JIPK. Volume 17 No 3 October 2025
Sinta 1 (Decree No: 158/E/KPT/2021)
e-ISSN:2528-0759; p-ISSN:2085-5842
Available online at https://e-journal.unair.ac.id/JIPK

JIPK
(JURNAL ILMIAH PERIKANAN DAN KELAUTAN)
Scientific Journal of Fisheries and Marine

Research Article

Uncovering Molluscs Diversity in Mandalika Coastal Through eDNA Metabarcoding

Dining Aidil Candri[1*], Mursal Ghazali[1], Tri Wahyu Setyaningrum[1], Aina Ul Mardiati[1] and Muhammad Syach Maulad Ichfa[2]

[1]Department of Biology, Faculty of Mathematic and Natural Science, Universitas Mataram, Mataram, 60115. Indonesia
[2]Department of Pharmacy, Faculty of Medicine and Health Science, Universitas Mataram, Mataram, 60115. Indonesia

OPEN ACCESS

ARTICLE INFO

Received: June 03, 2025
Accepted: July 30, 2025
Published: August 7, 2025
Available online: Sept 27, 2025

*) Corresponding author:
E-mail: aidilch@unram.ac.id

Keywords:
Coral Reef
Diversity
eDNA metabarcoding
Mandalika Coastal
Mollusca
Seagrass bed

Abstract

Molluscs are one of the main taxa in coastal ecosystems that play an important role in the food chain, bioindicators, and have high economic value. However, the limitations of conventional methods in detecting species that live hidden in complex ecosystems, such as seagrass and coral reefs, are a challenge in biodiversity monitoring. This study aimed to explore the diversity of mollusc species in the Mandalika coastal area using an eDNA metabarcoding approach. This study used the environmental DNA (eDNA) metabarcoding method to identify the diversity of molluscs in the coastal ecosystem of Mandalika, Central Lombok. The sites including Kuta Beach, Gerupuk Bay, and Aan Cape along the Mandalika coastal that contain both seagrass bed and coral reef ecosystems. Two samples were taken from each site in both the seagrass bed and coral reef ecosystems. Analysis of six water samples resulted in 99 ASVs and 116,611 final sequences, with 10 ASVs (50,960 sequences) identified as Mollusca taxa, all from the Gastropoda class. Four species were successfully identified, including Monetaria sp. M. obvelata, M. annulus and Phyllaplysia sp. that had not previously been reported through direct observation or conventional identification methods in Mandalika coastal area. Species diversity varied between locations and was influenced by environmental factors such as temperature, pH, salinity, phosphate, and anthropogenic pressure. These results show that eDNA metabarcoding is an effective tool in detecting mollusc species, even in hard-to-reach habitats, and support the urgency of scientific data-based mollusc conservation management.

Cite this as: Candri, D. A., Ghazali, M., Setyaningrum, T. W., Mardiati, A. U., & Ichfa, M. S. M. (2025). Uncovering Molluscs Diversity in Mandalika Coastal Through eDNA Metabarcoding. *Jurnal Ilmiah Perikanan dan Kelautan*, 17(3):684-697. https://doi.org/10.20473/jipk.v17i3.73791

1. Introduction

Molluscs are one of the largest phyla of eukaryotic organisms after Arthropoda, with a high level of diversity and wide distribution in various types of ecosystems, both aquatic and terrestrial. The morphological and ecological diversity they possess allows molluscs to occupy various habitats, from the intertidal zone to deep-sea waters, as pelagic and benthic organisms on various types of substrates (Gokoglu, 2021). The ecological role of molluscs is very important in aquatic ecosystems, because they are included in key taxonomic groups that support the sustainability of the life cycle. In particular, the classes Gastropoda and Bivalvia play an important role in the food chain in ecosystems such as seagrass beds and function as bioindicators of environmental quality, including in seagrass and coral reef habitats (Sangaji *et al*., 2023). However, the limited mobility and sensitivity of molluscs to environmental changes make them more vulnerable to the impacts of global warming and anthropogenic activities compared to other groups of marine organisms. This vulnerability makes molluscs an urgent group to monitor and protect their biodiversity. Some mollusc species are known to have very specific habitat preferences and are found in hard-to-reach locations, such as seagrass and coral reef ecosystems. In seagrass beds, molluscs can be found attached to seagrass leaves or hidden in the substrate, while on coral reefs, they often live attached to coral surfaces, hiding in narrow gaps, and even within the coral skeleton structure itself. These complex habitat conditions make direct observation and sampling difficult because they risk damaging ecosystems that have vital ecological roles.

Mollusc biodiversity is widespread throughout the waters of the archipelago, including in the coastal areas of Mandalika, Central Lombok, West Nusa Tenggara. The coastal area of Mandalika, which includes Kuta Beach, Aan Cape, and Gerupuk Bay, is an area with diverse coastal ecosystems, such as rivers, mangroves, seagrass beds, and coral reefs, and is rich in marine biodiversity. Currently, the Mandalika area is attracting attention because of its natural charm, which is still relatively natural, and the intensive development in the context of developing a Special Economic Zone (KEK) is a strategic mega–project prioritized by the Indonesian government, specially designated for economic development, with a primary focus on tourism. Its development is expected to contribute significantly to both the local and national economies. Although this development has made a positive contribution to local economic growth, this activity also has the potential to have a negative impact on the environment. Construction activities, increasing numbers of tourists, and infrastructure operations can cause increased water sedimentation due to erosion, which in turn can have a negative impact on coastal ecosystems and aquatic organisms, including molluscs that live in them.

Approximately 15,000 mollusc species have been identified in Indonesia, representing only 25% of the estimated global total of approximately 60,000 species (Matsuura *et al*., 2000). In Lombok Island, 37 molluscs species have been identified along the southern coast, dominated by *Cerithidea cingulata*, *Assminia lutea*, and *Chicorius capunicus* (Candri *et al.,* 2020). Additionally, 25 molluscs species, primarily *Clypeomorus moniliferus*, were found in the mangrove area of Pelangan, Sekotong (Candri *et al.*, 2022), while 97 molluscs species, including 65 species of Gastropods and 14 Bivalves, were recorded in Batu Kijuk, West Sekotong, with dominant species being *Pyrene versicolor*, *Hebra nigra*, and *Nassarius globosus* (Candri *et al.,* 2023b). The distribution of aquatic organisms, including molluscs, is highly heterogeneous both spatially and temporally, making monitoring challenging (Lamy *et al.,* 2012). Conventional methods for collecting and identifying species are often time-consuming and costly, requiring substantial taxonomic expertise for accurate identification, so they require more effective and efficient monitoring methods, such as eDNA metabarcoding. Environmental DNA (eDNA) metabarcoding is a potential tool that can provide a broader assessment of marine biodiversity than conventional visual consensus methods (Rey *et al.,* 2023; Hallam *et al.,* 2021; Madduppa *et al.,* 2021).

Several studies have shown the success of this method in identifying mollusk species. For example, Madduppa *et al.* (2021) successfully identified four classes of mollusks consisting of 17 orders, including two species of high economic value, namely *Tridacna maxima* and *T. crocea*, from nine rare coral reef ecosystems in Indonesia. In addition, Roussel *et al.* (2018) successfully identified eight species of mollusks in Arctic coastal waters using COI primers. Based on these results, the eDNA metabarcoding method is worth considering and developing for the identification of mollusk species, especially in hard-to-reach habitats such as seagrass beds and coral reefs, without having to damage these sensitive ecosystems.

Environmental DNA (eDNA) is considered an excellent alternative for overcoming limitations in species identification (Othman *et al*., 2023). The application of eDNA has significantly transformed biodiversity characterization standards in aquatic environments by utilizing the presence, location, and abundance of DNA collected from environmental

samples (Sahu *et al*., 2023; Huang *et al*., 2008). eDNA leverages DNA fragments from organisms to ascertain their presence and ecological characteristics (Kestel *et al.,* 2022; Harrison *et al.,* 2019). Some advantages of eDNA include its non-invasive nature, which minimizes or eliminates disturbance to target species or habitats during sampling (Zhang *et al.,* 2023), along with reduced time and cost requirements (Svenningsen *et al.,* 2022).

