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RESOURCE ARTICLE

Merging two eDNA metabarcoding approaches and citizen-science-based sampling to facilitate fish community monitoring along vast Sub-Saharan coastlines

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Abstract

The coastline of Sub-Saharan Africa hosts highly diverse fish communities of great conservation value, which are also key resources for local livelihoods. However, many costal ecosystems are threatened by overexploitation and their conservation state is frequently unknown due to their vast spatial extent and limited monitoring budgets. Here, we evaluated the potential of citizen science-based eDNA surveys to alleviate such chronic data deficiencies and assessed fish communities in Mozambique using two 12S metabarcoding primer sets. Samples were either collected by scientific personnel or trained community members and results from the two metabarcoding primers were combined using a new data merging approach. Irrespective of the background of sampling personnel, a high average fish species richness was recorded (38 ± 20 OTUs per sample). Individual sections of the coastline largely differed in the occurrence of threatened and commercially important species, highlighting the need for regionally differentiated management strategies. A detailed comparison of the two applied primer sets revealed an important trade-off in primer choice with MiFish primers amplifying a higher number of species but Riaz primers performing better in the detection of threatened fish species. This trade-off could be partly resolved by applying our new data-merging approach, which was especially designed to increase

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the robustness of multiprimer assessments in regions with poor reference libraries. Overall, our study provides encouraging results but also highlights that eDNA-based monitoring will require further improvements of, for example, reference databases and local analytical infrastructure to facilitate routine applications in Sub-Saharan Africa.

KEYWORDS
12S, artisanal fisheries, citizen science, environmental DNA (eDNA), fish communities, metabarcoding, South-Western Indian Ocean (SWIO)

1 | INTRODUCTION

The coastline of Sub-Saharan Africa (SSA) encompasses a wide range of highly diverse natural habitats (Stuart et al., 1990). Marine biodiversity hotspots include unique systems such as the Congo delta, the islands along the East African coast and the Mozambique Channel (Griffiths, 2005; Küper et al., 2004). Despite the very distinct fauna and flora hosted by these ecosystems, resource use patterns are very similar across most regions in SSA. In coastal regions, such as the Mozambican coastline, artisanal fisheries are a key contribution to local livelihoods (Sowman & Cardoso, 2010). Industrial fisheries target primarily offshore fish stocks although illegal near-shore activities are also systematically performed (Belhabib et al., 2020; Nielsen et al., 2004; Okafor-Yarwood et al., 2022; Zeeberg et al., 2006). At the same time, monitoring of both coastal and offshore fish communities is rudimentary in many regions (Kolding et al., 2016) and in many cases, even baseline community data are lacking. Consequently, effective and routine monitoring is a central requirement to address the many current and future management challenges in SSA.

Across SSA, human population is projected to almost double within the next 30 years (United Nations, 2021), which is expected to substantially increase the pressure on natural resources and habitats (Ramin, 2009). The resulting increased exploitation of fish stocks may be especially problematic when fish landings are poorly monitored and fishing regulations are insufficiently enforced as is frequently the case in SSA coastal regions (Belhabib et al., 2020; Okafor-Yarwood et al., 2022). Additionally, climate changes constitutes a major additional stressor exacerbating other anthropogenic pressures on marine ecosystems (Díaz et al., 2019; Doney et al., 2012; Harley, 2011; Lambers, 2015). Temperature rises and the increased incidence of marine heat waves have already had catastrophic global impacts on coral reef (Stuart-Smith et al., 2018), which provide highly valuable ecosystem services and support about a quarter of total marine biodiversity (Hughes et al., 2017). Additionally, rises in temperature lead to a higher frequency of tropical storms and alterations of marine nutrient cycling with large potential consequences for natural species assemblies (Behrenfeld, 2011; Doney et al., 2012). In face of these multiple threats, frequent and accurate monitoring of fish communities and general aquatic biodiversity is urgently required to implement adaptive management approaches and respond to early warning systems before the onset of catastrophic break-downs (Clements et al., 2017; Floros et al., 2013).

However, regular monitoring is a complex and challenging task (Jacquet et al., 2010). In Mozambique, the focal region of this study with ample sea grass meadows and coral reefs, fish monitoring relies on surveying methods such as electrofishing, netting, baited traps, as well as visual and acoustic surveys (Gell & Whittington, 2002; Marshall et al., 2011; Samoilys et al., 2019). These methods require substantial efforts of skilled personnel, and electrofishing and netting are ecologically invasive (Hänfling et al., 2016). Furthermore, these conventional monitoring approaches are hampered by systematic sampling bias, limits of morphological identification and elevated risks of false-negative results (i.e. the failure to detect species to be present; Wang et al., 2021; Yamamoto et al., 2016). This leads to frequent underestimation of species distributions and diversity as well as a knowledge bias towards larger, more charismatic and commercially important species. Hence, conventional methods generate without doubt valuable ecological data, but they are likely inadequate for regular monitoring of the estimated approximately 1500 fish species that occur along the 2700 km coastline of Mozambique (Schneider et al., 2005).

Molecular-based detection methods, such as environmental DNA (eDNA) metabarcoding, offer the potential for a robust, scalable and cost-effective alternative for the monitoring of fish communities (Gilbey et al., 2021; Ruppert et al., 2019; Thomsen et al., 2011). Environmental DNA metabarcoding refers to the amplification and sequencing of mixed-species DNA obtained from environmental samples such as water, sediment, or air (Lynggaard et al., 2022; Ruppert et al., 2019). A major advantage of eDNA-based monitoring is that field samples can be collected quickly and easily without requiring specialist survey skills (Larson et al., 2020). Furthermore, eDNA preservation techniques allow for the storage of samples without performance losses at room temperature for several months (Mauvisseau et al., 2021).

The relatively easy collection and storage of eDNA samples makes eDNA-based approaches very attractive for citizen-science and participatory, community-led monitoring programmes (Larson et al., 2020). The involvement of local communities in decentralized monitoring networks can not only lead to local empowerment and support conservation by increasing environmental awareness but also substantially reduce sampling costs (San Llorente Capdevila et al., 2020). Hence, community-based monitoring using eDNA approaches could be a game changer for evidence-based

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natural resource management in Sub-Saharan Africa and enable cost-effective environmental monitoring across large spatial scales.

