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Original Research

Aquatic eDNA Metabarcoding Reveals Biodiversity and Plankton Composition in River Ecosystems

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Abstract

Environmental DNA (eDNA) has the potential to be a viable approach for detecting the existence of aquatic organisms, such as plankton. This study aimed to assess the biodiversity and composition of plankton species in the Lower Ciliwung River, Jakarta, Indonesia. During the dry season (July 2022), the study was conducted upstream to downstream of the Lower Ciliwung River. Based on read sequences, the relative abundance, biological community composition, and diversity of plankton were evaluated. In this study, a total of 1,492,975 original reads filtered to 1,265,307 reads, belonging to 22 species of eukaryotic phytoplankton and 15 species of zooplankton. The taxa identified from the eDNA samples show that order Thalassiosirales from class Mediophyceae (eukaryotic phytoplankton) and order Ploima from class Monogononta (zooplankton) were the most commonly discovered plankton. The relative abundances of eukaryotic phytoplankton and zooplankton detected in eDNA water samples did not differ significantly (p-value>0.05). The most abundant species for all sites were *Stephanocyclus cryptica* (phytoplankton) and *Moina macrocopa* (zooplankton). The eukaryotic phytoplankton and zooplankton diversity index (H') were in the low to moderate category.

Keywords: composition, diversity index, eDNA, plankton, relative abundance

Introduction

Plankton organisms are an essential component of the trophic chain because they provide food for other organisms, such as a similar plankton species in some cases [1]. Climate change and other changes in the environment are threatening these planktonic communities as well as the loss of biodiversity. As a result, understanding plankton communities is critical for fisheries [2]. Plankton is classified into two types: phytoplankton (microscopic plant-like organisms) and zooplankton (animal-like traits) [3]. Phytoplankton are microorganisms that are directly involved in the process of primary productivity in the water, which are then consumed by zooplankton [4]. Zooplankton are important for regulating the availability of energy from phytoplankton to a higher trophic level, so the composition and presence of zooplankton support fish productivity [5].

The difficulty in plankton research is identifying species, so taxonomy expertise is required [6]. The existence of morphological similarities makes

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it troublesome for taxonomists and takes a long time in the identification process [6, 7]. Therefore, advanced methods offer Next Generation Sequencing (NGS), which can produce plankton taxonomic information rapidly and yield quite a lot only from environmental samples [8, 9]. The eDNA method is capable of detecting more than one species [10, 11], but also detects multispecies in various taxa, such as bacteria [12], viruses [13], fungi [14], plants [15], invertebrates [16], and vertebrates [17]. Seymour [18] introduced DNA metabarcoding to identify the multispecies of genetic components that are degraded in the environment. Environmental DNA applies the concept of metabarcoding DNA using environmental samples that can be obtained from water, soil, or air to detect the presence of an organism and measure its abundance in the environment [19].

eDNA metabarcoding is a non-invasive approach for recovering genetic materials generated by organisms and detached to their environment [20]. eDNA can produce more comprehensive biodiversity assessments than conventional methods [21]. eDNA could be a viable way to detect the existence of organisms such as plankton, fish, bacteria, amphibians, mammals, and other taxa without alienating the organism [22, 23]. They excrete a large amount of genetic material as lysed cells or feces, which degrade into tiny particles that could be stored in the water column [24] or settle in the sediment [25]. Environmental samples like water and soil can be easily extracted for genetic material. eDNA is made up of dead cells, dormant organisms, and molecules adsorbing on the surface of various mineral or organic materials [26].

The diversity of fish in the Ciliwung River from the 1910s decreased by 47.1% to 1930, then decreased to 92.5% in 2010 [27]. Ciliwung River is the largest river flowing in Jakarta, with a length of 119 km and 476 km^2 catchment area. It streams upstream from Bogor Regency, through Bogor City, Depok City, and Jakarta before reaching the Java Sea via Jakarta Bay. Development in the Ciliwung watershed has brought about various changes in the landscape, especially in the downstream area [28]. Land use for residential areas, offices, trade, and agriculture significantly impacts decreasing water quality [29]. The high human population in Jakarta also makes river flows more vulnerable due to the presence of domestic, industrial, agricultural, and livestock waste [29, 30]. Intensive monitoring is required to determine the potential of all species, particularly plankton.