Despite its growing global application, the use of eDNA metabarcoding to detect mollusk communities in coastal ecosystems in Indonesia is still limited, especially in rapidly developing areas where biodiversity is under increasing pressure. To date, no study has specifically applied eDNA metabarcoding to monitor mollusk diversity in the Mandalika Special Economic Zone (KEK), an area experiencing significant environmental change and tourism-driven development.

This study addresses the investigation of the hidden mollusk diversity in the Mandalika coastal ecosystem. The results are expected to provide important baseline data for biodiversity monitoring, conservation planning, and sustainable management of coastal environments under anthropogenic pressure.

2. Materials and Methods

2.1 Materials

2.1.1 The equipment

The tools used in this research included a 5L plastic bucket, 5L portable water container, funnel, vacuum pump, magnetic tube, magnetic filter, tweezers, 2 mL microtube, micro tube rack, petridish, scissors, Homogenization Bead-Beater, centrifuge, vortex, Qbit, micro pipettes and multichannel pipettes, writing tools, thermometer, digital pH, and handrefractometer.

2.1.2 The materials

The research materials used were consumables consisting of medical masks, latex gloves, bleach, membrane filter mix cellulose ester (MCE, 5.0 µm), DNA Shield, black insulation, label paper, plastic wrap, and QIAGEN DNA extraction KIT.

2.1.3 Ethical approval

This study does not require ethical approval because it does not use experimental animals.

2.2 Methods

2.2.1 Site description

The coastal area of Mandalika is utilized by the local community for various activities such as fishing, aquaculture, and seaweed farming. The Mandalika region receives international visitors due to its natural beauty and growing tourism infrastructure. The Mandalika coastline includes several marine areas, such as Kuta Beach, Aan Cape, and Gerupuk Bay. These areas are known to have relatively complete coastal ecosystems, such as mangrove, seagrass beds, and coral reefs. Kuta Beach and Gerupuk Bay are dominated by seagrass beds. In the seagrass bed at Kuta Beach, approximately eight seagrass species have been recorded, dominated by *Cymodocea rotundata* and *Syringodium isoetifolium* (Zulkifli *et al*., 2021). Identification of seagrass species in Kuta Bay and Gerupuk Bay revealed eight species, including: *Thalassia hemprichii, Enhalus acroides, Halodule uninervis, H. pinifolia, H. ovalis, Cymodocea rotundata, Syringodium isoetifolium*, and *Thalassodendron ciliatum* (Ahyadi *et al.,* 2021). The coral reef ecosystem at Kuta Beach hosts eight genera of hard corals and five genera of soft corals (Candri *et al*., 2023a). In 2023, research by Safrillah *et al.* (2023) identified only the abundance of Gastropod groups and successfully recorded 27 species using conventional survey methods in the seagrass bed of Kuta Beach. Due to the limited available information regarding the species richness, especially molluscs in the seagrass bed and coral reef along the Mandalika coastal region, there is a pressing need for ongoing and systematic scientific investigation.

2.2.2 Water sampling

This fieldwork was conducted during April 2024 in the Mandalika coastal area. Six monitoring sections were selected for this study; samples were taken in seagrass beds and coral reef ecosystems in Kuta Bay, Aan Cape, and Gerupuk Bay (Figure 1). A water collector was used to collect 5 L of water samples from each monitoring section. The samples were stored in a portable thermal insulation bag and brought back to the laboratory for filtration on the same day to minimize the risk of sample degradation. All samples were preserved and stored in the cool box; cold temperatures can help slow down degradation processes, but they cannot substitute for prompt processing. DNA and RNA are sensitive molecules that are prone to degradation, especially under warmer conditions (Kuncoro *et al*., 2023).

2.2.3 Filtration and DNA extraction

On the same day, the water samples were filtered using a vacuum pump DNA filter. The filter membrane was 45 mm in diameter; the pore size

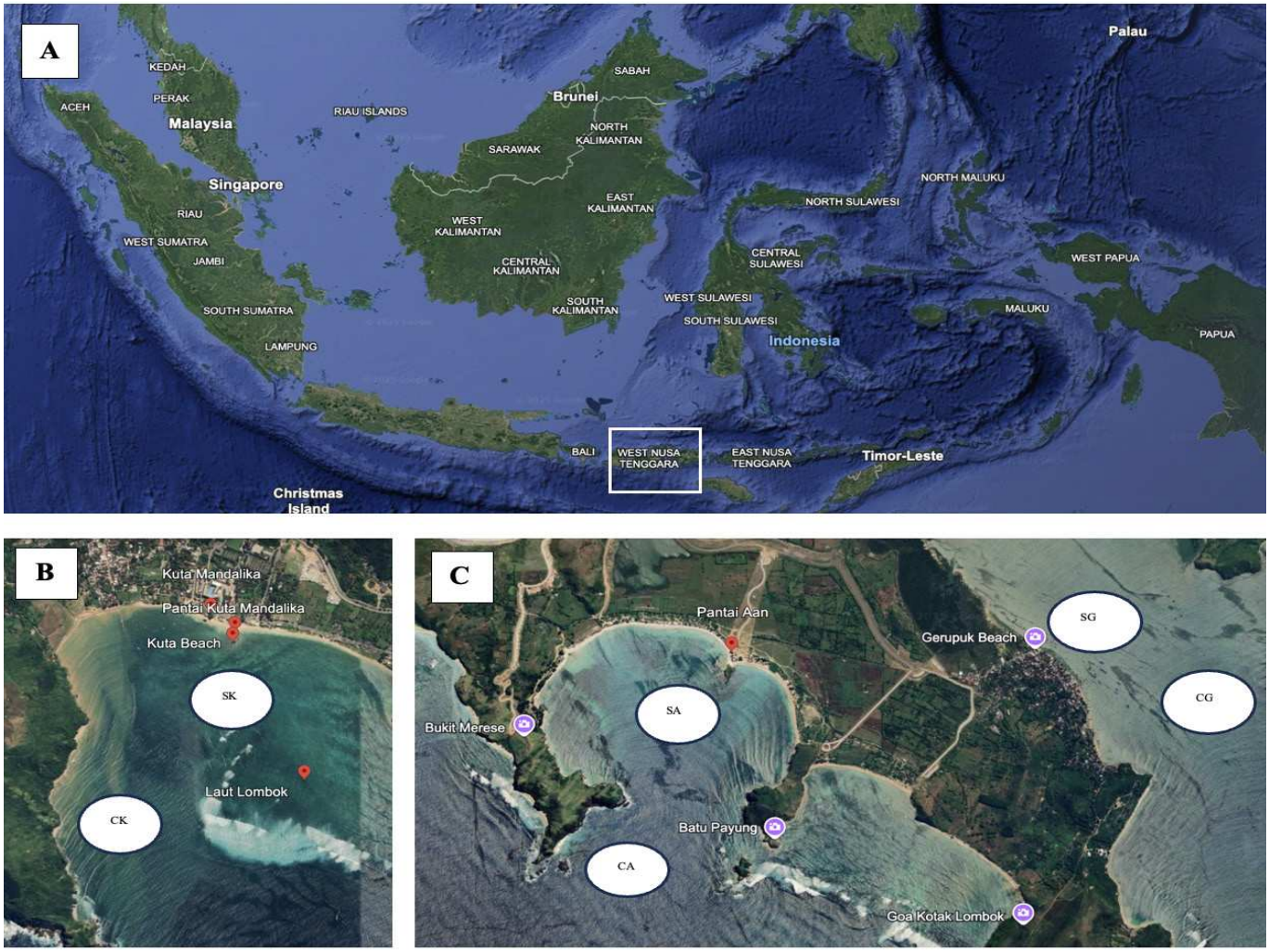

Figure 1. Partial map of Indonesia (A) indicating sampling area (B) in seagrass bed and coral reef Kuta, (C) seagrass bed and coral reef Aan and Gerupuk Mandalika coastal.

was 5,0 um, and the filtration volume was 5 L. After filtration, the filter membrane was removed and placed in a 1.5 mL DNA/RNA shield-filled 2 mL cryotube for storage until the DNA was extracted. Samples should be filtered within a few hours of collection to ensure the optimal preservation of DNA/RNA. It is important to note that the required time duration and temperature conditions may vary depending on the sample type and target analysis.

