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Integrating Direct Experimental Fishing and Environmental DNA Metabarcoding to Assess Fish Biodiversity in the Cibareno River, Indonesia, to Support Fishway Design

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ABSTRACT

1. A fishway is an engineered structure designed to help fish navigate past artificial barriers in rivers such as dams, weirs and regulators. An effective fishway design requires an understanding of fish biodiversity and migration requirements of the local community.
2. To support the conceptual design of a fishway at Caringin Weir in the Cibareno River in Indonesia, we conducted a fish biodiversity assessment using experimental fishing and an eDNA metabarcoding survey.
3. In early 2022, experimental fishing was conducted at six sampling sites upstream and downstream of the planned weir, and the sites were surveyed seven times during the wet and dry seasons. Environmental DNA sampling was conducted once during the wet season at each of the six sites.
4. A total of 72 aquatic species were detected, comprising 40 fish species and seven crustacean species captured during experimental fishing and 36 fish species identified through eDNA metabarcoding. Twelve fish species (18.46%) were detected using both methods. The total number of all species caught and species richness in experimental fishing varied significantly between upstream and downstream areas ($p < 0.05$), but there were no significant differences between seasons ($p > 0.05$). There

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was also a significant difference in the species richness upstream and downstream using eDNA metabarcoding ($p < 0.05$). There were also numerous diadromous species, further highlighting the need for an effective fishway design to enable these species to complete their migrations.

5. Based on the species present, migratory behaviour and economic importance, we prioritised six fish and crustacean species to be considered during fishway design: *Anguilla marmorata*, *Tor tambra*, *Barbodes binotatus*, *Sicyopterus* sp., *Rhyacichthys aspro* and *Macrobrachium* sp.

1 | Introduction

Inland waters are among the most vulnerable ecosystems directly impacted by human activities and climate change (Gozlan et al. 2019; Grafton et al. 2012). Globally, biodiversity in freshwater habitats has declined by at least 80% from 1970 to 2014 (Reid et al. 2019). Freshwater fish biodiversity has also become an important issue in Indonesia, where most aquatic freshwater resources are overexploited, and water pollution, ecosystem degradation, introduction of exotic species and habitat fragmentation have been linked to an increase in the number of vulnerable species (Gustiano, Kurniawan, and Haryono 2021; Hubert et al. 2015; Kurniawan et al. 2021). Habitat fragmentation in freshwater landscapes is predominantly caused by the installation of dams, weirs or regulators.

There are a total of 2363 dams, weirs and regulators across Indonesian rivers, with most of these structures located on the islands of Java and Sumatra (DGWR 2023). These structures reduce river connectivity, interrupt fish migrations and threaten fish biodiversity and the associated inland fisheries sector (Baumgartner and Wibowo 2018; Harris et al. 2017). In Indonesia, fish exhibit an enormous diversity in size, shape, biology and reproductive success in their natural habitat. Many species migrate to complete various stages of their life cycle. This includes adults undertaking spawning migrations; juveniles seeking nursery habitat; and all life stages seeking shelter during changing seasons (refugeeing), food foraging and finding a suitable environment for survival (Brönmark et al. 2014; Oliveira et al. 2020; Pollux et al. 2006; Worthington et al. 2022). To enable these migrations to continue in regulated river systems, fishways are often constructed to reinstate fish passage (Baumgartner et al. 2021; Katopodis, Kells, and Acharya 2001; Schletterer, Reind, and Thonhauser 2016).

The Cibareno River in West Java, Indonesia, forms a natural border between Banten and West Java Provinces. The upper reaches are located in the Halimun National Park area of West Java and the river flows approximately 27 km to the Pelabuhan Ratu Bay. The Cibareno River weir was constructed from early 2022 to 2023 to enable farmers to irrigate approximately 2000 ha of land, predominantly made up of rice fields. We conducted the fish survey during the construction of the irrigation weir, at a stage when the weir's foundation had been built to a height of approximately 1 m, out of the planned 3.25 m, extending from the left to the right of the riverbed. This weir construction creates challenges for fish migration, rendering passage feasible primarily during only high tide in the river. Before the river is totally fragmented by the weir, it is of importance to assess the fish biodiversity and composition both upstream and downstream of the Cibareno River. This preweir evaluation is a crucial foundation

for subsequent monitoring efforts. In addition, the fragmentation of fish populations may be occurring due to the changes in land use patterns surrounding the Cibareno River. Notable among these alterations are the transformation of forests into rice fields and plantations, the expansion of residential areas and the unregulated gold mining. These activities are inextricably linked to escalating concerns such as the influx of domestic waste, the contamination by pesticides and the discharge of pollutants from gold extraction processes into the river. There is no recent information available to evaluate the river's fish biodiversity. In 2002, there were ~22 species from 10 families recorded caught by an electrofishing method (Rachmatika, Sjafai, and Nurcahyadi 2002). But it is likely that fish biodiversity is much greater given that this river has never been intensively surveyed and the Island of Java is known to have very high fish species diversity with at least 408 species recorded (Wijaya et al. 2014). Furthermore, the Cibareno River flows into the sea, and thus, diadromous species with a requirement to move between freshwater and marine environments are likely to be common. Consequently, due to a highly variable local hydrology, a decision was made to install a vertical-slot fishway on the Caringin Weir to enable fish to complete their migrations.

The design of the fishway is integral to its performance given an inappropriate design will affect fish passage success (Atminarso et al. 2023; Harris et al. 2017; Sanchez-Perez et al. 2022). There are thousands of barriers to fish passage in Indonesia, and only four fishways have been installed to date (Baumgartner and Wibowo 2018). None of these four fishways were designed to meet the migratory needs of the local community. Furthermore, fishway effectiveness is poorly understood with only a single study examining fishway effectiveness at the Perjaya Dam on the Komerling River (Atminarso et al. 2023). This study found that the fish community was significantly different upstream and downstream of the Perjaya Dam, indicating that the fishway was not functioning effectively (Atminarso et al. 2023). Therefore, prior to the construction of the Caringin Weir fishway, there was a need to understand whether the fish community was already fragmented by the weir and to determine if the species were dominated by diadromous species given this would influence the fishway design (Rourke et al. 2018; Verhelst et al. 2021). Furthermore, it is also crucial that the fishway can cater to the migratory requirements of the species with high economic importance to enable the local community to derive a living from fishing. Therefore, the fishway needed to be highly functional and ensure the sustainability of the Cibareno River's fish resources.

Fish biodiversity is typically measured through traditional fish sampling methods that use both active and passive fishing gears. Given fish presence and abundance will vary according to

the location, time of day and season, it is important to conduct targeted fish surveys at a range of spatial and temporal scales (Baumgartner, Stuart, and Zampatti 2008; DSWEPC 2011). Traditional fish sampling methods are now often complemented by environmental DNA (eDNA) methods to assess fish biodiversity in a range of aquatic environments (Compson et al. 2020; Goutte et al. 2020). eDNA approaches are useful for describing biodiversity because they are non-invasive (Bergman et al. 2016; Dejean et al. 2012; Valentini et al. 2016), cost-effective (Anderson et al. 2018; Biggs et al. 2015; Evans et al. 2017; Miya et al. 2015; Takahara, Minamoto, and Doi 2013) and often more sensitive than conventional methods (Herve et al. 2022; Janosik and Johnston 2015; Sigsgaard et al. 2015). Specifically, eDNA has been shown to be an effective tool for monitoring and conservation (Rourke et al. 2021; Sahu et al. 2023; Zou et al. 2020). There have been few studies using eDNA metabarcoding to detect fish diversity in Indonesian freshwater habitats, but the studies to date indicate great promise for detecting fish species richness in this biodiverse environment (Roesma et al. 2021; Wibowo et al. 2022).

In this study, we combine conventional fishing surveys and an eDNA metabarcoding survey carried out during the construction of the Caringin Weir to (1) update the checklist of the freshwater organisms of the Cibareno River, with a particular emphasis on fish and migratory crustaceans, (2) determine whether the fish and crustacean communities of the Cibareno River have been fragmented by the changes induced by land use and by the constructed weir, (3) determine whether diadromous species have been disproportionately impacted by the weir and (4) make recommendations on the conceptual design of a future fishway in the Cibareno River.

