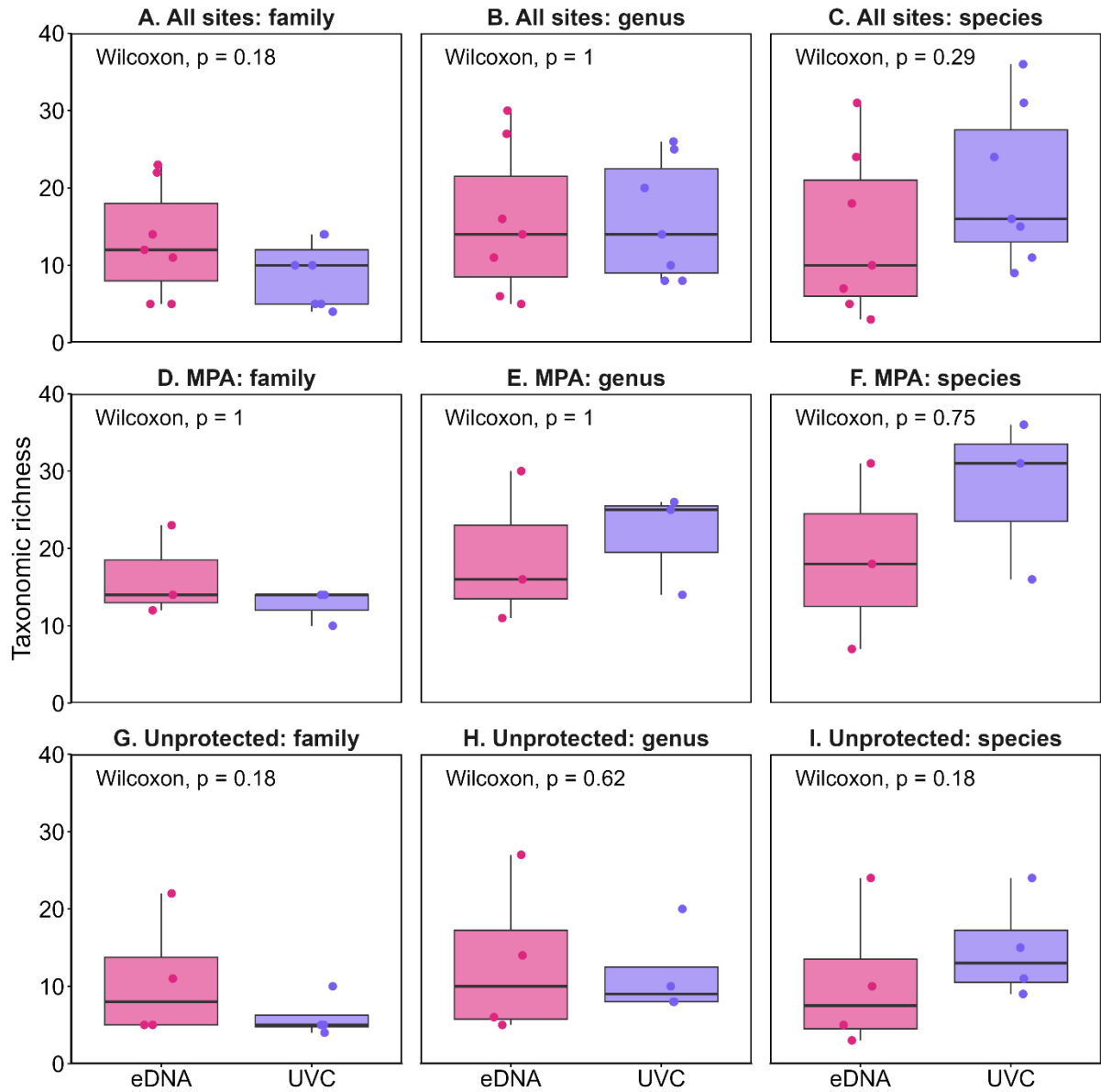


Figure 8. Boxplots illustrating differences in family, genus, and species richness between identification using environmental DNA (eDNA) and underwater visual census (UVC) surveys. Each panel includes the corresponding statistical test and p-value. Taxonomic richness was compared across (A-C) all sites, (D-F) marine protected areas (MPAs), and (G-I) unprotected reefs. Video-based data were collected using UVC surveys conducted along a 50 m transect simultaneously during eDNA sampling in coral reefs in Southern Kenya. For eDNA, data was derived from seawater samples collected in southern Kenya, amplified using a one-step and two-step polymerase chain protocol, and sequenced using Oxford Nanopore.

Differences between unique taxa identified by eDNA and UVC surveys were significant at all taxonomic levels, particularly genus and species. At the family level, eDNA identified 23 different families, UVC recovered five, and both methods shared 18 ($p = 0.049$; Figure 9A). A similar pattern occurred at genus level, where eDNA detected 37 different genera, UVC detected 21, and 24 were shared ($p < 0.001$; Figure 9B). Lastly at species-level, eDNA detected 63 different species, UVC 60, and both methods shared eight ($p < 0.001$; Figure 9C).

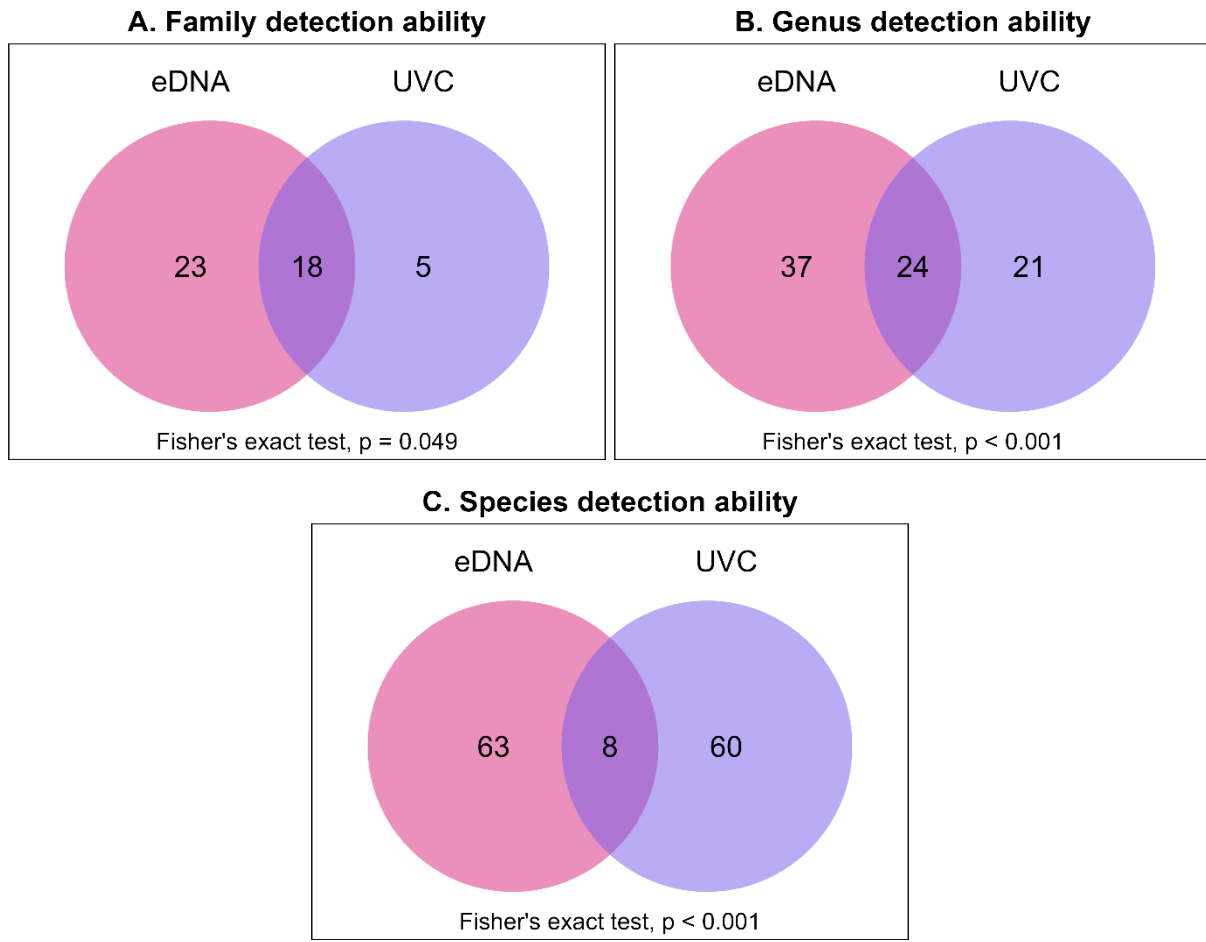


Figure 9. Venn diagrams comparing taxonomic detections between environmental DNA (eDNA) and underwater visual census (UVC) surveys at the (A) family, (B) genus, and (C) species levels. Each panel includes the corresponding test and p-value. Video-based data were collected using UVC surveys conducted along a 50 m transect simultaneously during eDNA sampling in coral reefs in Southern Kenya. For eDNA, data was derived from seawater samples collected in southern Kenya, amplified using a one-step and two-step polymerase chain protocol, and sequenced using Oxford Nanopore.

Survey method significantly affected fish species composition, with eDNA and UVC producing distinct community patterns ($R^2 = 0.18$, $F = 2.78$, $p = 0.001$; Figure 10). Greater within-group dispersion in eDNA surveys violated the assumption of homogeneity ($F = 9.47$, $p = 0.009$). In contrast, protection status had no significant effect on species composition when both survey methods were analysed together ($R^2 = 0.090$, $F = 1.19$, $p = 0.14$; Appendix J) and homogeneity of dispersion remained satisfied ($F = 0.65$, $p = 0.43$). The interaction of methodology and status also failed to reach statistical significance ($R^2 = 0.086$, $F = 1.36$, $p = 0.061$; Appendix J), although dispersion differed significantly among groups ($F = 4.093$, $p = 0.036$). Neither location ($R^2 = 0.20$, $F = 0.85$, $p = 0.87$; Appendix J) nor its interaction with methodology ($R^2 = 0.19$, $F = 0.93$, $p = 0.70$; Appendix J) significantly influenced species composition. However, the interaction analysis revealed greater within-group dispersion for eDNA surveys ($F < 0.001$, $p = 0.001$), while this was not the case with just geographic location ($F = 1.017$, $p = 0.44$).

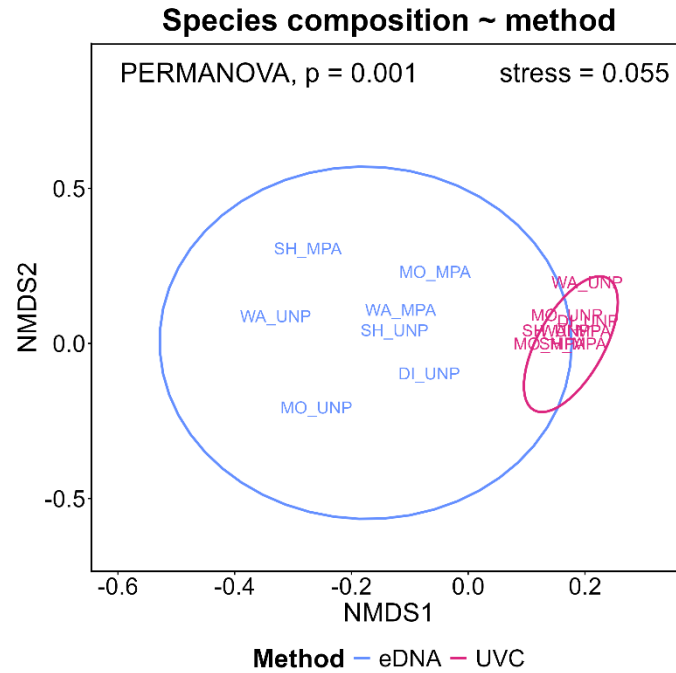


Figure 10. NMDS ordination plot of fish species community composition between environmental DNA (eDNA) and underwater visual census (UVC) surveys. The corresponding statistical test's p-value and stress score were provided. Video-based data were collected using UVC surveys conducted along a 50 m transect simultaneously during eDNA sampling in coral reefs in Southern Kenya. For eDNA, data was derived from seawater samples collected in southern Kenya, amplified using a one-step and two-step polymerase chain protocol, and sequenced using Oxford Nanopore.