River management aims to determine the river water quality, the ecological conditions, and the river's ability to maintain its biodiversity. Biodiversity management is one of the links in managing river sustainability. Conditions of good biodiversity can support river ecosystem services, namely providing food sources, pollutant regulators, supporting ecosystem balance, as well as providing recreational and research services [31]. The research was carried out during the dry season (July 2022). This research aimed to assess the biodiversity and composition of plankton species at several sites along the Lower Ciliwung River in Jakarta, Indonesia, using eDNA methods.

Material and Methods

eDNA Freshwater Sample Collection

To consider the effect of different ecosystems, the sampling locations (East Jakarta, Central Jakarta, and North Jakarta, separately) (Fig. 1) were chosen from upstream to downstream of the Lower Ciliwung River. The three ecosystems have different landscape conditions, so it is expected to have different water quality conditions and differences in plankton species. At each site, three replicate eDNA water samples were gathered for a total of nine eDNA freshwater samples. eDNA samples were taken directly from the surface and placed in 4 L water bottles. Each water sample was filtered through 0.45 µm Pall Corporation sterilized filter paper (47 mm diameter) using a peristaltic pump. The filtration process was halted if the flow was interrupted due to filter cluttering. To avoid contamination, a protocol was developed that sterilized all equipment between samples and sampling sites with distilled water and 10% bleach. Each filter paper was then located in a 2 mL cryotube containing 1 mL of Deoxyribo-Nucleic Acid/Ribo-Nucleic Acid (DNA/RNA) shield.

eDNA Laboratory Analysis

DNA extraction was performed after the field sampling utilizing gSYNC DNA extraction kits manufactured by Geneaid Biotech following the manufacturer's instructions. DNA amplification was carried out using the Polymerase Chain Reaction (PCR) technique with the target of the Cytochrome Oxidase subunit 1 (COI) gene. This step uses a combination of PCR primers, forward primer mlCOIintF and reverse primer jgHCO2198. This combination has been shown to work well for detecting metazoans down to the species level in the 313 bp COI fragment target [32]. The first PCR contained 13 µL bioline, 1 µL each of 10 nM primers (forward and reverse), 2 µL DNA template, and 8 µL ddH_2O. The following were the phases of the DNA amplification PCR profile: (1) pre-denaturation of the DNA template at 95ºC (5 minutes); (2) denaturation of the DNA template at 95ºC (30 seconds); (3) annealing at 42ºC (30 seconds); (4) primary extension at 72ºC (30 seconds); and (5) final extension at 72ºC (5 minutes) with 35 cycles of stages (2)-(4). To check for contamination, the 96 Universal peqStAR PCR machine (Peqlab Ltd, USA) was used with negative controls (blank templates). After passing the electrophoresis quality control, all PCR products were subjected to a second PCR for indexing. The PCR cycle began with

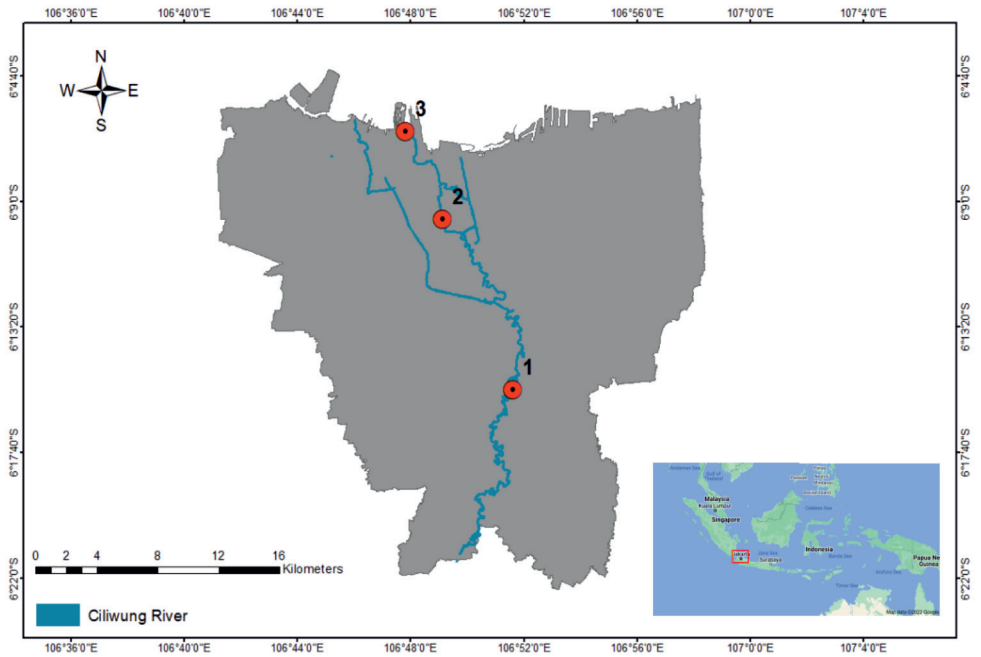

Fig. 1. Three eDNA freshwater sample collection sites across the Lower Ciliwung River: (1) East Jakarta; (2) Central Jakarta; and (3) North Jakarta.

