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Detection of the presence and distribution of invasive fish in the Progo River, Yogyakarta, Indonesia using the environmental DNA method

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Abstract. *Rahmi KA, Adharini RI, Sari DWK, Satriyo TB. 2023. Detection of the presence and distribution of invasive fish in the Progo River, Yogyakarta, Indonesia using the environmental DNA method. Biodiversitas 24: 276-281.* Invasive species are alien species that enter the ecosystem and can adapt to the ecosystem quickly. So, the presence of invasive fish can threaten endemic diversity in these ecosystems. This study aims to detect the presence and distribution of invasive fish in the Progo River, Yogyakarta, Indonesia, through the environmental DNA (eDNA) method. Water samples were taken at three observation points in the upstream area of the river (site 1), the middle of the river (site 2), and downstream of the river (site 3). The water samples were filtered using 0.45 µm MF-Milipore installed on the filtration pump and extracted using the ZymoBIOMICS DNA Miniprep Kit. Sequencing used nanopore sequencing. The primers used were FishF2 and FishR2. The sequencing results were analyzed using the mBRAVE software, which was then classified as an invasive species based on government regulations and journals. The results showed that 188 OTUs were detected in the upstream area (site 1), 227 OTUs in the middle area (site 2) and 154 OTUs downstream. *Lagocephalus* and *Sciaenops* were found in the upstream area (site 1). In the middle area of the river (site 2), *Gambusia* and *Lepomis* were found, while in the lower reaches of the river (site 3), *Gambusia* and *Sciaenops* were found. With the detection of these invasive species, appropriate management and conservation efforts must be carried out immediately to protect endemic species in the Progo River.

Keywords: *Gambusia, Lagocephalus, Lepomis,* nanopore sequencing, *Sciaenops*

INTRODUCTION

Alien fish are species that do not come from their natural habitat or geographical distribution. The presence of exotic fish in waters can occur through the process of introduction or naturally occurring. These introduced fish can be caused by human intervention directly or indirectly (Wahyudewantoro and Rachmatika 2016). According to Pastorino et al. (2018), the introduction of fish can be done to stock to manage fisheries. Fish introduction is also an effort to conserve endangered fish species. However, it is feared that the existence of these introduced fish will also impact the balance of the ecosystem, threatening the biodiversity of endemic fish in the waters. Jakubcinova et al. (2017) also stated that exotic species could cause various negative impacts, such as ecosystem disruption, health problems in humans, animals and plants, damage, and species extinction.

According to Arya et al. (2021), invasive species negatively impact biodiversity, reducing ecosystem diversity and value. In addition, invasive species also cause threats to native flora and fauna. Erarto and Getahun (2020) also explained that exotic aquatic organisms are organisms introduced in new habitats that are considered a threat to biodiversity after changing their original habitat. This is caused by predation, competition, hybridization with native species, and disruption of ecosystem processes and functions. The result of the entry of these alien or invasive species is causing harm to the economy, environment, and human health. Banks et al. (2021) stated that monitoring invasive or nuisance species could adversely affect non-target species when using sampling techniques. One method that can be used to monitor invasive species without causing negative impacts on non-target species is the use of environmental DNA.

The definition of environmental DNA, according to Dogdu and Turan (2018), is a method for detecting and identifying species from DNA found in water and soil. It is because organisms leave DNA fragments through their skin, urine, feces, or mucus in water or soil. According to Turner et al. (2015), environmental DNA sampling in liquid form can be used to identify rare aquatic macrofauna and can be used to determine the presence of species without isolating part of the target organism. So, environmental DNA analysis has been applied to detect one or several rare and endangered species in aquatic ecosystems (Takeuchi et al. 2019).

