BIODIVERSITAS
Volume 24, Number 7, July 2023
Pages: 4185-4191

ISSN: 1412-033X
E-ISSN: 2085-4722
DOI: 10.13057/biodiv/d240758

eDNA assessment of scleractinian diversity and distribution in Lemukutan Island, Indonesia

IWAN KUNCORO[1], NEVIATY PUTRI ZAMANI[1,♥], BEGINER SUBHAN[1], NI KADEK DITA CAHYANI[2]

[1]Department of Marine Science and Technology, Faculty of Fisheries and Marine Sciences, Institut Pertanian Bogor. Jl. Raya Dramaga, IPB Dramaga, Bogor 16680, West Java, Indonesia. Tel./fax.: +6285787115589, ♥email: neviaty@apps.ipb.ac.id

[2]Department of Biology, Faculty of Science and Mathematics, Universitas Diponegoro. Jl. Prof. Soedarto, SH Tembalang, Semarang 50275, Central Java, Indonesia

Manuscript received: 7 June 2023. Revision accepted: 29 July 2023.

Abstract. *Kuncoro I, Zamani NP, Subhan B, Cahyani NKD. 2023. eDNA assessment of scleractinian diversity and distribution in Lemukutan Island, Indonesia. Biodiversitas 24: 4185-4191.* Coral reefs are one of the most degraded and endangered tropical marine ecosystems. The study of biodiversity and its distribution is significant for biomonitoring. Lemukutan is an island that has a coral reef ecosystem with high biodiversity in Bengkayang District, West Kalimantan, Indonesia. This study aims to examine the biodiversity of coral species on Lemukutan Island using the Environmental DNA (eDNA) approach to survey scleractinian diversity across Lemukutan Island. Surface seawater samples were taken from five sites. eDNA sample extracted from the filter paper used for filtration Polymerase Chain Reaction (PCR) amplification was performed with Internal Transcribed Spacer (ITS2) primers, and Next Generation Sequencing was used to examine the results. The result found 2,413 Amplicon Sequence Variance (ASVs) and 275,000 reads from seawater samples from 5 sites. 87 ASVs from the eukaryotic group (146,378 reads) and 1,926 ASVs from unidentified taxa (128,622 reads) were identified. The highest phylum of eukaryotic taxa obtained was Phylum Cnidaria, with 332 ASVs (72% of the total eukaryotic ASVs) and 77,428 reads (85% of the total eukaryotic reads). There are 43 species of eukaryotes, including the Cnidaria group-based eDNA. The results show 14 species of scleractinian and different species compositions from each sampling location. In conclusion, the eDNA method has sensitive results in detecting 14 scleractinian species composition without destroying habitats and organisms. Knowledge regarding species diversity and distribution of taxa with eDNA assessment can be used as a reference in monitoring coral reef ecosystems on Lemukutan Island, Indonesia.

Keywords: Coral reef, diversity, ITS, metabarcoding, monitoring

INTRODUCTION

The biodiversity of the planet is experiencing a decline in all types of ecosystems (McGill et al. 2015; Visconti et al. 2016; Ceballos et al. 2017). At present, the global count of species remains uncertain. Within marine environments, biodiversity is diminishing as a result of climate events, water pollution, acidification, soil pollution, overfishing, resource extraction, and the intrusion of marine species (Jackson et al. 2001; Halpern et al. 2008; Richards & Day 2018). The coral reef ecosystem is identified as one of the marine ecosystems that contribute to the decline in biodiversity. Global records indicate a decrease in the biodiversity of coral reef ecosystems (Dugal et al. 2022). Changes in coral reef ecosystems can be caused by anthropogenic (Benkwitt et al. 2020). One-third of reef-forming corals, particularly the order Scleractinia, are categorized as high-risk on the International Union for Conservation of Nature's (IUCN) red list (Carpenter et al. 2008). Scleractinian corals have a good role in shallow marine ecosystems, which can help protect against coastal erosion (Harrison et al. 2019) and provide a source of food for marine biota (Bright et al. 2015; Welsh et al. 2015).

Indonesia is located within the coral triangle area in Southeast Asia and is well known for having high marine biodiversity. Several countries belong to this region, including Malaysia, Timor Leste, the Philippines, Papua New Guinea, and the Solomon Islands. This area has more than 500 species of coral, including Scleractinian (Veron 2009; Marwayana et al. 2022), and is designated as the most biologically diverse marine ecosystem in the world (Roberts et al. 2002; Veron 2009). Indonesian waters hosted 10% of the world's, including 574 coral species (Veron 2009), 13 seagrass species (Burke et al. 2002), and six species of turtles (Ario et al. 2016). More than 370 million people in the Coral Triangle region rely on the region's ecosystems and services, with 120 million benefiting from coastal and offshore fisheries and marine tourism (Foale et al. 2013). Due to coral reefs' high socio-economic and ecological impacts, several requirements for monitoring biodiversity exist. The effectiveness of monitoring coral reefs begins with the availability of data on the distribution and biodiversity or species richness of coral reefs (Deiner et al. 2017).

