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Exploring freshwater fish biodiversity using eDNA metabarcoding and traditional sampling to assess floodplain waters

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HIGHLIGHTS

- Fish biodiversity in Bangka floodplains requires diverse assessments.
- We found six endemic fish species among 22 fish species in two river systems.
- Floodplain water and fish behaviour impacts ecosystem genetics.

Abstract: The floodplains of Bangka Island, which are inhabited by endemic and native fish species, are increasingly threatened by several landscape change and waste pollution. Therefore, the investigation into fish diversity using environmental DNA (eDNA) metabarcoding and traditional sampling is required. eDNA samples were obtained from surface waters of Kurau and Bikang Rivers in Bangka Regency using a metaprobe with sterile gauze (10×10 cm). The extracted DNA products were amplified using Tele02 forward and reverse primers at annealing temperature of 54°C. The resulting polymerase chain reaction (PCR) products were cleaned, attached to adapters, and sequenced with the Next Generation Sequencing Illumina MiSeq platform. The total of 385,661 reads were initially generated using the Divisive Amplicon Denoising Algorithm 2 (DADA2). After the quality control (QC) process, 25.5% of the read (73,301) were retained for analysis. Results of the eDNA metabarcoding showed that species abundance and Shannon–Wiener

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diversity index were higher in the experiment fishing group (6 and 0.93) compared to those obtained from eDNA data (3 and 0.42). Additionally, both indices were higher in the Kurau River (4 and 0.82) than in the Bikang River (3 and 0.48). Species richness was significantly different between the two sampling methods ($p < 0.001$). Eighteen fish species were identified through experimental fishing, three using eDNA metabarcoding, and one species was common to both methods. PCA results revealed that the richness of species and effectiveness of sampling technique were significantly affected by water quality. The result of eDNA detection in this research was not better than experimental fishing. Hence, the development of reference for genetic database and optimisation of eDNA technique are required.

Keywords: Bangka Island, eDNA metabarcoding, experimental fishing, freshwater fish, metaprobe, water quality

INTRODUCTION

Freshwater ichthyofauna play an essential role in sustaining ecological equilibrium. The biodiversity of freshwater fish serves as a bioindicator of environmental health, contributing to nutrient cycles and food webs (Sarma *et al.*, 2024; Shaikh and Bansode, 2024). However, the global biodiversity loss – one of the most serious environmental crises in recent centuries, continues to significantly affect ecosystems (Pimm *et al.*, 2014). Despite several mitigation efforts, the rate of biodiversity loss remains largely unabated (Mace *et al.*, 2018). The main challenges in reducing tropical freshwater fish species include deforestation and pollution, with endemic species being particularly vulnerable due to their strong habitat dependency (Ahmed *et al.*, 2022). Furthermore, the large number of invasive freshwater fish from outside the region adds extra pressure on the extinction rate of endemic freshwater fish species (Ahmed *et al.*, 2022).

Floodplain lakes are water bodies of water that may be permanently or temporarily linked to various rivers, depending on flood pulses, and they support highly diverse and endemic fish communities (Reid, Delong and Thoms, 2012). Floodplain lakes exhibit pronounced seasonal dynamics, driven by substantial hydrological variability, as fish assemblages depend on hydrological connectivity to complete stages of their life cycles (Fernandes *et al.*, 2009). During periods of rising water levels, habitat areas become more interconnected, allowing fish communities to disperse randomly with a more uniform species distribution (Thomaz, Bini and Bozelli, 2007). In the high-water phase, increased inputs from river discharge, precipitation, and surface runoff lead to imperfect vertical mixing of stratified water masses and increase spatial similarities across aquatic habitats (Thomaz, Bini and Bozelli, 2007). As flood waters recede, connectivity between rivers and lakes diminishes, leading to habitat fragmentation. This process restricts movement, intensifies inter- and intra-specific interactions, and amplifies environmental filtering (He *et al.*, 2024).

Bangka Island has a high variety of freshwater fish. Akhrianti and Gustomi (2018) reported that 63 species from 24 families have been recorded on the island. Moreover, six endemic fish live in the Bangka Island floodplain (Helmizuryani *et al.*, 2024). Unfortunately, tin mining has contributed to floodplain degradation and alteration of native vegetation cover (Ibrahim, Zukhri and Rendy, 2019). Moreover, intense human activity and pollution around floodplain pose serious anthropogenic threats to freshwater fish biodiversity (Sarkar *et al.*, 2020). Addressing these issues requires a multifaceted approach, including pollution management and water quality monitoring, habitat restoration, and conservation efforts. Effective biodiversity monitoring is also essential to support the sustainable management of freshwater fish populations (Risjani *et al.*, 2020; Sarkar *et al.*, 2020; Hahshmi *et al.*, 2024).

Several conventional methods have been identified for analysing biodiversity, such as underwater surveys (Mathon *et al.*, 2022), cameras (Sales *et al.*, 2020), and experimental capture (Wibowo *et al.*, 2022). In Bangka Island, research on freshwater fish biodiversity has been conducted by Akhrianti and Gustomi (2018), using a conventional system based on morphological characteristics and colouring of fish. However, these methods are increasingly being phased out due to their time-consuming and costly nature (Hering *et al.*, 2018). Environmental deoxyribonucleic acid (eDNA) metabarcoding method has emerged as an effective tool for detecting aquatic animal biodiversity.

Biodiversity mapping using the eDNA metabarcoding method has been carried out in previous research (Sales *et al.*, 2020; Mathon *et al.*, 2022; Wibowo *et al.*, 2022). Environmental DNA (eDNA) metabarcoding involves the simultaneous sequencing of various DNA shed by organisms into the environment, such as excretions, sperm, mucus, and faeces – found in water, soil, and air. This non-invasive technique is considered a sensitive, cost-effective, and time-efficient approach for estimating the distribution and biodiversity of various aquatic organisms (Takeuchi *et al.*, 2019). Generally, DNA metabarcoding facilitate the identification of fish species, distinguish overlapping, and compare to traditional morphological taxonomy to determine species boundaries (Elsaied *et al.*, 2021). Moreover, in turbid habitats and areas with high activity near the riverbed, water filtration is not optimal due to the large amount of material clogging filter paper, hindering the detection of fish species (Barnes *et al.*, 2014). In such conditions, the use of a metaprobe, a gauze tied to a hollow, round probe – offers a fast and easy alternative for eDNA metabarcoding (Maiello *et al.*, 2022). The use of metaprobe can reduce the risk of filter clogging during filtering water and can yield results that complement those obtained from conventional surveys (Maiello *et al.*, 2022).