2 | Methods

2.1 | Study Area

The Cibareno River is one of the eight rivers flowing into the Pelabuhan Ratu Bay. The Cibareno River supports a diverse range of diadromous (catadromous and amphidromous) fish species and heavily harvested species by inland fisheries (Hakim et al. 2015). There is a single weir (Caringin Weir, S 6.8783°, E 106.4200°) that may interfere with fish migration in the Cibareno River. The weir was under construction in early 2022 including during the 7 months of the surveys during which the weir's foundation was elevated by 1 m (the final height will be 3.25 m high) above the riverbed, extending from one bank to the other. To mitigate the impacts of the weir on fish migration, a fishway will be installed to enable fish to move freely between upstream and downstream locations and to ensure river connectivity, particularly for species migrating downstream as larvae (i.e., amphidromy) or adults (i.e., catadromy). Six sampling locations were selected upstream ($n=3$) and downstream ($n=3$) of the Caringin Weir. Upstream and downstream sampling sites were located at 0, 5 and 10 km from the weir (Figure 1). At the upstream sites, Upstream 1 is surrounded by rice fields, plantations and unregulated gold mining areas on both the left and right sides of the river. Similarly, Upstream 2 includes forest, rice fields, plantations, small-scale gold mining areas, fish ponds and a wood-cutting factory that discharges sawdust into

the river. Upstream 3 and Downstream 1 are neighbouring areas but are separated by the Caringin Weir. Upstream 3 is an upstream area of the weir, dominated by rice fields and plantations, whereas Downstream 1, downstream of the weir, is a relatively well-preserved plantation and forest area. Downstream 2 is primarily characterised by rice fields, plantations and residential areas. Downstream 3 is an area adjacent to the estuary (1 km), dominated by rice fields, plantations and settlements and featuring a small-scale sand mine. The coordinates, altitudes, slopes, widths and discharges of the sampling locations along the Cibareno River are detailed in Table 1.

2.2 | Experimental Fishing

Over a 7-month period, fish were collected during daytime in the dry season (February–May 2022) and the rainy season (June–September 2022) using a range of fishing gears including cast nets, gillnets, fyke nets, bait traps and bamboo traps.

Experimental fishing with cast nets was carried out in three operational trials, each of which was repeated 30 times of effort (90 times in total). Two sets of gill nets with six different mesh sizes (1.9, 2.5, 3.8, 5.0, 7.6 and 10.2 cm; each mesh panel was 400 cm long by 100 cm high) were set up on opposite sides of the river. Two sets of fyke nets were installed on opposite sides of the river, with one set opposite to the current and the other set in the same direction as the current. Eight sets of Jarvis traps (40 × 20 cm) and bamboo traps (80 × 25 cm) were set at the vegetated areas of the river. All fishing gears including gillnets, fyke nets, Jarvis and bamboo traps were checked after 4 h, conducted once per day. Experimental fishing was conducted on the same day every month from February 2022 to September 2022.

Fish were anaesthetised using 2-phenoxyethanol (0.5 mL/L of water) before being identified, photographed, measured (fork length or total length) and weighed. When systematic knowledge was insufficient for reliable identification of the species, all adult fish caught were identified to genus level following (Kottelat et al. 1993; Rachmatika, Sjafei, and Nurcahyadi 2002).

2.3 | eDNA Collection and Laboratory Methods

eDNA samples were collected from the six sampling locations only in the first survey (February 2022). Triplicate 1-L water samples were collected at the sampling site and stored in an insulated container prior to filtering. Water samples were filtered within 4–6 h of collection using a mixed cellulose ester membrane filter (pore size: 0.45 μm; Whatman) in a sterile filter housing connected to a vacuum pump. Each of the three water samples collected at each site was filtered through the same filter, resulting in six filters per site. The filter paper was cut in half with sterile scissors and tweezers to create two technical replicates. Each filter paper half was placed in a 2-mL cryotube (Cryo Tubes, presterilised) containing 2 mL of DNA shield liquid (ZymoBIOMICS DNA Research) and stored in a plastic cryobox and stored at −20°C (Biologix, 100 wells) (Gelis et al. 2021; Zamani, Zuhdi, and Maddupa 2022).

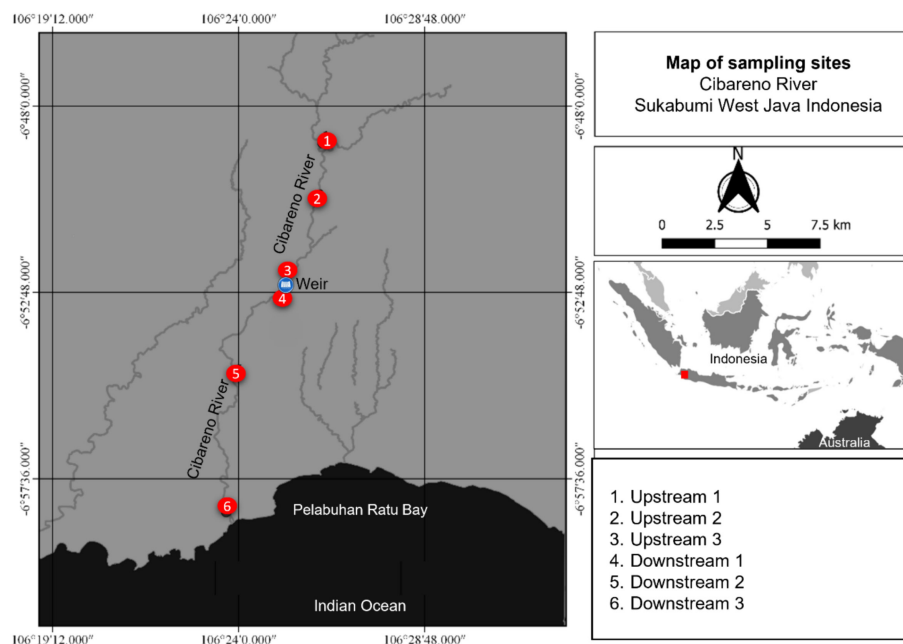


FIGURE 1 | Map of the study location in the Cibareno River, Sukabumi, West Java Indonesia.

TABLE 1 | Coordinates, altitudes, river slopes, river widths and mean discharges of the sampling locations of Cibareno River, West Java, Indonesia.

Coordinate	Sampling site	Altitude (m)	Slope (%)	Width (m)	Discharge (m ³ /s)
S 6.8157°, E 106.4361°	Upstream 1	499	3.07	15	8.001
S 6.8409°, E 106.4338°	Upstream 2	435	2.73	24	10.129
S 06.8808°, E 106.4185°	Upstream 3	310	3.60	22	13.247
S 06.8768°, E 106.4200°	Downstream 1	278	3.49	22	18.911
S 06.9161°, E 106.3985°	Downstream 2	137	2.08	32	17.221
S 06.9743°, E 106.3946°	Downstream 3	25	0.91	36	15.469

The metabarcoding work was conducted at the Genetika Science Laboratory (Banten, Indonesia). DNA concentration and quality were determined using NanoDrop spectrophotometers and a Qubit fluorometer. Genomic DNA from each filter paper was extracted using a ZymoBIOMICS DNA Miniprep Kit (Zymo Research, D4300). The MiFish primer pairs (MiFish-U-F, 5'-GCCGGTAAACTCGTGCCAGC-3'; MiFish-U-R, 5'-CATAGTAGGTATCTAATCCTA-GTTG-3') were used to target a ~172-bp amplicon from a variable region of the 12S rRNA mitochondrial gene (Miya et al. 2015; Taberlet et al. 2018). Following extraction, there were a total of six samples and one PCR blank. The PCRs contained a total volume of 24 μ L composed of 12.5 μ L of MyTaq Red Mix (Bioline), 1 μ L of the 5- μ M premixed forward and reverse primers (Macrogen), 3 μ L of DNA (10–15 ng/ μ L) and 7.5 μ L of sterile water. Thermal cycling conditions included an initial denaturing step of 95°C for 10 min, 40 cycles of 95°C for 30 s, 60°C for 45 s and 72°C for 30 s and a final extension step of 72°C for 5 min. PCR products were visually examined on a 1.2% agarose gel, stained with GelRed Nucleic Acid Gel Stain using a 100-bp ladder (Invitrogen) to verify that the PCR products were of the expected size.