4. Discussion

Environmental DNA (eDNA) successfully described shallow coral reef fish communities along the southern Kenyan coast and complemented underwater visual census (UVC) surveys. Successful amplification of fish DNA enabled taxonomic assignment at the family, genus, and species levels. These findings demonstrate the potential of eDNA metabarcoding for coral reef biodiversity assessments while highlighting the need to consider both its strengths and limitations.

4.1. Technical performance

Both sequencing runs recovered unique molecular operational taxonomic units (MOTUs), although their performance differed considerably. The first run retained 32 MOTUs, which were assigned to 16 families, 15 genera, and nine species, whereas the second retained 128 MOTUs representing 38 families, 56 genera, and 67 species. Most accepted MOTUs in the second run met the predefined 95 % identity threshold for species-level assignment, resulting in greater taxonomic resolution than in the first run. While both runs successfully detected fish taxa, neither achieved optimal sequencing and assignment performance. Even so, the improved recovery observed in the second run demonstrates that the chosen protocol strongly influences taxonomic recovery.

The first run analysed only two-step polymerase chain reaction (PCR) products and experienced extensive barcode loss. Of the 4.55 million generated reads, 85.93 % could not be classified and

required manual demultiplexing where a mismatch of three base pairs was allowed. At the end of the bioinformatic pipeline, only 34 accepted MOTUs remained. The second run included one-step PCR products of which barcodes were identified with more efficiency but was constrained by the availability of only 25 active nanopores, resulting in lower sequencing output. Nevertheless, it recovered substantially more MOTUs and achieved greater taxonomic recovery at the family, genus, and species levels. This contrast suggests that sequencing performance is influenced more strongly by library preparation quality and PCR-protocol than by sequencing output alone. Improved barcode recovery, potentially associated with the one-step PCR protocol, likely increased the proportion of usable reads and ultimately, biodiversity recovery.

The MiFish-U primers recovered 15 out of the 18 indicator fish families associated with shallow coral reefs in the Western Indian Ocean (WIO), while additionally detecting the cryptic Gobiidae family. This broad taxonomic recovery demonstrates the ability of the primers to amplify DNA of a diverse array of reef-associated fish families and capture overall community composition. The recovery of Gobiidae individuals also highlights one of the main advantages of eDNA metabarcoding since cryptic taxa are frequently underrepresented in UVC surveys. The only indicator families not recovered were Caesionidae, Chaetodontidae, Nemipteridae, and the cryptic Blenniidae. Rather than indicating true absence from the ecosystems, this likely resulted from a combination of ecological and methodological factors. Potential explanations include low population densities, low DNA shedding, limited sampling, primer mismatches, or incomplete reference databases (DiBattista et al., 2017).

Curated MOTUs were first compared against a subset of the GenBank reference database containing only fish mitochondrial sequences. To identify non-fish taxa, MOTUs were also compared against the complete mitochondrial GenBank database, which identified microbial taxa, primarily diatoms and unicellular algae, but also human DNA, sea sponges, echinoderms, and other taxa. Such detections are not unsurprising since eDNA represents a composite mixture of DNA released by all organisms present in the water column (Taberlet et al., 2018). Furthermore, although the MiFish-U primers were designed for fish metabarcoding, they are not entirely fish-specific and can amplify non-target taxa, while still outperforming alternative primer sets (Bylemans et al. 2018; Collins et al., 2019; Zhang et al. 2020).

One MOTU in the fish dataset was assigned to a freshwater species endemic to Lake Tanganyika, while four others were assigned to marine species with no documented occurrence in the WIO. These unexpected assignments most likely reflect incomplete reference databases, insufficient marker resolution, sequencing errors, or misidentified reference sequences (Chorlton, 2024). Alternatively, false-positive detections may result from anthropogenic sources such as coastal wastewater discharge, which can introduce non-local DNA into aquatic environments. Even with well-established infrastructure, treated wastewater can still retain DNA since even advanced treatment processes are not able to completely remove all DNA from the effluent (Xiong et al., 2024; Zhan, 2025). Since wastewater pollution has been documented along the Mombasa coastline, where wastewater treatment is minimal at best (Okuku et al., 2011), this suggests another possible explanation for some unexpected detections.

4.2. Ecological interpretations

After adapting the dataset to possible bias, only three locations remained yet geographic location still seemed to affect fish community more strongly than protection status. Nonetheless, the Watamu Marine National Park consistently supported the highest taxa richness across levels, which is consistent with its long history of protection. Established in 1968 and recognised as a UNESCO Biosphere Reserve in 1979, the MPA covers approximately 10 km² and protects coral reefs and seagrass habitats that form part of East Africa's longest fringing reef systems (Muthiga 2009; Tuda & Omar, 2012; Cowburn et al. 2018). However, unprotected reefs in Shimoni also exhibited relatively high taxonomic richness and community composition comparable to those observed within the Watamu MPA. In contrast, reefs in Watamu Marine National Reserve were characterised by the lowest species richness. The similarity in species composition between protected reefs in Watamu and unprotected reefs in Shimoni likely reduced the contrast between protection categories. While this difference between protection categories was not deemed significant, this should be interpreted with caution since the assumption of homogeneity of dispersion was not met.

The observed spatial variation may reflect environmental differences rather than differences in conservation management. In particular, live hard coral has been reported to have a stronger influence on multiple trophic groups than fishing pressure (Russ et al., 2020). Even though attention was paid to the selection of sampling sites as to avoid this type of environmental difference, i.e. only fringing reefs along the coast were sampled, differences in benthic composition are likely to influence fish communities. Benthic composition was recorded along with fish community composition for video analyses, but this was not fully analysed yet. Differences in benthic composition might explain the spatial variation encountered.

The distribution of eDNA in marine environments can also be influenced by ocean currents, which can transport genetic material over great distances. Through this process, DNA between neighbouring habitats may be homogenised (Westgaard et al., 2024). Given the close proximity of the unprotected reefs in Shimoni to the Kisite Mpunguti Marine Park, eDNA transport may have reduced differences between MPAs and adjacent reefs. Alternatively, such a pattern is consistent with the spillover effect associated with MPAs, where protected populations contribute to biomass to surrounding fishing grounds through the active movement of fish or the dispersal of eggs and larvae (Grüss et al., 2011).

In addition to taxonomic composition, functional trophic composition provides complementing insights into the ecological differences among reef sites. The dominance of grazers and detritivores across all sampling locations, together with no detected corallivores, is consistent with the widespread transition from coral to macroalgal reef systems, also reported in Indo-Pacific reefs (McClanahan et al., 2001; Ledlie et al., 2007; Chong-Seng et al., 2014). Since macroalgae compete with corals for available substrate, this shift is commonly interpreted as evidence of reef degradation (Clements & Hay, 2023). Excavators were also quite scarce and can contribute less to controlling algal growth through bioerosion and grazing. Again, a future analysis of benthic composition may help to explain our data in this respect. The lack of piscivores detections in the Watamu Marine National Park was unexpected, since no-take zones are associated with increases in abundance of top predators (Farina et al., 2022). This

unexpected pattern may partly reflect the limited number of MPA samples that were successfully sequenced following one-step PCR amplification.

A limitation of the eDNA dataset is that it solely provided presence-absence information, restricting analyses to species richness. In contrast, abundance and biomass have proved to be more effective for detecting the ecological effects of MPAs. Specifically, biomass has consistently shown stronger and more reliable responses to protection efforts than abundance (Soykan & Lewison, 2015). This likely reflects the capacity of MPAs to promote increases in fish body size and aid in the restoration of complex trophic structures through the accumulation of large predators (Graham et al. 2003; García-Charton et al. 2008; Harborne et al. 2008; Lester et al. 2009).

Technical limitations should also be considered when interpreting these patterns. While all samples were successfully amplified using the two-step PCR and sequenced, the new PCR protocol was introduced later in the study. Only samples gathered from the unprotected reefs in Diani, reefs in Mombasa Marine National Park, reefs in Mombasa Marine National Reserve, unprotected reefs in Shimoni, reefs in Watamu Marine National Park, and reefs in Watamu Marine National Reserve amplified using the one-step PCR protocol were sequenced. However, not all of them yielded reads due to a limited availability of available pores. Due to logistical constraints, fewer sequencing runs than originally planned could be conducted, resulting in a comparatively small one-step PCR dataset. Including successfully sequenced one-step PCR products from all sampling locations could have enabled a more comprehensive evaluation of all sampling locations.

4.3. Comparing monitoring approaches

Although eDNA and UVC surveys recovered comparable taxonomic richness across the family, genus, and species levels, species detection overlap was limited. Only eight detected species were proven to be shared between both methods: *Abudefduf sparoides* (False-eye sergeant), *Abudefduf vaigiensis* (Indo-Pacific sergeant), *Acanthurus triostegus* (Convict surgeonfish), *Halichoeres hortulanus* (Checkerboard wrasse), *Kyphosus cinerascens* (Blue sea chub), *Platax teira* (Longfin batfish), *Sargocentron diadema* (Crown squirrelfish), and *Zanclus cornutus* (Moorish idol). Despite this overlap, each survey method identified approximately 60 different species, reflecting their complementary nature.

The two methods differ fundamentally in the way they characterise fish communities. Whereas eDNA captures genetic material released by organisms in their environment, UVC surveys depend on direct visual observations within the observer's field of view. This difference enables eDNA to recover taxa that are difficult to observe visually, such as the cryptic Gobiidae family. In addition, it also recovered several species not typically associated with coral reefs, such as *Chanos chanos* (Milkfish), *Gazza minuta* (Toothpony), *Mobula eregoodoo* (Longhorned pygmy devil ray), and *Psenes cyanophrys* (Freckled driftfish). These detections most likely reflect genetic material transported from neighbouring habitats by oceanic currents. In contrast, UVC surveys rely on direct visual observation and are not affected by other constraints such as incomplete reference databases. Nevertheless, these surveys are inherently biased against cautious, highly mobile, small, and cryptic species, while environmental conditions such as

water depth and visibility can further reduce detection success (MacNeil et al., 2008; Bozec et al., 2011).

Differences in community compositions most likely reflect differences in detection mechanisms of the two survey methods, although greater within group dispersion observed for the eDNA dataset requires careful interpretation. Together, these findings help support the growing consensus that eDNA and visual surveys are complementary and are most effective when applied together (McElroy et al., 2020; Oka et al., 2020; West et al., 2020; Valdivia-Carrillo et al., 2021).

4.4. Optimising future research

Methodological variation remains widespread since eDNA research lacks a universally accepted protocol. Differences in sampling strategy, filter pore size, DNA extraction protocols, primer choice, and bioinformatic processing can all affect taxonomic recovery and study outcomes.

Metabarcoding workflows are often optimised by maximising species richness, but successful species detection is not solely determined by the amount of target DNA captured. Environmental samples collected to detect larger organisms will inevitably contain large quantities of non-target DNA, particularly from microbial organisms. This may reduce the detection efficiency of target taxa (Power et al., 2023). One way to improve on results is to reduce the proportion of non-target DNA collected during sampling. In this study, we collected samples with 0.45 µm filters, which are considered the standard choice for eDNA field sampling (Tsuji et al., 2019). However, larger pore sizes, such as 5 µm, capture less microorganisms and may improve DNA recovery of larger taxa by retaining larger animal cells or tissue fragments suspended in the water column (Andruszkiewicz Allan et al., 2025).

The MiFish-U primers set was selected due to their consistently demonstrated superior performance in comparison with other primers (Bylemans et al. 2018; Collins et al., 2019; Zhang et al. 2020). Mismatches between primers and target DNA can reduce amplification success and can result in false negatives. This limitation has been detected for the amplification of elasmobranch DNA and motivated further refinements to the MiFish-U primers (Miya et al., 2015; Miya et al., 2020). Although elasmobranch detection was not a specific objective, similar primer bias could have contributed to the absence of several expected reef-associated families in the dataset. Despite the established performance of the MiFish primers, the relatively conserved nature of the targeted 12S region still limits discrimination among certain closely related species. This can increase the likelihood of ambiguous taxonomic assignments, leading to biodiversity underestimations (Evans et al., 2017; Miya et al., 2020). Future studies may benefit from a multi-marker approaches or multiplex primer cocktails to minimise primer bias while improving taxonomic coverage.

