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Research Article

Environmental DNA Metabarcoding Reveals the Eukaryotes Diversity in Marine Protected Area of Lombok Island, Indonesia

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ABSTRACT

Biodiversity assessment surveys are necessary for establishing conservation areas. However, such surveys are typically expensive, primarily if they cover a large area and take a long time. The survey difficulty increases when applied to cryptic, sparse, and fast-moving organisms. In addition, it requires expertise in taxonomic-biota classification. The breakthrough environmental DNA (eDNA) metabarcoding technique promises to overcome all the hurdles of assessing the potential for marine biodiversity in a non-invasive, rapid, extensive, and more effective way. We evaluated the ability of the eDNA survey to reveal the potential diversity and character of marine eukaryotes in the Lombok Island Marine Protected Area. A sampling of seawater and sediment eDNA in pore size fractions of 0.4-12 µm and >12 µm was carried out in the Core Zone, Non-Core Zone, and Non-Conservation Area, in east, north, and west Lombok, respectively. The detection and classification of eukaryotes using bioinformatics analysis were accomplished following extraction, amplification, and DNA sequencing. We identified 20,478 unique sequences of potential species classified in five kingdoms to 654 marine eukaryotes families. The comparison results show differences in community structure between locations, as well as differences in diversity between media and factions. The eDNA survey can assess marine biodiversity at a macro level and has implications for management in conservation areas.

Keywords: Biodiversity, Marine eukaryotes, environmental DNA, the Primary V9-SSU 18S rRNA gene

ABSTRAK

Survei penilaian keanekaragaman hayati penting bagi penetapan kawasan konservasi. Namun survei ini umumnya tidak murah, apalagi bila mencakup area yang luas dan waktu yang lama. Kesulitan survei meningkat terhadap biota yang kriptik, jarang, dan bergerak cepat, serta memerlukan keahlian klasifikasi taksonomi-biota. Terobosan teknik metabarcoding DNA lingkungan (eDNA) menjanjikan mengatasi semua rintangan penilaian potensi keragaman hayati laut secara non invasif, cepat, luas, dengan cara lebih efektif. Kami mengkaji kemampuan survei eDNA mengungkap potensi keragaman jenis dan karakter komunitas Eukaryota laut di Kawasan Konservasi Perairan Pulau Lombok. Pengambilan sampel eDNA air laut dan sedimen dalam fraksi ukuran-pori 0.4-12 µm dan >12 µm dilakukan di Zona Inti, Luar Zona Inti, dan Luar kawasan konservasi, masing-masing di Lombok Timur, Utara, dan Barat. Analisis bioinformatika untuk mendeteksi dan mengklasifikasi Eukaryota dilakukan setelah proses ekstraksi, amplifikasi dan sekeunsing DNA. Kami mendeteksi 20,478 sekuens unik berpotensi spesies yang terklasifikasi dalam lima kingdom hingga 654 famili Eukaryota laut. Hasil perbandingan menunjukkan perbedaan struktur komunitas antar lokasi serta perbedaan karakter keragaman antar media dan fraksi. Survei eDNA mampu menilai keragaman hayati laut secara makro dan berimplikasi terhadap pengelolaan dalam kawasan konservasi.

Kata kunci: Keragaman jenis, Eukaryota laut, DNA lingkungan, primer V9-SSU 18S rRNA

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1. Introduction

Marine biodiversity has the potential benefits of goods and services for the fulfillment of welfare, food, and community nutrition (Beaumont et al., 2007; Bernhardt & O'Connor, 2021; Cisneros-Montemayor et al., 2016), a source of pharmaceuticals and cosmetics (Dayanidhi et al., 2021; Malve, 2016), and various biotechnology products that have high prospects in the future (Kim, 2015). According to Fisher et al. (2015) estimation, the biodiversity in coral reef ecosystems (excluding single-celled biota) ranges from 550,000 - 1,330,000, and only 10% are specified. Habitat degradation due to natural and anthropogenic factors, either locally or globally, causes marine biodiversity helpless to lose and decrease (Alabia et al., 2020; Gray, 1997). Generally, vulnerable habitat area protection is involved in efforts to prevent these impacts (Venter et al., 2014).

Designing and prioritizing marine protected areas usually requires species diversity information, mainly through inventory surveys and monitoring (Beger et al., 2015; Strassburg et al., 2020). However, such surveys are not cheap, primarily if they cover a large area and take a long time. In addition, the difficulty increases when the biota surveyed is micro, cryptic, and sparse (Lynch & Neufeld, 2015; Montes et al., 2021; Pearman et al., 2016). Overcoming these difficulties, environmental DNA metabarcoding (eDNA) has become a promising alternative method in assessing the potential for marine biodiversity in a non-invasive, fast, extensive, and cost-effective compared to traditional methods (Deiner et al., 2017; Madduppa et al., 2021). In addition, this method is relatively easy because it does not rely on observational expertise to detect unknown species (Ruppert et al., 2019). eDNA is genetic material released into the environment from various biota through feces, exfoliated cell tissue, mucus layer, and other secretory processes (Rees et al., 2014). The detecting taxa in eDNA uses marker genes (primary). Commonly used primers are nuclear gene markers and cell organelles (mitochondria and plastid chloroplasts) to reveal various biota at all taxonomic levels (Amaral-Zettler et al., 2009; Decelle et al., 2018; Gelis et al., 2021; Stoeck et al., 2010).

As the world's marine mega-biodiversity and part of the coral triangle area (Gray, 2001; Hoeksema, 2007; Veron et al., 2009), Indonesia has set a marine conservation area of 12.9 million hectares of a reserved area of about 28 million hectares with a target of 10% of all marine areas by 2030 (Rusandi et al., 2021). The establishment of this marine conservation area aims to protect biodiversity, save and improve essential habitats, and use the sea for sustainable community welfare, especially in fisheries and tourism (CEA 2018). The potential application of eDNA metabarcoding for biodiversity studies has been carried out in Indonesia using the cytochrome c oxidase subunit I (COI) primary method to detect chordates, mollusks, and echinoderms taxa (Gelis et al., 2021; Madduppa et al., 2021).