Utilising eDNA for biomonitoring offers a promising approach to assess fish diversity in floodplains, particularly when accounting for the effects of high- and low-water phases (He *et al.*, 2024). However, eDNA metabarcoding has several limitations, including incomplete taxonomic resolution, variability in sample stability, and the potential for false positives due to the detection of non-target species (Poyntz-Wright *et al.*, 2024). The persistence and detectability of eDNA are affected by environmental factors such as water quality (pH, light, oxygen, salinity, and substrate type) and the physiological state of the target organisms, including extracellular enzymes (Barnes *et al.*, 2014). Therefore, this study compared eDNA metabarcoding with

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conventional capture methods to provide a comprehensive overview of fish diversity in the Bangka Islands. This research aimed to: 1) map the biodiversity of fish in floodplain waters of Bangka Island, Indonesia, by comparing experimental fishing and eDNA metabarcoding methods, and 2) assess the effectives of biomonitoring methods in relation to water quality and characteristics of floodplain waters. The results are expected to contribute valuable insights for future freshwater fish biodiversity evaluation and the development of biomonitoring strategies on Bangka Island.

STUDY MATERIALS AND METHODS

RESEARCH LOCATION

This research was conducted from August 2023 to August 2024 on Bangka Island. The activities were carried out in two locations: the Kurau River Basin in Central Bangka Regency, and the Bikang River Basin in South Bangka Regency. The length of the Kurau River is approximately 60.16 km, with a basin area of 657.69 km^2 (Sari, Utami and Umroh, 2018), while Bikang River Basin covers 199.04 km^2 (Peraturan, 2018). The first location is adjacent to a community tin mining area and is bordered by *Tristaniopsi* sp. forest, serving as a land cover buffer. Tin mining activities are concentrated upstream, while the downstream area is adjacent to oil palm plantations.

The second location had no mining activities either upstream or downstream, but its downstream area was adjacent to oil palm plantations. Sampling was conducted at a total of seven stations, four in the Kurau River (PL01, PL03, PL04, and PL05) and three in the Bikang River (BK01, BK04, and BK05), as shown in Figure 1. Sampling was carried out starting from downstream to upstream to avoid contamination. After the survey, a field blank was collected by soaking gauze in mineral water only; this served as a control to detect potential contamination.

EXPERIMENTAL FISHING AND eDNA SAMPLING

eDNA samples were collected using a passive filtration method known as the metaprobe, a 3D-printed, hollow, perforated plastic spherical probe. We built two custom-made rolls of gauze, rolling 1 g of pharmacy sterilised cotton inside two sterile 10×10 cm sterile gauze compresses (mesh-size: 1 mm) (Maiello *et al.*, 2022). Originally developed for biodiversity survey in trawl fisheries, the metaprobe has proven effective in detecting "biodiversity bonus" (Maiello *et al.*, 2022). In this study, we explored its potential as an innovative tool for eDNA collection in freshwater environments. Compared to vacuum pumps and Sterivex techniques, which are often costly in tropical countries like Indonesia – the methaprobe offers an inexpensive and simple alternative. Moreover, limited access to reliable electricity and the high risk of DNA degradation due to elevated temperatures in Indonesia make on-site filtration challenging. Therefore, this research also aimed to evaluate the effectiveness of the metaprobe as an alternative of existing eDNA collection methods.

In this research, gauze rolls were securely fixed inside the methaprober using plastic cable ties and attached to a 1–5 meter rope. A sinker was added to prevent the device from floating, and the methaprobe was submerged in water.