Bioinformatic processing decisions represent another important source of variation in eDNA metabarcoding studies. Most high-throughput sequencing workflows apply minimum quality score thresholds between 20 and 30 to remove short reads, chimeras, and singletons (Xiong et al., 2022). In this study, this threshold was reduced to 10 since stricter filtering would have resulted in the loss of a high number of sequences. Even though Oxford Nanopore sequencing precision is steadily improving, it is still generally lower than that of other high throughput

platforms. Applying appropriate bioinformatic filtering, however, Oxford Nanopore output generates reliable results when compared to Illumina output (Egeter et al., 2022). We are confident we applied a similar, self-developed Nanopore custom-made bioinformatic pipeline capable of generating perfectly reliable barcoding data derived from Nanopore sequencing. Reads were clustered into MOTUs and subsequent taxonomic assignment relied on the completeness of reference databases, making them a major limitation of identification success. Assignments are inherently restricted to the taxa represented within the database and results may vary with database coverage and composition (Mendoza et al., 2015). Public databases such as GenBank provide wide taxonomic coverage, whereas specialised databases such as MitoFish provide curated fish mitochondrial sequences (Zhu et al., 2023). Although large databases can increase coverage, they may reduce species-level confidence for short eDNA sequences, increasing ambiguous assignments and false positives (Xiong et al., 2022).

This limitation became visible with the taxonomic assignment of *Abudefduf saxitalis*, an Atlantic species with no documented occurrence in Kenyan coral reefs (Froese & Pauly, 2026). Rather than reassigning this sequence to closely related Indo-Pacific species, it was retained as an unidentified *Abudefduf* MOTU. Six species have been reported to occur in the sampled locations, including the identified *A. sparoides*, and *A. vaigiensis* species (Heemstra et al., 2022). However, the MOTU could be assigned with confidence to either of these species either through the online BLAST tool, suggesting insufficient taxonomic resolution or incomplete 12S references. Establishing curated local reference databases would improve taxonomic assignments by increasing the representation of locally occurring reef fishes (Gold et al., 2021; Valdivia-Carrillo et al., 2021). However, using only locally occurring references may fail to capture invasive or cryptic species. For this reason, combining local reference libraries with large public databases is likely to maximise taxonomic coverage and identification accuracy (Xiong et al., 2022). In addition, dedicated bioinformatic platforms such as the MiFish pipeline can simplify metabarcoding analyses and improve accessibility for researchers (Sato et al., 2018).

Further methodological optimisation is needed to maximise the performance of the eDNA metabarcoding workflow. This includes evaluating alternative filtration strategies, the use of complementary primer sets, expansion of regional reference databases, and further refinement of bioinformatic pipelines. Efforts should also be made to minimise logistical constraints to ensure that all samples can be processed using identical conditions and sequencing effort, thereby improving the robustness of the results.

5. Conclusion

This study investigated the applicability of nanopore-based environmental DNA (eDNA) metabarcoding for assessing fish biodiversity in shallow coral reefs along the southern Kenyan coast. Specifically, it evaluated the performance of the 12S genetic marker, examined ecological patterns in fish community structure, and compared the resulting eDNA data with underwater visual census (UVC) surveys.

Despite technical challenges, amplification of the 12S mitochondrial region with the MiFish-U primers enabled successful detection of several reef fish taxa. Comparison of two sequencing

runs using differently prepared libraries further demonstrated that library preparation quality influenced barcode recovery which was more influential than sequencing output alone. The resulting dataset was sufficient to detect ecological patterns, revealing that communities were more strongly structured by geographic location than protection status. The predominance of grazing fishes compared to the limited representation of coral-associated specialists were consistent with global patterns associated with coral reef degradation. Future research should examine whether benthic composition forms an explanation for the regional differences encountered in our dataset. Lastly, comparison with UVC surveys demonstrated that neither method alone provides a complete overview of reef fish biodiversity. Instead, they offer complementary perspectives by detecting different aspects of fish communities.

Further refinement of eDNA metabarcoding workflows will increase its reliability and strengthen its accessibility for long-term coral reef monitoring. Rather than replacing UVC surveys, its greatest value lies in complementing those surveys to provide a more complete understanding of reef biodiversity. Integrating both survey methods has the potential to provide more comprehensive assessments of reef fish communities and to support conservation and management of these threatened ecosystems.

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Appendix A

Use of generative AI

The tool ChatGPT was used in this report to aid with grammar checks and paraphrasing. The prompts for grammar checks were “Can you check for UK spelling and grammar?”, while paraphrasing was done using the prompt “Can you rewrite this three times?”. Additionally, it was also used to aid with scripts during the bioinformatic pipeline and statistical analysis. The latter included instructions on how to make certain graphs, but it also included aiding with correcting errors.

The generated responses were used to analyse the issues, but they were not copy pasted into the report. The different replies were analysed and applied in the correct manner.

These applications were also declared in the AI code of conduct disclosure.

Appendix B

Sample collection data

Summary of sampling information, including collection date, sampling location, filtered seawater volume, geographic coordinates, protection status, and distance between marine protected areas (MPAs) and their adjacent marine reserves or unprotected reefs. Since multiple unprotected reefs were sampling within a 1 km radius in Shimoni, the distance to the associated MPA is reported as the average distance across these reefs with the corresponding standard deviation. Diani was the sole location lacking an established no-take MPA.

Table B1

Sampling metadata

Date	Site	Volume (L)	Latitude	Longitude	Status	Distance between MPA and adjacent marine reserve (km)
18/09/2025	Watamu	4.5	-3.406222	39.973194	Marine reserve	4.17
18/09/2025	Watamu	4.5	-3.406222	39.973194	Marine reserve	4.17
18/09/2025	Watamu	4.5	-3.406222	39.973194	Marine reserve	4.17
19/09/2025	Watamu	4.5	-3.378194	39.997444	MPA	4.17
19/09/2025	Watamu	4.5	-3.378194	39.997444	MPA	4.17
19/09/2025	Watamu	4.5	-3.378194	39.997444	MPA	4.17
21/09/2025	Diani	4.5	-4.336583	39.575528	Marine reserve	NA
21/09/2025	Diani	4.5	-4.336583	39.575528	Marine reserve	NA
21/09/2025	Diani	4.5	-4.336583	39.575528	Marine reserve	NA
22/09/2025	Diani	4.5	-4.336833	39.575528	Marine reserve	NA
22/09/2025	Diani	4.5	-4.336833	39.575528	Marine reserve	NA
22/09/2025	Diani	4.5	-4.336833	39.575528	Marine reserve	NA
23/09/2025	Shimoni	4.5	-4.660139	39.389500	Unprotected	5.44
23/09/2025	Shimoni	4.5	-4.660139	39.389500	Unprotected	5.44
23/09/2025	Shimoni	3.8	-4.660139	39.389500	Unprotected	5.44
24/09/2025	Shimoni	4.5	-4.659833	39.384056	Unprotected	5.42
24/09/2025	Shimoni	4.5	-4.659833	39.384056	Unprotected	5.42
24/09/2025	Shimoni	4.5	-4.659833	39.384056	Unprotected	5.42
24/09/2025	Shimoni	4.5	-4.650139	39.383222	Unprotected	6.44
24/09/2025	Shimoni	4.5	-4.650139	39.383222	Unprotected	6.44
24/09/2025	Shimoni	4.5	-4.650139	39.383222	Unprotected	6.44
7/10/2025	Mombasa	4.5	-3.990861	39.750556	MPA	9.02
7/10/2025	Mombasa	4.5	-3.990861	39.750556	MPA	9.02
7/10/2025	Mombasa	4.5	-3.990861	39.750556	MPA	9.02
8/10/2025	Mombasa	4.5	-4.062139	39.710806	Marine reserve	9.02
8/10/2025	Mombasa	4.5	-4.062139	39.710806	Marine reserve	9.02
8/10/2025	Mombasa	3.2	-4.062139	39.710806	Marine reserve	9.02
9/10/2025	Shimoni	2.8	-4.707639	39.378972	MPA	5.77 ± 0.51
9/10/2025	Shimoni	4.5	-4.707639	39.378972	MPA	5.77 ± 0.51
9/10/2025	Shimoni	4.5	-4.707639	39.378972	MPA	5.77 ± 0.51
9/10/2025	Shimoni	2.4	-4.707639	39.378972	MPA	5.77 ± 0.51

Appendix C

Barcode sequences

Sequences of the barcodes used in both polymerase chain reaction (PCR) protocols. Regular barcodes were used in the two-step PCR, while the one-step included primers with incorporated barcodes.

Table C1

Barcode sequences used in the two-step PCR protocol

Barcode type	Sequences
Barcode 1	GATACAAGAAAGTTGTCGGTGTCTTTGTG
Barcode 2	GATACTCGATTCCGTTTGTAGTCGTCTGTG
Barcode 3	GATACGAGTCTTGTGTCCCAGTTACCAGGG
Barcode 4	GATACTTCGGATTCTATCGTGTTCCTAG
Barcode 5	GATACCTTGTCAGGGTTTGTGTAACCTTG
Barcode 6	GATACTTCTCGCAAAGGCAGAAAGTAGTCG
Barcode 7	GATACGTGTTACCGTGGAATGAATCCTTG
Barcode 8	GATACTTCAGGGAACAAACCAAGTTACGTG
Barcode 9	GATACAAC TAGGCACAGCGAGTCTTGTTG
Barcode 10	GATACAAGCGTTGAAACCTTTGTCCTCTCG
Barcode 11	GATACGTTTCATCTATCGGAGGGAATGGAG
Barcode 12	GATACGTTGAGTTACAAAGCACCGATCAGG
Barcode 13	GATACAGAACGACTCCATACTCGTGTGAG
Barcode 14	GATACAACGAGTCTCTTGGGACCCATAGAG
Barcode 15	GATACAGGTCTACCTCGCTAACACCACTGG
Barcode 16	GATACCGTCAACTGACAGTGGTTCGTA CTG
Barcode 17	GATACACCCTCCAGGAAAGTACCTCTGATG
Barcode 18	GATACCCAAACCCAACAACCTAGATAGGCG
Barcode 19	GATACGTTCCCTCGTGCAGTGTCAAGAGATG
Barcode 20	GATACTTGCGTCCTGTTACGAGAACTCATG
Barcode 21	GATACGAGCCTCTCATTGTCCGTTCTCTAG
Barcode 22	GATACACCACTGCCATGTATCAAAGTACGG
Barcode 23	GATACCTTACTACCCAGTGAACCTCCTCGG
Barcode 24	GATACGCATAGTTCTGCATGATGGGTTAGG

Table C2

Barcode sequences used in the one-step PCR protocol

Barcode type	Sequences
Forward tail barcode 1	GATACAAGAAAGTTGTCGGTGTCTTTGTGGTTCGGTAAAACTCGTGCCAGC
Forward tail barcode 2	GATACTCGATTCCGTTTGTAGTCGTCTGTGTCGGTAAAACTCGTGCCAGC
Forward tail barcode 3	GATACGAGTCTTGTGTCCCAGTTACCAGGGTCGGTAAAACTCGTGCCAGC
Forward tail barcode 4	GATACTTCGGATTCTATCGTGTTCCTAGTCGGTAAAACTCGTGCCAGC
Forward tail barcode 5	GATACCTTGTCAGGGTTTGTGTAACCTTGTTCGGTAAAACTCGTGCCAGC
Forward tail barcode 6	GATACTTCTCGCAAAGGCAGAAAGTAGTCGTTCGGTAAAACTCGTGCCAGC

