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Combining eDNA metabarcoding and conventional method in rapid screening of fish diversity in Maros-Pangkep Global Geopark, South Sulawesi, Indonesia

AMBENG[1,♥], MUHAMMAD IQRAM[1], MAGDALENA LITAAY[1], ZOHRAH HASYIM[1], WILMA J. C. MOKA[2], GEORGINA MARIA TINUNGKI[3]

[1]Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Hasanuddin. Jl. Perintis Kemerdekaan Km.10, Makassar 90245, South Sulawesi, Indonesia. Tel.: +62-411-586200, Fax.: +62-411-585188, ♥email: ambeng85@gmail.com
[2]Faculty of Marine Science and Fisheries, Universitas Hasanuddin. Jl. Perintis Kemerdekaan Km.10, Makassar 90245, South Sulawesi, Indonesia
[3]Department of Statistics, Faculty of Mathematics and Natural Sciences, Universitas Hasanuddin. Jl. Perintis Kemerdekaan Km.10, Makassar 90245, South Sulawesi, Indonesia

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Abstract. *Ambeng, Iqram M, Litaay M, Hasyim Z, Moka WJC, Tinungki GM. 2024. Combining eDNA metabarcoding and conventional method in rapid screening of fish diversity in Maros-Pangkep Global Geopark, South Sulawesi, Indonesia. Biodiversitas 25: 819-828.* A newly designated geopark by UNESCO, Maros-Pangkep Global Geopark, South Sulawesi, Indonesia owns great geodiversity, exceptional cultural heritage, and high biodiversity. However, the global biodiversity crisis that has hit the earth might also occur in Maros-Pangkep river systems, potentially threatening endemic freshwater fish and all other biota. It has been reported that there is high pressure on the environment along the watershed in the Maros-Pangkep rivers, including a high sedimentation rate downstream, which is linked to extreme erosion upstream and could eventually affect fish populations such as endemic ones. This should prompt a call for the authorities to build a strategy for monitoring and documenting aquatic biota such as fish to preserve biodiversity. Environmental DNA (eDNA) could be considered a method for monitoring fish species based on the DNA traces they shed in the water. Hence, this study aims to use eDNA to survey fish biodiversity in several rivers in the Maros-Pangkep Global Geopark and compare the result against the traditional gillnet method. The results showed that using two methods, 29 fish species in 19 families and 15 orders were obtained from the three rivers. Despite the majority of detected species, 25 species, as the result of using eDNA, 4 species were only detected using a traditional method which eDNA failed to identify. Among those species, several endemic and non-native species were successfully identified by either one or both methods. This could eventually be a suggestion for responsible authorities in biodiversity conservation to combine both approaches to monitor and conserve local biodiversity.

Keywords: Biodiversity, eDNA, freshwater fish, karst, Maros-Pangkep

INTRODUCTION

The recent designation of Maros-Pangkep Global Geopark, South Sulawesi, Indonesia, by UNESCO has admitted the uniqueness of this landscape, including the biodiversity it holds, which has high endemicity of flora and fauna (UNESCO 2023). However, one thing to be concerned about is the global biodiversity crisis, which might threaten biodiversity in the area. A short time ago, a report by the Intergovernmental Science-Policy Platform on Biodiversity and Ecosystem Services (IPBES) highlighted the unprecedented decline of global biodiversity; thus immediate policy and management are highly demanded (IPBES 2019). The declining trend of biodiversity might directly affect nearly all ecosystems in the globe, but freshwater ecosystems should be highly concerned since various land based-activities such as dam construction, land use change, climate change, and anthropogenic and industrial pollution could eventually deteriorate river systems (Reid et al. 2019), and all living creatures in the water, including fish and other macroinvertebrates. These global circumstances are not only happening in a certain region but could also potentially exist in some rivers in the Maros-Pangkep karst landscape. Therefore, regarding freshwater fish, the region's main rivers have been reported to be home to at least 18 fish species which 5 species among them are endemic (Nur et al. 2019; Omar et al. 2021). The deteriorated ecological condition in the rivers within the landscape can potentially be observed and eventually affect the biota if further preventive measures are not taken. It has been reported that there is high pressure on the environment along the watershed in Maros-Pangkep rivers, including a high sedimentation rate in the downstream which is linked to the extreme erosion in the upstream (Salam et al. 2021). This condition has altered the morphometric of the rivers which in some parts are becoming narrow and eventually change the hydrological flow (Salam et al. 2021). Consequently, the alteration of river flow patterns and water quality could adversely impact the fish population and, on the worst occasion, account for the loss of 80% of aquatic biodiversity (Janse et al. 2015; Desrita et al. 2020).