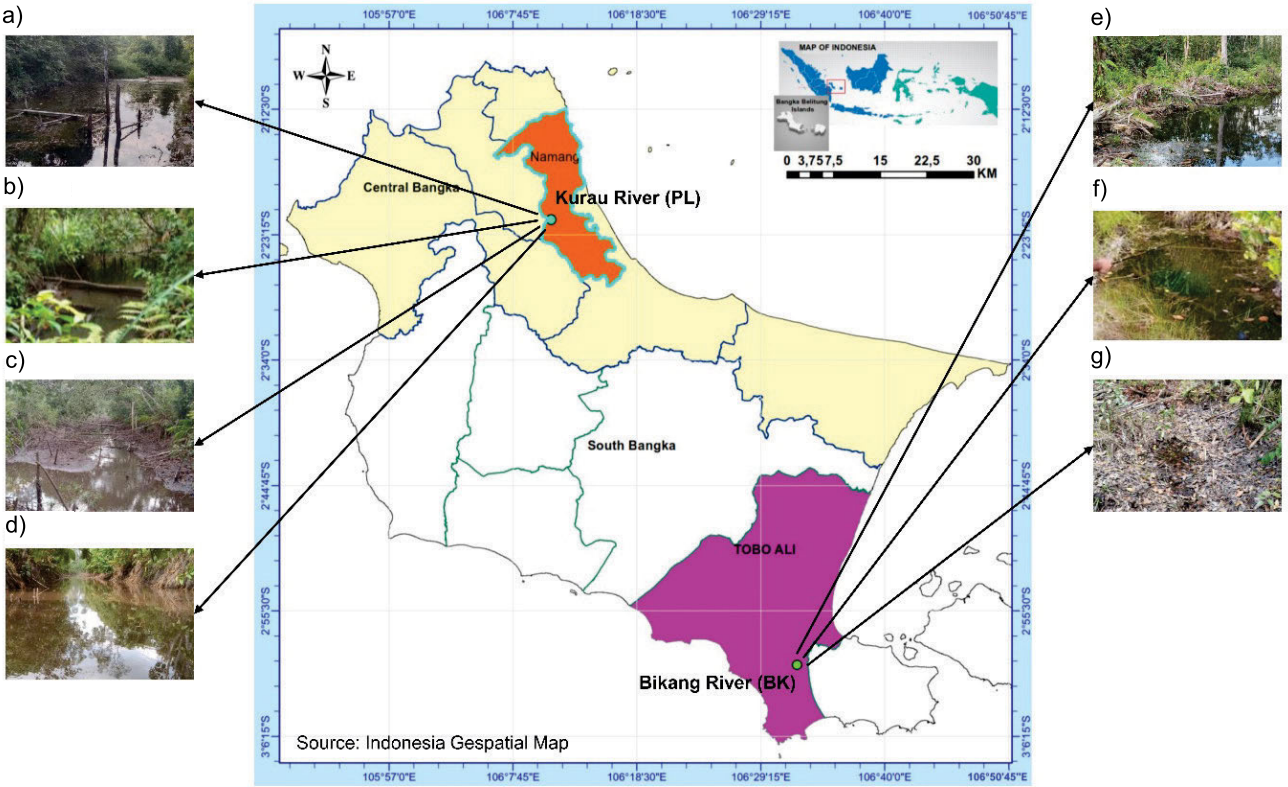

Fig. 1. Map of eDNA material sampling and fishing ground of fish in Bangka Island – the Kurau River (PL) and the Bikang River (BK): a) PL01 2°21'42.5"S, 106°11'27.7"E, b) PL03 2°22'00.7"S, 106°10'57.6"E, c) PL04 2°22'01.9"S, 106°10'58.1"E, d) PL05 2°22'01.1"S, 106°10'57.5"E, e) BK01 2°54'37.0"S, 106°27'32.5"E, f) BK04 2°54'37.3"S, 106°27'34.2"E, g) BK05 2°54'01.1"S, 106°27'44.5"E; source: own study and Indonesia geospatial map

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After 10 min, the metaprobe was retrieved, and the gauze rolls were preserved in 50 cm^3 Falcon tubes filled with 98% ethanol. Samples were stored at –20°C for further analysis. After eDNA sampling (Photo 1a), experimental fishing was conducted using fishing traps (Photo 1b), with crushed fish feed put inside as an attractor.

Additionally, fishing activities were conducted to validate the findings of eDNA metabarcoding. Fish species identification was based on various morphological characteristics, including body shape, mouth type, scale type, total and standard length, colour, tail type, number of lateral lines, and fin rays (Saanin, 1984; Kottelat and Whitten, 1996; Rainboth, 1996). Fish specimens were collected using fishing gear and traps at each documented, and re-released. Productivity of experimental fishing and eDNA metabarcoding will be influenced by condition of water quality (Stoeckle *et al.*, 2017; Leblanc and Farrell, 2023). Hence, we did in-situ water quality measurements using the AZ instrument 86031 water quality meter. The observed water quality parameters included temperature, dissolved oxygen (DO), pH, and total dissolved solids (TDS). Water quality measurements were conducted on seven occasions in the Kurau River and three occasions in the Bikang River.

GENOMIC DNA EXTRACTION

The gauze was preserved with 98% ethanol and stored in a freezer at a temperature of –20°C and the gauze was transported to a special room for environmental sample extraction at the Genomics Laboratory, KST Soekarno, Cibinong. Subsequently, the gauze gets squeesed until the ethanol is expelled and the cable tying the gauze rolls were cut into representative subsamples from all side of the gauze. Subsamples were taken from the right, left, middle, top, and bottom of the gauze using small scissors with a total weight of approximately 250 mg. Genomic DNA (gDNA) isolation was processed using the DNeasy® PowerSoil® Pro Kit by following the manufacturer's instructions.

PCR AMPLIFICATION AND LIBRARY PREPARATION

After successful extraction, DNA was amplified with Tele02 primers (Taberlet *et al.*, 2018) with the target 129-209 bp of 12S region. The base sequence of Tele02 forward and reverse primers were AAACTCGTGCCAGCCACC and GGGTATCTAATCC-CAGTTTG, respectively. polymerase chain reaction (PCR) mix used the following composition: 10 mm^3 AmpliTaq Gold Master Mix (applied biosystems), 0.16 mm^3 BSA (bovine serum albumin), 1 mm^3 for each primer, 5.84 mm^3 nuclease-free water, and 2 mm^3 DNA template, producing a total volume of PCR product 20 mm^3. The stages of PCR include: pre-denaturation at 94°C for 3 min, denaturation at 94°C for 3 s repeated for 30 cycles, annealing at 54°C for 30 s, initial extension at 72°C for 90 s, and final extension at 72°C for 8 min. The amplification was validated using 1% gel electrophoresis with a 50 bp DNA ladder marker. The Illumina MiSeq technology was used to sequence the PCR products at a commercial service provider, PT. Genetika Science.

DEVELOPING REFERENCES DATABASE

A sequence reference database from fish samples in the Bangka Islands has been developed. The extraction procedure for gel electrophoresis was examined at the Genomics Laboratory, KST Soekarno, Cibinong. Fish tissue samples weighing 200 mg were obtained from the pinna dorsalis consisted of the *Betta chloropharynx*, *B. schalleri*, *B. burdigala*, *Desmopuntius hexazona*, *Luciocephalus pulcher*, and *Sphaerichthys osphromenoides*. The DNA extraction kit employed was GENEAID gSYNC, and the primer utilised was Tele02. The PCR parameters for pre-denaturation, final extension, and validation of amplification via gel electrophoresis are identical to those outlined in the sub-chapter on PCR amplification and library preparation. The PCR product is subsequently dispatched to a commercial service (PT. Genetika Science) for Sanger sequencing, and the sequencing results are submitted to NCBI to get an accession number.

Photo 1. Two different sampling techniques, eDNA sampling with: a) "metaprobe", b) experimental fishing using trap (phot.: *A.P. Prasetyo* and *Helmizuryani*)

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BIOINFORMATIC AND DATA ANALYSIS

PCR products were cleaned to be attached with adapters and sequenced with a sequencer machine (Prasetyo *et al.*, 2023). Adapter and PCR primer sequences from the paired-end reads were removed using Cutadapt (Martin, 2011). The sequencing results would be processed bioinformatically by Divisive Amplicon Denoising Algorithm 2 (DADA2) to obtain the number of reads. The DADA2 was used to correct sequencing errors, remove low-quality sequences and chimera (Callahan *et al.*, 2016). We filtered out all reads with ambiguous nucleotides (max*N* = 0). The max*EE* parameter sets the maximum number of "expected errors" allowed in a read (max*EE* = 2). Truncate reads at the first instance of a quality score less than or equal to 2 (trunc*Q* = 2) and remove reads with length less than 120 bp (minLen = 120). The resulting ASVs data was used for taxonomic classification against Basic Local Alignment Search Tool nucleotide (BLASTn) on NCBI database to determine the type of non-fish reads and fish reads. Non-fish reads were excluded, and fish reads were utilised for Operational Taxonomic Unit (OTU) classification. The classification of classes and families of ASVs was refined according to NCBI BLASTn results, utilising the % identify parameter for filtering. A minimum threshold value of 97% was employed for OTU identification based on BLASTn results.