An important requirement for eDNA-based monitoring to live up to its potential is the robust representation of natural community composition, which strongly depends on the choice of primer sets. Here, we refer to robust as the capacity to reduce false negative detection as much as possible without increasing false-positive detection rates. Primers define the phylogenetic identity of the taxa that can be detected, and they often differ in their power to discriminate among species and their sensitivity for detecting target groups (Hajibabaei et al., 2019; Schenekar et al., 2020). Most fish eDNA metabarcoding surveys to date use primers amplifying a short fragment of the mitochondrial 12S gene, since these have generally been shown to have a higher specificity and record a greater fish diversity than primers targeting 16S rRNA or COI genes (Collins et al., 2019; Jackman et al., 2021; Kelly et al., 2014; Milan et al., 2020; Miya et al., 2015; Polanco et al., 2021; Schenekar et al., 2020; Zhang et al., 2020). Although the 12S region is generally a preferential target when conducting metabarcoding surveys targeting fishes (Zhang et al., 2020), different 12S primers are available linked to specific strength and weaknesses. For example, different primer pairs may vary in (1) DNA amplification efficiency, (2) the ability to discriminate between closely-related species and (3) the availability of reference sequences to enable confident identification to species level (Polanco et al., 2021). These performance indicators can vary even for the same primer among taxonomic groups (e.g. families), resulting in trade-offs, which complicate the process of primer choice.

A possibility to resolve such trade-offs is the use of multiple primer pairs for analysing the same set of samples (e.g. Blackman et al., 2021). Such multiprimer approaches can be especially advantageous in tropical regions as primers tests are foremost implemented in temperate regions and optimal primer choice in often highly diverse tropical environments are largely underexplored (Jerde et al., 2019). However, the use of multiple primer sets can complicate data interpretation, especially when biodiversity patterns and the occurrence of priority species differ among the generated data sets. A possibility to overcome such difficulties is the use of data merging techniques that can combine the gathered information and therefore help to generate more complete community data supporting the management of extensive tropical coastlines.

In this study, we evaluate the potential to use eDNA metabarcoding and community-based sampling for surveying tropical and subtropical fish communities in costal zones of Mozambique. Samples were collected by experienced scientific personnel as well as by trained local community members along a 600 km stretch of the Mozambican coastline including marine habitats as well as some costal inland waters. In order to assess fish diversity and community composition, we applied two 12S primer sets and combined the resulting data sets with a newly developed merging-algorithm designed to operate also on incomplete references databases common to SSA. Furthermore, we used the data for a detailed performance comparison of the two used primer sets, the 'MiFish-U' (Miya et al., 2015) and the 'Riaz' primers (Kelly et al., 2014; Riaz et al., 2011), providing a methodological baseline for future eDNA-based monitoring and conservation efforts in Mozambique.

2 | METHODS

2.1 | Field sampling and training of citizens for eDNA sampling

Water samples were collected from 47 locations spread across 600 km of coastline in the southern part of Mozambique between July and September 2020 (10 inland-water, 37 marine samples from Inhamabane Bay as well as Maputo bay and more southern marine habitats; Figure 1; Table S2). Sample sites included marine (both open sea and bay water where coral reefs can be found) as well as brackish and some freshwater sites. For simplicity, brackish and freshwater are subsequently referred to as inland-water sites. Samples were collected either by scientific personal (18 locations) or by trained citizens (29 locations, all of them in the province of Inhambane).

Involved citizens were trained in a two-step process. First, a one-day workshop was jointly organized by our local partners, Ocean Revolution (a local nongovernmental organization) and the Inhambane Bay Community Conservation Network. This workshop was split in a training part and a discussion part. In the training part, potential applications of eDNA-based survey methods were explained by eDNA specialists from Nature Metrics with translations in English and the local language Gitonga. Afterwards, an extensive question and answer session was held followed by a live demonstration and praxis sampling by involved community members. In the discussion part of the workshop, views on current measures to protect fish nursery grounds were exchanged. This discussion resulted in a co-design of a sampling strategy for Inhambane Bay. It was agreed upon that sampling should be repeated twice to compare fish communities in different seasons (early dry seasons in June and July with mid-late dry season in September).

After this initial workshop, seven community champions from four different communities were chosen. These community champions attended a second training workshop, which was provided by Ocean Revolution staff. Additionally, a WhatsApp eDNA group was established to provide further possibilities for information exchange and technical assistance. When necessary, sampling by community champions was assisted by Ocean Revolution staff providing their boat to access sampling locations. Community members were not paid for their work. Four of the seven selected community champions were female to support women empowerment as their participation helped them to gain a voice in fishing councils and strengthen their engagement in decision-making processes.

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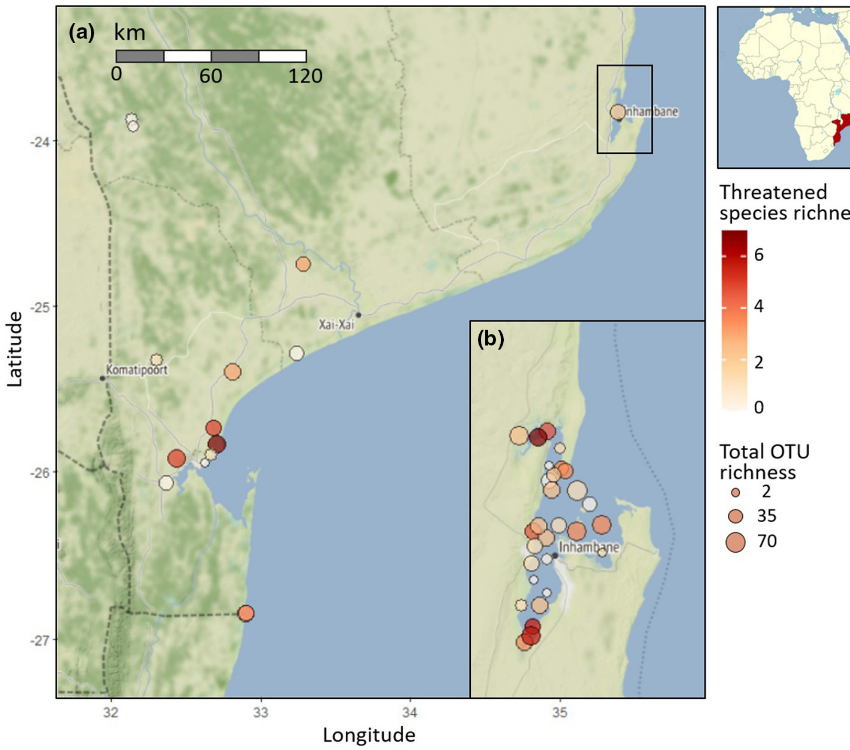

FIGURE 1 Location of sampling sites along the Southern coastline of Mozambique ($n=47$). The number of recorded OTUs is reflected by the size of the dots and the number of threatened species that have been recorded is indicated by colour intensity. A focus area of our study was Inhambane bay, which is highlighted by a black rectangular in (a) and in greater detail in (b).