2.2.4 PCR amplification and sequencing process

DNA extraction, PCR amplification, and sequencing were performed in dedicated sterile labs while wearing nitrile gloves to minimize the risk of DNA contamination. The Qiagen Blood and Tissue DNA Extraction Kit was used to extract the DNA from each sample, according to the manufacturer's instructions. The first PCR amplified the target region of cytochrome c oxidase subunit I (COI) using mlCOIintF and dgHCO2198 primers (Leray *et al.*, 2013). The first PCR reaction contained 10 µL of Phanta Flash Master Mix (2x), 1 µL each of 10 nM primers (F and R), 11 µL ddH2O, and 2 µL DNA Template. The phases of the DNA amplification PCR profile were as follows: (1) pre-denaturation of the template DNA at 95°C for 5 minutes; (2) denaturation of the template DNA at 98°C for 30 seconds; (3) annealing at 56ºC for 30 seconds; (4) primary extension at 72°C for 30 seconds and (5) final extension (post extension) at 72°C for 5 minutes with 35 cycles of stages (2)-(4). To check for contamination, the 96 Universal peqStAR PCR machine was used with negative controls (blank template). PCR product quality was visualized using electrophoresis on 2% agarose gel (100 mL TAE buffer and 1 g agarose). A 2 µL aliquot of PCR product was then inserted into each agarose well with a 100 bp DNA ladder in one of the wells. The electrophoresis machine was run at 100 volts for 30 minutes, and the results were visualized using a UV Fluorescent via an Alpha Imager Mini Gel Documentation System. All

triplicate PCR products that passed the electrophoresis quality control underwent a second PCR for indexing purposes. The IDT double index and Illumina sequencing adapter for Illumina - Nextera DNA Unique Dual Index, Set B were added to the target amplicon in the second PCR. The PCR cycle comprised an initial denaturation at 95°C (3 minutes), then 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. PCR purification was conducted on the first and second PCR products using an AMPure XP before proceeding to the next step. The amplicon library was quantified using a Qubit fluorometer and then normalized to an equal concentration. DNA sequencing was performed on a NovaSEQ6000. The entire process of eDNA analysis was conducted at the Oceanogen Laboratory in Bogor, Indonesia, which specializes in molecular environmental research.

2.3 Analysis Data

The sequenced data obtained from NGS (Next Generation Sequencing) results will then be processed using bioinformatics analysis methods, imported into QIIME2 software (Quantitative Insight into Microbial Ecology 2) (https://qiime2.org/) for quantitative analysis (Bolyen *et al.,* 2018). The analytical pipeline included: removal of forward and reverse primer sequences using Cutadapt (Martin, 2011), sequence denoising, error correction, and chimera filtering with DADA2 (Callahan *et al*., 2016), and clustering of sequences based on similarity thresholds to generate Amplicon Sequence Variance (ASVs). Identification of DNA sequences in the taxonomic annotation process was performed using the CRUX (Creating Reference Libraries Using eXisting tools) reference database, the taxa are shorted based on the order of 97% similarity with the database, then followed by R/R studio (/https://www.r-project.org/) for further analysis related to biological composition, relative abundance, biodiversity indexation included alpha biodiversity, and data visualization (Zinger *et al*., 2021). Several R packages were employed in this study, the phyloseq package (https://joey711.github.io/phyloseq/) served as the primary tool for importing, storing, analyzing, and graphically displaying taxonomically organized sequencing data (ASVs), tidyverse package (https://ggplot2.tidyverse.org/) was used for data management, including data import or export, cleaning, transformation, and visualization, ensuring datasets were tidy and analysis-ready. For community ecology analyses using Shannon-Wiener (H') and Simpson (D) indices, the vegan package (https://github.com/vegandevs/vegan/) was applied to assess biodiversity metrics. For each of the six sites, taxonomic identification by family and species was visualized with graphics. To clarify the taxonomic identification of mollusc taxa carried out through comparison with the World Register of Marine Species (WORMS) reference database (https://www.marinespecies.org/).

3. Results and Discussion

3.1 Results

3.1.1 Result of PCR amplification of COI genes

The results of agarose gel electrophoresis visualization of PCR products showed that all samples (SA, SK, SG, CA, CK, and CG) successfully experienced target gene amplification as indicated by the appearance of a clear and uniform DNA band at the same position (Figure 2). The length of the COI gene sequence obtained ranged from 312 to 343 bp. This indicates that the PCR reaction is optimal and produces DNA fragments of the appropriate size. Meanwhile, the negative control (-ve) did not show any DNA bands, indicating that there was no contamination during the amplification process.

3.1.2 Taxonomic composition of eDNA data in seagrass beds and coral reef ecosystems on the Mandalika coastal

Based on the research that has been conducted, a total of 116,611 final reads of aquatic organisms were successfully grouped into 99 ASVs. The highest number of ASVs was found in the coral Aan sample (37 ASVs), followed by seagrass Kuta (32 ASVs), coral Gerupuk (20 ASVs), seagrass Aan (15 ASVs), coral Kuta (14 ASVs), and the lowest in seagrass Gerupuk with 9 ASVs. All ASVs produced were further annotated to obtain their taxonomic identities using the CRUX (Creating Reference libraries Using eXisting tools) database. Of all the data, based on the number of sequences read, the mollusc phylum is one of the phyla with the most reads (50,960 reads), with the largest number of sequences read in the Coral Aan sample (Figure 3).

3.1.3 Taxonomic composition of molluscs eDNA data in seagrass beds and coral reef ecosystems on the Mandalika coast

Taxonomic annotation using the CRUX database successfully detected 10 ASVs and 50,960 reads from the six samples belonging to the Molluscs phylum; all of these data were grouped in the same class, namely Gastropoda. There were 3 taxa at the family level, namely Cypraeidae, Strombidae, and Aplysiidae, dominated by the Cypraeidae family, which was detected in all samples (Figure 4A). There

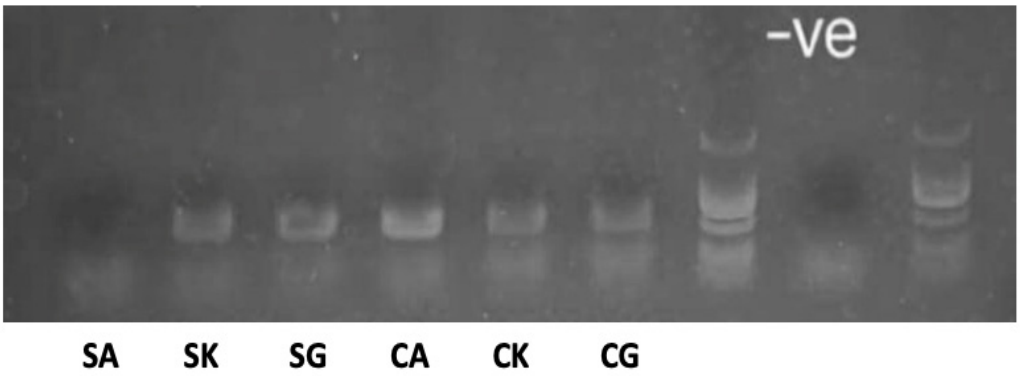

Figure 2. Result of PCR amplification of COI genes (SA: Seagrass Aan; SK: Seagrass Kuta; SG: Seagrass Gerupuk; CA: Coral Aan; CK: Coral Kuta; CG: Coral Gerupuk).