West Nusa Tenggara Province has allocated its waters covering an area of 341,641, 5 hectares as a marine protected area (MPA) (Hernawati et al., 2020. Here, we present the potential for marine biodiversity through eDNA metabarcoding using a primer in the V9 hypervariable region of the small-subunit 18S ribosomal gene (V9 SSU 18S rRNA) to see the potential and characterize eukaryote communities inside and outside the MPA of Lombok Island.

2. Material and methods

2.1. Study sites

The research was conducted in the coral reef ecosystem around Lombok Island, West Nusa Tenggara Province, Indonesia. The sites represent in the zoning and outside the marine protected area (MPA). The criteria for determining the zoning have been regulated and defined in official documents based on the decision of the local government or minister. The MPA includes the Gili Meno, Air, and Trawangan Island Marine Tourism Park (TWP GILIMATRA), North Lombok Regency; MPA of Gili Tangkong, Gili Nanggu, Gili Sudak, and the surrounding waters (KKP GITANADA), West Lombok Regency; and MPA of Gili Sulat and Gili Lawang, East Lombok Regency (Hernawati et al., 2020; Gelis et al., 2021; Santoso et al., 2021) (Figure 1).

2.2. Collection of DNA environmental

We use the location and method of sample collection as has been done by Pratomo et al., (2022). By scuba diving, eDNA samples (seawater and sediment) were collected from six reef stations within each coastal area (west Lombok, east Lombok, and north Lombok). From

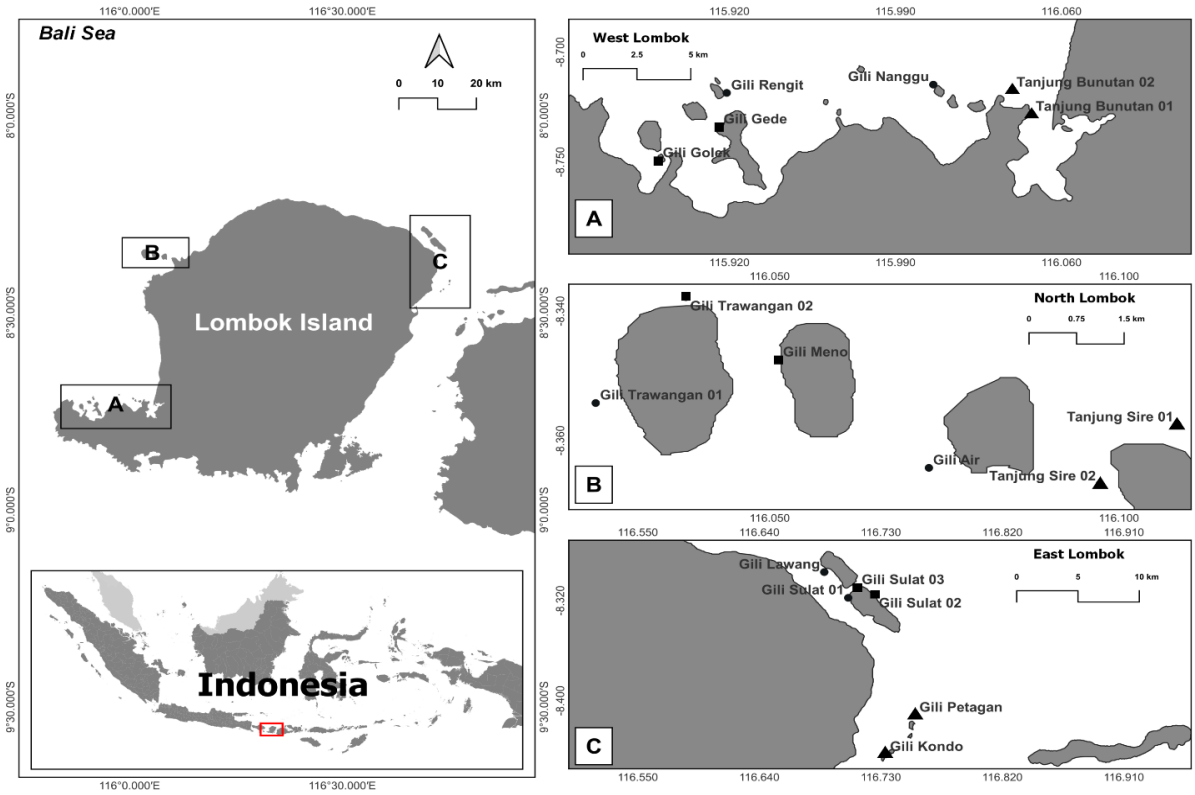

Figure 1. Map of the research sites around Lombok Island, Indonesia. (A) West Lombok. (B) North Lombok. (C) East Lombok. The sampling point marked with rounds are the core zone, cubes are the non-core zone, and triangles are the non-conservation area

18 stations and 72 samples in Lombok, the samples (one seawater and one sediment) per station were collected per day from three stations. (Fig. 1 and Table 1). 4 L of seawater sample each from the water column (~2 m above the reef substrate) and the sediment sample (water + sediment in 1:1 ratio) at each reef station, were collected in a sterilized bottle. The collected eDNA samples were stored in a cool box with ice and brought to base camp at Lombok Island in less than 12 hours. Through 47 mm diameter polycarbonate membrane filters (Sterlitech), each sample was filtered twice using a peristaltic pump (Fisher Scientific) with two different pore sizes: 12 µm first and then 0.4 µm. Each filter was cut into two, and each half was placed in a 1.5 mL vial prefilled with DNA Shield as a preservative. At the end of all eDNA survey activities, all the samples were transported to the Marine Biodiversity and Biosystematics laboratory at IPB University, Indonesia, via commercial courier service and then stored at −20 °C until DNA extraction.

