

Aquatic eDNA for monitoring fish biodiversity in Ciliwung River, Indonesia

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Abstract. The economic development in the Ciliwung watershed has brought about various changes in the downstream area. Without consideration for the environment, development in urban areas will have a negative impact on water quality and aquatic life. Environmental DNA (eDNA) is a technology that can assist humans in producing more comprehensive biodiversity data than the conventional technology that has been widely used. This study aimed to assess the biodiversity of fish species at several points in the Downstream Ciliwung River, Jakarta, Indonesia. Among the 69 analyzed fish specimens, there were detected 11 species from 8 genera, 8 families, and 7 orders. The Cichlidae family was represented by the highest number of species. The diversity index (H') ranged from 0 to 1.59 (low to moderate diversity), while the dominance index (D) ranged from 0.24 to 1 (low to high dominance). The native species were not detected in Ciliwung River by the eDNA method. The eDNA method can be an effective tool for the next generation biodiversity monitoring technology.

Key Words: diversity index, dominance index, water quality, aquatic life, urban river.

Introduction. The river water quality degradation has a high relationship with the ecosystem damage, especially in urban areas. The high level of development and the urbanization rate in urban areas reduce vegetation cover and increase the rate of waste (both domestic and industrial) flowing into the rivers (Cheng et al 2018). This condition causes a high risk of runoff, sedimentation, and pollutants flow into the river (Kimijima et al 2020). Without consideration for the environment, development in urban areas will have a negative impact on water quality and aquatic life.

The condition of water quality in an ecosystem has an important role in maintaining aquatic life (Josué et al 2021; Sremački et al 2020). The high pollution in the river ecosystem cause oxygen depletion in the aquatic environment, consequently, for the intolerant native species it will be hard to survive. On the other hand, non-native and invasive species tend to be highly adaptable to oxygen depletion. This situation eventually triggered the extinction of the river's native species. Usually, invasive species are dispersed unintentionally due to human activities (Prasetyo et al 2022), being one of the biggest causal factors in the loss of native species in an area and have an impact on the natural ecosystems, human health, and economic losses (Wondie 2018). Their presence can change the trophic chain and the abundance of species in the ecosystem. Although initially changes to the environment might be insignificant, this condition can turn into an ecological disaster.

Ciliwung is one of the important rivers in Indonesia because it crosses various strategic areas on the Java island and flows into Jakarta, the nation's capital (Effendi 2016; Permatasari et al 2017). In the last few decades, development in the Ciliwung watershed has brought about various changes in the landscape, especially in the downstream area. The high human population in the Jakarta Metropolitan Area also increases the vulnerability of river flows due to the presence of domestic and industrial waste. Moreover, 75.6% or as many as 102 species of fish in the Ciliwung and Cisadane watersheds have experienced extinction. The extinction of most fish species in Ciliwung reduces their ability to support

ecosystem sustainability and economic value in people's lives (Jakarta Provincial Environmental Service 2019).

To determine the existing condition of fish species in the Ciliwung River, intensive monitoring is needed so that the potential of all species can be known. Environmental DNA (eDNA) is a technology that can assist humans in producing more comprehensive biodiversity data than conventional technology that has been widely used (Pawlowski et al 2020). Analysis of eDNA from water samples is an effective method for determining the presence of aquatic organisms such as fish, because they release a lot of genetic material in the form of lysed cells or faeces; these break down into small fragments which can be retained in the water column (Takahara et al 2013). This study aimed to assess the biodiversity of fish species at several points in the Lower Ciliwung River, Jakarta, Indonesia.

Material and Method

Description of the study sites. This study was conducted at Downstream of Ciliwung River, Jakarta. The three sampling locations (East Jakarta, Central Jakarta, and North Jakarta) were selected from upstream to downstream to study the effect of different ecosystems on fish biodiversity (Figure 1). The three ecosystems have different landscape conditions so that it is expected to have different conditions of water quality and diversity of fish species. The study was conducted in the dry season (July 2022) when the weather was sunny.