The fish assemblages analysed in this study were categorised into two distinct groups: the catch results alongside eDNA findings, and the fish population counts in the Kurau River and the Bikang River. The Shannon–Wiener index was employed for statistical analysis of both groups. Multivariate analysis used were permutational multivariate ANOVA (PERMANOVA), non-metric multidimensional scaling (NMDS) and principal component analysis (PCA). The analysis of species abundance and diversity across various monitoring techniques (eDNA vs experimental fishing) and various river populations was conducted using index of richness, Shannon–Wiener, and PERMANOVA (Euclidean method). The NMDS was used to plot fish communities, while PCA biplot was used for plotting environmental factors and fish community. The NMDS is a visual tool to illustrate relationships among groups across various habitats (Borisova *et al.*, 2023), facilitating a comparison between eDNA vs experimental fishing. The distance-based ordination technique was for NMDS, whereas PCA used Euclidean distance. The correlation between species abundance, location, water type (lotic and lentic), and water quality (temperature, DO, pH and TDS) either from sampling techniques of eDNA and experimental fishing was analysed using PCA. The PCA data were performed for transformation. These results were analysed and visualised using vegan, phyloseq and PCA test packages in R (McMurdie and Holmes, 2013; Camargo, 2022).

RESULTS AND DISCUSSION

ABUNDANCE AND DIVERSITY

The amplification results were checked by gel electrophoresis, as shown in supplementary material (Photo S1). The 12S gene target was obtained with a length ranging from 129–209 kb (line 153–154). The number of reads from the 54 ASVs sequencing results was 385,661 reads from seven water samples obtained at each station, producing a total of 298,827 reads (Fig. 2). One-fourth of the total sequencing reads was family of Actinopterygii by 76,301 reads, followed by Aves (22.4%), Mammalia (44.7%), and none (7.4%). We retained 15 ASVs of family Actinopterygii and removed thirty-nine (39) ASVs non-fish reads.

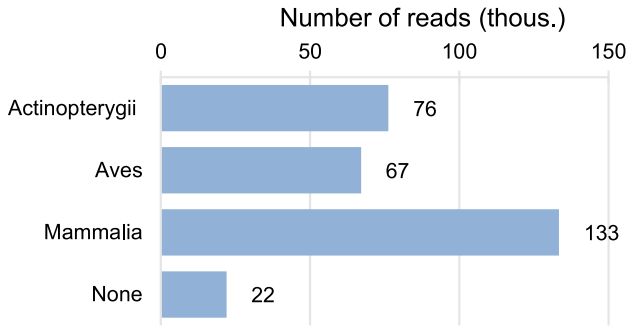

Fig. 2. Number of reads group of Actinopterygii and non-Actinopterygii (Aves, Mammalia and none) from 54 ASV: source: own study

The eDNA analysis using Tele02 primers has demonstrated efficacy in quantifying fish species in both marine and freshwater environments (Cananzi *et al.*, 2022). However, this study observed a higher prevalence of non-fish reads compared to fish reads (Fig. 2). This phenomenon is attributed to the Tele02 primer's capacity to detect not only Actinopterygii but also various non-target taxa (Xu *et al.*, 2023), resulting in an increased detection of non-fish organisms in this investigation. Additionally, Zhang, Zhao and Yao (2020) emphasised the necessity for primer validation through in-silico and in vitro methods, particularly in peat swamp ecosystems, which are characterised as sub-optimal habitats.

The fishes total reads that passed the quality control were 76,301 (25.5%), while the Kurau River had 10,828 reads and the Bikang River had 65,477 reads. Boxplot for fish species abundance and Shannon–Wiener diversity index from eDNA and experiment fishing as well as among river population systems (Bikang and Kurau) is presented in Figure 3. Species abundance and Shannon–Wiener indices were higher than experimental fishing compared to eDNA sampling (Fig. 3a, b). Additionally, both species abundance and Shannon–Wiener diversity were higher in the Kurau River than in the Bikang River (Fig. 3c, 3d). This difference may be attributed to hydrological conditions of water flow. Water from the Bikang River is characterised by limited river flow which is restricted by the new road development, while the Kurau River has fairly stable flow and is located in the protected area.

PERMANOVA analysis results of degrees of freedom (*df*) on fish community with R^2 (0.227) and *F*-value (3.529). They revealed a significant difference between eDNA metabarcoding and experimental fishing ($p < 0.001$).

Fish species identified from eDNA and experimental fishing results are illustrated in Figure 4. Eighteen fish species were recorded through experimental fishing, while three species were detected through eDNA analysis. One species was classified as *Betta* sp. based on BLASTn results showing similarity <97% (Fig. 4), and was grouped at the genus *Betta* level. The species accumulation curve comparing experimental fishing and eDNA results revealed an exponential trend (Fig. 5). However, the addition of eDNA samples did not correspond to the increase in the number of detected species.

