Fish Biodiversity Monitoring in Singkarak Lake, West Sumatra: Comparison of Fish Detections Using Environmental DNA and Conventional Methods

D. I. Roesma(✉), D. H. Tjong, Syaifullah, Nofrita, M. N. Janra, F. D. L. Prawira, V. M. Salis, and D. R. Aidil

Department of Biology, Faculty of Mathematics and Natural Sciences, Andalas University. Limau Manis, Pauh, Padang, West Sumatra, Indonesia
dewiroesma@sci.unand.ac.id

Abstract. Singkarak lake is the largest lake in West Sumatra that is experiencing a decline in fish species. The main factors are constructing hydroelectric power plants, degradation, overfishing, and invasive species. Therefore, a reliable non-invasive survey method is needed to detect fish species. A monitoring study was conducted to compare the conventional recording of Singkarak Lake fish biodiversity with the eDNA method. Water samples were taken as much as one L with two replications to filtering and amplified using universal fish primers with the NGS technique. The study detected as many as 147 species from 80 genera, 18 families, and nine orders. The study detected ten species of the total (30) previously reported in Singkarak Lake. Four species were found in all studies: *Barbonymus schwanefeldii*, *Clarias batrachus, Mystacoleucus padangensis*, and *Osteochilus vittatus/Osteochilus hasseltii*. The study also detected the species that have never been previously reported in Singkarak Lake (135 species). The study showed the eDNA method could be used for fish monitoring by considering some factors: DNA quality, contamination, the use of specific primers, and the availability of sequence in Genbank. The results can be useful for biomonitoring the other taxa in Singkarak Lake, West Sumatra, using the eDNA method.

Keywords: Singkarak lake · monitoring · environmental DNA · Next generation sequencing · specific primer

1 Introduction

Aquatic resources, such as lakes, which have various attractions and benefits, are experiencing declining quality and quantity [1]. The management of water areas was carried out through monitoring that begins with identifying the characteristics of the components that make up the ecosystem [1]. Before the significant changes, monitoring and evaluating native communities from the waters provided information about biodiversity, which is needed to resolve anthropogenic stress and determine conservation strategies [2, 3]. One of the ecosystem components of the lake's water is the freshwater fish group.

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Based on the available data, freshwaters worldwide, especially lakes, lack the basic data on fish biodiversity, which is important for local people [4, 5].

For a long time, fish biodiversity survey has been conducted using conventional methods (direct capture) with various fishing gear. However, the differences in the time collections, fishing gear, and habitat conditions for each capture resulted in different species detection, which may reduce detection accuracy and fish diversity estimates [6, 7]. Thus, reliable capture methods are needed to complete fish diversity data [8]. Singkarak Lake is one of the aquatic ecosystems that have experienced changes in habitat conditions continuously, which affect the fish biodiversity. Singkarak is the largest lake in West Sumatra and the second largest in Sumatra after Toba Lake. Singkarak Lake is one of the lakes formed due to tectonic processes influenced by the Sumatran Fault [9]. Singkarak Lake directly or indirectly becomes the main support for the local community's economy due to the lake water being used for washing and bathing, water sources to irrigate rice fields and plantations, sources of community livelihood through fish catches, recreational areas, and water sources for the Singkarak hydropower plant [10].

Although Singkarak is the largest lake in West Sumatra, the diversity of native fish is relatively lower than the other lakes. It occurs because the mesotrophic conditions of Singkarak Lake do not support the development and growth of plankton and benthos as a source of fish food [11]. Currently, the number of fish found in Singkarak Lake continues to decline. The first data reported that 26 fish live in Singkarak Lake [12, 13]. However, future studies reported only found 19 species [14, 15], and in 2011 declined to 16 species [16].