This could be an alarming condition in Maros-Pangkep rivers, which should prompt a call for the authorities to be more aware to build a strategy for monitoring and documenting aquatic biota in the region to preserve the freshwater biodiversity. In supporting this effort,

environmental DNA (eDNA) could be considered a method for detecting organisms based on the DNA traces they shed in the water or other environments (Taberlet et al. 2012). Using eDNA metabarcoding will offer a minimally invasive and fast technique that is reliable for detecting multiple species, including non-indigenous, rare, and elusive species from any localities (Jerde et al. 2011; Deiner et al. 2017; Pawlowski et al. 2018). This method has expanded within the last decade, giving a promising potential for biodiversity assessment at spatial and temporal conditions (Pawlowski et al. 2018). eDNA as a non-destructive method has widely been used in fish biodiversity surveys, demonstrating its effectiveness in detecting and monitoring fish in freshwater ecosystems (Evans et al. 2017; He et al. 2022; Shen et al. 2022). Several studies revealed that eDNA outperforms established conventional methods in detecting both rare and common freshwater fish species, yet gill net could still offer more insights when combined with eDNA to obtain a complete overview of species composition (Piggott et al. 2021; Golpour et al. 2022). Thus, this study aims to use eDNA in combination with gill net to rapidly screen fish diversity in several rivers in the Maros-Pangkep Global Geopark in an attempt to examine a potential mean of monitoring aquatic biota in the area. In some instances, the MiFish-U and Teleo primer pairs were most effective for fish diversity monitoring with eDNA (Xiong et al. 2022). Despite both primer pairs are commonly used in eDNA for fish biodiversity monitoring, they are still argued which one performs better. A study examined their in-situ efficiency and showed a higher recovered species richness of Teleo than MiFish-U (Polanco et al. 2021). On the contrary, a review of eDNA methods recommended the MiFish-U since the primer provides higher species detection probabilities than the Teleo primer set (Shu et al. 2020). Hence, this study evaluated two primer sets, MiFish-U and Teleo, to compare their performance in detecting fish diversity within the Maros-Pangkep river system. The results are expected to give another instance of eDNA use in fish diversity monitoring and to inform the local authority regarding eDNA as the potential option to monitor and preserve regional biodiversity.

MATERIALS AND METHODS

Study area

Water samples were collected from three rivers in the Maros-Pangkep area, South Sulawesi, Indonesia, including Pangkajene River, which is administratively located in the Pangkep regency, and Pute as well as Bantimurung River, located in Maros administrative area. Three sites were surveyed in each river in April 2023, representing upstream, midstream, and downstream, as seen in Figure 1. Apart from the region's status as UNESCO Global Geopark, the rivers were chosen since they are major rivers in Maros-Pangkep karst area, which are home to several endemic species but have experienced various environmental disturbances from the surrounding activities such as industrial, agricultural, and anthropogenic pressure.

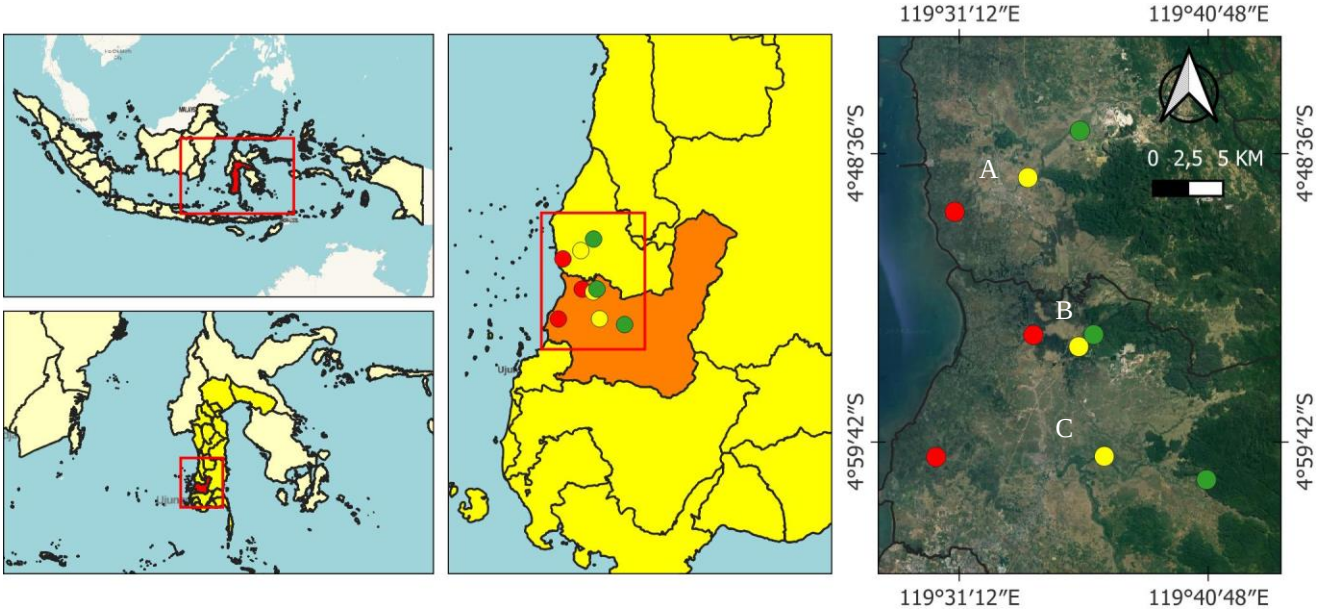

Figure 1. A map shows sampling sites in three main rivers in Maros-Pangkep karst landscape: A) Pangkajene River; B) Pute River; C) Bantimurung River. Coloured circles represent different zones from each river. Red circles present all sites located in the downstream of the river. Yellow circles depict locations in the midstream, while green ones are upstream area.

