Environmental DNA biomonitoring in biodiversity hotspots: A case study of fishes of the Okavango Delta

von der Heyden, Sophie ; Neef, Gotz; Grevesse, Thomas; Cwecwe, Yandisa; Sado, Tetsuya; Miya, Masaki; Mosie, Ineelo; Creer, Simon; Skelton, Paul; von Brandis, Rainer

Environmental DNA

DOI:
10.1002/edn3.494

Published: 28/11/2023

Publisher's PDF, also known as Version of record

Received: 20 June 2023 | Revised: 6 November 2023 | Accepted: 8 November 2023

DOI: 10.1002/edn3.494

Environmental DNA
Open Access
Dedicated to the study and use of environmental DNA for basic and applied sciences

WILEY

ORIGINAL ARTICLE

Environmental DNA biomonitoring in biodiversity hotspots: A case study of fishes of the Okavango Delta

Sophie von der Heyden[1,2] | Götz Neef[1,3] | Thomas Grevesse[4] | Yandisa Cwecwe[1] | Tetsuya Sado[5] | Masaki Miya[5] | Ineelo Mosie[6] | Simon Creer[7] | Paul Skelton[3,8] | Rainer von Brandis[3]

[1]Department of Botany and Zoology, Stellenbosch University, Matieland, South Africa

[2]School of Climate Studies, University of Stellenbosch, Matieland, South Africa

[3]National Geographic Okavango Wilderness Project, Wild Bird Trust, Sandton, South Africa

[4]Department of Biology, Concordia University, Montreal, Quebec, Canada

[5]Natural History Museum and Institute, Chiba, Chiba, Japan

[6]Okavango Research Institute, University of Botswana, Maun, Botswana

[7]Molecular Ecology and Evolution Group, School of Natural Sciences, Bangor University, Gwynedd, UK

[8]NRF-SAIAB, Makhanda, South Africa

Correspondence
Sophie von der Heyden, Department of Botany and Zoology, Stellenbosch University, Private Bag X1, Matieland 7602, South Africa.
Email: svdh@sun.ac.za

Funding information
Royal Society, Grant/Award Number: NI170129; Wild Bird Trust

Abstract

The Okavango Delta is the largest freshwater wetland in southern Africa and a recognized biodiversity hotspot and UNESCO World Heritage Site. The region is extremely rich in floral and faunal diversity, including a fish fauna of ~90 species in 15 families, that also support recreational and subsistence fishing. Anthropogenic pressures and invasive species threaten the unique biodiversity and ecosystem services that the Delta provides, necessitating biomonitoring tools that can provide broad community-level diversity insights. Here, we utilize environmental DNA metabarcoding of aquatic eDNA using the MiFish 12S rRNA primers, to investigate fish communities and also sequenced 211 mtDNA 12S barcodes for 74 species across 36 genera of fishes from the region. Metabarcoding recovered 11 of 15 families, with 40 species detected across 23 genera, representing ~50% of known diversity, with the mtDNA 12S fragment able to delineate all genera (except for the cichlid genera *Serranochromis* and *Pharyngochromis* that comprised a single clade) and most species, except for some in the *Clarias*, *Enteromius*, *Labeo*, *Lacustricola*, and *Petrocephalus* genera. Generally, abundant and wide-spread taxa such as *Clarias* spp. and *Marcusenius altisambesi*, amongst others, were often detected in the surveys, with other species, including *Zaireichthys kavangoensis*, *Schilbe intermedius*, and *Labeo* sp. detected less frequently. Dissolved oxygen, temperature, and dissolved organic solids were positively correlated with community diversity, highlighting the influence of environmental factors in shaping fish communities in the region. Further, there was strong variability in the eDNA signal across only 1000 m, suggesting that future surveys need to consider spatio-temporal aspects of sample collection. Our study highlights the potential of eDNA metabarcoding for surveying aquatic biodiversity in the Okavango Delta, particularly within the context of baseline biodiversity inventories, that underpin conservation and management initiatives. As such, we provide a number of recommendations that can help structure future sampling efforts in the region.

1 | INTRODUCTION

Freshwater ecosystems are amongst the most threatened globally and face numerous pressures including catchment alterations through development and urbanization, pollution, changes in runoff, and flow regimes and water abstraction (Dudgeon et al., 2006; Reid et al., 2019). Such anthropogenic stressors in turn impacts a range of ecosystem services that affect freshwater-associated biodiversity and human-wellbeing (Dodds et al., 2013; Lin et al., 2021; Scherer et al., 2023). The Okavango Delta, around 16,000 km^2 in extent, is the largest freshwater wetland system in southern Africa (McCarthy, 2013) and a major environmental and socioeconomic resource for Botswana and its people. The Delta is an alluvial fan formed by seismic faulting across the drainage line of the river, part of what is likely the south-western extension of the East African Rift System (Daly et al., 2020). The Delta provides an extremely rich spectrum of aquatic habitats ranging from permanent flowing channels, lagoons and swamps to seasonally flooded swamps, and floodplains of sedge- and grasslands (Murray-Hudson et al., 2011: Figure 1) and is recognized through the UNESCO World Heritage Sites and RAMSAR Wetlands programs.

The Delta is extremely rich in floral and faunal diversity including a fish fauna of ~90 species in 15 families (Bruton et al., 2018). The fish community of the Delta is not only dominated by mostly small cyprinids (24%; 21 species including minnows, yellowfishes, and labeos), catfishes (23%; 20 species), and the bream-like cichlids (21%; 19 species), but also includes mormyrids (13%; 11 species) and a host of other afro-tropical families. Iconic angling species like the Tigerfish (*Hydrocynus vittatus*), Southern African pike (*Hepsetus cuvieri*), large catfishes (*Clarias* spp.), and the largemouth cichlids (*Serranochromis* spp.) sustain a popular angling economy (Bruton et al., 2018). The fish fauna and other aquatic resources also provide for an open subsistence and small-scale commercial fishery traditionally practiced by local communities (Mmopelwa et al., 2009). Acknowledging the notable biodiversity and socioeconomic importance of the Delta, it is under threat from anthropogenically induced climate change, but more directly from a rapidly rising human population and development of the catchment and the immediate environs of the Delta itself (Mendelsohn et al., 2010; Skelton, 2019). The most evident threats include alien invasive species like *Salvinia molesta* and Nile tilapia, *Oreochromis niloticus*, channel clearing, riparian and floodplain burning, pollution from pesticides and human settlements, and unregulated overfishing. However, larger scale threats include potential damming for hydropower and large-scale water abstraction in the catchment for agriculture and rapidly expanding urban spaces, including distant cities such as Windhoek (King & Chonguiça, 2016;