Information related to species distribution is essential for designing an area for conservation, especially for MPAs. Therefore, this study examines marine biodiversity, especially in coral reef ecosystems, and the distribution of scleractinian corals found on Lemukutan Island. Lemukutan Island has a high ecological value due to the existence of coral reefs, coral fish, and seaweed, and it has the potential for reef fish resources. The coral reef community is one of the determining factors for the

sustainability of existing benthic communities. The environmental DNA (eDNA) metabarcoding method is a rapidly developing method for studying biodiversity in an ecosystem area (Deiner et al. 2017), a non-invasive method that determines whether or not aquatic species are present by looking for the DNA components that organisms make and release into their habitats (Boussarie et al. 2018; Garlapati et al. 2019). This method has been used to provide insight into species diversity and was recently combined with high-throughput sequencing (HTS) technology to investigate the distribution of aquatic macroorganism species (Fraija-Fernández et al. 2020; Pukk et al. 2021; Suter et al. 2021). This study aims to detect, identify and explain the biodiversity pattern of scleractinian on Lemukutan Island, especially for coral reef conservation efforts.

MATERIALS AND METHODS

Study area

Five samples were collected in 3 L water bottles from 5 locations on Lemukutan Island, Bengkayang District, West Kalimantan, Indonesia (Figure 1). In the five sites selected, an attempt was made to choose sites that could represent the diversity of ecosystems on the island. The samples taken from these sites included enough variation to achieve the research objectives. The sampling location is included in an area with high scleractinian diversity. Water samples were collected directly from the surface of seawater. All samples were preserved and kept in the cool box until they were brought into the laboratory and cooled until filtering. Based on previous research, it is generally recommended to filter samples as soon as possible after collection to minimize the risk of sample degradation. Cold temperatures can help slow down degradation processes, but they are not a substitute for timely processing. For DNA/RNA analysis, it is especially crucial to minimize the delay between collection and filtering. DNA and RNA are sensitive molecules that can degrade over time, particularly in warmer conditions. Cold temperatures can help preserve the integrity of the samples to some extent, but it is still advisable to filter them as soon as practically possible. Samples should be filtered within a few hours of collection to ensure the best preservation of DNA/RNA. However, if immediate filtration is not feasible, the samples should be stored at low temperatures (such as in a cool box or refrigerator) to slow down degradation until they can be filtered. It is important to note that the specific time duration and temperature conditions may vary depending on the sample type and target analysis, so it is always recommended to consult relevant literature or protocols for specific guidelines. The vacuum pump is used, and each sample has to be filtered using a 0.45 m Pall Company sterilized filter paper (47 mm in diameter). After filtering, each filter paper was placed in a 1.5 ml zymoBIOMICS DNA/RNA shield-filled 2 ml cryotube.

Laboratory processing

All eDNA extraction, quantification, and sequencing were performed in dedicated sterile labs while wearing nitrile gloves to minimize the risk of DNA contamination at the Fisheries Laboratory of Pontianak Polytechnic, Indonesia. Quantitative polymerase chain reaction (qPCR) was conducted in specialized UV-sterilized hoods. DNA was extracted from each filtered water sample (DNeasy Blood and Tissue kit). Extraction controls were performed on each processed batch to look for any cross-contamination. With minimal exceptions, the manufacturer's extraction process was always followed. ATL Buffer and Proteinase K volumes were increased. The digested supernatant was put into a QIAcube for automated DNA extraction sample preparation (Qiagen; Venlo, Netherlands). The type and quantity of DNA in each extract and dilution were determined using quantitative PCR.

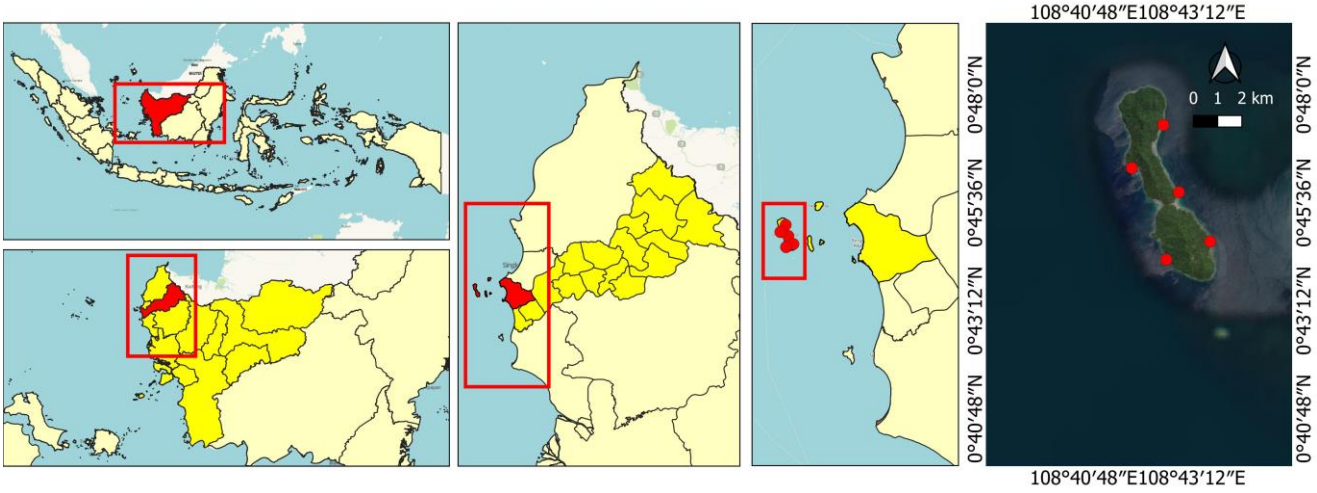

Figure 1. eDNA Metabarcoding sampling locations in Lemukutan Island, Bengkayang District, West Kalimantan, Indonesia. ST-1: Tanjung Porok, ST-2: Teluk Melano Timur, ST-3: Teluk Surau, ST-4: Teluk Panjang, and ST-5: Teluk Tanjung Meruhum