2.3. DNA extraction, amplification, and sequencing

The filtered eDNA samples were extracted and amplified at the Marine Biodiversity and Systematic Laboratory of IPB University. From the filters, DNA was extracted using ZymoBiomics Miniprep Kit (Zymo Research, Irvine, CA, USA) following the manufacturer's instructions. V9 hypervariable regions of the eukaryotic SSU 18S rRNA were amplified using a polymerase chain reaction (PCR) platform and prepared for 2×250 bp paired-end Illumina MiSeq sequencing (Illumina, San Diego, CA, United States) using V9 primer set 1389F: 5'-TTG TAC ACA CCG CCC-3' and 1510R: 5'-CCT TCY GCA GGT TCA CCT AC-3', (Stoeck et al., 2010). The PCR profile used was as follows: 3 min at 94 °C, followed by 35 cycles of 94 °C for 45 s, 48 °C for 30 s, and 72 °C for 30 s, and a final extension at 72 °C for 5 min. Each 49 µL of PCR reaction comprised 25 µL of MyTM HS red mix (Bioline Ltd., London, UK), 1 µL of (10 µM) forward primer, 1 µL of (10 µM) reverse primer, and 1 µL of DNA template. The final volume was adjusted to 49 µL using ddH2O.

Table 1. Coordinates of the sampling stations around Lombok Island

Coastal area	Station	Date	Depth (m)*	Position	
				South	East
East Lombok	Gili Sulat 01	5 July 2018	< 1	08°19.069'	116°42.355'
	Gili Lawang	6 July 2018	1.2	08°17.833'	116°41.290'
	Gili Sulat 02	5 July 2018	> 10	08°18.900'	116°43.519'
	Gili Sulat 03	5 July 2018	< 1	08°18.574'	116°42.767'
	Gili Petagan	6 July 2018	2.8	08°24.698'	116°45.324'
	Gili Kondo	6 July 2018	< 1	08°26.572'	116°44.016'
North Lombok	Gili Trawangan 01	11 July 2018	8.46	08°21.253'	116°01.505'
	Gili Air	12 July 2018	< 1	08°21.854'	116°04.369'
	Gili Trawangan 02	11 July 2018	1.4	08°20.271'	116°02.280'
	Gili Meno	11 July 2018	> 10	08°20.852'	116°03.077'
	Tanjung Sire 01	12 July 2018	4.8	08°21.455'	116°06.506'
	Tanjung Sire 02	12 July 2018	8.3	08°22.001'	116°05.840'
West Lombok	Gili Nanggu	8 July 2018	< 1	08°42.887'	116°00.362'
	Gili Rengit	9 July 2018	< 1	08°43.114'	115°55.135'
	Gili Golek	9 July 2018	< 1	08°44.967'	115°53.405'
	Gili Gede	9 July 2018	< 1	08°44.045'	115°54.945'
	Tanjung Bunutan 01	8 July 2018	> 10	08°43.693'	116°02.848'
	Tanjung Bunutan 02	8 July 2018	> 10	08°43.039'	116°02.363'

*) In lowest low water level based on Hydrographic and Oceanographic Center, The Indonesian Navy (2007) and mean tidal range is 187 cm.

1x reaction was 0.2 µM. The PCR product was inspected through electrophoresis final master mix concentration in 1× reaction was 0.8×, and the final primer concentration of 5 µL of aliquots on 1% agarose gel in 0.5X TBE buffer. Library preparation and sequencing were performed at the University of Rhode Island (URI) Genomics and Sequencing Center, United States of America. Using Kapa HotStart HiFi 2x ReadyMix DNA polymerase (Kapa Biosystems Ltd., London UK.), a second PCR was performed to add the dual indices and Illumina sequencing adapters from the TruSeq PCR-Free LT kit to the target amplicons. Using paramagnetic Kapa pure beads (bead-to-sample volumetric ratio in 1.6:1), successful amplicons were then purified. In equal concentrations, prepared samples were combined and then pooled with a 20% denatured and diluted PhiX Illumina control library. The final pooled library was sequenced on an Illumina MiSeq with the MiSeq v2 500-cycle kit (Illumina, San Diego, CA, United States).

2.4. Data processing and bioinformatic analyses

The obtained forward and reverse raw sequence data were converted to demultiplexed fastq files (see additional information on data availability). Cutadapt v.1.18 (Martin, 2011) was applied to trim the reverse and forward primer sequences and remove short reads with lengths < 100 bp and low-quality reads with a Phred Q score of < 20. Qiime2.2019.10 pipeline (Bolyen et al., 2019; Caporaso et al., 2010) was employed for DADA2 v.2018.11.0 (Callahan et al., 2016)(via q2-dada2) processing for denoising, joining denoised paired-end reads, filtering out chimeric sequences and singletons, and dereplicating sequences to produce amplicon sequence variants (ASVs).

2.5. Identification of eukaryotes

Eukaryotes were identified from the eDNA sequences by classifying all ASVs using the q2-feature-classifier (Bokulich et al., 2018) classify-sklearn Fit-Naïve Bayes taxonomy classifier against the 18S NR SILVA (release 123 Qiime compatible) at 99% similarity level of operational unit taxonomy reference sequences (https://www.arb-silva.de/download/archive/qiime/). Based on the NCBI database (https://www.ncbi.nlm.nih.gov/) and WORMS (https://www.marinespecies.org/), obtained eukaryotes taxonomy was further taxonomic analyzed in getting the levels of kingdom, phylum, class, order, and family (see supplement file). The non-marine-taxa were excluded. The final taxonomic result was visualized by a Krona diagram (https://github.com/marbl/Krona/wiki)

2.6. Statistical analyses

The data on the relative abundance of eukaryote taxa, composition, and distribution

were generated from DADA2 results. All statistical analyses were carried out on Qiime2.2019.10 pipeline (Bolyen et al., 2019; Caporaso et al., 2010). Alpha diversity (Shannon's entropy) and beta diversity (Bray–Curtis dissimilarity) were estimated using q2-diversity after the samples were rarefied (subsampled without replacement) to 34,280 sequences per sample. The comparison of all samples grouped by location, zone, medium, and fraction to examine differences in abundance and alpha diversity employing the Kruskal–Wallis test (Kruskal & Wallis, 1952) via q2-diversity alpha-group-significance and beta diversity applying the permutational analysis of variance (Permanova) test (Anderson, 2001) in 9999 permutations via q2-diversity beta-group-significance and visualized by principal of coordinate analysis (PCoA) plots (Halko et al., 2010).