MinION sequencing libraries were prepared following the native barcoding genomic DNA protocol and consisted of end-prep, barcoding ligation and adapter ligation (Toxqui Rodriguez, Vanhollebeke, and Derycke 2023). Modifications to the protocol included the use of a Bioanalyzer (Agilent) to check the size and to quantify the library at the end of each step (end-prep, barcoding ligation and sequencer adapters) and DNA quantification using the Qubit fluorometer. The libraries were sequenced using a flow cell (which was quality-checked in order to ensure the presence of at least 800 active biological nanopores for each run) and using MinKNOW v20.10.3 to run MinKNOW core v4.1.2 and Guppy 4.0.11 in high-accuracy mode (Wick, Judd, and Holt 2019). The quality of fastq files was visualised using NanoPlot (De Coster et al. 2018). Filtered reads were classified using a Centrifuge classifier (Kim et al. 2016). The consensus of reads was extracted using Medaka v1.5.0 (<https://github.com/nanoporetech/medaka>). The extracted consensus sequence was aligned against the NCBI database using BLAST (<https://blast.ncbi.nlm.nih.gov>). The DNA barcoding threshold used to confirm species assignment was defined as a sequence similarity of greater than 97%.

2.4 | Fish Migration and Fish Size

To determine which fish migrate from upstream to downstream and vice versa, as well as to assess the size of fish that could pass through Caringin Weir, we categorised fish based on whether they were found exclusively upstream, downstream or in both locations, with the weir serving as the reference point. The specific type of fish migration, namely, catadromous (adults migrate downstream to the sea to spawn and juveniles return upstream), amphidromous (adults live in freshwater, larvae drift downstream to the estuary or sea and juveniles return upstream) or potamodromous (i.e., adults perform migrations in freshwater for reproduction), was determined through a review of relevant literature. Additionally, we employed heat map analysis to visualise the distribution of fish migration at each station, spanning from upstream to downstream. For further analysis, the captured fish were grouped according to their minimum and maximum lengths, serving as a valuable reference for sizing

and designing fishways (O'Connor et al. 2022). These findings were then compared to existing literature for a comprehensive assessment.

2.5 | Data Analysis

Taxonomic identification data of fish were arranged by order, family, genus and species. The distribution and relative abundance of fish species for each sampling location were visualised on maps using bar charts. A Venn diagram was constructed to illustrate the number of fish species detected upstream and downstream of the weir using experimental fishing and metabarcoding results using an online program (<http://bioinformatics.psb.ugent.be/webtools/venn/>). Species richness, Shannon diversity and Simpson dominance index (D) analysis were analysed using the Biodiversity Pro 2 package (McAleece et al. 1997). Subsequently, statistical analysis (PERMANOVA)

TABLE 2 | Fish biodiversity collected by experimental fishing in upstream and downstream of the weirs in Cibareno River, Sukabumi, West Java, Indonesia from February to September 2022.

Order	Family	Genus	Species	Upstream		Downstream		Seasons
				<i>n</i>	%	<i>n</i>	%	
Anguilliformes	Anguillidae	1	3	0	0	33	2.68	Wet and dry
	Muraenidae	1	1	0	0	1	0.08	Dry
	Ophichthidae	1	1	0	0	1	0.08	Wet
Centrarchiformes	Kuhliidae	1	1	0	0	155	12.57	Wet and dry
Cichliformes	Cichlidae	2	2	20	3.37	6	0.49	Wet and dry
Cypriniformes	Cyprinidae	3	3	476	80.27	196	15.90	Wet and dry
Cyprinodontiformes	Poeciliidae	2	2	54	9.11	11	0.89	Wet and dry
Gobiiformes	Eleotridae	2	2	0	0	24	1.95	Wet and dry
	Gobiidae	4	5	7	1.18	70	5.68	Wet and dry
	Oxudercidae	3	8	28	4.72	386	31.31	Wet and dry
	Rhacichthyidae	1	1	5	0.84	108	8.76	Wet and dry
Mugiliformes	Mugilidae	1	1	0	0	1	0.08	Wet
Perciformes	Ambassidae	1	1	0	0	27	2.19	Wet
	Carangidae	1	1	0	0	1	0.08	Wet
	Lutjanidae	1	1	0	0	1	0.08	Wet
Scorpaeniformes	Tetrarogidae	1	1	0	0	3	0.24	Wet and dry
Siluriformes	Clariidae	1	2	2	0.34	0	0	Wet
	Sisoridae	1	1	1	0.17	1	0.08	Dry
Syngnathiformes	Syngnathidae	1	2	0	0	63	5.11	Wet and dry
Tetraodontiformes	Tetraodontidae	1	1	0	0	1	0.08	Wet
Decapoda	Atyidae	1	1	0	0	32	2.60	Wet and dry
	Gecarcinucidae	1	1	0	0	4	0.32	Wet
	Palaemonidae	2	5	0	0	108	8.76	Wet and dry
Total		34	47	593	100	1233	100	

was implemented to evaluate the significance of differences in the distribution of total fish caught, species richness, diversity and dominance indices at each sampling location using the PRIMER v7 package (Clarke and Gorley 2015) for both direct fishing and eDNA survey. This application was also used to generate a heat map to illustrate fish species that were found upstream and downstream of the weir for direct fishing.

3 | Results

3.1 | Experimental Fishing

There were 47 aquatic species representing 13 orders caught during experimental fishing. This included 40 fish species from 20 families and seven crustaceans from three families (Table 2). Gobiiformes was the most represented order with 16 species from four families followed by Decapoda with seven species from three families and Anguilliformes with five species from three families, and all the remaining orders had less than four species. Seven fish families were found both upstream and downstream of the weir, 15 families were found only downstream of the weir, and one family was found only upstream of the weir. Cyprinidae was the most common family of fish upstream of the weir (80.27%), followed by Poeciliidae (9.11%), Oxudercidae (4.72%), Cichlidae (3.37%) and the remaining four families (2.53%). The number of fish-based families was greater downstream (22 families) than upstream (eight families) (Table 2). Similarly, downstream sites had 35 more species than upstream sites, which had seven species (Figure 2).

The number of fish species and species richness caught were significantly different between upstream and downstream sites ($p < 0.05$), but there was no difference between seasons ($p > 0.05$)

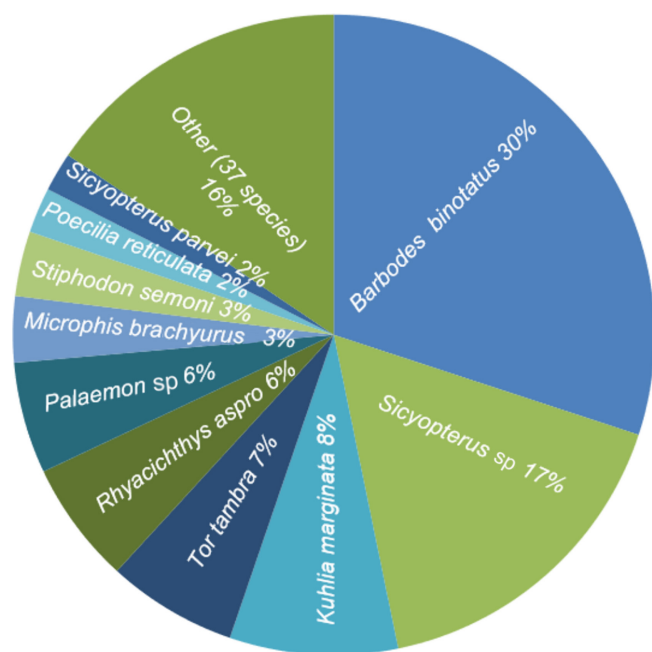


FIGURE 2 | Species proportion in all sites ranked by abundance during the 7-month survey from February to September 2022 on the Cibareno River, Sukabumi, West Java.