According to Herder et al. (2014), the eDNA method has several advantages compared to traditional methods. These advantages include the possibility of detection, which is higher than traditional methods, especially for unknown species or species that occur at low densities. In addition, less effort is required to detect a species, and it is more cost-effective than traditional methods. The eDNA method does not need to catch species to detect their presence, so this method is non-invasive and does not damage habitats. Moreover, misidentification can also be excluded using validated species-specific primers. The eDNA method can be used in some cases as it provides a higher taxonomic resolution, especially in the case of species that cannot be differentiated based on morphological characteristics. However, Herder et al. (2014) mentioned several disadvantages of the eDNA method compared to traditional methods. These weaknesses include the eDNA method cannot provide absolute density because the sampling strategy dramatically affects the amount of eDNA in the sample. In addition, the eDNA method only collects information about the presence or absence of the target species. Thus, this method does not provide information on factors such as a species' life stage, reproduction and fitness.

This research was conducted to determine the presence and distribution of invasive species in the Progo River, Yogyakarta, Indonesia using environmental DNA analysis. This research is essential because invasive fish can threaten the existence of endemic fish in waters that threaten aquatic biodiversity, so this research is expected as a step in the conservation and management of fisheries in the Progo River, Yogyakarta, Indonesia.

MATERIALS AND METHODS

Sampling site

Water sampling was carried out at the Progo River, Kulon Progo Regency, Yogyakarta, Indonesia, in February 2022. Sampling stations are selected based on the river section, namely upstream, middle and downstream. Water sampling was carried out at 3 station points, upstream (7˚38'31.919975" S and 110˚15'13.513184" E), middle (7˚49'57.205925" S and 110˚13'38.756104" E), and downstream (7˚58'52.356834" S and 110˚12'21.577148" E) (Figure 1). Furthermore, each station took one sample of 2L, the sample is a mixture of water on the banks of the river and the middle of the river and filtered. The molecular analysis process was carried out at the Aquatic Resources Management Laboratory Department of Fisheries, Faculty of Agriculture, Gadjah Mada University.

Procedure

Sampling and preservation of samples

Water samples at each station were taken as much as 2L at a depth of approximately 3 m of the water column. The next process is filtration to separate the water from the suspended particles in the sample. Filtration was carried out using a vacuum filtration pump with 0.45 µm filter paper, Durapore filter membrane (Millipore, MA, USA). After all the water has been filtered, the filter paper is then inserted into a 1.5 mL microtube, and 96% alcohol is added until all the filter paper is submerged.

DNA extraction

DNA extraction was carried out using a commercial kit, namely the ZymoBIOMICS DNA Miniprep Kit from Zymo Research. There are several stages in the extraction process, namely lysis, DNA binding, washing and elution. This process is carried out according to the ZymoBIOMICS DNA Miniprep Kit procedure (Roesma et al. 2021). After extraction, the genomic DNA was checked using electrophoresis with 1% agarose gel.