a 3 minute denaturation at 95ºC, followed by 9 cycles of 95ºC (30 seconds), 55ºC (30 seconds), 72ºC (30 seconds), and 72ºC (30 seconds) (5 minutes). The first and second PCR products were purified with AMPure XP before proceeding to the next step (Beckman Coulter, Inc). For DNA sequencing, the Illumina NovaSeq 6000 with Illumina MiSeq 16S metagenomic sequencing library protocol was used. Oceanogen Environmental Biotechnology Laboklinikum (Oceanogen) in Bogor, Indonesia, performed the molecular identification of eDNA samples.

Bioinformatics and Data Analysis

The sequenced data were then imported into the Quantitative Insights into Microbial Ecology 2 software (QIIME2, https://qiime2.org) for quantitative analysis [33]. The process in QIIME2 includes: (a) deletion of forward and reverse primer sequences with cut-adapt [34], (b) detection and correction of amplicon sequences with the DADA2 pipeline [35], (c) grouping sequences based on their proportion of similarity (clustering) to produce an Operational Taxonomic Unit (OTU). COI sequence taxonomic identification to the species level using the CRUX database (Creating Reference Libraries Using the eXisting tool).

Based on read sequences, the biological composition, relative abundance, and diversity of plankton were evaluated. A read is the DNA sequence from a single fragment (a small section of DNA). For each of the three sites, taxonomic identification by class was visualized on maps with pie charts. The ggplot2 package in R v. 4.2.1 (http://r-projekt.org) was used to analyze and visualize the relative abundance and composition of the identified eukaryotic phytoplankton and zooplankton [36]. The results of relative abundance were evaluated using the Analysis of Similarities (ANOSIM) test on PAST (PAleontological STatistics) v. 4.11. ANOSIM test was executed to assign which levels differed significantly (p-value≤0.05). The ANOSIM-R value shows the extent to which groups differed, i.e., barely separated (R < 0.25), separated but strongly overlapping (R = 0.25-0.50), separated but overlapping (R = 0.50-0.75), and well separated groups (R>0.75) [37].

SIMPER (Similarity of Percentages) was used to examine plankton species contributing to the plankton composition from all sites. This analysis breaks down each species' contribution to the reported similarity (or dissimilarity) among samples. As a result, we will be able to recognize the most significant species in the occurrence of similarity [38]. The Shannon-Wiener (H') and Simpson Index (D) were projected by the vegan package in R v. 4.2.1 to assess species diversity and dominance [39]. Non-metric multidimensional scaling (NMDS) was used in R Studio to analyze the difference in read sequence composition between sites based on the Bray-Curtis distance index [40].

Results and Discussion

Eukaryotic Phytoplankton and Zooplankton Composition

A genetic approach with different stages, both in the process of DNA collection, DNA extraction, PCR, and bioinformatics sorting would potentially be

