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Hydrological Connectivity Enhances Fish Biodiversity in Amazonian Mining Ponds: Insights From eDNA and Traditional Sampling

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ABSTRACT

Artisanal and small-scale gold mining (ASGM) expansion in the Madre de Dios region of the Peruvian Amazon has transformed primary forests into a novel wetland complex of thousands of abandoned mining ponds. Despite their ecological relevance, post-mining recovery of these systems remains understudied, particularly regarding fish biodiversity and recolonisation. In this study, we evaluate fish community richness and composition in mining ponds of different dimensions, years post abandonment, physicochemical properties and degree of pulse flood connectivity using traditional collection-based methods and environmental DNA (eDNA) with the 12S and COI markers. We compared these two methods of biodiversity inventory and contrasted results from ASGM waterbodies with those obtained from nearby pristine oxbow lakes. Overall, we registered more fish richness at all sites using eDNA versus traditional methods, especially with the 12S marker. We identified 14 and 13 unique genera using traditional methods and eDNA, respectively, with 40 genera detected by both approaches, evidencing their complementarity. Notably, we found that the degree of pulse flooding connectivity was the main predictor of species richness among the abandoned mining ponds (p-value <0.05). We registered 11–22, 23–71 and 56 morphospecies in non-flooded mining ponds, pulse flooded mining ponds and nearby oxbow lakes, respectively. Furthermore, the fish community composition of mining ponds most influenced by pulse flooding was similar to that of pristine lakes. Our findings highlight the role of hydrological connectivity in ecological recovery within mining-impacted wetlands. Future restoration efforts should enhance aquatic connectivity to accelerate recovery in post-mining environments.

1 | Introduction

The Amazon basin is recognised for its exceptional biological diversity and indigenous communities, standing as one of the most vital and ecologically significant regions on Earth (Cassemiro et al. 2023; Heilpern et al. 2022). Notwithstanding its ecological relevance, artisanal and small-scale gold mining (ASGM) is occurring in many regions within the Amazon, severely affecting the environment as well as the economy, public health and contributing to local and transnational crime (Dethier, Silman, Leiva, et al. 2023; Esdaile and Chalker 2018; Gasparinnetti et al. 2024; Keane et al. 2023). ASGM, which typically occurs along rivers and primary forests, is a significant driver of the extensive and ongoing deforestation within the Amazon basin, increases in sediment loads in rivers, water siltation, mercury pollution (used for gold amalgamation) and the alteration of hydro-geological processes, among other negative effects (Cheng et al. 2023; Dethier, Silman, Leiva, et al. 2023; Swenson et al. 2011; Veiga et al. 2006).

Within the Amazon basin, the Madre de Dios Region in Perú is a biodiversity hotspot that has been particularly impacted by ASGM activities (Asner and Tupayachi 2016; Barocas et al. 2023; Caballero et al. 2018; Dethier et al. 2019; Diringer et al. 2020; Paiva et al. 2023). Mining activities in Madre de Dios have resulted in more than 120,000 ha of primary forest cleared and transformed into an artificial wetland system and bare or naturally revegetating land (Caballero et al. 2018, 2020; Camalan et al. 2022; Dethier, Silman, Leiva, et al. 2023) where, due to extensive soil degradation and uncertain land tenure, reforestation efforts have been difficult to deploy (Román-Dañobeytia et al. 2021; Garate-Quispe et al. 2021; Herrera-Machaca et al. 2024). The Madre de Dios post-mining landscape presents thousands of small mining pits or ponds ranging from 0.1 to 28.8 ha in size, created to extract alluvial deposits of gold (Alvarez-Berríos and Aide 2015; Asner and Tupayachi 2016; Caballero et al. 2020). These ponds are frequently left abandoned and represent a significant risk due to their potential for mercury methylation (Gerson et al. 2020, 2021). Aquatic communities have been able to recolonise these new artificial ecosystems, facilitated by their connectivity with rivers and streams during the rainy season (Araújo-Flores et al. 2021). Annual river flood pulses during the rainy season serve as a main driver of species accumulation in a river floodplain (Baumgartner et al. 2018; Melack and Coe 2021; Pereira et al. 2017) by providing them with nutrients and individuals (Araújo-Flores et al. 2021; Fernandes et al. 2014; Strahler 1952). In addition, isolated mining ponds may support aquatic communities through fish introductions by birds, human stocking or occasional climate-driven connections (Cooke et al. 2022; Green et al. 2023). In any case, post-mining recovery and aquatic communities' recolonisation of these systems remain understudied, particularly concerning fish biodiversity.

To improve restoration and management strategies in these aquatic environments, extensive and continuous monitoring is necessary to assess their dynamics and behaviour over time and space (Deiner et al. 2017; Kelly et al. 2014; Magurran et al. 2010). Traditional methods, based on species collection through nets and taxonomic identification using morphological keys, present limitations such as cost, time, labour and the need for taxonomic reference collections that are sparse or nonexistent (Blattner et al. 2021; Cristescu and Hebert 2018; Evans et al. 2017; van der Sleen and Albert 2017; Wilcox et al. 2013). Alternatively, environmental DNA (eDNA), which refers to the detection of genetic material left by organisms in the sampled environment (Deiner et al. 2017) has proven to be a complementary method in wildlife research in general (Keck et al. 2022; Klymus et al. 2017; Munian et al. 2024; Ruppert et al. 2019; Takahashi et al. 2023; Veron et al. 2023), and in fish communities in the Neotropics in particular (Cilleros et al. 2019; Coutant et al. 2023; de Santana et al. 2021; Jackman et al. 2021; Mariac et al. 2022; Sales et al. 2021), including in this post-mining environment (Timana-Mendoza et al. 2024). Particularly, eDNA has the potential to be a powerful tool for gathering aquatic biodiversity data across extensive aquatic landscapes that are difficult to access and survey (Carvalho et al. 2022; Harper et al. 2019; Takahashi et al. 2023), though its ability to capture total aquatic biodiversity, or even total fish biodiversity, remains to be tested, as does the simple congruence between eDNA-based and collections-based surveys.

In this study, we assessed the richness and composition of fish communities in mining ponds of varied surface areas and years post abandonment, affected by various degrees of seasonal flooding, within the Madre de Dios Region in Southern Peru. Our aim was to explore what the main factors impacting the recolonisation dynamics in these novel ecosystems. We used both eDNA and traditional biological monitoring methods simultaneously and looked into the taxonomic resolution of two DNA markers commonly used for eDNA analysis of ichthyofauna. We included nearby unmined oxbow lakes in the study to serve as local references for richness and community composition.

2 | Materials and Methods

2.1 | Study Area

We evaluated nine abandoned mining ponds and two nearby unmined oxbow lakes within the Madre de Dios River basin, located in the Madre de Dios region, at the headwaters of the tropical Amazon and the foothills of the Andes (11°37′46.60″ S, 70°32′25.84″ O) in southeastern Peru (Figure 1). In this area, annual precipitation varies from 1700 to 3300 mm per year, depending on proximity to the Andes (Torre-Marin Rando et al. 2021). Intense local precipitation and poor drainage result in seasonally recurrent short-duration fluvial floods (often < 1–2 weeks), generating floodplains between December and April. These changing conditions contribute to the Madre de Dios River basin's unique ecological importance related to its high fish diversity (ca. 700 sp.) and for fish spawning migrations within the Amazon system (Araújo-Flores et al. 2021).

Among the mining ponds surveyed, five belong to a floodplain ecosystem, and four to a recently deforested *terra firme* or non-flooded ecosystem (Figure 1). The mining ponds within the floodplain ecosystem (flooded [abandoned mining] ponds, FP) are influenced by the nearby rivers 'Madre de Dios' and 'Malinowski' through rainy season flooding (see Figure 1 and

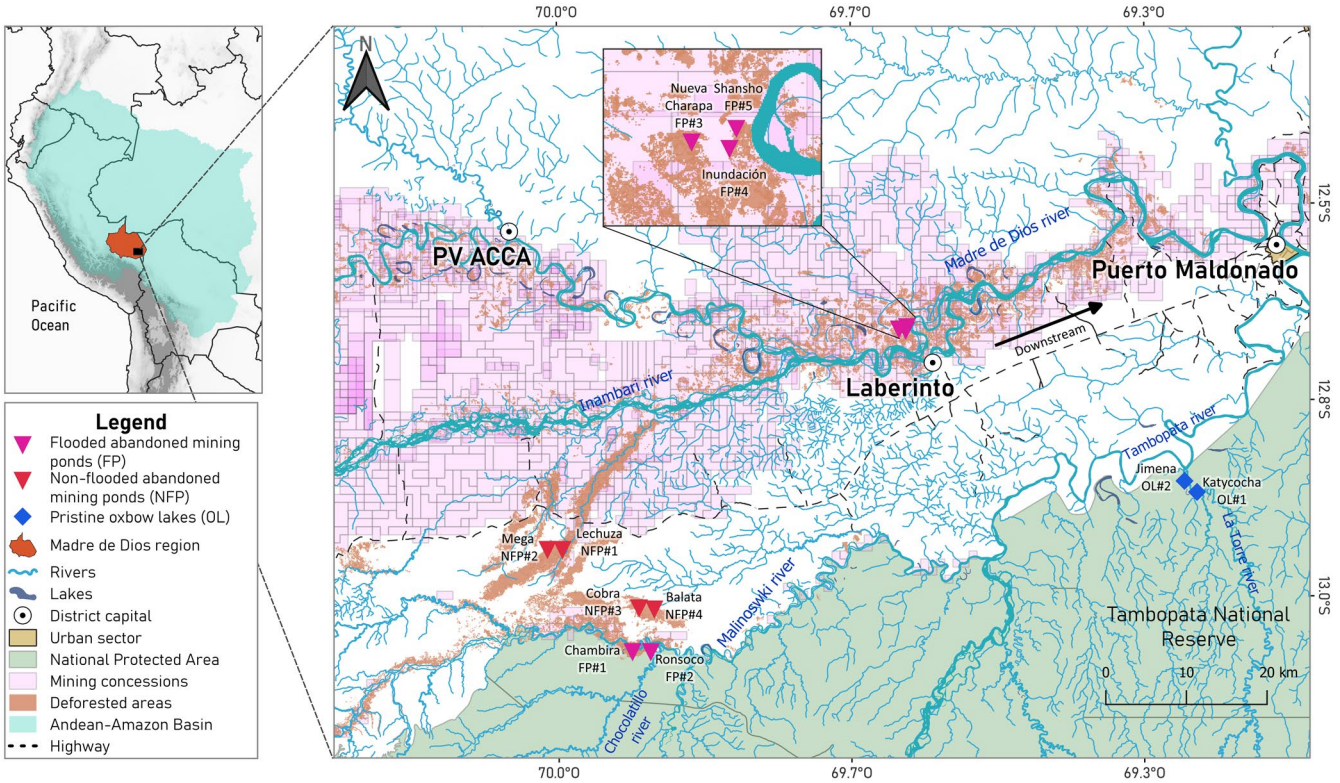

FIGURE 1 | Study area within the Madre de Dios River basin showing the 11 lentic systems surveyed in the study: Five flooded abandoned mining ponds (FP), four non-flooded abandoned mining ponds (NFP) and two pristine oxbow lakes (OL).

TABLE 1 | Main characteristics of the abandoned mining ponds and unmined oxbow lakes surveyed in the study.