The decline in fish populations, especially native species, is influenced by various factors, including; hydropower development, invasive capture methods, overfishing, degradation in the system, and invasive species [9, 17]. Therefore, efforts to remove fish resources from threat must be made immediately with a fish diversity survey using non-invasive (environmentally friendly) methods. For a long time, biodiversity waters survey was conducted using the conventional method (observation and direct capture organisms) [18]. However, some native species in Singkarak Lake are difficult to find using direct capture because of the population decline. Therefore, a reliable capture method is needed to monitor and evaluate fish diversity to improve management and conservation strategies [19, 20].

The development in the survey method makes environmental DNA (eDNA) a promising method for monitoring and evaluating fish diversity in a short time [21]. The eDNA is a sample of DNA from an organism that is released into the environment, either living or dead [22–25]. The eDNA method has been increasingly used because able to overcome the shortcomings of the survey using the conventional method (direct capture), which consume a lot of time, used the invasive method (disturbs, captures, and kills organisms) and is more cost-effective for large-scale monitor [26–29].

The DNA obtained directly from environmental samples is translated into DNA sequences using the High-throughput sequencing (HTS) technique, Next Generation Sequencing (NGS). The NGS technique can read the multiple sequences (at one time), which is more efficient than Sanger sequencing [30]. The eDNA study has been applied in other countries for quite a long time [6, 21]. Many studies have directly assessed the

ability of the eDNA method compared with the conventional method (direct capture) [6, 21, 31–33]. Comparisons between methods showed that the eDNA method has a similar or slightly different performance than that conventional method [6, 21, 34]. Recently, the eDNA method has been applied to biodiversity surveys from various taxa in Indonesia, including; the fish diversity in Pondok Dadap, Malang [35], marine fish detection in the Pelabuhan Ratu Bay, Indonesia [36], diversity studies of coral reefs (different species) in Indonesia [37], freshwater vertebrates monitoring in Maninjau Lake, West Sumatra [38, 39], and biomonitoring of coral reef fish communities [40].

The previous study [38, 39] showed that the eDNA method detected fewer fish species in Maninjau Lake than previously reported studies using conventional methods. Those results related to using non-specific primers and unavailable sequence data species in Genbank. Recently, some fish sequences in West Sumatra from the previous studies [16, 41–44] have been reported to Genbank, which can be used as references to the eDNA study. Although the previous study [38, 39] in Maninjau Lake was not 100% successful, the study showed that the eDNA method could be applied to fish biodiversity surveys in other waters by considering the factors affecting detection accuracy. Therefore, a fish biodiversity monitoring study in Singkarak Lake using eDNA and NGS techniques was designed. The study compares the conventional recording of Singkarak Lake fish biodiversity with the eDNA method. The results can be used as references for biomonitoring the other taxa in Singkarak Lake, West Sumatra, using the eDNA method.

2 Material and Methods

2.1 Sampling

All equipment that needs to be sterilized is prepared for sample collection to avoid contamination. eDNA samples were carried out in May 2022 in Singkarak Lake, West Sumatra. Water sample collection using the eDNA method following the protocol [45, 46]. The water samples were collected as much as 2 L using a sterilized bottle. The bottles are labeled with the location name and collection number, then placed into the cool box. The sample was carried out to the laboratory for rapid filtration using a vacuum machine. Ecological data consisting of temperature, hydrogen power (pH), humidity, and Global positioning System (GPS) were recorded.

2.2 Water Sample Filtration

Water samples, vacuum machines, analytical funnel filters, membrane filters, vacuum flasks, bleach solution, and distilled water were prepared for the filtration process. First, the water sample was put into a disposable analytical test filter funnel (250 mL) (Nalgene, USA) with 0.22 μm pore size (47 mm diameter) Durapore filter membrane (Millipore, MA, USA) for filtration using a vacuum machine [47, 48]. During filtration, ensure that the vacuum pressure is maintained (if a pump gauge monitor is available) or check the water level to ensure that water flows between the filter funnel and the vacuum flask. After filtration, sterile forceps removed the filter membrane from the analytical test funnel filter. The filter membrane was folded and placed into a sterile 2 ml microtube

containing absolute ethanol (PA). The tube was closed tightly and labeled with sample ID and the date filtration using an ethanol marker, and stored the sample at −20 °C until the DNA isolation process [45, 46].