2.2 | Water collection

At each site, water was collected from the surface. Filtration volume ranged from 800 to 2000mL due to differences in water turbidity. Sampling was performed using sampling kits that were designed to support also sampling by nonspecialists with main targets to minimize contamination risks and guarantee ease of handling (for details, see Appendix S1, Sections S1 and S3, Table S1). Water samples were immediately filtered by pressure filtration using a 100mL syringe. The sterile encapsulated 0.8-μm pore size PES filter (50mm diameter) contained a 5-μm glass fibre prefilter (NatureMetrics, UK). The filter was air-dried using the syringe and the capsule subsequently filled with 1.5mL of Longmire's buffer for preservation (Mauvisseau et al., 2021). Disposable gloves were used for sample collection and replaced for each new sample. Sterile and disposable equipment was used at each collection site to avoid potential cross-contamination. Filters were stored in the dark at room temperature until DNA extraction, which was implemented in less than 4 months after sampling.

2.3 | Sample analysis

Samples were analysed using MiFish and Riaz primers for DNA amplification in two different metabarcoding runs. Whilst MiFish primer are specifically designed to target fish communities (Miya et al., 2015), Riaz primers target vertebrates more generally. Nonetheless, Riaz primers often perform well in characterizing fish communities (Zhang et al., 2020). We therefore compared their potential to characterize and monitor fish communities, which was a primary goal of this study.

DNA was extracted following Spens et al. (2017), using the DNeasy Blood and Tissue kit (QIAGEN, Germany). DNA lysis was performed inside filter capsules to avoid DNA loss and contamination following a protocol scaled for larger lysate volumes. The large volume of lysate was successively passed through the spin column, replacing the collection tube after every spin through to reduce the risk of contamination. Two distinct primer sets were investigated to optimize DNA recovery of a hypervariable region of the 12S rRNA in a two-step PCR process. First, purified DNA was amplified using (i) a cocktail of slightly modified 12S MiFish primers from (Díaz et al., 2020; Miya et al., 2015) amplifying a 172bp fragment (see Section S4 and Table S2 for additional details regarding the primer sequences and modification justification) and (ii) 12S Riaz primers (Kelly et al., 2014; Riaz et al., 2011) amplifying a 106bp fragment. Subsequently, tails were added at the 5′ end to be complementary with Illumina Nextera index primers. DNA amplifications with both primer sets were performed with 12 replicates per water sample each in a final volume of 8μL containing 1× Phusion Green Hot Start II High Fidelity PCR Master Mix (ThermoScientific, USA), 0.4μM of each Forward and Reverse tailed primers, 0.6μg/μL of BSA (ThermoScientific), 3% of DMSO (ThermoScientific), 1.5mM of $MgCl_2$ (Invitrogen), 0.9μL of template DNA and PCR grade water (ThermoScientific). PCR conditions were set to an initial denaturation at 98°C for 3min followed by 45 cycles at 98°C for 20s, 69°C for 15s and 72°C for 15s, and a final elongation at 72°C for 5min (Díaz et al., 2020). Negative control and positive control consisting of a mock community with a known composition of non-native fish species (equal volumes of DNA from nine fin clips from fish collected in Sierra Leone, not occurring in Mozambique) were used to assess first-stage PCR

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success and were also sequenced to evaluate sequencing efficiency. Moreover, negative control consisting of PCR grade water were analysed alongside eDNA samples. The presence of PCR products was evaluated by gel electrophoresis, and all PCR replicates for each sample were pooled and purified using MagBind TotalPure NGS (Omega Biotek, USA) magnetic beads with a 0.8:1 beads-DNA ratio to remove primer dimers.

Purified amplicons were indexed in a second PCR following the Illumina 16S Metagenomics Sequencing Preparation protocol in a final volume of 20µL containing 1× Phusion Green Hot Start II High-Fidelity PCR Master Mix (ThermoScientific), 2µL of Nextera XT i7 Index Primer (Illumina, USA), 2µL of Nextera XT i5 Index Primer (Illumina), 4µL of PCR grade water (ThermoScientific), and 2µL of pooled and purified first-round PCR product. After the second amplification, PCR products were purified similarly as above (1:1 beads:DNA ratio), quantified using a Qubit dsDNA HS Assay Kit, sized using a TapeStation D1000 ScreenTape System (Agilent, USA) and normalized to 4nM (Díaz et al., 2020). Libraries were pooled in equimolar concentrations and sequenced on a MiSeq platform (Illumina) with a V2 2×250bp kit. The final library was loaded at 12pM with a 10% PhiX control spike (Díaz et al., 2020).

2.4 | Bioinformatic and statistical analyses

Samples were demultiplexed based on the combination of the i5 and i7 index tags. Paired-end reads for each sample were merged using USEARCH (Edgar, 2010) requiring a minimum of 80% agreement in the overlap. Forward and reverse primers were trimmed from the merged sequences using cutadapt (Martin, 2011) and filtered by length to retain sequences of the expected size for each primer set. Sequences were filtered using USEARCH to assess their quality and retain only those with an expected error rate per base of 0.01 or below and dereplicated by sample, retaining singletons. Unique reads from all samples were denoised in a single analysis using UNOISE (Edgar, 2016), requiring retained ZOTUs (zero-radius Operational Taxonomic Units, also known as Amplicon Sequence Variance) to have a minimum abundance of 8 in at least one sample. The UNOISE algorithm reports all correct biological sequences in the reads. Indeed, some species might be split into several ZOTUs due to intra-specific variations, allowing a differentiation between potential different phenotypes which can be clustered together. ZOTUs were then clustered at 99% similarity with USEARCH to form OTUs (Operational Taxonomic Units; Edgar, 2016). In USEARCH, the algorithms report OTUs which are correct biological sequences, making a clear connection to organism. A taxon-by-sample table was generated by mapping all dereplicated reads for each sample to the OTU representative sequences using USEARCH with an identity threshold of 97% (Edgar, 2016).

Operational Taxonomic Units were identified via BLASTN (Altschul et al., 1990; Camacho et al., 2009) searches of the representative sequences against the nucleotide database of NCBI and a curated local reference database of 12S fish sequences (Lawson Handley et al., 2019), requiring an e-score of 1e-20 and a hit length of at least 90% of the query sequence. Species-, genus- and higher level assignments required at least one hit of ≥99%, ≥95%, and ≥92% similarity, respectively. In case of multiple hits meeting these criteria, public records in the Global Biodiversity Information Facility (GBIF; accessed 29/03/2021 with R package rgbif; Chamberlain et al., 2023; Chamberlain & Boettiger, 2017) for Mozambique were used to resolve the conflicts where possible. Low abundance detections were omitted using a filter threshold of 0.02%, or 10 reads, whichever was the greater threshold for the sample. Common contaminant species (human and domestic animals) and unidentified OTUs were also excluded.