Site name	Code	Coordinates		Influencing lotic system	Abandonment year	Elevation (m.a.s.l.)	Area (ha)	Depth (m)
		East	North					
Lechuza	NFP#1	12°55′36.4″ S	70°0′37.7″ W	Not influenced	2011	254	4.11	5.63
Mega	NFP#2	12°55′36.1″ S	70°1′38.9″ W	Not influenced	2011	257	5.30	0.80
Cobra	NFP#3	12°59′33.4″ S	69°55′23.3″ W	Stream	2017	248	1.53	1.50
Balata	NFP#4	12°59′37.9″ S	69°54′20.4″ W	Stream	2017	246	2.41	1.60
Chambira	FP#1	13°2′29.8″ S	69°55′50.5″ W	Malinoskwi River	2016	248	2.32	2.00
Ronsoco	FP#2	13°2′30.1″ S	69°54′35.6″ W	Malinoskwi River	2012	243	1.92	2.27
Nueva Charapa	FP#3	12°40′51.8″ S	69°37′30.8″ W	Madre de Dios River	2017	220	0.12	1.13
Inundación	FP#4	12°40′56.6″ S	69°37′2.9″ W	Madre de Dios River	2014	208	0.56	1.80
Shansho	FP#5	12°40′42.1″ S	69°36′57.8″ W	Madre de Dios River	2009	215	0.28	1.00
Katycocha	OL#1	12°51′49.1″ S	69°17′5.7″ W	La Torre River	NA	203	5.09	1.70
Jimena	OL#2	12°51′4.4″ S	69°17′57.2″ W	La Torre River	NA	198	1.04	1.77

Abbreviations: FP, flooded (abandoned mining) ponds; NFP, non-flooded (abandoned mining) ponds; OL, (pristine) Oxbow lakes.

Table 1). In contrast, the abandoned mining ponds within the *terra firme* (non-flooded abandoned mining ponds, NFP) are located inside the mining area known as 'La Pampa', south of the Interoceanic Highway (12°59′26.88″ S, 69°56′36.42″ O) and are either not influenced by any streams or slightly influenced by small and oligotrophic Amazonian blackwater streams tightly

linked with local rainfall regimes (see Figure 1 and Table 1). Flood zones were delimited using ArcGIS Pro 2.8.7 and a Digital Elevation Model (DEM). Elevation values ('Z') and geomorphological criteria were analysed to identify flood levels in the two floodplain ecosystem study sites: 243–248 m.a.s.l. in the FP#1 and FP#2 within the 'PVC Azul' area, and 215–220 m.a.s.l. in the FP#3, FP#4 and FP#5 within the 'Laberinto' area (Figure 1). These results were validated with high-resolution PlanetScope images (3 m) captured during the seasonal flooding peak, in February. Additionally, the abandonment date was estimated through a multitemporal analysis of Landsat 8 satellite images using supervised classification algorithms to determine the last documented mining impact in each area. This approach enabled tracking land cover disturbances and establishing abandonment periods up to the evaluation date. The two nearby unmined oxbow lakes (OL)—used as reference—are located within the Tambopata National Reserve (see Figure 1 and Table 1). All these lentic systems were sampled during the dry season transition from May to June 2022 to avoid recent contributions from lotic systems.

2.2 | eDNA Sampling and Purification

We collected water samples for eDNA analysis at each site as follows: We took three to five 4 L water samples across the mining pond or oxbow lake using a clean 4 L plastic bucket and a small boat. These 4 L water samples were pooled into a clean 20 L plastic bucket to make a single composite sample. Two such composite samples were generated for each site surveyed. Both the 4 L and the 20 L buckets used to collect environmental samples were pre-cleaned by washing them with commercial powder detergent, rinsed thoroughly with tap water, then treated by submerging them in 1% v/v chlorine bleach solution for at least 1 h and then rinsed thoroughly with bottled drinking water. Buckets were rinsed with the pond or lake's water at least three times before collecting the water samples used for the analysis. From each 20 L composite sample, we took a 1 L water subsample with a clean 1 L high-density polyethylene (HDPE) plastic bottle. These bottles were previously cleaned by washing them with commercial detergent, thoroughly rinsed with tap water, submerged in 1% v/v chlorine bleach solution for at least 1 h, rinsed thoroughly with distilled water (Direct-Q 3 UV, Merck Millipore, Massachusetts, United States), let dry in a UV-irradiated bench and finally irradiated for 30 min with UV-C light. They were packed in a clean cabinet to be opened only in the field for sample collection. We obtained two 1 L subsamples per site (one from each 20 L composite sample) to make a total of 22 environmental water samples from the 11 sites surveyed. The 1 L plastic bottles were then stored in a cooler at < 8°C and transported to the field station, where they were immediately filtered (within 6–12 h after sample collection). We filtered 400–950 mL from these 1 L subsamples (see Table S1) through a glass fibre membrane of 1.2 μm pore size (Merck Millipore, Massachusetts, United States) using a MF5Pro Magnetic Filter Holder and Funnel (Rocker Scientific Co., Taiwan) with a vacuum pump (KNF Group, Freiburg, Germany), until the filter was clogged. We then passed 5 mL pure ethanol (Sigma-Aldrich, Missouri, United States) through the filters and stored moist filters in 5 mL centrifuge tubes (Eppendorf, Hamburg, Germany). A field station control was obtained by filtering bottled drinking water alongside the environmental water samples, to assay for possible environmental DNA contamination in the field station.

We obtained total environmental DNA using the NucleoSpin eDNA Water Kit (Macherey-Nagel, Düren, Germany), following manufacturer guidelines without modifications. We eluted total DNA in 100 μL Elution Buffer, provided in the kit. We performed eDNA extractions in a DNA extraction chamber dedicated to eDNA work. Before each extraction batch, we carefully wiped the bench surface, pipettes, scissors, tweezers and all other equipment using DNA/RNA-ExitusPlus (PanReac AppliChem) followed by ultraviolet exposure for 30 min. We used filtered tips for all steps involving pipetting. We included DNA extraction blanks, consisting of ultra-pure water (Direct-Q 3 UV, Merck Millipore) processed alongside the samples, as laboratory negative controls. Total DNA samples were stored at −20°C until further processing.

Complementary to water sampling for eDNA analysis, we assessed water quality in the surface layer of the ponds or lakes (Table S1) by collecting at least three measurements from the central area of each pond. A multiparameter probe HI-98129 (Hanna Instruments, Woonsocket, RI, USA) was used to measure pH, conductivity (μS/cm) and temperature (°C). Transparency was calculated as the percentage ratio of Secchi depth to total depth, and dissolved oxygen (DO) was determined using a colorimetric Dissolved Oxygen Test Kit (LaMotte Company, Chestertown, MD, USA), as described by Araújo-Flores et al. (2021).

2.3 | Library Preparation and Sequencing

We prepared eDNA libraries targeting regions within the mitochondrial cytochrome oxidase I gene (COI) and the mitochondrial 12S ribosomal RNA (12S rRNA) gene, using the Illumina MiSeq dual-barcoded two-step PCR amplicon sequencing protocol, following guidelines described in the Illumina 16S Metagenomic Sequencing Library Preparation Manual (Illumina Technology 2013), with the following modifications: The COI region was PCR-amplified using the primer set MK1-F1 5′-TCG TCGGCAGCGTCAGATGTGTATAAGAGACAG-TCHACHAA YCAYAAAGAYATYGGYACYCT-3′ and MK1- R1 5′-GTCTCG TGGGCTCGGAGATGTGTATAAGAGACAG-ACYATRAARA ARATYATYACRAADGC-3′ described in Mariac et al. (2022), which yields 185 bp fragments (including primers). Four PCR reaction replicates were set per eDNA sample in 11 μL total reaction volume (Galan et al. 2018). PCR reactions contained 1× DreamTaq Green PCR Master Mix (ThermoFisher Scientific, Waltham, Massachusetts, USA), 0.4 μM of each primer, 0.8 mg/mL BSA, 3 mM $MgCl_2$ and 1 μL template DNA. Amplification parameters included an initial denaturation at 95°C for 3 min, followed by 40 cycles of 95°C for 30 s, 51°C for 30 s and 72°C for 1 min and a final extension step of 72°C for 5 min. The 12S region was PCR-amplified using the primer set 12S-V5-F1 5′-TC GTCGGCAGCGTCAGATGTGTATAAGAGACAG-ACTGGG ATTAGATACCCC-3′ and 12S-V5-R1 5′-GTCTCGTGGGCTC GGAGATGTGTATAAGAGACAG-TAGAACAGGCTCCTCTA G-3′ described in Kelly et al. (2014) (Riaz et al. 2011 in Kelly et al. 2014). Twelve PCR reaction replicates were set for each eDNA sample, in 8 μL total reaction volume (Timana-Mendoza

et al. 2024). PCR reactions contained 1× DreamTaq Green PCR Master Mix, 0.4 μM of each primer, 0.8 mg/mL BSA, 1.5 mM $MgCl_2$, 3% DMSO and 0.9 μL template DNA. Amplification parameters included an initial denaturation at 95°C for 15 s, followed by 45 cycles of 95°C for 15 s, 57°C for 30 s, 72°C for 30 s and a final extension step of 72°C for 5 min (Timana-Mendoza et al. 2024). In both cases, PCR reaction replicates per eDNA sample were pooled and verified by agarose gel electrophoresis using 5 μL of pooled PCR product. Pooled amplification products were purified using sample purification beads (SPB, Illumina, San Diego, California, USA). Index PCR reactions were carried out using 1× DreamTaq Green PCR Master Mix, 2 μL of clean amplicon PCR product as a template and indexes from the Nextera XT Index Kit v2 (Illumina) in a final volume of 20 μL. Index PCR amplification parameters included an initial denaturation at 95°C for 3 min followed by eight cycles of 95°C for 30 s, 55°C for 30 s, 72°C for 30 s, and a final extension step of 72°C for 5 min. Index-PCR products were analysed by agarose gel electrophoresis and cleaned using the same SPB procedure described above. Samples were then quantified using the Qubit fluorometer 4 (Invitrogen, Waltham, Massachusetts, USA) with the Qubit dsDNA HS kit (Invitrogen), and then normalised by diluting all indexed libraries to 4 nM in nuclease-free water. Libraries were denatured and sequenced at 8 pM final concentration (20% Phi-X control added) in the Illumina MiSeq sequencing system using the MiSeq Reagent Kit v3 (2 × 300 bp) following the standard run protocol (Illumina).