Forward tail barcode 7	GATACGTGTACCGTGGAATGAATCCTTGTCGGTAAAACTCGTGCCAGC
Forward tail barcode 8	GATACTTCAGGGAACAAACCAAGTTACGTGTCGGTAAAACTCGTGCCAGC
Forward tail barcode 9	GATACAACTAGGCACAGCGAGTCTTGTTGTCGGTAAAACTCGTGCCAGC
Forward tail barcode 10	GATACAAGCGTTGAAACCTTTGTCCTCTCGTCGGTAAAACTCGTGCCAGC
Forward tail barcode 11	GATACGTTTCATCTATCGGAGGGAATGGAGTCGGTAAAACTCGTGCCAGC
Forward tail barcode 12	GATACGTTGAGTTACAAAGCACCGATCAGGTCGGTAAAACTCGTGCCAGC
Forward tail barcode 13	GATACAGAACGACTTCCATACTCGTGTGAGTCGGTAAAACTCGTGCCAGC
Forward tail barcode 14	GATACAACGAGTCTCTTGGGACCCATAGAGTCGGTAAAACTCGTGCCAGC
Forward tail barcode 15	GATACAGGTCTACCTCGCTAACACCACTGGTCGGTAAAACTCGTGCCAGC
Forward tail barcode 16	GATACCGTCAACTGACAGTGGTTCGTAAGTCGGTAAAACTCGTGCCAGC
Forward tail barcode 17	GATACACCCTCCAGGAAAGTACCTCTGATGTCGGTAAAACTCGTGCCAGC
Forward tail barcode 18	GATACCCAAACCCAAACCTAGATAGGCGTCGGTAAAACTCGTGCCAGC
Forward tail barcode 19	GATACGTTCCCTCGTGCAAGTGTCAAGAGATGTCGGTAAAACTCGTGCCAGC
Forward tail barcode 20	GATACTTGCGTCTGTTACGAGAACTCATGTCGGTAAAACTCGTGCCAGC
Forward tail barcode 21	GATACGAGCCTCTCATTGTCCGTTCTCTAGTCGGTAAAACTCGTGCCAGC
Forward tail barcode 22	GATACACCACTGCCATGTATCAAAGTACGGTCGGTAAAACTCGTGCCAGC
Forward tail barcode 23	GATACCTTACTACCCAGTGAACCTCCTCGGTCGGTAAAACTCGTGCCAGC
Forward tail barcode 24	GATACGCATAGTTCTGCATGATGGGTTAGGTCGGTAAAACTCGTGCCAGC
Reverse tail barcode 1	GATACAAGAAAGTTGTCGGTGTCTTTGTGCATAGTGGGGTATCTAATCCCAGT
Reverse tail barcode 2	GATACTCGATTCCGTTTGTAGTCGTCTGTCATAGTGGGGTATCTAATCCCAGT
Reverse tail barcode 3	GATACGAGTCTTGTGTCCCAGTTACCAGGCATAGTGGGGTATCTAATCCCAGT
Reverse tail barcode 4	GATACTTCGGATTCTATCGTGTTCCTACATAGTGGGGTATCTAATCCCAGT
Reverse tail barcode 5	GATACCTTGTCAGGGTTTGTGTAACCTTCATAGTGGGGTATCTAATCCCAGT
Reverse tail barcode 6	GATACTTCTCGCAAAGGCAGAAAGTAGTCCATAGTGGGGTATCTAATCCCAGT
Reverse tail barcode 7	GATACGTGTACCGTGGAATGAATCCTTCATAGTGGGGTATCTAATCCCAGT
Reverse tail barcode 8	GATACTTCAGGGAACAAACCAAGTTACGTCATAGTGGGGTATCTAATCCCAGT
Reverse tail barcode 9	GATACAACTAGGCACAGCGAGTCTTGTTTCATAGTGGGGTATCTAATCCCAGT
Reverse tail barcode 10	GATACAAGCGTTGAAACCTTTGTCCTCTCCATAGTGGGGTATCTAATCCCAGT
Reverse tail barcode 11	GATACGTTTCATCTATCGGAGGGAATGGACATAGTGGGGTATCTAATCCCAGT
Reverse tail barcode 12	GATACGTTGAGTTACAAAGCACCGATCAGCATAGTGGGGTATCTAATCCCAGT
Reverse tail barcode 13	GATACAGAACGACTTCCATACTCGTGTGACATAGTGGGGTATCTAATCCCAGT
Reverse tail barcode 14	GATACAACGAGTCTCTTGGGACCCATAGACATAGTGGGGTATCTAATCCCAGT
Reverse tail barcode 15	GATACAGGTCTACCTCGCTAACACCACTGCATAGTGGGGTATCTAATCCCAGT
Reverse tail barcode 16	GATACCGTCAACTGACAGTGGTTCGTAAGTCATAGTGGGGTATCTAATCCCAGT
Reverse tail barcode 17	GATACACCCTCCAGGAAAGTACCTCTGATCATAGTGGGGTATCTAATCCCAGT
Reverse tail barcode 18	GATACCCAAACCCAAACCTAGATAGGCCATAGTGGGGTATCTAATCCCAGT
Reverse tail barcode 19	GATACGTTCCCTCGTGCAAGTGTCAAGAGATCATAGTGGGGTATCTAATCCCAGT
Reverse tail barcode 20	GATACTTGCGTCTGTTACGAGAACTCATCATAGTGGGGTATCTAATCCCAGT
Reverse tail barcode 21	GATACGAGCCTCTCATTGTCCGTTCTCTACATAGTGGGGTATCTAATCCCAGT
Reverse tail barcode 22	GATACACCACTGCCATGTATCAAAGTACGCATAGTGGGGTATCTAATCCCAGT
Reverse tail barcode 23	GATACCTTACTACCCAGTGAACCTCCTCGCATAGTGGGGTATCTAATCCCAGT
Reverse tail barcode 24	GATACGCATAGTTCTGCATGATGGGTTAGCATAGTGGGGTATCTAATCCCAGT

Appendix D

DNA extractions data

Extraction yields of the Kenyan samples and internal negative controls (INCs) using a NanoDrop™ 2000 spectrophotometer (Thermo Fisher Scientific Inc., Waltham, Massachusetts, USA). Data included sample name, date and time of measurement, nucleic acid content, purity ratios 260/280 and 260/230, and sample type.

Table D1

DNA extraction results

Sample ID	Date and Time	Nucleic Acid (ng/μL)	260/280	260/230	Sample Type
SH_004	22/10/2025 16:19	4.0	2.06	-5.87	DNA
SH_005	22/10/2025 16:20	7.7	1.42	6.09	DNA
SH_006	22/10/2025 16:20	13.5	1.11	1.50	DNA
INC	22/10/2025 16:21	4.3	1.52	1.25	DNA
SH_002	29/10/2025 15:41	27.7	1.34	2.06	DNA
MO_002	29/10/2025 15:42	39.0	1.54	1.48	DNA
MO_003	29/10/2025 15:43	-20.5	1.55	0.41	DNA
MO_003	29/10/2025 15:44	-22.4	1.57	0.43	DNA
MO_004	29/10/2025 15:45	85.7	1.36	1.63	DNA
MO_005	29/10/2025 15:46	130.8	1.57	1.60	DNA
INC1	29/10/2025 15:46	8.6	1.84	0.67	DNA
INC2	29/10/2025 15:47	4.4	1.74	2.64	DNA
KI_001	18/11/2025 16:25	106.2	1.99	3.08	DNA
KI_002	18/11/2025 16:26	15.2	0.66	-0.70	DNA
KI_002	18/11/2025 16:27	95.5	1.99	4.10	DNA
KI_004	18/11/2025 16:28	112.2	1.89	2.59	DNA
MO_001	18/11/2025 16:28	141.0	1.85	2.52	DNA
MO_006	18/11/2025 16:29	250.2	1.94	2.40	DNA
SH_008	18/11/2025 16:30	63.1	1.90	5.77	DNA
INC1	18/11/2025 16:31	1.9	1.41	-0.14	DNA
INC2	18/11/2025 16:32	7.4	1.88	8.08	DNA
WA_006	19/11/2025 16:58	76.3	1.84	1.51	DNA
DI_003	19/11/2025 16:59	91.5	1.85	1.86	DNA
DI_005	19/11/2025 17:00	147.8	1.57	1.01	DNA
SH_003	19/11/2025 17:01	87.1	1.84	1.75	DNA
SH_007	19/11/2025 17:01	101.9	1.86	1.83	DNA
KI_003	19/11/2025 17:02	189.6	1.99	2.11	DNA
INC1	19/11/2025 17:03	57.7	0.82	0.56	DNA
INC1	19/11/2025 17:04	5.7	1.64	0.36	DNA
INC2_70	19/11/2025 17:04	2.9	2.19	0.39	DNA
WA_002	21/11/2025 16:30	79.6	1.85	1.59	DNA
WA_003	21/11/2025 16:31	74.5	1.80	1.95	DNA
WA_005	21/11/2025 16:32	14.9	1.66	26.65	DNA
DI_001	21/11/2025 16:33	43.0	1.83	2.81	DNA
DI_002	21/11/2025 16:35	35.7	1.66	2.36	DNA

DI_006	21/11/2025 16:36	9.0	0.55	-0.69	DNA
DI_006	21/11/2025 16:36	44.0	1.84	1.54	DNA
INC1	21/11/2025 16:37	3.1	2.33	0.42	DNA
INC2	21/11/2025 16:38	5.7	2.06	0.55	DNA
WA_004	9/12/2025 16:26	91.6	1.91	1.90	DNA
W_001	9/12/2025 16:26	83.5	1.86	2.26	DNA
DI_004	9/12/2025 16:27	78.6	1.84	2.14	DNA
SH_001	9/12/2025 16:28	50.9	1.88	2.22	DNA
SH_009	9/12/2025 16:28	50.4	1.85	1.60	DNA
INC1	9/12/2025 16:29	11.1	1.89	0.44	DNA
INC2	9/12/2025 16:30	2.8	1.98	0.30	DNA

Appendix E

Taxa summary sequencing runs

Overview of all taxa identified using the 12S mitochondrial marker between the two sequencing runs, summarising family, genus, species, number of reads, and mean and max identity percentages. Taxa that did not occur in Kenyan coastal waters were denoted to a level with a known occurrence, noted as molecular operational taxonomic unit (MOTU).

Table E1

Identified taxa across the two sequencing runs

Run	Family	Genus	Species	Reads	Mean identity (%)	Max identity (%)
1	Acanthuridae	<i>Acanthurus</i>	<i>Acanthurus</i> MOTU3	1	95.71	95.71
1	Acanthuridae	<i>Acanthurus</i>	<i>Acanthurus triostegus</i>	2	94.13	94.25
1	Apogonidae	<i>Pristiapogon</i>	<i>Pristiapogon kallopterus</i>	1	96.70	96.70
1	Atherinidae	<i>Hypoatherina</i>	<i>Hypoatherina</i> MOTU1	1	93.84	93.84
1	Balistidae	<i>Pseudobalistes</i>	<i>Pseudobalistes flavimarginatus</i>	1	93.81	93.81
1	Chanidae	<i>Chanos</i>	<i>Chanos chanos</i>	1	97.61	97.61
1	Holocentridae	<i>Myripristis</i>	<i>Myripristis</i> MOTU1	3	94.59	95.30
1	Kyphosidae	<i>Kyphosus</i>	<i>Kyphosus</i> MOTU2	2	94.64	95.09
1	Labridae	<i>Novaculoides</i>	<i>Novaculoides macrolepidotus</i>	1	93.75	93.75
1	Lethrinidae	<i>Lethrinus</i>	<i>Lethrinus</i> MOTU1	1	93.33	93.33
1	Mobulidae	<i>Mobula</i>	<i>Mobula eregoodoo</i>	1	95.22	95.22
1	Muraenidae	<i>Gymnothorax</i>	<i>Gymnothorax rueppelliae</i>	1	89.52	89.52
1	Pempheridae	<i>Pempheris</i>	<i>Pempheris</i> MOTU1	1	88.10	88.10
1	Pempheridae	<i>Pempheris</i>	<i>Pempheris</i> MOTU2	1	96.77	96.77
1	Pempheridae	<i>Pempheris</i>	<i>Pempheris</i> MOTU3	1	90.40	90.40
1	Plotosidae	<i>Plotosus</i>	<i>Plotosus</i> MOTU1	1	85.71	85.71
1	Plotosidae	<i>Plotosus</i>	<i>Plotosus</i> MOTU2	1	90.00	90.00
1	Pomacentridae	<i>Abudefduf</i>	<i>Abudefduf vaigiensis</i>	3	95.11	96.26
1	Pomacentridae	<i>Stegastes</i>	<i>Stegastes</i> MOTU1	3	88.29	92.23
1	Siganidae	<i>Siganus</i>	<i>Siganus luridus</i>	1	91.36	91.36
1	Siganidae	<i>Siganus</i>	<i>Siganus sutor</i>	2	95.75	96.64
1	Sillaginidae	<i>Sillago</i>	<i>Sillago</i> MOTU1	1	82.93	82.93
1	Spratelloididae	<i>Spratelloides</i>	<i>Spratelloides delicatulus</i>	2	84.94	86.55
1	Tripterygiidae	<i>Enneapterygius</i>	<i>Enneapterygius</i> MOTU1	1	84.23	84.23
2	Acanthuridae	<i>Acanthurus</i>	<i>Acanthurus</i> MOTU1	4	96.15	97.36
2	Acanthuridae	<i>Acanthurus</i>	<i>Acanthurus</i> MOTU2	1	96.04	96.04
2	Acanthuridae	<i>Acanthurus</i>	<i>Acanthurus</i> MOTU3	4	98.33	99.54
2	Acanthuridae	<i>Acanthurus</i>	<i>Acanthurus</i> MOTU4	1	93.61	93.61
2	Acanthuridae	<i>Acanthurus</i>	<i>Acanthurus nigrofuscus</i>	6	96.67	99.54
2	Acanthuridae	<i>Acanthurus</i>	<i>Acanthurus triostegus</i>	1	96.88	96.88
2	Acanthuridae	<i>Naso</i>	<i>Naso brevirostris</i>	3	98.76	99.07
2	Acanthuridae	<i>Naso</i>	<i>Naso</i> MOTU1	1	93.18	93.18
2	Apogonidae	<i>Apogonidae</i> MOTU1	<i>Apogonidae</i> MOTU1	1	88.64	88.64
2	Apogonidae	<i>Ostorhinchus</i>	<i>Ostorhinchus</i> MOTU1	1	91.89	91.89