3. Results

3.1. Sequences and Amplicon Sequence Variants

As obtained by Pratomo et al., (2022), only 41 out of 72 samples from 16 stations were sufficiently high quality for sequencing (Table 2), yielding a total of 3,168,655 raw sequences and about 30,205–240,604 sequences per sample with a mean sequence length of 127.81 ± 22.03. The low quality of some libraries may be due to eDNA degradation during sample transport and extraction. After removing all non-marine-taxa (Table 3), the obtained sequence became 2,737,734 sequences consisting of 20,478 unique sequences (ASVs). The non-marine taxa were only 0.06% of the total obtained sequences. Each ASV obtained can be assumed to be an individual representative of a particular species, variant, or phylotype.

3.2. Identification, classification, composition and distribution of eukaryotes

Identification of eukaryotes in ASVs by classifier showed an average confidence level of 94%. Further classification illustrated that Lombok waters eDNA includes five kingdoms with composition Chromista (35%), Animalia (32%), Fungi (3%), Plantae (2%), and Protozoa (2%), while 26% ASV could not be determined at the kingdom level (Figure 2A). In addition, there were 57 phyla, 154 classes, 381 orders, and 654 families, while 43.34% of ASV were not identified (Figure 2B). The closer to the species, the smaller the percentage of ASV that can be determined (Figure 2C).

Based on the total abundance across location, zoning, medium, and fraction; the distribution and composition of eukaryote diversity showed the top seven classes with the highest percentage abundance, namely Dinophyceae, Bacillariophyceae, Hydrozoa, Mediophyceae, Demospongiae, Coscinodisco-

Table 2. Successfully amplified eDNA samples by sample type and filter pore size. EB356–EB396 are the sample codes; n.a. (not available) indicates the eDNA samples were not successfully amplified

Location	Station	Seawater fraction		Sediment fraction	
		0.4–12 µm	>12 µm	0.4–12 µm	>12 µm
East Lombok	Gili Sulat 1	n.a.	EB356	EB357	EB358
	Gili Lawang	EB367	EB368	EB369	EB370
	Gili Sulat 2	EB359	EB360	EB361	EB362
	Gili Sulat 3	EB363	EB364	EB365	EB366
	Gili Petagan	n.a.	EB371	EB372	EB373
	Gili Kondo	n.a.	EB374	EB375	EB376
West Lombok	Gili Nanggu	n.a.	n.a.	EB377	n.a.
	Gili Rengit	n.a.	n.a.	EB379	n.a.
	Gili Golek	n.a.	n.a.	EB380	EB381
	Gili Gede	EB382	n.a.	EB383	n.a.
	Bunutan 1	n.a.	n.a.	EB378	n.a.
	Bunutan 2	n.a.	n.a.	n.a.	n.a.
North Lombok	Gili Trawangan 1	EB384	EB385	EB386	EB387
	Gili Air	EB396	n.a.	n.a.	n.a.
	Gili Trawangan 2	EB388	EB389	EB390	EB391
	Gili Meno	EB392	EB393	EB394	EB395
	Tanjung Sire 1	n.a.	n.a.	n.a.	n.a.
	Tanjung Sire 2	n.a.	n.a.	n.a.	n.a.

phyceae, and Spirotrichea, respectively (Figures 3 and 4). The label 'No Rank' in the figure means that the ASVs have no rank class. This study found 15,707 (76.7%) total ASVs in only one sample, but only represents 19.4% of all sequence data. These ASVs indicate the rare eukaryote taxa.

3.3. Eukaryote alpha diversities and the comparison

The total alpha diversity (Shannon H' entropy) of the eukaryote communities ranged from {min-max (median)} 4.90 to 9.59 (7.63). A comparison of eukaryotic alpha diversities did not

Table 3. Non-marine* eukaryote sequences found in the sample

ASV Taxa	Sequence frequencies
Neoptera (Winged insects)	1237
Gallus gallus (Chickens)	103
Homo sapiens (Humans)	4
Hyperamoeba sp. (Protozoa)	65
Protosteliida (Protozoa)	37
Glomeromycetes (Fungi)	219
Vermamoeba vermiformis (Protozoa)	12
Unindentified freshwater Cercozoan (Chromista)	31
Total	**1708**

*) Biota environmental attributes and habitat were based on WORMS (https://www.marinespecies.org/)