Following denaturation at 95ºC for 5 minutes, there were 40 cycles of 95ºC for 30 seconds, 30 seconds at a temperature suitable for annealing the primers, and an extension of 45 seconds at the ultimate extension of 72ºC for 10 minutes. Denaturation at 95ºC for 5 minutes was followed by 40 cycles of 30 seconds at 95ºC, 30 seconds at a temperature sufficient for annealing the primers, and 45 seconds at 72ºC for 10 minutes. Through electrophoresis on a 2% agarose gel, the quality of the PCR result was seen (100 mL TAE buffer and 2 g agarose). Then, a 100 bp DNA ladder was placed in one of the agarose wells along with a 3 l aliquot of the PCR result. The results were observed using a UV Fluorescent with an Alpha imager Mini Gel documentation System after running the electrophoresis machine at 50 Volts for 60 minutes (Protein Simple Ltd, California, USA).

Every PCR result that passed the quality check by electrophoresis conducted a second PCR for indexing reasons. In the second PCR, 12.5 l of Kapa HotStart HiFi 2 ReadyMix DNA polymerase was used to add the IDT double index and Illumina sequencing adaptor for Illumina - Nextera DNA Unique Dual Index, Set A (catalog number 20027213) (Illumina, San Diego, USA) to the target amplicon (Kapa Biosystems Ltd., London, UK) and the PCR product that is 2 µl. In the PCR cycle, there were 9 cycles of 95°C for 30 seconds, 55°C for 30 seconds, 72°C for 30 seconds, and a final extension at 72°C for 5 minutes. The initial denaturation was placed at 95 °C for 3 minutes. The first and second PCR products were purified using AMPure XP from Beckman Coulter, Inc) before proceeding to the next step. The Illumina NovaSeq 6000 was used to sequence DNA in accordance with the Illumina MiSeq 16S metagenomic sequencing library technique.

Data analysis

The Quantification Insights Into Microbial Ecology 2 tool (https://qiime2.org/), was used to analyze forward and reverse FASTQ sequences for further analysis (Bolyen et al. 2019). The DADA2 software (Divisive Amplicon Denoising Algorithm 2) was incorporated into QIIME2 for the quality filter, trim, de-noise, and merge of the data (Callahan et al. 2017). Contaminating mitochondrial and chloroplast sequences were filtered from the resulting feature Amplicon Sequence Variants (ASVs) table. Including data from Genbank and the Basic Local Alignment Search Tool (BLAST) (Ismail, 2022; Altschul et al. 1990) of the National Center for Biotechnology Information, taxonomy was assigned to the ASVs (NCBI). DNA amplicon data were analyzed and visualized using an even sampling (rarefaction) depth per sample locale using the core-metrics pipeline from the Phyloseq package (McMurdie & Holmes 2014) plugin in R (R development core team). The taxonomy barplots were constructed using RStudio. The Venn diagram was created using RStudio. Alpha and Beta Diversity were calculated using RStudio (R development core team).

RESULTS AND DISCUSSION

The study found 2,413 ASVs, and 275,000 readings from five different seawater sample sites, with each site taking 3 L of water. From those data, 487 ASVs from the eukaryote group (146,378 reads) were identified and 1,926 ASVs of unidentified taxa (128,622 reads). All samples up to an even depth of 33,678 sequences per sample were refined to guarantee that variations in sequencing depth did not affect the study of downstream diversity. Then refines, filters for quality, and exclusion chimeras, 168,390 reads were obtained and 2,199 Amplicon Sequence Variants (ASVs). The new dataset consists of 458 ASVs of eukaryote taxa (91,575 reads) and 1,728 ASVs of unidentified taxa (76,815 reads). The eukaryote taxa cover 21% taxa from the total ASVs, and 54% reads from the total reads from the results. The highest phylum from eukaryote taxa is Cnidaria, with 332 ASVs (72% of total eukaryote ASVs) and 77,428 reads (85% of total eukaryote reads). The dominant taxa followed by *Porifera* (20% of eukaryote ASVs and 14% of eukaryote reads), *Placozoa* (5% of eukaryote ASVs and 1% of eukaryote reads), and *Stenophora* (2% of eukaryote ASVs and 0.2% of eukaryote reads). After being assigned to the NCBI database, the results showed that the ASVs covered 22 families, 31 genera, and 44 species.