2	Atherinidae	<i>Atherinomorus</i>	<i>Atherinomorus lacunosus</i>	2	96.52	97.21
2	Balistidae	<i>Pseudobalistes</i>	<i>Pseudobalistes flavimarginatus</i>	1	97.72	97.72
2	Balistidae	<i>Rhinecanthus</i>	<i>Rhinecanthus aculeatus</i>	1	98.65	98.65
2	Belonidae	<i>Tylosurus</i>	<i>Tylosurus melanotus</i>	1	98.14	98.14
2	Callionymidae	<i>Callionymidae</i> MOTU1	<i>Callionymidae</i> MOTU1	1	87.88	87.88
2	Carangidae	<i>Caranx</i>	<i>Caranx</i> MOTU1	1	100.00	100.00
2	Cirrhitidae	<i>Cirrhitichthys</i>	<i>Cirrhitichthys oxycephalus</i>	1	96.52	96.52
2	Diodontidae	<i>Diodontidae</i> MOTU1	<i>Diodontidae</i> MOTU1	1	98.43	98.43
2	Echeneidae	<i>Echeneis</i>	<i>Echeneis naucrates</i>	1	94.69	94.69
2	Ephippidae	<i>Platax</i>	<i>Platax teira</i>	2	95.18	98.16
2	Epinephelidae	<i>Cephalopholis</i>	<i>Cephalopholis argus</i>	4	96.34	99.51
2	Gerreidae	<i>Gerres</i>	<i>Gerres</i> MOTU1	2	94.69	94.88
2	Gobiidae	<i>Gobiidae</i> MOTU1	<i>Gobiidae</i> MOTU1	1	90.87	90.87
2	Gobiidae	<i>Valenciennea</i>	<i>Valenciennea</i> MOTU1	1	96.74	96.74
2	Haemulidae	<i>Plectorhinchus</i>	<i>Plectorhinchus</i> MOTU1	2	95.46	95.54
2	Haemulidae	<i>Plectorhinchus</i>	<i>Plectorhinchus</i> MOTU2	2	92.24	92.41
2	Haemulidae	<i>Plectorhinchus</i>	<i>Plectorhinchus sordidus</i>	1	92.76	92.76
2	Hemiramphidae	<i>Hemiramphus</i>	<i>Hemiramphus far</i>	3	97.21	98.60
2	Holocentridae	<i>Myripristis</i>	<i>Myripristis berndti</i>	1	100.00	100.00
2	Holocentridae	<i>Myripristis</i>	<i>Myripristis</i> MOTU1	29	97.61	100.00
2	Holocentridae	<i>Myripristis</i>	<i>Myripristis</i> MOTU2	1	96.76	96.76
2	Holocentridae	<i>Sargocentron</i>	<i>Sargocentron diadema</i>	3	98.76	99.54
2	Kyphosidae	<i>Kyphosus</i>	<i>Kyphosus cinerascens</i>	2	98.86	99.08
2	Kyphosidae	<i>Kyphosus</i>	<i>Kyphosus</i> MOTU1	2	96.85	98.66
2	Kyphosidae	<i>Kyphosus</i>	<i>Kyphosus</i> MOTU2	4	98.77	99.09
2	Labridae	<i>Calotomus</i>	<i>Calotomus carolinus</i>	2	96.00	98.12
2	Labridae	<i>Calotomus</i>	<i>Calotomus spinidens</i>	5	97.11	99.54
2	Labridae	<i>Cheilinus</i>	<i>Cheilinus</i> MOTU1	7	97.59	98.20
2	Labridae	<i>Cheilinus</i>	<i>Cheilinus oxycephalus</i>	1	98.10	98.10
2	Labridae	<i>Coris</i>	<i>Coris</i> MOTU1	2	94.03	94.57
2	Labridae	<i>Halichoeres</i>	<i>Halichoeres hortulanus</i>	5	96.13	98.70
2	Labridae	<i>Halichoeres</i>	<i>Halichoeres scapularis</i>	1	93.27	93.27
2	Labridae	<i>Labroides</i>	<i>Labroides dimidiatus</i>	1	90.71	90.71
2	Labridae	<i>Leptoscarus</i>	<i>Leptoscarus vaigiensis</i>	3	96.22	98.16
2	Labridae	<i>Novaculoides</i>	<i>Novaculichthys taeniourus</i>	1	96.46	96.46
2	Labridae	<i>Novaculoides</i>	<i>Novaculoides macrolepidotus</i>	3	98.62	99.09
2	Labridae	<i>Oxycheilinus</i>	<i>Oxycheilinus bimaculatus</i>	7	94.83	96.38
2	Labridae	<i>Scarus</i>	<i>Scarus</i> MOTU1	1	99.10	99.10
2	Labridae	<i>Scarus</i>	<i>Scarus psittacus</i>	1	95.48	95.48
2	Labridae	<i>Stethojulis</i>	<i>Stethojulis</i> MOTU1	6	96.65	99.07
2	Labridae	<i>Thalassoma</i>	<i>Thalassoma amblycephalum</i>	1	93.72	93.72
2	Labridae	<i>Thalassoma</i>	<i>Thalassoma</i> MOTU1	1	94.06	94.06
2	Leiognathidae	<i>Gazza</i>	<i>Gazza minuta</i>	1	96.77	96.77
2	Lethrinidae	<i>Lethrinus</i>	<i>Lethrinus harak</i>	11	98.03	99.54
2	Lethrinidae	<i>Lethrinus</i>	<i>Lethrinus lentjan</i>	3	97.13	97.76
2	Lethrinidae	<i>Lethrinus</i>	<i>Lethrinus</i> MOTU1	5	95.30	97.26
2	Lethrinidae	<i>Lethrinus</i>	<i>Lethrinus nebulosus</i>	2	93.30	93.58
2	Lutjanidae	<i>Lutjanus</i>	<i>Lutjanus fulvus</i>	1	86.30	86.30

2	Lutjanidae	<i>Lutjanus</i>	<i>Lutjanus gibbus</i>	1	97.72	97.72
2	Lutjanidae	<i>Pristipomoides</i>	<i>Pristipomoides multidentatus</i>	1	86.15	86.15
2	Lutjanidae	<i>Pterocaesio</i>	<i>Pterocaesio marri</i>	1	97.28	97.28
2	Monacanthidae	<i>Cantherhines</i>	<i>Cantherhines pardalis</i>	2	99.07	99.54
2	Mullidae	<i>Parupeneus</i>	<i>Parupeneus</i> MOTU1	3	96.53	99.03
2	Mullidae	<i>Upeneus</i>	<i>Upeneus</i> MOTU1	1	94.95	94.95
2	Muraenidae	<i>Echidna</i>	<i>Echidna nebulosa</i>	1	98.19	98.19
2	Muraenidae	<i>Gymnothorax</i>	<i>Gymnothorax chilospilus</i>	1	100.00	100.00
2	Muraenidae	<i>Gymnothorax</i>	<i>Gymnothorax flavimarginatus</i>	2	91.37	92.04
2	Muraenidae	<i>Gymnothorax</i>	<i>Gymnothorax</i> MOTU1	1	99.09	99.09
2	Muraenidae	<i>Gymnothorax</i>	<i>Gymnothorax pictus</i>	1	91.27	91.27
2	Nomeidae	<i>Psenes</i>	<i>Psenes cyanophrys</i>	2	98.05	99.06
2	Opistognathidae	<i>Opistognathus</i>	<i>Opistognathus</i> MOTU1	1	85.07	85.07
2	Ostraciidae	<i>Lactoria</i>	<i>Lactoria fornasini</i>	1	97.75	97.75
2	Pempheridae	<i>Pempheris</i>	<i>Pempheris</i> MOTU1	6	94.26	98.61
2	Pempheridae	<i>Pempheris</i>	<i>Pempheris</i> MOTU2	2	91.84	92.73
2	Pempheridae	<i>Pempheris</i>	<i>Pempheris</i> MOTU3	1	95.63	95.63
2	Plesiopidae	<i>Plesiops</i>	<i>Plesiops</i> MOTU1	3	94.67	97.21
2	Pomacanthidae	<i>Centropyge</i>	<i>Centropyge</i> MOTU1	1	85.84	85.84
2	Pomacentridae	<i>Abudefduf</i>	<i>Abudefduf</i> MOTU1	8	94.94	98.14
2	Pomacentridae	<i>Abudefduf</i>	<i>Abudefduf sparoides</i>	1	98.07	98.07
2	Pomacentridae	<i>Abudefduf</i>	<i>Abudefduf vaigiensis</i>	63	97.71	100.00
2	Pomacentridae	<i>Chromis</i>	<i>Azurina lepidolepis</i>	1	96.44	96.44
2	Pomacentridae	<i>Chromis</i>	<i>Chromis</i> MOTU1	1	87.50	87.50
2	Pomacentridae	<i>Chromis</i>	<i>Chromis</i> MOTU2	1	88.43	88.43
2	Pomacentridae	<i>Chromis</i>	<i>Chromis ternatensis</i>	4	96.74	97.32
2	Pomacentridae	<i>Chrysiptera</i>	<i>Chrysiptera brownriggii</i>	1	96.43	96.43
2	Pomacentridae	<i>Pomacentridae</i> MOTU1	<i>Pomacentridae</i> MOTU1	2	96.10	99.51
2	Pomacentridae	<i>Pomacentrus</i>	<i>Pomacentrus trilineatus</i>	1	98.61	98.61
2	Pomacentridae	<i>Stegastes</i>	<i>Stegastes</i> MOTU1	3	93.42	94.27
2	Scorpaenidae	<i>Scorpaenopsis</i>	<i>Scorpaenopsis</i> MOTU1	1	85.65	85.65
2	Siganidae	<i>Siganus</i>	<i>Siganus luridus</i>	2	97.30	99.10
2	Siganidae	<i>Siganus</i>	<i>Siganus</i> MOTU1	4	96.40	99.10
2	Siganidae	<i>Siganus</i>	<i>Siganus</i> MOTU2	1	91.82	91.82
2	Siganidae	<i>Siganus</i>	<i>Siganus sutor</i>	24	97.83	99.54
2	Sphyraenidae	<i>Sphyraena</i>	<i>Sphyraena</i> MOTU1	1	84.93	84.93
2	Sphyraenidae	<i>Sphyraena</i>	<i>Sphyraena</i> MOTU2	2	99.29	99.53
2	Spratelloididae	<i>Spratelloides</i>	<i>Spratelloides delicatulus</i>	2	86.57	86.67
2	Spratelloididae	<i>Spratelloides</i>	<i>Spratelloides gracilis</i>	11	92.11	94.67
2	Tetraodontidae	<i>Arothron</i>	<i>Arothron hispidus</i>	2	97.11	97.77
2	Tetraodontidae	<i>Arothron</i>	<i>Arothron stellatus</i>	1	93.75	93.75
2	Zanclidae	<i>Zanclus</i>	<i>Zanclus cornutus</i>	1	97.72	97.72

Appendix F

Top 10 species run 1 and run 2

Relative read proportions of the top 10 detected fish species between the first sequencing run and the second. Species that did not occur in Kenyan coastal waters were denoted to a taxa level with a known occurrence, noted as molecular operational taxonomic unit (MOTU). Data was derived from seawater samples collected in southern Kenya, amplified using a one-step and two-step polymerase chain protocol, and sequenced using Oxford Nanopore sequencing across two separate runs. The first run contained two-step products, while the second run contained products from both protocols.