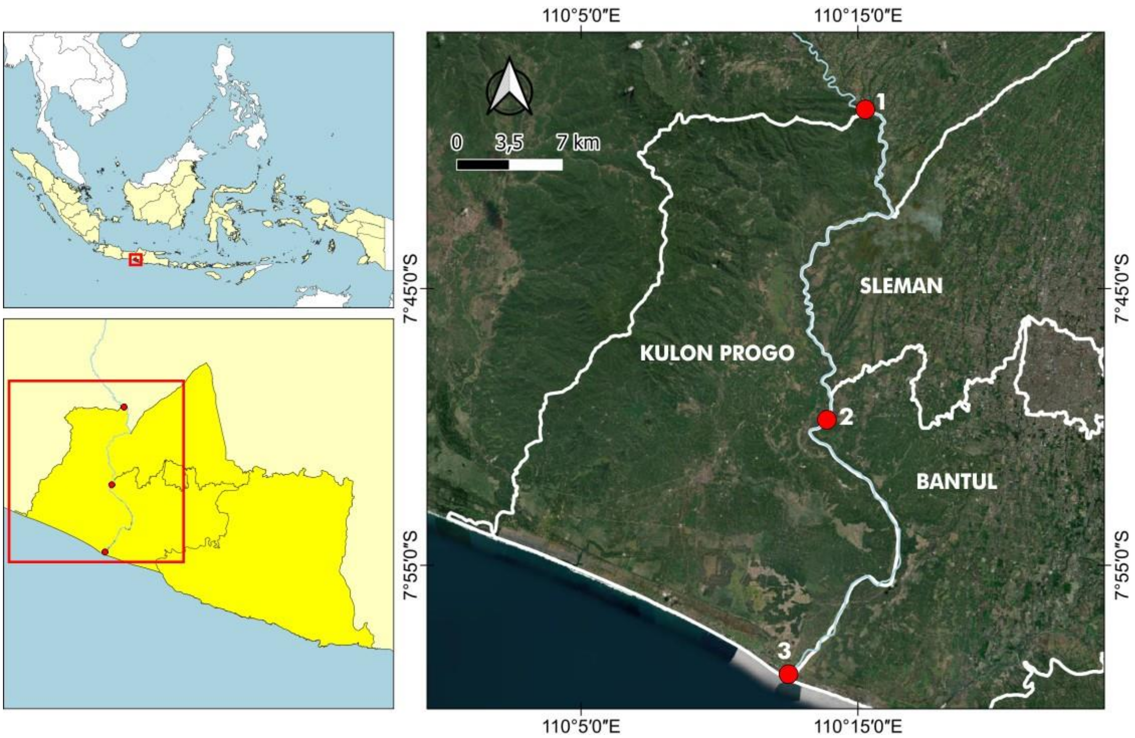

Figure 1. Water sampling locations for the detection of invasive species using the environmental DNA method in the Progo River, Yogyakarta, Indonesia

Polymeration Chain Reaction (PCR) amplification and sequencing

PCR amplification and sequencing were carried out by sending the DNA extraction product to the 1st base sequencing service through PT. Genetika Science. Sequencing, Tangerang City, Banten. Sequencing used in this study is nanopore sequencing, the primers used were FishF2-5'TCG ACT AAT CAT AAA AGA TAT CGG CAC3' and FishR2-5'ACT TCA GGG TGA CCG AAG AAT CAG AA3' designed by Ward et al. (2005).

Data analysis

Sequencing results data were analyzed through the website www.mbrave.net. mBRAVE (multiplex Barcode Research and Visualization Environment) is a platform that supports the storage, validation, and analysis of multiplexed projects based on high-throughput sequencing (HTS) instruments. The results of the analysis from mBRAVE are then BLAST (basic local alignment search tool) through NCBI. Species found that were classified as invasive were then matched based on Zenetos et al. (2005), Zenetos et al. (2010), and Wahyudewantoro and Rachmatika (2016). Definition of an invasive species, according to Veennvliet (2021), is a species introduced to a new habitat whose distribution has threatened or negatively impacted biodiversity and ecosystems. The negative impacts of the spread of invasive species include threatening ecosystems, reducing the number of endemic species, and impacting socioeconomic aspects (Kodiran et al. 2020).

RESULTS AND DISCUSSION

Results

The sequencing results that had been analyzed using mBRAVE were then identified with the NCBI BLAST. Based on the results of the analysis, four invasive species were found in the Progo River, Yogyakarta, namely *Lagocephalus, Lepomis, Gambusia,* and *Sciaenops* (Figure 2).