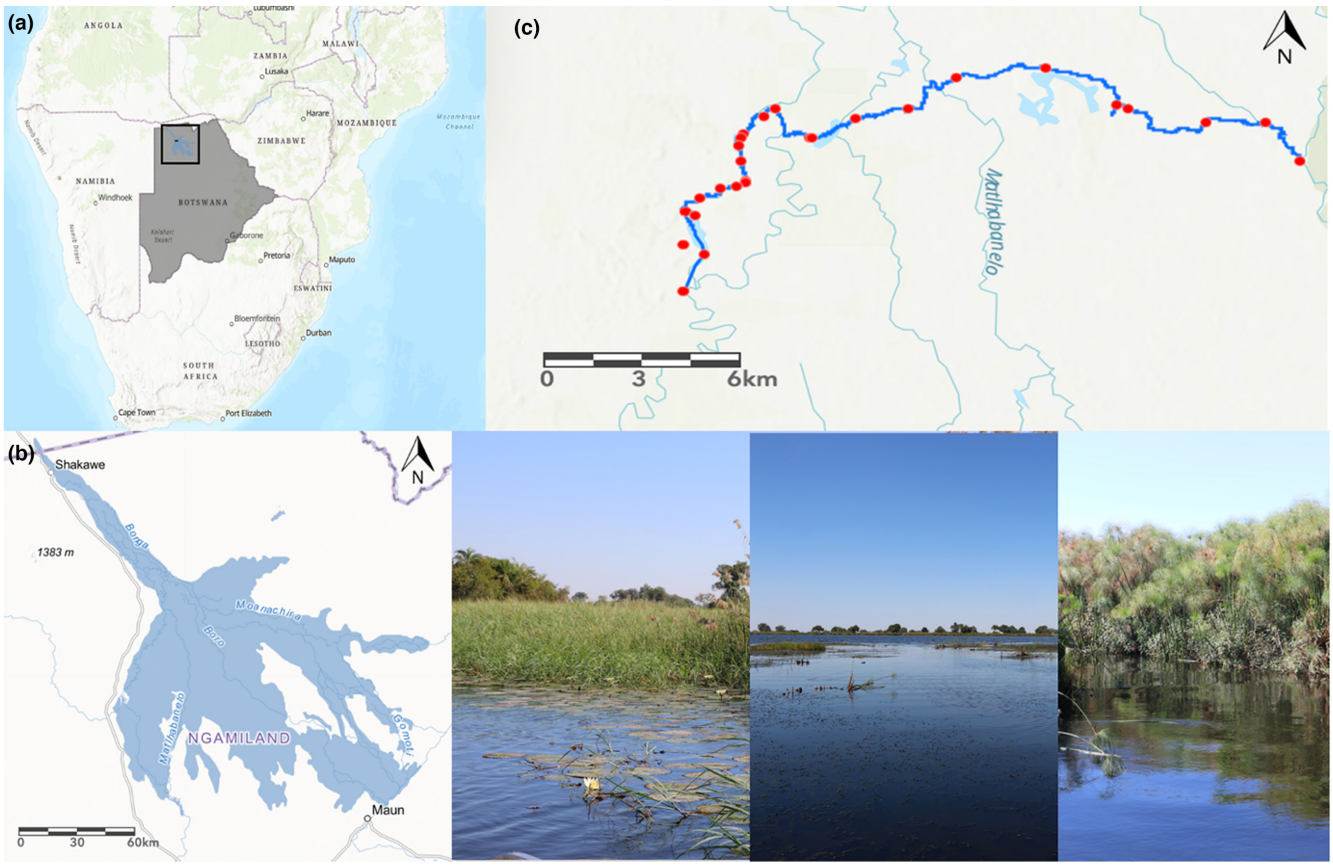

FIGURE 1 Map of study area and of the 27 sampling sites in the panhandle of the Okavango Delta. The upper left insert shows the location of Botswana in southern Africa (a), with the location of the Okavango Delta shown in (b). Red circles on the top left map (c) denote sampling locations. Photographs of some different habitat types that were targeted throughout sample collection (papyrus lined channels, open lagoon systems, and lagoon fringes) are included for visualization of the ecosystem.

Mendelsohn et al., 2010; Mendelsohn & Martins, 2018; Mosepele & Kolding, 2003; Skelton, 2019; Tweddle & Hay, 2013; van Wilgen et al., 2022).

The biodiversity of the Delta is strongly influenced by seasonal climatic factors, and so the need for sensitive and efficient monitoring of the spatio-temporal patterns of biodiversity is immense. However, the logistical difficulties of working in the system, as well as high levels of biodiversity, provide numerous challenges when accounting for species distributions. The advance of environmental DNA (eDNA) metabarcoding has provided a viable alternative for non-invasive and rapid monitoring of community diversity (Deiner et al., 2017; Gilbey et al., 2021; Ruppert et al., 2019), including for freshwater environments (Carraro et al., 2020; Hallam et al., 2021; Laporte et al., 2022; Leese et al., 2021), although the application of eDNA metabarcoding in Africa remains limited (Schenekar, 2022; von der Heyden, 2023). Environmental DNA studies on fishes are particularly advanced and numerous examples from across a wide range of environments show that eDNA is a suitable tool for estimating the diversity of fish communities (Miya et al., 2020), although studies remain geographically uneven, particularly toward the global south (Belle et al., 2019; Schenekar, 2022; von der Heyden, 2023). Further, for the power of eDNA to be realized within the context of biodiversity monitoring, a well curated database is crucial, as reliable databases facilitate the accurate identification of species from within a sample (Berry et al., 2021; de Santana et al., 2021; Gaither et al., 2022). Within the context of this study and the Okavango representing an important biodiversity hotspot, only six cytochrome oxidase (COI) barcodes were readily available for fishes (boldsystems.org, accessed 12 February 2021), highlighting the need for further barcode efforts in the region.

When considering the detection of species via eDNA analyses, the 'ecology' of eDNA, or the relationship between the organism and eDNA trace material, that is, the origin, state, fate, persistence, and transport of eDNA (Barnes & Turner, 2016) requires careful consideration. For lotic systems in particular, it is predicted that the persistence, rate of degradation, and transport are likely the overarching factors that govern the connection between eDNA and the organism. Empirical studies have demonstrated levels of detection of lotic fish eDNA from between 100–5000 m (Jane et al., 2015; Laporte et al., 2022; Wood et al., 2021) and even up to 100 km (Pont et al., 2018), or within 43 h of release (Seymour et al., 2018) in temperate systems. As elsewhere, the persistence of eDNA in the Delta is likely governed by temperature, UV levels, biological (e.g., microbial and enzymatic) and chemical activity (e.g., adhering and bonding to substrates; Mauviesseau et al., 2022). As temperature has been shown as an important determinant of eDNA degradation (Strickler et al., 2015; Tsuji et al., 2017), it is expected that in a tropical environment, eDNA will either persist for an equivalent or reduced duration, resulting in a finer level of geographical resolution between the living community and that of the eDNA. In contrast, varying flow rates, between, for example, still or low flow lagoon environments versus rapidly flowing channels, may also determine the link between origin and detection of eDNA.

Despite the presence of substantial biodiversity and growing challenges pertaining to environmental change and anthropogenic pressures, environmental DNA studies from Africa remain in the minority (Belle et al., 2019; Miya et al., 2020; Rourke et al., 2022; Schenekar, 2022; Takahashi et al., 2023; von der Heyden, 2023), with no studies to date carried out in Botswana (von der Heyden, 2023). In this study, we generated a comprehensive partial 12S RNA database for fishes from the Okavango Delta and surrounding areas, in combination with an investigation of the utility of aquatic eDNA analysis for the detection of fish communities in the region. Specifically, (1) we tested the overlap between barcodes generated from fishes and OTUs generated from water eDNA using fish-specific primers; (2) we used the MiFish primers (Miya et al., 2015, 2020) to characterized fish communities from 27 sites in the panhandle region of the upper Okavango Delta (Figure 1); (3) tested environmental variation as predictors of fish community structure and (4) deployed a fine-scale sampling strategy to better understand eDNA signals in relatively higher flow channel environments to test eDNA transport. Overall, our paper provides first insights into the power of environmental DNA metabarcoding in the Okavango Delta, specifically within the context of monitoring the substantial biodiversity of this iconic ecosystem and thus providing future directions for conducting eDNA surveys in the region.

2 | MATERIALS AND METHODS

2.1 | 12S barcoding of Okavango and other southern African freshwater fish species

In order to maximize the identification of species from reads generated through metabarcoding, a comprehensive alignment of species level sequences associated with the Okavango, in addition to associated drainage systems was created. Tissue was kindly supplied through the South African Institute of Aquatic Biodiversity tissue collection (species inventory available at www.github.com/vonderheydenlab), with DNA extractions performed using the Macherey-Nagel DNA extraction kit, with 12S barcodes generated using the primers MiFish-U-F and MiFish-U-R and protocol following Miya et al. (2015). Post-amplification PCR products were gel purified and sequenced bi-directionally on an ABI 3730xl at the Central Analytical facility in Stellenbosch. Sequences were checked and aligned using Geneious v11 (https://www.geneious.com).

2.2 | Environmental DNA sampling and extraction protocol

The field study took place in May 2019, with samples collected at 27 sites over four days in the northern Okavango Delta. We targeted a large variety of habitats, including fast-flowing channels, shallow pans, and inundated grasslands, as well as larger lagoon systems (Figure 1). At each site, three independent replicate water samples

were collected directly from the water, about 20–30 cm below the water surface. In addition to the broader sampling, we also sought to understand whether downstream transport in channel systems would play a significant role in the distribution of eDNA. As such, we collected triplicate samples downstream at intervals of 50, 100, 500 and 1000 m (total of five sites) from a starting point at the mouth of Qhwaxa (Figures 1 and 4). At each of the 27 sites, we used an In-Situ Aqua TROLL 600 Multiparameter Sonde to collect a comprehensive suite of physicochemical environmental variables including pH, Oxidation Reduction Potential, Total suspended Solids, Dissolved Oxygen, Conductivity, Turbidity, Salinity, Resistivity, Temperature, and Water Density.

Samples were collected directly from a boat, minimizing human contact with the water. To further limit contamination, fresh gloves were worn for every sampling procedure. Immediately after sampling, water samples were pushed through a 0.22 μm Sterivex (Merck) filter, using a 50 mL piston syringe or a Geotech Peristaltic field pump, until no additional water could be passed through (volumes of water ranged from 320 to 1550 mL depending on factors such as turbidity, with an average of 910 mL sampled per site). Upon completing filtering, all remaining water was removed from the Sterivex filter, via air injection and the filter preserved with 2 mL of ATL buffer (Qiagen) prior to sealing with Helapet Combicaps (Helapet, UK) and parafilm. All samples were stored at room temperature until extraction. Ten negative controls were deployed in the field by filtering 500 mL of bottled spring water using the same protocol amongst the empirical samples, in order to account for possible in-field contamination (field blanks were taken through the workflow as for the samples). DNA extraction utilized the DNEasy blood and tissue kit (Qiagen), following the modified protocol of Czachur et al. (2022). DNA was extracted directly from the Sterivex filters, including from all negative controls. To prevent contamination, all extractions were carried out in a laboratory reserved only for eDNA extractions in the Department of Botany and Zoology, Stellenbosch University; surfaces and equipment were exposed to UV for 30 min prior to extractions and wiped with 10% sodium hypochlorite solution. Negative controls were included in each extraction batch to monitor potential contamination. Extracted DNA was stored at −20°C.