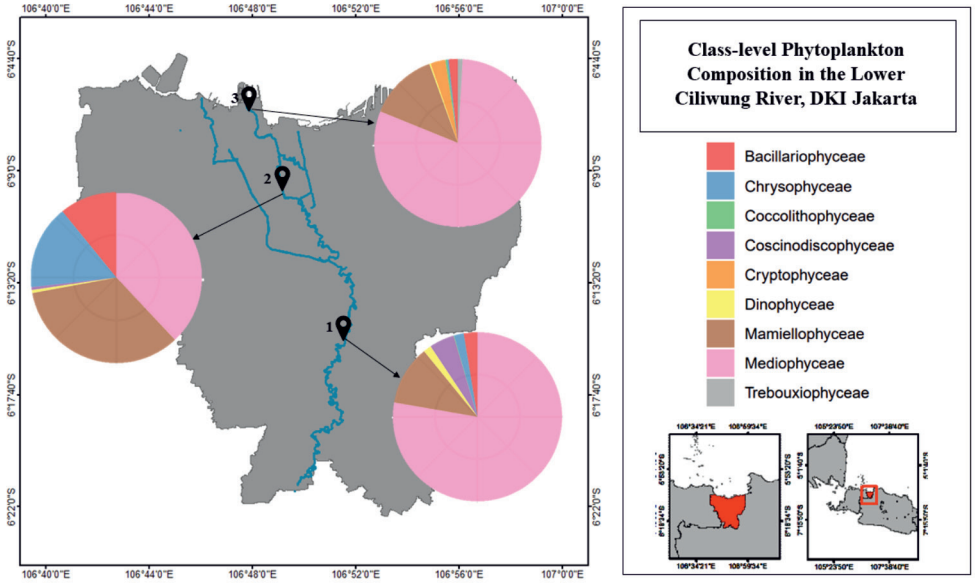

Fig. 2. Class-level eukaryotic phytoplankton structure at 3 sites in the Lower Ciliwung River, Jakarta.

biased [41]. Therefore, this study was further classified based on the type of plankton. The next-generation sequencing of amplicons from 9 samples collected from 3 sites yielded 1,492,975 original reads, which were then filtered to 1,265,307 reads. The eukaryotic phytoplankton taxa identified from the eDNA samples included 22 species representing 16 genera, 15 families, 13 orders, 9 classes, and 6 phyla. Meanwhile, the identified zooplankton included 15 species from 12 genera, 10 families, 7 orders, 4 classes, and 3 phyla. This research shows the benefit of eDNA metabarcoding in illuminating freshwater plankton biodiversity in the Lower Ciliwung River, Indonesia. We emphasized its prospect for improving assessment and conservation of ecologically valuable taxa. This method can be used in a variety of situations where traditional census methods (such as morphology-based identification and visual census) produce poor results or necessitate a large sampling effort [42]. This is the case when we assess invasive, threatened, or shrouded harmful species [43].

Environmental patterns primarily influence the abundance of aquatic organisms (including plankton) [44]. It should be noted that numerous sequen ces were generated, some of which corresponded to other organisms (e.g., fish, benthos, and other macro-microorganisms) or species that could not be identified because of technical difficulties (e.g., inadequate guidance databases). Thus, when interpreting data for ecosystem monitoring, these factors must be considered [45]. However, this study detected more phytoplankton phyla (Bacillariophyta, Chlorophyta, Cryptista, Haptophyta, Ochrophyta, and Miozoa) than the 5 phytoplankton phyla (Bacillariophyta, Chlorophyta, Charophyta, Ochrophyta (former Chrysophyta), Cyanobacteria (former Cyanophyta), and Rhodophyta) reported by Pambudi et al. [46].

This study did not detect Cyanobacteria as found by Pambudi et al. [46]. This is thought to be caused by the primer used, namely the universal primer for metazoans. In genetic analysis, each organism has a unique DNA sequence. Therefore, forward and reverse primers must be designed with specific sequences for the organism to be detected or identified in the eDNA sample. A properly designed primer will maximize amplification efficiency and produce accurate and specific results [32]. The results of this study (3 phyla: Arthropoda, Cnidaria, and Rotifera) also complement Rahmatia et al. [47], who found 5 zooplankton phyla (Protozoa, Rotifera, Mollusca, Nematoda, and Arthropoda) in the Ciliwung River. According to these findings, eDNA metabarcoding can be potential to identify complex parts of freshwater biodiversity that reside in hidden areas and are often inaccessible using conventional methods [48].

In general, the taxa identified show that the most commonly discovered plankton classes across whole sites were Mediophyceae (eukaryotic phytoplankton) (Fig. 2) and Monogononta (zooplankton) (Fig. 3). All sites had high between-site variability in class composition. Thalassiosirales from Mediophyceae class (Fig. 4) and Ploima from Monogononta class (Fig. 5) had the highest relative abundance. The relative abundance of eukaryotic phytoplankton and zooplankton detected in eDNA water samples not differed significantly (p-value>0.05) between the three sites (ANOSIM-R value by site: R = 0.32 (eukaryotic phytoplankton), R = 0.05 (zooplankton)). The eukaryotic phytoplankton compositions were separated but strongly overlapped (ANOSIM-R = 0.25-0.50), meanwhile the zooplankton compositions were barely separated between sites (ANOSIM-R<0.25). According to Effendi et al. [49], numerous factors can obscure quantitative inferences from eDNA water samples, causing practical