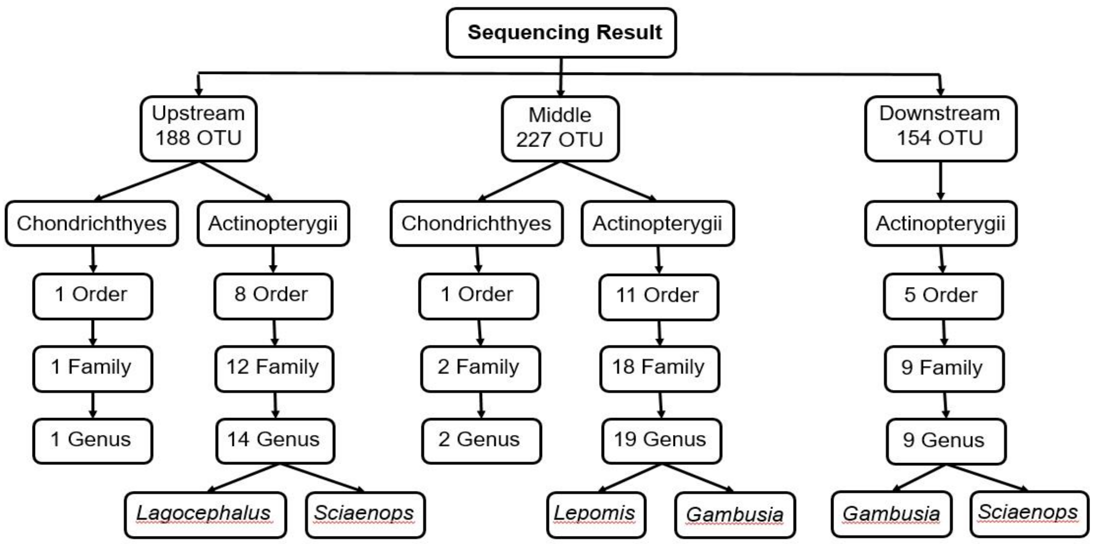

Figure 2. Schematic analysis of the results of sequencing using mBRAVE. Note: OTU (Operational Taxonomy Unit) is an operational definition used to classify closely related groups of individuals

Table 1. Result of invasive species identification with BLAST

OUT code	Species	Query cover (%)	Percent identity (%)	Acession number	Distribution
57	*Gambusia affinis*	81%	85.51%	MK628400.1	Downstream
71	*Gambusia affinis*	82%	89.83%	MK628400.1	Middle
91	*Sciaenops ocellatus*	80%	85%	KP641519.1	Downstream
96	*Gambusia affinis*	81%	85.19%	MK628400.1	Downstream
107	*Gambusia affinis*	41%	95.12%	MK628400.1	Downstream
109	*Lagocephalus spadiceus*	72%	87.50%	KY130423.1	Upstream
116	*Gambusia affinis*	81%	93.51%	MK628400.1	Middle
129	*Gambusia affinis*	82%	89.02%	MK628400.1	Downstream
141	*Sciaenops ocellatus*	81%	85.54%	KP641519.1	Downstream
144	*Gambusia affinis*	43%	95.53%	KM220898.1	Downstream
186	*Sciaenops ocellatus*	60%	90%	KP641519.1	Upstream
219	*Lepomis macrochirus*	83%	87.95%	KM220897.1	Middle

Note: OTU (Operational Taxonomy Unit) is an operational definition used to classify closely related groups of individuals

Identification results can be said to be the same species if they have a minimum similarity level of 70% and a percent identity of more than 97% (Choi et al. 2020). According to Drancourt et al. (2000), a similarity level of 99% can be said to be a species, and a similarity level of 97% is said to be a genus level. Based on this explanation, the results of the analysis using mBrave cannot be said up to the same species level but can only be said up to the genus. Based on the sequencing analysis conducted in this study, invasive fish from the genera *Lagocephalus* and *Sciaenops* have been detected in the upstream river. In the middle area, invasive fish of the genera *Gambusia* and *Lepomis* were detected, while in the river downstream, invasive fish of the genera *Gambusia* and *Sciaenops* were detected (Table 1).

Discussion

Environmental DNA is a technique for detecting and measuring biodiversity that is superior to traditional survey techniques because it is non-invasive. In addition, the eDNA technique can also detect more than one species in one sample (Beng and Corlett 2020). According to Lin et al. (2021), nanopore technology refers to nanoscale holes embedded in a thin membrane structure to detect changes in potential when a smaller charged biological molecule passes through the nanoholes. Therefore, nanopore technology has the potential to detect and analyze single-molecule amino acids, DNA, RNA, and others.