Figure F1

Top 10 species of the first run

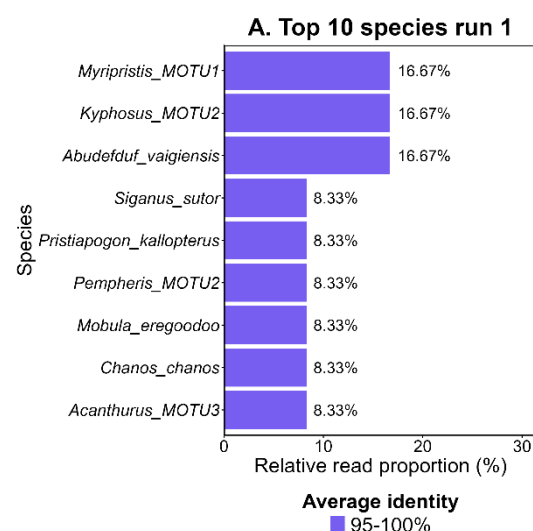
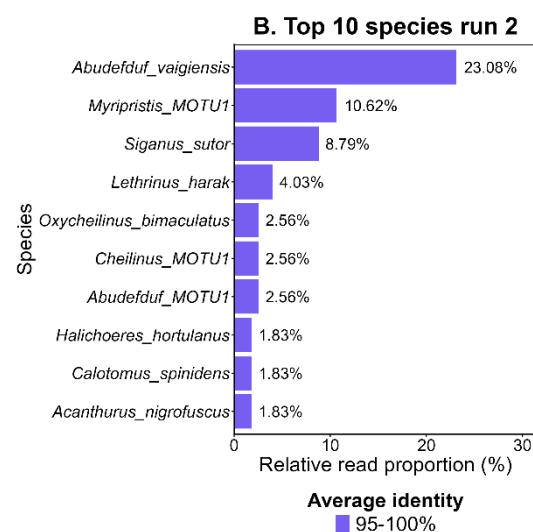


Figure F2

Top 10 species of the second run



Appendix G

Identified taxa across all locations

Overview of all taxa identified through the 12S mitochondrial marker across all sampling sites based on one-step polymerase chain reaction (PCR) products from the second sequencing run. Summarising geographic locations, protection status, family, genus, species, amount of reads, and mean and max identity percentage. Taxa that did not occur in Kenyan coastal waters were denoted to a level with a known occurrence, noted as molecular operational taxonomic unit (MOTU). Marine reserves were referred to as unprotected. Data was derived from seawater samples collected in southern Kenya, amplified using a one-step polymerase chain reaction protocol, and sequenced using Oxford Nanopore.

Table G1

Taxa found across all locations

Location	Status	Family	Genus	Species	Reads	Mean identity (%)	Max identity (%)
Diani	Unprotected	Acanthuridae	<i>Acanthurus</i>	<i>Acanthurus</i> MOTU1	4	96.15	97.36
Diani	Unprotected	Acanthuridae	<i>Acanthurus</i>	<i>Acanthurus</i> MOTU3	4	98.33	99.54
Diani	Unprotected	Acanthuridae	<i>Acanthurus</i>	<i>Acanthurus</i> MOTU3	1	95.71	95.71
Diani	Unprotected	Acanthuridae	<i>Acanthurus</i>	<i>Acanthurus nigrofuscus</i>	1	92.23	92.23
Diani	Unprotected	Acanthuridae	<i>Acanthurus</i>	<i>Acanthurus triostegus</i>	2	94.13	94.25
Diani	Unprotected	Apogonidae	Apogonidae MOTU1	Apogonidae MOTU1	1	88.64	88.64
Diani	Unprotected	Cirrhitidae	<i>Cirrhitichthys</i>	<i>Cirrhitichthys oxycephalus</i>	1	96.52	96.52
Diani	Unprotected	Holocentridae	<i>Myripristis</i>	<i>Myripristis</i> MOTU1	1	94.98	94.98
Diani	Unprotected	Labridae	<i>Halichoeres</i>	<i>Halichoeres hortulanus</i>	1	95.95	95.95
Diani	Unprotected	Labridae	<i>Novaculoides</i>	<i>Novaculoides macrolepidotus</i>	1	93.75	93.75
Diani	Unprotected	Labridae	<i>Stethojulis</i>	<i>Stethojulis</i> MOTU1	2	99.05	99.07
Diani	Unprotected	Lethrinidae	<i>Lethrinus</i>	<i>Lethrinus harak</i>	2	95.45	95.85
Diani	Unprotected	Muraenidae	<i>Gymnothorax</i>	<i>Gymnothorax</i> MOTU1	1	99.09	99.09
Diani	Unprotected	Pempheridae	<i>Pempheris</i>	<i>Pempheris</i> MOTU2	2	91.84	92.73
Diani	Unprotected	Plesiopidae	<i>Plesiops</i>	<i>Plesiops</i> MOTU1	1	92.82	92.82
Diani	Unprotected	Pomacentridae	<i>Abudefduf</i>	<i>Abudefduf vaigiensis</i>	27	96.25	100.00
Diani	Unprotected	Pomacentridae	Pomacentridae MOTU1	Pomacentridae MOTU1	2	96.10	99.51
Diani	Unprotected	Pomacentridae	<i>Pomacentrus</i>	<i>Pomacentrus trilineatus</i>	1	98.61	98.61
Diani	Unprotected	Pomacentridae	<i>Stegastes</i>	<i>Stegastes</i> MOTU1	6	90.86	94.27
Diani	Unprotected	Spratelloididae	<i>Spratelloides</i>	<i>Spratelloides delicatulus</i>	1	86.46	86.46

Shimoni	Unprotected	Acanthuridae	<i>Naso</i>	<i>Naso brevirostris</i>	3	98.76	99.07
Shimoni	Unprotected	Acanthuridae	<i>Naso</i>	<i>Naso</i> MOTU1	1	93.18	93.18
Shimoni	Unprotected	Atherinidae	<i>Atherinomorus</i>	<i>Atherinomorus lacunosus</i>	2	96.52	97.21
Shimoni	Unprotected	Callionymidae	Callionymidae MOTU1	Callionymidae MOTU1	1	87.88	87.88
Shimoni	Unprotected	Gerreidae	<i>Gerres</i>	<i>Gerres</i> MOTU1	1	94.88	94.88
Shimoni	Unprotected	Gobiidae	Gobiidae MOTU1	Gobiidae MOTU1	1	90.87	90.87
Shimoni	Unprotected	Haemulidae	<i>Plectorhinchus</i>	<i>Plectorhinchus</i> MOTU1	1	95.54	95.54
Shimoni	Unprotected	Labridae	<i>Calotomus</i>	<i>Calotomus spinidens</i>	1	95.02	95.02
Shimoni	Unprotected	Labridae	<i>Cheilinus</i>	<i>Cheilinus</i> MOTU1	1	98.04	98.04
Shimoni	Unprotected	Labridae	<i>Cheilinus</i>	<i>Cheilinus oxycephalus</i>	1	98.10	98.10
Shimoni	Unprotected	Labridae	<i>Coris</i>	<i>Coris</i> MOTU1	1	94.57	94.57
Shimoni	Unprotected	Labridae	<i>Halichoeres</i>	<i>Halichoeres hortulanus</i>	4	96.31	98.70
Shimoni	Unprotected	Labridae	<i>Leptoscarus</i>	<i>Leptoscarus vaigiensis</i>	1	97.71	97.71
Shimoni	Unprotected	Labridae	<i>Oxycheilinus</i>	<i>Oxycheilinus bimaculatus</i>	6	94.71	96.38
Shimoni	Unprotected	Leiognathidae	<i>Gazza</i>	<i>Gazza minuta</i>	1	96.77	96.77
Shimoni	Unprotected	Lethrinidae	<i>Lethrinus</i>	<i>Lethrinus harak</i>	5	98.26	99.49
Shimoni	Unprotected	Lethrinidae	<i>Lethrinus</i>	<i>Lethrinus lentjan</i>	3	97.13	97.76
Shimoni	Unprotected	Lutjanidae	<i>Pristipomoides</i>	<i>Pristipomoides multidentatus</i>	1	86.15	86.15
Shimoni	Unprotected	Lutjanidae	<i>Pterocaesio</i>	<i>Pterocaesio marri</i>	1	97.28	97.28
Shimoni	Unprotected	Monacanthidae	<i>Cantherhines</i>	<i>Cantherhines pardalis</i>	2	99.07	99.54
Shimoni	Unprotected	Mullidae	<i>Parupeneus</i>	<i>Parupeneus</i> MOTU1	1	96.90	96.90
Shimoni	Unprotected	Mullidae	<i>Upeneus</i>	<i>Upeneus</i> MOTU1	1	94.95	94.95
Shimoni	Unprotected	Muraenidae	<i>Echidna</i>	<i>Echidna nebulosa</i>	1	98.19	98.19
Shimoni	Unprotected	Muraenidae	<i>Gymnothorax</i>	<i>Gymnothorax pictus</i>	1	91.27	91.27
Shimoni	Unprotected	Ostraciidae	<i>Lactoria</i>	<i>Lactoria fornasini</i>	1	97.75	97.75
Shimoni	Unprotected	Pempheridae	<i>Pempheris</i>	<i>Pempheris</i> MOTU1	1	92.86	92.86
Shimoni	Unprotected	Pomacanthidae	<i>Centropyge</i>	<i>Centropyge</i> MOTU1	1	85.84	85.84
Shimoni	Unprotected	Pomacentridae	<i>Abudefduf</i>	<i>Abudefduf</i> MOTU1	1	92.79	92.79
Shimoni	Unprotected	Pomacentridae	<i>Abudefduf</i>	<i>Abudefduf vaigiensis</i>	2	97.49	98.61
Shimoni	Unprotected	Pomacentridae	<i>Chromis</i>	<i>Chromis ternatensis</i>	4	96.74	97.32
Shimoni	Unprotected	Scorpaenidae	<i>Scorpaenopsis</i>	<i>Scorpaenopsis</i> MOTU1	1	85.65	85.65
Shimoni	Unprotected	Siganidae	<i>Siganus</i>	<i>Siganus luridus</i>	1	99.10	99.10
Shimoni	Unprotected	Siganidae	<i>Siganus</i>	<i>Siganus</i> MOTU1	1	96.30	96.30
Shimoni	Unprotected	Siganidae	<i>Siganus</i>	<i>Siganus sutor</i>	3	98.64	99.54