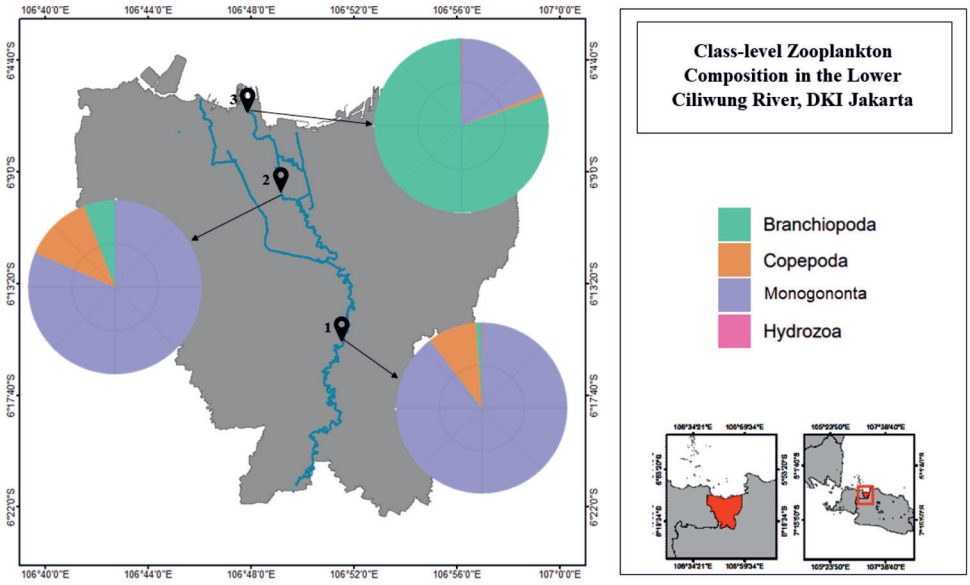

Fig. 3. Class-level zooplankton structure at 3 sites in the Lower Ciliwung River, Jakarta.

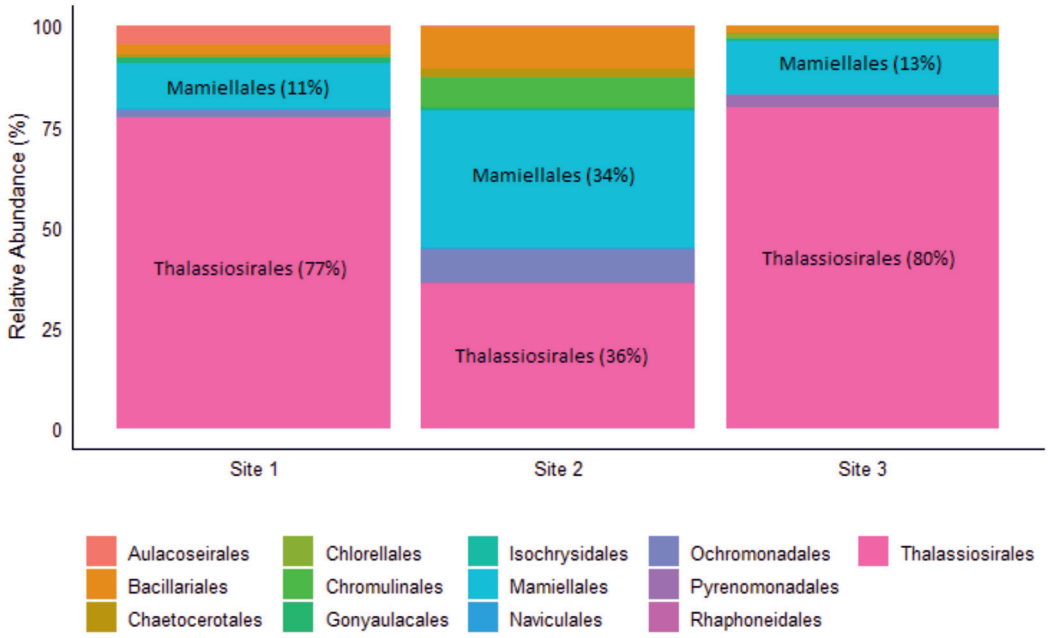

Fig. 4. Eukaryotic phytoplankton order composition and relative abundance at 3 sites in the Lower Ciliwung River, Jakarta.

implementation of read abundances from eDNA kind of chancy. Nevertheless, seasonal data can serve useful insights on species distribution and diversity. As with environmental issues, eDNA sampling could be incredibly beneficial because the method is effective and simple to standardize all over time and location [11].