2.3 | Library preparation and sequencing

2.3.1 | Paired-end library preparation and MiSeq sequencing

For this study, we chose to utilize a primer that amplifies a portion of the mtDNA 12S gene, given that this has been suggested as a more effective metabarcoding marker with broader applicability and resolution for assessing fish communities, compared to, for example, mtDNA COI (Collins et al., 2019; Miya, 2022; Miya et al., 2020; Xing et al., 2022). Library preparation and sequencing were carried out at the Natural History Museum and Institute, Chiba, with the triplicate samples pooled into one reaction prior to library preparation. A two-step PCR was employed to prepare paired-end libraries for the MiSeq platform (Illumina, CA, USA) and generally followed the methods developed by Miya et al. (2015). For the first-round PCR (first PCR), we used a mixture of the following PCR primers: MiFish-U-forward (5′-ACA CTC TTT CCC TAC ACG ACG CTC TTC CGA TCT NNN NNN GTC GGT AAA ACT CGT GCC AGC-3′) and MiFish-U-reverse (5′-GTG ACT GGA GTT CAG ACG TGT GCT CTT CCG ATC TNN NNN NCA TAG TGG GGT ATC TAA TCC CAG TTT G-3′). These primer pairs co-amplify a hypervariable region of the fish mitochondrial 12S rRNA gene (around 172 bp; hereafter called MiFish sequence) and append primer-binding sites (5′ ends of the sequences before six Ns) for sequencing at both ends of the amplicon. Six random bases (Ns) in the middle of those primers were used to enhance cluster separation on the flow cells during initial base call calibrations on the MiSeq platform.

The first PCR was carried out with 35 cycles of a 12 μL reaction volume containing 6.0 μL 2× Platinum™SuperFi™II PCR Master Mix (Thermo Fisher Scientific, Waltham, MA, USA), 1.4 μL of each MiFish primer (5 μM primer F/R), 1.2 μL sterile distilled H_2O, and 2.0 μL eDNA template (diluted 10×). To minimize PCR dropouts, eight technical replications were performed for the 1st PCR using a 0.2 mL 8-strips tube. The thermal cycle profile after an initial 3 min denaturation at 95°C was as follows: denaturation at 98°C for 20 s, annealing at 60°C for 15 s, and extension at 72°C for 15 s, with the final extension 72°C for 5 min. The first PCR products from the eight tubes were pooled in a single 1.5 mL tube and the pooled products were purified using a GeneRead Size Selection kit (Qiagen, Hilden, Germany) to remove dimers and monomers following the manufacturer's protocol. Subsequently, the purified products were quantified using a TapeStation 2200 (Agilent, Tokyo, Japan), and the quantified products were diluted to 0.1 ng/μL using Milli-Q water, which was used as a template for the second-round PCR (second PCR).

For the second PCR, we used the following two primers to append the dual-index sequences (eight nucleotides indicated by Xs) and flowcell-binding sites for the MiSeq platform (5′ ends of the sequences before eight Xs): second-PCR-forward (5′-AAT GAT ACG GCG ACC ACC GAG ATC TAC ACX XXX XXX XAC ACT CTT TCC CTA CAC GAC GCT CTT CCG ATC T-3′); and second-PCR-reverse (5′-CAA GCA GAA GAC GGC ATA CGA GAT XXX XXX XXG TGA CTG GAG TTC AGA CGT GTG CTC TTC CGA TCT-3′). The second PCR was conducted with 10 cycles of a 15 μL reaction volume containing 7.5 μL 2× KAPA HiFi HotStart ReadyMix, 0.9 μL of each primer (5 μM), 3.9 μL sterile distilled H_2O, and 1.9 μL template. The thermal cycle profile after an initial 3 min denaturation at 95°C was as follows: denaturation at 98°C for 20 s, annealing and extension combined at 72°C (shuttle PCR) for 15 s, with the final extension at 72°C for 5 min. To monitor contamination during the PCR process, three blank samples (negative controls) were prepared. In addition to FB, first and second PCR blanks (1B and 2B, respectively) with 2.0 μL Milli-Q water instead of template eDNA.

All libraries containing the target region and the adapter sequences were mixed in equal volumes, and the pooled libraries

were size-selected from approximately 340 bp using a 2% E-Gel Size Select agarose gel (Invitrogen, CA, USA). The concentration of the size-selected libraries was measured using a Qubit dsDNA HS assay kit and a Qubit fluorometer (Life Technologies, CA, USA), diluted to 11.0 pM with HT1 buffer (Illumina, CA, USA), and sequenced on the MiSeq platform using the MiSeq v2 Reagent Kit Mini for 2 × 150 bp paired-end (Illumina) with a PhiX Control library (v3) spike-in (expected at 5%) following the manufacturer's protocol. All raw DNA sequence data and associated information are deposited in the DNA Databank of Japan under Accession number DRA016590.

2.3.2 | Data preprocessing and taxon assignment

Data preprocessing and analysis of MiSeq raw reads from 30 samples (27 sites and three blanks) were performed using USEARCH v10.0.240 (Edgar, 2010) according to the following steps: (1) Forward (R1) and reverse (R2) reads were merged by aligning both reads using the "fastq mergepairs" command. During this process, low-quality tail reads with a cut-off threshold set at a quality (Phred) score of 2, reads too short (<100 bp) after tail trimming, and those paired reads with too many differences (>5 positions) in the aligned region (around 65 bp) were discarded; (2) primer sequences were removed from those merged reads using the "fastx truncate" command; (3) those reads without the primer sequences underwent quality filtering using the "fastq filter" command to remove low-quality reads with an expected error rate of >1% and reads too short (<120 bp); (4) the preprocessed reads were dereplicated using the "fastx uniques" command, and all singletons, doubletons, and tripletons were removed from the subsequent analyses to avoid false positives following the recommendation by the program's author (Edgar, 2010); (5) the dereplicated reads without single- to tripletons were denoised using the "unoise3" command to generate amplicon sequence variants (ASVs) that remove all putatively chimeric and erroneous sequences (Callahan et al., 2017); (6) the ASVs were rarefied to the minimum read number and (7) finally ASVs were assigned to taxon, that is, species names (thereby forming molecular operational taxonomic units; MOTUs, derived from the ASV taxonomy assignments) using the "usearch global" command with a sequence identity of >98.5% with the reference sequences (two nucleotide differences allowed).

ASVs with sequence identities of 80%–98.5% were tentatively added U98.5 labels before the corresponding species names with the highest identities (e.g., U98.5_*Pagrus_major*) and were subjected to clustering at the level of 0.985 using the "cluster smallmem" command. An incomplete reference database needs this clustering step that enables the detection of multiple MOTUs under the same species name. Such multiple MOTUs were annotated with "gotu1, 2, 3…" and all of these outputs (MOTUs and U98.5 MOTUs) were tabulated with read abundances. Those ASVs with sequence identities of <80% (saved as "no hit") were excluded from the above taxon assignments and downstream analyses because all of them were found to be non-fish organisms (see Section 3).

To refine the above taxon assignments, family-level phylogenies were reproduced with MiFish sequences and novel sequences generated in this study from MOTUs, U98.5 MOTUs plus the reference sequences (contained in MiFish DB ver. 36) from those families. For each family, representative sequences (most abundant reads) from MOTUs and U98.5 MOTUs were assembled, and all reference sequences from that family were added in a fasta format. The combined fasta-formatted sequences were subjected to multiple alignment using MAFFT 7 (Katoh & Standley, 2013) with a default set of parameters. A neighbor-joining (NJ) tree was constructed with the aligned sequences in MEGA7 (Kumar et al., 2016) using the Kimura two-parameter distances (Kimura, 1980). Distances were calculated using pairwise deletion of gaps and the amongst-site rate variations modeled with gamma distributions (shape parameter = 1) and midpoint rooting was performed on the resulting NJ tree.

Family-level trees were visually inspected, and taxon assignments revised as follows: For those U98.5 MOTUs placed within a monophyletic group consisting of a single genus, the unidentified MOTUs were named that genus plus sp. with sequential numbers (e.g., *Pagrus* sp. 1, sp. 2, sp. 3, …). For the remaining MOTUs ambiguously placed in the family-level tree, the unidentified MOTUs were named that family plus sp. with sequential numbers (e.g., Cichlidae sp. 1, sp. 2, sp. 3, …).

2.4 | Statistical analyses

To test the effect of sample volume on OTU richness at each site, a Pearson Correlation test was carried out. To build the OTU presence-absence table, we considered an OTU present in a sample if at least one read was mapped to this OTU and absent if no reads were mapped. A Principal component analysis (PCA) was performed on the presence/absence table using the rda() function from the *vegan* package in R. To follow how the richness of the fish communities changed over environmental gradients, the richness in each sample was calculated as the number of OTUs present and plotted as a function of various environmental parameters collected at the time of sampling. Parameters measured included distance between sites, temperature, density, turbidity, dissolved oxygen, pH, and total dissolved solids. Community turnover between two samples was calculated as the Jaccard distance based on the presence/absence OTU table using the funcClassJacc function from the *ConNEct* package in R. Community turnover was plotted as a function of the difference of environmental variables values between two samples. The relationship between the community turnover and difference in environmental variables values was fitted with a linear model, displaying a 95% confidence level interval based on predictions from the linear model. A Mantel test was performed between the matrix of Jaccard dissimilarity and each of the matrices containing the difference in environmental variables between sites. The Mantel tests were performed using the mantel.rtest() function from the ade4 package in R.