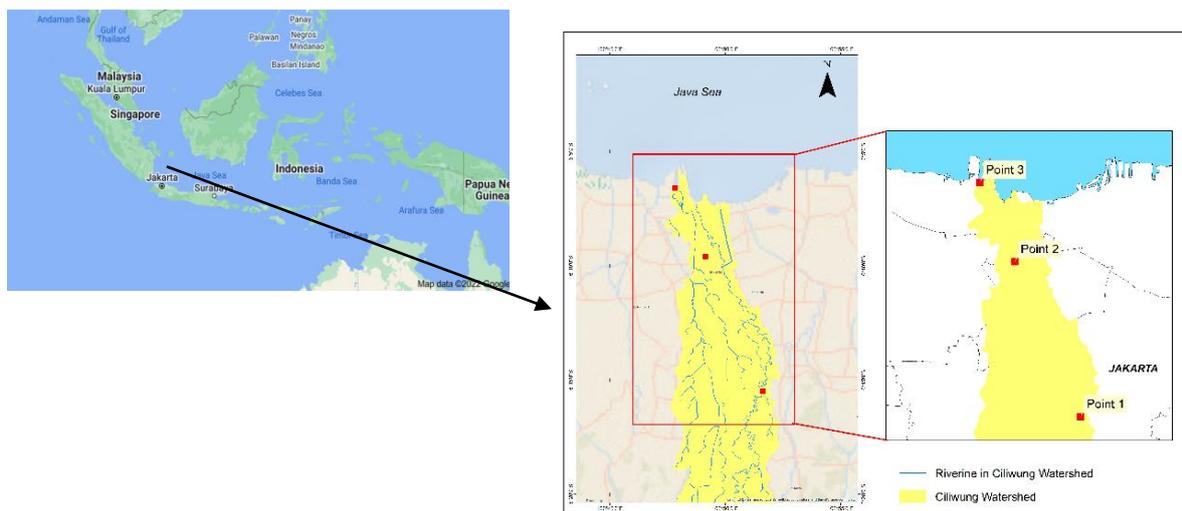


Figure 1. The sampling locations of eDNA.

eDNA sampling and laboratory analysis. Sampling of eDNA was carried out by the Oceanogen Laboratory in July 19, 2022. River water samples were taken at the top of the water column and collected in 4 L bottles. Each water sample was filtered through a Pall Corporation sterile filter paper of 0.45 m (47 mm diameter) using a peristaltic pump. After the filtration was completed, each filter paper was placed in a 2 mL cryotube filled with a 1.5 mL ZymoBIOMICS DNA/RNA shield. The flow chart for the eDNA analysis can be seen in Figure 2.

DNA extraction was carried out after a field sampling trip using the ZymoBIOMICS DNA extraction kit, manufactured by Zymo Research Corporation, by following the manufacturer's guidelines. The approximately 313 bp segment of the COI mtDNA region was amplified using a universal COI primer pair. After the analysis, the taxonomy of aquatic biota in the ecosystem will be known from the kingdom to the type of level. The process of eDNA laboratory analysis can be seen in Figure 3.

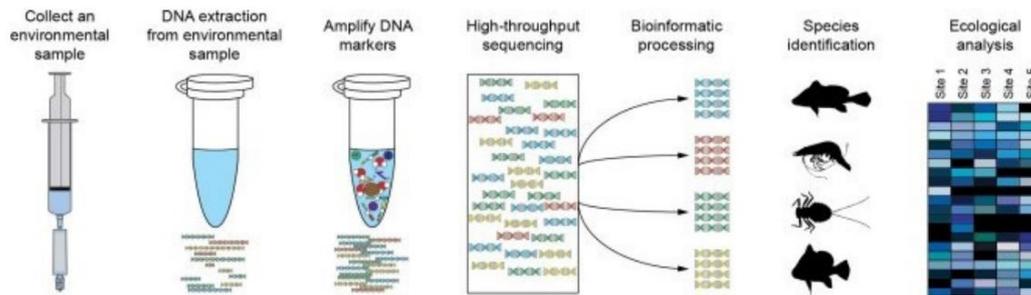


Figure 2. eDNA analysis flowchart (Madduppa et al 2021).