The study investigates 63 ASVs of Order Scleractinia with a total of 1,926 reads. There are seven family levels identified from the samples, including Acroporidae, Merulinidae, Fungiidae, Poritidae, Agariciidae, Mussidae, and Fungiacyathidae. There were 14 species identified as belonging to scleractinian from Lemukutan Island using eDNA metabarcoding as follows *Montipora tuberculosa*, *Gardineroseris planulata*, *Mussa angulosa*, *Porites lutea*, *Scapophyllia cylindrica*, *Danafungia scruposa*, *Astreopora listeri*, *Porites* sp., *Goniastrea* sp., *Anacropora* sp., *Alveopora* sp., *Fungiacyathus* sp., *Montipora angulata*, and *Acropora millepora* (Figure 2).

The Venn diagram shows that most of the taxa are unique to every sampling location, and there are no shared taxa between locations. Sample from station 3 has 60 unique ASVs, station 4 has 51 ASVs, station 5 has 58 ASVs, station 1 has 76 ASVs, and station 2 has 109 unique ASVs (Figure 3). Compared to all the sampling locations, station 2 has the highest richness with 698 ASVs, followed by station 5 (582 ASVs), station 1 (538 ASVs), and station 3 (461 ASVs). Station 4 has the lowest richness with 330 ASVs. Beta diversity shows that station 2 and station 4 are different in composition. Meanwhile, station 3, station 5, and station 1 were grouped together and separated from both station 2 and station 4.

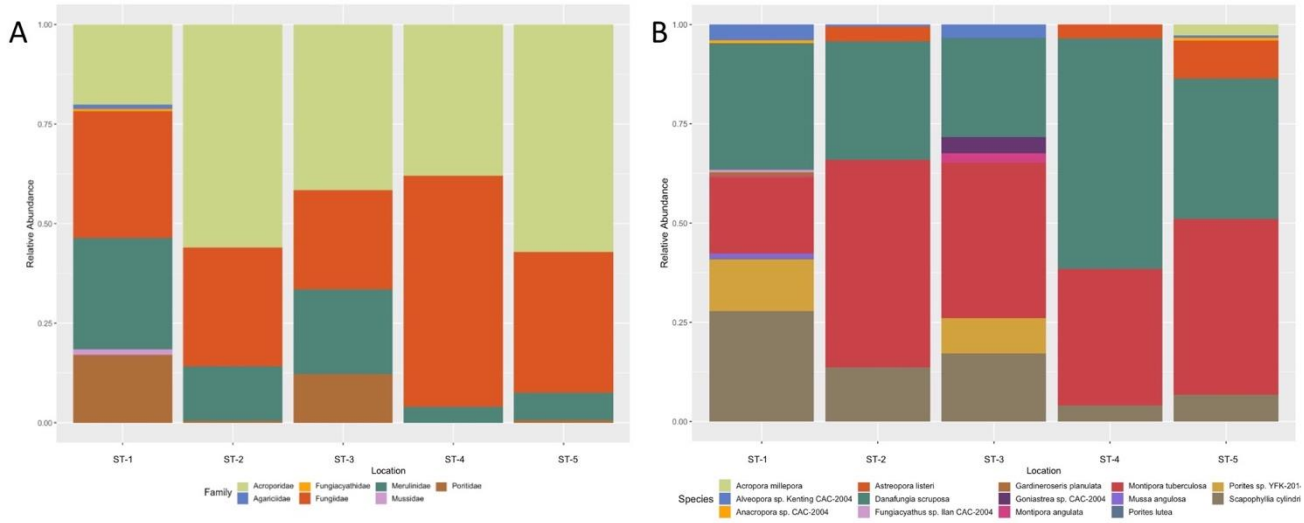

Figure 2. Taxonomy barplot based on the reads relative abundance of: A) Family and, B) Species level obtained from five sampling locations. The five sampling points are ST-1: Tanjung Porok, ST-2: Teluk Melano Timur, ST-3: Teluk Surau, ST-4: Teluk Panjang, and ST-5: Teluk Tanjung Meruhum

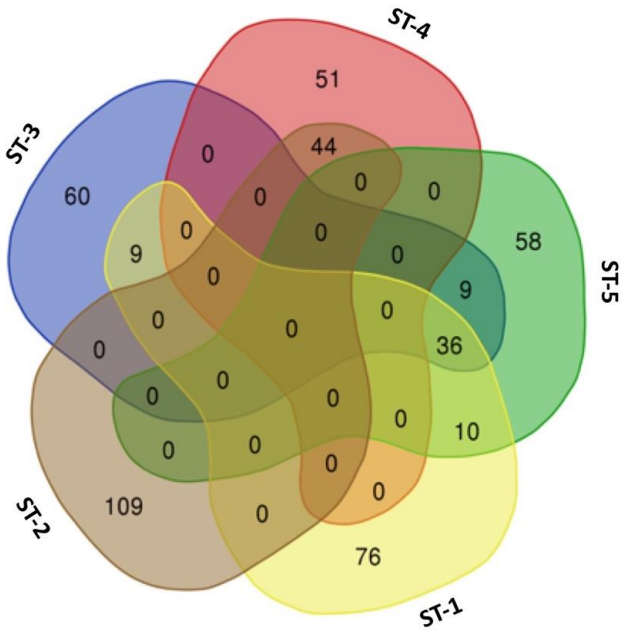

Figure 3. The Venn diagram shows the number of unique and shared ASVs (Amplicon Sequence Variance) from five sampling locations. The five sampling points are ST-1: Tanjung Porok, ST-2: Teluk Melano Timur, ST-3: Teluk Surau, ST-4: Teluk Panjang, and ST-5: Teluk Tanjung Meruhum