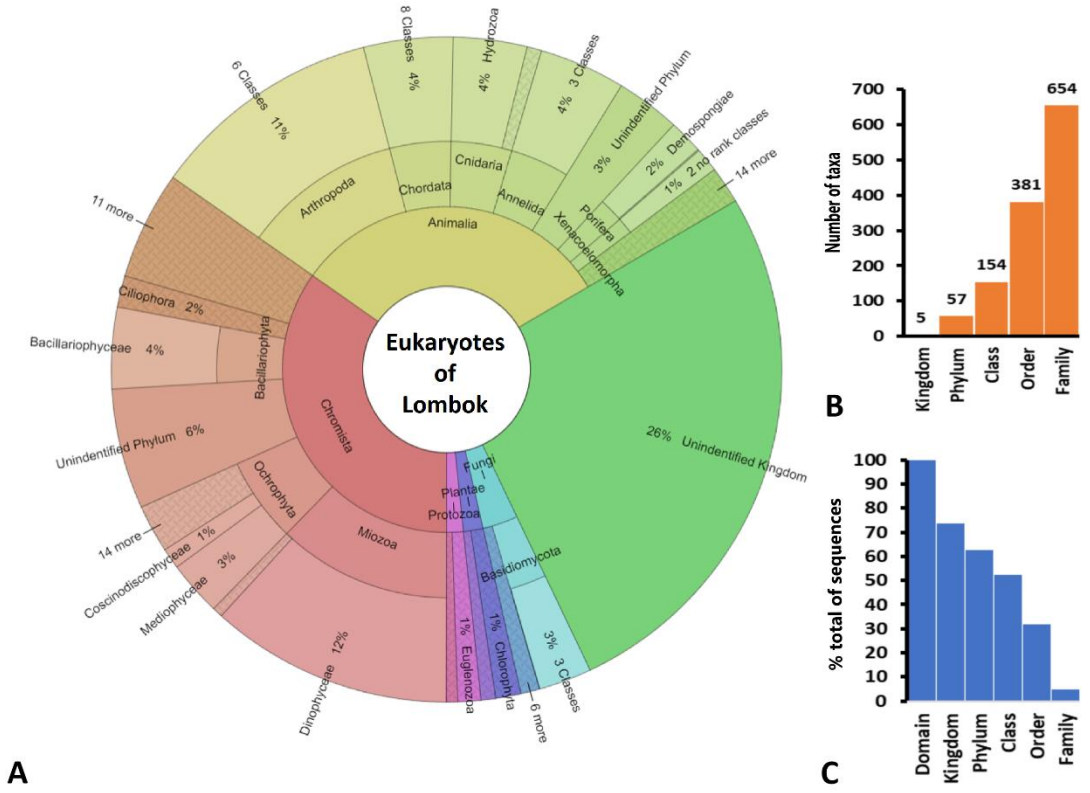

Figure 2. Classification and composition of eukaryote eDNA in Lombok Waters. (A) Krona diagram visuals the taxonomic composition of eukaryotes from kingdom rank to class, (B) The number of classified taxa of eukaryotes from kingdom rank to family, and (C) Percentage of total classified sequences according to taxon rank.

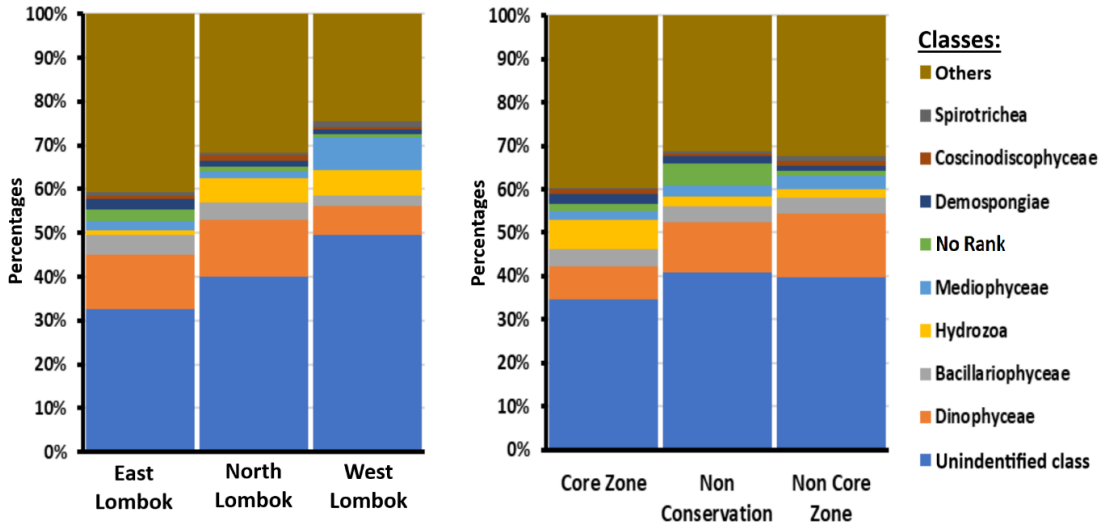

Figure 3. Distribution and composition of eukaryote class in location (A) and zoning (B) of Lombok waters. Only the top ten groups of abundance are shown.

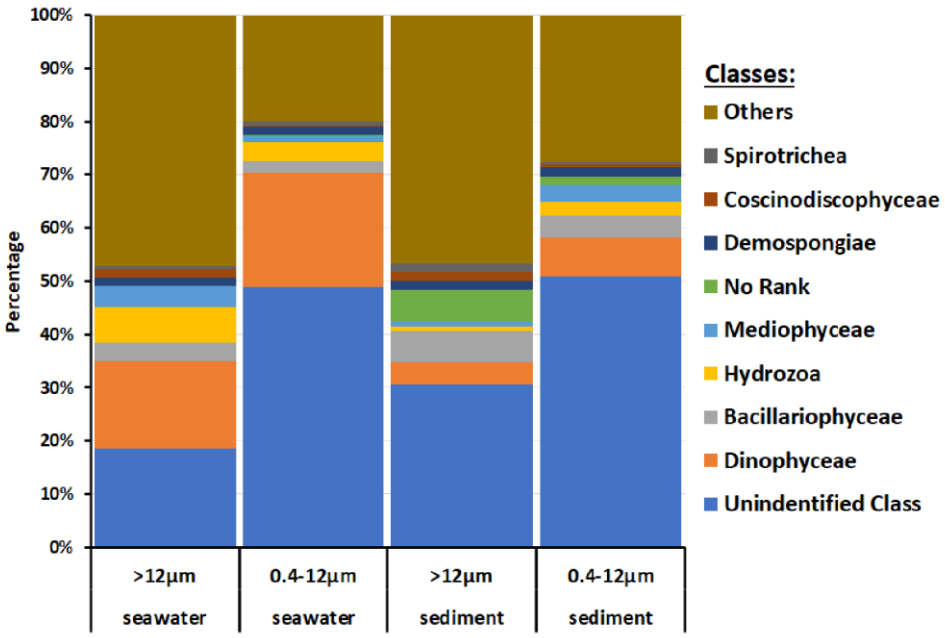

Figure 4. Composition of eukaryote class in media and fractions of Lombok waters. Only the top ten groups of abundance are shown.

show significant differences between locations and zoning. Those indicate that no factors based on local geography and marine protected areas affect the alpha diversity of eukaryotes. However, a comparison of the alpha diversity of eukaryotes between media and fractions demonstrated differences significantly (Figure 5), in which the differences in the pore size fractions were more prominent than in media. The alpha diversity found in sedimentary media was higher than in seawater, and the alpha diversity found in the 0.4-12 µm fraction was higher than the >12 µm fractions. These results indicate that natural factors related to habitat medium and technical aspects linked to the distribution of the pore size fraction of eDNA samples affect the acquirement of eukaryote alpha diversity.