3 | RESULTS

3.1 | A novel custom database of mtDNA 12S for fishes from the Okavango

We generated 211 novel mtDNA 12S barcodes for 74 species found throughout the Okavango ecosystem. As a reference, we used the work of Bruton et al. (2018), the most comprehensive reference of fishes in the region. Our sequencing effort covered all known families present except one (Nothobranchidae, represented by a single species *Nothobranchius capriviensis* for which no samples were available), 36 genera (from 39), and 74 species (from 87 species in total). All sequences are available as a downloadable alignment available through GitHub (www.github.com/vonderheydenlab) or as part of the MiFish pipeline (PMiFish ver. 2.4; the latest version is available from https://github.com/rogotoh/PMiFish.git).

3.2 | Metabarcoding fish communities

Post sequencing, filtering, and removal of non-fish reads, 4, 434, 451 reads were retained. The number of reads ranged from 84,792 to 253,019 per sampling site, with an average of 164, 239 (±35, 200) reads per site. Reads in three negative controls ranged from 0 to 35 reads and were removed from the OTU counts for each site. Our metabarcoding of aquatic eDNA recovered 11 of 15 families, with 40 species detected across 23 genera, representing ~50% of all known Okavango fish diversity. The most commonly detected species across all sites were the *Clarias gariepinus* complex, *Marcusenius altisambesi*, and an unassigned species of cichlid (Cichlid sp. 1). Other commonly detected (>20 sites) species include *Brycinus lateralis*, *Microctenopoma intermedium*, *Coptodon rendalli*, *Petrocephalus okavangensis-longicapitis* complex, and *Lacustricola katangae*. The least detected (<5 sites) species included *Zaireichthys kavangoensis*, an unassigned species of cichlid (Cichlid sp. 2), three species of *Enteromius* (including *E. poechii* and *E. bifrenatus*), *Labeo* sp. (either *L. lunatus* or *cylindricus*), *Cyphomyrus cubangoensis*, and *Schilbe intermedius*. There was generally strong overlap between the barcodes generated from identified fish samples, with only five OTUs (Cichlid 1, Cichlid 2, Cichlid 3, Tilapia 1 and Tilapia 2) not assigned to a specific barcode. The mtDNA 12S fragment was able to delineate all genera (except for the cichlid genera *Serranochromis* and *Pharyngochromis* that comprised a single clade) and most species, except for some in the *Clarias*, *Enteromius*, *Labeo*, *Lacustricola*, and *Petrocephalus* genera.

3.3 | Species richness and community composition

Species richness ranged between six and 31 (average = 20) per site, with the highest number of species recovered from site 1 in the Mopiri lagoon. There was no association between community composition per site and geographic distance (Mantel test, rM = 0.137, $p = 0.109$). The PCA analysis (Figure 2) revealed a separation along the first axis between sites with a species richness <17 (5–9, 11,

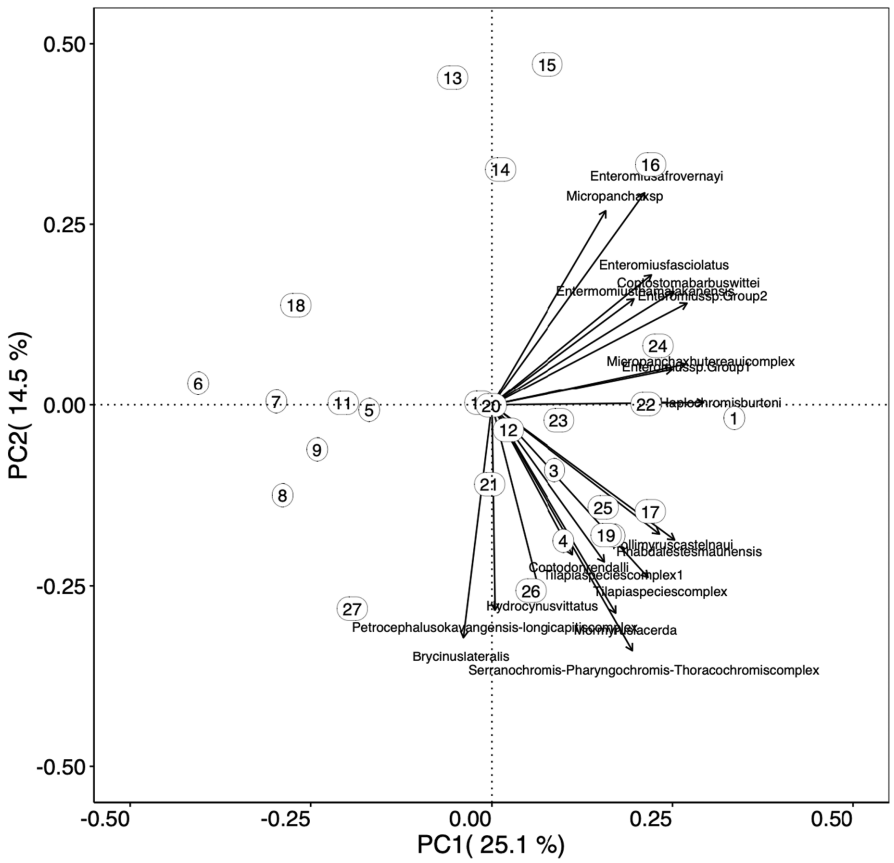

FIGURE 2 Principal Component Analysis of all 27 sampling sites showing association of sites (provided as numbers) with fish taxa, suggesting that species composition at some sites are dominated by single taxonomic groups.

18, 27) and the sites with a species richness >16. Sites with a richness >16 species, (lower right quadrant) were characterized by the presence of a diverse suite of fishes, while sites 15, 16, and 24 were linked to members of the cyprinid family. Many of the sites (sites 5, 6, 7, 8, 9, 11, and 18) did not show strong associations with particular taxonomic groups (Figure 2).

3.4 | Influence of environmental parameters on community composition and richness

The richness of fish communities was most influenced by dissolved oxygen concentration, reaching a maximum around 3 mg/L (Figure 3), and increased significantly with pH (from 10 to 25 within a pH range of 6.0 to 7.5; Figure 3). Community richness increased significantly with dissolved organic solids until reaching a plateau of 17 species around 0.05 ppt (Figure 3). The only environmental variable that statistically influenced the community composition was dissolved oxygen concentration (Mantel test, rM = 0.38, $p = 0.0029$). In addition, temperature (Mantel test, rM = 0.196, $p = 0.065$) and dissolved organic solids (Mantel test, rM = 0.227, $p = 0.0507$) were positively correlated with community diversity (Figure 3).

3.5 | Fine-scale analyses of eDNA signals

In order to provide preliminary insights into potential transport of eDNA between sites connected by strong flowing channels, we sampled eDNA from sites along a 1000 m transect and compared the fish eDNA signals for all species found at those sites. Our findings reveal no clear pattern with, for example, decreasing numbers of OTUs between sites downstream of each other, and show that the eDNA signal is site-specific, even at fine spatial scales (Figure 4). For example, some species such as *Hydrocynus vittatus* are found at the start and furthest downstream site (1000 m apart), but at no intermediate sites. In contrast, species including *Labeo* sp. or Cichlid sp. 2, were detected in the middle of, but not at the beginning or end of the transect (Figure 4).

4 | DISCUSSION

This study represents the first attempt at utilizing aquatic eDNA metabarcoding to capture community diversity of fishes in the Okavango Delta. By adopting a combined approach of generating mtDNA 12S rRNA barcodes for a large number of fish taxa with distributions in the Okavango, and sampling eDNA from 27 environmentally diverse sites in the northern section of the Delta, we detected ~50% of known Okavango fish species diversity, mostly to species level. The MiFish (Miya et al., 2015) primers were able to detect differences in eDNA signals, even at fine (50–1000 m) spatial scales and we show that environmental variation influences fish community composition. Our findings are an important step in building the foundations for biomonitoring using aquatic eDNA of the natural diversity of this iconic ecosystem, particularly in the light of ongoing climatic changes and anthropogenic pressures.