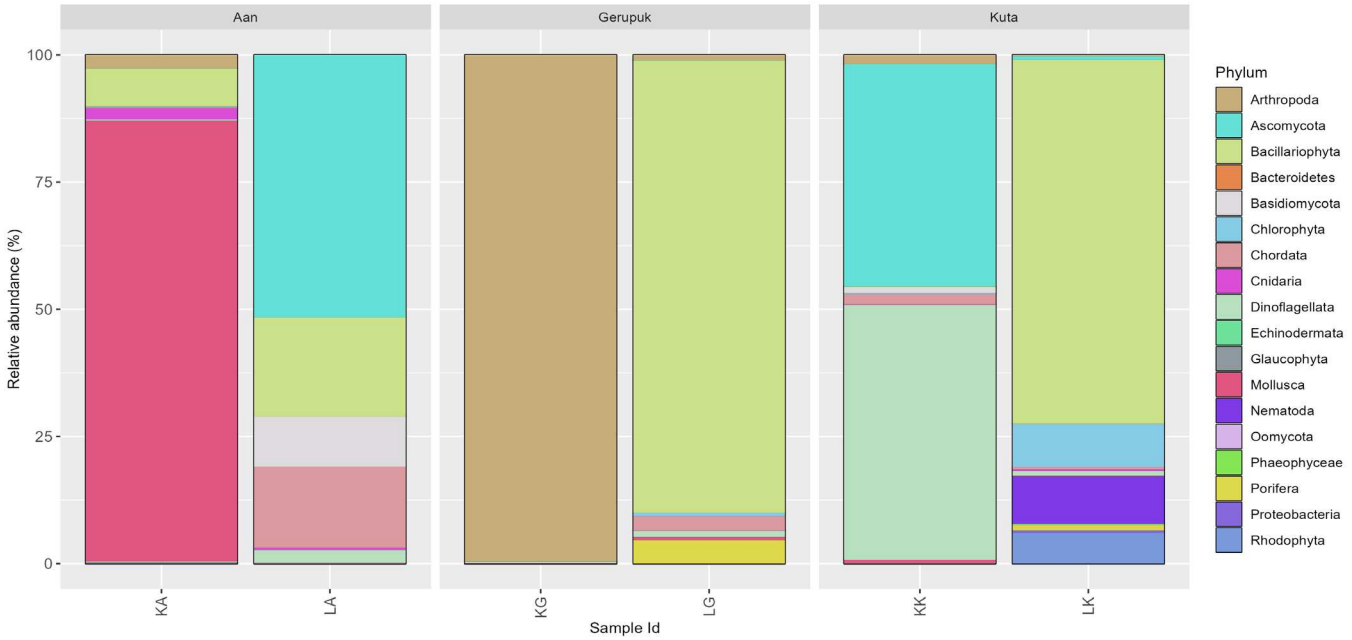

Figure 3. Taxonomy at phylum level based on the read relative abundance obtain from six sampling locations. The six sampling points are: KK: Coral Aan, LA: Seagrass Aan, KG: Coral Gerupuk, LG: Seagrass Gerupuk, KK: Coral Kuta, LK: Seagrass Kuta.

were 4 mollusc taxa that were successfully identified at the species level, namely *Monetaria* sp. (15 reads), *M. annulus* (50021 reads), *M. obvelata* (10 reads), and *Phyllaplysia* sp. (890 reads) (Figure 4B). Based on the reads abundant of all taxa dominated by *M. annulus*.

3.1.4 Environmental parameter measurements in seagrass bed and coral reef ecosystems on the Mandalika coastal

The results of measurements of several physical and chemical parameters of the environment in the seagrass and coral reef ecosystems in the coastal area of Mandalika showed differences that were not very significant even tended to be the same (Table 1) for temperature parameters, which ranged from 29-30°C, with pH ranging from 7.7 to 8.5, salinity ranging from 32 to 34 ppt. The results of nitrate concentration measurements also showed the same results in all samples. However, there were quite significant differences in the results of phosphate concentration measurements at the three research locations. The phosphate values measured in seagrass beds and coral reef ecosystems in Aan Cape and Gerupuk Bay tended to be uniform (0.25 mg/L); in contrast, quite high phosphate concentrations were measured in Kuta Bay samples, both in seagrass and coral reef ecosystems. The high phosphate value at this location is thought

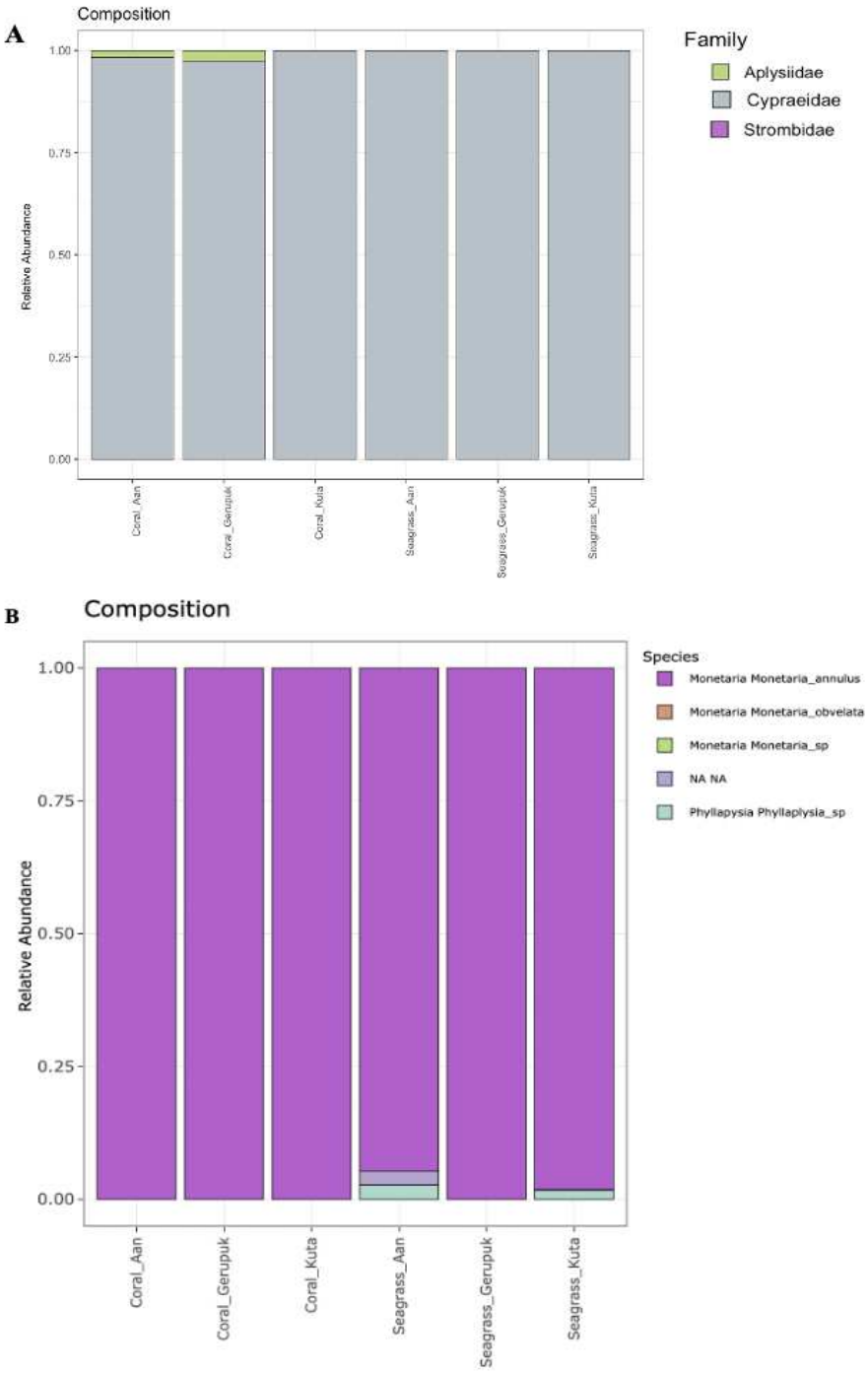

Figure 4. The relative abundance of family level (A), and species level (B). The bar plot constructed based on the read abundance dataset.

to be influenced by the results of anthropogenic waste activities, the coastal conditions of Kuta Bay, which is the center of industrial and hotel infrastructure development, and the intensity of tourism activities that tend to be centered or dominated in Kuta Bay compared to Aan Cape and Gerupuk Bay. These factors allow for an increase in nutrient loads, especially phosphate, in the waters of Kuta Bay.