2.3 DNA Isolation, Polymerase Chain Reaction (PCR), and Sequencing

The genomic DNA (gDNA) isolation was conducted using the gSYNC™ DNA Extraction Kit (Geneaid, GS300) DNA isolation kit following the protocol. The isolate quantities, such as DNA purity and concentration, were examined using a NanoDrop spectrophotometer (IMPLEN, CA, USA) and the Qubit dsDNA Assay Kit, Qubit 2.0 Fluorometer (Life Technologies, CA, USA). gDNA isolate was amplified using the MyTaq HS Red Mix PCR kit, 2X (Bioline, BIO-25048). Amplification of gDNA was performed with custom primers FISH F1 (5' TCAACCAACCACA AAGACATTGGCAC 3') forward and FISH R1 (5' TAGACTTCTGGGTGGCCAAAGAATCA 3') reverse [49]. The PCR quality was checked using electrophoresis on 1% agarose gel. Library preparations were conducted using Kits from Oxford Nanopore Technology. Sequencing was started with the attachment of rapid 1D sequencing adapters. Samples were primed and loaded onto the GRIDION machine.

2.4 Bioinformatic Analysis

GridION sequencing was performed by operating MinKNOW software version 20.06.9. Base calling with highly accurate mode was performed using Guppy version 4.0.11 [50]. The quality of FASTQ files was visualized using NanoPlot [51]. The filtered FASTQ data were classified using the Centrifuge classifier [52]. The consensus of reads was extracted using Medaka v1.5.0 (https://github.com/nanoporetech/medaka). Finally, the extracted consensus sequence was aligned against the NCBI nucleotide database using BLAST (https://blast.ncbi.nlm. Nih.gov).

3 Result

The gDNA samples from Singkarak Lake have been successfully isolated with purity levels ranging from 1.8 to 2.0 and DNA concentrations of 30 ng/ul and five ng/ul with nanodrop and qubit readings, respectively. A total of 124.750.000 reads were obtained from a single run on the GridION sequencing, Oxford Nanopure. Sequence raw data from water samples had a mean read length: of 810 and a median read length: of 760. All fragment reads detected as many as 147 freshwater fish from 80 genera, 18 families, and nine orders. The total species detected using the eDNA method were divided into two groups; 1) species previously reported in Singkarak Lake and 2) species that have never been reported in Singkarak Lake but detected using the eDNA method. Based on the distribution area, the second group is divided into three sub-groups; 1) species detected live in the other rivers and lakes in Sumatra, 2) species detected, however, never live in Sumatra Island and are found in other Indonesia Islands, and 3) species never detected present in Indonesia.

The results of the eDNA study were compared with recordings that have been reported in the previous studies using conventional methods, which are presented in Table 1. Based on Table 1, as many as 30 species have been reported in Singkarak Lake since the first data in 1913. Monitoring using the eDNA method detected as many as ten species (33.3% species) of the total species that have been previously reported in Singkarak Lake using conventional methods; there are *Barbonymus schwanefeldii* (Kapiek), *Cyprinus carpio* (Mas), *Mystacoleucus padangensis* (Bilih), *Oreochromis niloticus* (Nila), *Osteochilus vittatus/Osteochilus hasseltii* (Nilem), *Rasbora argyrotaenia* (Bada), *Rasbora jacobsoni* (Bada), *Channa striata* (Gabus), *Clarias batrachus* (Lele), and *Mastacembelus erythrotaenia* (Tilan). *Rasbora jacobsoni* is the highest abundance species (248 individuals) detected using the eDNA method. Among these species, there are introduced and invasive species such as; *Cyprinus carpio*, *Oreochromis niloticus*, and *Channa striata.*