4.1 | Barcoding fish diversity of the Okavango Delta

A critical facet of successfully matching OTUs generated through eDNA metabarcoding is a reference database of identified specimens and associated barcodes (Cilleros et al., 2019; Evans & Lamberti, 2018; Gaither et al., 2022; Schenekar, 2022). However, well curated, targeted, and comprehensive reference databases with a regional focus remain rare (Gaither et al., 2022; Jerde et al., 2019; Xing et al., 2022), thus hampering the elucidation of spatio-temporal dynamics of species distributions. Our barcoding efforts resulted in a high coverage at family (14/15 families), genus (36/39), and species (74/87) level, generating the most extensive mtDNA 12S barcode dataset for the region to date. However, using the MiFish 12S primer region does not always realize full resolution to species level, particularly for members of *Enteromius* (Cyprinidae), *Petrocephalus* (Mormyridae), or *Lacustricola* (Procatopodidae). As such, from a metabarcoding perspective, species-specific primers will provide additional resolution of the distribution of these species. Finally, we could match all OTUs generated through metabarcoding to barcodes generated from identified specimens except for two species of cichlids and three of *Enteromius*. The latter in particular tend to be small bodied, with some intra-specific morphological variability, making it likely that their full diversity has not been accounted for. However, several *Enteromius* and other species favour habitats not represented in the Okavango Delta, such as rocky outcrops or rapids, such as those found at Popa in Namibia, approximately 35 km upstream above our sampling site (G. Neef, personal communication). As such, these OTUs may alternatively represent long distance eDNA transport (including through deceased individuals), dropped prey items, or predator fecal deposits. Alternative explanations also include that the *Enteromius* reads represent species missing from our barcode database, or novel species not yet described from the system and provides additional impetus to continue sampling, collection, and barcoding efforts in the region. Overall, our data also suggest that even in a well-studied system like the Okavango, it is imperative to truth data generated through metabarcoding with species lists and inventories from the region, in order to not only validate the data, but to highlight novel biodiversity not yet described.

4.2 | Environmental DNA metabarcoding detects a wide range of fish species

Metabarcoding of aquatic eDNA detected ~50% of known fish diversity from the Okavango Delta, with only three of the 15 described families not detected (Claroteidae, Mastacembelidae, and Nothobranchidae). This is not unexpected given that *Nothobranchius*

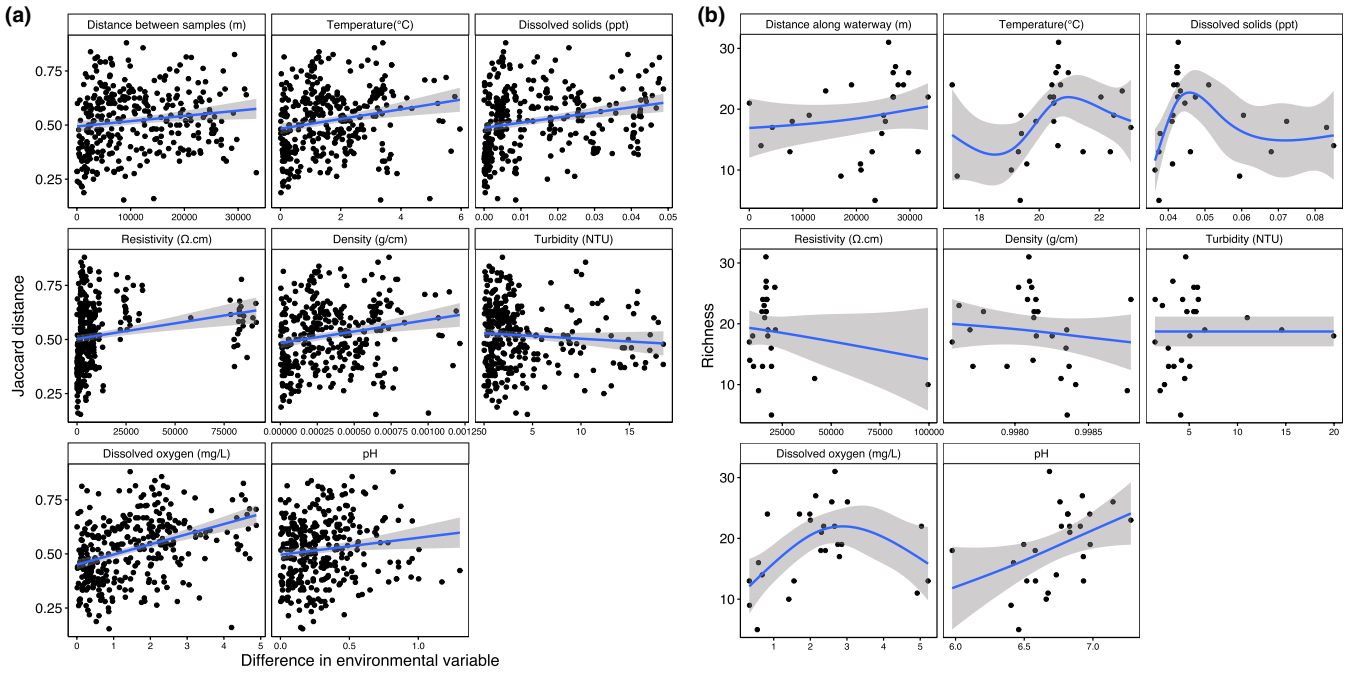

FIGURE 3 Evolution of community dissimilarity as a function of difference of difference in environmental variables (a) and richness against environmental variables (b). Environmental variables were collected in situ at the same time as filtering water samples.

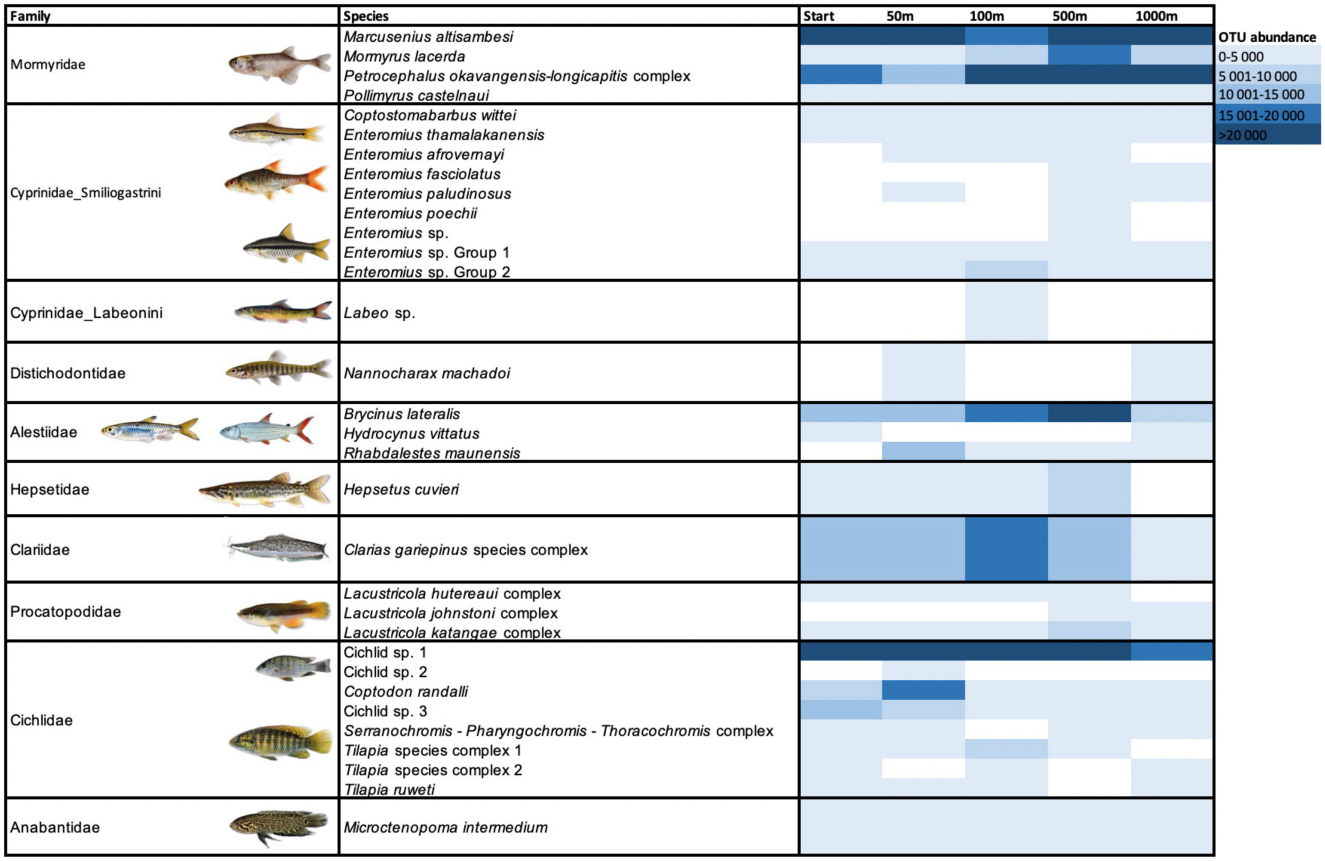

FIGURE 4 Distribution of OTUs per species along a 1000 m transect. Note the distances between sampling stations, which range from 50 to 1000 m.

do not generally occur in the area in which we sampled, with the Claroteidae and Mastacembelidae favoring more rocky areas and outcrops and both taxonomic groups are not numerous in the swamps (Bruton et al., 2018). Further, a comparison of their 12S sequences at the priming sites match completely with the MiFish primer sequences (M. Miya, personal communication), providing further evidence that their absence from our data set is likely due to biological reasons, or that their eDNA was present in such low concentrations, it would take an increased sequencing depth in order for them to be detected.