A comparison of recorded biodiversity was an essential part of the primer comparison. In order to guarantee the robustness of this approach, we rarefied all samples to the same threshold level (8000 reads of fish DNA) and discarded sites that did not meet this requirement for both data sets in the primer comparison, leading to 44 retained location out of the 47 sampled. A total of 8000 reads per sample was chosen as threshold for this part of the analysis as it provides a reasonable compromise between (a) reliable richness assessments in rarefied samples and (b) avoid the exclusion of too many samples because of high threshold values (Figure S1). In order to assess differences in alpha diversity (richness and evenness per sample) between primer-sets, we performed paired *t*-tests after data were log-transformed (if necessary) to achieve statistical requirements such as variance homogeneity and normality. We also used a paired *t*-test (or Mann–Whitney *U*-test in case of non-parametric data) to test for differences in taxonomic coverage, which was defined as the fraction of genera that were recorded by one primer-set and were also present in the second primer set. Furthermore, differences between the alpha diversity of habitat types (inland-water, southern marine habitats and central marine habitats) were tested using ANOVA after performing a log-transformation required to achieve variance homogeneity. Additionally, we tested for systematic differences among samples taken by scientific personal and trained citizens (*t*-test, after checking statistical assumptions) and invested primer performance along diversity gradients using a model 2 major-axis regression based on the lmodel2 package in R (Legendre, 2018). A model 2 regression was also used to test for common patterns in beta-diversity (calculated as Bray–Curtis dissimilarity) recorded with the two primer approaches by pairwise comparing similarity values.

Finally, we developed an approach to create a joined data set based on the data provided by the two individual primer sets. The main purpose of this algorithm is to capitalize on the availability of two different data sets, which helps to detect false negative results and improve the robustness of biodiversity estimates by reducing such negative detection as much as possible without increase false-positive detection rates. The main operation principle of the approach is to compare records of occurrence and the taxonomic assignments from both data sets (See Box 1 for method explanation) and combine taxa to one joint feature table. However, if reference databases are incomplete, it is not always

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possible to determine whether entries in two data sets represent two different or the same species (e.g. if a species is missing in one of the two reference databases). We chose conservative procedures (Box 1) to account for such conditions that are frequently encountered in SSA and to avoid the artificial inflation of taxon richness as a result of data merging procedures. A detailed discussion of potential sources of error that could not be eliminated, and an in-depth description of the merging algorithm are provided in the Appendix S1, Section S2. An annotated R-Script to implement data merging procedures, which can in principle be applied to merge any metabarcoding data set, is included as an annex. Definition of threatened species, whose detection was one criteria for the primer comparison, were based on the IUCN's redlist (IUCN, 2021). All data processing and statistical analyses were performed in R version 4.1.0 (R Core Team Foundation).

3 | RESULTS

Sample analysed with MiFish and Riaz primers reached a sequencing depth of 62,139 ± 15,015 (values after ± signify standard deviations) and 57,228 ± 20,088, respectively (Figure S1) and no fish DNA

BOX 1 The problem of interpreting multi-primer data.

Trade-offs in the specificity of primers can emerge especially in tropical regions, which are frequently underrepresented during primer development but rich in fish diversity. The application of multiple primers is a promising approach to resolve such trade-offs (Blackman et al., 2021). If reference libraries are complete and taxonomic assignment consistently reaches the species level, the resulting data sets can simple be combined by merging species lists. However, in case of incomplete reference databases, which are commonly encountered in SSA, multiple challenges can emerge during data merging and interpretation. For example, if both primers result at one site in the detection of multiple OTUs, which are lacking species affiliation, the simple combination of data sets might either reduce the risk of false negative detection or result in an artificially inflate species numbers.

A potential solution: The application of systematic data-merging approaches represents a promising option to reduce false negative detection whilst controlling for the risk of artificial species inflation. We developed here a new data merging approach that is designed to merge different data sets that are hampered by low success rates of taxonomic assignments. The presented approach applies largely conservative procedures by following three simple rules (Figure B1). However, the application of data-merging procedures may sill result in distortion of read counts per species and a certain risk to inflate beta-diversity, which should be considered in the application of such approaches (see also Appendix S1, section S2).

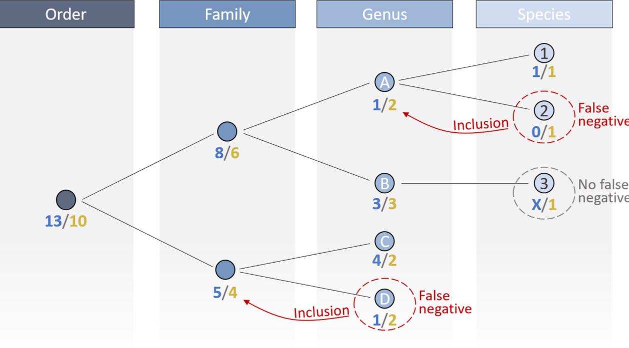

FIGURE B1 Schematic overview of the framework of the newly developed merging procedure to one sample that was analysed with two primers resulting in two separate data sets. Circles represent taxa, numbers below indicate the OTUs per taxon recorded by primer 1 (blue) and primer 2 (yellow). Taxonomic assignment in this example is incomplete due to incomplete reference libraries. The first step of the merging procedure is to identify the data set that contains the highest number of OTUs for a given order. This data set is then used as primary data set and the secondary data set is used in two subsequent steps to identify false negatives and complement the primary data set. One procedure to complement the primary data set is based on species that are assigned to OTUs in both data sets. If the secondary data set contains such a species, which is not listed in the primary data set, it is considered as a false negative detection and corrected in the primary data (see Species 2 as an example). An X signifies that a species is missing in a reference taxonomy and hence e.g. species 3 cannot be diagnosed as false negative because of the incomplete taxonomic assignment for primer 1. The second procedure to detect false negatives is based on the number of OTUs assigned to a taxon. For example, see genus 4: even though none of the OTUs are identified to species level, the secondary data set contains more OTUs of that genus indicating again, a false negative in the primary data set that is corrected. Further methodological details and an r-script for are provided in the Appendix S1.