3.1.5 Alpha biodiversity index based on COI gene

The alpha biodiversity index is used to describe the level of diversity of taxa composition at a location. The biodiversity index based on ASV from NGS data of the COI gene in the seagrass and coral reef ecosystems in three different locations on the Mandalika coast is presented in Figure 5. The highest number of ASVs detected (observed) was recorded in the coral Aan sample (7 ASVs), while the lowest number of ASV was in the seagrass Aan

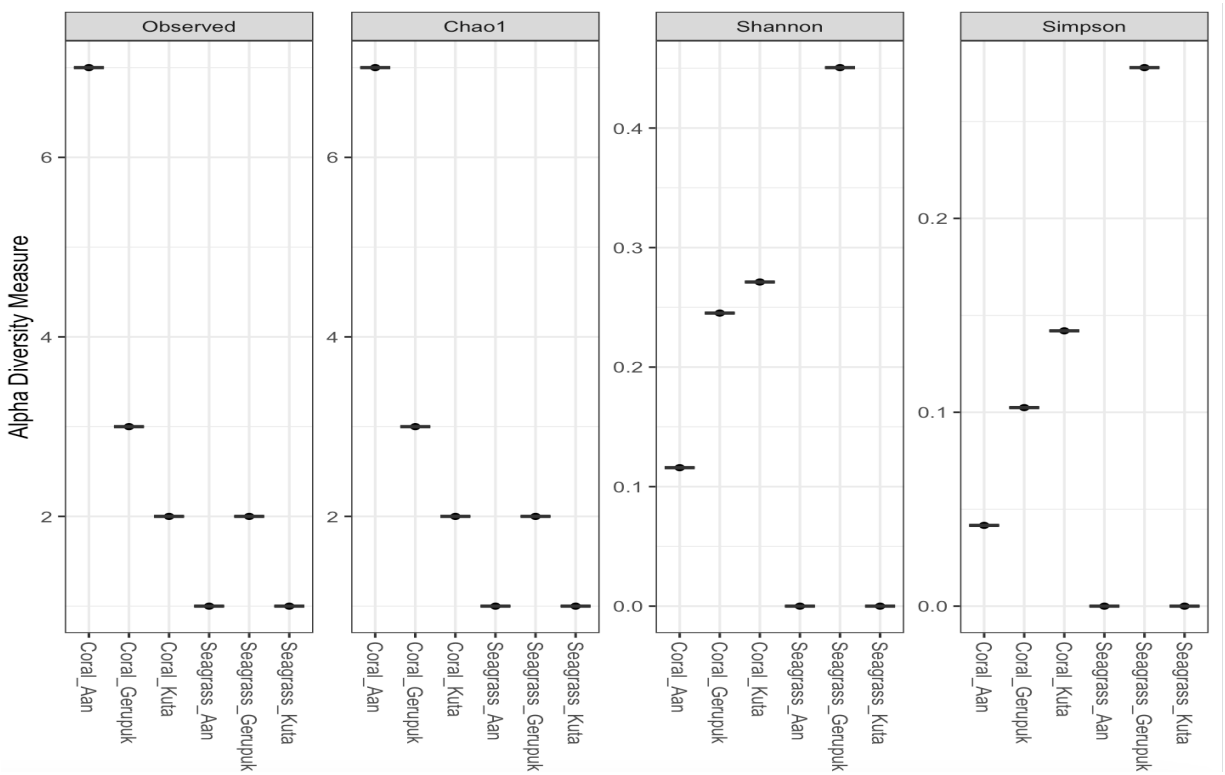

Figure 5. The relative abundance of family level (A), and species level (B). The bar plot were constructed based on the read abundance dataset.

Table 1. The average of water quality on seagrass beds and coral reef ecosystems in Mandalika coastal area

Water quality parameter	Coral Aan	Coral Gerupuk	Coral Kuta	Seagrass Aan	Seagrass Gerupuk	Seagrass Kuta
Temperature (°C)	32	32	29	32	32	29
pH	7.7	8.2	8.5	7.7	8.2	8.5
Salinity (ppt)	32	33	34	32	33	34
Nitrate (mg/L)	0	0	0	0	0	0
Phosphat (mg/L)	0.25	0.25	2.5	0.25	0.25	2.5

and Kuta samples (1 ASV). The results of measuring the Shannon diversity and Simpson dominance index values showed different patterns.

3.2 Discussion

3.2.1 Molluscs species composition in the Mandalika coastal area based on NGS of the COI gene

A total of 10 ASVs and 50,960 reads belonging to the phylum Mollusca were successfully detected, consisting of four species: *Monetaria* sp., *M. annulus*, *M. obvelata*, and *Phyllaplysia* sp., as well as two other taxa that were only identified to the family level, namely Strombidae and Cypraeidae. The *M. annulus* species, which dominated all samples, is known to have high adaptability to various types

of habitats and environmental conditions, including temperature and available substrates, according to the results of environmental parameter measurements on the Mandalika coast, which are within the optimal range for the survival of this species. Irie and Morimoto (2016) reported that the classification process of *Monetaria* shells takes place optimally at temperatures of 21–33°C, but slows down drastically above 34°C. At this research location, the highest temperature was recorded at 30°C, and the lowest temperature was 29°C in seagrass Kuta, which is the ideal range for this species. This is in accordance with the high number of *Monetaria* reads found at the location. The seagrass and coral reef ecosystems in Mandalika show relatively uniform environmental characteristics in terms of temperature (29–30°C), salinity (32–34 ppt), and pH (7.7–8.5). However, differences in phosphate concentrations, especially the high values in Kuta Bay, indicate anthropogenic pressures due to intensive development and tourism that have the potential to change the dynamics of the biota community.

The results of this study confirm that the environmental DNA (eDNA) metabarcoding method is a fairly successful approach in detecting the diversity of mollusc taxa in the seagrass and coral reef ecosystems of the Mandalika coast. This method allows the identification of species that were previously undetected through conventional observations, such as *M. obvelata* which was identified in the coral reef ecosystem in Aan cape, and *M. annulus* which was identified in all sampling locations both in the seagrass and coral reef ecosystems, the results of this identification are confirmed by previous studies by Katoh (1989) and Irie (2006) which explained that the *M. annulus* species is generally found living in seagrass and coral reef ecosystem areas. Previously, the *M. annulus* species was only reported in the Gili Trawangan, West Lombok, and East Lombok areas (Mujiono, 2020), so this result is a new record for the Mandalika coastal area.

Another important contribution of this study is the identification of *Phyllaplysia* sp. in coral reef Aan and Gerupuk samples. This species is generally known as an inhabitant of seagrass ecosystems, usually attached to seagrass leaves or macroalgae thallus. There has never been a previous report of its presence in Mandalika coral reefs, so this finding enriches information on mollusc diversity in the region. Its undetectability in previous conventional surveys is likely due to unrepresentative sampling times or limited identification methods due to the difficulty of access for sample collection and observation in coral reef ecosystems.