As a first study encompassing an array of habitat types (see examples in Figure 1), we sampled only a small fraction of the entire Delta yet showed a significant spread in species diversity between sites, with a maximum of 31 and a minimum of six species detected. Species prevalence varied significantly, with some, such as *Clarias* spp. and *Marcusenius altisambesi* detected across all 27 sites, with other common species detected through aquatic eDNA including *Brycinus lateralis*, *Coptodon rendalli*, and *Microctenopoma intermedium*, all of which have wide geographic distributions (Bruton et al., 2018). Interestingly, fish abundance data from Mosepele et al. (2017) highlights *Clarias* spp. and *M. altisambesi* as contributing significantly to variations in fish assemblages and being commonly encountered in samples. Other species, such as *Enteromius bifrenatus*, *E. poechii*, and *Schilbe intermedius* were rarely detected, suggesting that they may have more specific habitat requirements. One species detected, likely *Zaireichthys kavangoensis*, has not previously been sampled from the Delta, although Bruton et al. (2018) state "*…not as yet recorded from the Okavango Delta, although it may occur there*" suggesting that eDNA metabarcoding may detect even rare species and those with limited distribution ranges.

Fish assemblages in the Okavango Delta are known to vary with broader hydrological variables such as the annual flood pulse, which brings with it hypoxic conditions as flood waters move through the system (Edwards et al., 2020; Mosepele et al., 2017). Our approach of collecting environmental data alongside eDNA suggests that several environmental parameters, such as dissolved oxygen in particular, as well as pH and temperature influence both community composition and richness. Over a 10-year period, oxygen availability was shown as a strong determinant of fish community assemblages throughout the annual hydrological cycle (Mosepele et al., 2017). In particular, the annual flood pulse is strongly linked to decomposition with instances of local hypoxia reported as the flood moves through the Delta. Interestingly, although some fishes can withstand periods of hypoxic stress, they do so through changes in their physiology, with significant impacts in overall health (Edwards et al., 2020). Given potential impacts on health, fishes may well avoid low oxygen areas with increased community richness in well oxygenated habitats. However, the extent of environmental variation in shaping the distribution of fish communities across different habitat types will require a combination of ecological, as well as metabarcoding approaches. Further, climate change forced changes in temperature and other environmental variables may well reorganize functional and phylogenetic diversity of freshwater fishes (Scherer et al., 2023; Woods et al., 2023) through shifts in the distribution of species, which could profoundly impact fish community structures in the Delta.

4.3 | Signals of eDNA vary even across small spatial scales

The transport of DNA in lotic systems may disconnect the origin of eDNA to where it is collected, with potential movement of eDNA at scales of tens of kilometers (Deiner et al., 2016; Harrison et al., 2019; Jo & Yamanaka, 2022; Laporte et al., 2022; Pont et al., 2018; Seymour et al., 2018; Shogren et al., 2017). However, the impact of eDNA transport on the community composition may be limited and only a significant component close to the origin of the eDNA (Laporte et al., 2022). Notably, through fine spatial sampling, across a 1000 m transect, our results suggest that eDNA signals for fishes are heterogeneous and have a patchy rather than continuous distribution. The sample design incorporated collection of water at different intervals along a continuous channel, however, if eDNA were being continuously distributed downstream, we would have detected similar communities in adjacent sites. In contrast, even at the closest sites, only 50 m apart, we detected vastly different fish eDNA signals and numbers of OTU read proportions, suggesting that the eDNA signal is likely spatially representative of the underlying fish communities; a finding that is increasingly prevalent from lotic eDNA studies from various catchments globally (Blackman et al., 2022; Li et al., 2022; Pont et al., 2018; Valentini et al., 2016; Zhang et al., 2022). A lateral transect across the channel and adjacent well vegetated channel margins could provide the answers to such microscale variation (see for example, Momota et al., 2022), especially as the vegetated margins and open channel likely differ strongly in terms of dissolved oxygen and other environmental parameters, that we did not have the opportunity to fully characterize during this sampling series.

4.3.1 | Conclusions and recommendations for future eDNA metabarcodings efforts in the Okavango

Our work shows the potential of eDNA metabarcoding for surveying aquatic biodiversity in the Okavango Delta, particularly for setting baseline biodiversity inventories, which are crucial for conservation and management initiatives given the ongoing and increasing anthropogenic pressures in the region, which threaten to disturb the ecosystem balance of one of the world's iconic freshwater biodiversity hotspots. Even with our limited geographic coverage, our findings allow for some recommendations to consider for future and ongoing sampling efforts:

1. The eDNA signal at small spatial scales (50–1000 m) does not appear continuous, suggesting that denser, multiple sampling, including replication for each sampling event, will be required in order to adequately capture the biodiversity of an area. Temporal variability in the eDNA signal will also require further investigation.
2. Given the environmental heterogeneity associated with the annual flood pulse, that also affects community diversity (including the life history of species such as *Nothobranchius capriviensis*), further investigation into flood versus non-flood community turnover are essential.
3. In our study, we utilized only aquatic eDNA, thus providing a recent snapshot of the fish community. Given that sediment and aquatic eDNA can shed lights on biodiversity at different temporal scales (Nevers et al., 2020; Sakata et al., 2020; Turner et al., 2015) analyses of both aquatic and sediment eDNA will likely provide a more comprehensive overview of local biodiversity.
4. In our case of fishes in the Okavango, the resolution of the 12S rRNA fragment amplified by the MiFish primers was not always variable enough to distinguish between species. Species-specific primers, or additional primer sets, will help detect and identify additional taxa. Ongoing taxonomic work to identify potentially novel species will provide a more holistic overview of fish diversity and its patterns in the Delta.
5. Our proof of concept shows the power of eDNA metabarcoding for continuous assessment of the biodiversity of the Okavango Delta and that this could easily be extended to include other taxonomic groups, with regular monitoring potentially being able to detect invasive aquatic species. Given the logistical challenges of working in this region, a citizen-science based approach, that involves local communities as well as commercial lodges, could provide additional spatio-temporal coverage for detecting biodiversity in this region.

AUTHOR CONTRIBUTIONS

Sophie von der Heyden: project coordination, conceptualized study, provided research funding, laboratory work, collected data, contributed toward data analyses, wrote first draft, edited the manuscript. Götz Neef: collected data, edited the manuscript. Thomas Grevesse: conceptualized study, contributed toward data analyses, edited manuscript. Yandisa Cwecwe: laboratory work. Tetsuya Sado: laboratory work, contributed to data analyses. Masaki Miya: contributed to data analyses, edited the manuscript. Ineelo Mosie: collected data. Simon Creer: conceptualized study, collected data, edited the manuscript. Paul Skelton: edited the manuscript. Rainer von Brandis: provided research funding, edited the manuscript.

ACKNOWLEDGMENTS

We gratefully acknowledge the help from the following individuals as fieldwork support: Gobonamang Kgetho, Oreneil Yeni, and Water Setlabosha and the Ministry of Environment, Natural Resources Conservation & Tourism of Botswana, for permit EWT8/36/4/XXXVII under which samples were collected. This work was funded through the Wild Bird Trust Okavango Wilderness Project. A Royal Society-Newton Mobility grant (NI170129) awarded to SvdH and SC is gratefully acknowledged. SC would like to acknowledge support from the UK Natural Environment Research Council (NERC) Highlight Topic grant (NE/N006216/1), with SvdH thanking the National Research Foundation of South Africa (Grant UID 105842).

FUNDING INFORMATION

This work was funded through (1) The Wild Bird Trust Okavango Wilderness Project; (2) Royal Society-Newton Mobility grant (NI170129).

CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

All data are available through www.github.com/vonderheydenlab (OUT tables, sequence alignments, etc.) or https://github.com/rogotoh/PMiFish.git. Raw DNA sequence data are deposited in the DNA Databank of Japan under Accession number DRA016590.

COLLECTION PERMITS

The samples were collected under permit EWT 8/36/4 XXXVII (2)—Okavango Wilderness Project issued by the Ministry of Environment, Natural Resources Conservation and Tourism of the Republic of Botswana.

ORCID

Sophie von der Heyden https://orcid.org/0000-0001-9166-976X
Masaki Miya https://orcid.org/0000-0002-9791-9886

REFERENCES

Barnes, M. A., & Turner, C. R. (2016). The ecology of environmental DNA and implications for conservation genetics. *Conservation Genetics*, *17*(1), 1–17.

Belle, C. C., Stoeckle, B. C., & Geist, J. (2019). Taxonomic and geographical representation of freshwater environmental DNA research in aquatic conservation. *Aquatic Conservation: Marine and Freshwater Ecosystems*, *29*(11), 1996–2009.

Berry, O., Jarman, S., Bissett, A., Hope, M., Paeper, C., Bessey, C., Schwartz, M. K., Hale, J., & Bunce, M. (2021). Making environmental DNA (eDNA) biodiversity records globally accessible. *Environmental DNA*, *3*(4), 699–705.

Blackman, R. C., Ho, H.-C., Walser, J.-C., & Altermatt, F. (2022). Spatiotemporal patterns of multi-trophic biodiversity and food-web characteristics uncovered across a river catchment using environmental DNA. *Communications Biology*, *5*(1), 259.