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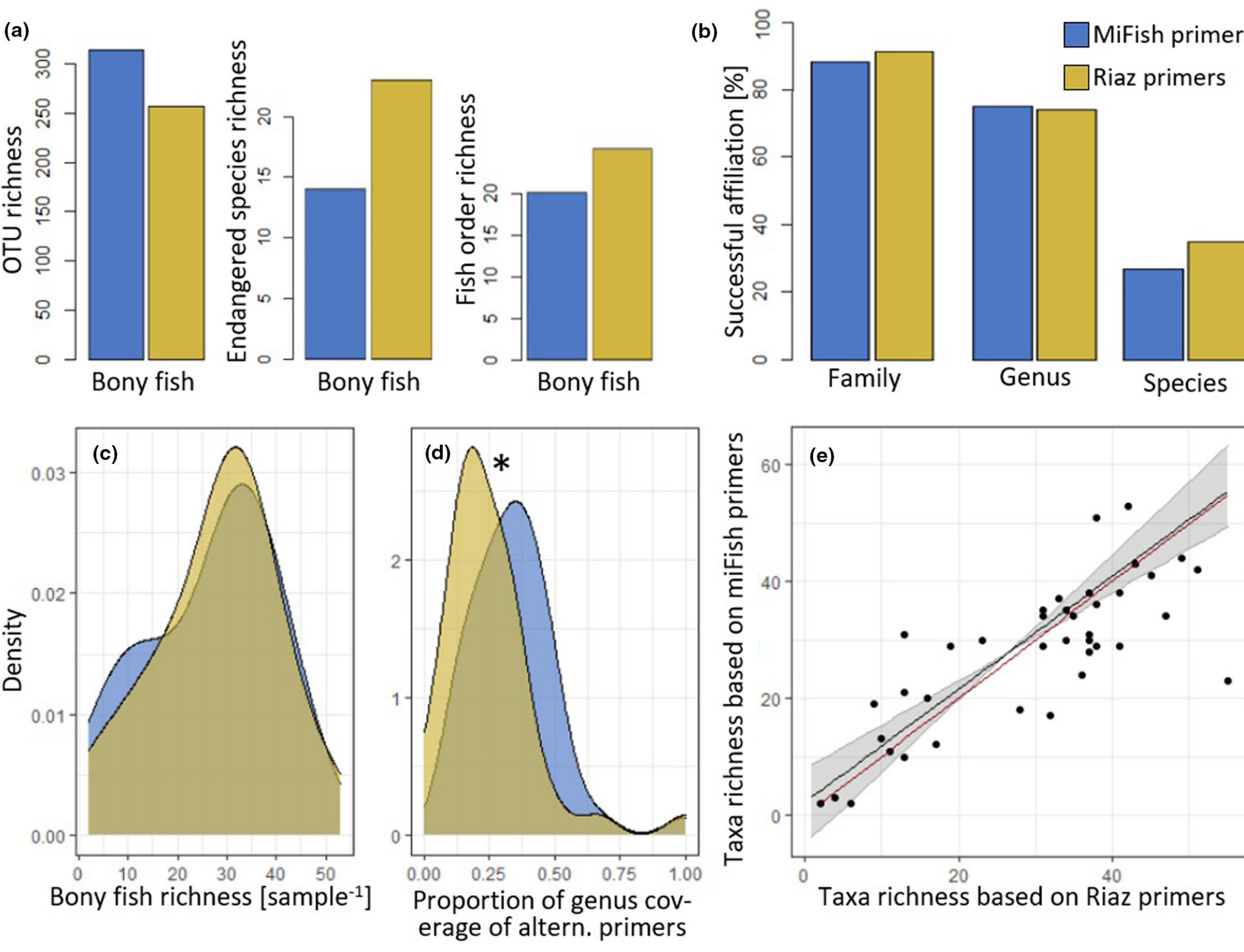

FIGURE 2 Comparison of richness and success of species assignment in data sets generated with MiFish and Riaz primers. Displayed are the number of bony fish OTUs, threatened species (including fish and other vertebrates) and fish taxa at the order levels (a) and the assignment success of the two primer sets at the family, genus, and species level (b). In (c), the OTU richness per sample and in (d), the comparative taxonomic coverage at the genus level for the two primer sets is presented. The comparative taxonomic coverage is thereby defined as the proportion of genus that were recorded by both primers at a specific site. Asterisks indicates a significant difference between primers. In (e), the OTU richness per sample is compared between the two primer sets with the red line indicating the 1:1 ratio and the black line representing the model 2 regression slope. The confidence interval of the regression slopes included 1 (0.74–1.24) and is indicated as a grey band. (c–e) are all based on samples that are amplified by both primer sets and on rarefied data to account for a potentially confounding effect of differential sequencing depth.

was detected in the negative controls. Both Riaz and MiFish primers recovered generally a high fish diversity per sample (Figure 2). Yet, the identity of fish species and the overall performance differed between assays. Whist the MiFish primers detected a higher number of fish OTUs across all samples (317 vs. 263 OTUs), the Riaz data set encompassed a higher number of fish orders and threatened species (Figures 2 and 3; Figures S2–S4). Riaz primers by design also facilitate the detection of other vertebrate species (53 OTUs, 23% of all reads, 98% of non-fish taxa belonging to birds), which can be valuable auxiliary data for ecosystem management. Though, a high contribution of bird eDNA to total vertebrate eDNA decreases the number of fish target reads and resulted here in the exclusion of five samples (<3000 reads of fish sequences per sample, decreasing the reliability of biodiversity comparisons).

The average OTU richness per rarefied sample was very similar between the two primer sets (t-test, $p = .16$) and a pairwise comparison of OTU richness demonstrated their consistent coupling (slope of model 2 regression was not significantly different from 1; confidence interval 0.66–1.13). However, MiFish primers had a significantly better taxonomic coverage at the genus level (i.e. MiFish primers had a lower probability to miss genera that were detect by Riaz primers than vice-versa; Mann–Whitney U-test, $p < .01$; Figure 2). The rate of successful taxonomic assignment was generally low and only 28% of MiFish and 37% of Riaz OTUs could be identified to the species level. These values were in the range of a GenBank-based in-silico analysis, which revealed that 12S sequences were available for 37% of fish species listed for Mozambique (see MiFish OTU table and Riaz OUT table for species-by-species results). Assignment success increased