2.4 | Bioinformatic Analysis

Bioinformatic analysis was performed via a custom pipeline from NatureMetrics, United Kingdom. In brief, sequences were demultiplexed with bcl2fastq based on the combination of the i5 and i7 index tags. Paired-end FASTQ reads for each sample were merged with 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) with a length filter of 125–135 bp for COI primer and 80–120 bp for 12S primer (post primer removal). Sequences were quality filtered with USEARCH to retain only those with an expected error rate per base of 0.01 or below and dereplicated by sample, retaining singletons. Unique sequences from all samples were denoised in a single analysis with UNOISE (Edgar 2016) requiring retained zOTUs (zero-radius Operational Taxonomic Units) to have a minimum abundance of 8 in at least one sample. Consensus taxonomic assignments were made for each zOTU using sequence similarity searches against NCBI nucleotide (NCBI nt) for 12S primer, and NCBI and BOLD (Ratnasingham and Hebert 2007) for COI primer. Searches against databases were made using blastn (Altschul et al. 1990; Camacho et al. 2009) and required hits to have a minimum e-score of 1e-20 and cover at least 90% of the query sequence. The taxonomic identification associated with all hits was converted to match the Global Biodiversity Information Facility (GBIF) taxonomic backbone. Assignments were made to the lowest possible taxonomic level where there was consistency in the matches, with minimum similarity thresholds of 99%, 97% and 95% for species, genus and higher level assignments, respectively. zOTUs were clustered at 97% similarity with USEARCH to obtain OTUs. Automated identifications were sense-checked against GBIF occurrence records for presence in the sampling country, and elevated to higher taxonomic levels where required (rgbif; Chamberlain et al. 2023), as well as the CAS Eschmeyer's Catalog of Fishes database (Eschmeyer et al. 2024), and the reports in Peru (Chuctaya et al. 2022; Meza-Vargas et al. 2021; Ortega et al. 2012).

Following taxonomic assignment, zOTUs were clustered into OTUs to minimise the number of sequence variants for a species (that may be present due to intraspecific variations, or amplification or sequencing artefacts). Supervised clustering was done using a combination of USEARCH UPARSE (Edgar 2013) and a custom pipeline that takes into account sequence similarity, co-occurrence patterns, abundance profiles and taxonomy to prevent the over-clustering of distinct, closely related species. Chimeric sequences were excluded, and an OTU-by-sample table was generated by mapping all dereplicated reads for each sample to the OTU representative sequences with USEARCH at an identity threshold of 97%. The OTU table was filtered to remove low abundance OTUs from each sample. The minimum read count for a detection of an OTU within a sample was set at approximately 20 reads. To do this, the percentage threshold across all samples was identified within the dataset that most closely achieved this and applied this threshold across all samples. Unassigned OTUs and OTUs identified to human and domesticated mammals were removed from the dataset for subsequent analyses. All raw sequencing data in FastQ format is available at Zenodo (https://doi.org/10.5281/zenodo.15000947).

2.5 | Traditional Fish Sampling

Fish samples were collected at the same time as eDNA samples or within 2 weeks after eDNA sample collection. We used a 10 mm seine net (30 m long and 2 m high with a 0.6 cm mesh opening) and a gill net of 0.4 mm nylon (40 m long and 2 m high composed of three nets of mesh sizes 5, 7.5 and 10 mm) to catch individual fish. Sampling with the seine net was done three times at three sites (randomly picked) within each lentic system, starting from the middle of the waterbody towards the shoreline (Araújo-Flores et al. 2021). The gill net was employed overnight (sunset to sunrise) and placed parallel to the shore. Individual fish were identified at the species level when possible and otherwise to the genus level using morphological observations and a variety of keys and checklists together with the nomenclatural assignments of Eschmeyer et al. (2024).

2.6 | Data Analysis

To analyse species richness among the sites surveyed, we compared taxonomic groups at the morphospecies level—that is, unique MOTUS mapped at the genus or species level, when available, for eDNA, and unique morphological assignments when using traditional methods. The dataset used in the analysis, listing the number of reads mapped to a given order, family, genera and species per sampling site, is presented as Table S2 for the 12S marker, and as Table S3 for the COI marker.

When using the COI marker, there were four instances where more than one species was mapped to a unique MOTU with

100% similarity. In these cases, the species reported for Peru (Meza-Vargas et al. 2021; Chuctaya et al. 2022; Ortega et al. 2012; Eschmeyer et al. 2024) among the mapped set was selected. This was done as follows: Between *Astyanax bimaculatus* and *Psellogrammus kennedyi*, *Astyanax bimaculatus* was selected. Between *Prochilodus nigricans* and *Prochilodus rubrotaeniatus*, *Prochilodus nigricans* was selected. *Between Hypoptopoma gulare* and *Hypoptopoma steindachneri, Hypoptopoma gulare* was selected. However, between *Auchenipterus ambyiacus* and *Auchenipterus nuchalis*, both species have been reported for Peru, so this unique MOTU was taken as a single morphospecies referred to as *Auchenipterus ambyiacus/Auchenipterus nuchalis* in the analysis. For other MOTUs' assignments to fish not reported for Peru, we modified the taxonomic designations as follows: *Acestrorhynchus lacustris* (99.06% similarity) was relabelled as A. aff. *lacustris*, *Leporinus friderici* (100% similarity) as L. aff. *friderici* and *Eigenmannia trilineata* (99.21% similarity) as E. gr. *trilineata* (see Table 3).

To assess the effect of different ecological contexts or lentic system characteristics (flooding status, year of abandonment and pond dimensions) on species richness among the sites surveyed, we performed a multifactorial analysis of variance (ANOVA). The analysis was performed using the *'aov'* function from the *'stats'* package (R Core Team 2024), and validated with the Shapiro–Wilk test to confirmed the normality of residuals, as well as Levene's test to verify homogeneity of variances among groups. Then, to assess the effect of flooding on the composition of fish communities between the three conditions described previously: FP, NFP and OL, we used the Jaccard dissimilarity index (Baselga 2012). Briefly, we created a presence–absence matrix comprising all identified morphospecies by traditional methods and eDNA (considering both the 12S and COI markers) (Table 3). We, then, ran the Jaccard dissimilarity index between sites using the '*vegdist*' function from the '*vegan*' package (Oksanen et al. 2023), and performed a Principal Coordinate Analysis (PCoA) to visualise the patterns in two-dimensional plots using the Jaccard dissimilarity matrix with the *'cmdscale'* function from the *'stats'* package (R Core Team 2024), extracting two-dimensional ordination scores. We then tested for population assemblages' similarities between the three conditions (FP, NFP and OL) using a permutational multivariate analysis of variance (PERMANOVA), comparing assemblage variation with an analysis of homogeneity of multivariate dispersion using the *'adonis'* function from the *'vegan'* package (Oksanen et al. 2023). The significance was assessed with 999 permutations.

Finally, to assess the relationships between environmental variables and community composition, we conducted a distance-based redundancy analysis (dbRDA) using the Jaccard dissimilarity matrix. Environmental predictor variables, including area, depth, pH, conductivity, temperature, dissolved oxygen, transparency, flooding condition (NFP, FP and OL) and Strahler stream order (Venticinque et al. 2016), were included in the analysis (see the variables in Table S1). The db-RDA was performed using the 'capscale' function from the 'vegan' package (Oksanen et al. 2023). To select the most influential predictor variables, we applied stepwise selection using the 'ordistep' function in the package vegan with both directions and 999 permutations. The significance of the final model was tested using the 'anova' function with 999 permutations. All data analyses were conducted in R version 4.3.1., using RStudio 2023.06.2 as the front end (Allaire 2012), and Python 3.9 (Van Rossum and Drake 2009).

3 | Results

3.1 | Contrast Between the 12S and COI Markers for eDNA Analysis

Contrasting results were obtained when using either the 12S or the COI marker for eDNA analysis. With the 12S marker, we obtained 2,588,108 processed reads, of which 1,617,483 were mapped to class Actinopterygii, with an average of 147,043 reads per sample (range = 1012–235,431). With the COI marker, we obtained 1,783,519 processed reads, of which only 218,614 were mapped to class Actinopterygii, with an average of 19,874 reads per sample (range = 1160–80,732). In relation to taxa identification, with the 12S marker, we obtained 63 MOTUs; all of which were mapped at the order level, 61 at the family level, 43 at the genera level and only 20 at the species level. They belong to 5 orders, 21 families, 39 genera, and were grouped into 41 morphospecies (i.e., MOTUs mapped at the genus or species level, when available) (see Table S2). In contrast, with the COI marker, we obtained 61 MOTUs; all of which were mapped at the order level, 58 at the family level, 46 at the genera level, and 30 at the species level. They belong to 4 orders, 16 families, 38 genera and were grouped into 44 morphospecies (see Table S3). Overall, the 12S marker registered more MOTUs than the COI marker at all sites (Figure 2). At the family level, the 12S marker identified five families not recorded by the COI marker, whereas the COI marker identified only one family not recorded by the 12S marker. At the genera level, the 12S marker identified 15 genera not recorded by the COI marker, whereas the COI marker recorded 14 genera not recorded by the 12S marker. At the morphospecies level, both markers shared 15 morphospecies, the 12S marker identified 26 non-shared morphospecies and the COI marker identified 29 non-shared morphospecies, while 20 and 15 MOTUs could not be identified at the morphospecies level with the 12S and COI markers, respectively (Figure 3). At the species level, the COI marker allowed us to identify 10 more species than the 12S marker considering all sites surveyed (Table S3).

Differences in fish community composition were observed across the sites surveyed when using either DNA marker (Figure 4). The 12S marker identified more families than the COI marker at all sites (Table S4), detecting a total of 5 and 1 unique families, respectively (Figure 3). Families exclusively detected by the 12S marker include: Acestrorhynchidae, Iguanodectidae (found only in Balata NFP#4 and Cobra NFP#3), Parodontidae, Synbranchidae (found only in Jimena OL#2) and Heptapteridae (found only in Inundación FP#4). In contrast, the COI marker was able to identify the Triportheidae family, not registered when using the 12S marker. In addition, the 12S marker was unable to resolve (i.e., assign to lower taxonomic levels) several MOTUs mapped at the order Characiformes in Lechuza NFP#1, Cobra NFP#3, Balata NFP#4 and Chambira FP#1, where the COI marker did not show any unassigned MOTUs at the family level. Both markers consistently detected the family Cichlidae across all sites (Figure 4, Table S4). Similarly, the families