Furthermore, SIMPER analysis was found to be effective in identifying the most contributed species across all sites (Table 1). The SIMPER analysis was used to determine the most contributed species from all sites. *Stephanocyclus cryptica* is a diatom from the class Mediophyceae, phylum Bacillariophyta. Bacillariophyta is the most common phylum found in the Lower Ciliwung River. These findings corroborate Pambudi et al. [46], who observed that the group of phytoplankton that dominates freshwaters consists primarily of Bacillariophyta (diatoms) due to their high adaptability to the environment and rapid reproduction. According to Sirait et al. [50], dominance of Bacillariophyta shows competition in resource utilisation and unbalanced or stressed aquatic environmental conditions.

Moina macrocopa is a type of zooplankton species that has the potential to be used as live food (natural feed) for fish and shrimp. Moina are found throughout freshwater, such as rivers, lakes, swamps, reservoirs,

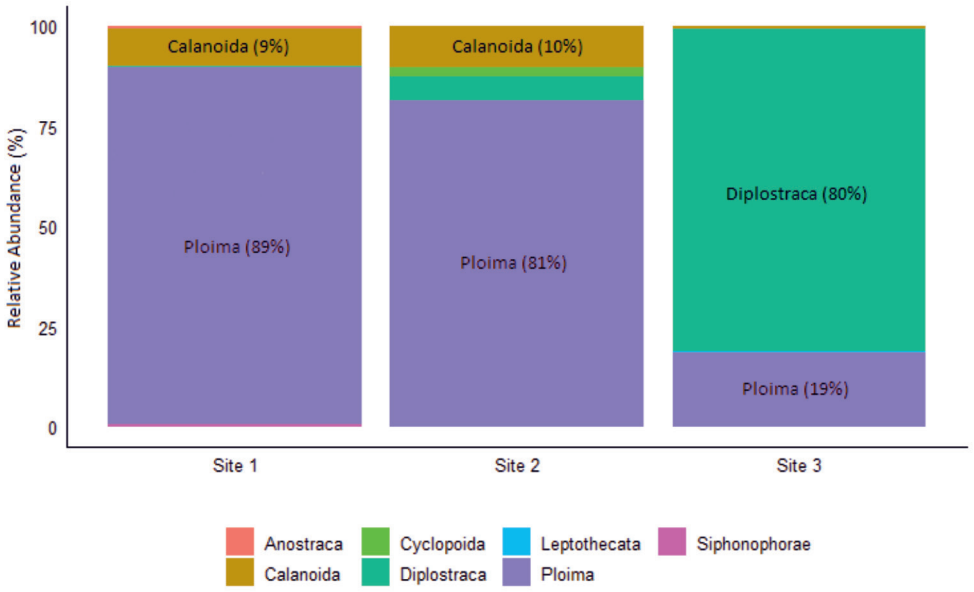

Fig. 5. Zooplankton order composition and relative abundance at 3 sites in the Lower Ciliwung River, Jakarta.

and ponds. *Moina macrocopa* has a high protein content and the right size for the mouth opening of fish or shrimp and can easily be digested in the digestive tract of fish and shrimp [51]. *Brachionus plicatilis* is a zooplankton from phylum Rotifera, which plays a vital role as food for various types of cultivated fish. *B. plicatilis* can provide higher survival to crab larvae and accelerate the moulting process. In addition, *B. plicatilis* is a good feed for the larvae of tiger grouper (*Epinephelus fuscoguttatus*), barramundi (*Lates calcarifer*), and mullets (*Mugil cephalus*). *B. plicatilis* is small (150-220 µm) and swims slowly, making it easy for larvae to prey. They have a high reproductive rate, easy to digest, easy to breed, and have relatively high nutritional value content [52]. These results were supported by Rahmatia et al. [47] who suggested that *Asplanchna* sp. and *Brachionus* sp. are the most common zooplankton in the Ciliwung River.

Table 1. The most contributing species (up to 80%) at the study site.