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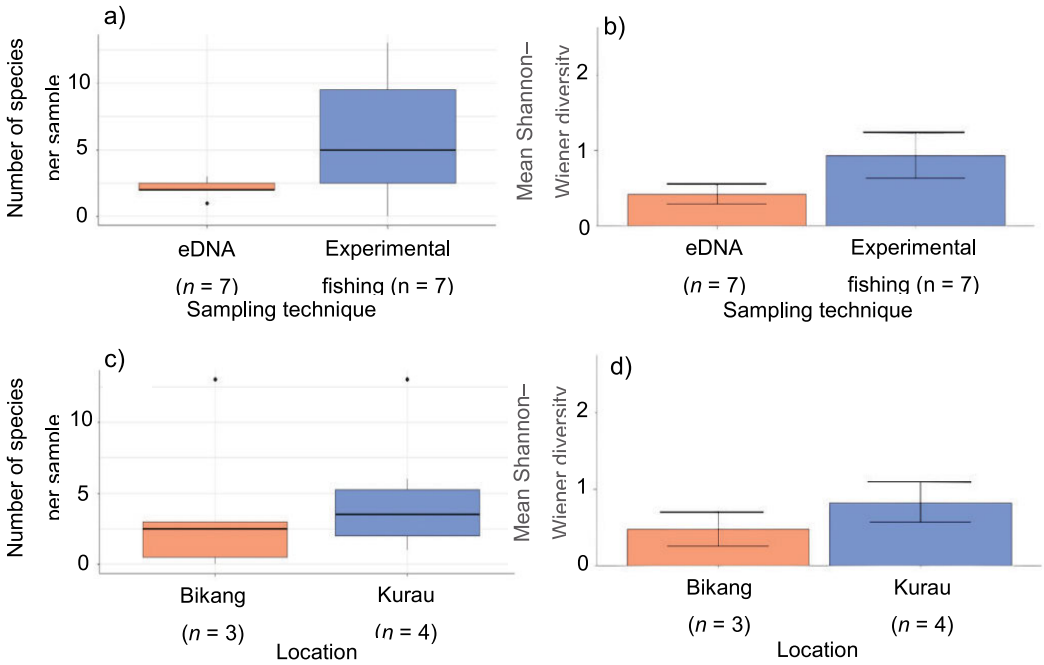

Fig. 3. Boxplots of richness, Shannon–Wiener, for the fish assemblages' difference between eDNA metabarcoding and experimental fishing (a and b), and difference among locations i.e. Bikang and Kurau (c and d); source: own study

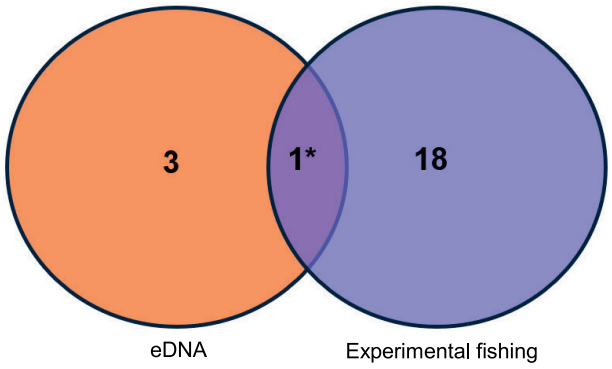

Fig. 4. Number of fish species were identified by eDNA, experimental fishing, and both methods; source: own study

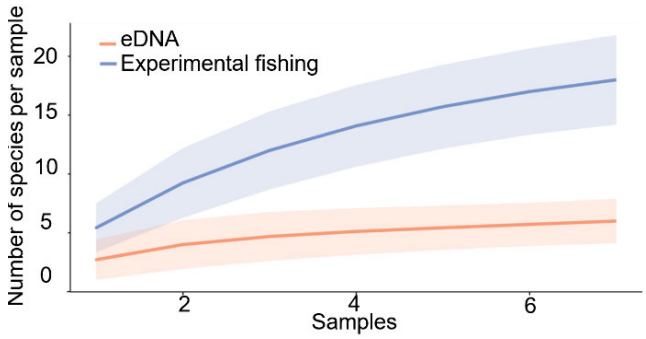

Fig. 5. Species accumulation curves for fish assemblages recorded by eDNA metabarcoding and experimental fishing in Bangka Island; source: own study

The eDNA sampling with the "metaprobe" was less effective in assessing fish communities in environments characterised by low DO, acidic water (<7 pH), and elevated TDS. Species accumulation through eDNA was lower compared to that from experimental fishing (Fig. 4). This may be attributed to the high organic matter content typical of floodplain habitats, such as litter, leaves, and roots – which can interfere with DNA extraction and reduce detection efficiency (Kirtane, Wilder and Green, 2019).

A total of 22 species were detected from eDNA metabarcoding and experimental fishing consisting of eight families, i.e. family Osphronemidae (*Betta burbigala*, *B. chloroparynx*, *Parosphronemus deissneri*, *B. schalleri*, *Luciocephalus puncher*, *Sphaerichtys osphromenoides*, *B. edithae*, *B. simprum*, and *B. coccina*), Cyprinidae (*Trigonopoma gracile, T. pauci perforatum, Brevibora cheeya, Osteochilus spilurus, Neolissochilus soroides,* and *Barbodes binotatus*), Clariidae (*Clarias leiacanthus and Encheloclarias tapeinopterus*), Bagridae (*Hemibagrus nemurus*), Nandidae (*Nandus nebulosus*), Nemacheilidae (*Nemacheilus fasciatus*), Barbinae (*Desmopuntius puntius hexazona*), and Channidae (*Channa bankanensis*) – Table 1.

The grouping of fish species from the catch was based on morphological characteristics using fish identification guides (Saanin, 1984; Kottelat and Whitten, 1996; Rainboth, 1996). Morphological identification is documented in the supplementary material (Photo S2–S17, Fig. S1). Accession numbers from Sanger sequensing are as follows: *B. chloropharynx* (PP849944.1), *B. schalleri* (PP849943.1), *B. burdigala* (PP849940.1), *Desmopuntius hexazona* (PP849948.1), *L. pulcher* (PP849945.1), and *S. osphromenoides* (PP849941.1). *Betta* sp. was result of ASV with annotation of two species (*B. burdigala* and *B. coccina*) under low similarity. It is assumed that *B. coccina* may have been detected on Bangka Island, as its distribution includes the floodplains of Sumatra and surrounding regions (Fahmi *et al.*, 2020). The family Osphronemidae was the most frequently detected, likely due to their possession of a labyrinth organ that stores oxygen reserves (Tate *et al.*, 2017), enabling survival in low-oxygen environments.

Betta sp. is known as cupang fish or Tempalak, which is an endemic fish on Bangka Belitung, comprising *B. burbigala*, *B. edithae*, *B. schalleri*, and *B. chloropharynx* (Hui and Ng, 2005a; Hui and Ng, 2005b; Syarif *et al.*, 2020; Ramadhanu *et al.*, 2024).