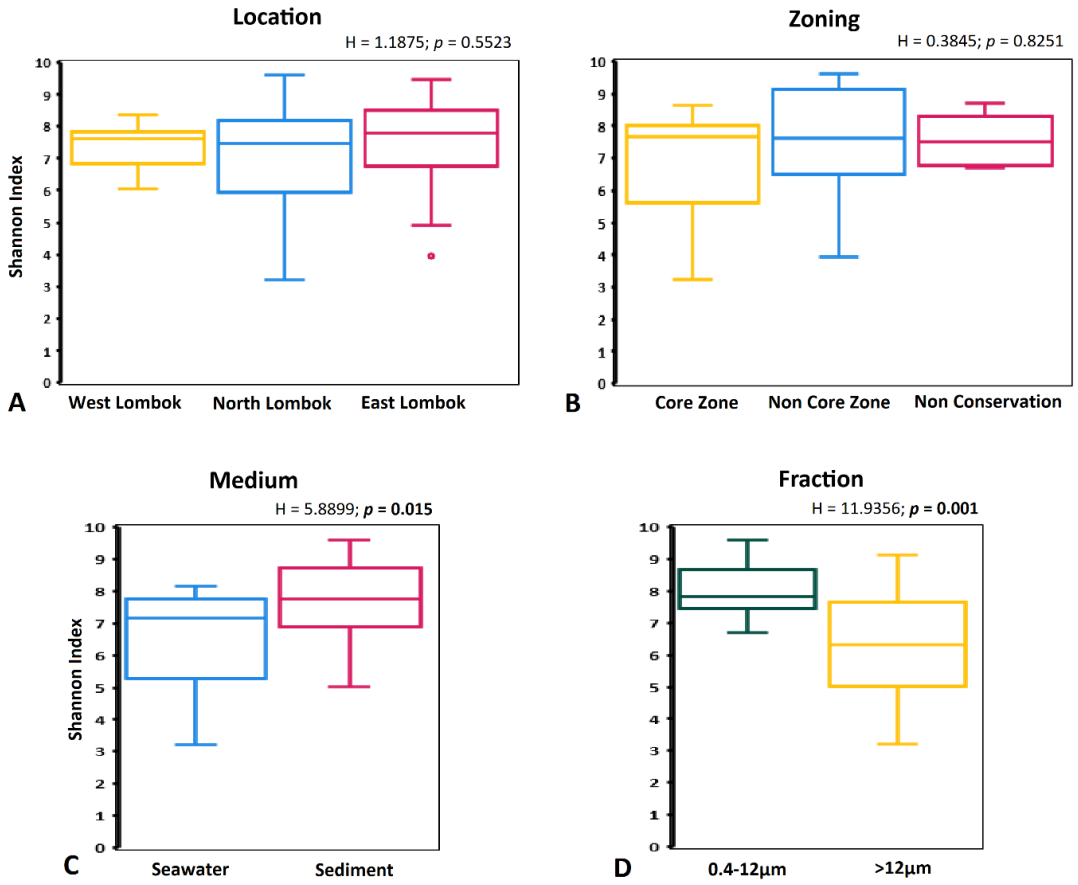

Figure 5. Boxplot diagram of the eukaryote alpha diversity comparison (Shannon). Based on the Kruskal-Wallis test, no significant differences existed between locations (A) and zoning (B). A considerable difference occurred across media (C) and fractions (D). The lines in the media indication box and the lower and upper bounds of the box indicate the 25% and 75% quartiles. The upper and lower fin lines are the 95% confidence interval limits. Outer points are considered outlier data. The bold p indicates significance.

3.4. Eukaryote beta diversities and the comparison

The total beta diversity of the eukaryote communities based on the Bray-Curtis dissimilarity {min-max (median)} ranged from 0.72 to 0.99 (0.92). These indicate the high heterogeneity of the eukaryote communities in Lombok waters. Yet, the eukaryote communities in west Lombok waters were slightly heterogeneous (Figure 6A). Based on Permanova on 9999 permutations, a comparison of the beta diversity of the eukaryote communities showed no significant difference between zoning but significant differences between locations, media, and fractions (Figure 6). These showed that management factors related to the determination of marine protected areas do not affect the heterogeneity of the eukaryote communities in Lombok. Pairwise Permanova revealed no significant difference between east Lombok and north Lombok (Figure 6A). The pattern of differences in community structure was seen clearly on the plot in the PCoA diagram by location, medium, and fraction, except zoning (Figures 7A and 7B).

4. Discussions

4. 1. The power of environmental DNA in revealing marine biodiversity

This study demonstrates the power of eDNA surveys in revealing marine eukaryote diversity. Broader taxa acquisition showed the sensitivity of eDNA able to indicate five kingdoms in Lombok Island waters. Similar yields will be hard to perform when using traditional methods (Smart et al., 2015). The reliability and accuracy of the eDNA survey results depend on the completeness of the available databases of diversity and reference DNA sequences. A large number of unclassified eDNA sequences (>50%)