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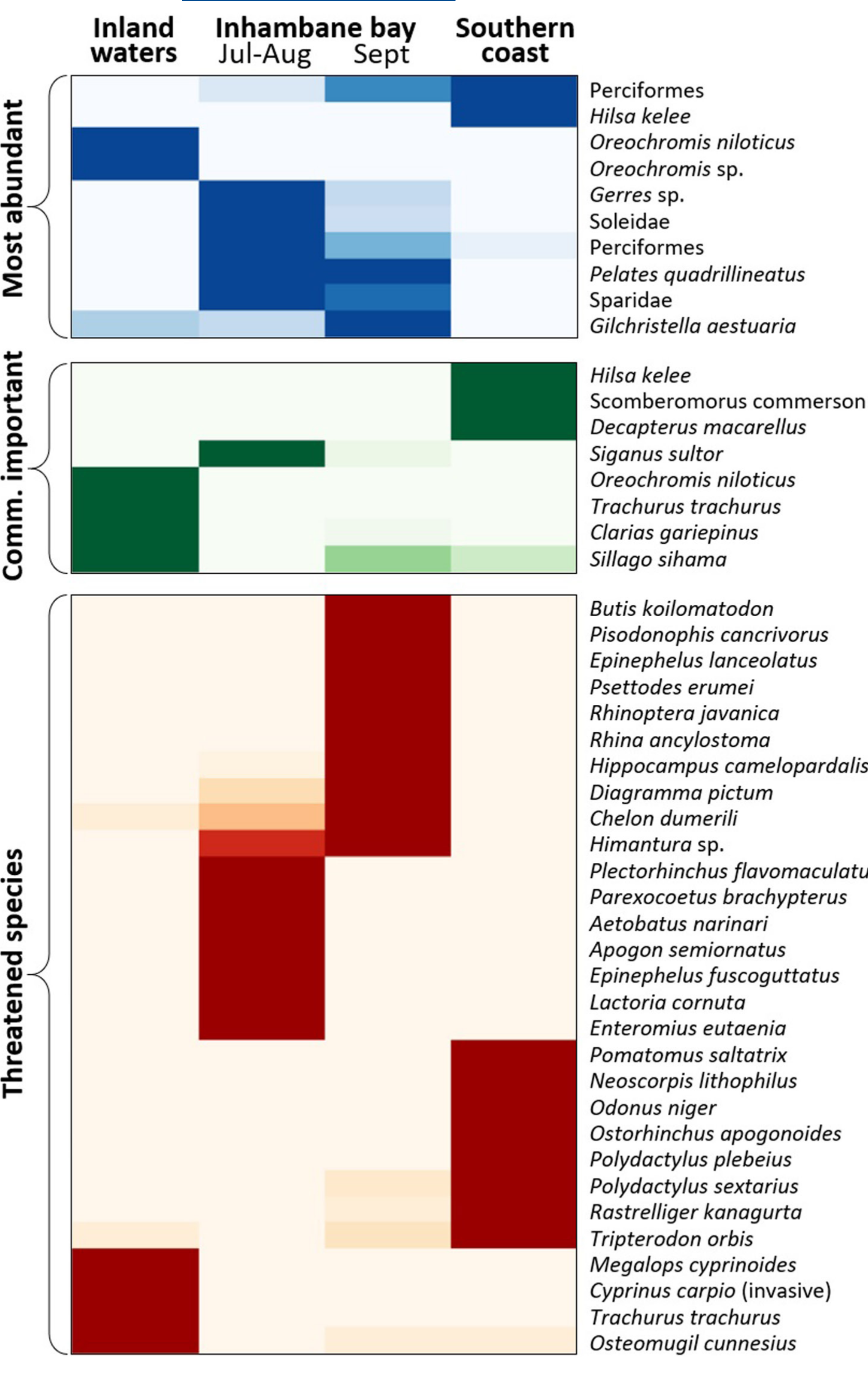

FIGURE 3 Heat map based on relative abundance data of merged OTU data displaying differences in species occurrence in non-marine sites, in Inhambane bay, and in the most Southern part of the Mozambican coast (between the South African border and Maputo). Displayed are the 10 most abundant species (blue), species of high commercial interest (green), and threatened species (red). Abundant OTUs that were not assigned to species level are displayed with the lowest taxonomic resolution available. Note that *Cyprinus carpio* is globally threatened but not endemic to the study region.

at lower taxonomic resolution, but even at the family level more than 10% of MiFish OTUs remained unassigned.

Patterns in community composition showed a clear distinction among fish communities from Inhambane Bay, the southern Mozambican coastline, and inland water habitats (Figure 4). This differentiation was consistent for the data sets generated with Riaz and MiFish primers, and a pairwise comparison of beta-diversity showed a good accordance among the two assays (model 2 regression, confidence interval of slope: 0.95–1.02; Figure 4c). Nonetheless, less than 50% of all fish genera per sample were identified by both assays, indicating that detected taxa differed substantially between assays.

In order to capitalize on the strength of both approaches and to mediate trade-offs between primer sets, we developed a data set merging algorithm (Box 1) and applied this procedure to assess the biodiversity of target sites. The merging procedure resulted in a highly significant increase of recorded fish species richness by 9.4 ± 6.4 and 7.7 ± 6.4 taxa per sample compared to sole applications of MiFish and Riaz primers, respectively (paired *t*-test; $p < .001$; Figure 5). Overall fish OTU richness was 35.9 ± 18 taxa per sample and no significant differences were found between samples collected by either local community members or research personnel (*t*-test, $p = .29$). Moreover, we recorded 29 different threatened fish species with an average of two threatened species per sample. The highest number of threatened species was found

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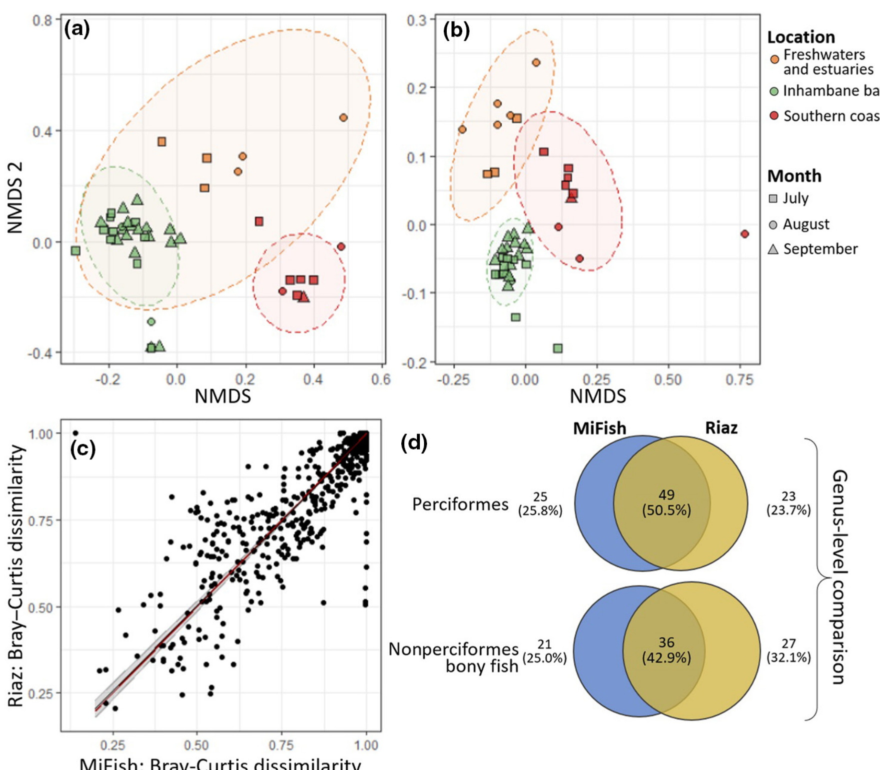

FIGURE 4 Comparison among results generated with Riaz and MiFish primer sets to capture patterns in fish community composition. Displayed are results of a non-metric dimension scaling (NMDS) of OTU tables based on MiFish (a) and Riaz primers (b) reflecting similarity in community composition within specific environments. Ellipses represent data ellipses, predicted to contain 95% of the data associated with on group. The relationship of community similarities obtained by the two primer sets is depicted in (c), with the red line representing the prediction of a model 2 regression. In (d), the number of genera in perciform and non-perciform fish are presented for both primer sets in a Venn-diagram. We chose these presentation as perciforms were better covered by MiFish and non-perciform were better covered by Riaz primers, highlighting primer-specific differences in group resolution.