No.	Species	Contribution (%)	Cumulative (%)
Phytoplankton			
1	*Stephanocyclus cryptic*a	66.47	66.47
2	Micromonas commoda	12.31	78.78
3	Aulacoseira ambigua	3.37	82.16
Zooplankton			
1	Moina macrocopa	38.52	38.52
2	Brachionus plicatilis	34.70	73.22
3	Brachionus calyciflorus	13.05	86.27

Eukaryotic Phytoplankton and Zooplankton Diversity

The Shannon-Wiener diversity index (H') was found to be generally inversely related to the Simpson dominance index (D). The values of these indices represent the species composition for every site. The eukaryotic phytoplankton diversity index (H') was in the low to moderate category (ranged from 0.90 to 1.82), whereas H' of zooplankton is slightly lower (ranged from 0.69 to 0.98) and falling into the low diversity. The dominance index (D) classified from low to high dominancy for eukaryotic phytoplankton and high dominancy for zooplankton (ranged from 0.22 to 0.60 and 0.57 to 0.72, respectively) (Fig. 6). The general Simpson Dominance Index (D) was near to zero, indicating that no taxon dominated the entire study location. The results of statistical analysis of plankton at each sampling site from the NMDS results showed a stress value of 0.12 (Fig. 7). The stress value on the NMDS graph ranges from 0 to 1. The lower the stress value, the more reliable the graph is [40].

The ordination on the NMDS tends to be grouped for each site, with some of the data for Site 1 being closer to those of Site 2. This is thought to be related to the environmental conditions of the waters, which tend to be the same between Site 1 and Site 2 so that the biota also found not significantly different. Many environmental factors, which including physical, chemical, and biological parameters, can cause significant variation in the presence of eDNA particles between sites [49, 53]. The topographic conditions influencing water transport processes were most likely one of the factors that caused disparities in eDNA diversity and abundance. Environmental factors such as pH, water temperature, water currents, dissolved oxygen, organic matter, UV radiation, as well as the quantity and type of material

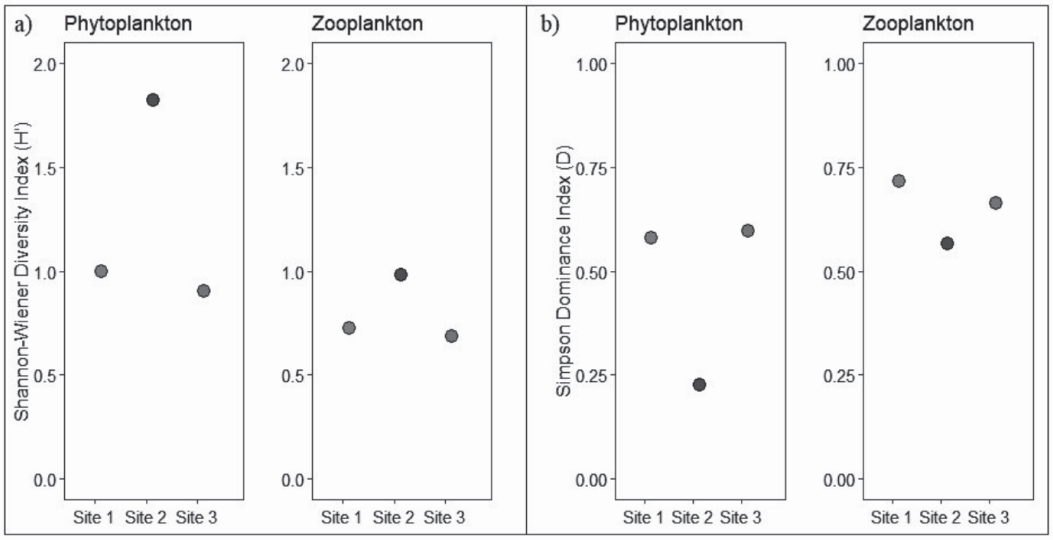

Fig. 6. a) Shannon-Wiener Diversity Index (H') and b) Simpson Dominance Index (D) values for plankton identified.