Based on the analysis carried out in this study, there were two classes detected, namely Actinopterygii and Chondrichthyes. These results are then classified into invasive and non-invasive species based on Wahyudewantoro and Rachmatika (2016), Zenetos et al. (2010), and Zenetos et al. (2005). In this study, only invasive species were taken. In the Actinopterygii class, four genera were found that had the potential to become invasive species, namely *Lagocephalus, Lepomis, Gambusia,* and *Sciaenops*. In the upstream area, *Lagocephalus* and *Sciaenops* were found. *Lepomis* and *Gambusia* were found in the middle area, and in the downstream area, *Gambusia* and *Sciaenops* were found. *Lagocephalus* is found in the upstream area, which has freshwater conditions. According to Alshawy et al. (2019), *Lagocephalus* can be found on Mediterranean coasts. This proves that this species has a high tolerance for water conditions. Matsuura (2015) also mentions that *Lagocephalus spadiceus* is a member of the Tetraodontidae family, which is spread across tropical and temperate seas and is found in fresh waters all over the world.

Lagocephalus is a genus with five recorded species, namely *L. lagocephalus, L. sceleratus, L. spadiceus, L. suezensis,* and *L. guentheri* (Kleitou et al. 2019). *Lagocephalus* detected during the NCBI BLAST was *Lagocephalus spadiceus*. According to Rousou et al. (2014), *Lagocephalus* migrated to reproduce and build a population from the Indo-Pacific to the Mediterranean Sea. *Lagocephalus spadiceus* is a species that swims in the middle of the water but descends to the bottom to eat benthic organisms. Fishermen's trawls or purse seines often catch this species, and originally *L. spadiceus* had a wide distribution in the Indo-Pacific (Basusta et al. 2020). Morphological characteristics of *L. spadiceus* according to Chakraborty et al. (2020), this species has a long body and a small mouth with two large teeth in each jaw. According to Basusta et al. (2020), this *L. spadiceus* species is a puffer fish considered an invasive species in the Mediterranean Sea. *Lagocephalus spadiceus* can be found in various habitats with sandy, rocky substrates, and seagrass beds. This fish has no commercial value because it has poison in its flesh.

Gambusia detected in this study was *Gambusia affinis*. *Gambusia affinis* originates from the southeastern coastal region of the United States and has been widely introduced to the world for mosquito control (Chabet et al. 2022). According to Wahyudewantoro and Rachmatika (2016), *Gambusia affinis* is relatively small, approximately 4 cm long, with a slightly curved back. *Gambusia affinis* is an invasive species because it has fast growth and high reproductive potential besides its tolerance to environmental changes (Gao et al. 2017). The characteristics of invasive species, according to Wahyudewantoro and Rachmatika (2016) and Andriyono and Fitrani (2021), are highly adaptable to new habitats and can reproduce quickly. Therefore, *G. affinis* can be considered invasive because of its high tolerance and ability to reproduce quickly.

Gambusia is found in the middle area of the river with freshwater conditions and the downstream regions with brackish water conditions. According to Fernandez-Delgado (1989), *Gambusia affinis* is a small species originating from the USA, which was widely introduced in fresh and brackish waters around the world as a mosquito controller. In their research, Thien and Khanh (2019) found *Gambusia affinis* in fresh waters and stated that this species is an invasive species registered in the 2018 Global Invasive Species Database.