Shimoni	Unprotected	Sphyraenidae	<i>Sphyraena</i>	Sphyraena MOTU2	2	99.29	99.53
Shimoni	Unprotected	Spratelloididae	<i>Spratelloides</i>	<i>Spratelloides</i> <i>gracilis</i>	11	92.11	94.67
Shimoni	Unprotected	Zanclidae	<i>Zanclus</i>	<i>Zanclus</i> <i>cornutus</i>	1	97.72	97.72
Watamu	MPA	Acanthuridae	<i>Acanthurus</i>	<i>Acanthurus</i> MOTU2	1	96.04	96.04
Watamu	MPA	Acanthuridae	<i>Acanthurus</i>	<i>Acanthurus</i> <i>nigrofuscus</i>	4	98.24	98.67
Watamu	MPA	Acanthuridae	<i>Acanthurus</i>	<i>Acanthurus</i> <i>tristegus</i>	1	96.88	96.88
Watamu	MPA	Apogonidae	<i>Ostorhinchus</i>	<i>Ostorhinchus</i> MOTU1	1	91.89	91.89
Watamu	MPA	Balistidae	<i>Rhinecanthus</i>	<i>Rhinecanthus</i> <i>aculeatus</i>	1	98.65	98.65
Watamu	MPA	Carangidae	<i>Caranx</i>	<i>Caranx</i> MOTU1	1	100.00	100.00
Watamu	MPA	Diodontidae	Diodontidae MOTU1	Diodontidae MOTU1	1	98.43	98.43
Watamu	MPA	Gerreidae	<i>Gerres</i>	<i>Gerres</i> MOTU1	1	94.50	94.50
Watamu	MPA	Gobiidae	<i>Valenciennea</i>	<i>Valenciennea</i> MOTU1	1	96.74	96.74
Watamu	MPA	Haemulidae	<i>Plectorhinchus</i>	<i>Plectorhinchus</i> MOTU1	1	95.37	95.37
Watamu	MPA	Haemulidae	<i>Plectorhinchus</i>	<i>Plectorhinchus</i> MOTU2	2	92.24	92.41
Watamu	MPA	Haemulidae	<i>Plectorhinchus</i>	<i>Plectorhinchus</i> <i>sordidus</i>	1	92.76	92.76
Watamu	MPA	Hemiramphidae	<i>Hemiramphus</i>	<i>Hemiramphus</i> <i>far</i>	2	98.14	98.60
Watamu	MPA	Holocentridae	<i>Sargocentron</i>	<i>Sargocentron</i> <i>diadema</i>	1	99.10	99.10
Watamu	MPA	Kyphosidae	<i>Kyphosus</i>	<i>Kyphosus</i> <i>cinerascens</i>	2	98.86	99.08
Watamu	MPA	Kyphosidae	<i>Kyphosus</i>	<i>Kyphosus</i> MOTU1	2	96.85	98.66
Watamu	MPA	Kyphosidae	<i>Kyphosus</i>	<i>Kyphosus</i> MOTU2	3	98.45	98.64
Watamu	MPA	Labridae	<i>Calotomus</i>	<i>Calotomus</i> <i>spinidens</i>	4	99.19	99.54
Watamu	MPA	Labridae	<i>Cheilinus</i>	<i>Cheilinus</i> MOTU1	6	97.13	98.20
Watamu	MPA	Labridae	<i>Coris</i>	<i>Coris</i> MOTU1	1	93.49	93.49
Watamu	MPA	Labridae	<i>Halichoeres</i>	<i>Halichoeres</i> <i>scapularis</i>	1	93.27	93.27
Watamu	MPA	Labridae	<i>Leptoscarus</i>	<i>Leptoscarus</i> <i>vaigiensis</i>	1	92.79	92.79
Watamu	MPA	Labridae	<i>Novaculoides</i>	<i>Novaculoides</i> <i>macrolepidotus</i>	2	99.08	99.09
Watamu	MPA	Labridae	<i>Oxycheilinus</i>	<i>Oxycheilinus</i> <i>bimaculatus</i>	1	95.07	95.07
Watamu	MPA	Labridae	<i>Scarus</i>	<i>Scarus</i> <i>psittacus</i>	1	95.48	95.48
Watamu	MPA	Labridae	<i>Stethojulis</i>	<i>Stethojulis</i> MOTU1	4	95.45	97.73
Watamu	MPA	Labridae	<i>Thalassoma</i>	<i>Thalassoma</i> <i>amblycephalum</i>	1	93.72	93.72
Watamu	MPA	Labridae	<i>Thalassoma</i>	<i>Thalassoma</i> MOTU1	1	94.06	94.06
Watamu	MPA	Lethrinidae	<i>Lethrinus</i>	<i>Lethrinus</i> <i>harak</i>	3	98.63	99.07
Watamu	MPA	Lethrinidae	<i>Lethrinus</i>	<i>Lethrinus</i> MOTU1	4	96.00	97.26

Watamu	MPA	Lethrinidae	<i>Lethrinus</i>	<i>Lethrinus nebulosus</i>	2	93.30	93.58
Watamu	MPA	Mullidae	<i>Parupeneus</i>	<i>Parupeneus</i> MOTU1	2	96.15	99.03
Watamu	MPA	Muraenidae	<i>Gymnothorax</i>	<i>Gymnothorax chilospilus</i>	1	100.00	100.00
Watamu	MPA	Muraenidae	<i>Gymnothorax</i>	<i>Gymnothorax flavimarginatus</i>	2	91.37	92.04
Watamu	MPA	Nomeidae	<i>Psenes</i>	<i>Psenes cyanophrys</i>	2	98.05	99.06
Watamu	MPA	Opistognathidae	<i>Opistognathus</i>	<i>Opistognathus</i> MOTU1	1	85.07	85.07
Watamu	MPA	Pempheridae	<i>Pempheris</i>	<i>Pempheris</i> MOTU1	4	96.44	98.61
Watamu	MPA	Pempheridae	<i>Pempheris</i>	<i>Pempheris</i> MOTU3	1	95.63	95.63
Watamu	MPA	Plotosidae	<i>Plotosus</i>	<i>Plotosus</i> MOTU1	1	85.71	85.71
Watamu	MPA	Pomacentridae	<i>Abudefduf</i>	<i>Abudefduf vaigiensis</i>	4	96.64	99.54
Watamu	MPA	Pomacentridae	<i>Chromis</i>	<i>Chromis</i> MOTU2	1	88.43	88.43
Watamu	MPA	Pomacentridae	<i>Chrysiptera</i>	<i>Chrysiptera brownriggii</i>	1	96.43	96.43
Watamu	MPA	Siganidae	<i>Siganus</i>	<i>Siganus luridus</i>	1	91.36	91.36
Watamu	MPA	Siganidae	<i>Siganus</i>	<i>Siganus</i> MOTU1	2	97.33	99.10
Watamu	MPA	Siganidae	<i>Siganus</i>	<i>Siganus sutor</i>	17	96.67	99.52
Watamu	MPA	Spratelloididae	<i>Spratelloides</i>	<i>Spratelloides delicatulus</i>	1	86.67	86.67
Watamu	MPA	Tetraodontidae	<i>Arothron</i>	<i>Arothron hispidus</i>	2	97.11	97.77
Watamu	MPA	Tetraodontidae	<i>Arothron</i>	<i>Arothron stellatus</i>	1	93.75	93.75
Watamu	Unprotected	Labridae	<i>Calotomus</i>	<i>Calotomus carolinus</i>	1	93.87	93.87
Watamu	Unprotected	Labridae	<i>Leptoscarus</i>	<i>Leptoscarus vaigiensis</i>	1	98.16	98.16
Watamu	Unprotected	Labridae	<i>Novaculoides</i>	<i>Novaculichthys taeniourus</i>	1	96.46	96.46
Watamu	Unprotected	Labridae	<i>Novaculoides</i>	<i>Novaculoides macrolepidotus</i>	1	97.72	97.72
Watamu	Unprotected	Lethrinidae	<i>Lethrinus</i>	<i>Lethrinus</i> MOTU1	1	94.60	94.60
Watamu	Unprotected	Muraenidae	<i>Gymnothorax</i>	<i>Gymnothorax rueppelliae</i>	1	89.52	89.52
Watamu	Unprotected	Pempheridae	<i>Pempheris</i>	<i>Pempheris</i> MOTU1	1	91.28	91.28
Watamu	Unprotected	Siganidae	<i>Siganus</i>	<i>Siganus luridus</i>	1	95.50	95.50
Watamu	Unprotected	Siganidae	<i>Siganus</i>	<i>Siganus sutor</i>	5	97.27	98.68

Appendix H

Relative taxa composition across all sites

Relative composition of fish families, genera, and species across sampling sites Diani-Chale Marine Reserve (DI_UNP), unprotected reefs in Shimon (SH_UNP), Watamu Marine National Park (WA_MPA), and Watamu Marine National Reserve (WA_UNP). Taxonomic assignments were accepted at minimum identity thresholds of 85 % for family, 90 % for genus, and 95 % for species. Taxa that did not occur in Kenyan coastal waters were denoted to a taxa level with a known occurrence, noted as molecular operational taxonomic unit (MOTU). Data was derived from seawater samples collected in southern Kenya, amplified using a one-step polymerase chain protocol, and sequenced using Oxford Nanopore.