Based on the lists of species found between the conventional and the eDNA methods showed does not always find the same species. Only four species were found in all studies there are *Barbonymus schwanefeldii*, *Clarias batrachus*, *Mystacoleucus padangensis,* and *Osteochilus vittatus/Osteochilus hasseltii.* Based on the total number of species reported using the conventional methods, as many as 20 species were undetected using the eDNA method. In between 20 undetected species that are native and important economic species, there are *Barbonymus belinka* (Balingkah), *Tor tambroides* (Gariang), *Tetraodon leiurus* (Jabuih/Buntal), and *Gobiopterus* cf. *Brachypterus* (Rinuak). Besides, among the undetected species, three species (*Homaloptera gymnogaster*, *Rasbora spilotaenia*, and *Nemacheilus olivaceus*) are only found in the first data survey and unreported again in further studies using the conventional method or the eDNA method. *Anabas testudineus* (Puyu), *Channa Lucius* (Gabus), *Cyclocheilichthys armatus* (Catua), *Hampala macrolepidota* (Sasau/Barau), *Mastacembelus unicolor* (Tilan), and *Tetraodon leiurus* (Jabuih/Buntal) are the species detected in all studies using conventional methods, however undetected using the eDNA. Species not detected using the eDNA but present in the system are classified as false negatives.

The second group is species that have never been reported in Singkarak Lake based on data since 1913, but detected using the eDNA method as many as 137 species (data not shown). Based on the distribution region, as many as 15 species are present in Sumatra. Two species are found in other lakes and rivers in West Sumatra; there are *Barbonymus goniotus* and *Rasbora sumatrana.* While 13 species are present on other rivers and lakes in Sumatra and are not found in West Sumatra, i.e., *Carassius auratus, Carrasius carrasius, Chromobotia macracanthus, Cyclocheilichthys repasson, Desmopuntius hexazona, Eirmotus* cf. *Furvus, Poecilia reticulata, Rasbora cephalotaenia, Rasbora elegans, Rasbora meinkeni, Rasbora tornieri, Trigonostigma heteromorpha,* and *Pangio cuneovirgata.* A total of 20 species were detected live on other Indonesian islands and not found on Sumatra Island. Between 20 species, as many as 14 as native and introduced species to Indonesia, there are *Boraras urophthalmoides*, *Danio kerri*, *Danio rerio*, *Dawkinsia denisonii*, *Eirmotus octozona*, *Elopichthys Bamboosa, Hypophthalmichthys molitrix, Kottelatia brittani, Labeo calbasu, Labeo rohita, Puntius titteya, Rasbora rubrodorsalis*, and *Schizothorax labiatus.* Meanwhile, six species are only found on certain islands in Indonesia, such as; in Borneo (*Boraras brigittae, Cyclocheilichthys janthochir,* and

Desmopuntius rhomboocellatus), Belitung (*Eirmotus* cf. *Insignis*), and Java and Bali (*Rasbora aprotaenia*, and *Rasbora baliensis*). A total of 102 other species detected were never reported found in Indonesia and are present in other Asia, Africa, and America. The detection of species that have never been reported in Singkarak Lake using the eDNA method is classified as a false positive.

4 Discussion

The first eDNA study in Singkarak Lake successfully detected fish species and other taxa. Other taxa detected in this study were not using for further analysis. The success of the eDNA method in detecting freshwater fish species for various purposes has been reported in previous studies [6, 21, 32, 33]. Table 1 compares the number and species found using the conventional and eDNA methods. The eDNA study successfully detects as many as ten species (33.3%) of the total species previously reported in Singkarak Lake since the first data in 1913. The results of detection using the eDNA method and the last survey using the conventional method [16] proved that the number of fish species in Singkarak Lake that can be found continuously decreased. The study [21] using eDNA metabarcoding only detected an average of 39% of the total species of the known community across all water bodies. The data presented low species detection because only using a single gene for taxonomic assignments [21]. Many eDNA studies [32–34] used multiple genes to increase the number of species detected.