Procedures

Water sampling

Before going to the field, all nalgene bottles were cleaned with 10% sodium hypochlorite and sterilized under UV Light. The sterile gloves were used throughout the process to avoid contamination. One liter of surface water was collected in the sterile nalgene bottle and immediately preserved at 4°C in the cool box for transport to the laboratory. Three duplicates of 1 L from one site were collected for eDNA analysis, which will then be filtered through a 0.45 µm nylon filter membrane with a vacuum peristaltic pump. The funnel and filter head were thoroughly disinfected with 10% sodium hypochlorite before use. The filter membranes of each replicate were immediately stored in sterile 2 mL tubes containing Zymo Research DNA/RNA Shield and stored in the fridge/ at room temperature. At each site, filtration blank was conducted by filtering 1 L of distilled water in the same way to check for contamination.

Conventional method of catching fish

In addition to eDNA sample, a traditional method of catching fish using a gillnet was conducted. It is such a way to compare the effectiveness of eDNA to the conventional practice of identifying fish. Some studies have also compared the effectiveness of eDNA in identifying fish diversity against gillnetting (Golpour et al. 2022; Li et al. 2023). The gillnet was deployed at exactly the same point where the water was taken. For identification, all fish caught were photographed alive in a small chamber before releasing them back into the water. One specimen was too sensitive and died as soon as brought up out of the water after gillnetting. The list of species caught using gillnet can be seen in Table 1.

DNA extraction, amplification, and sequencing

DNA retained on the filter was extracted using the Qiagen Blood and Tissue DNA Extraction Kit (QIAGEN, Hilden, Germany), following the manufacturer's instructions. A total of 27 filters were extracted, representing three replicates from each site. Filtration blanks as negative control were also co-extracted alongside the sample with the same protocol. DNA was checked and visualized using 2.0% agarose gel. Thick bands were observed in all samples, while no bands were observed for the negative controls.

Two different processes were done to amplify the target region using different primers. The primer MiFish-U F (5′-GTCGGTAAAACTCGTGCCAGC-3′) and MiFish-U R (5′-CATAGTGGGGTATCTAATCCCAGTTTG- 3′) used to target the 12S rRNA gene (Miya et al. 2015). Another primer is Teleo - F (5'- ACACCGCCCGTCACTCT-3') and Teleo-R (5'- CTTCCGGTACACTTACCRTG - 3') (Valentini et al. 2016). The first PCR reaction contained 12 Kapa HotStart HiFi 2 × ReadyMix DNA polymerase, 1 µL each of 10 nM primers (F and R), 8 µL ddH2O, and 2 µL DNA Template. The phases of the DNA amplification PCR profile were as follows: (1) pre-denaturation of the template DNA at 95°C for 5 minutes; (2) denaturation of the template DNA at 98°C for 30 seconds; (3) annealing at 67°C (MiFish-U) and 55°C (Teleo) for 30 seconds; (4) primary extension at 72°C for 30 seconds and (5) final extension (post extension) at 72°C for 5 minutes with 35 cycles of stages (2)-(4). Therefore, the 96 Universal peqStAR PCR machine was used with negative controls (blank template) to detect contamination. PCR product quality was visualized using electrophoresis on 2% agarose gel (100 mL TAE buffer and 2 g agarose). A 3 µL aliquot of PCR product was inserted into each agarose well with a 100 bp DNA ladder in one of the wells. The electrophoresis machine was run at 50 Volts for 60 minutes, and the results were visualized using a UV Fluorescent via an Alpha imager Mini Gel Documentation System.

All triplicate PCR products passed the electrophoresis quality control and underwent a second PCR for indexing purposes. The IDT double index and Illumina sequencing adapter for Illumina - Nextera DNA Unique Dual Index, Set B were added to the target amplicon in the second PCR, using 12.5 µL of 12 MyFi 2 × ReadyMix and 2 µL of PCR product. The PCR cycle comprised an initial denaturation at 95°C (3 minutes), then 9 cycles of 95°C for 30 seconds, 55°C for 30 seconds, 72°C for 30 seconds, and a final extension at 72°C for 5 minutes. PCR purification was conducted on the first PCR and second PCR products using AMPure XP before proceeding to the next step. DNA sequencing was performed on an Illumina iSeq100.

Bioinformatics and data analysis

Qiime2 pipeline was used to process all the fastq. Cutadapt was deployed to trim the primer sequences of Teleo01 and MiFish-U at the first stage (Martin 2011). All samples were processed in two different running batches based on the primer used in this study. The DADA2 was used for filtering, merging, and denoising all sequences (Callahan et al. 2016). The chimeric sequences were also removed using the consensus method in DADA2, resulting in Amplicon Sequence Variance (ASVs). The ASV sequences were then assigned for taxonomy using BLAST+ with ≥97% similarity value at the species level (Camacho et al. 2009) against the MitoFish database (Iwasaki et al. 2013). Moreover, the taxonomy nomenclature was evaluated through the World Registered of Marine Species (WoRMS) (https://www.marinespecies.org/). The eDNA dataset was combined with the conventional method dataset in the absence and presence format. Alpha diversity was calculated based on observed species, Shannon, Chao1 using Phyloseq (McMurdie and Holmes 2013). The Analysis of Variance (ANOVA) was conducted to examine the differentiation of fish species richness obtained from different methods used in this study. Venn diagram of overlapping fish species was visualized using InteractiVenn (Heberle et al. 2015).