The primer Coral ITS2 was used in this research approach to detect scleractinian corals. From the results obtained, the metazoan range of taxa was seen. This shows that the eDNA approach can be used to monitor coral reef ecosystems. Scleractinian detection using ITS 2 primer is the first in Indonesia country. In Indonesia, there was a lack of information and databases on scleractinian detection using ITS2 in Indonesia. In the past, studies have been conducted using ITS2 primers on the Cocos (Keeling) Islands, located between the shores of Indonesia and Australia. The findings of the Coral ITS2 study revealed the presence of 49 Operational Taxonomic Units (OTUs) belonging to 11 families and 16 genera (Alexander et al. 2020). The current research investigates the application of eDNA metabarcoding as a method for monitoring biodiversity. A total of 2,413 ASVs and 275,000 reads were recovered in this research. There were 14 species identified as belonging to scleractinian from Lemukutan

Island using Environmental DNA (eDNA) metabarcoding as follows *M. tuberculosa, G. planulata, M. angulosa, P. lutea, S. cylindrica, D. scruposa, A. listeri, Porites* sp., *Goniastrea* sp., *Anacropora* sp., *Alveopora* sp., *Fungiacyathus* sp., *M. angulata,* and *A. millepora.* Differences in the number of reads and community composition of eDNA are caused by various factors, including water quality, biological circumstances, chemical oceanography, and physical oceanography (Anton et al. 2019; Holman et al. 2019). The phylum Cnidaria, which has 21 orders consisting of 98 families and 145 genera, was discovered through previous research in Jakarta Bay using eDNA metabarcoding (Maqbul et al. 2021). The results obtained showed fewer results compared to the research locations in Jakarta Bay. This is because Lemukutan Island is outside the coral triangle zone. However, Lemukutan Island is included in the marine protected area by having several coral reef ecosystem areas that must be preserved. The success of detecting scleractinian corals through surface seawater samples, this study demonstrates that the eDNA metabarcoding method effectively identifies biodiversity in the sea (Guardiola et al. 2015; Cowart et al. 2018). Nevertheless, the test (barcoding area) and the availability of sequence reference data influence the accuracy of the metabarcoding results (Pompanon et al. 2012).

The existence of an abundance of taxa that cannot be identified shows that the taxa obtained from this study have not been recorded in a global database (Madduppa et al. 2021). However, this shows the high marine biodiversity in the region. Some ASVs that cannot be identified can also be described as marine organisms with unknown scientific names, which have the potential to be further specified in the future. The accuracy of reference sequences and databases impacts the metabarcoding eDNA results, which can improve taxonomic resolution (Zinger et al. 2019). eDNA approach is still relatively new. However, it has the potential to quickly and accurately identify species to determine the biodiversity of an ecosystem area. In this study, we have successfully detected several scleractinian taxa found on Lemukutan Island. This shows the parts of the essential components of marine biodiversity. Environmental DNA emerges as a solution, useful for biodiversity assessment surveys. This method relies on a molecular approach that includes high-level genetic markers, which results in more precise identification findings. It is based on the division of organisms into species according to the arrangement of their nucleotide bases. As a result, the genetic code of the species is revealed. Identified in the database (e.g., National Center for Biotechnology Information) and recognized as a barcode (Sani et al. 2021).

The type of substrate, as well as the biological and physical characteristics of taxonomic identification, are some of the elements that contribute to the variation in the composition of the community structure as determined by eDNA. In investigating marine life using eDNA, the type of substrate is a crucial factor (Turner et al. 2015). Because these taxa spend their whole lives in the substrate, samples from sediments are ideal for identifying benthic macrofaunae like sponges, echinoderms, and cnidarians (Turner et al. 2015; Leduc et al. 2019). Sediments will therefore accumulate genetic material that has been released into the environment. Nevertheless, the genetic material will be stirred into the water column due to physical causes causing the silt to mix (Turner et al. 2015).

The eDNA approach also has several limitations, including reliance on the availability and concentration of eDNA in the seawater sample, sample interference (such as inhibition), assay sensitivity, and sample capture and extraction (Goldberg et al. 2016). Seawater eDNA samples can degrade beyond the detection threshold within one day to weeks (Dejean et al. 2011; Thomsen et al. 2012). High temperatures, a high pH, and UV-B radiation all tend to accelerate the destruction of eDNA in water (Strickler et al. 2015). In metabarcoding research, a common issue is the unavailability of a comprehensive reference sequence database. GenBank reference sequences' quality and amount also limit how well eDNA data may be interpreted (Strickler et al. 2015; Alexander et al. 2020). As observed in eDNA studies from ecosystems with lower diversity, a comprehensive database would enable eDNA to collect and detect local variety in biodiversity hotspots with greater efficiency (Thomsen et al. 2012; Kelly et al. 2014; Miya et al. 2015; Andruszkiewicz et al. 2017), increases the usefulness of eDNA for ecosystems monitoring. The potential of marine eDNA research to provide more diversity than conventional survey methods in comparisons is a common characteristic (Kelly et al. 2017). However, the focus of our study is on eDNA in various scleractinian. The discovery of these other species demonstrates how eDNA can improve the conventional visual survey, regardless of the sampling effort (Marwayana et al. 2022).