Based on Table 1, each study not always found the same species. Only four species were found in all studies there are *Barbonymus schwanefeldii*, *Clarias batrachus*, *Mystacoleucus padangensis*, and *Osteochilus vittatus/Osteochilus hasseltii*. The low number of the same species found in all studies is related to differences in the collection time, sampling locations, methods and fishing gear used, and the species that inhabited Singkarak lake.

Sampling collection at the same time and locations will increase the percentage found the same species using both methods. The study [6] showed a high percentage of overlapping species (70%) between captures using the conventional method and the eDNA at the same time and location.

Mystacoleucus padangensis (Bilih) was initially assigned as endemic fish and became an important economic fish in Singkarak Lake. However, to maintain the declining population since 2003, Bilih fish seeds stocked in Toba Lake have grown and developed well. Since then, Bilih has been classified as native fish in Singkarak Lake because it can grow and develop in other waters in a limited area. Besides living in Toba Lake, Bilih fish also can be found in Maninjau Lake, West Sumatra. Various factors have contributed to the decline in *Mystacoleucus padangensis* populations [53], including overfishing using fishing gear that is not environmentally friendly (KJA, Bagan/lift nets, Langling/gill nets with small mesh sizes) [54–56] and the presence of invasive fish in Singkarak Lake. The fish catching using gill nets with a mesh size of <1 inch will catch fish that are smaller than the size of the first maturity of the gonads (immature), resulting in disruption of the spawning process [56]. Bagan (lift nets) can produce large catches; however, a lot of fish catch is wasted because the size of the fish is too small, which has an impact on the decline in the Bilih fish population.

Table 1. Comparison of fish detection using the eDNA method with the previous studies using the conventional method

No.	Ordo	Family	Genus	Species	[12-13]	[14-15]	[16]	eDNA method detection	note
1	Cypriniformes	Balitorida	Homaloptera	*Homaloptera*	✓				Type locality: Maninjau lake
2		Cyprinida	Barbonymus	*Barbonymus belinka*	✓	✓			Type locality: Singkarak lake
3				*Barbonymus schwanefeldii*	✓	✓	✓	✓	Type locality: Singkarak lake
4			Cyclocheilichthys	*Cyclocheilichthys apogon*	✓		✓		Type locality: Java
5				*Cyclocheilichthys*	✓	✓	✓		Type locality: Java
6			Cyprinus	*Cyprinus carpio*	✓			✓	introduction from Europa
7			Hampala	*Hampala bimaculata*	✓		✓		Type locality: Borneo
8				*Hampala macrolepidota*	✓	✓	✓		Type locality: Java
9			Mystacoleucus	*Mystacoleucus padangensis*	✓	✓	✓	✓	Type locality: Sumatra
10			Osteochilus	*Osteochilus vittatus/ Osteochilus hasseltii*	✓	✓	✓	✓	Type locality: Java
11			Tor	*Tor tambroides*	✓	✓			Type locality: Sumatra
12		Danionina	Rasbora	*Rasbora argyrotaenia*	✓			✓	Type locality: Java
13				*Rasbora jacobsoni*	✓			✓	Type locality: Singkarak lake
14				*Rasbora spilotaenia*	✓				Type locality: Sumatra
15		Nemacheil	Nemacheilus	*Nemacheilus olivaceus*	✓				Type locality: Borneo
16		Gobiidae	Gobiopterus	*Gobiopterus* cf. *brachypterus*	✓	✓			Type locality: Java
17	Perciformes	Anabantid	Anabas	*Anabas testudineus*	✓	✓	✓		introduction from Japan
18		Channidae	Channa	*Channa Lucius*	✓	✓	✓		Type locality: Java
19				*Channa striata*		✓	✓	✓	introduction from India (invasive species)
20		Cichlidae	Oreochromis	*Oreochromis nossambicus*	✓	✓			introduction from Mozambique
21				*Oreochromis niloticus*	✓	✓		✓	introduction from Nile river
22		Osphrone	Osphronemus	*Osphronemus goramy*	✓	✓			introduction from china
23			Trichopodus	*Trichopodus trichopterus*	✓	✓			introduction from india
24	Siluriformes	Bagridae	Hemibagrus	*Hemibagrus planiceps*	✓	✓			Type locality: Java
25				*Hemibagrus velox*			✓		Type locality: Sumatra
26		Claridae	Clarias	*Clarias batrachus*	✓	✓	✓	✓	Type locality: Java
27		Sisoridae	Glyptothorax	*Glyptothorax platypogon*	✓		✓		Type locality: Java
28	Synbranchiformes	Mastacembelidae	Mastacembelus	*Mastacembelus erythrotaenia*	✓		✓	✓	Type locality: Borneo
29				*Mastacembelus unicolor*	✓	✓	✓		Type locality: Java
30	Tetraodontifor	Tetraodont	Tetraodon	*Tetraodon leiurus*	✓	✓	✓		Type locality: Java