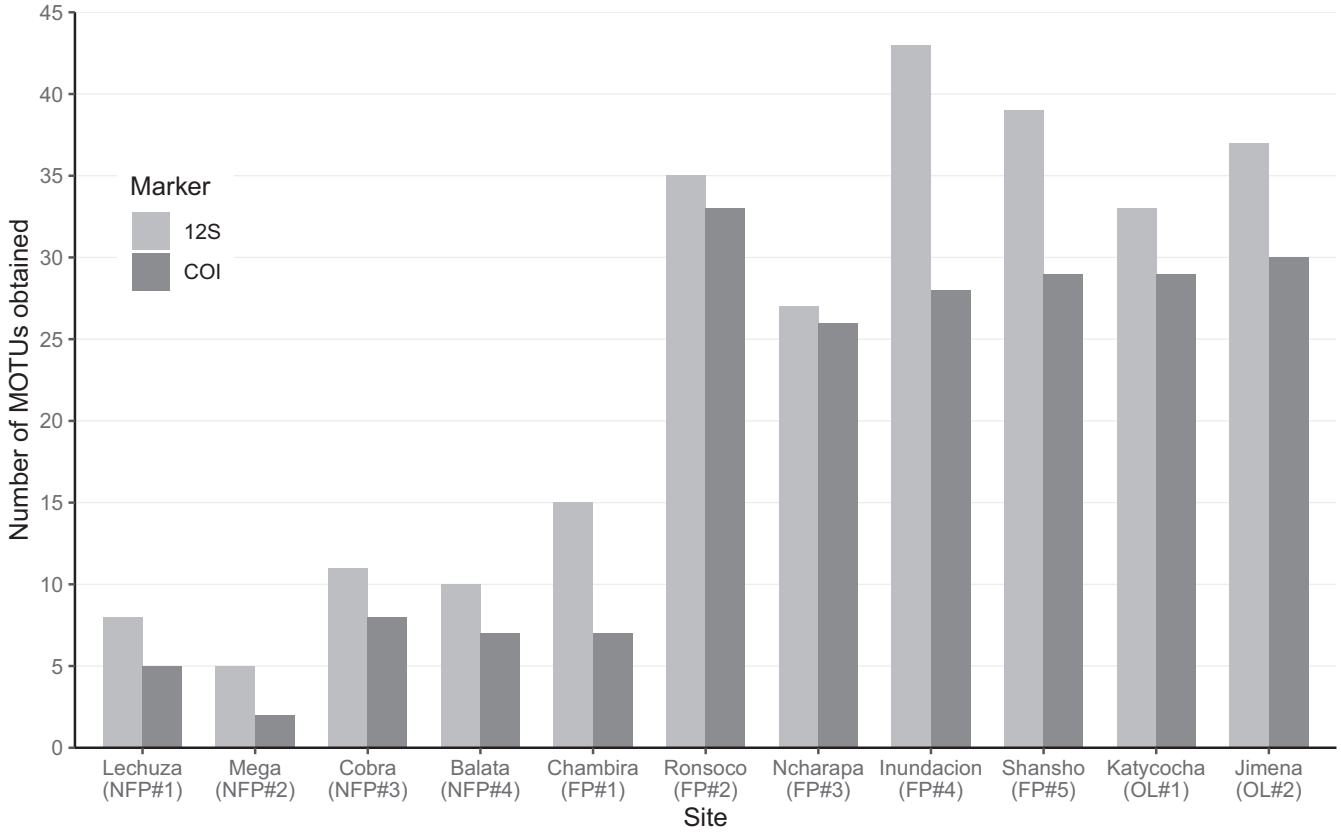

FIGURE 2 | Number of MOTUs belonging to Actinopterygii obtained using the 12S (Riaz et al. 2011 in Kelly et al. 2014) and COI (Mariac et al. 2022) markers in the abandoned mining ponds and nearby pristine oxbow lakes surveyed in the study. FP, flooded (abandoned mining) ponds; NFP, non-flooded (abandoned mining) ponds; OL, (pristine) Oxbow lakes.

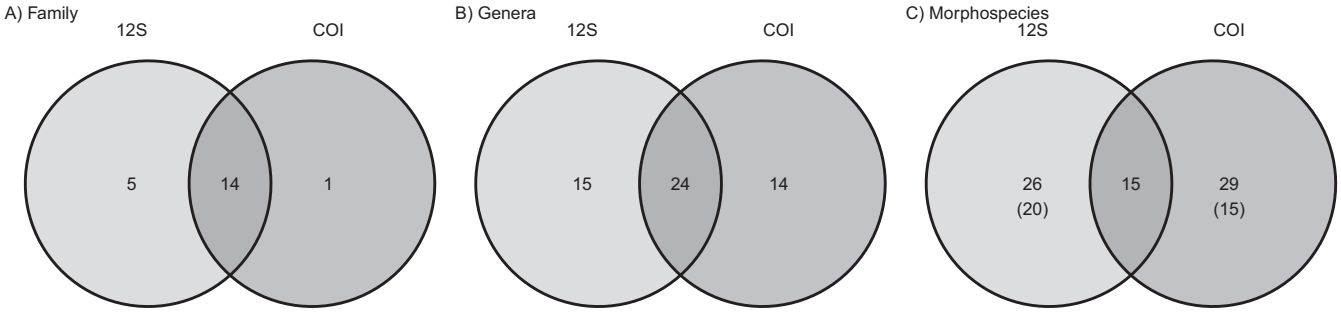

FIGURE 3 | Venn diagrams showing the shared (and not shared) number of families (A), genera (B) and morphospecies (C) by the 12S (Riaz et al. 2011 in Kelly et al. 2014) and COI (Mariac et al. 2022) markers across all sites. Numbers in parenthesis are MOTUS not mapped at morphospecies level.

Erythrinidae and Characidae were present at all locations except for Mega NFP#2. At this site, neither molecular marker registered these families, although traditional sampling did confirm the presence of *Hoplias malabaricus* from the family Erythrinidae.

3.2 | Contrast Between eDNA and Traditional Methods for Biodiversity Monitoring

Considering all the sites surveyed, we detected a total of 123 morphospecies using eDNA and traditional methods (TM) combined, which comprised 68 genera, 26 families and 6 orders (Table 3). With the eDNA approach, using both markers combined, we identified a total of 70 morphospecies, belonging to 54 genera, 21 families and 5 orders. In contrast, when using TM, we recorded 76 morphospecies belonging to 53 genera, 20 families and 6 orders (see Table S5). Notably, at the species level, TM identified 61 species, whereas the eDNA method identified 41 species. However, a substantial number of MOTUS remained unassigned at the species level, with 43 and 31 unassigned MOTUs for the 12S and COI markers, respectively (Table 3).

When considering the sites surveyed independently, we could identify more morphospecies when using the eDNA approach with both markers combined than with TM only, with the exception of

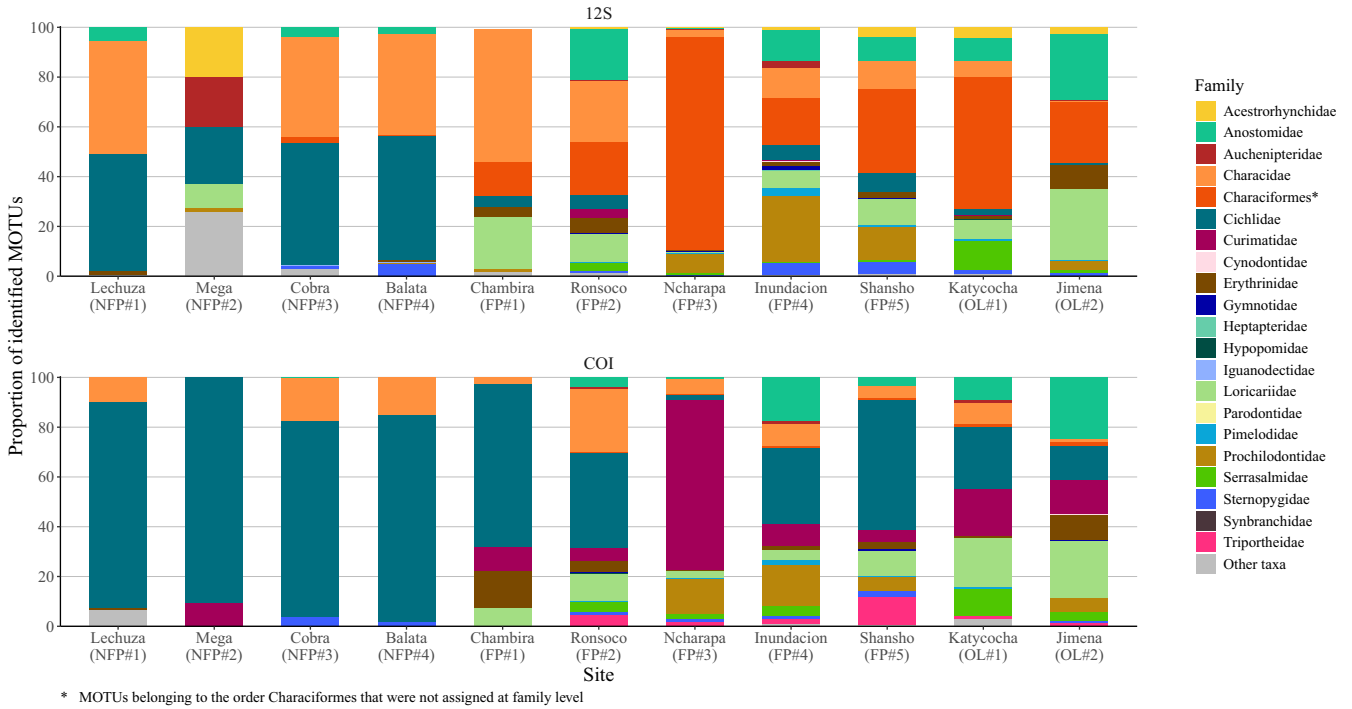

FIGURE 4 | Family composition of fish communities in the abandoned mining ponds and oxbow lakes identified with eDNA, using the 12S marker (Riaz et al. 2011 in Kelly et al. 2014, top panel) and the COI marker (Mariac et al. 2022, bottom panel). Other taxa, represented by grey colour (birds, amphibians and reptiles), were identified at all sites, although at lower proportions (see Tables S6 and S7). FP, flooded (abandoned mining) ponds; NFP, non-flooded (abandoned mining) ponds; OL, (pristine) Oxbow lakes.

Chambira FP#1, a flooded abandoned mining pond, where TM identified three additional morphospecies in contrast to the eDNA method (Figure 5). It is important to note that at this site, and others, many MOTUs were not mapped at the morphospecies level given the (well-documented) limited genetic reference data available (Table 2). Interestingly, it would appear that greater species richness was obtained by combining eDNA and TM (Figure 5). However, given that the identity of the fish obtained with TM was not verified using 12S and COI markers, it cannot be ruled out that some taxonomically close morphospecies detected by either method actually correspond to the same species.

At the family level, both approaches identified five families not detected by the other approach (Figure 6). At the genera level, the eDNA method identified 14 genera not found by the traditional method, whereas the traditional approach found 13 genera not registered by eDNA (Figure 6). Finally, at the morphospecies level, the eDNA method identified 47 morphospecies not found by the TM, whereas the TM identified 53 morphospecies not registered by eDNA (Figure 6). Both methods identified 15 shared families, 40 shared genera and 23 shared morphospecies (Figure 6).

Regarding commercial species, we identified more diversity with eDNA than with traditional approaches (Table 3). With the 12S marker we registered seven species: *Hydrolycus scomberoides* (common name, chambira or cachorro), *Hemisorubim platyrhynchos* (common name, pico pato or toa), *Pseudoplatystoma tigrinum* (common name, puma zungaro or tiger sorubim), *Pseudoplatystoma* sp. (common name, doncella or barred sorubim), *Prochilodus lineatus* (common name, bocachico or curimata), *Leporinus* sp. and *Schizodon* sp. (common name, both as lisa or araçu). With the COI marker, we registered 10 species: *Schizodon fasciatus*, *Leporinus* aff. *friderici*, *Leporinus* sp. (common name, both as lisa or araçu), *Megaleporinus trifasciatus* (common name, lisa or araçu), *Potamorhina altamazonica* (common name, yahuarachi or llambina), *Psectrogaster rutiloides* (common name, chio-chio or branquinha), *Prochilodus nigricans* (common name, bocachico or curimata), *Mylossoma albiscopum* (common name, palometa or pacupeba), *Pimelodus tetramerus* (common name, bagre-cunchi or bloch's catfish) and *Sorubim lima* (common name, pico pato—shiripira or duckbill catfish). In addition, with the eDNA method we were able to identify species that were difficult to capture by TM such as the electric fishes *Gymnotus carapo*, *Brachyhypopomus* sp., *Brachyhypopomus pinnicaudatus* and *Eigenmannia limbata* from the Order Gymnotiformes which are usually found around trees, and beneath branches and leaf litter from macrophytes and riparian vegetation in the ponds.