The research successfully utilized the eDNA technique, explicitly employing the Coral ITS2 primer, to detect scleractinian corals. The findings significantly enhance our understanding and monitoring of the biodiversity of coral reefs on Lemukutan Island. The eDNA metabarcoding method demonstrated its efficacy in species identification and showcased its potential for conducting surveys to assess biodiversity. However, it is essential to acknowledge certain limitations associated with the eDNA approach, such as the availability and concentration of eDNA, potential sample interference, assay sensitivity, and the process of capturing and extracting samples. In conclusion, this study offers valuable insights into the biodiversity and distribution patterns of coral reefs on Lemukutan Island. It underscores the significance of preserving these ecosystems and emphasizes the promising prospects of utilizing the eDNA approach for future monitoring endeavors.

ACKNOWLEDGEMENTS

This study has received funding from Indonesia Endowment Fund for Education (LPDP). We are grateful from our hearts to thank the Ministry of Finance and the Republic of Indonesia for providing research funding assistance so that this research can be completed properly.

REFERENCES

Alexander JB, Bunce M, White N, Wilkinson SP, Adam AAS, Berry T, Stat M, Thomas L, Newman SJ, Dugal L, Richards ZT. 2020. Development of a multi-assay approach for monitoring coral diversity using eDNA metabarcoding. Coral Reefs 39 (1): 159-171. DOI: 10.1007/s00338-019-01875-9.

Andruszkiewicz EA, Starks HA, Chavez FP, Sassoubre LM, Block BA, Boehm AB. 2017. Biomonitoring of marine vertebrates in Monterey Bay using eDNA metabarcoding. PLoS ONE 12 (4): 1-20. DOI: 10.1371/journal.pone.0176343.

Anton A, Geraldi NR, Lovelock CE, Apostolaki ET, Bennett S, Cebrian J, Krause-Jensen D, Marbà N, Martinetto P, Pandolfi JM, Santana-Garcon J, Duarte CM. 2019. Global ecological impacts of marine exotic species. Nat Ecol Evol 3 (5): 787-800. DOI: 10.1038/s41559-019-0851-0.

Ario R, Wibowo E, Pratikto I, Fajar S. 2016. Pelestarian habitat penyu dari ancaman kepunahan di Turtle Conservation and Education Center (TCEC), Bali. Jurnal Kelautan Tropis 19 (1): 60-66. DOI: 10.14710/jkt.v19i1.602. [Indonesian]

Benkwitt CE, Wilson SK, Graham NAJ. 2020. Biodiversity increases ecosystem functions despite multiple stressors on coral reefs. Nat Ecol Evol 4 (7): 919-926. DOI: 10.1038/s41559-020-1203-9.

Boussarie G, Bakker J, Wangensteen OS, Mariani S, Bonnin L, Juhel JB, Kiszka JJ, Kulbicki M, Manel S, Robbins WD, Vigliola L, Mouillot D. 2018. Environmental DNA illuminates the dark diversity of sharks. Sci Adv 4 (5). DOI: 10.1126/sciadv.aap9661.

Bright AJ, Cameron CM, Miller MW. 2015. Enhanced susceptibility to predation in corals of compromised condition. Peer J 2015 (9): 1-12. DOI: 10.7717/peerj.1239.

Burke L, Selig E, Spalding M. 2002. Reefs at risk in Southeast Asia. World Fishing. http://www.citeulike.org/group/342/article/484868.

Carpenter KE, Abrar M, Aeby G, Aronson RB, Banks S, Bruckner A, Chiriboga A, Cortes J, Delbeek JC, et al. 2008. One-third of reef-building corals face elevated extinction risk from climate change and local impacts. Science 321 (5888): 560-563. DOI: 10.1126/science.1159196.

Ceballos G, Ehrlich PR, Dirzo R. 2017. Biological annihilation via the ongoing sixth mass extinction signaled by vertebrate population losses and declines. Proc Nat Acad Sci U S A 114 (30): E6089-E6096. DOI: 10.1073/pnas.1704949114.

Cowart DA, Murphy KR, Cheng CHC. 2018. Metagenomic sequencing of environmental DNA reveals marine faunal assemblages from the West Antarctic Peninsula. Mar Genomics 37, 148-160. DOI: 10.1016/j.margen.2017.11.003.

Deiner K, Bik HM, Mächler E, Seymour M, Lacoursière-Roussel A, Altermatt F, Creer S, Bista I, Lodge DM, de Vere N, Pfrender ME, Bernatchez L. 2017. Environmental DNA metabarcoding: Transforming how we survey animal and plant communities. Mol Ecol 26 (21): 5872-5895. DOI: 10.1111/mec.14350.

Dejean T, Valentini A, Duparc A, Pellier-Cuit S, Pompanon F, Taberlet P, Miaud C. 2011. Persistence of environmental DNA in freshwater ecosystems. PLoS ONE 6 (8): 8-11. DOI: 10.1371/journal.pone.0023398.