RESULTS AND DISCUSSION

Fish diversity

Fish diversity obtained from the three rivers using two methods consisted of 29 species in 19 families and 15 orders (Table 1). The conventional method detected 8 orders and 10 families from 12 species caught and observed in the field. In comparison, eDNA identified 12 orders and 15 families out of 25 species from two primers (Table 1). MiFish-U has

identified more families and orders of the two primers than Teleo. Moreover, 12 orders and 15 families were identified using MiFish-U, whereas only 9 orders and 10 families came out of the Teleo. Beloniformes have the most species members with four identified species, followed by Cypriniformes and Gobiiformes with three species for each of them. Most of the species observed can survive from marine brackish water to freshwater. *Encrasicholina punctifer* is the only species in the list that is only found in marine water, according to WoRMS (https://www.marinespecies.org/). *Marosatherina ladigesi, Trichopodus leerii, Leiopotherapon plumbeus, Barbodes binotatus, Barbonymus gonionotus,* and *Oryzias celebensis* are among the species that are narrowly confined in freshwater ecosystems. *M. ladigesi* and *O. celebensis* are two species listed as endemic species in Maros-Pangkep rivers. In addition, other species of non-native fishes but widely spread were also retrieved from this study, such as *Cyprinus carpio, Gambusia affinis,* and *Oreochromis niloticus.*

Comparison between eDNA metabarcoding and conventional method

As for the comparison of the effectiveness of both methods in discriminating freshwater species from three rivers in Maros-Pangkep, out of 29 fish species (Table 1), including overlapped species obtained by both methods, 8 (27.6%) species were detected by both eDNA and gillnet, 4 (13.8%) species were observed only with the gillnet, while 17 (58.6%) of total species were identified solely using eDNA metabarcoding (Figure 2.A). This result has put a clear emphasize to some extent that eDNA outperforms the traditional method using gillnet in species-level identification. Despite the majority of species identified with eDNA, four species were observed with the traditional method but eDNA failed to detect their presence. For instance, *Marosatherina ladigesi* was abundantly available upstream of Pangkajene River, but no information was retrieved after eDNA metabarcoding. Likewise, *Toxotes jaculatrix* was also present during the sampling but was not detected through eDNA.

Table 1. List of species identified using different methods from three rivers in Maros-Pangkep Karst. eDNA with two different primers and a conventional method using gillnet. The black dot represents the presence of species in the site by using different methods

Class	Order	Family	Species	MiFish-U	Teleo	Gillnet
Actinopterygii	Anabantiformes	Channidae	*Channa striata*	●	●	
		Osphronemidae	*Trichopodus leerii*	●		
	Atheriniformes	Telmatherinidae	*Marosatherina ladigesi*			●
	Beloniformes	Zenarchopteridae	*Dermogenys pusilla*	●		●
		Adrianichthyidae	*Oryzias celebensis*	●	●	●
		Adrianichthyidae	*Oryzias javanicus*	●	●	●
		Zenarchopteridae	*Zenarchopterus dispar*	●	●	
	Carangaria	Latidae	*Lates calcalifer*			●
		Toxotidae	*Toxotes jaculatrix*			●
	Centrarchiformes	Terapontidae	*Leiopotherapon plumbeus*	●		
	Cichliformes	Cichlidae	*Oreochromis niloticus*	●	●	
		Cichlidae	*Oreochromis sp.*		●	
		Cichlidae	*Sarotherodon galilaeus*		●	
	Clupeiformes	Engarulidae	*Encrasicholina punctifer*	●	●	
	Cypriniformes	Cyprinidae	*Barbodes binotatus*	●		
		Cyprinidae	*Barbonymus gonionotus*	●	●	
		Cyprinidae	*Cyprinus carpio*		●	
		Cyprinidae	*Osteochilus sp.*	●	●	●
	Cyprinodontiformes	Poeciliidae	*Gambusia affinis*	●		●
		Poeciliidae	*Poecilia velifera*		●	
	Elopiformes	Megalopidae	*Megalops cyprinoides*	●		
	Gerreiformes	Gerreidae	*Gerres microphthalmus*	●	●	
	Gobiiformes	Gobiidae	*Butis sp.*	●		●
		Gobiidae	*Glossogobius aureus*		●	●
		Gobiidae	*Mugilogobius cavifrons*	●		
		Oxudercidae	*Pseudogobius sp.*	●		
	Gonorynchiformes	Chanidae	*Chanos chanos*	●	●	
	Ovalentaria	Ambassidae	*Ambassis interrupta*	●		●
	Syngnathiformes	Syngnathidae	*Ichthyocampus carce*			●

ANOVA test was performed to see any significant difference between eDNA metabarcoding and gillnetting using alpha diversity measure from all sites in the three rivers. In this study, it was observed that there is a differentiation in the extent of distinguishing fish species in different rivers (Figure 3). There is a clear significant difference in alpha diversity between all methods in Bantimurung River (Figure 3), but it could not simply be interpreted that the traditional method is far better than eDNA since there could be another reason underlying this result in Bantimurung River such as strong river flows or seasonal change in water condition (Curtis et al. 2021). Meanwhile, despite no significant difference (p-value = 0.34) in Pute River, in which sampling was conducted in normal conditions, clear and slow stream flow, eDNA metabarcoding performed better than gillnetting.