According to the Ministry of Environment and Forestry of Republic of Indonesia (KLHK 2016), the *Lepomis* that is invasive but has not yet been found in Indonesia is *Lepomis auritus*. Meanwhile, *Lepomis macrochirus* has been registered as a disease carrier species (KKP 2020). Besides *L. auritus* and *L. macrochirus*, *L. gibbosus* is also an invasive species (Lambea-Camblor et al. 2022). Redear sunfish or *Lepomis microlophus* is a species originating from the United States which is reported to be invasive because it reduces the snail population and overpowers the native fish population in the United States (Whitney et al. 2021). The *Lepomis* detected in this study was *Lepomis macrochirus*. *Lepomis macrochirus* was first introduced in South Africa from the United States in 1938 as a feed for bass species. This species forms populations in the Eastern Cape, Western Cape, and KwaZulu-Natal areas and has been evaluated as an invasive species (Ndaleni et al. 2018). According to Kizuka et al. (2014), *Lepomis macrochirus*, or bluegill, is a fish native to North America and is listed among the 100 worst invasive species in the world, according to the IUCN. *Lepomis macrochirus* is invasive because it reproduces in several locations in South Africa. In addition, this species has been shown to reduce the abundance of certain invertebrates and can outcompete native fish species in finding food (Ndaleni et al. 2018).

Lepomis was detected in the middle area of the Progo River, which has freshwater conditions. These results are to the statement of Takahara et al. (2013) that the bluegill sunfish or *Lepomis macrochirus* is one of the most widely distributed species in freshwater ecosystems in Japan. This species is also listed as invasive in the Invasive Alien Species Act of Japanese Law. Yamamoto and Shiah also stated that bluegill *Lepomis macrochirus* is a freshwater fish in Japan whose habitat is in curved water bodies such as lakes, ponds, and the lower reaches of rivers. This species was introduced as a game fish, which is game fish for fishing areas.

According to Lin et al. (2020), the red drum is a marine fish that belongs to the family Sciaenidae, which naturally inhabits estuaries and coastal waters. *Sciaenops*, which has the potential to become an invasive species, is *Sciaenops ocellatus*. According to Liao et al. (2010), *S. ocellatus* meets the requirements as an invasive species because this species can survive well in Taiwanese waters. After all, these waters have sandy or muddy bottoms according to their area of origin. In addition, Taiwan waters have abundant food, such as crustaceans, mollusks and other fish. *Sciaenops ocellatus* also has a high-temperature tolerance. Wang et al. (2022) stated that *S. ocellatus* had invaded and formed ecosystems in western Taiwan and the Indo-Pacific waters. In addition, this species can survive well and breed in the waters of China, Korea, Singapore, and Thailand. Lin et al. (2020) also added that as a predatory fish, *S. ocellatus* caught off the coast of China ate a variety of prey, including Pisces, Macrura, Brachyura, Cephalopods, Stomatopods, and Polychaeta.

Sciaenops is found in the upstream area of the Progo River with freshwater conditions and the downstream area of the Progo River with brackish water conditions. According to Vela et al. (2019), the red drum or *S. ocellatus* is a species found in estuaries that target commercial and recreational fishing in the Gulf of Mexico and the southeastern United States. Liao et al. (2010) also stated that *S. ocellatus* is a predatory marine fish from Atlantic America. *Sciaenops ocellatus* can be found in marine, brackish and fresh waters because this species is euryhaline, meaning it can maintain relatively constant blood osmolality in various aquatic environments (Vela et al. 2019).

Table 1 above shows that the downstream area of the Progo River with brackish water conditions is the point where most invasive species are found. According to Kume et al. (2021), estuaries and beaches are important habitats for fish. This area is used as a nursery habitat for freshwater or seawater adaptations when they migrate upstream/downstream, while other species use it for foraging, growing, and laying eggs. Clarke et al. (2020) stated that brackish waters have a high potential for brackish water species or species with a high salinity tolerance to survive. According to Paavola et al. (2005) estuary areas are areas where there are many invasive species because salinity is an important factor for most aquatic species. In brackish waters, salinity determines the limit for species to roam the waters, and in this area, a species has the potential to colonize.

In conclusion, the invasive species in the Progo River are as follows: in the river's upper reaches (station 1), genera *Lagocephalus* and *Sciaenops* were detected. The middle area of the river (station 2) is found *Gambusia* and *Lepomis*. The lower river area (station 3) is detected *Gambusia* and *Sciaenops*. With the detection of these invasive species, appropriate management and conservation efforts must be carried out immediately to protect endemic species in the Progo River.

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