Dugal L, Thomas L, Meenakshisundaram A, Simpson T, Lines R, Colquhoun J, Jarman S, Meekan M. 2022. Distinct coral reef habitat communities characterized by environmental DNA metabarcoding. Coral Reefs 42 (1): 17-30. DOI: 10.1007/s00338-022-02301-3.

Foale S, Adhuri D, Aliño P, Allison EH, Andrew N, Cohen P, Evans L, Fabinyi M, Fidelman P, Gregory C, Stacey N, Tanzer J, Weeratunge N. 2013. Food security and the coral triangle initiative. Mar Pol 38, 174-183. DOI: 10.1016/j.marpol.2012.05.033.

Fraija-Fernández N, Bouquieaux MC, Rey A, Mendibil I, Cotano U, Irigoien X, Santos M, Rodríguez-Ezpeleta N. 2020. Marine water environmental DNA metabarcoding provides a comprehensive fish diversity assessment and reveals spatial patterns in a large oceanic area. Ecol Evol 10 (14): 7560-7584. DOI: 10.1002/ece3.6482.

Garlapati D, Charankumar B, Ramu K, Madeswaran P, Murthy RMV. 2019. A review on the applications and recent advances in environmental DNA (eDNA) metagenomics. Rev Environ Sci Biotechnol 18 (3): 389-411. DOI: 10.1007/s11157-019-09501-4.

Goldberg CS, Turner CR, Deiner K, Klymus KE, Thomsen PF, Murphy MA, Spear SF, McKee A, Oyler-McCance SJ, Cornman RS, Laramie MB, Mahon AR, Lance RF, Pilliod DS, Strickler KM, Waits LP, Fremier AK, Takahara T, Herder JE, Taberlet P. 2016. Critical considerations for the application of environmental DNA methods to detect aquatic species. Methods Ecol Evol 7 (11): 1299-1307. DOI: 10.1111/2041-210X.12595.

Guardiola M, Uriz MJ, Taberlet P, Coissac E, Wangensteen OS, Turon X. 2015. Deep-sea, deep-sequencing: Metabarcoding extracellular DNA from sediments of marine canyons. PLoS ONE 10 (10). DOI: 10.1371/journal.pone.0139633.

Halpern BS, Walbridge S, Selkoe KA, Kappel CV, Micheli F, D'Agrosa C, Bruno JF, Casey KS, Ebert C, Fox HE, Fujita R, Heinemann D, Lenihan H. S, Madin EMP, Perry MT, Selig ER, Spalding M, Steneck R, Watson R. 2008. Global map impact marine. Science 319: 948-952. DOI: 10.1126/science.1149345.

Harrison JB, Sunday JM, Rogers SM. 2019. Predicting the fate of eDNA in the environment and implications for studying biodiversity. Proc R Soc B Biol Sci 286 (1915). DOI: 10.1098/rspb.2019.1409.

Holman LE, de Bruyn M, Creer S, Carvalho G, Robidart J, Rius M. 2019. Detection of introduced and resident marine species using environmental DNA metabarcoding of sediment and water. Sci Rep 9 (1): 1-10. DOI: 10.1038/s41598-019-47899-7.

Jackson JBC, Kirby MX, Berger WH, Bjorndal KA, Botsford LW, Bourque BJ, Bradbury RH, Cooke R, Erlandson J, Estes JA, Hughes TP, Kidwell S, Lange CB, Lenihan HS, Pandolfi JM, Peterson CH, Steneck RS, Tegner MJ, Warner RR. 2001. Historical overfishing and the recent collapse of coastal ecosystems. Science 293 (5530): 629-637. DOI: 10.1126/science.1059199.

Kelly RP, Closek CJ, O'Donnell JL, Kralj JE, Shelton AO, Samhouri JF. 2017. Genetic and manual survey methods yield different and complementary views of an ecosystem. Front Mar Sci 3: 1-11. DOI: 10.3389/FMARS.2016.00283.

Kelly RP, Port JA, Yamahara KM, Crowder LB. 2014. Using environmental DNA to census marine fishes in a large mesocosm. PLoS ONE 9 (1). DOI: 10.1371/journal.pone.0086175.

Leduc N, Lacoursière-Roussel A, Howland KL, Archambault P, Sevellec M, Normandeau E, Dispas A, Winkler G, McKindsey CW, Simard N, Bernatchez L. 2019. Comparing eDNA metabarcoding and species collection for documenting Arctic metazoan biodiversity. eDNA 1 (4): 342-358. DOI: 10.1002/edn3.35.

Madduppa H, Cahyani NKD, Anggoro AW, Subhan B, Jefri E, Sani LMI, Arafat D, Akbar N, Bengen DG. 2021. eDNA metabarcoding illuminates species diversity and composition of three phyla (chordata, mollusca and echinodermata) across Indonesian coral reefs. Biodivers Conserv 0123456789. DOI: 10.1007/s10531-021-02237-0.

Maqbul I, Fahrezi FY, Bakhri ENA, Verawati I, Sani LMI, Subhan B, Zamani NP, Madduppa H. 2021. Environmental DNA biomonitoring revealed species diversity of Cnidarian and Poriferan across Jakarta Bay and Seribu Islands National Park. Researchsquare. DOI: 10.21203/rs.3.rs-1167046/v1.