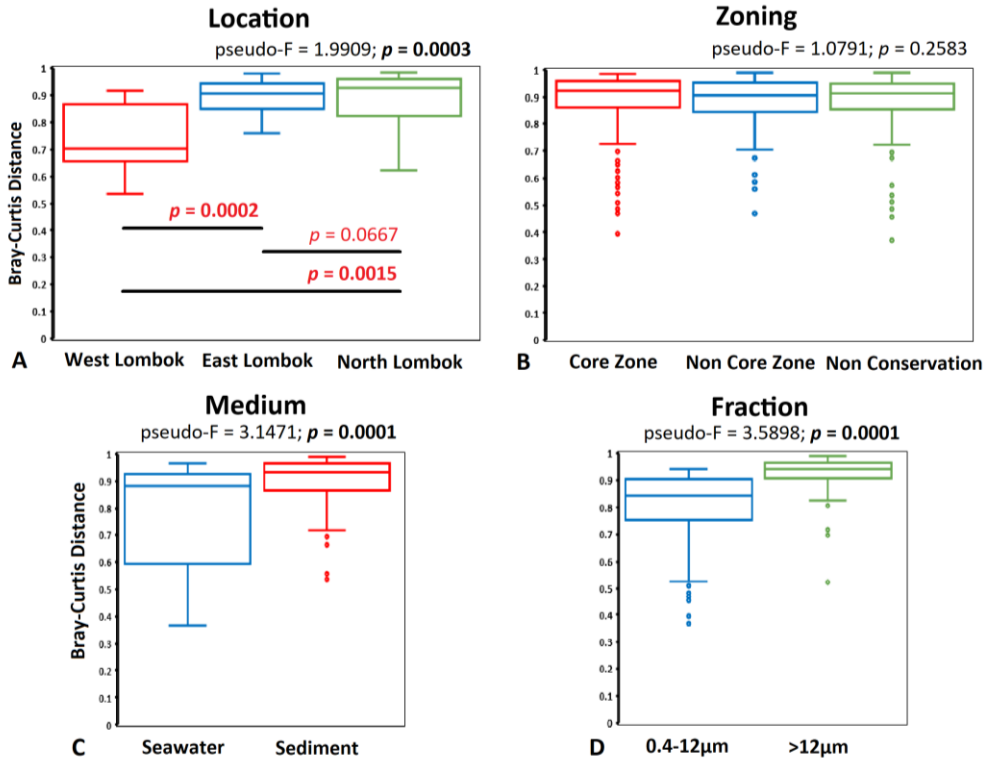

Figure 6. Box plot diagram of beta diversity comparison (Bray-Curtis dissimilarity) eukaryotes. Based on permanova on 9999 permutations, there were significant differences between locations (A), media (C), and fractions (D) but not between zoning (B). The lines in the median indication box and the lower and upper bounds of the box indicate the 25% and 75% quartiles. The upper and lower fin lines are the 95% confidence interval limits. Points are considered outlier data. The bold p indicates significance.

in this study indicate that many new taxa had unregistered. On the other hand, new species registrations in global databases like NCBI tend to increase (Schoch et al., 2020). To date, 496,735 species from 692,822 eukaryote sequence data registered at NCBI (Accessed June 14, 2022). According to LON-LIPI (Suharsono, 2014), the total diversity of marine species in Indonesia consists of 910 families or 11,133 species (excluding associated biota from land). With an eDNA survey, we could expect its potential to dig deeper into species richness.

This study also reveals the magnitude of rare biota (76.7%). This phenomenon is typical in the marine microbes of both prokaryotes and eukaryotes plankton and occurs at almost all ranks of the eukaryote taxonomy (de Vargas et al., 2015; Sunagawa et al., 2015). In general, this indicates that the main characteristics of marine communities consist of a small number of common but abundant biota though most dominated by rare biota in a broad range. This phenomenon is known as the “rare biosphere” that has now been discovered and may be a crucial community to the ecosystem (Lynch & Neufeld, 2015). Increasing the detection of rare biota can be done by adding sample replications during the PCR process (Ficetola et al., 2015). The primer of the SSU 18S rRNA gene is commonly used in molecular analysis to detect the presence of eukaryote taxa because this primer contains ribosomal gene regions that exist in all eukaryote taxa (Neefs et al., 1993; Wang et al., 2014). This study used the V9-SSU 18S rRNA gene primer, the same primer used in the TARA Ocean Expedition for revealing oceanic eukaryotes plankton (de Vargas et al., 2015). The application of different primers in other eDNA surveys in Lombok waters gave different results. The use of COI primers (f mlCOIintF and r dgHCO2198) resulted in a more diverse diversity of marine fish species, but the identified eukaryota taxa were slightly lower (43%) than in the current study (Gelis et al., 2021; Madduppa et al., 2021).

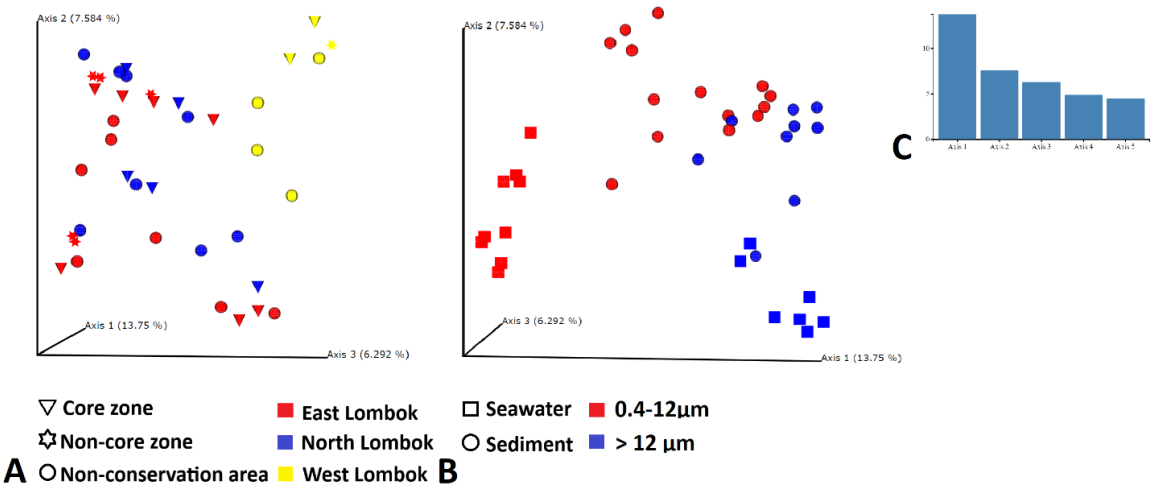

Figure 7. Visualization of the eukaryote bray-curtis dissimilarity in the PCoA plot diagram in Lombok Waters. (A) PCoA plot diagram based on zone and location. (B) PCoA plot diagram based on media and fraction. (C) Graph of scree plot percentage of variation explained from axes 1 to 5.