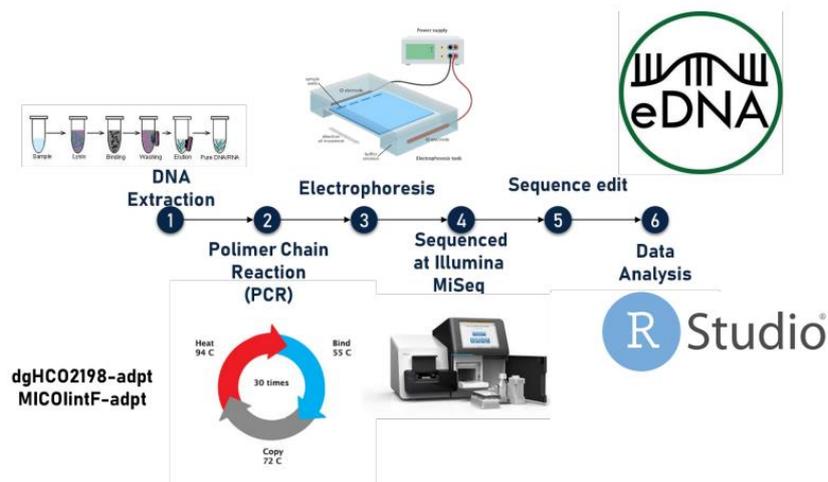


Figure 3. eDNA laboratory analysis (Madduppa et al 2021).

Data analysis. The results of the eDNA analysis, for the riverine ecosystem of each location, will show different biodiversity structures, according to location conditions. By using R, species richness can be assessed based on taxonomic criteria (Mächler et al 2019). The results of the analysis will also describe the potential of these species to live in various sampling point conditions. Bioinformatic analysis was performed using the software Quantitative Insights into Microbial Ecology 2 (QIIME2, <https://qiime2.org/>) for further quantitative analysis. All statistical analyses were conducted and visualized in R Studio v. 4.2.1 software. The species composition and relative abundance of the fish were analyzed and visualized using the ggplot2 package (Wickham 2009), then a one-way Anova (Analysis of Variance) was conducted to evaluate the difference in total abundance between each sampling station. The Shannon–Wiener Index (H') and the Simpson Index (D) were calculated to evaluate the species diversity and dominance at each sampling station. These calculations were performed in the vegan R package (Oksanen et al 2013).

Results and Discussion. The eDNA method of monitoring has successfully detected fish specimens from the water samples from the Lower Ciliwung, Jakarta, Indonesia. The results of the 69 sequencing reads identified 11 species from 8 genera, 8 families, and 7 orders. The result of fish identification using the eDNA method is shown in Table 1.

Among the 69 fish specimens identified based on the sequencing reads, the highest number of detected species was found in Cichlidae with three species. While the other families detected were represented by single species. Monitoring using the eDNA method only detected 8 families, 8 genera, and 11 species in Ciliwung Lake, which were also previously reported using the conventional method. The native fish groups from Ciliwung River, such as *Glyptothorax platypogon* and *Puntius binotatus*, which were found in a previous study (Hadiaty 2011) were not detected by the eDNA method. Species detection in eDNA samples can be influenced by the primer to amplify a specific mtDNA region. Zhang et al (2020) found that eDNA primers targeting the 12S rRNA gene identified more fish diversity than those targeting 16S rRNA or COI genes.

Table 1

Fish identification using the eDNA method

Order	Family	Genus	Species	No of reads sequences
Cypriniformes	Cyprinidae	Carassius	<i>Carassius auratus</i>	2
Gobiiformes	Gobiidae	Glossogobius	<i>Glossogobius giuris</i>	3
Cichliformes	Cichlidae	Oreochromis	<i>Oreochromis aureus</i>	4
			<i>Oreochromis niloticus</i>	3
			<i>Oreochromis urolepis</i>	24
Perciformes	Nototheniidae	Patagonotothen	<i>Patagonotothen tessellata</i>	2
	Scorpaenidae	Scorpaenodes	<i>Scorpaenodes littoralis</i>	4
Characiformes	Serrasalminidae	Piaractus	<i>Piaractus brachypomus</i>	6
			<i>Piaractus mesopotamicus</i>	6
Cyprinodontiformes	Poeciliidae	Poecilia	<i>Poecilia reticulata</i>	4
Siluriformes	Loricariidae	Pterygoplichthys	<i>Pterygoplichthys pardalis</i>	11
Total number of reads sequences				69