Another test was done to compare the results of each method in one river with the other rivers. No significant difference in the alpha biodiversity was showed among all rivers where gillnet was deployed. On the contrary, MiFish-U and Teleo showed performance variation in detecting fish in each river, leading to a significant difference in alpha biodiversity among the rivers.

A general comparison to see the difference between total fish identified using both methods showed that regardless of the significant difference among all methods (p-value = 0.16), there is a slightly different in which more species richness could be observed using eDNA than the conventional method. Moreover, the Shannon-Wiener index (Figure 4) emphasized an obvious difference among the methods in which eDNA metabarcoding is more favorable for delineating biodiversity.

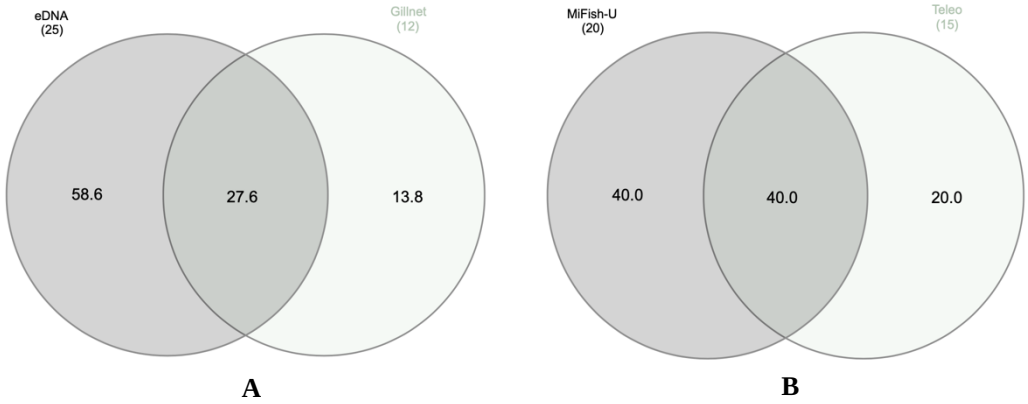

Figure 2. Venn diagram shows the number of overlapping species identified using either method (A) and a comparison of the effectiveness of two different primers in identifying species from three different rivers in Maros-Pangkep Karst Landscape (B); the number inside the Venn diagram is represented in percentage

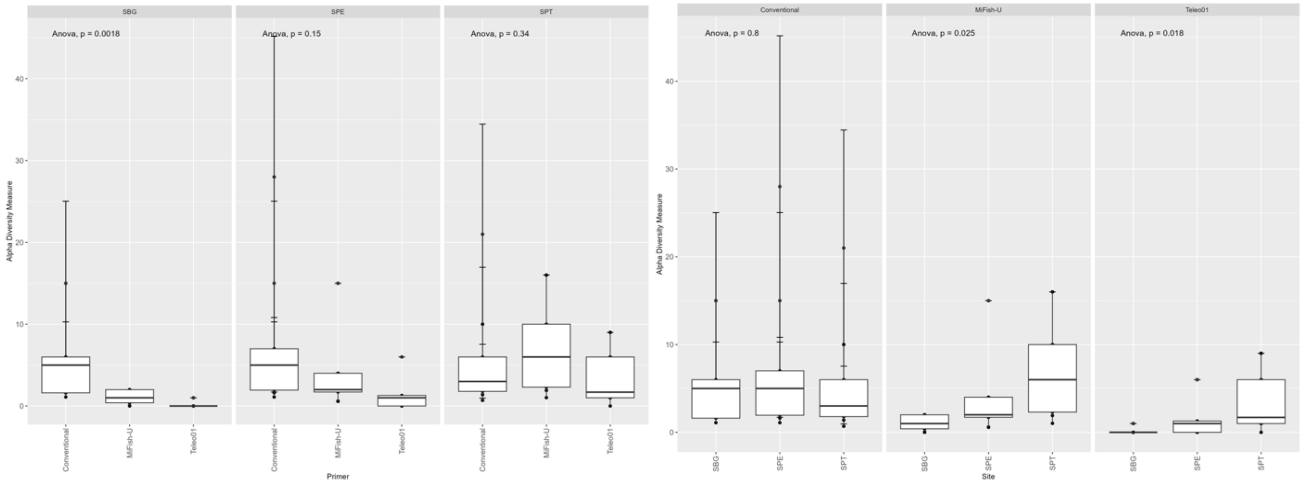

Figure 3. The box plot compares each method's performance detecting fish species in all studied rivers. (Left) A comparison between methods in one river, and (Right) a test to differentiate the performance of each method in one river concerning the other