Bruton, M., Merron, G., & Skelton, P. (2018). *Fishes of the Okavango Delta & Chobe River, Botswana* (p. 120). Struik Nature Publishers.

Callahan, B. J., McMurdie, P. J., & Holmes, S. P. (2017). Exact sequence variants should replace operational taxonomic units in marker-gene data analysis. *The ISME Journal*, *11*(12), 2639–2643.

Carraro, L., Mächler, E., Wüthrich, R., & Altermatt, F. (2020). Environmental DNA allows upscaling spatial patterns of biodiversity in freshwater ecosystems. *Nature Communications*, *11*(1), 3585.

Cilleros, K., Valentini, A., Allard, L., Dejean, T., Etienne, R., Grenouillet, G., Iribar, A., Taberlet, P., Vigouroux, R., & Brosse, S. (2019). Unlocking

biodiversity and conservation studies in high-diversity environments using environmental DNA (eDNA): A test with Guianese freshwater fishes. *Molecular Ecology Resources*, *19*(1), 27–46.

Collins, M. K., Spear, S. F., Groves, J. D., Williams, L. A., & Kuchta, S. R. (2019). Searching for a salamander: Distribution and habitat of the mudpuppy (*Necturus maculosus*) in Southeast Ohio using eDNA as a rapid assessment technique. *The American Midland Naturalist*, *182*(2), 191–202.

Czachur, M. V., Seymour, M., Creer, S., & Heyden, S. (2022). Novel insights into marine fish biodiversity across a pronounced environmental gradient using replicated environmental DNA analyses. *Environmental DNA*, *4*(1), 181–190.

Daly, M. C., Green, P., Watts, A. B., Davies, O., Chibesakunda, F., & Walker, R. (2020). Tectonics and landscape of the central African plateau and their implications for a propagating southwestern rift in Africa. *Geochemistry, Geophysics, Geosystems*, *21*(6), e2019GC008746.

de Santana, C. D., Parenti, L. R., Dillman, C. B., Coddington, J. A., Bastos, D. A., Baldwin, C. C., Zuanon, J., Torrente-Vilara, G., Covain, R., Menezes, N. A., Datovo, A., Sado, T., & Miya, M. (2021). The critical role of natural history museums in advancing eDNA for biodiversity studies: A case study with Amazonian fishes. *Scientific Reports*, *11*(1), 18159.

Deiner, K., Bik, H. M., Mächler, E., Seymour, M., Lacoursière-Roussel, A., Altermatt, F., Creer, S., Bista, I., Lodge, D. M., Vere, N., Pfrender, M. E., & Bernatchez, L. (2017). Environmental DNA metabarcoding: Transforming how we survey animal and plant communities. *Molecular Ecology*, *26*(21), 5872–5895.

Deiner, K., Fronhofer, E. A., Mächler, E., Walser, J.-C., & Altermatt, F. (2016). Environmental DNA reveals that rivers are conveyer belts of biodiversity information. *Nature Communications*, *7*(1), 12544.

Dodds, W. K., Perkin, J. S., & Gerken, J. E. (2013). Human impact on freshwater ecosystem services: A global perspective. *Environmental Science & Technology*, *47*(16), 9061–9068.

Dudgeon, D., Arthington, A. H., Gessner, M. O., Kawabata, Z.-I., Knowler, D. J., Lévêque, C., Naiman, R. J., Prieur-Richard, A.-H., Soto, D., Stiassny, M. L. J., & Sullivan, C. A. (2006). Freshwater biodiversity: Importance, threats, status and conservation challenges. *Biological Reviews*, *81*(2), 163–182.

Edgar, R. (2010). *USEARCH*. Lawrence Berkeley National Lab.

Edwards, T. M., Mosie, I. J., Moore, B. C., Lobjoit, G., Schiavone, K., Bachman, R. E., & Murray-Hudson, M. (2020). Low oxygen: A (tough) way of life for Okavango fishes. *PLoS One*, *15*(7), e0235667.

Evans, N. T., & Lamberti, G. A. (2018). Freshwater fisheries assessment using environmental DNA: A primer on the method, its potential, and shortcomings as a conservation tool. *Fisheries Research*, *197*, 60–66.

Gaither, M. R., DiBattista, J. D., Leray, M., & Heyden, S. (2022). Metabarcoding the marine environment: From single species to biogeographic patterns. *Environmental DNA*, *4*(1), 3–8.

Gilbey, J., Carvalho, G., Castilho, R., Coscia, I., Coulson, M. W., Dahle, G., Derycke, S., Francisco, S. M., Helyar, S. J., Johansen, T., Junge, C., Layton, K. K. S., Martinsohn, J., Matejusova, I., Robalo, J. I., Rodríguez-Ezpeleta, N., Silva, G., Strammer, I., Vasemägi, A., & Volckaert, F. A. M. (2021). Life in a drop: Sampling environmental DNA for marine fishery management and ecosystem monitoring. *Marine Policy*, *124*, 104331.

Hallam, J., Clare, E. L., Jones, J. I., & Day, J. J. (2021). Biodiversity assessment across a dynamic riverine system: A comparison of eDNA metabarcoding versus traditional fish surveying methods. *Environmental DNA*, *3*(6), 1247–1266.

Harrison, J. B., Sunday, J. M., & Rogers, S. M. (2019). Predicting the fate of eDNA in the environment and implications for studying biodiversity. *Proceedings of the Royal Society B: Biological Sciences*, *286*(1915), 20191409.

Jane, S. F., Wilcox, T. M., McKelvey, K. S., Young, M. K., Schwartz, M. K., Lowe, W. H., Letcher, B. H., & Whiteley, A. R. (2015). Distance, flow and PCR inhibition: EDNA dynamics in two headwater streams. *Molecular Ecology Resources*, *15*(1), 216–227.

Jerde, C. L., Wilson, E. A., & Dressler, T. L. (2019). Measuring global fish species richness with eDNA metabarcoding. *Molecular Ecology Resources*, *19*(1), 19–22.

Jo, T., & Yamanaka, H. (2022). Meta-analyses of environmental DNA downstream transport and deposition in relation to hydrogeography in riverine environments. *Freshwater Biology*, *67*(8), 1333–1343.

Katoh, K., & Standley, D. M. (2013). MAFFT multiple sequence alignment software version 7: Improvements in performance and usability. *Molecular Biology and Evolution*, *30*(4), 772–780.

Kimura, M. (1980). A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *Journal of Molecular Evolution*, *16*(2), 111–120.

King, J., & Chonguiça, E. (2016). Integrated management of the Cubango-Okavango River basin. *Ecohydrology & Hydrobiology*, *16*(4), 263–271.

Kumar, S., Stecher, G., & Tamura, K. (2016). MEGA7: Molecular evolutionary genetics analysis version 7.0 for bigger datasets. *Molecular Biology and Evolution*, *33*(7), 1870–1874.

Laporte, M., Berger, C. S., García-Machado, E., Côté, G., Morissette, O., & Bernatchez, L. (2022). Cage transplant experiment shows weak transport effect on relative abundance of fish community composition as revealed by eDNA metabarcoding. *Ecological Indicators*, *137*, 108785.

Leese, F., Sander, M., Buchner, D., Elbrecht, V., Haase, P., & Zizka, V. M. A. (2021). Improved freshwater macroinvertebrate detection from environmental DNA through minimized nontarget amplification. *Environmental DNA*, *3*(1), 261–276.

Li, H., Yang, F., Zhang, R., Liu, S., Yang, Z., Lin, L., & Ye, S. (2022). Environmental DNA metabarcoding of fish communities in a small hydropower dam reservoir: A comparison between the eDNA approach and established fishing methods. *Journal of Freshwater Ecology*, *37*(1), 341–362.

Lin, J., Huang, J., Prell, C., & Bryan, B. A. (2021). Changes in supply and demand mediate the effects of land-use change on freshwater ecosystem services flows. *Science of the Total Environment*, *763*, 143012.

Mauviesseau, Q., Harper, L. R., Sander, M., Hanner, R. H., Kleyer, H., & Deiner, K. (2022). The multiple states of environmental DNA and what is known about their persistence in aquatic environments. *Environmental Science and Technology*, *56*, 5322–5333.

McCarthy, T. S. (2013). The Okavango Delta and its place in the geomorphological evolution of southern Africa. *South African Journal of Geology*, *116*(1), 1–54.

Mendelsohn, J., & Martins, A. (2018). *River catchments and development prospects in South-Eastern Angola* (p. 106). https://www.researchgate.net/profile/John-Mendelsohn/publication/341652220_South-eastern_Angola_-_A_synthesis_of_information_RAISON_2019/links/5ecd44734585152945144c0e/South-eastern-Angola-A-synthesis-of-information-RAISON-2019.pdf

Mendelsohn, J., van der Post, C., Ramberg, L., Murray-Hudson, M., Wolski, P., & Mosepele, K. (2010). *Okavango Delta: Flood of life*. RAISON.

Miya, M. (2022). Environmental DNA metabarcoding: A novel method for biodiversity monitoring of marine fish communities. *Annual Review of Marine Science*, *14*(1), 161–185.