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Table 1. Species composition based son two different sampling techniques, namely eDNA (orange) and experimental fishing (blue) on two different locations

No.	Species	Status	eDNA							Experimental fishing							Detection	
			BK01	BK04	BK05	PL01	PL03	PL04	PL05	BK01	BK04	BK05	PL01	PL03	PL04	PL05	eDNA	experimental fishing
1	*Betta burdigala*	endemic								x								x
2	*Betta chloroparynx*	endemic								x				x				x
3	*Betta edithae*	endemic								x				x	x	x		x
4	*Betta schalleri*	endemic												x				x
5	*Parosphromenus deissneri*	endemic												x				x
6	*Encheloclarias tapeinopterus*	endemic								x								x
7	*Betta simorum*	native								x								x
8	*Luciocephalus pulcher*	native								x				x	x	x		x
9	*Sphaerichthys osphromenoides*	native								x				x				x
10	*Channa bankanensis*	native								x								x
11	*Nandus nebulosus*	native								x				x				x
12	*Barbodes binotatus*	native		x	x		x										x	
13	*Desmopuntius hexazona*	native								x					x	x		x
14	*Neolissochilus soroides*	native	x					x	x								x	
15	*Osteochilus spilurus*	native											x					x
16	*Brevibora cheeya*	native											x	x				x
17	*Trigonopoma gracile*	native											x	x				x
18	*Trigonopoma pauciperforatum*	native								x			X	x	x	x		x
19	*Nemacheilus fasciatus*	native			x		x								x	x	x	
20	*Hemibagrus nemurus*	native											x	x				x
21	*Clarias leiacanthus*	native								x			x	x				x
22	*Betta* sp. [*Betta burdigala*, *Betta coccina*]		x	x	x	x		x	x	x				x	x	x	x	x
Species diversity			**2**	**2**	**3**	**1**	**2**	**2**	**2**	**12**	**0**	**0**	**6**	**12**	**5**	**5**	**4**	**19**

Explanations: BK01–BK05 = the Bikang River sampling points, PL01–PL05 = the Kurau River sampling points.
Source: own study.

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According to the IUCN (2024) Red List database, these fish are considered endangered. Fahmi *et al.* (2020) reported that Indonesia had the largest distribution of wild *Betta* species, naturally abundant across several regions. Based on distribution data, these species have been found on the islands of Kalimantan, Bangka Belitung, and Bintan (Icas *et al.*, 2019). *Betta* spp. comprises 55 species (Syarif *et al.*, 2021) and has CR (critically endangered) status IUCN (2024), as supported by Prianto *et al.* (2017). *Parasphnonemus deissneri*, *L. puncher*, and *S. osphromenoides* were species of Osphoronemidae family found in waters of the Bangka River (Ng and Kottelat, 1998). Traditional fish traps (bubu) are passive, non-selective fishing tools that have no impact on both target species and their habitats. As such, they are commonly used to catch several fish species in Indonesia (Jayanto *et al.*, 2018), with reported efficiency rates of up to 80% (Vivi *et al.*, 2023). In this study, the number of fish caught using traditional tools was higher than that detected through eDNA sampling, likely due to the use of bait. Larungkondo *et al.* (2022), also reported that baited bubu traps with bait could optimise the result of fish catch.

The result of 54 ASVs was grouped into Class Actinopterygii (25.53%), whereas Aves and Mammalia were categorised as contaminated from water samples (22.44% and 46.67%, respectively), and unidentified (7.36%), according to NCBI database. Furthermore, 18.6% of the Actinopterygii class comprises freshwater fish. The low detection of endemic species with eDNA in this research could be attributed to the unavailability of DNA database in the gene database (Hatzenbuhler *et al.*, 2017). Contamination was caused by rapid extracellular degradation in the sampling environment, such as increasing temperatures in tropical waters (Gonzalez Colmenares *et al.*, 2023). The number of endemic fish caught was less than that of native fish, as shown in Table 1. Based on previous research, the decrease in occurrence rate was due to environmental damage and habitat degradation caused by human activities such as mining, settlements, plantations, pollution, and climate change (Kottelat and Whitten, 1996; Thushari and Senevirathna, 2020). Therefore, the natural existence of this species is threatened, causing a significant impact on survival (Wood *et al.*, 2017; Spikmans *et al.*, 2020).

The result of NMDS analysis among these three dimensions with the R^2 and stress value of 0.1311 (Fig. 6), represented the strong observed dissimilarity intergroup (Dexter, Rollwagen-Bollens and Bollens, 2018). Plot group experimental fishing had abundant richness species compared to eDNA (Fig. 7) and showed distinct clusters. Experimental fishing detected fairly similar fish communities, while eDNA sampling detected unique species across sampling sites. The activity of experimental fishing and eDNA results are influenced by environmental factors. Factors affecting distribution and productivity in the fishing of freshwater fish in floodplain are flood area and duration bringing feed source, and the connectivity of between swamps and river channels (Moses, 1987; Leblanc and Farrell, 2023). The relation-

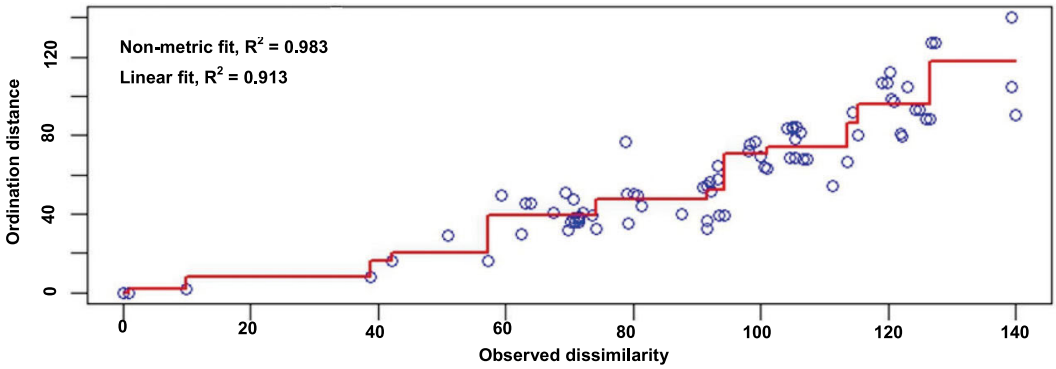

Fig. 6. Stress plot of non-metric multidimensional scaling (NMDS) based on Euclidean similarity index between two sampling types in different locations; source: own study