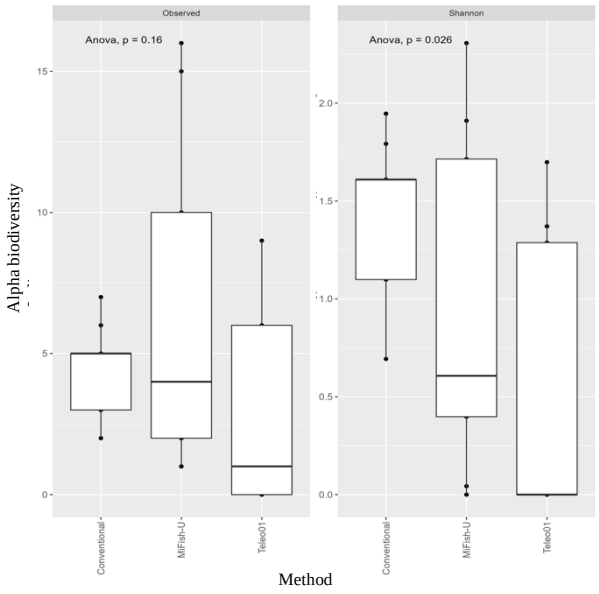

Figure 4. The box plot shows the difference between methods of detecting fish richness when all species from rivers were merged

Comparison between MiFish-U and Teleo Primer

In this study, we attempted to test the effectiveness of two commonly used primers, MiFish-U and Teleo, in discriminating freshwater species from rivers in Maros-Pangkep region. MiFish-U has obtained more species than Teleo, identifying 20 and 15 species, respectively. There is a 40% overlap between the two primers, implying that 10 species were successfully identified using both methods. While only five species (20%) were solely identified by the Teleo primer, there are ten species (40%) that have been listed using MiFish-U (Figure 2.B). Briefly, the study showed that MiFish-U might be efficient in determining number of fishes identified from the eDNA samples. The ability of the two primers for fish identification can be seen from the reads proportion of identified taxa at the site level (Figure 5). From the bar chart (Figure 5), there seems to be a discrepancy between the two primers in detecting fish from each site. No fish is found in sites 2 and 3 of Bantimurung River and sites 3 in Pangkajene River, which could raise a question about Teleo's performance. Despite working out in some sites, Teleo could only detect one fish from site 1, Bantimurung River, site 2 Pangkajene River, and site 1 in Pute River. Meanwhile, MiFish-U showed a better resolution by at least detecting species in every site.

Furthermore, the ANOVA test (Figure 6) has showed no significant difference between the performance of both primers in screening the presence of fish in the site, particularly for Pangkajene and Pute River. However, a significant difference (p-value = 0.034) is seen in Bantimurung River when comparing the ability of MiFish-U and Teleo. It is clear that it is because lack of fish identified in the river using Teleo.

Discussion

Fish is one of the major components in aquatic ecosystems that have a pivotal role in creating ecosystem balance and could also be considered as an indicator in evaluating the biological integrity of water bodies, particularly in the case of Maros-Pangkep river systems where some disturbances have started to occur (Salam et al. 2021). Fish are also broadly recognized as a biological indicator of water quality following their sensitivity to pollutants and other environmental changes due to anthropogenic activities (Plessl et al. 2017; Ali et al. 2020). Therefore, it is inevitable to keep monitoring the fish population as a bioindicator or as a biodiversity constituent, particularly in areas such as Maros-Pangkep geopark. There were 25 fish species retrieved after using eDNA from the three rivers in Maros-Pangkep region, which is more than the number of fish caught using gillnet. This result is in concordant with some studies that show higher detection rate of fish compared to traditional methods (Hänfling et al. 2016; Valentini et al. 2016). This study has thus demonstrated the evidence of the potential alternative of fish monitoring using eDNA metabarcoding in the region.

Comparison between eDNA metabarcoding and conventional method

Two endemic fish out of five species that have been published (Omar et al. 2021) were successfully screened in this study. *O. celebensis* were detected using both methods, while *M. ladigesi* was only retrieved by gillnetting since eDNA failed to detect them despite being abundant in the water when sampling was done. Another similar instance was when two species of elasmobranchs were observed while water sampling but failed to be detected using eDNA (Williford et al. 2023). This has given another instance that eDNA might still have issues to be solved. Despite being widely published that eDNA has various benefits such as cost-effectiveness, broader geographic coverage, early detection of invasive species, and non-lethal stock assessments, it is pivotal to carefully evaluate the eDNA signal, assay design, experimental design, quality assurance, and quality control checks to build more confidence in the eDNA metabarcoding (Jerde 2021). One possible answer for the undetected *M. ladigesi* is due to the incomplete MitoFish database.

A manual search was done on the MitoFish database by typing the species name *M. ladigesi*, but the species is not yet on the database. The consequences of not having a complete database for eDNA analysis include the potential for failure to detect certain fish species that seem to be abundant in the river. The MitoFish database contains genetic information, which is important for identifying fish species through eDNA analysis. Incomplete databases can cause inaccurate taxonomic assignments, affecting the reliability of the results (Sato et al. 2018). Moreover, the completeness of reference databases used for taxonomic classification is crucial to avoid false negatives in eDNA metabarcoding analyses (Petit-Marty et al. 2023). Furthermore, other non-indigenous species, such as *Cyprinus carpio, Gambusia affinis,* and *Oreochromis niloticus,* were successfully detected using eDNA. The detection of endemic and non-native species in the river could suggest the local authority to consider eDNA metabarcoding as a tool for biodiversity monitoring of aquatic biota, complementing traditional methods.