4. 2. Characteristics of diversity and community structure of eukaryotes in Lombok waters

Generally, this study illustrates the relatively high alpha and beta diversity of eukaryotes in Lombok waters, similar to the marine plankton study of the Ocean Tara Expedition (de Vargas et al., 2015; Sunagawa et al., 2015). Alpha diversity analysis indicates that the diversity of eukaryotes in Lombok waters does not vary across locations in Lombok waters, both inside and outside the marine protected area. On the other hand, beta diversity indicates variations in community structure between locations. Beta diversity characterized by high dissimilarity means high heterogeneity (Socolar et al., 2016), and consequently, the composition of the biota species assemblage everywhere in these waters tends to be different. The absence of variation in beta diversity between zones indicates that the application of the zoning system tends to make the heterogeneity pattern of eukaryotes similar between zones.

The high beta diversity indicates that the eDNA of eukaryotes tends to cluster and scatter unevenly throughout Lombok waters. These characteristics suggest that the source of eDNA is affected by local factors and sources. However, eDNA materials are inclined to degradation (Barnes & Turner, 2016). Presuming the source rate is constant, so they tend to accumulate around the source. That hints that there are niche diversities of various eukaryote taxa. Therefore, this supports the ability of eDNA surveys to show the condition of local ecosystems. However, interpreting eDNA data needs to evaluate possible sources outside the sample area. The presence of contaminant eDNA in this study shows the possibility of exogenous sources due to biological and human activity factors as well as sources of errors in methods both in the field and in the laboratory (Goldberg et al., 2016; Nguyen et al., 2015; Port et al., 2016).

The alpha and beta diversity showed significant differences in eukaryote eDNA between seawater and sediment media (see Figures 4 and 6). That shows the differences clearly in the characteristics of the eukaryote community structure among media. This finding supports previous studies that the diversity of eDNA in sediment is higher than in seawater because eDNA degrades about 57 times slower in deposition than in seawater (Sakata et al., 2020; Turner et al., 2015). That causes eDNA tends to accumulate in sedimentary media.

Comparison between the 0.4-12 µm and >12 µm fractions also showed the difference where is the high diversity in the 0.4-12 µm fraction. This fact may reflect the general condition of the high diversities of piconanoplankton ranging from 0.8 to 5 µm (Decelle et al., 2018). Technically, the difference in the size of the particles filtered from dead and live tissues will affect the characteristics of the obtained eDNA. Therefore, this will be an essential issue in the eDNA sampling strategy. In addition, the observations also show that the smaller fractions give higher diversity. The study of Turner et al. (2014) on the particle size distribution of eDNA samples found that the particles filtered in the smaller pore size fraction had a higher concentration. Technically, the

smaller the filter pores, the easier it will be to clog, and the longer the filtering time. If the desired eDNA material is filtered as much as possible with good operational time, it is supposed that there is an optimum point based on these two variables (Turner et al., 2014).

4. 3. Implications of the environmental DNA survey on biodiversity and marine protected area management

In addition to assessing the potential for marine biodiversity, eDNA surveys are promising as a noninvasive, fast, vast, and cost-effective monitoring tool for marine biota compared to traditional methods (Deiner et al., 2017; Madduppa et al., 2021). eDNA metabarcoding allows for more reliable data and can overcome the challenges of surveying rare, invasive, high-range migratory biota (Goldberg et al., 2015; Rees et al., 2014; Valsecchi et al., 2020). eDNA metabarcoding can also be applied but is not limited to predicting the abundance and biomass of biota (Doi et al., 2015; Fukaya et al., 2021), seasonal activity patterns, and spawning of biota (Bylemans et al., 2017; De Souza et al., 2016). Diversity analysis through eDNA can provide an overview of the compositional pattern of species diversity at the macro level. Guidance for marine protected area management likely can be acquired when eDNA analysis is on specific taxa. For instance, in the study of Karkarey et al. (2022), the selection of the management patterns for marine protected areas is based on changes in alpha and beta diversity in reef fish class taxa due to the influence of environmental conditions and human activities. Further exploration can provide a complete ecological meaning regarding various associations or interactions among marine biota and their environments (Deiner et al., 2017). For example, some of them were eDNA studies of eukaryote diversity as an indication of anthropogenic stress in coastal ecosystems (DiBattista et al., 2020) and metagenomic studies of bacterial communities in coral waters of Kham Island, Thailand (Somboonna et al., 2017)

4. 4. Limitations of research

There were no available data for all Station Bunutan 2, Tanjung Sire 1, and Tanjung Sire 2, likely due to eDNA degradation during sample transport and extraction. Several factors affect the success of eDNA data acquisition, such as the dependence on the presence and concentration of eDNA in the water sample, capture efficacy, extraction efficacy, sample interference (e.g., inhibition), and assay sensitivity (see Goldberg et al., 2016). The eDNA samples can degrade beyond the detection threshold within one day (Dejean et al., 2011; Thomsen et al., 2012). Environmental quality conditions, such as high temperatures, neutral pH, and highly UV-B, tend to increase the eDNA degradation rate (Strickler, Fremier & Goldberg, 2014). However, the nature and proportion of minerals, organic substances, and charged particles adsorbing eDNA fragments influence the rate of eDNA degradation in media and protect them from further destruction (Torti, Lever & Jørgensen, 2015).

This study did not employ sterile filter paper samples or distilled water extraction as negative control samples. These samples can detect the presence of an eDNA source of cross-contamination, and they need to be out during the analysis (see N. H. Nguyen et al., 2015; Port et al., 2016). These conditions can increase the results of false positive data. Even so, research has taken action to suppress the source of contaminants using sterilized equipment during the sampling, extraction process, and disposal of exotic biota identified in the taxonomic analysis. The study also did not do repetition and pooling of samples during PCR. This condition potentially reduces target eDNA concentration, thereby reducing the detectability of biota in the study area (Ficetola et al., 2015), and may cause some of the PCR samples in this study did not meet the sequencing quality threshold on the next generation sequencing (NGS) platform, even though they passed the electrophoresis test.

5. Conclusions

The eDNA survey revealed the biodiversity of marine eukaryotes covering five kingdoms in the marine protected area of Lombok. The alpha and beta diversity are relatively high, but no difference in diversity and community structure between the core zone, non-core zone, and non-conservation area. Likewise, there is no difference in species diversity between locations, but there are differences in community structure between locations and between media and factions in Lombok waters. The application of eDNA surveys is promising for assessing marine biodiversity and its implications for marine protected area management in Indonesia on a large scale.

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