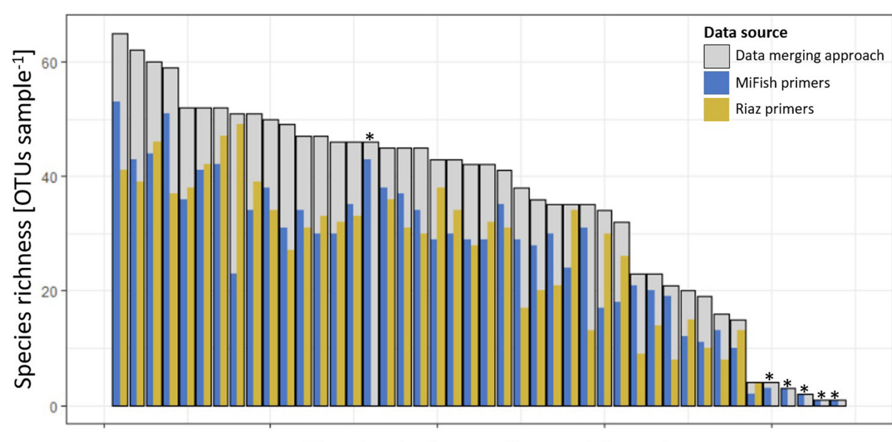

FIGURE 5 Operational Taxonomic Unit richness attained by the data-merging approach (grey bars) compared to values based on single primer-sets (coloured bars) for each sampling site covered in our field campaign. All samples have been rarefied to facilitate richness comparison and * denote Riaz-primer based samples that lay below threshold for the reads per samples (i.e. 8000 reads sample^{-1}).

at the end points of bays and in estuaries characterized by both marine and freshwater influences (Figure 1; Figures S5 and S6).

The samples taken by trained citizens contained included four of the five samples with highest OTU richness recorded in our study. Further, the variance in OTU richness among samples within a region, which can also partly indicate the robustness of the sampling process, was not significantly different for regions sampled by trained citizens or scientific personal (Levine Test, $p = .41$). Operational Taxonomic Unit richness also did not differ among target-ecosystems (ANOVA, $p = .32$) but an assessment of the most abundant OTUs, as well as of species with high commercial and conservation value revealed large regional differences (Figure 5). As expected, species occurrence and abundance differed substantially between the southernmost Mozambican coastline and Inhambane Bay. However, we also found large differences in the threatened species composition recorded in Inhambane Bay in July–August compared to September, highlighting the importance of an appropriate temporal resolution to support conservation planning in the region.

4 | DISCUSSION

Recurrent monitoring of the vast seascapes of SSA will be crucial for their protection and to respond to the growing anthropogenic pressures these habitats are currently facing (Ibe & Amikuzuno, 2019; Ramin, 2009). Here, we have evaluated the potential of eDNA-based monitoring techniques using two different primer sets to survey

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coastal fish communities in Mozambique. Environmental DNA-based surveys which were partly implemented by local communities, were able to reveal a high species richness per sample, reflecting its sensitivity and potential to complement conventional surveying methods. However, several improvements are still required to aid routine applications. First, reference databases for Mozambique show large gaps and even some of the most abundant species failed to match known reference sequences. Furthermore, we found substantial trade-offs in species-recovery between primer sets. However, we demonstrated that these trade-offs can be substantially mitigated by combining data sets in a data-merging approach. Hence, our results highlight that once methodological hurdles are overcome, eDNA-based monitoring techniques can be powerful tools to address the diverse conservation challenges encountered in coastal habitats of SSA.

4.1 | Primer selection

In this study, we used two 12S primer sets, which have previously been shown to outperform assays targeting other gene regions for fish community assessments (Collins et al., 2019; Kelly et al., 2014; Milan et al., 2020; Miya et al., 2015; Polanco et al., 2021; Schenekar et al., 2020; Zhang et al., 2020). Although the two primer sets reached similar results in some regards (e.g. alpha diversity, community characterization), we found also substantial trade-offs in primer performance. Similar trade-offs among metabarcoding primer sets have already been encountered in previous studies evaluating a wider primer range (Polanco et al., 2021). For example, 'teleo' primers targeting 12S mtDNA (Valentini et al., 2016) have been shown to recover higher species richness compared to MiFish primers, despite the higher power of the latter to discriminate among taxa at species, genus and family levels (Polanco et al., 2021). In our application in Mozambique, MiFish primers detected a substantially higher number of fish OTUs across all samples, whilst Riaz primers facilitated the detection of a 50% higher number of threatened fish species. Whether this finding also holds true in other regions with different sets of endangered species requires further testing. Moreover, Riaz primers are designed more generally to target vertebrate taxa, and have the advantage of providing data on water birds and other taxa of potential interest. Additionally, the Riaz primers amplify a shorter amplicon (106 bp) compared to the MiFish primers (172 bp; Kelly et al., 2014; Miya et al., 2015; Riaz et al., 2011). In Mozambique, this may help to detect e.g., Dugongs and other critically endangered marine vertebrates. However, disadvantages such as reducing sequencing depth for fish through replication of none-target eDNA might outweigh benefits (Zhang et al., 2020). In our study, bird eDNA resulted in substantially reduced fish sequence reads, which translates into a lower detection power and a higher risk of false negatives. Hence, the optimal choice of the primers for fish community characterization depends on specific conservation and research targets and will need to be determined on a case-by-case basis.

A possibility to capitalize on the strengths of individual primer sets is to merge data generated by different approaches following, e.g., the procedure developed in our study (Box 1). However, the procedure is not trivial, as uncertainties and errors during taxonomic assignment can potentially result in both under and overestimation of species richness during the data-merging process. One potential challenge is that cross-contamination or tag jumps during MiSeq sequencing runs (Bohmann et al., 2021; Schnell et al., 2015) can potentially be amplified by data merging and lead to an inflation of the species richness per sample. Furthermore, our merging approach has been especially developed to function also when taxonomic assignment is imperfect, but the robustness of procedures nonetheless increase when reference databases are improved. Indeed, an increase coverage of biodiversity in the public databases will lead to a better resolution of the taxonomic assignment, and a higher number of identified OTUs. Despite these methodological challenges, data merging approaches have the power to resolve primer-related trade-offs. Hence, this can substantially improve data quality and the reliability of biodiversity assessments, translating into a more robust basis for conservation planning.