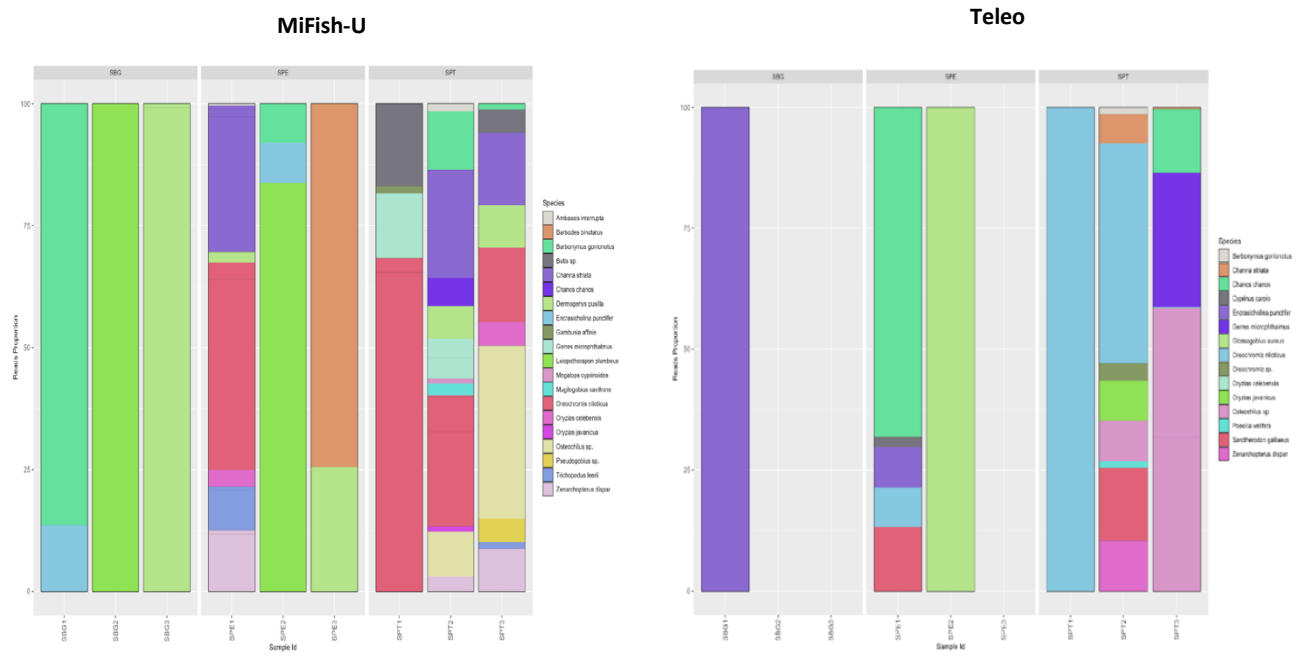

Figure 5. The bar chart displays the proportion of identified taxa in each site based on reads proportion using MiFish-U (*left*) and Teleo (*right*). SBG is short for Bantimurung River; SPE stands for Pangkajene River; and SPT is Pute River. The number represents each site at the end of each river's name

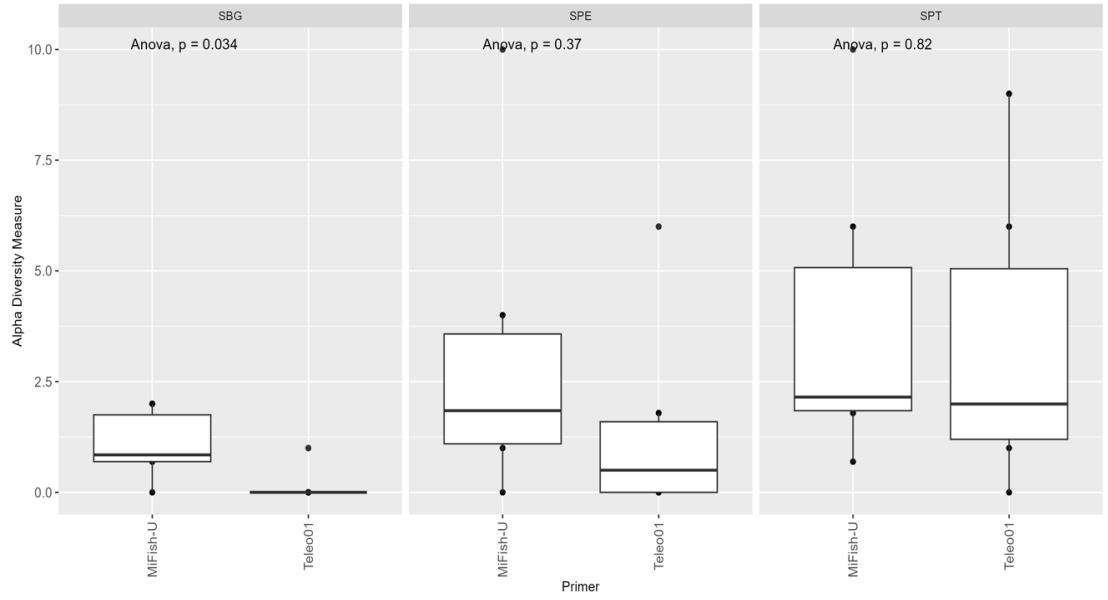

Figure 6. Boxplot shows the different abilities of both primers in delineating fish diversity from the three rivers. SBG is short for Bantimurung River; SPE stands for Pangkajene River; and SPT is Pute River