The presence of *M. obvelata*, known as a typical species of coral reef ecosystems, and *Phyllaplysia* sp., which is generally found living in seagrass ecosystems but was detected in coral reef ecosystems, indicates habitat flexibility and the possibility of ecological shifts. This distribution pattern is greatly influenced by environmental variables such as substrate, nutrients, and interactions between species. Molluscs are known to inhabit the littoral and intertidal zones (Nurhayati *et al*., 2021), attach to seagrass roots and leaves (Rahman *et al*., 2021), or bury themselves in sandy substrates or coral rubble (Bula *et al*., 2017; Saleky *et al*., 2019). Small species such as *Monetaria* camouflage under the sand or often attach to coral rubble to survive the waves. The presence of molluscs in Mandalika is reinforced by substrate conditions and other supportive environmental factors, including seagrass photosynthetic activity which contributes to the availability of nutrients and protective structures.

Mollusks' diversity analysis based on eDNA data revealed notable variations across different sites and habitat types (Figure 5). Overall, the coral Aan site recorded the highest number of mollusc species (observed and Chao1 = 7 ASVs). However, the Shannos (0.11) and Simpson (0.0042) diversity indices were relatively low, indicating an uneven distribution of species, possibly dominated by one or two dominant species based on their read abundance. In contrast, coral Gerupuk and Kuta showed lower species richness (Chao 1 = 2 – 3 ASVs) but higher diversity indices. Coral Kuta had the highest Shannon index among coral reef sites (0.27), while coral Gerupuk exhibited the highest Simpson value (0.71), suggesting a more balanced community structure despite the lower species count. Compared to coral reefs, mollusc diversity in seagrass habitats was generally lower. Nonetheless, seagrass Gerupuk displayed relatively high diversity (Shannon = 0.45; Simpson = 0.28) with only two species present, indicating a relatively even species distribution. Meanwhile, seagrass Aan and Kuta had the lowest diversity, with only one species detected (Observed and Chao1 = 1), and Shannon and Simpson indices equal to 0. This suggests extremely simplified communities, likely influenced by habitat degradation, anthropogenic disturbances, or suboptimal environmental conditions for mollusc populations. These differences in diversity reflect the importance of local habitat conditions and community structure in shaping mollusk distribution. They also highlight the potential of eDNA as a sensitive tool for uncovering patterns of biodiversity in coastal ecosystems.

The sustainability of molluscs is increasingly under threat due to human activities. Traditional practices like "madak" harvesting marine organisms

during low tide for consumption or sale, and high demand for economically valuable genus such as *Monetaria* (Aji *et al*., 2018) have placed significant pressure on mollusc populations. Overexploitation can lead to population declines, reduce distribution ranges, and loss of biodiversity within mollusk communities. Molluscs play vital roles in ecosystems, including nutrient recycling (Yap *et al*., 2024), serve as bioindicators of environmental quality (Chahouri *et al*., 2023), and offer medical and economic benefits (Ngandjui *et al*., 2024; Varis, 2024). To address these threats, sustainable science-based management is essential. This includes regular population monitoring, regulated harvesting, and conservation police grounded in scientific evidence.

3.2.2 Limitations and complementary role of eDNA metabarcoding in mollusc biodiversity assessment

Although the eDNA metabarcoding method offers an efficient and non-invasive approach to detecting the presence of species, there are some limitations that need to be considered. In this study, the number of mollusc taxon detected was relatively small compared to the results of previous studies using conventional methods. For example, the study by Berliana *et al.* (2024) in the mangrove and seagrass ecosystems of Gerupuk Bay managed to identify 55 mollusc species from 22 families through sample collection and direct observation methods. However, several species detected through eDNA metabarcoding were not found in previous studies. This difference can be caused by various factors, including different sampling times that affect environmental conditions and the presence of certain species, considering that each mollusc species has specific habitat preferences. In addition, the use of universal COI primers in this study allows DNA amplification from various taxa, so it is not specific to molluscs. eDNA analysis through multiple markers, preferably from different genes, could increase the technique's sensitivity and overcome the specificity issues of a single pair of universal primers (Stat *et al.,* 2017). There are numerous markers that are frequently used to detect mollusk diversity there are *cytochrome b* region (Kim *et al.,* 2019) for early detection of New Zealand mudsnails (*Potamopyrgus antipodarum*) (Goldberg *et al.,* 2013), mitochondrial 16S rDNA (Jaman *et al.,* 2006; Petters *et al.,* 2015), *NADH* for monitoring freshwater mussel (Sansom and Sassoubre, 2017), and Cytochrome c oxidase subunit 1 (COI gene) (Chen *et al.,* 2020). Detecting Chepalopoda group in marine ecosystems using Chep18S, but this primer is not suitable for the detection of octopus, and should be cautious on pepiids, myopsides, octopotheuthids, and gonatids (de Jonge *et al.,* 2021). However, 16S rRNA, unionioda and veneroida primers were applied in the analysis of freshwater bivalves but not in marine molluscs (Elbrecht and Leese, 2017). Therefore, the eDNA metabarcoding approach with multiple markers, the strengths and limitations of which are tested is a suitable complementary method for further studies into mollusc biodiversity patterns. Another factor may be the limited genetic baseline data used in the identification process, which may not provide comprehensive DNA readings for all mollusk species. Another factor is that during the sample delivery process to the laboratory or during the laboratory process, DNA is very sensitive and may easily degrade under unfavorable conditions. Conducting Next Generation Sequencing-based eDNA metabarcoding research requires careful attention to precision and sterilization standards in the field and in the laboratory. Therefore, the eDNA metabarcoding method should be used in a complementary manner with conventional methods to produce more accurate and comprehensive biodiversity data. Despite its limitations, this approach remains promising as a method for early monitoring of community changes, detection of rare species, and evaluation of sustainable conservation success.

4. Conclusion

Based on the results of this study, the environmental DNA (eDNA) method has proven to have advantages in identifying mollusc diversity in the Mandalika coastal ecosystem. eDNA allows the detection of species that are not observed through conventional methods, such as *Monetaria obvelata* and *Phyllaplysia* sp., which were each identified outside their common habitats, namely *M. obvelata* and *Phyllaplysia* sp. in coral reefs, although they are usually found in seagrass. In addition, the dominance of *M. annulus,* which was successfully detected in all locations, shows the high sensitivity of this method to environmental variations and the presence of species. Another advantage is its ability to reveal the distribution of taxa to the family level, such as Strombidae and Cypraeidae, and supports diversity calculations through sequence data. With the ability to detect hidden, difficult-to-observe, or small-sized species, eDNA has proven to be a more sensitive, efficient, and informative approach. In this study, some mollusc taxa were only identified at higher taxonomic levels, likely due to the universal primers used and the completeness of the reference database. For future research related to mollusc diversity monitoring, careful preparation is essential, particularly regarding

the primers and databases that will be utilized.

Acknowledgement

The authors would like to thank the Ecology Laboratory of the Biology Department for their assistance and for providing space and equipment during the research.

Authors' Contributions

DAC conceptualized and designed the experiments, received the research funding, analyzed the data, prepared figures and tables, wrote or reviewed the paper draft, and approved the final version. MG, TWS, AUM, and MSMI collected and analyzed the data and wrote or reviewed drafts of the paper. All authors read and approved the manuscript.

Conflict of Interest

The authors declare that they have no competing interests.

Declaration of Artificial Intelligence (AI)

The author(s) acknowledge the use of Grammarly for language refinement in preparing this manuscript. All AI-generated content was rigorously reviewed, edited, and validated to ensure accuracy and originality. Full responsibility for the manuscript's final content rests with the author(s). To ensure transparency and support the review process, a comprehensive delineation of the tool's application is furnished in the "Introduction" or "Materials and Methods" section of this manuscript in compliance with the publisher's ethical guidelines.