In contrast to *Mystacoleucus padangensis* and *Barbonymus schwanefeldii*, native fish of Singkarak Lake, *Osteochilus hasseltii* is an introduced fish regularly stocked by the Department of Marine and Fisheries, West Sumatra, to increase the catch of fishermen [17]. The introduction of *Osteochilus hasseltii* also aims to improve environmental quality [57]. *Osteochilus hasseltii* has a wide distribution area and inhabits various habitats. Therefore, this fish can be detected using conventional and eDNA methods.

Three species were detected using the eDNA method previously only reported in the first survey in 1913 [12]; *Cyprinus carpio*, *Rasbora argyrotaenia*, and *Rasbora jacobsoni*. *Cyprinus carpio* is an introduced fish with important economic value in Singkarak Lake. The sampling collection centered in the middle of the lake and adjacent to the cultivation area is suspected to be the reason detected of these species. The same case was also reported [21], which found *Pimephales promelas* detected in Trout Lake in 1981 (outside the most recent five years of monitoring). *Homaloptera gymnogaster, Rasbora spilotaenia,* and *Nemacheilus olivaceus* were only found in the first survey in 1913 and

were never reported in further studies using conventional methods or eDNA methods. The undetected species is suggested because of the decreasing populations due to being prey for invasive species. In addition, because these fish inhabit the bottom of the waters with sand/gravel substrates, it won't be easy to find them on the surface of the water lake. The study [21] reported that not detecting some species using the eDNA in Backwater lakes may result from a limited amount of eDNA on the lake's surface because the species inhabit the sediment or sand bed of the lakes.

Barbonymus belinka (Balingkah), *Tor tambroides* (Gariang), *Tetraodon leiurus* (Jabuih/Buntal), and *Gobiopterus* cf. *Brachypterus* (Rinuak) are native fish and have an important economic value which was not detected using the eDNA method. Therefore, undetectable targeted species inhabiting the system can be said to the false negative. False negatives were also reported in various eDNA studies [6, 21, 38, 39, 47]. For example, the previous study using the eDNA method in Maninjau Lake failed to detect native species such as *Rasbora*, *Hampala*, *Cyclocheilichthys*, and *Tor* genera [38, 39]. Another study [6] also reported were not detected three fish species using eDNA were previously found using the direct capture method (*Letenteron* sp., *Ctenopharyngodon idella*, and *Hypomesus nipponensis*). Rinuak is one of the economically important fish in Singkarak Lake, which still not has an exact scientific name. Morphologically, Rinuak is similar to *Gobiopterus brachypterus*, the only genus of transparent fish in Gobiidae. Genetic studies [43] showed that Rinuak has high sequence divergences from *Gobiopterus brachypterus* at different genus levels. However, *Gobiopterus* is the only transparent genus in Gobiidae; thus, Rinuak cannot place in clear taxa within Gobiidae. Therefore, [43] stated that Rinuak is a different species from *Gobiopterus brachypterus.*