(Tables 3 and 4). In the downstream area, 19 fish and crustacean species were not represented in the other five sampling sites, namely, *Ambassis buruensis*, *Anguilla bicolor*, *Anguilla* sp., *Caridina brevicarpalis*, *Caranx sexfasciatus*, *Eleotris* sp., *Glossogobius giuris*, *Kuhlia marginata*, *Lutjanus fuscescens*, *Liza macrolepis*, *Awaous ocellaris*, *Schismatogobius insignis*, *Schismatogobius* sp., *Macrobrachium australe*, *Macrobrachium rosenbergii*, *Palaemon* sp., *Microphis brachyurus*, *Tetraodon* sp. and *Vespicula* sp. Only nine of the 41 fish species were identified both upstream and downstream of the weir, including *Barbodes binotatus*, *Glyptothorax platypogon*, *Poecilia reticulata*, *Rhyacichthys aspro*, *Sicyopterus parvei*, *Sicyopterus* sp., *Tilapia niloticus* and *Tor tambra* (Figure 3). We also found six species, namely, *Andinoacara rivulatus*, *Clarias batrachus*, *Clarias gariepinus*, *Cyprinus carpio*, *Lentipes ikeae* and *Xiphophorus hellerii* represented only in upstream areas.

The relative abundance of fish per species varied across the six sampling sites, with *B. binotatus*, for example, having a relatively high abundance ranging from 0.52% at Downstream 3 to 82.46% at Upstream 2 (Figure 3a). Furthermore, when fish were ranked by abundance (Figure 2), two species dominated all sites: *B. binotatus* (30% of all specimens) and *Sicyopterus* sp. (17% of all specimens).

3.2 | eDNA Survey

The one-off eDNA survey detected 36 species from nine orders and 20 families (Table 4). Gobiiformes was the most abundant order, with nine species from four families followed by Cypriniformes with five species from three families and Perciformes with four species from three families, with the remaining orders having less than four species. There were two new orders discovered: Anabantiformes (four species from three families) and Elopiformes (one species from one family). We detected nine families both upstream and downstream of the weir, whereas three families were detected only upstream, and five families were detected only downstream. The largest number of raw reads of the fish families in the upstream area was Cyprinidae (23.86%) followed by Osphronemidae (17.50%), Channidae (14.77%) and Anabantidae (6.82%), and the remaining was 13 families (37.50%). The number of fish families was greater upstream (17 families) than downstream (15 families) (Table 5).

3.3 | Integrated Findings

The result of the fish biodiversity assessment integrating experimental fishing and eDNA surveys is shown in Table 6 and Figure 3. In total, 72 fish and crustacean species were detected in the Cibareno River including 65 fish species from 26 families and seven crustacean species from three families. We identified 40 fish species and seven crustaceans using direct fishing and detected 36 fish species using eDNA. A total of 12 species (18.46%) detected in the eDNA survey were also found in direct fishing (12 species from the present study), whereas the remaining 19 species have not been previously recorded from the Cibareno River. In addition, the eDNA results showed that a further 12 fish species were found upstream

TABLE 3 | The statistical comparison of total fish caught between upstream and downstream sampling sites of the Cibareno River using the PERMANOVA test.

Source	df	SS	MS	Pseudo-F	p (perm)	Perms
Location	1	22,749	22,749	12.29	0.0001	9939
Season	1	1318.9	1318.9	0.71	0.6168	9938
Location vs. season	1	1695.6	1695.6	0.92	0.4546	9926
Residual	38	70,346	1851.2			
Total	41	99,111				

Note: Significant value ($\alpha=0.05$); p values are indicated in bold font. Abbreviations: df, degree of freedom; MS, mean squares; Perms, number of permutations; SS, sum of squares.

TABLE 4 | The statistical comparison of species richness (based on direct fishing) between upstream and downstream (location) of the Cibareno River using the PERMANOVA test.

Source	df	SS	MS	Pseudo-F	p (perm)	Perms
Source	1	3447.4	3447.4	8.4	0.0003	9924
Location	1	88.2	88.2	0.2	0.9273	9951
Season	1	118.5	118.5	0.3	0.8169	9943
Location vs. season	38	15,662	412.2			
Residual	41	19,844				

Note: Significant value ($\alpha=0.05$); p values are indicated in bold font. Abbreviations: df, degree of freedom; MS, mean squares; Perms, number of permutations; SS, sum of squares.

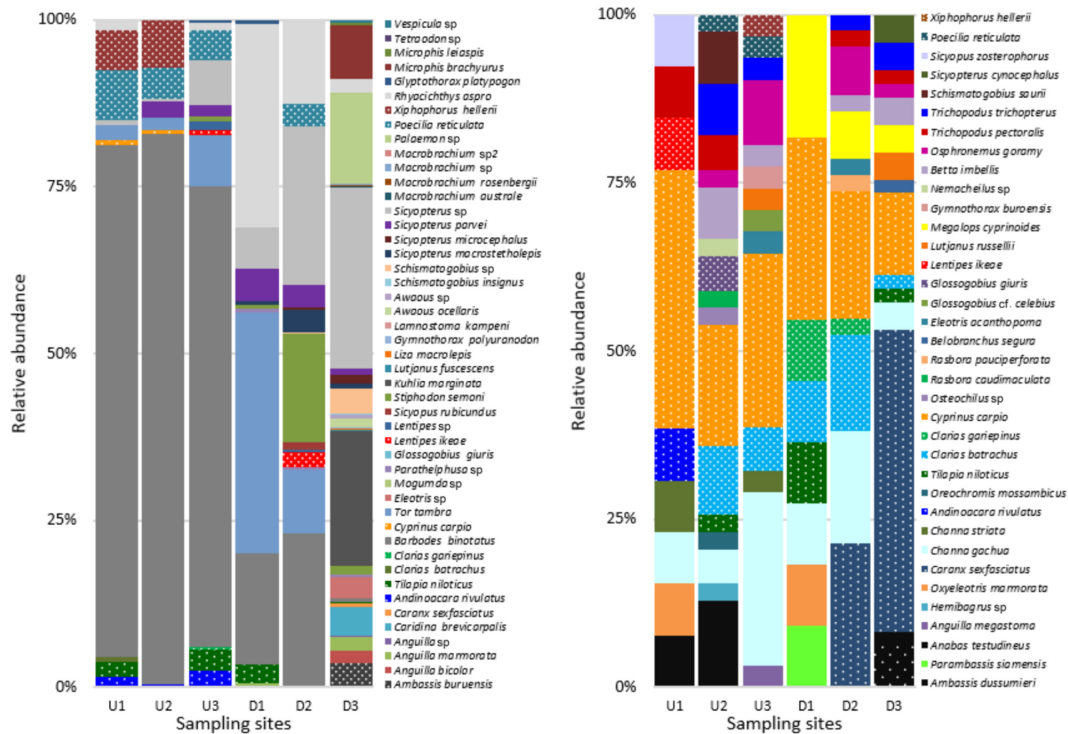


FIGURE 3 | Relative abundance of fish species identified using experimental fishing (a) and eDNA survey (b) in upstream and downstream sampling sites. The species similarity ratio between eDNA and fishing is 18.46% (12/65). Experimental fishing was conducted during the 7-month survey from February to September 2022, and the eDNA survey was conducted once in February 2022 at Cibareno River, Sukabumi, West Java. U (upstream) and D (downstream) followed by a number (1–3) refer to the location of sampling sites in relation to the Caringin Weir.

TABLE 5 | Fish biodiversity collected by eDNA survey (raw reads) upstream and downstream of the Caringin Weir in the Cibareno River, Sukabumi, West Java, Indonesia, in February 2022.