Funding Information

This research was partially supported by Universitas Mataram through PNBP with grant number: 1250/UN18.L1/PP/2024.

References

Ahyadi, H., Erdin, E., Candri, D. A., Farista, B., Astuti, S. P., & Virhota, A. (2021). Species diversity and health status of seagrass meadows in the Mandalika Coastal area, Central Lombok Regency. *Prosiding Saintek LPPM Universitas Mataram*, 3(2021):1-5.

Aji, L. P., Widyastuti, A., & Capriati, A. (2018). Community structure of mollusca in Seagrass Beds Padaido and Aimando Islands Biak Numfor Regency, Papua. *Oseanologi dan Limnologi di Indonesia,* 3(3):219-234.

Berliana, C. G., Candri, D. A., & Ahyadi, H. (2024). Mollusca biodiversity as a quality bioindicator waters in Central Lombok Gerupuk Bay Coastal area. *Jurnal Pijar MIPA*, 19(2):267-272.

Bolyen, E., Rideout, J. R., Dillo, M. R., Bokulich, N. A., Abnet, C. C., Al-Ghalith, G. A., Alexander, H., Alm, E. J., Arumugam, M., Asnicar, F., Bai, Y., Bisanz, J. E., Bittinger, K., Brejnrod, A., Brislawan, C. J., Brown, C. T., Callahan, B. J., Rodriguez, A. M. C., Chase, J., Cope, E. K., Silva, R. D., Diener, C., Dorrestein, P. C., Douglas, G. M., Durall, D. M., Duvallet, C., Edwardson, C. F., Ernst, M., Estaki, M., Fouquier, J., Gauglitz, J. M., Gibbons, S. M., Gibson, D. L., Gonzalez, A., Gorlick, K., Guo, J., Hillmann, B., Holmes, S., Holste, H., Huttenhower, C., Huttley, G. A., Janssen, S., Jarmusch, A. K., Jiang, L., Kaehler, B. D., Kang, K. B., Keefe, C. R., Keim, P., Kelley, S. T., Knights, D., Koester, I., Kosciolek, T., Kreps, J., Langgille, M. G. I., Lee, J., Ley, R., Liu, Y. X., Loftfield, E., Lozupone, C., Maher, M., Marotz, C., Martin, B. D., McDonald, D., Mclver, L. J., Melnik, A. V., Metcalf, J. L., Morgan, S. C., Morton, J. T., Naimey, A. T., Molina, J. A. N., Nothias, L. F., Orchanian, S. B., Pearson, T., Peoples, S. L., Petras, D., Preuss, M. L., Pruesse, E., Rasmussen, L. B., Rivers, A., Robeson, M. S., Rosenthal, P., Segata, N., Shaffer, M., Shiffer, A., Sinha, R., Song, S. J., Spear, J. R., Swafford, A. D., Thompson, L. R., Torres, P. J., Trinh, P., Tripathi, A., Turnbaiugh, P. J., Hasan, S., Hooft, J. J., Vargas, F., Baeza, Y. V., Vogtmann, E., Hippel, M., Walters, W., Wan, Y., Wang, M., Warren, J., Weber, K. C., Williamson, C. H. D., Willis, A. D., Xu, Z. Z., Zaneveld, J. R., Zhang, Y., Zhu, Q., Khight, R., & Caporaso, J. G. (2018). Reproducible, interactive, scalable, and extensible microbiome data science using QIIME 2. *Nature Biotechnology,* 37(7):852-857.

Bula, W., Leiwakabessy, F., & Rumahlatu, D. (2017). The influence of environmental factors on the diversity of Gastropods in Marsegu Island, Maluku. *Jurnal Biosaintifika,* 9(3):483-491.

Callahan, B. J., Mcmurdie, P. J., Rosen, M. J., Han, A. W., Johnson, A. J. A., & Holmes, S. P. (2016). Dada2: Highresolution sample inference from illumina amplicon data. *Nat Methods*, 13(7):581-583.

Candri, D. A., Hakimi, B., Ahyadi, H., Suana, I. W.,

Prasedya, E. S., Ambarwati, K., & Mardiati, A. U. (2023a). Condition of coral reef diversity in Kuta Mandalika Coastal, Central Lombok Regency. *Jurnal Biologi Tropis*, 23(2):15-26.

Candri, D. A., Hidayati, L. J., Ahyadi, H., & Zamroni, Y. (2023b). Community structure of Molluscs at Batu Kijuk Village. *Jurnal Penelitian Pendidikan IPA,* 9(2):856-863.

Candri, D. A., Rahmani, M. S., Ahyadi, H., & Zamroni, Y. (2022). Diversity and distribution of Gastropoda and Bivalvia in mangrove ecosystem of Pelangan, Sekotong, West Lombok. *Jurnal Biologi Tropis,* 22(3):1092-1100.

Candri, D. A., Sani, L. H., Ahyadi, H., & Farista, B. (2020). Mollusk community structure in natural and rehabilitated mangrove areas on the south coast of Lombok Island. *Jurnal Biologi Tropis,* 20(1):139-147.

Chahouri, A., Yacoubi, B., Moukrim, A., & Banaoiu, A. (2023). Bivalve molluscs as bioindicators of multiple stressors in the marine environment: Recent advances. *Continental Shelf Research*. 264(13):1-14.

Chen, Z., Song, N., Minamoto, T., Wu, Q. Q., & Gao, T. X. (2020). The eDNA collection method of Zhoushan coastal waters. *Acta Hydrobiologica Sinica,* 44(1):50-58.

de Jonge, D. S. W., Merten, V., Bayer, T., Puebla, O., Reusch, T. B. H., & Hoving, H. J. T. (2021). A novel metabarcoding primer pair for environmental DNA analysis of Cephalopoda (Mollusca) targeting the nuclear 18S rRNA region. *Royal Society Open Science*, 8(2):1-16.

Elbrecht, V., & Leese, F. (2017). PrimerMiner: An R package for development and in silico validation of DNA metabarcoding primers. *Methods in Ecology and Evol*ution, 8(5):622-626.

Gokoglu, N. (2021). Shellfish processing and preservation. Springer.

Goldberg, C. S., Sepulveda, A., Ray, A., Baumgardt, J., & Waits, L. P. (2013). Environmental DNA as a new method for early detection of New Zealand mudsnails (*Potamopyrgus antipodarum*). *Freshwater Science*, 32(3):792-800.

Hallam, J., Clare, E. L., Jones, J. I., & Day, J. J. (2021). Biodiversity assessment across a dynamic riverine system: A comparison of eDNA metabarcoding versus traditional fish surveying methods. *Environmental DNA*, 3(6):1247-1266.

Harrison, J. B., Sunday, J. M., & Rogers, S. M. (2019). Predicting the fate of eDNA in the environment and implications for studying biodiversity. *Proceedings of the Royal Society B*, 286(1915):1-9.

Huang, C., Zhu, S. X., Lin, J., & Dong, Q. (2008). Imposex of *Mauritia arabica* on the south-eastern coast of China. *Journal of the Marine Biological Association of the United Kingdom*, 88(7):1451-1457.

Irie, T. (2006). Geographical variation of shell morphology in *Cypraeae annulus* (Gastropoda: Cypraeidae). *Journal of Molluscan Studies*, 72(1):31-38.

Irie, T., & Morimoto, N. (2016). Intraspecific variations in shell calculation across thermal window and within constant temperatures: Experimental study on an intertidal Gastropod *Monetaria annulus*. *Journal of Experimental Marine Biology and Ecology*, 483(10):130-138.

Jarman, S. N., Redd, K. S., & Gales, N. J. (2006) Group specific primers for amplifying DNA sequences that identify Amphipoda, Cephalopoda, Echinodermata, Gastropoda, Isopoda, Ostracoda and Thoracica. *Molucar Ecology Notes,* 6(1):268-271.