Ten species found in the last survey [16] using the conventional method were not detected using eDNA, including; *Cyclocheilichthys apogon*, *Cyclocheilichthys armatus, Hampala bimaculata*, *Hampala macrolepidota, Anabas testudineus, Channa lucius*, *Hemibagrus velox, Glyptothorax platypogon*, *Mastacembelus unicolor,* and *Tetraodon leirus*. Undetected species that inhabit Singkarak Lake as a natural population, which classified into the false negatives suggest because several factors, including; the failure to collect target DNA in water samples, low DNA quality/the presence of inhibitors during the PCR process, unavailability of target sequences in Genbank, and not used the specific primers. Taxa determination from eDNA samples was based on the similarity of the nucleotide bases species to the sequences registered in Genbank. When the target sequence is not registered in Genbank, the determination of taxa is based on the highest nucleotide base similarity with the sequence in Genbank. Therefore, the absence of target sequences in Genbank will become one of the reasons for miss identification of species. False-negative detection has been reported in various eDNA studies [20, 58, 59] and maybe result due to the absence of DNA in water samples [48], low DNA quality, or the presence of inhibitors in the PCR process [27, 28, 61], unavailability of specific primers, and unregistered sequences target in Genbank [58, 59].

The second group is the detection of species that have never been reported in Singkarak Lake based on data survey since 1913. However, among these species, two species have been reported to be present in other lakes and rivers in West Sumatra there are *Barbonymus gonionotus* and *Rasbora sumatrana.* Although not reported in previous studies, this species has a natural distribution in the Sumatran, especially West

Sumatra. Thus, the DNA could have originated from a nearby waterbody connected to Singkarak Lake, or this species was present in Singkarak Lake but undetected using conventional methods. These species' detection could be true positive detections for species in Singkarak Lake. *Barbonymus gonionotus* and *Rasbora sumatrana* has been reported present in Diatas Lake, West Sumatra. Based on the geography, Diatas Lake is hydrologically connected to Singkarak Lake [16, 41], allowing *Barbonymus gonionotus* and *Rasbora sumatrana* to be present in Singkarak Lake through a river that connects the two lakes. The study [21] detected the presence of *Umbra limi* species, which has never been observed in Trout Lake since 1981. However, Trout Lake is hydrologically connected to Trout Bog, which could be the source of *Umbra limi*. Therefore, suspected that *Umbra limi* originated from the Trout Bog population.

As many as 102 other species that did not live in Indonesia but were detected in Singkarak Lake were classified as false positives. Various studies have also reported false positives in monitoring using the eDNA method [21, 38, 39]. Previous eDNA studies in Maninjau Lake [38, 39] detected as many as 35% native species to Europe, America, and Africa, which were never found in Indonesia, especially Maninjau Lake. The study [21] also detected eight species that had not been previously reported at three sites observation. False positive detection can occur due to several factors, including; contamination in the field/ laboratory [20, 62], competition between target DNA and non-target DNA [20], the specificity of the low primer for detecting species [21, 32], and unavailability of target sequences references in Genbank database [31].

The study showed that the eDNA method could detect species almost the same as conventional ones. However, various factors need to be considered for detection success to obtain maximum results, such as using specific primers and providing the target sequences submitted in Genbank as sequence references. Thus miss identification can be avoided in taxa determining.

5 Conclusion

1. The eDNA method using the NGS technique detected as many as 147 species from 80 genera, 18 families, and nine orders in Singkarak Lake.
2. The eDNA method detected ten species that have been previously reported in Singkarak Lake.
3. There are found 20 species not detected using the eDNA method (false negative), including the native species and important economic fish in Singkarak lake are *Barbonymus belinka* (Balingkah), *Tor tambroides* (Gariang), *Tetraodon leiurus* (Jabuih/Buntal), and *Gobiopterus* cf. *Brachypterus* (Rinuak).
4. As many as 135 species were detected using the eDNA, which was never found in Singkarak Lake (false positive).

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