Taxonomic level collected by eDNA				DNA raw reads			
Order	Family	Genus	Species	Upstream		Downstream	
				Number of reads	%	Number of reads	%
Anabantiformes	Anabantidae	1	1	6	6.90	1	0.94
	Channidae	1	2	15	17.24	12	11.32
	Osphronemidae	3	4	15	17.24	12	11.32
Anguilliformes	Anguillidae	1	1	1	1.15	0	0.00
	Muraenidae	1	1	1	1.15	0	0.00
Cichliformes	Cichlidae	2	3	3	3.45	2	1.89
Cypriniformes	Cyprinidae	2	2	21	24.14	17	16.04
	Danionidae	1	2	1	1.15	1	0.94
	Nemacheilidae	1	1	1	1.15	0	0.00
Cyprinodontiformes	Poeciliidae	2	2	5	5.75	2	1.89
Elopiformes	Megalopidae	1	1	0	0.00	7	6.60
Gobiiformes	Butidae	1	1	1	1.15	1	0.94
	Eleotridae	2	2	1	1.15	2	1.89
	Gobiidae	2	3	4	4.60	0	0.00
	Oxudercidae	3	3	4	4.60	2	1.89
Perciformes	Ambassidae	2	2	0	0.00	4	3.77
	Carangidae	1	1	0	0.00	31	29.25
	Lutjanidae	1	1	1	1.15	2	1.89
Siluriformes	Bagridae	1	1	1	1.15	0	0.00
	Clariidae	1	2	6	6.90	10	9.43
Total		30	36	87	100	106	100

and downstream of the weir, namely, *Anabas testudineus*, *Oxyeleotris marmorata*, *Channa gachua*, *Oreochromis niloticus*, *C. batrachus*, *C. carpio*, *Eleotris acanthopoma*, *Lutjanus russellii*, *Betta imbellis*, *Osphronemus goramy*, *Trichopodus pectoralis* and *Trichopodus trichopterus* (Table 5). The number of fish species overlapping from the experimental fishing and eDNA metabarcoding survey upstream and downstream of Caringin Weir can be found in Figure 4.

Estimates of species richness differed significantly between upstream and downstream sites of the river in both direct fishing and eDNA surveys ($p < 0.05$) (Figure 5a). The Shannon–Wiener index (H') and Simpson dominance index were significantly different upstream and downstream in experimental fishing ($p < 0.05$), whereas there were no significant differences in the Shannon–Wiener index (H') and Simpson dominance index in the eDNA survey. The diversity index H' of direct fishing ranged from 0.32 to 1.05 categorised as low to moderate level; similarly, the eDNA survey ranged from 0.85 to 1.13, indicating low to moderate criteria. The dominance index (D) of direct fishing

ranged from 0.15 to 0.69 categorised dominance, whereas the eDNA survey ranged from 0.07 to 0.13. The diversity index (H') index in both direct fishing and the eDNA survey is negatively correlated to the dominance index (D) ($r = -0.94$, $p < 0.05$) (Figure 5b,c). The downstream area has significantly more diverse fish communities than the upstream location in both direct fishing and eDNA survey.

3.4 | Fish Migration and Size

The fish species present at the Cibareno River migrate upstream and downstream at a variety of life stages, including juveniles and adults. We collected catadromous and amphidromous species, which were commonly associated with freshwater–marine migration patterns. Three species categorised as catadromous species were identified, namely, *A. bicolor*, *Anguilla marmorata*, and *Anguilla* sp. (Table 6). The freshwater eel *A. megastoma* was also detected in the eDNA survey. Based on the experimental fishing, adult fish at the Cibareno River could vary in

TABLE 6 | The list of fish species detected at Cibareno River, Sukabumi, West Java, Indonesia, collected based on experimental fishing (Rachmatika, Sjafei, and Nurcahyadi 2002) and eDNA survey.

No.	Family	Species	Identification method	Fish collection	Remark
A	Fish				
1	Ambassidae	<i>Ambassis buruensis</i>	Morphological identification	Present study	Native
2		<i>Ambassis dussumieri</i>	eDNA/blast	Present study	Native
3		<i>Parambassis siamensis</i>	eDNA/blast	Present study	Native
4	Anabantidae	<i>Anabas testudineus</i>	eDNA/blast	Present study	Native
5	Anguillidae	<i>Anguilla bicolor</i>	Morphological identification	Present study	Native
6		<i>Anguilla marmorata</i>	Morphological identification	Present study	Native
7		<i>Anguilla</i> sp.	Morphological identification	Present study	Native
8		<i>Anguilla megastoma</i>	eDNA/blast	Present study	Native
9	Bagridae	<i>Hemibagrus</i> sp.	eDNA/blast	Present study	Native
10	Butidae	<i>Oxyeleotris marmorata</i>	eDNA/blast	Present study	Native
11	Carangidae	<i>Caranx sexfasciatus</i>	Morphological identification and eDNA/blast	Present study	Native
12	Channidae	<i>Channa gachua</i>	Morphological identification and eDNA/blast	Previous study (Rachmatika, Sjafei, and Nurcahyadi 2002) and eDNA/blast	Native
13		<i>Channa striata</i>	eDNA/blast	Present study	Native
14	Cichlidae	<i>Andinoacara rivulatus</i>	Morphological identification and eDNA/blast	Present study	Exotic
15		<i>Oreochromis mossambicus</i>	Morphological identification and eDNA/blast	Previous study (Rachmatika, Sjafei, and Nurcahyadi 2002) and eDNA/blast	Exotic
16		<i>Tilapia niloticus</i>	Morphological identification and eDNA/blast	Present study	Exotic
17	Clariidae	<i>Clarias batrachus</i>	Morphological identification and eDNA/blast	Present study	Native
18		<i>Clarias gariepinus</i>	Morphological identification and eDNA/blast	Present study	Exotic
19	Cyprinidae	<i>Cyprinus carpio</i>	Morphological identification and eDNA/blast	Present study	Exotic
20		<i>Tor tambra</i>	Morphological identification	Present study	Native
21		<i>Osteochilus</i> sp.	eDNA/blast	Present study	Native
22	Danionidae	<i>Rasbora caudimaculata</i>	eDNA/blast	Present study	Native
23		<i>Rasbora pauciperforata</i>	eDNA/blast	Present study	Native
24	Eleotridae	<i>Eleotris</i> sp.	Morphological identification	Present study	Native
25		<i>Eleotris acanthopoma</i>	eDNA/blast	Present study	Native
26		<i>Mogurnda</i> sp.	Morphological identification	Present study	Native
27		<i>Belobranchius segura</i>	eDNA/blast	Present study	Native

(Continues)

TABLE 6 | (Continued)

No.	Family	Species	Identification method	Fish collection	Remark
28	Gobiidae	<i>Glossogobius giurii</i>	Morphological identification and eDNA/blast	Present study	Native
29		<i>Lentipes ikeae</i>	Morphological identification and eDNA/blast	Present study	Native
30		<i>Lentipes</i> sp.	Morphological identification	Present study	Native
31		<i>Sicyopterus rubicundus</i>	Morphological identification	Present study	Native
32		<i>Sicyopterus zosterophorus</i>	Morphological identification and eDNA/blast	Present study	Native
33		<i>Stiphodon semoni</i>	Morphological identification	Present study	Native
34		<i>Glossogobius cf. celebius</i>	eDNA/blast	Present study	Native
35	Kuhliidae	<i>Kuhlia marginata</i>	Morphological identification	Present study	Native
36	Lutjanidae	<i>Lutjanus fuscescens</i>	Morphological identification	Present study	Native
37		<i>Lutjanus russellii</i>	eDNA/blast	Present study	Native
38	Megalopidae	<i>Megalops cyprinoides</i>	Morphological identification and eDNA/blast	Previous study (Rachmatika, Sjafei, and Nurcahyadi 2002) and eDNA/blast	Native
39	Mugilidae	<i>Liza macrolepis</i>	Morphological identification	Present study	Native
40	Muraenidae	<i>Gymnothorax polyuranodon</i>	Morphological identification	Present study	Native
41		<i>Gymnothorax buroensis</i>	eDNA/blast	Present study	Native
42	Oxudercidae	<i>Awaous ocellaris</i>	Morphological identification	Present study	Native
43		<i>Awaous</i> sp.	Morphological identification	Present study	Native
44		<i>Schismatogobius insignis</i>	Morphological identification	Present study	Native
45		<i>Schismatogobius saurii</i>	eDNA/blast	Present study	Native
46		<i>Schismatogobius</i> sp.	Morphological identification	Present study	Native
47		<i>Sicyopterus microcephalus</i>	Morphological identification	Present study	Native
48		<i>Sicyopterus macrostolepis</i>	Morphological identification	Present study	Native
49		<i>Sicyopterus parvei</i>	Morphological identification	Present study	Native
50		<i>Sicyopterus cynocephalus</i>	Morphological identification and eDNA/blast	Present study, previous study (Rachmatika, Sjafei, and Nurcahyadi 2002) and eDNA/blast	Native
51		<i>Sicyopterus</i> sp.	Morphological identification	Present study	Native
52	Nemacheilidae	<i>Nemacheilus</i> sp.	eDNA/blast	Present study	Native