4.2 | Ecological considerations and reference databases

In Mozambique, coastal habitats of the exclusive economic zone host the resources sustaining approximately half of the country's population living in coastal areas (Hoguane et al., 2018). In our study, the application of eDNA-metabarcoding facilitated the detection of a large range of economically important as well as threatened fish taxa. However, there were also several gaps in our taxonomic assessment. A major shortcoming of currently available fish metabarcoding primers is their poor coverage of elasmobranch species (Asbury et al., 2021). MiFish primers are known to rarely detect elasmobranch taxa (Miya et al., 2020). Riaz primers revealed the occurrence of the critically endangered bowmouth guitarfish *Rhina ancylostoma*, as well as the threatened cownose ray *Rhinoptera jayakari*, and whiptail stingray *Himantura* spp., but their overall coverage of elasmobranchs was still poor. Only 11 of the in total 1225 presence records were elasmobranchs and the detected species represent merely a fraction of the elasmobranch communities present in the target regions (Ebert et al., 2021; Schneider et al., 2005). Hence, metabarcoding primers targeting bony fish should ideally be complemented by primers targeting ray and shark species to attain a comprehensive assessment of threatened fish communities (Asbury et al., 2021).

Moreover, we found large temporal turn-over of the threatened fish species recorded with both primer sets in Inhambane Bay. These differences might result from changes in habitat use as the sampling period between July and September marks the transition from the rainy to the dry season. However, there might also be other factors contributing to the observed differences. First, threatened species are often rare and difficult to detect. The resulting low eDNA concentrations shed by these rare species increases

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the stochasticity of their detection (Currier et al., 2018) and make sample comparisons based on rare species less comparable, especially when field replication is low (Mauvisseau et al., 2019). Second, sampling conducted by local community members was not repeated at the same locations in both time periods. In July/August more sampling points from the outer Bay were recorded, whereas in September sampling focused more on the endpoints of the lagoons (Figure S5). Hence, spatial differences might have been a confounding factor of temporal effects on community composition recorded in our study. Nonetheless, large spatiotemporal differences in the occurrence of threatened species are an important finding for the implementation of temporary fishing restrictions and other habitat management strategies.

A critical measure that will increase the ecological insight generated through eDNA applications in Mozambique and in many Sub-Saharan countries is the improvement of available reference databases. In our study, species level assignment was merely 26.5%, a very low rate compared to what can be achieved with fish reads in well-studied temperate regions (for example, up to >90% in Collins et al., 2019). The main reason for this low assignment rate is the insufficient availability of sequencing facilities in Mozambique and the limited effort that is invested into the sequencing of the local flora and fauna. Additionally, Mozambique is expected to still host a relatively high number of undescribed fish species (Gell & Whittington, 2002), which of course also contribute to unassigned taxa in metabarcoding field campaigns. A better coverage of currently known species would therefore unlock a major secondary benefit of metabarcoding approaches, which under such conditions can also help to support in the detection of new species and cryptic species complexes (Zinger et al., 2020).

4.3 | Possible future improvements

The regular and reliable monitoring of coastal fish communities in SSA will require a cost-effective, scalable, highly sensitive and robust surveying method. Our results demonstrate that the eDNA-based approach used in this study can fulfil these requirements if it is appropriately adjusted to management needs. Sensitivity of assessments could for example be further increased if the highly heterogeneously distribution of eDNA in natural habitats (Troth et al., 2021) is accounted for by an increase in the number of field replicates (Capo et al., 2019; Mauvisseau et al., 2019). Implementation of field replication would also enable the application of occupancy models and other postprocessing tools to increase the robustness of data interpretation and decision-making processes (Burian et al., 2021). Another exciting possibility is to combine metabarcoding with the quantification of eDNA in a sample (Everts et al., 2021; Shelton et al., 2019). Such quantification using for example qPCR and droplet digital PCR (ddPCR), can be used to infer fish biomass (Brys et al., 2020; Mauvisseau et al., 2021), which would provide very valuable additional information for the sustainable management of fish stocks.

Finally, current monitoring strategies in most Sub-Saharan countries rely strongly on centralized implementation structures, which are linked to substantial travel and field sampling costs. Our results and earlier studies have shown that eDNA-based tools provide an excellent basis for decentralized community-based monitoring and citizen science initiatives (Aylagas et al., 2020; Biggs et al., 2015; Howell et al., 2021). Such community-based programmes can rely on numerous volunteers and easily be conducted on large scale due to the development of collaborative technology and social media (Larson et al., 2020). With proper training, involvement of professional scientists and access to suitable technologies, these programmes can allow to cover large geographic areas that would otherwise be unavailable due to field conditions or limited funding. In Mozambique and other regions of SSA, such initiatives would be very well suited to complemented centralized survey efforts and thereby help to reduce associated costs for flights, per diems, boat time, fuel, etc. The freed-up resources could then be used to extent eDNA survey efforts and the coverage of remote and currently often marginalized regions. Environmental eDNA preservation techniques can play an important role in supporting such efforts as they facilitate the storage of samples at ambient temperatures without eDNA degradation for several months (Mauvisseau et al., 2021). Furthermore, earlier studies have shown that a reliance on community member is unlikely to decrease data quality or lead to potential biases, and that community members can be as efficiently trained as conservation professionals (Agersnap & Thomsen, 2022; Larson et al., 2020). Instead, a stronger integration of local communities into environmental monitoring often leads to increased agency and environmental awareness (Trimble & Berkes, 2013; Wiber et al., 2009). Especially, Kelly et al. (2020) highlighted that citizen science is a promising tool to enhance engagement in marine conservation worldwide. Sub-Saharan Africa, costal resources are often primarily managed and administrated by local communities and such 'side-effects' of community-based monitoring can be of equal importance for conservation as the availability of reliable monitoring data. Consequently, eDNA-based methods can provide powerful tools that help to attain a sustainable use of costal ecosystems. Critical further steps to attain this goal will be the development of local analytical infrastructure, the complementation of local genetic reference databases, and the establishment of suitable organizational structures to facilitate routine monitoring.

AUTHOR CONTRIBUTIONS

Kat Bruce and Vere Ross-Gillespie designed the study and facilitated training of local stakeholders. Hugo M. Costa, Antonio de Sacramento, Luisa Simbine and Naseeba Sidat, conducted and organized field sample collection. Laura Balcells, Judith Bakker performed laboratory analysis. Alex Crampton-Platt and Sarah Chordekar undertook bioinformatics assessments. Alfred Burian, Quentin Mauvisseau and Alex Crampton-Platt performed the data analysis and Alfred Burian and Quentin Mauvisseau wrote the first draft of the manuscript. All co-authors provided comments and helped revising the manuscript.

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CONFLICT OF INTEREST STATEMENT

Kat Bruce, Cuang Tong, Vere Ross-Gillespie, Laura Balcells, Sarah Chordekar, Alex Crampton-Platt, and Judith Bakker are employed by NatureMetrics, a for profit company providing DNA-based solutions for biodiversity monitoring.

OPEN RESEARCH BADGES

This article has earned an Open Data badge for making publicly available the digitally-shareable data necessary to reproduce the reported results. The data is available at https://doi.org/10.5281/zenodo.8074781.

DATA AVAILABILITY STATEMENT

Processed data and R-script are provided in Appendix S2. Raw sequencing data are available in the following Zenodo Repository (https://doi.org/10.5281/zenodo.8074781).

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