Katoh, M. (1989). Life history of the golden ring cowry *Cypraea annulus* (Mollusca: Gastropoda) on Okinawa Island, Japan. *Marine Biology*, 101(1):227-233.

Kestel, J. H., Field, D. L, Bateman, P. W., White, N. E., Allentoft, M. E., Hopkins, A. J. M., Gibberd, M., & Nevill, P. (2022). Applications of environmental DNA (eDNA) in agricultural systems: Current uses, limitations and future prospects. *Science of the Total Environment*, 874(11):1-21.

Kim, E. B., Lee, S. R., Lee, C. I., Park, H., & Kim, H. W. (2019). Development of the cephalopod-specific universal primer set and its application for the metabarcoding analysis of planktonic cephalopods in Korean waters. *PeerJ,* 7(1):7140-7151.

Kuncoro, I., Zamani, N. P., Subhan, B., & Cahyani, N. K. D. (2023) eDNA assessment of

Sclerentinian diversity and distribution in Lemukutan Island, Indonesia. *Biodiversitas*, 24(7):4185-4191.

Lamy, T., Pointer, J. P., Jarne, P., & David, P. (2012). Testing metapopulation dynamics using genetic, demographic and ecological data. *Molecular Genetics*, 21(6):1394-1410.

Leray, M., Yang, J. Y., Meyer, C. P., Mills, S. C., Agudelo, N., Ranwez, V., Boehm, J. T., & Machida, R. J. (2013). A new versatile primer set targeting a short fragment of the mitochondrial COI region for metabarcoding metazoan diversity: Application for characterizing coral reef fish gut contents. *Frontiers in zoology*, 10(1):1-14.

Madduppa, H., Cahyani, N. K. D., Anggoro, A. W., Subhan, B., Jefri, E., Sani, L. M. I., Arafat, D., Akbar, N., & Bengen, D. G. (2021). eDNA metabarcoding illuminates species diversity and composition of three phyla (Chordata, Mollusca and Echinodermata) across Indonesian coral reefs. *Biodiversity and Conservation*, 30(11):3087-3114.

Martin, M. (2011). Cutadapt removes adapter sequence from high-troughput sequencing reads. *EMBnet Journal,* 17(1):1-10.

Matsuura, K., Sumadhiharga, O. K., & Tsukamoto, K. (2000). Field guide to Lombok Island: Identification guide to marine organisms in seagrass beds of Lombok Island, Indonesia. Tokyo: Ocean Research Institute, University of Tokyo.

Mujiono, N. (2020). Checklist of Gastropoda from Lombok Island and its satellite islands. *Jurnal Biologi Udayana*, 24(2):72-86.

Ngandjui, Y. A. T., Kereeditse, T. T., Kamika, I., Madikizela, L. M., & Msagati, T. A. M. (2024). Nutraceutical and medicinal importance of marine Molluscs. *Marine Drugs*, 22(5):1-28.

Nurhayati, P. A., Affandi, M. & Nurinsiyah, A. S. (2021). Diversity and abundance of terrestrial Gastropods on the slopes of Mount Arjuna-Welirang, East Java, Indonesia. *Biodiversitas*, 22(10):4193-4220.

Othman, N., Munian, K., Haris, H., Ramli, F. F., Sariyati, N. H., Najmuddin, M. F., & Latief, M. A. B. A. (2023). A review on next-generation wildlife monitoring using environmental DNA (eDNA) detection and next-generation sequencing in Malaysia. *Sains Malaysiana,* 51(1):17-33.

Peters, K. J., Ophelkeller, K., Herdina, Bott, N. J., & Goldsworthy, S. D. (2015). PCR-based techniques to determine diet of the Australian sea lion (*Neophoca cinerea*): A comparison with morphological analysis. *Marine Ecology,* 36(4):1428-1439.

Rahman, M. H., Hossain, M. B., Habib, A., Noman, M. A., & Mondal, A. (2021). Mangrove associated macrobenthos community structure from an estuarine island. *Biodiversitas*, 22(1):247-252.

Rey, A., Viard, F., Lize, A., Corre, E., Valentini, A., & Thiriet, P. (2023). Coastal rocky reef fish monitoring in the context of the marine strategy framework directive: Environmental DNA metabarcoding complements underwater visual census. *Ocean and Coastal Management*, 241(11):1-14.

Roussel, A. L., Howland, K., Normandeau, E., Grey, E. K., Archambault, P., Deiner, K., Lodge, D. M., Hernandez, C., Leduc, N., & Bernatchez, L. (2018). eDNA metabarcoding as a new surveillance approach for Coastal artic biodiversity. *Ecology and Evolution*, 8(16):7763-7777.

Safrillah, A., Karnan, & Japa, L. (2023). Species diversity of gastropoda in seagrass ecosystems at Mandalika Beach. *Jurnal Biologi Tropis,* 23(3):231-237.

Sahu, A., Kumar, N., Singh, C. P., & Singh, M. (2023). Environmental DNA (eDNA): Powerful technique for biodiversity conservation. *Journal for Nature Conservation*, 71(1):1-14.

Saleky, D., Leatimea, S. P. O., Yuanike, Rumengan, I., & Putra, I. N. G. (2019). Temporal distribution of Gastropods in rocky intertidal area in North Manokwari, West Papua. *Jurnal Sumberdaya Akuatik Indopasifik*, 3(1):1-10.

Sangaji, M., Hulopi, M., Sahetapy, J. M. F., Sirajuddin, N. T., Lokollo, F. F., & Lestari, F. (2023). The potential of Mollusca communities in seagrass ecosystems on Buntal Island Waters West Seram Regency, Maluku Province. *International Journal of Science and Environment*, 3(3):1-7.

Sansom, B. J., & Sassoubre, L. M. (2017). Environmental DNA (eDNA) shedding and decay rates to model freshwater mussel eDNA transport in a river. *Environmental Science and Technology*, 51(24):14244-14253.

Stat, M., Huggett, M. J., Bernasconi, R., Dibattista, J. D., Berry, T. E., Newman, S. J., Harvey, E. S., & Bunce, M. (2017). Ecosystem biomonitoring with eDNA: Metabarcoding across the tree of life in a tropical marine environment. *Scientific Reports,* 7(1):1-11.

Svenningsen, A. K. N., Pertoldi, C., & Bruhn, D. (2022). eDNA metabarcoding benchmarked towards conventional survey methods in amphibian monitoring. *Animals*, 12(6):1-15.

Varis, M. (2024). Economic importance of molluscan. *ShodhKosh: Journal of Visual and Performing Arts*, 5(6):120-124.

Yap, C. K., Aguol, K. A., Ong, M. C., Syazwan, W. M., Nulit, R., Okamura, H., Horie, Y., Ismail, M. S., Setyawan, A. D., Kumar, K., Cheng, W. H., & Leow, C. S. (2024). The role of Molluscs in monitoring marine pollution and its connection to climate change and ESG. *Current World Environment,* 19(3):1-14.

Zhang, M., Zou, Y., Xaio, S., & Hou, J. (2023). Environmental DNA metabarcoding serves as a promising method for aquatic species monitoring and management: A review focused on its workflow, applications, challenges and prospects. *Marine Pollution Bulletin*, 194(9):1-17.

Zinger, L., Lionnet, C., Benoiston, A. S., Donald, J., Mercier, C., & Boyer, F. (2021). MetabaR: An R package for the evaluation and improvement of DNA metabarcoding data quality. *Methods in Ecology and Evolution,* 12(1):586-592.

Zulkifli, L., Patech, L. R., Lestari, A., Fidiantara, F., Idrus, A. A., & Syukur, A. (2021). The sustainability of the diversity of marine macrofauna associated with seagrass through ecotourism in the Mandalika Exclusive Economic Zone, Lombok Island, Indonesia. *IOP Conf. Series*: *Earth and Environmental Science,* 913(012053):1-11.