(Continues)

TABLE 6 | (Continued)

No.	Family	Species	Identification method	Fish collection	Remark
53	Osphronemidae	<i>Betta imbellis</i>	eDNA/blast	Present study	Native
54		<i>Osphronemus goramy</i>	eDNA/blast	Present study	Native
55		<i>Trichopodus pectoralis</i>	eDNA/blast	Present study	Native
56		<i>Trichopodus trichopterus</i>	Morphological identification and eDNA/blast	Previous study (Rachmatika, Sjafei, and Nurcahyadi 2002) and eDNA/blast	Native
57	Ophichthidae	<i>Lamnostoma kampeni</i>	Morphological identification	Present study	Native
58	Poeciliidae	<i>Poecilia reticulata</i>	Morphological identification and eDNA/blast	Present study	Exotic
59		<i>Xiphophorus hellerii</i>	Morphological identification and eDNA/blast	Present study	Exotic
60	Rhyacichthyidae	<i>Rhyacichthys aspro</i>	Morphological identification	Present study	Native
61	Sisoridae	<i>Glyptothorax platypogon</i>	Morphological identification	Present study	Native
62	Syngnathidae	<i>Microphis brachyurus</i>	Morphological identification	Present study	Native
63		<i>Microphis leiaspis</i>	Morphological identification	Present study	Native
64	Tetraodontidae	<i>Tetraodon</i> sp.	Morphological identification	Present study	Native
65	Tetrarogidae	<i>Vespicola</i> sp.	Morphological identification	Present study	Native
B	Crustacean				
66	Palaemonidae	<i>Macrobrachium australe</i>	Morphological identification	Present study	Native
67		<i>Macrobrachium rosenbergii</i>	Morphological identification	Present study	Native
68		<i>Macrobrachium</i> sp. 1	Morphological identification	Present study	Native
69		<i>Macrobrachium</i> sp. 2	Morphological identification	Present study	Native
70		<i>Palaemon</i> sp.	Morphological identification	Present study	Native
71	Atyidae	<i>Caridina brevicarpalis</i>	Morphological identification	Present study	Native
72	Gecarcinucidae	<i>Parathelphusa</i> sp.	Morphological identification	Present study	Native

(Continues)

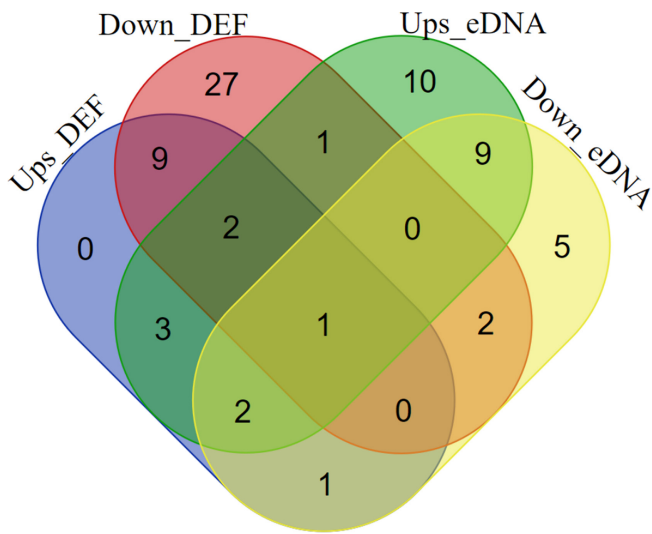


FIGURE 4 | Venn diagram showing the number of fish species overlapping from the experimental fishing and eDNA metabarcoding survey upstream and downstream of the Caringin Weir, Sukabumi, West Java, Indonesia. Down = Downstream, Ups = Upstream, DEF = direct experimental fishing.

size from 10 to >600 mm long. We also found that many fish species in the Cibareno River were gobies (Gobiidae = 7 species and Oxudercidae = 11 species), which are likely amphidromous. Gobies migrating upstream and downstream had a minimum size of 22 mm and a maximum size of 148 mm (Table 7). Besides the catadromous and amphidromous species, there were migratory freshwater species that are also presented in Figure 6, where seven fish can be found upstream and downstream, namely, *T. tambra*, *B. binotatus*, *A. rivulatus*, *T. niloticus*, *P. reticulata*, *R. aspro* and *G. platypogon*. These species may migrate from upstream to downstream for spawning, feeding and refuge. The largest size in this group is observed for *T. tambra* (256 mm). The maximum size of *T. tambra* can be even larger than observed here as we observed that *T. tambra* cultivated at the research station for freshwater aquaculture in Bogor can reach 500–700 mm long (5 kg). Although most fishes were collected in the downstream area, which is closed to the estuary system, many small-bodied fish are likely to migrate up into the freshwater environments; therefore, juvenile and small fish species (10–100 mm long) also migrate upstream and downstream and need to use the fishway at various times. These small fish form the basis of the food chain for larger fish species and also make up an important component of the subsistence livelihood-based species essential for food and nutritional security for local communities. Therefore, all sizes of fish that we collected (i.e., 10–600 mm long) and crustaceans will need to be considered in the fishway design.

4 | Discussion

The findings of this study offer a significant update on the biodiversity of fish in the Cibareno River. The comprehensive use of diverse fishing gears and eDNA surveys has yielded more robust results compared to prior studies that relied solely on an electrofishing method conducted by Rachmatika, Sjafei, and Nurcahyadi (2002). Monitoring fish biodiversity based on

direct fishing and eDNA metabarcoding revealed a significant difference in species richness upstream and downstream of the Caringin Weir, indicating that the weir is limiting fish passage.

Using the combined methods, we collected a greater variety of fish species (72 species) than was observed 20 years ago by Rachmatika, Sjafei, and Nurcahyadi (2002) (22 species). This includes 14 species that have never been recorded from the Cibareno River while being known from other areas in Java islands (Dahrudin et al. 2017). However, we did not detect five species previously recorded in the river. The failure to detect these five species could be due to them no longer being present, insufficient spatial or temporal sampling and sample replication (Altermatt et al. 2023), unsuitable primer pairs (Blackman et al. 2024) or a lack of a reference database (Takahashi et al. 2023). The primary challenges in eDNA monitoring in Indonesia revolve around the limitations of the reference library within the Indonesian public database. Furthermore, the unavailability of multiple mitochondrial and nuclear markers, which are instrumental in identifying the most effective targets for species identification, exacerbates these issues (Lecaudey et al. 2019; Wibowo et al. 2022). Nevertheless, our integrated methods provide a better understanding of the fish community in the Cibareno River than either method on its own. The eDNA metabarcoding approach detected either more fish taxa (~47%) or the same species (~42%) in 89% of the studied sites compared to traditional methods, suggesting the combined methods can identify more species than each method on its own (Valentini et al. 2016). The combined data set revealed the detection of 30 species not captured by gillnets in the previous 3 years (Gillet et al. 2018). A similar study in Boardman Lake in Michigan, United States, using traditional fishing methods captured 12 species of fish, but 40 taxa were detected using eDNA metabarcoding, which included all of the species observed using traditional fishing methods (Gehri et al. 2021).