Miya, M., Gotoh, R. O., & Sado, T. (2020). MiFish metabarcoding: A high-throughput approach for simultaneous detection of multiple fish species from environmental DNA and other samples. *Fisheries Science*, *86*(6), 939–970.

Miya, M., Sato, Y., Fukunaga, T., Sado, T., Poulsen, J. Y., Sato, K., Minamoto, T., Yamamoto, S., Yamanaka, H., Araki, H., Kondoh, M., & Iwasaki, W. (2015). MiFish, a set of universal PCR primers for metabarcoding environmental DNA from fishes: Detection of more than 230 subtropical marine species. *Royal Society Open Science*, *2*(7), 150088.

Mmopelwa, G., Mosepele, K., Mosepele, B., Moleele, N., & Ngwenya, B. (2009). Environmental variability and the fishery dynamics of the Okavango delta, Botswana: The case of subsistence fishing. *African Journal of Ecology*, *47*, 119–127.

Momota, K., Hosokawa, S., & Komuro, T. (2022). Small-scale heterogeneity of fish diversity evaluated by environmental DNA analysis in eelgrass beds. *Marine Ecology Progress Series*, *688*, 99–112.

Mosepele, K., & Kolding, J. (2003). Fish stock assessment in the Okavango delta, Botswana—Preliminary results from a length based analysis. In *Environmental monitoring of tropical and subtropical wetlands*. Okavango report (pp. 363–390). University of Botswana, Maun and University of Florida.

Mosepele, K., Kolding, J., & Bokhutlo, T. (2017). Fish community dynamics in an inland floodplain system of the Okavango Delta, Botswana. *Ecohydrology & Hydrobiology*, *17*(2), 89–102.

Murray-Hudson, M., Combs, F., Wolski, P., & Brown, M. T. (2011). A vegetation-based hierarchical classification for seasonally pulsed floodplains in the Okavango Delta, Botswana. *African Journal of Aquatic Science*, *36*(3), 223–234.

Nevers, M. B., Przybyla-Kelly, K., Shively, D., Morris, C. C., Dickey, J., & Byappanahalli, M. N. (2020). Influence of sediment and stream transport on detecting a source of environmental DNA. *PLoS One*, *15*(12), e0244086.

Pont, D., Rocle, M., Valentini, A., Civade, R., Jean, P., Maire, A., Roset, N., Schabuss, M., Zornig, H., & Dejean, T. (2018). Environmental DNA reveals quantitative patterns of fish biodiversity in large rivers despite its downstream transportation. *Scientific Reports*, *8*(1), 10361.

Reid, A. J., Carlson, A. K., Creed, I. F., Eliason, E. J., Gell, P. A., Johnson, P. T. J., Kidd, K. A., MacCormack, T. J., Olden, J. D., Ormerod, S. J., Smol, J. P., Taylor, W. W., Tockner, K., Vermaire, J. C., Dudgeon, D., & Cooke, S. J. (2019). Emerging threats and persistent conservation challenges for freshwater biodiversity. *Biological Reviews*, *94*(3), 849–873.

Rourke, M. L., Fowler, A. M., Hughes, J. M., Broadhurst, M. K., DiBattista, J. D., Fielder, S., Wilkes Walburn, J., & Furlan, E. M. (2022). Environmental DNA (eDNA) as a tool for assessing fish biomass: A review of approaches and future considerations for resource surveys. *Environmental DNA*, *4*(1), 9–33.

Ruppert, K. M., Kline, R. J., & Rahman, M. S. (2019). Past, present, and future perspectives of environmental DNA (eDNA) metabarcoding: A systematic review in methods, monitoring, and applications of global eDNA. *Global Ecology and Conservation*, *17*, e00547.

Sakata, M. K., Yamamoto, S., Gotoh, R. O., Miya, M., Yamanaka, H., & Minamoto, T. (2020). Sedimentary eDNA provides different information on timescale and fish species composition compared with aqueous eDNA. *Environmental DNA*, *2*(4), 505–518.

Schenekar, T. (2022). The current state of eDNA research in freshwater ecosystems: Are we shifting from the developmental phase to standard application in biomonitoring? *Hydrobiologia*, *850*, 1263–1282.

Scherer, L., Boom, H. A., Barbarossa, V., & Van Bodegom, P. M. (2023). Climate change threats to the global functional diversity of freshwater fish. *Global Change Biology*, *29*(13), 3781–3793.

Seymour, M., Durance, I., Cosby, B. J., Ransom-Jones, E., Deiner, K., Ormerod, S. J., Colbourne, J. K., Wilgar, G., Carvalho, G. R., de Bruyn, M., Edwards, F., Emmett, B. A., Bik, H. M., & Creer, S. (2018). Acidity promotes degradation of multi-species environmental DNA in lotic mesocosms. *Communications Biology*, *1*(1), 4.

Shogren, A. J., Tank, J. L., Andruszkiewicz, E., Olds, B., Mahon, A. R., Jerde, C. L., & Bolster, D. (2017). Controls on eDNA movement in streams: Transport, retention, and resuspension. *Scientific Reports*, *7*, 5065.

Skelton, P. (2019). The freshwater fishes of Angola. In B. J. Huntley, V. Russo, F. Lages, & N. Ferrand (Eds.), *Biodiversity of Angola: Science & conservation: A modern synthesis* (pp. 207–242). Springer International Publishing.

Strickler, K. M., Fremier, A. K., & Goldberg, C. S. (2015). Quantifying effects of UV-B, temperature and pH on eDNA degradation in aquatic microcosms. *Biological Conservation*, *183*, 85–92.

Takahashi, M., Saccò, M., Kestel, J. H., Nester, G., Campbell, M. A., Van Der Heyde, M., Heydenrych, M. J., Juszkiewicz, D. J., Nevill, P., Dawkins, K. L., Bessey, C., Fernandes, K., Miller, H., Power, M., Mousavi-Derazmahalleh, M., Newton, J. P., White, N. E., Richards, Z. T., & Allentoft, M. E. (2023). Aquatic environmental DNA: A review of the macro-organismal biomonitoring revolution. *Science of the Total Environment*, *873*, 162322.

Tsuji, S., Ushio, M., Sakurai, S., Minamoto, T., & Yamanaka, H. (2017). Water temperature-dependent degradation of environmental DNA and its relation to bacterial abundance. *PLoS One*, *12*, e0176608.

Turner, C. R., Uy, K. L., & Everhart, R. C. (2015). Fish environmental DNA is more concentrated in aquatic sediments than surface water. *Biological Conservation*, *183*, 93–102.

Tweddle, D., & Hay, C. J. (2013). *A transboundary fisheries management plan for the Okavango/Kavango/Cubango Basin*.

Valentini, A., Taberlet, P., Miaud, C., Civade, R., Herder, J., Thomsen, P. F., Bellemain, E., Besnard, A., Coissac, E., Boyer, F., Gaboriaud, C., Jean, P., Poulet, N., Roset, N., Copp, G. H., Geniez, P., Pont, D., Argillier, C., Baudoin, J. M., … Dejean, T. (2016). Next-generation monitoring of aquatic biodiversity using environmental DNA metabarcoding. *Molecular Ecology*, *25*(4), 929–942. https://doi.org/10.1111/mec.13428

van Wilgen, B. W., de Klerk, H. M., Stellmes, M., & Archibald, S. (2022). An analysis of the recent fire regimes in the Angolan catchment of the Okavango Delta, Central Africa. *Fire Ecology*, *18*, 13.

von der Heyden, S. (2023). Environmental DNA surveys of African biodiversity: State of knowledge, challenges, and opportunities. *Environmental DNA*, *5*(1), 12–17.

Wood, Z. T., Lacoursière-Roussel, A., LeBlanc, F., Trudel, M., Kinnison, M. T., Garry McBrine, C., Pavey, S. A., & Gagné, N. (2021). Spatial heterogeneity of eDNA transport improves stream assessment of threatened Salmon presence, abundance, and location. *Frontiers in Ecology and Evolution*, *9*, 650717.

Woods, T., Freeman, M. C., Krause, K. P., & Maloney, K. O. (2023). Observed and projected functional reorganization of riverine fish assemblages from global change. *Global Change Biology*, *29*(13), 3759–3780.

Xing, Y., Gao, W., Shen, Z., Zhang, Y., Bai, J., Cai, X., Ouyang, J., & Zhao, Y. (2022). A review of environmental DNA field and laboratory protocols applied in fish ecology and environmental health. *Frontiers in Environmental Science*, *10*, 725360.

Zhang, S., Zheng, Y., Zhan, A., Dong, C., Zhao, J., & Yao, M. (2022). Environmental DNA captures native and non-native fish community variations across the lentic and lotic systems of a megacity. *Science Advances*, *8*, eabk0097.

How to cite this article: von der Heyden, S., Neef, G., Grevesse, T., Cwecwe, Y., Sado, T., Miya, M., Mosie, I., Creer, S., Skelton, P., & von Brandis, R. (2023). Environmental DNA biomonitoring in biodiversity hotspots: A case study of fishes of the Okavango Delta. *Environmental DNA*, *5*, 1720–1731. https://doi.org/10.1002/edn3.494