3.3 | Effect of Ecological Context on Fish Diversity

The degree of flood pulse had a statistically significant effect on fish richness ($p < 0.05$), whereas the year of abandonment ($p = 0.804$) and surface area ($p = 0.183$) did not, according to the ANOVA test. We identified a total of 123 morphospecies across all sites using traditional and eDNA monitoring combined (Table 3), of which 32 were found in NFP, 101 in FP and 76 in OL. NFPs exhibit lower species richness (16 ± 5 SD per site) compared to both FP (50 ± 17 SD per site) and OL (56 ± 0 SD per site) (Figure 5 and Table 2). We also observed lower family diversity at the NFP compared to FP and OL, with a predominance of the families Cichlidae, Characidae and Curimatidae (Figure 4, Table S4).

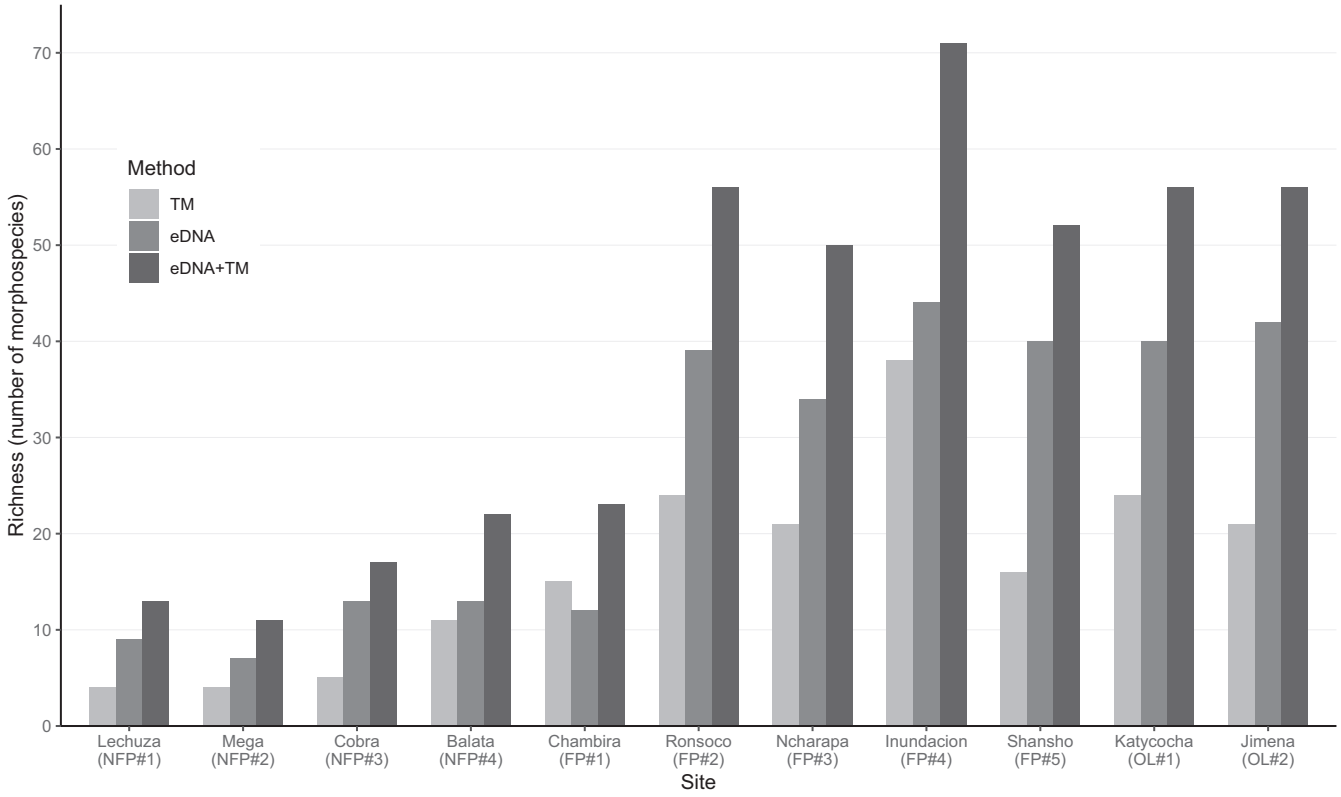

FIGURE 5 | Total number of morphospecies registered at each site using only traditional methods (TM), using eDNA—compiling results obtained with the 12S and COI markers (eDNA)—and using traditional methods and eDNA combined (TM + eDNA). MOTUs not mapped at morphospecies level are not included in the graph. FP, flooded (abandoned mining) ponds; NFP, non-flooded (abandoned mining) ponds; OL, (pristine) Oxbow lakes.

TABLE 2 | Number of morphospecies identified with the 12S marker, the COI marker, both markers combined (12S+COI), traditional methods (TM) and both eDNA and traditional methods combined (eDNA+TM).

Site name	Code	12S	COI	12S+COI	TM	eDNA+TM	Shannon index (*H*)
Lechuza	NFP#1	5 (8)	5 (5)	9	4	13	1.53
Mega	NFP#2	5 (5)	2 (2)	7	4	11	0.62
Cobra	NFP#3	7 (11)	7 (8)	13	5	17	1.07
Balata	NFP#4	8 (10)	6 (7)	13	11	22	2.12
Chambira	FP#1	9 (15)	7 (7)	12	15	23	2.5
Ronsoco	FP#2	25 (35)	25 (33)	39	24	56	2.95
Nueva Charapa	FP#3	20 (27)	21 (26)	34	21	50	3.05
Inundación	FP#4	29 (43)	24 (28)	44	38	71	4.07
Shansho	FP#5	26 (39)	23 (29)	40	16	52	2.36
Katycocha	OL#1	26 (33)	20 (29)	40	24	56	3.32
Jimena	OL#2	25 (37)	25 (30)	42	21	56	2.52

Note: Numbers in parentheses represent the total number of MOTUs for each eDNA marker. The Shannon–Wiener diversity index (*H*) shown was calculated using the data obtained with the traditional sampling method only.
Abbreviations: FP, flooded (abandoned mining) ponds; NFP, non-flooded (abandoned mining) ponds; OL, (pristine) Oxbow lakes.

Following the ANOVA results, we decided to further test the effect of flood pulse on fish community composition using a PERMANOVA test. We found statistically significant differences in fish species composition among ponds affected by different flood pulse conditions ($p < 0.005$). When plotting the Jaccard dissimilarity index in a PCoA, we could see that the fish community composition among OL and FP had high similarity—excluding FP#1—in contrast to the NFP (Figure 7).

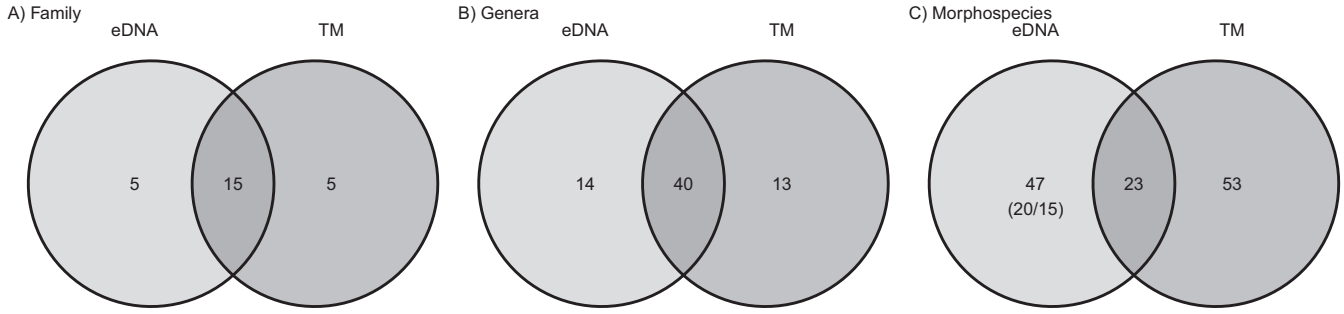

FIGURE 6 | Venn diagram showing the number of families, genera and morphospecies shared by both methods: eDNA and traditional methods (TM). Numbers in parentheses indicate MOTUS not mapped at the morphospecies level by the 12S/COI markers, respectively.

The NFP#1 displayed a distinct ordination pattern among NFPs, possibly due to its central location within La Pampa, being the least connected to streams or forest (see Figure 1).

The db-RDA revealed a significant relationship between environmental variables and the biodiversity of aquatic communities in small-scale mining-impacted ecosystems ($p < 0.005$) (Figure S1). The model explained 80.56% of the variation in biodiversity. The db-RDA identified transparency and flood condition (NFP, FP and OL) as the most influential variables, with transparency showing a strong negative association and flood condition playing a role in differentiating the community assemblage across different environments (Figure S1), as suggested by the PCoA. The biplot further demonstrated the contribution of individual variables, with pH and temperature showing weaker associations compared to transparency and flood condition.

The NFPs, located in La Pampa, had similar fish compositions among them, with a predominance of the Cichlidae and Erythrinidae families (Figure 4). The genus *Saxatilia* was predominant in the NFP ecosystems, with a greater proportion of reads per sample (see Tables S2 and S3), and a higher abundance found with TM (see Table S5). While the FP and OL share more genera and morphospecies between them, compared to NFP and OL, or between NFP and FP. When looked at proportion levels, the NFP shared 62.5% of their diversity with both conditions, while FP and OL shared only 19.8% and 26.3%, respectively (Figure 8). This is possibly due to an important proportion of morphospecies found in NFPs being species that probably can survive in almost all mining ponds and OL (Figure 8). These include *Saxatilia lepidota*—formerly *Crenicichla lepidota*, *Cichlasoma bimaculatum*, Leporinus sp., *Ctenobrycon hauxwellianus*, *Astyanax bimaculatus*, Saxatilia sp. and the species *Hoplias malabaricus*, from the Erythrinidae family, which was found at all sites surveyed using either method and marker (Table S8). Interestingly, the FP, which are influenced by the Madre de Dios and Malinowski Rivers (see Table 1), have a higher richness of fish when compared to the OL, influenced by the La Torre River, a smaller hierarchy river (Figure 5).

4 | Discussion

In this study, we assessed fish diversity (i.e., richness) and community composition in abandoned mining ponds using both eDNA and traditional monitoring to understand factors that could be influencing the recolonisation dynamics of post-ASGM landscapes within the Madre de Dios region. We studied nine mining ponds with different degrees of pulse flood connectivity with extant waterways, classified as FP, located in a seasonally flooded ecosystem, and NFPs, located in a *terra firme* or non-flooded ecosystem, with and without connection to lotic systems, with different ages since the abandonment and different dimensions; and two nearby unmined oxbow lakes (OL) used as references (see Table 1). Overall, our results highlight the value of eDNA as a cost-efficient tool for rapid biodiversity monitoring (Andres et al. 2023; Pukk et al. 2021), especially in remote and hard-to-access areas (Timana-Mendoza et al. 2024).