Land use in the vicinity of the Cibareno River predominantly comprises forestry, rice fields and plantations. However, various human activities occur at each surveyed point. The potential for habitat fragmentation in the Cibareno River is not solely attributed to the initiation of river damming in early 2022. A significant contributing factor is the presence of unregulated gold mining operations in the areas of Upstream 1 and 2, which have persisted for the past decade. These mining activities discharge their washing waste into the river, leading to pollution that deters fish from this region. The measurements of mercury (Hg), lead (Pb) and cadmium (Cd) in the upstream Cibareno River were <0.020, 0.237 and <0.001 mg/L, respectively, signifying that the river was lightly polluted (Vamellia 2014). However, at a gold mine site with a holding pond that released waste into the Cikidang River, one of the Cibareno River's tributaries, Hg concentrations in fish reached 1.66 ppm, whereas spinach and soil contained 4.61 and 0.0127 ppm, respectively (Pamungkas, Thayib, and Inswiasri 2015). Further statistical analysis has revealed that the two surveyed points exhibit similar fish diversity, which significantly differs from other points. This suggests that only specific fish species can thrive in areas where gold washing waste is regularly discharged. The disparity in fish diversity between sites Upstream 3 and Downstream 1, which are separated by the construction of the Caringin Weir, is anticipated to manifest in early 2022 once the river is dammed. Fish within the Cibareno

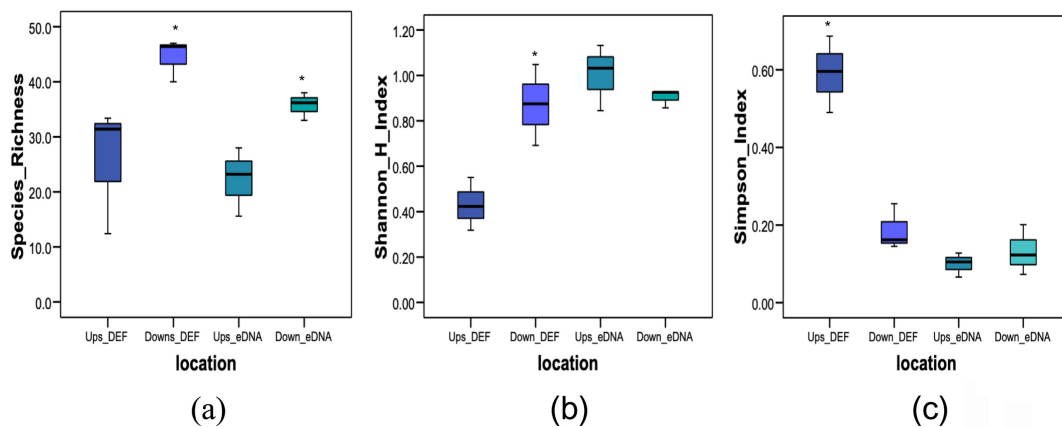


FIGURE 5 | Boxplots showing species richness (a), Shannon–Wiener diversity index (H') (b) and Simpson dominancy index (D) (c) of fish detected using direct environmental fishing and eDNA metabarcoding collected from upstream and downstream locations at the Cibareno River, Sukabumi, West Java, Indonesia. The bottom and top boundary lines of each box represent the first and the third quartile values (Q1 and Q3), respectively; the lines of each box indicate median (Q2) values, and the dots represent outlier values. Asterisks inside the figures denote significant differences ($p < 0.05$). (Down = downstream, Ups = upstream, DEF = direct experimental fishing).

River may encounter difficulties passing through the newly constructed 1-m-high barrier. Survey Downstream 2, located in a rice field area, is hampered by residents disposing of pesticides and conducting domestic activities in the river, potentially limiting the aquatic life. In contrast, Survey Point 6, adjacent to the estuary, stands out as the area with the richest variety of fish species. This is attributed to the estuary's unique characteristics, fostering a habitat where marine and freshwater ecosystems intermingle, resulting in a diverse and flourishing fish population (de Moura, Vieira, and Garcia 2012; Gillanders et al. 2022).

The current fish biodiversity assessment of the Cibareno River provides an excellent understanding of the importance of river network connectivity in maintaining fish biodiversity. Historically, there were no barriers constructed in the river, and overall fish biodiversity remained well preserved at the Cibareno River, indicated by the relatively unchanged number of species compared to the last two decades (2002–2022). In the Citarum River, West Java, a large dam, the Djuanda Reservoir, was constructed in 1968 for hydropower purposes. Prior to the construction of the dam, 31 species were detected in the reservoir. But in 2008, after 40 years of operation, the number of species had declined to 18 (Kartamihardja 2008). Understanding the connection between fish diversity and river network connectivity is crucial to species survival, ecosystem integrity and human well-being (Shao et al. 2019).

The presence of catadromous and amphidromous species in this study indicates the crucial need to maintain the connectivity of the Cibareno River to enable species to complete the crucial migratory components of their life cycle. Although the overall species diversity detected in the current study was high, there were clearly more species detected downstream of the weir, suggesting some species were not able to migrate past the weir. Therefore, managing waterway connectivity for migratory species is crucial for their conservation and warrants the careful consideration of fish passage development in dam infrastructures (Franklin and Gee 2019). We also detected 21 fish species found in both upstream and downstream areas of the river, emphasising the importance of river connectivity in

preserving fish biodiversity. These findings can help to guide water resource authorities in sustainably managing the river for irrigation and constructing fishways in weirs/dams to facilitate species migration. Additionally, ongoing research should explore the interconnectedness among riverine habitats to better understand how spatial mechanisms shape riverine fish assemblages (Fullerton et al. 2010).

The Cibareno River hosts many species with complex migratory habits. Often, both adults and juveniles are involved in migration. Therefore, for the purposes of the fishway concept design, we assume that both adults and juveniles will require passage (i.e., 10–600 mm long). The main objectives of fish passage development are to facilitate the migration of multispecies, both upstream and downstream, thereby contributing to the preservation of fish biodiversity (O'Connor et al. 2022). The development of selective passage is founded on interspecific variations in physical capabilities, body shape, sensory capacities, behaviour and movement patterns. These distinctions have been studied in an effort to create selective passage (Rahel and McLaughlin 2018). The selection of targeted species for fish passage design primarily hinges on factors such as migration pattern, fish size, population abundance and economic consideration.

The target fish species of the Cibareno River for fishway design are *A. marmorata*, *T. tambra*, *B. binotatus*, *Sicyopterus* sp. and *R. aspro* plus crustaceans. The fishway design needs to accommodate the size of *T. tambra* and *A. marmorata*, which are the two largest fish in the river. The larger fish will need appropriate depth and space, which should be considered in determining the size and depth of the fishway pools. Species like mahseer (*Tor genera*) typically engage in an upstream spawning migration at the onset of the rainy season as they search clear water areas for spawning and their larvae drift downstream after hatching (Amanda et al. 2023; Jaafar et al. 2021). Elvers (juvenile eels) migrate upstream during the dry season and early rainy season (Wahju et al. 2021; Wibowo et al. 2021). The smaller fish such as *B. binotatus*, larvae and juveniles, require low water velocity and reduced turbulence when navigating the fishway. Therefore, the channel, baffles

TABLE 7 | Fish and crustacean species collected based on experimental fishing in the Cibareno River and their expected minimum and maximum migrating sizes.