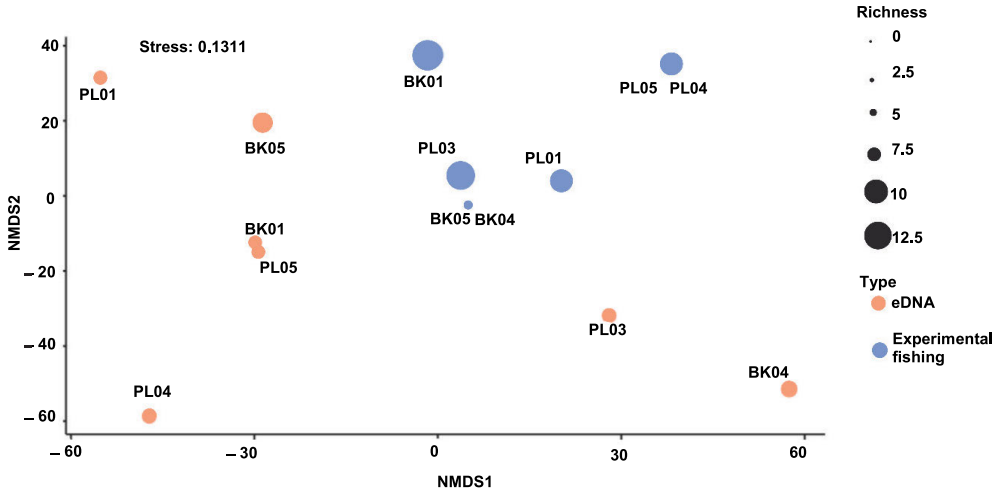

Fig. 7. The NMDS plot of two sampling types in detecting fish species in Bangka Island; BK01–BK05 and PL01–PL05 as in Tab. 1; source: own study

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ship between samples from different environments and the species caught using experimental fishing was associated with significant changes in fish species composition, distribution, and diversity (Cahyono, Budiharjo and Sugiyarto, 2018). In general, eDNA has been shown to effectively detect freshwater fish communities in floodplain ecosystems compared to experimental fishing (He *et al.*, 2024), However, in this study, its effectiveness was limited due to several environmental conditions and water quality factors. These factors include the presence of substances that inhibit eDNA detection, such as sediment, humic compounds, and algae, which can bind to or degrade DNA (Stoeckle *et al.*, 2017), thereby reducing detection efficiency. Other factors that may limit eDNA performance in floodplain are tide in floodplain and the selection of primary type (He *et al.*, 2024).

WATER QUALITY AND FISH DETECTION

The relation between species abundance, sampling location, water type, and water quality parameters (temperature, DO, pH, and TDS) based on both eDNA and experimental fishing is presented in Figure 8. Species abundance was primarily influenced by TDS (–0.8) and DO (–0.6). Sample variation across different locations was affected by pH (–0.2), while water type (lotic and lentic) was influenced by TDS (–0.8) and DO (–0.6). Ion stability in fish metabolism, osmoregulation, and the potential for stress or mortality, canbe significantly affected by TDS (Sarkar *et al.*, 2020). Variation in TDS, influenced by differences in water type, clarity, and nutrient availability, play a very important role in the feed chain and fish reproduction. Hence, the composition of fish species in catches tends to vary accordingly (Mohamed *et al.*, 2008).

The collection of fish samples from different locations is affected by pH. Variations in pH during tidal processes can be significant and may affect distribution and survival (Engel, 2003). Long-term changes in pH can lead to shifts in spawning zones and impact fish diversity (Mitra and Zaman, 2021). This is consistent with the findings of this study, which recorded differences in species numbers between freshwater habitants (lotic and lentic). The results showed that the reduction in diversity of fish in water was due to changes in ecosystem conditions from original lotic to lentic, leading to fish selection (Wang *et al.*, 2011; Hasseb *et al.*, 2016). Higher oxygen will support the life, both fishes and other organism which was consumed by fish (Beamish, Beamish and Lim, 2003). However, DO was negatively correlated to with species richness. This may be due to the limitations of the sampling technique using the metaprobe, which may have captured less environmental DNA. The metaprobe relies on active water flow or movement to collect DNA material and can become saturated in stagnant environments such as peatlands. Additionally, the low efficiency of the Teleo02 primer may have reduced DNA amplification success by amplifying more non-target taxa.

The results of sampling variables and water quality observations for eDNA and experimental fishing are presented in Figure 9. Overall, the sampling technique was affected by water quality parameters (temperature, DO, pH and TDS) and strongly correlated to the dynamic of water quality in floodplain water environmental of Bangka. These findings align with those of Sarkar *et al.* (2020), who reported a positive correlation between these four parameters as the indicator of environmental carrying capacity for sustaining fish populations in floodplain ecosystems. Water quality parameters were measured to determine whether the conditions were suitable for fish farming. In the Bikang River, the average values recorded were: temperature (28.10 ±0.24°C), pH (5.66 ±0.12), DO (6.07 ±0.90 mg·dm^{-3}), and TDS (60.33 ±8.22 mg·dm^{-3}). In the Kurau River, the average values were: temperature (27.28 ±0.54°C), pH (6.40 ±0.43°C), DO (6.07 ±0.81 mg·dm^{-3}), and TDS (64.33 ±2.05 mg·dm^{-3}). Regarding acidity, the pH in the Bikang River dropped as low as 4, indicating increased acid levels likely influenced by the surrounding swamps. The pH, DO, and temperature values observed in this study were higher than those reported by Ramadhanu *et al.* (2023) for *Betta* sp. habitats (2–3 mg·dm^{-3}, and 26.9°C). Meanwhile, wild family Nemacheilidae and Cyprinidae can survive in environments with pH ranging 3.8–4.0, DO 0.49–6.5 mg·dm^{-3}, and water temperatures ranging between 28.07 and 33.25°C (Sofarini *et al.*, 2019).