Traditional methods might not detect as many species as eDNA did. Still, it is quite interesting that four species such as *M. ladigesi, T. jaculatrix, Ichthyocampus carce,* and *Lates calcalifer*, were detected, which eDNA failed to detect them leading to a false negative (type II error) detection. It is important to note that traditional methods may still have advantages in certain occasions which could basically complement the weakness of eDNA. For instance, a study comparing eDNA and traditional sampling methods observed that some species such as *Pseudorasbora parva, Carassius carassius* and *Lota lota* were only detected using traditional sampling gears, while eDNA unsuccessfully detected the presence of these fishes (Golpour et al. 2022). Another study reported that some fish species have been detected using gill nets but not with eDNA, including rare and invasive fishes such as non-native species like

Hypophthalmichthys nobilis (Feng et al. 2023). This discrepancy of results between the two methods, in one hand adding the complementary value of gillnetting on eDNA detectability. On the other hand, it shows the limitation of eDNA in detecting fish which could potentially be associated with the limited amount of eDNA in the water released by rare species (Golpour et al. 2022). This instance could be seen on *I. carce* in Pangkajene river which is rarely found during the sampling, detected by using gillnet but undetected with eDNA, giving a false negative detection. As mentioned in the previous paragraph, the incompleteness of database could also explain another reason the inability of eDNA detected *M. ladigesi* despite it was abundantly present in the sampling site. Furthermore, gillnetting may offer the advantage of capturing and observing live specimens, providing additional morphological information that eDNA analysis alone. However, it should not instantly be interpreted that the traditional method is still preferable over eDNA. One should be noted that several factors including observer bias, distinct visible morphological traits, misidentification bias, and environmental conditions, could influence the accuracy of traditional methods in detecting fish species. Understanding these factors is essential to improve the accuracy of traditional fish methods (Kirsch et al. 2018; Czeglédi et al. 2021).

Comparison between MiFish-U and Teleo Primer

The two primer sets used in this study are designed to amplify fish DNA for eDNA analysis. However, both primers-seemed to have showed different performance and inconsistency in fish detection throughout the sites, particularly Teleo's detection ability in sites located upstream of Bantimurung and Pangkajene rivers. Meanwhile, it worked out and successfully discriminated some fish species in other sites (Figure 5). A study has also showed that Teleo primer covered fewer species than MiFish-U could (Duhamet et al. 2023). Despite more orders and families detected using MiFish-U primer, a note remains in which Teleo primer could complement the MiFish-U. Five species were identified using Teleo, but MiFish-U primer could not detect them which could give another instance of the complementary trait between the two primers. Previous studies have suggested that combining Teleo and MiFish-U could show the best performance in recovering fish diversity and thus suggested using this combination to comprehensively detect fish assemblages (Polanco et al. 2021). The inconsistent results when using the Teleo and MiFish-U primer for eDNA have been observed in various studies. The evaluation of five primer pairs for eDNA metabarcoding of Central European fish species based on mock communities discovered differences in primer efficiency depending on the species and primer preferential species in different fish (Macher et al. 2023). Furthermore, the genetic coverage of both primers by depth and ocean was assessed, highlighting potential differences in their performance (Duhamet et al. 2023). Using different primer pairs can lead to variations in amplification efficiency, taxonomic coverage, and preferential species, potentially contributing to inconsistencies in eDNA analysis results. Thus, the selection of primers and the completeness of reference databases are pivotal in eDNA to ensure accurate and reliable detection of fish species (Shu et al. 2021).

Overall, this study has added another example of using eDNA in identifying fish biodiversity in combination with gillnetting in the river. Despite detecting more species both endemic and invasive fishes from three rivers in Maros-Pangkep area, the results given by eDNA should be carefully interpreted since there are still some drawbacks on eDNA use (Evans et al. 2017). And regardless of detecting less species than eDNA, traditional methods can still be a complement for the eDNA. Some studies have suggested that the combination of eDNA and fish capturing can reliably provide a real condition of communities in freshwater habitat (Lacoursière-Roussel et al. 2016; Hering et al. 2018). Further studies are needed to cover more sites in the region so that it can comprehensively verify the eDNA use in monitoring fish diversity in Maros-Pangkep karst landscape. It is also important to keep exploring DNA barcoding species that are still not on the database, such as MitoFish or other DNA databases often used as references. This will eventually improve the detection ability of eDNA as a potential technique for biodiversity monitoring and discovery amidst the global biodiversity crisis. The result could also inform the local authority to start looking at the eDNA method as a complementary approach to the traditional methods for freshwater biodiversity monitoring in an attempt to preserve unique biodiversity in Maros-Pangkep karst landscape.

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