No.	Family	Scientific name	Upstream of the weir	Downstream of the weir	Fish number	Min. length (mm)	Max. length (mm)
A	Fish						
1	Ambassidae	<i>Ambassis buruensis</i>	-	+	27	25	81
2	Anguillidae	<i>Anguilla bicolor</i>	-	+	14	227	600
3		<i>Anguilla marmorata</i>	-	+	11	224	451
4		<i>Anguilla</i> sp.	-	+	2	235	286
5	Carangidae	<i>Caranx sexfasciatus</i>	-	+	5	60	102
6	Cichlidae	<i>Andinoacara rivulatus</i>	+	+	9	55	148
7		<i>Tilapia niloticus</i>	+	+	17	55	136
8	Clariidae	<i>Clarias batrachus</i>	+	-	1	190	190
9		<i>Clarias gariepinus</i>	+	-	1	271	271
10	Cyprinidae	<i>Barbodes binotatus</i>	+	+	550	20	120
11		<i>Cyprinus carpio</i>	+	-	2	145	300
12		<i>Tor tambra</i>	+	+	120	47	285
13	Eleotridae	<i>Eleotris</i> sp.	-	+	22	23	111
14		<i>Mogurnda</i> sp.	-	+	1	85	85
15	Gobiidae	<i>Glossogobius giuris</i>	-	+	1	101	101
16		<i>Lentipes ikeae</i>	+	+	9	35	47
17		<i>Lentipes</i> sp.	+	+	4	43	47
18		<i>Sicyopus rubicundus</i>	-	+	3	43	48
19		<i>Sicyopus zosterophorus</i>	-	+	1	0	0
20		<i>Stiphodon semoni</i>	-	+	60	22	51
21	Kuhliidae	<i>Kuhlia marginata</i>	-	+	155	10	146
22	Lutjanidae	<i>Lutjanus fuscescens</i>	-	+	1	93	93
23	Mugilidae	<i>Valamugil</i> sp.	-	+	1	106	106
24	Muraenidae	<i>Gymnothorax polyuranodon</i>	-	+	1	480	480

(Continues)

TABLE 7 | (Continued)

No.	Family	Scientific name	Upstream of the weir	Downstream of the weir	Fish number	Min. length (mm)	Max. length (mm)
25	Oxudercidae	<i>Awaous ocellaris</i>	-	+	10	70	92
26		<i>Awaous</i> sp.	-	+	4	75	95
27		<i>Schismatogobius insignis</i>	-	+	2	63	90
28		<i>Schismatogobius</i> sp.	-	+	16	74	130
29		<i>Sicyopterus microcephalus</i>	-	+	8	106	106
30		<i>Sicyopterus parvei</i>	+	+	36	60	138
31		<i>Sicyopterus</i> sp.	+	+	306	24	147
32		<i>Sicyopterus macrostetholepis</i>	-	+	17	40	115
33	Ophichthidae	<i>Lamnostoma kampeni</i>	-	+	1	30	30
34	Poeciliidae	<i>Poecilia reticulata</i>	+	+	42	15	47
35		<i>Xiphophorus hellerii</i>	+	-	23	25	68
36	Rhyacichthyidae	<i>Rhyacichthys aspro</i>	+	+	113	40	145
37	Sisoridae	<i>Glyptothorax platypogon</i>	+	+	2	54	76
38	Syngnathidae	<i>Microphis brachyurus</i>	-	+	61	55	155
39		<i>Microphis leiaspis</i>	-	+	2	151	151
40	Tetraodontidae	<i>Tetraodon</i> sp.	-	+	1	85	85
41	Tetrarogidae	<i>Vespicola</i> sp.	-	+	3	48	105
B	Crustaceans						
1	Atyidae	<i>Caridina brevicarpalis</i>	-	+	28	20	40
2	Palaemonidae	<i>Macrobrachium australe</i>	-	+	3	70	92
3		<i>Macrobrachium rosenbergii</i>	-	+	1	106	106
4		<i>Macrobrachium</i> sp.	-	+	2	50	70
5		<i>Palaemon</i> sp.	-	+	103	17	36
6	Gecarcinucidae	<i>Parathelphusa</i> sp.	-	+	4	30	75
		Total number of fish			1830		

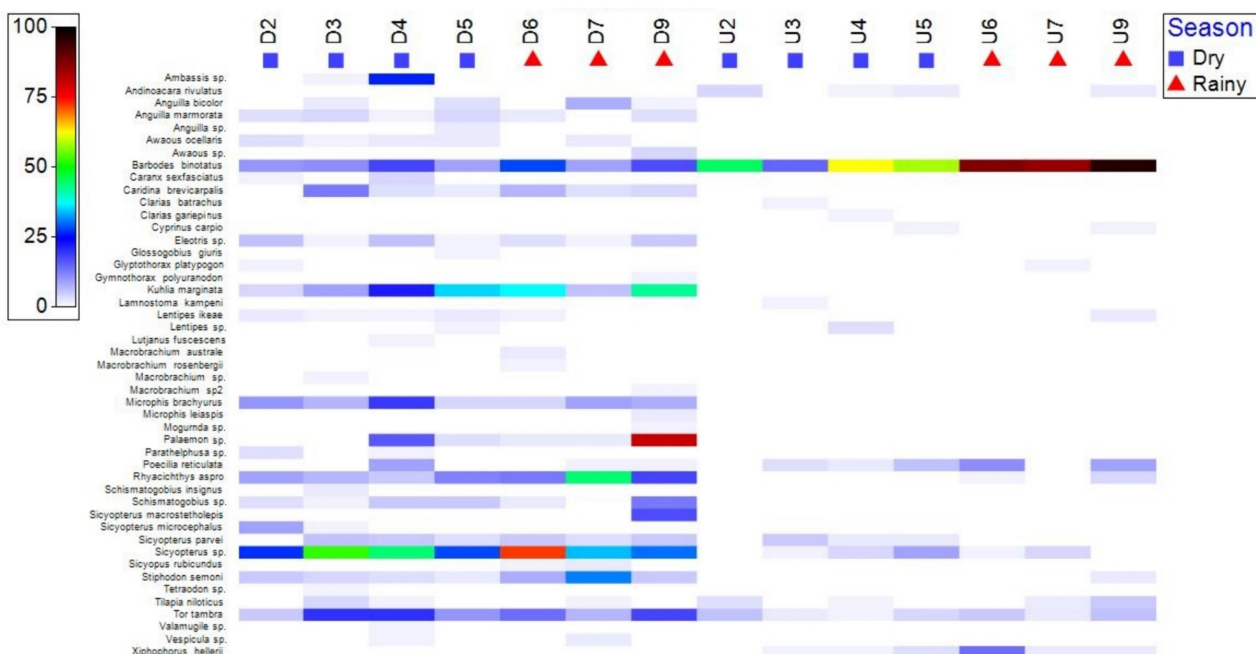


FIGURE 6 | Heat map showing the relative read abundance for fish species at downstream and upstream sites in the Cibareno River. U (upstream) and D (downstream) followed by a number (2–9) referring to months February–September; triangles for the wet season and squares for the dry season.

and gradient/slope of the passage can be appropriately designed to accommodate the migration of these smaller fish. The artificial rock substrate on the channel bottom of the fishway must be designed to accommodate the high abundance of demersal fish from the Gobiidae family such as *Sicyopterus* sp. and *R. aspro*, which inhabit the rock areas and may also improve crustacean passage. Utilising less invasive culvert remediation techniques to accommodate small-bodied fish species can contribute to their sustained survival in the region for the future (Knapp et al. 2019).

5 | Conclusion

Fish biodiversity assessment surveys are an essential phase in fishway design. They provide critical insights into fish biodiversity, abundance and migration characteristics, which are invaluable for developing an appropriate conceptual model fishway. The distribution of fish in both downstream and upstream areas serves as a guide and aids in studying changes in biodiversity, including planning for artificial barriers such as dams or other physical structures. Out of a total of 47 aquatic species caught through experimental fishing in the Cibareno River, 13 inhabited both upstream and downstream sites, indicating their potential for migration in both areas. Among this group, the two most dominant species are *T. tambra* and *B. binotatus*, consistently found in nearly every sampling event. On the other hand, some species are exclusively found either downstream or upstream. The findings from this study highlight the significance of combining experimental fishing and eDNA surveys, providing vital information to support the planning of weir construction for the design of fishways. The design and implementation of a fishway will potentially improve the natural migration patterns of several fish species.

Author Contributions

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data used in this manuscript are organised by the first author. All sequences were submitted to <https://www.ncbi.nlm.nih.gov/genbank/> (PP352528–PP352565).

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