According to Yanti and Haryadi (2019), Cyprinidae and Osphronemidae species are often found around aquatic vegetation in calm and sheltered river environments. Aquatic plants play a crucial role in peatland ecosystems by providing breeding habitats, supporting nutrient cycling, and helping to maintain water quality (Quiroga *et al.*, 2022). The aquatic habitats of the Kurau and Bikang Rivers are characterised by the presence of aquatic vegetation. The Kurau River Basin is dominated by *Tristaniopsi* sp. trees. These water ecosystems offer low pH, high

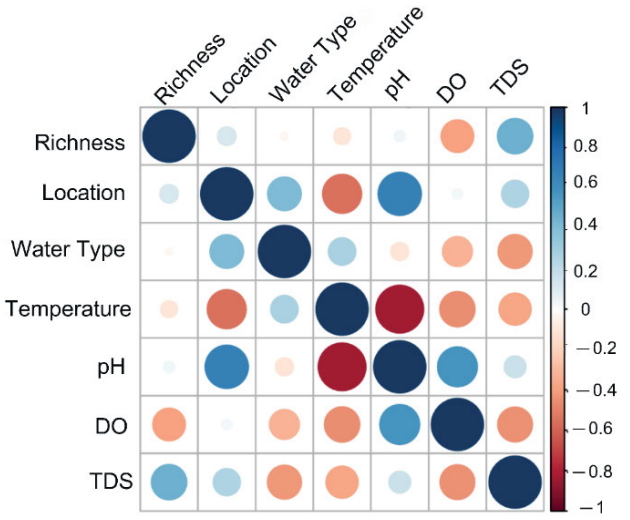

Fig. 8. Correlation plot of sampling variables and water qualities; pH = potential hydrogen, DO = dissolved oxygen, TDS = total dissolved solids; source: own study

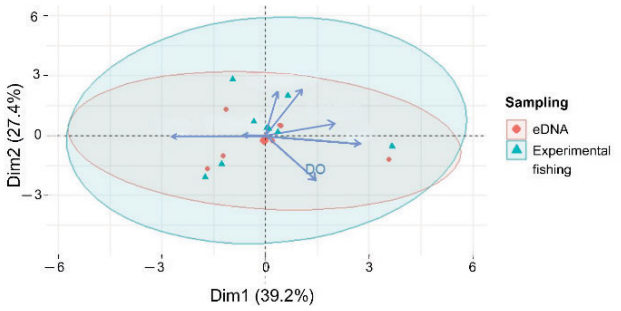

Fig. 9. Biplot distribution two different sampling techniques in relation with sampling variables and water qualities; pH = potential hydrogen, DO = dissolved oxygen, TDS = total dissolved solids; source: own study

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organic matter concentration, and fluctuating dissolved oxygen levels, which are essential for the survival of specific aquatic organisms (Erniaty *et al.*, 2023). Meanwhile, the Bikang River Basin is dominated by *Gluta renghas* trees. These forested areas serve distinct ecological functions, including preserving water quality and serving as breeding grounds for fish, both of which are crucial for the viability of unique fish populations (Posa, Wijedasa and Corlett, 2011).

Physical, chemical, and biological water quality is significantly affected by resource-related activities both on land and in water (Yanti and Haryadi, 2019). The transformation of peatlands into agricultural areas, such as oil palm plantations, negatively affects water quality by exacerbating pollution and nutrient loss (Sule *et al.*, 2018). These changes can alter the behaviour and reduce food availability for species such as Cyprinidae (Aliu, 2016). Fish diversity is further threatened by activities such as peat mining, which contribute to additional water quality degradation. Although Cyprinidae continue to dominate fish species in mining regions, overall fish diversity is lower in these areas compared to protected environments (Haryono and Tjakrawidjaja, 2000). Therefore, water quality, as indicated by various parameters, indirectly supports sustainable development efforts in the fisheries sector (Berthet, Vincent and Fleury, 2021). Based on this research, the water quality values in the Kurau and Bikang Rivers were still within a suitable range for supporting fish life. Despite the conformity of the aquatic environment, the eDNA sampling technique used in this study was less effective than that of experimental fishing in floodplain habitats. Several factors may influence the sensitivity of eDNA analysis. One such factor is the use of 12S rDNA primer, which can lead to misidentification at the species, genus, or family levels (Yang, Liu and Yu, 2018). Additionally, eDNA degradation may occur due to low pH, particularly in environments containing pyrite, which can delay the release of eDNA. Other environmental factors affecting eDNA stability and detection include turbidity caused by suspended particles, dissolved oxygen, temperature, water flow, UV radiation, microbial activity, and nutrient status (Barnes *et al.*, 2014; Eichmiller, Best and Sorensen, 2016; Stoeckle *et al.*, 2017; Salter, 2018; Fremier *et al.*, 2019; McCartin *et al.*, 2022; Holmes *et al.*, 2024). Moreover, the development of a comprehensive genetic database for freshwater ecosystems is crucial for genetic conservation efforts and the advancement of molecular-based monitoring approaches. The application of eDNA metabarcoding in this study was suboptimal; therefore, future research should include a comprehensive assessment of environmental conditions, preliminary testing, alternative sampling procedures, and repeated sampling in floodplain environments.

CONCLUSIONS

This study demonstrated that fish diversity in floodplain environments with extreme water quality can be assessed using either eDNA technique or experimental fishing. eDNA detected three species, experimental fishing detected 18 species, and one species was identified by both methods. The mean values of species abundance and the Shannon–Wiener diversity index were higher in the experimental fishing group compared to the eDNA results. Additionally, both indices were higher in the Kurau River than the Bikang River. The effectiveness of each sampling technique, in relation to species richness and influenced by environmental variation and water type, was affected by water quality parameters including pH, DO, temperature, and TDS. Optimisation of the eDNA technique for use in extreme floodplain water conditions, along with the development of a genetic reference database for fish species in Bangka Island, Indonesia, is essential.

SUPPLEMENTARY MATERIAL

Supplementary material to this article can be found online at https://www.jwld.pl/files/Supplementary_material_65_Helmizur-yani.pdf.

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CONFLICT OF INTERESTS

All authors declare that they have no conflict of interests.

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