Figure H1

Relative family composition

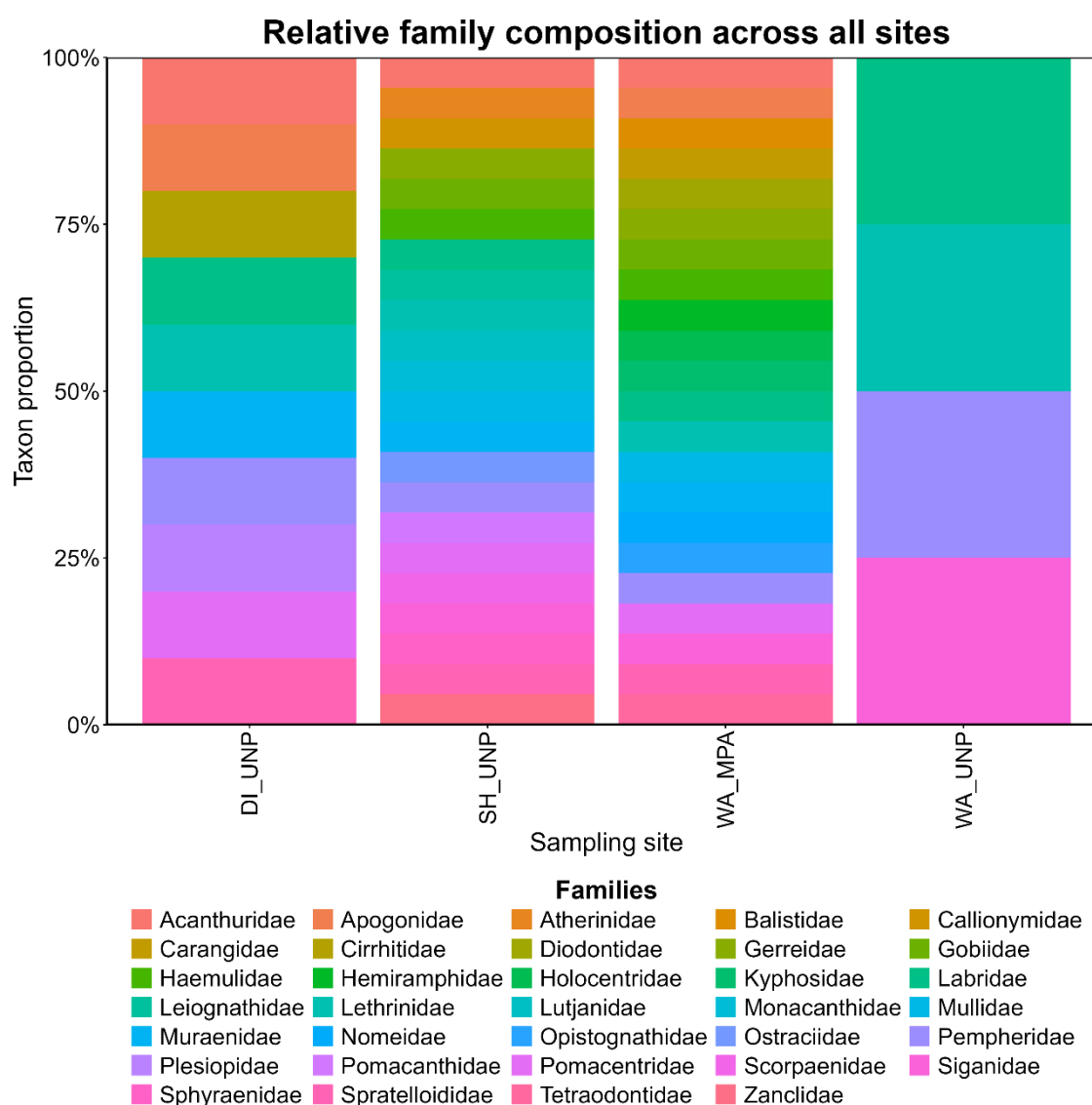


Figure H2

Relative genus composition

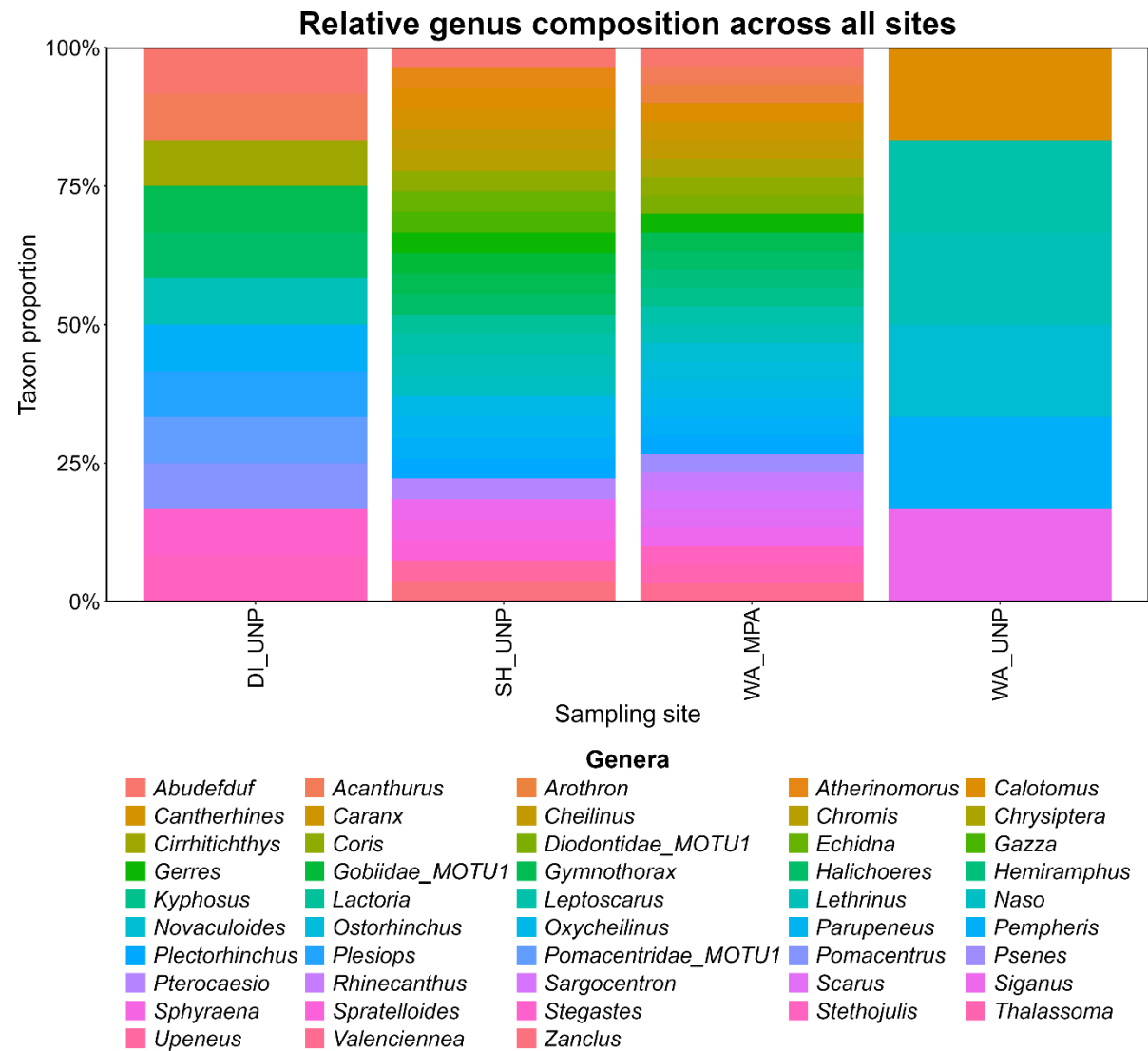
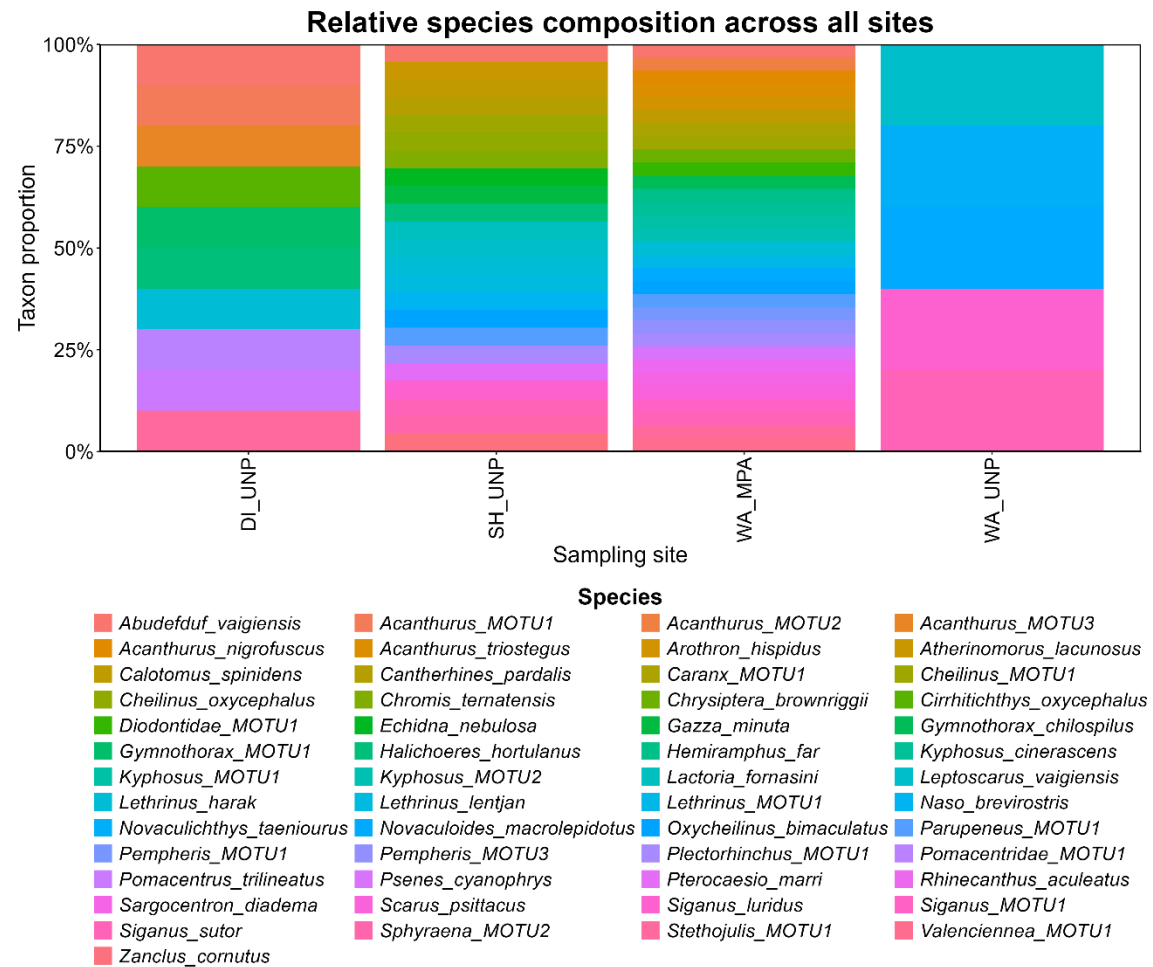


Figure H3

Relative species composition



Appendix I

Location richness comparison between methodologies

Family, genus, and species richness comparisons between environmental DNA (eDNA) and underwater visual census (UVC) surveys across the sampled locations. These include Diani-Chale Marine Reserve (DI_UNP), Mombasa Marine National Park (MO_MPA), Mombasa Marine National Reserve (MO_UNP), Kisite Mpunguti Marine Park (SH_MPA), unprotected reefs in Shimoni (SH_UNP), Watamu Marine National Park (WA_MPA), and Watamu Marine National Reserve (WA_UNP). Video-based data were collected using UVC surveys conducted along a 50 m transect simultaneously during eDNA sampling in coral reefs in Southern Kenya. For eDNA, data was derived from seawater samples collected in southern Kenya, amplified using a one-step and two-step polymerase chain protocol, and sequenced using Oxford Nanopore.

Table I1

Richness comparison

Sample	eDNA family richness	eDNA genus richness	eDNA species richness	UVC family richness	UVC genus richness	UVC species richness
DI_UNP	11	14	10	5	10	15
MO_MPA	14	16	18	14	25	31
MO_UNP	5	5	3	4	8	11
SH_MPA	12	11	7	14	26	36
SH_UNP	22	27	24	10	20	24
WA_MPA	23	30	31	10	14	16
WA_UNP	5	6	5	5	8	9

Figure J2

Interaction of protection status and survey method

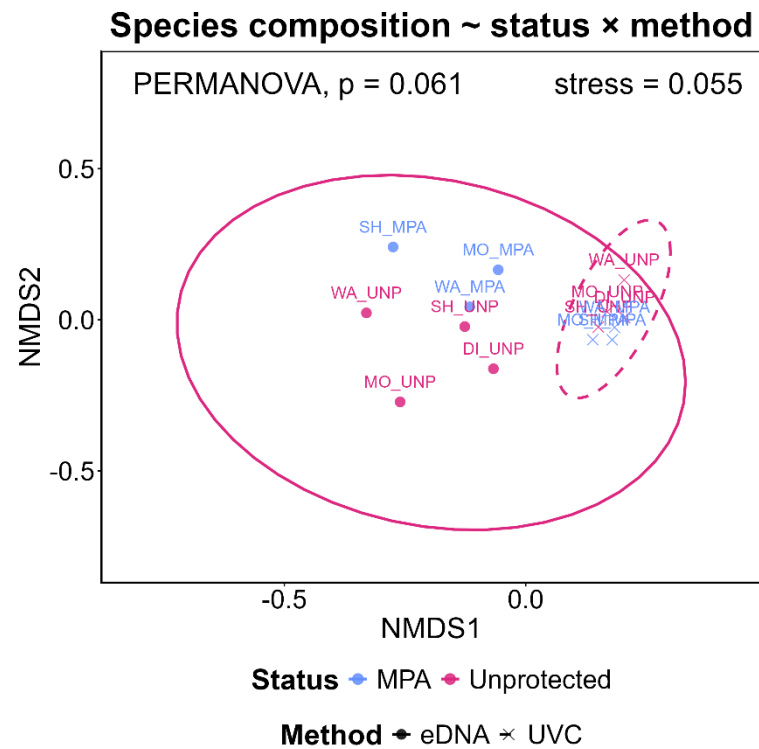


Figure J3

Species composition driven by location with combined datasets

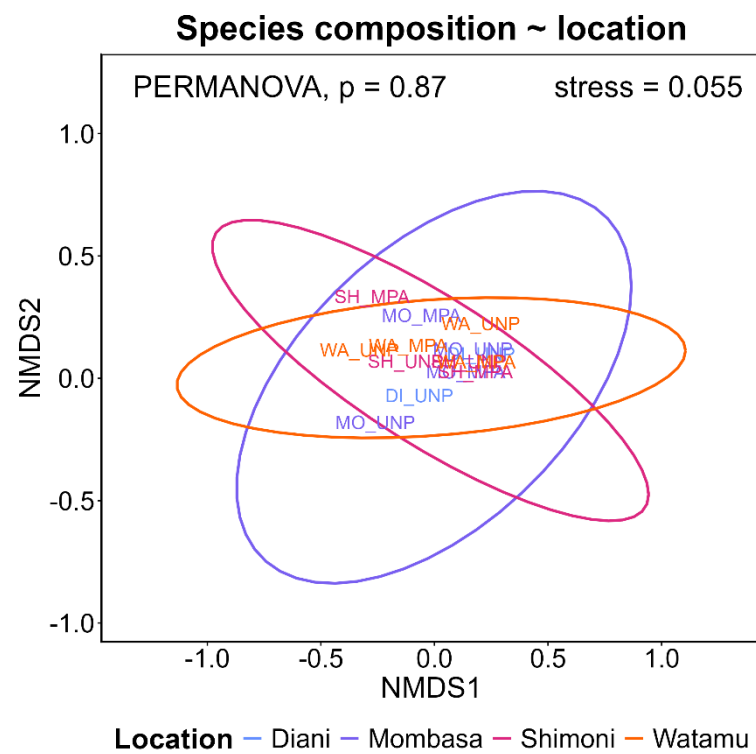


Figure J4

Interaction of location and survey method