4.1 | eDNA Fish Primers Performance

We used two molecular markers for detecting fish diversity: The COI marker targeting Amazonian fishes (Mariac et al. 2022) and the 12S marker targeting vertebrates (Riaz et al. 2011 in Kelly et al. 2014), which has been successfully used to assess fish diversity in this Amazonian ecosystem (Timana-Mendoza et al. 2024). The COI gene is a well-established marker for assessing animal diversity, particularly supported by its extensive database (Collins et al. 2019; Teixeira et al. 2023). While its high interspecific variability makes it suitable for differentiating closely related species, it requires highly degenerate primers, which limits its effectiveness in broader biodiversity detection (Kumar et al. 2022; Teixeira et al. 2023; Zhang et al. 2020). In contrast, the 12S gene is widely utilised in metabarcoding due to its conserved regions that facilitate primer design and its variable regions that allow genus- or species-level resolution, and has been effective for the detection of fish taxa (Jackman et al. 2021; Kumar et al. 2022; Thomsen et al. ; Valentini et al. 2016; Milan et al. 2020; Teixeira et al. 2023; Blackman et al. 2021).

In our study, both molecular markers successfully characterised fish diversity, detecting a similar number of morphospecies across all sites, despite some differences. For instance, the 12S marker outperformed the COI marker by identifying more MOTUs per location (Figure 2), although the increased number of amplicon PCR replicates in the library preparation procedure (12 vs. 4 for the COI marker) could have somewhat contributed to this result (Ficetola et al. 2015). In contrast, using the COI marker, we were able to identify 10 additional MOTUs at the species level compared to the 12S marker (Table S3), indicating higher taxonomic resolution, possibly due to the availability of a more extensive database (Collins et al. 2019; Jackman et al. 2021; Milan et al. 2020, among others).

TABLE 3 | Prevalence of morphospecies registered by eDNA (12S and COI) and traditional methods (TM) among the 11 sites surveyed.

Family	Genus	Species	eDNA		TM
			12S	COI	
Acestrorhynchidae	*Acestrorhynchus*	*Acestrorhynchus* aff. *lacustris*	7	0	0
Acestrorhynchidae	*Acestrorhynchus*	*Acestrorhynchus falcatus*	0	0	4
Anostomidae	*Leporinus*	*Leporinus* aff. *friderici*	0	5	0
Anostomidae	*Leporinus*	*Leporinus* cf. *subniger*	0	0	6
Anostomidae	*Leporinus*	*Leporinus* sp.	9	2	0
Anostomidae	*Megaleporinus*	*Megaleporinus trifasciatus*	0	3	0
Anostomidae	*Schizodon*	*Schizodon fasciatus*	0	6	2
Anostomidae	*Schizodon*	*Schizodon* sp.	6	0	0
Auchenipteridae	*Auchenipterus*	*Auchenipterus ambyiacus*	0	0	2
Auchenipteridae	*Auchenipterus*	*Auchenipterus ambyiacus/Auchenipterus nuchalis*	0	2	0
Auchenipteridae	*Auchenipterus*	*Auchenipterus* sp.	2	0	0
Auchenipteridae	*Trachelyopterus*	*Trachelyopterus galeatus*	3	1	0
Auchenipteridae	*Trachelyopterus*	*Trachelyopterus porosus*	0	0	1
Belonidae	*Potamorrhaphis*	*Potamorrhaphis* cf. *eigenmanni*	0	0	1
Cetopsidae	*Cetopsis*	*Cetopsis coecutiens*	0	0	1
Characidae	*Aphyocharax*	*Aphyocharax avary*	0	0	6
Characidae	*Aphyocharax*	*Aphyocharax* sp.	0	2	0
Characidae	*Aphyocheirodon*	*Aphyocheirodon* sp.	0	6	0
Characidae	*Astyanax*	*Astyanax bimaculatus*	6	9	5
Characidae	*Brachychalcinus*	*Brachychalcinus* aff. *nummus*	0	0	1
Characidae	*Brachychalcinus*	*Brachychalcinus copei*	0	0	2
Characidae	*Charax*	*Charax gibbosus*	0	2	4
Characidae	*Charax*	*Charax pauciradiatus*	0	0	1
Characidae	*Compsura*	*Compsura* sp.	2	0	0
Characidae	*Ctenobrycon*	*Ctenobrycon hauxwellianus*	9	0	7
Characidae	*Cynopotamus*	*Cynopotamus* sp.	3	0	0
Characidae	*Galeocharax*	*Galeocharax gulo*	0	0	1
Characidae	*Gymnocorymbus*	*Gymnocorymbus ternetzi*	8	0	1
Characidae	*Gymnocorymbus*	*Gymnocorymbus thayeri*	0	0	1
Characidae	*Hemigrammus*	*Hemigrammus sp*	2	0	3
Characidae	*Jupiaba*	*Jupiaba anteroides*	0	0	1
Characidae	*Knodus*	*Knodus* sp. 1	0	0	1
Characidae	*Knodus*	*Knodus* sp. 2	0	0	1
Characidae	*Knodus*	*Knodus* sp. 3	0	0	1
Characidae	*Knodus*	*Knodus* sp. 4	0	0	1
Characidae	*Knodus*	*Knodus* sp. 5	0	0	3
Characidae	*Knodus*	*Knodus* sp. 6	0	0	4

(Continues)

TABLE 3 | (Continued)

Family	Genus	Species	eDNA		TM
			12S	COI	
Characidae	*Moenkhausia*	*Moenkhausia bonita*	0	0	1
Characidae	*Moenkhausia*	*Moenkhausia lepidura*	0	0	1
Characidae	*Moenkhausia*	*Moenkhausia madeirae*	0	0	7
Characidae	*Moenkhausia*	*Moenkhausia oligolepis*	0	0	1
Characidae	*Moenkhausia*	*Moenkhausia sanctaefilomenae*	2	0	0
Characidae	*Moenkhausia*	*Moenkhausia* sp.	2	6	0
Characidae	*Phenacogaster*	*Phenacogaster* sp.	4	0	0
Characidae	*Poptella*	*Poptella compressa*	1	0	0
Characidae	*Roeboides*	*Roeboides afinnis*	0	0	1
Characidae	*Roeboides*	*Roeboides biserialis*	0	0	1
Characidae	*Roeboides*	*Roeboides descalvadensis*	0	3	0
Characidae	*Roeboides*	*Roeboides* sp.	5	2	0
Characidae	*Serrapinnus*	*Serrapinnus piaba*	7	0	0
Characidae	*Serrapinnus*	*Serrapinnus* sp. 1	0	0	6
Characidae	*Serrapinnus*	*Serrapinnus* sp. 2	0	0	1
Characidae	*Tetragonopterus*	*Tetragonopterus argenteus*	0	2	1
Characidae	*Tyttobrycon*	*Tyttobrycon* sp.	0	0	1
Cichlidae	*Aequidens*	*Aequidens tetramerus*	0	0	2
Cichlidae	*Apistogramma*	*Apistogramma* sp.	0	0	3
Cichlidae	*Bujurquina*	*Bujurquina eurhinus*	0	0	2
Cichlidae	*Cichlasoma*	*Cichlasoma bimaculatum*	0	10	0
Cichlidae	*Cichlasoma*	*Cichlasoma boliviense*	0	0	5
Cichlidae	*Cichlasoma*	*Cichlasoma* sp.	6	1	0
Cichlidae	*Mesonauta*	*Mesonauta festivus*	0	2	2
Cichlidae	*Satanoperca*	*Satanoperca jurupari*	0	0	5
Cichlidae	*Satanoperca*	*Satanoperca* sp.	0	5	0
Cichlidae	*Saxatilia*	*Saxatilia lepidota*	10	0	0
Cichlidae	*Saxatilia*	*Saxatilia semicincta*	0	0	5
Cichlidae	*Saxatilia*	*Saxatilia* sp.	0	9	0
Crenuchidae	*Characidium*	*Characidium* sp.	0	0	1
Curimatidae	*Curimatella*	*Curimatella meyeri*	0	0	1
Curimatidae	*Curimatella*	*Curimatella* sp.	0	2	0
Curimatidae	*Cyphocharax*	*Cyphocharax* sp.	6	5	0
Curimatidae	*Cyphocharax*	*Cyphocharax spiluropsis*	0	0	3
Curimatidae	*Potamorhina*	*Potamorhina altamazonica*	0	8	5
Curimatidae	*Psectrogaster*	*Psectrogaster rutiloides*	0	2	2
Curimatidae	*Steindachnerina*	*Steindachnerina aff. dobula*	0	0	4

(Continues)

TABLE 3 | (Continued)

Family	Genus	Species	eDNA		TM
			12S	COI	
Curimatidae	*Steindachnerina*	*Steindachnerina guentheri*	0	2	3
Curimatidae	*Steindachnerina*	*Steindachnerina* sp.	0	0	1
Cynodontidae	*Cynodon*	*Cynodon gibbus*	0	0	1
Cynodontidae	*Cynodon*	*Cynodon* sp.	0	1	0
Cynodontidae	*Hydrolycus*	*Hydrolycus scomberoides*	1	0	0
Erythrinidae	*Hoplerythrinus*	*Hoplerythrinus unitaeniatus*	0	2	2
Erythrinidae	*Hoplias*	*Hoplias malabaricus*	9	8	9
Gymnotidae	*Gymnotus*	*Gymnotus carapo*	6	4	0
Hemiodontidae	*Anodus*	*Anodus elongatus*	0	0	1
Heptapteridae	*Pimelodella*	*Pimelodella* sp.	1	0	0
Hypopomidae	*Brachyhypopomus*	*Brachyhypopomus pinnicaudatus*	0	1	0
Hypopomidae	*Brachyhypopomus*	*Brachyhypopomus* sp.	3	0	0
Iguanodectidae	*Bryconops*	*Bryconops* sp.	1	0	0
Iguanodectidae	*Bryconops*	*Bryconops melanurus*	0	0	1
Loricariidae	*Ancistrus*	*Ancistrus* sp.	5	4	0
Loricariidae	*Hypoptopoma*	*Hypoptopoma* cf. *bianale*	0	0	1
Loricariidae	*Hypoptopoma*	*Hypoptopoma gulare*	0	4	1
Loricariidae	*Hypoptopoma*	*Hypoptopoma* sp.	5	0	0
Loricariidae	*Hypostomus*	*Hypostomus* sp.	8	7	0
Loricariidae	*Hypostomus*	*Hypostomus* sp. 1	0	0	5
Loricariidae	*Hypostomus*	*Hypostomus* sp. 2	0	0	2
Loricariidae	*Loricariichthys*	*Loricariichthys platymetopon*	7	7	4
Loricariidae	*Loricariichthys*	*Loricariichthys* sp.	0	0	1
Loricariidae	*Pterygoplichthys*	*Pterygoplichthys disjunctivus*	0	0	1
Pimelodidae	*Hemisorubim*	*Hemisorubim platyrhynchos*	1	0	0
Pimelodidae	*Hypophthalmus*	*Hypophthalmus edentatus*	2	0	1
Pimelodidae	*Hypophthalmus*	*Hypophthalmus* sp.	0	2	0
Pimelodidae	*Pimelodus*	*Pimelodus* sp.	4	0	0
Pimelodidae	*Pimelodus*	*Pimelodus tetramerus*	0	2	0
Pimelodidae	*Pseudoplatystoma*	*Pseudoplatystoma* sp.	1	0	0
Pimelodidae	*Pseudoplatystoma*	*Pseudoplatystoma tigrinum*	3	0	0
Pimelodidae	*Sorubim*	*Sorubim lima*	2	1	1
Prochilodontidae	*Prochilodus*	*Prochilodus lineatus*	8	0	0
Prochilodontidae	*Prochilodus*	*Prochilodus nigricans*	0	5	4
Serrasalmidae	*Mylossoma*	*Mylossoma albiscopum*	0	1	1
Serrasalmidae	*Serrasalmus*	*Serrasalmus maculatus*	0	0	5
Serrasalmidae	*Serrasalmus*	*Serrasalmus rhombeus*	0	3	3

(Continues)

TABLE 3 | (Continued)

Family	Genus	Species	eDNA		TM
			12S	COI	
Serrasalmidae	*Serrasalmus*	*Serrasalmus* sp.	6	0	0
Serrasalmidae	*Serrasalmus*	*Serrasalmus spilopleura*	0	0	2
Sternopygidae	*Eigenmannia*	*Eigenmannia* cf. *macrops*	0	0	1
Sternopygidae	*Eigenmannia*	*Eigenmannia* gr. *trilineata*	0	1	0
Sternopygidae	*Eigenmannia*	*Eigenmannia limbata*	0	1	0
Sternopygidae	*Eigenmannia*	*Eigenmannia* sp.	3	2	1
Sternopygidae	*Eigenmannia*	*Eigenmannia virescens*	0	0	1
Sternopygidae	*Sternopygus*	*Sternopygus macrurus*	8	6	2
Synbranchidae	*Synbranchus*	*Synbranchus marmoratus*	1	0	1
Triportheidae	*Triportheus*	*Triportheus angulatus*	0	0	4
Triportheidae	*Triportheus*	*Triportheus rotundatus*	0	0	1
Triportheidae	*Triportheus*	*Triportheus* sp.	0	6	0

Note: Values in the table indicate the number of sites where a given morphospecies was encountered using either method.