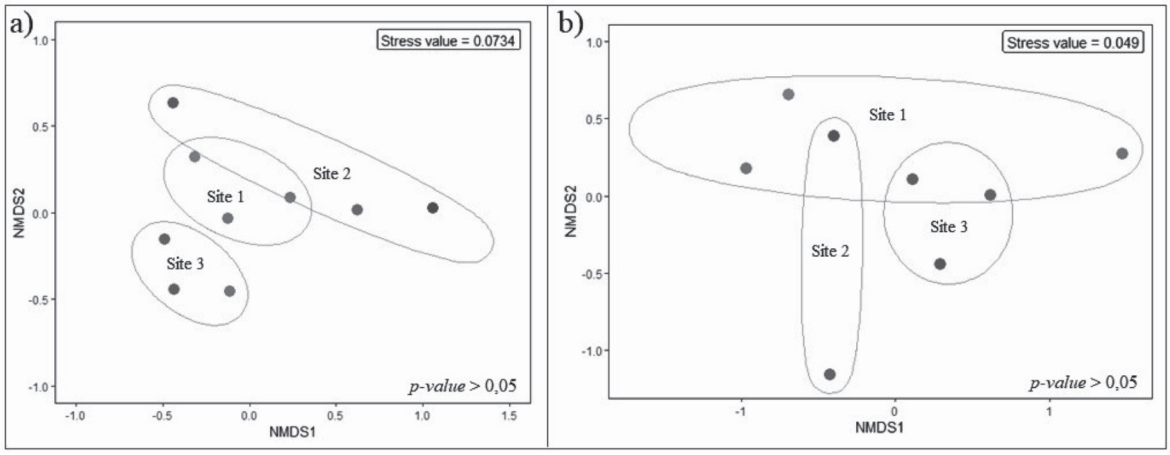

Fig. 7. NMDS analysis of a) eukaryotic phytoplankton and b) zooplankton reads sequences based on Bray-Curtis distance.

used for sampling can all have an impact on eDNA quality and retention [54]. The marine organisms that were also detected in this study are thought to be related to the sampling location, which is at the mouth of the river. eDNA from aquatic organisms can travel long distances depending on water conditions, for example fish and invertebrates found up to 10 km from their original habitat [55]. Thus, differences in species composition and abundance observed across all sites could be attributed to rates of chemical-physical components and organic material decomposition, which therefore influence DNA persistence and degradation rates [56, 57].

One challenge with using eDNA-based biomonitoring strategies in streams is that organisms' DNA would be transferred downstream, frequently over unspecified distances and across least understood processes [55]. Another feasible limitation of this research is the inability to identify the species express. Primer sensitivity, lack of DNA template, and DNA degradation can contribute to the inability to amplify DNA from collected samples. Furthermore, for eDNA studies, determining the authentic DNA template concentration within the aquatic environment at the collection time is difficult [49]. It is becoming clear that eDNA sampling has massive benefits, including the capacity to strive sampling without being (or only minimally) impacted by changing site conditions or a limitation of taxonomic specialist [58].

Environmental DNA monitoring could be a massive benefit to underfunded national services. eDNA metabarcoding, in particular, can be advantageous for observing communities that contain multiple conservation-sensitive species. It is possible to respond more quickly if plankton dominance occurs or newly invasive fish species are discovered if surveys are undertaken on a regular schedule (e.g., every 6 months) [48, 59]. eDNA, on the other hand, cannot be applied to distinguish between dead and alive biota or to examine demographic parameters that are important

in environmental studies [59]. The Ciliwung River's management efficiency must be improved by ensuring high-level compliance with regulations through stakeholder involvement, robust surveillance, and enforcement. Understanding plankton abundance and distribution is also important for improving management efficiency and directing resource usage, especially in high biodiversity locations [60]. The information on plankton composition, abundance, and diversity presented in this study represents a snapshot of the current state and can be used to state river ecosystem management. As a result, these data can be used as a benchmark for regular inspection of Ciliwung River fisheries.

Conclusions

Environmental DNA is a delicate and convenient method for investigating aquatic organisms with broad geographical distribution patterns, so it can be used to supplement traditional methods. The original reads were filtered from 1,492,975 to 1,265,307 reads. The eukaryotic phytoplankton taxa found in the eDNA samples included 22 species from 16 genera, 15 families, 13 orders, 9 classes, and 6 phyla. Meanwhile, 15 species were identified from 12 genera, 10 families, 7 orders, 4 classes, and 3 phyla of zooplankton. The taxa identified from the eDNA samples show that order Thalassiosirales from class Mediophyceae (eukaryotic phytoplankton) and order Ploima from class Monogononta (zooplankton) were the most commonly discovered plankton across entire sites. The relative abundances of eukaryotic phytoplankton and zooplankton detected in eDNA water samples did not differ significantly (p-value>0.05).

The most abundant species for all sites were *Stephanocyclus cryptica* (phytoplankton) and *Moina macrocopa* (zooplankton). The eukaryotic phytoplankton diversity index (H') was in the low to moderate (ranged from 0.90 to 1.82), whereas the zooplankton diversity index (H') is in low category (ranged from 0.69 to 0.98). eDNA has potential benefits, but it is limited by the inability to identify species expressed (dead or living organisms), primer sensitivity, lack of DNA template, and DNA degradation. It is becoming increasingly difficult to ensure that DNA is transferred downstream, over unspecified distances and across less understood processes. Additionally, determining the authentic DNA template concentration within the aquatic environment is difficult.

Acknowledgments

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Conflict of Interest

The authors declare no conflict of interest.

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