Marwayana ON, Gold Z, Meyer CP, Barber PH. 2022. Environmental DNA in a global biodiversity hotspot: Lessons from coral reef fish diversity across the Indonesian archipelago. eDNA 4 (1): 222-238. DOI: 10.1002/edn3.257.

McGill BJ, Dornelas M, Gotelli NJ, Magurran AE. 2015. Fifteen forms of biodiversity trend in the anthropocene. Trends Ecol Evol 30 (2): 104-113. DOI: 10.1016/j.tree.2014.11.006.

McMurdie PJ, Holmes S. 2014. Waste not, want not: Why rarefying microbiome data is inadmissible. PLoS Comput Biol 10 (4). DOI: 10.1371/journal.pcbi.1003531.

Miya M, Sato Y, Fukunaga T, Sado T, Poulsen JY, Sato K, Minamoto T, Yamamoto S, Yamanaka H, Araki H, Kondoh M, Iwasaki W. 2015. MiFish, a set of universal PCR primers for metabarcoding environmental DNA from fishes: Detection of more than 230 subtropical marine species. R Soc Open Sci 2 (7). DOI: 10.1098/rsos.150088.

Pompanon F, Deagle BE, Symondson WOC, Brown DS, Jarman SN, Taberlet P. 2012. Who is eating what: Diet assessment using next generation sequencing. Mol Ecol 21 (8): 1931-1950. DOI: 10.1111/j.1365-294X.2011.05403.x.

Pukk L, Kanefsky J, Heathman AL, Weise EM, Nathan LR, Herbst SJ, Sard NM, Scribner KT, Robinson JD. 2021. eDNA metabarcoding in lakes to quantify influences of landscape features and human activity on aquatic invasive species prevalence and fish community diversity. Divers Distrib 27 (10): 2016-2031. DOI: 10.1111/ddi.13370.

Richards ZT, Day JC. 2018. Biodiversity of the Great Barrier Reef- how adequately is it protected? PeerJ 2018 (5): 1-26. DOI: 10.7717/peerj.4747.

Roberts CM, McClean CJ, Veron JEN, Hawkins JP, Allen GR, McAllister DE, Mittermeier CG, Schueler FW, Spalding M, Wells F, Vynne C, Werner T. B. 2002. Marine biodiversity hotspots and conservation priorities for tropical reefs. Science 295 (5558): 1280-1284. DOI: 10.1126/science.1067728.

Sani LMI, Husna AK, Subhan B, Madduppa H. 2021. Environmental DNA (eDNA) reveals endangered narrow sawfish across Indonesian Reefs. IOP Conf Ser Earth Environ Sci 944 (1). DOI: 10.1088/1755-1315/944/1/012020.

Strickler KM, Fremier AK, Goldberg CS. 2015. Quantifying effects of UV-B, temperature, and pH on eDNA degradation in aquatic microcosms. Biol Conserv 183, 85-92. DOI: 10.1016/j.biocon.2014.11.038.

Suter L, Polanowski AM, Clarke LJ, Kitchener JA, Deagle BE. 2021. Capturing open ocean biodiversity: Comparing environmental DNA metabarcoding to the continuous plankton recorder. Mol Ecol 30 (13): 3140-3157. DOI: 10.1111/mec.15587.

Thomsen PF, Kielgast J, Iversen LL, Møller PR, Rasmussen M, Willerslev E. 2012. Detection of a diverse marine fish fauna using environmental DNA from seawater samples. PLoS ONE 7 (8): 1-9. DOI: 10.1371/journal.pone.0041732.

Turner CR, Uy KL, Everhart RC. 2015. Fish environmental DNA is more concentrated in aquatic sediments than surface water. Bio Conserv 183, 93-102. DOI: 10.1016/j.biocon.2014.11.017.

Veron JEN, Devantier LM, Turak E, Green AL, Kininmonth S, Stafford-Smith M, Peterson N. 2009. Delineating the coral triangle. Galaxea, J Coral Reef Stud 11 (2): 91-100. DOI: 10.3755/galaxea.11.91.

Visconti P, Bakkenes M, Baisero D, Brooks T, Butchart SHM, Joppa L, Alkemade R, Di Marco M, Santini L, Hoffmann M, Maiorano L, Pressey RL, Arponen A, Boitani L, Reside AE, van Vuuren DP, Rondinini C. 2016. Projecting global biodiversity indicators under future development scenarios. Conserv Lett 9 (1): 5-13. DOI: 10.1111/conl.12159.

Welsh JQ, Bonaldo RM, Bellwood DR. 2015. Clustered parrotfish feeding scars trigger partial coral mortality of massive Porites colonies on the inshore Great Barrier Reef. Coral Reefs 34 (1): 81-86. DOI: 10.1007/s00338-014-1224-4.

Zinger L, Bonin A, Alsos IG, Bálint M, Bik H, Boyer F, Chariton AA, Creer S, Coissac E, Deagle BE, De Barba M, Dickie IA, Dumbrell AJ, Ficetola GF, Fierer N, Fumagalli L, Gilbert MTP, Jarman S, Jumpponen A, Taberlet P. 2019. DNA metabarcoding - Need for robust experimental designs to draw sound ecological conclusions. Mol Ecol 28 (8): 1857-1862. DOI: 10.1111/mec.15060.