Hadiaty (2011) research on the Ciliwung River (upstream, middle, and downstream) found that 547 fish specimens were found, consisting of 20 species, 13 families, and 4 orders. The Cyprinidae family dominates the river, followed by the Bagridae, Channidae, and Poeciliidae. Fish native to the Ciliwung River, namely sisorid catfish (*G. platypogon*) and spotted barb (*P. binotatus*) were the most commonly found species, with 220 and 116 specimens, respectively. The third highest yield was an introduced guppy fish (*Poecilia reticulata*), with as many as 45 specimens.

Fish species composition and relative abundance. Each site has a different species composition and richness (Figure 4).

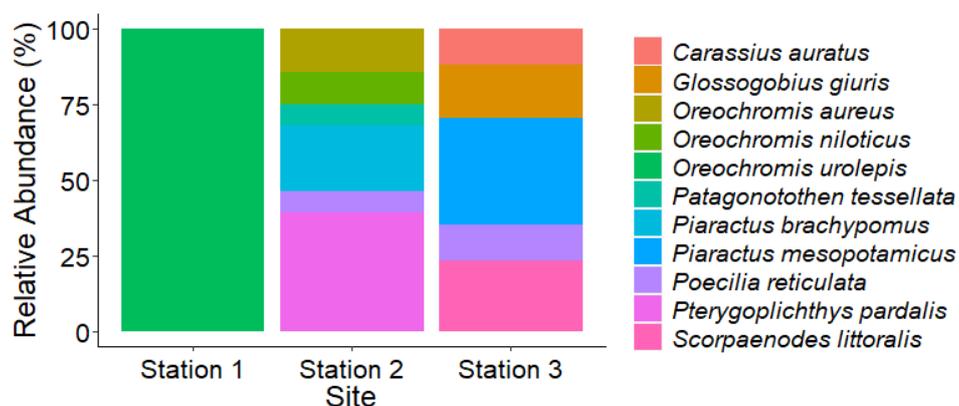


Figure 4. Fish species composition and relative abundance at each sampling station.

At station 1, only Wami tilapia, *Oreochromis urolepis*, species were found. At station 2, the species with the highest relative abundance was the Amazon sailfin catfish,

Pterygoplichthys pardalis (39.29%); followed by the red-bellied pacu, *Piaractus brachypomus* (21.43%); blue tilapia, *Oreochromis aureus* (14.29%); Nile tilapia, *Oreochromis niloticus* (10.71%); black southern cod, *Patagonotothen tessellata* and guppy, *P. reticulata* (7.14% each). At station 3, the small-scaled pacu, *Piaractus mesopotamicus* (35.29%) had the highest relative abundance, followed by the cheekspot scorpionfish, *Scorpaenodes littoralis* (23.53%); the tank goby, *Glossogobius giuris* (17.65%); the goldfish, *Carassius auratus* and *P. reticulata* (11.76%, respectively). In general, based on the sequencing reads, 24 fish specimens were found at station 1, 28 fish specimens at station 2, and 17 fish specimens at station 3, thus, there were no significant differences in the total abundance between stations (p -value >0.05).

In Indonesia, *Oreochromis* species are generally spread in mainland waters where aquaculture activities have been underway for some time. *O. niloticus* is a tilapia native to the Nile (Africa) and now introduced to numerous nations around the world for aquaculture (Ishikawa et al 2012). *O. urolepis* is a species of tilapiine cichlids, native to Morogoro Region of Tanzania. *O. aureus* is an euryhaline species, tolerant to a wide range of salinity, native to Northern and Western Africa (Setyawan et al 2012). *Oreochromis* exhibits fast growth (Qiang et al 2014) and tolerance to new habitats (Kulac et al 2012), therefore it has the potential to become an invasive species and to compete with other fish species for resources (Gu et al 2015), especially in Ciliwung River.