Nevertheless, the COI marker exhibited some limitations, including four instances where a single identified MOTU was assigned to two different species with 100% sequence similarity, reflecting its reduced ability to differentiate between these species (Zhang et al. 2020). Furthermore, the COI marker registered 1,564,905 reads (87.7% of total processed reads), grouped into 601 MOTUs not assigned to Actinopterygii, which were unassigned (84.6%) or assigned to other taxonomic groups (3.1%) such as Arthropoda, Mollusca, Gastrotricha, Rotifera and other vertebrates. While the COI primers used in this study were reportedly optimised to target fish species (Mariac et al. 2022), our results do indicate certain selectivity for fish, but a high proportion of unassigned MOTUs, possibly due to amplification artefacts derived from using highly degenerated primers (Collins et al. 2019; Elbrecht et al. 2019; Kumar et al. 2022; Zhang et al. 2020). In contrast, the 12S marker had only 970,625 reads (37.5% of total processed reads), grouped into 50 MOTUs not assigned to Actinopterygii, but to other vertebrates such as Mammalia, Aves, Reptilia (23.7%) or unassigned reads (13.8%). This suggests that the 12S primers used in this study are more robust for eDNA analysis, and that they can also cover a high proportion of fish diversity. Despite these differences, each marker provided unique fish diversity information (Figures 3 and 4), highlighting the importance of employing multiple primer sets to improve taxonomic coverage (García-Machado et al. 2023; Munian et al. 2024), particularly in megadiverse regions (Jackman et al. 2021; Zhang et al. 2020).

4.2 | eDNA And Traditional Approach Monitoring

When comparing the two monitoring approaches used in the study, eDNA identified a greater number of morphospecies at nearly all sites (Figure 5), highlighting its advantages for detecting fish diversity (Munian et al. 2024; Pont et al. 2023; Thomsen et al. ; Yao et al. 2022). Additionally, it required fewer resources than TM, as noted by Timana-Mendoza et al. (2024). eDNA also detected the presence of some species that are usually not recorded using TM due to their elusive habits or the extensive fishing effort required, such as *Trachelyopterus galeatus*, *Pseudoplatystoma tigrinum*, *Sternopygus macrurus*, *Gymnotus carapo*, *Saxatilia* spp., *Hypoptopoma* spp., *Ancistrus* spp. and *Brachypopomus* spp. (Table 3).

Nevertheless, species detection via eDNA in Amazon aquatic environments remains challenging due to limitations in taxonomic resolution, which can prevent some MOTUs from being identified at the species level or result in erroneous assignments due to misannotations in genomic databases (Beng and Corlett 2020; Collins et al. 2019; Mariac et al. 2022; Yao et al. 2022). For instance, in our study, the 12S marker identified *Prochilodus lineatus*, a species not found in the Madre de Dios River basin or the Beni-Madeira River system (Carvajal-Vallejos and Zeballos-Fernández 2011; Queiroz et al. 2013; Ortega et al. 2012). In contrast, *Prochilodus nigricans* (commonly known as bocachico or curimata), an important species for subsistence fishing throughout the Amazon (García-Dávila et al. 2018; Goulding et al. 2003; Silvano 2020), was identified using the COI marker and TM, and may represent the same species found with the 12S marker (*P. lineatus*).

Similarly, the COI marker identified *Satanoperca* sp., while TM identified *Satanoperca jurupari*; thus, this MOTU could either be an unrecorded species in genomic databases or a potential new species (Willis et al. 2012). Additionally, the genus *Moenkhausia* was represented by one morphospecies with the COI marker (*Moenkhausia* sp.), two morphospecies with the 12S marker (*Moenkhausia* sp. and *Moenkhausia sanctaefilomenae*) and four species by TM (*Moenkhausia bonita*, *Moenkhausia lepidura*, *Moenkhausia madeirae* and *Moenkhausia oligolepis*). Notably, *Moenkhausia sanctaefilomenae*, identified with the 12S marker, is not registered for Peru, suggesting a possible misidentification in the genomic database. This MOTU possibly corresponds to one of the species identified using TM. Overall, our results agree with previous reports for Neotropical

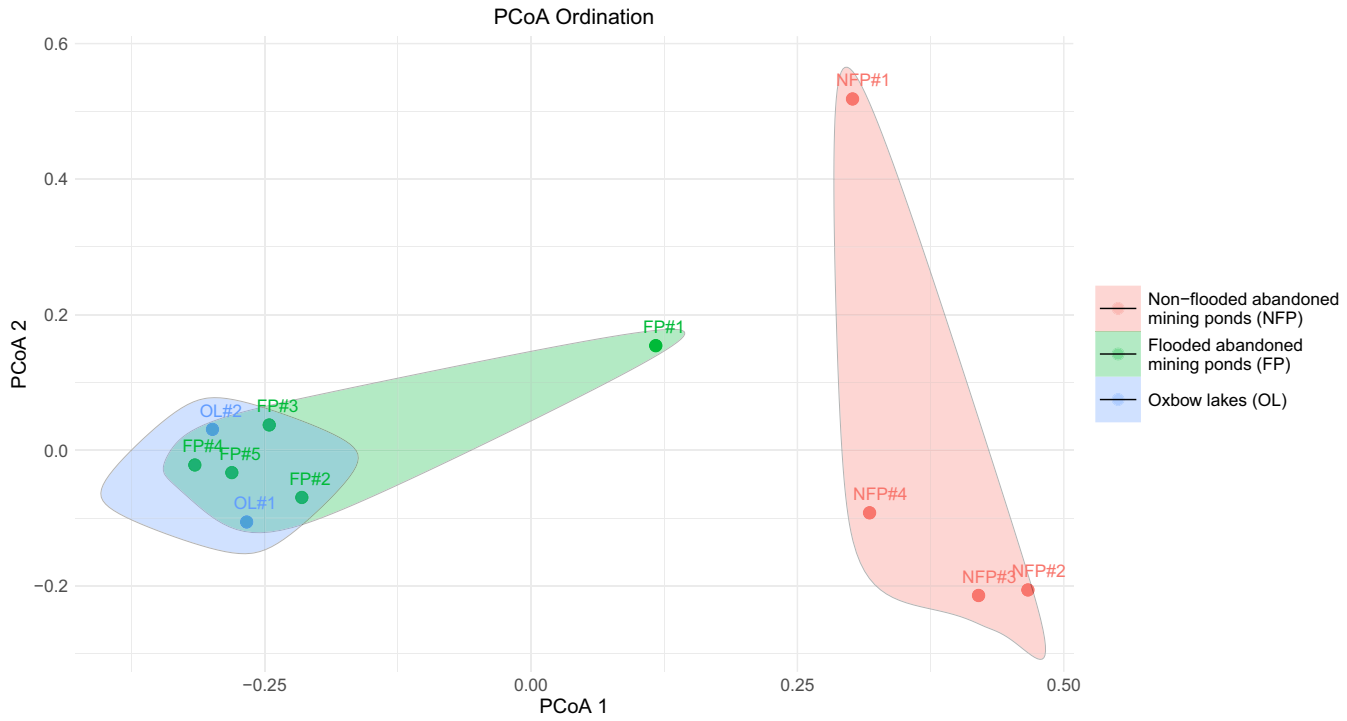

FIGURE 7 | PCoA Ordination using the Jaccard dissimilarity index.

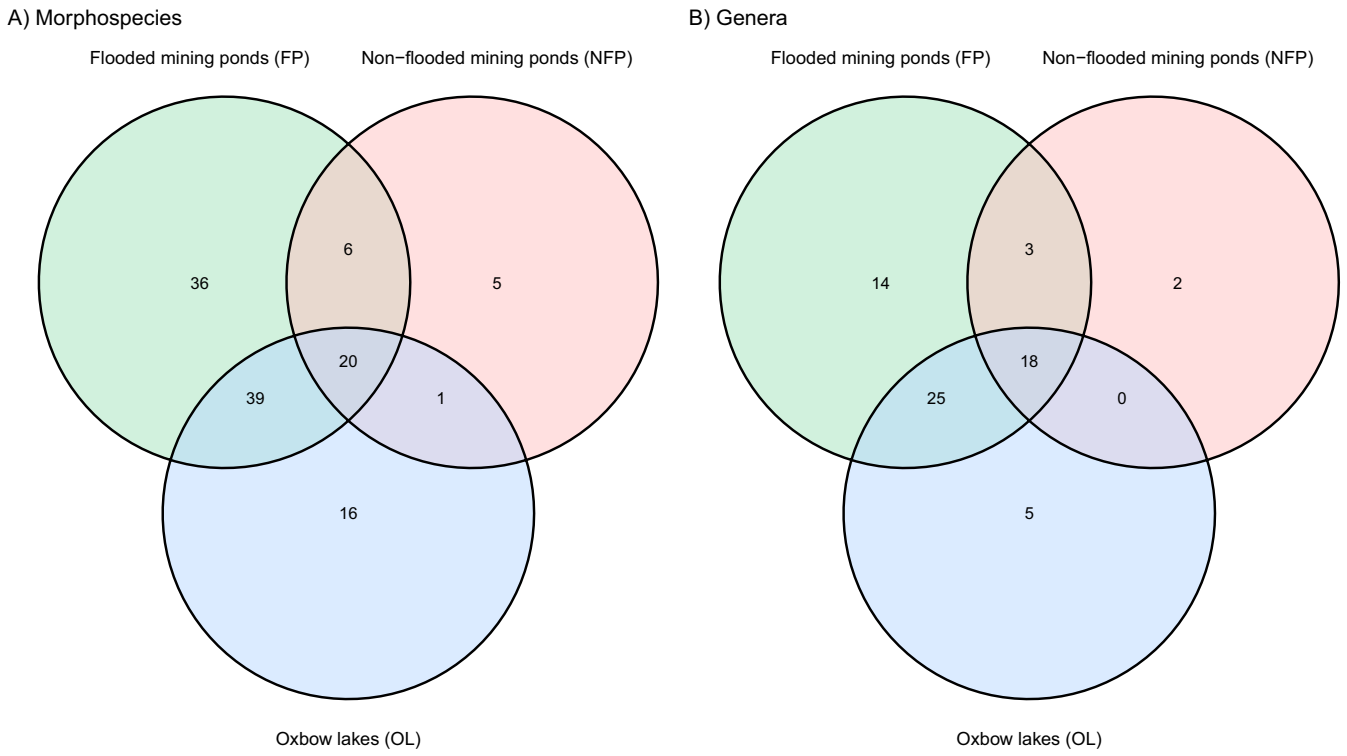

FIGURE 8 | Venn diagram showing the number of morphospecies and genera shared by the three different conditions (NFP, FP and OL), considering both approaches eDNA and TM combined.

freshwater ecosystems in that the insufficient reference databases for fish limit taxonomic resolution and restrict widespread adoption of molecular tools for biodiversity monitoring (Coutant et al. 2023; Hilário et al. 2023; Milan et al. 2020; Sales et al. 2021).