C. auratus is a freshwater fish in the family Cyprinidae of the Cypriniformes order, native to East Asia. It is commonly kept as a pet in indoor aquariums, and is one of the most prevalent aquarium fish species. Goldfish released into the wild have become an invasive pest (Takada et al 2010), especially in Ciliwung River. *G. giuris*, a commercial fish belonging to the family Gobiidae, is an elongated-bodied fish broadly distributed in coastal and estuarine waters of the Indo-Pacific regions (Dinh et al 2017). *S. littoralis* is a predator fish. *P. tessellata*, is widely distributed in the channel and exhibits a high degree of plasticity for adapting to inhabit different environments influenced by oceanic and continental conditions (Hüne & Vega 2015). *P. brachypomus* is a large species of pacu, a close relative of piranhas and silver dollars. It is native to the Amazon basin in tropical South America, while *P. mesopotamicus* is a South American ray-finned fish that is native to the Paraguay-Paraná River basin, but it has been introduced by aquaculture activities in a wider area (Aguar et al 2011).

P. reticulata originating from the United States, are introduced fish into the waters of Venezuela, Barbados, Trinidad, Brazil. *P. reticulata* and *P. pardalis* are the two most resistant types of fish to degraded aquatic environments; they can live in waters with low dissolved oxygen levels and high organic matter. *P. pardalis* is very dangerous to the preservation of native fish. Its body consists of a hard plate so that no predator can eat it. Thus, these fish gradually dominate the waters and become competitors of native fish for food, as well as for survival space (Hadiaty 2011), which is facilitated by its dual-organ respiratory system consisting of gills and labyrinth. The gills are used for breathing in clear water, while the labyrinth is used by biota that live in mud or cloudy water (Puspitasari et al 2017).

Fish diversity. In general, the Shannon–Wiener diversity index (H') was positively correlated with the species richness and negatively correlated with the Simpson dominance index (D) (Gelis et al 2021). The diversity index (H') ranged from 0 to 1.59, in the low to moderate category, while the dominance index (D) ranged from 0.24 to 1. The values of the community structure indices reflect the species composition at each site, with different sequencing reads. The highest diversity index (H') was 1.59, in the medium category, at station 2. Station 1 had high dominance index (D) values because only *O. urolepis* species were found. The uniformity and dominance index values (D) are always inversely correlated.

Data of detected species are fundamental to determine the value of biodiversity richness in monitoring studies. In any case, data can produce biased results known as false positive and false negative detection. False-positive may be a false discovery where the found DNA isn't the target or not present within the framework. In contrast, a false negative could be a condition where there's target DNA within the framework, but it isn't identified

amid monitoring (Roesma et al 2021). No native fish of Ciliwung River have been reported by the monitoring using the conventional method (Hadiaty 2011), which can be attributed to a false negative detection.

According to previous studies (Bohmann et al 2014; Rees et al 2014; Shelton et al 2016), the presence of false negatives in the monitoring using the eDNA method may happen for three reasons. First, the collection of water samples is not sufficient to represent all the eDNA targets. Second, there is contamination in the sample collection process. Third, there is no complete database available for identification. Based on the references, it can be stated that the native fish of Ciliwung River were not detected by the eDNA method. DNA target was not carried in the collected water samples because the amount of DNA concentration was low, so it could not be continued for the next process.

Conclusions. This study demonstrated the eDNA method's ability for monitoring fish biodiversity in Lower Ciliwung River, Jakarta, Indonesia. The results detected 69 fish specimens, belonging to 11 species from 8 genera, 8 families, and 7 orders. The highest number of detected species belonged to the Cichlidae family. The diversity index (H') ranged from 0 to 1.59 (low to moderate diversity), while the dominance index (D) ranged from 0.24 to 1 (low to high dominance). The native species were not detected in Ciliwung River by the eDNA method. The eDNA method can be an effective tool for the next generation of biodiversity monitoring technology.

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Conflict of interest. The authors declare no conflict of interest.

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