Taxonomic identification of fish using TM also has some hurdles. For instance, TM is more time-consuming and often yields controversial results, especially for morphologically challenging ichthyofauna or species lacking reliable taxonomic keys (e.g., *Brachyopomus*, *Eigenmannia*, *Loricariichthys*, *Hypostomus*,

Serrapinnus, *Moenkhausia*, *Knodus* and others) (Araújo-Flores 2016; van der Sleen and Albert 2017). For example, TM recorded six different species from the *Knodus* genera, but no taxonomic keys are available to distinguish them. Fish biodiversity monitoring using TM in the study area can be risky, especially when using techniques such as electrofishing, dip nets, seines and gillnets, which require extended stays at the monitoring sites. Within the study area, there have been reports of social disturbances related to mining activities (Dethier, Silman, Fernandez, et al. 2023) that currently prevent us from accessing locations that were previously considered safe and had the potential to host long-term ecological studies, such as Lechuza (NFP#1), Mega (NFP#2), Cobra (NFP#3) and Balata (NFP#4) (Araujo-Flores personal communication). Nevertheless, TM still has some advantages. We were able to detect species not found with the eDNA approach, possibly due to their position in the water column (i.e., mid-depth or deep layers not targeted in our eDNA surveillance procedure) (Rehill et al. 2024; Rourke et al. 2022), small size or low frequency (e.g., *Aequidens tetramerus*, *Anodus elongatus*, *Cetopsis coecutiens*, *Pterygoplichthys disjunctivus*, *Potamorrhaphis* cf. *eigenmanni*, *Bryconops melanurus* and *Apistogramma* spp.) (Galvis et al. 2006; van der Sleen and Albert 2017). All these genera and species were commonly reported using traditional fishing methods yet remained undetected by the 12S and COI markers in this study (Table 3).

Multiple studies have highlighted the effectiveness of molecular techniques for detecting invasive fish species, particularly in temperate regions (Duarte et al. 2023; Van Nynatten et al. 2023; Takahashi et al. 2023). However, in our study, we did not detect invasive species previously reported in the Madre de Dios river basin, such as *Arapaima* (Paiche or Pirarucu), *Oreochromis* (Tilapia) in the floodplain and *Oncorhynchus* (Trout or Trucha) in the Andean foothills (Goulding et al. 2014; Araújo-Flores 2016; Pelicice et al. 2021; Catâneo et al. 2022). The continuous development of molecular techniques for diversity monitoring in these new wetlands is still a challenge but represents a promising opportunity to effectively manage this vast and ever-changing territory, transformed by mining activities.

Finally, it is important to highlight that the eDNA approach was also able to identify other vertebrate taxa, 10 and 29 MOTUs with the COI and 12S marker, respectively, within the classes Mammalia, Reptilia, Amphibians and Aves (Tables S6 and S7). Notably, *Lontra longicaudis* ('river otter', Near Threatened), *Podocnemis unifilis* ('yellow-headed sideneck turtle' or 'taricaya', Vulnerable), *Tapirus terrestris* ('tapir', Vulnerable) and *Alouatta seniculus* ('red howler monkeys', Least Concern) were found in mining ponds as well as pristine lakes. The latter species was detected in Mega NFP#2, an area with significant mining activity, raising safety concerns. These findings underscore the importance of management strategies to protect vulnerable and near-threatened species within these aquatic ecosystems.

4.3 | Species Richness and Fish Communities

Our study revealed that seasonal flooding plays a crucial role in fish recolonisation of mining ponds, as previously suggested (Araújo-Flores et al. 2021). We found significant differences in fish species richness (Figure 5) and community composition (Figure 7) when analysing all three conditions of study sites with different degrees of connectivity to lotic systems (i.e., FP, NFP and OL), in contrast to the abandonment time and the dimensions of the pond or lake. We found that the FP (five mining ponds) exhibited the highest number of morphospecies and MOTUs, followed by OL (two lakes) and NFP (four mining ponds). Although longer abandonment periods might facilitate ecosystem recovery and recolonisation, our results indicate that connectivity to lotic systems is a more influential factor. NFPs, which lack such connections, do not benefit from incoming aquatic communities, nutrients and resources (Pander et al. 2018). For example, Lechuza NFP#1 and Mega NFP#2, located in La Pampa and not connected to lotic systems or forests, had the lowest richness among the study sites despite being the largest mining ponds and having been abandoned since 2011. Similarly, the Cobra NFP#3 and Balata NFP#4 sites, also located in La Pampa, though slightly influenced by a small stream from the Malinowski River, also presented relatively low diversity (Table 2) due to the lack of connection with a river main channel. Moreover, the similarity in species composition between Chambira FP#1 and NFP (Figure 7 and Table S1) suggests less restoration in this mining pond, which was also observed during field sample collection. Furthermore, the disappearance of migratory fish, many of which are of commercial interest, from the mining ponds of the Malinowski River (FP#1 and FP#2), a tributary heavily impacted by mining, suggests the deterioration of these ecosystems due to poor connectivity with the rest of the basin, hindering access to these areas for spawning (Arana and Chang 2005; Duponchelle et al. 2021).

Additionally, the dbRDA suggested a similar pattern in community composition (Figure S1), reinforcing that condition and transparency are key factors shaping aquatic communities. The clustering of NFP sites suggests that transparency plays a dominant role in structuring these environments, likely due to the absence of riverine inputs and their predominantly oligotrophic nature. In contrast, temperature, pH and Strahler stream order appear to be more influential in FP and OL sites, potentially reflecting differences in hydrological connectivity, nutrient dynamics and habitat complexity. However, given the limited number of sites in this study, further research with a broader dataset is needed to confirm these patterns and their ecological implications. This is further supported by the species accumulation curve (Figure S2), which suggests that additional sampling may be necessary to capture the full extent of species diversity in these environments.

Notably, families such as Cichlidae, Characidae, Erythrinidae and Curimatidae were present across almost all sites, which could indicate their role in recolonisation and adaptation to various conditions. Furthermore, the majority of the taxa found in NFP were present in all three conditions (Figure 8), indicating that the species in this ecosystem could be the first to recolonise environments with limited nutrients and resources, such as the genera Saxatilia and Cichlasoma, which are dominant in NFP sites.

Finally, FP exhibits even higher richness than OL (Table 2), such as the Inundacion FP#4 and the Ronsoco FP#2 sites, influenced by the Madre de Dios River and the Malinowski River,

respectively. This suggests potential ecosystem recovery, offering a hopeful perspective for post-mining restoration efforts. The lower richness in OL may result from their connection to the smaller La Torre River, which provides fewer nutrients and species compared to the Madre de Dios River, known for its critical role in seasonal flooding (Fernandes et al. 2014; Strahler 1952; Timana-Mendoza et al. 2024). Our study highlights the need for more pristine lakes to improve our understanding of landscape ecology (Terborgh et al. 2018).

5 | Conclusions

Our study demonstrates the efficacy of eDNA as a powerful, cost-efficient tool for monitoring fish diversity in post-ASGM landscapes, complementing traditional monitoring methods. Both 12S and COI molecular markers provided valuable insights, with the 12S marker showing more robustness and coverage in terms of the number of distinct MOTUs assigned to Actinopterygii across locations. In contrast, the COI marker offered higher species-level resolution possibly due to a more extensive reference database. We showed that using multiple molecular markers, along with traditional monitoring, provides a more comprehensive understanding of species richness in both mining ponds and oxbow lakes. While eDNA identified a higher number of taxa, traditional methods still detected unique species not captured by molecular techniques. Our results emphasise the need for continued sequencing efforts to expand genomic reference databases for Amazonian fishes in order to accurately identify species in post-mining ecosystems, including threatened species, to better inform conservation and restoration strategies. Notably, we found that the connectivity of the mining ponds to lotic systems plays a significant role in species richness and community composition in mining ponds. Flooded mining ponds exhibited species richness and assemblages almost comparable to those of unimpacted oxbow lakes, suggesting potential ecosystem recovery, which can be a hopeful perspective for post-mining restoration efforts. In contrast, mining ponds disconnected from lotic systems showed drastically reduced biodiversity, despite being abandoned for more years and with a greater pond dimension. However, we observed that some mammals use these areas, highlighting their importance for certain species and the need for ecosystem management. Moreover, our results revealed that transparency is a key driver in shaping community composition in NFP sites, while temperature, pH and Strahler stream order play a more significant role in influencing assemblages in FP and OL sites. These findings align with our broader results, reinforcing the influence of environmental factors on post-mining ecosystem recovery and species distribution. Overall, our findings provide critical insights into the recovery and biodiversity of post-ASGM ponds and support the integration of both eDNA and traditional methods for monitoring freshwater ecosystems in the Amazon basin.

Author Contributions

C.T.-M.: Experimental design, fieldwork, analysis and interpretation of the data, manuscript preparation and manuscript revision. A.R.-C.: Laboratory work and manuscript preparation. P.V.: Interpretation of the data and manuscript revision. R.B.: Taxonomic identification assistance and manuscript revision. M.C.S.-M.: Experimental design, analysis and interpretation of the data, manuscript preparation and manuscript revision. J.M.A.-F.: Secured funding, experimental design, fieldwork, interpretation of the data and manuscript revision. M.S. and L.E.F.: Secured funding, project administration and manuscript revision.

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Ethics Statement

This study was conducted under research permits RESOLUCIÓN DIRECTORAL No. 00476-2022-PRODUCE/DGPCHDI and RESOLUCIÓN DIRECTORAL No. 00812-2023-PRODUCE/DGPCHDI, both granted by the Ministerio de Produccion (PRODUCE, Peru), as well as RESOLUCIÓN JEFATURAL DE LA RESERVA NACIONAL TAMBOPATA No. 09-2022-SERNANP-JEF, granted by the Servicio Nacional de Áreas Naturales Protegidas por el Estado (SERNANP, Peru). All fieldwork and sampling were carried out in accordance with ethical guidelines for biodiversity research and conservation.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The raw sequencing data in FASTQ format used in this study is available at Zenodo (https://doi.org/10.5281/zenodo.15000947). Additional data used in the analyses is available as Supporting Information.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section.