C7. Imam Bachtiar

by Imam Bachtiar

Submission date: 14-Feb-2023 07:39PM (UTC-0600)
Submission ID: 2014440773
File name: C7_Environmental biomonitoring.pdf (1.73M)
Word count: 9010
Character count: 47420

Environ Biol Fish
https://doi.org/10.1007/s10641-021-01118-3

Environmental biomonitoring of reef fish community structure with eDNA metabarcoding in the Coral Triangle

Ester Restiana Endang Gelis · M. Mukhlis Kamal · Beginer Subhan · Imam Bachtiar · Lalu M. Iqbal Sani · Hawis Madduppa

Received: 16 December 2020 / Accepted: 28 June 2021

Abstract Coral reef fishes perform a range of vital ecosystem functions, and can serve as indicators of ecological stress and resilience. However, many species are not observed when using Underwater Visual Census (UVC) during biomonitoring, and therefore overall biodiversity is often underestimated. Environmental DNA (eDNA) is proposed as an advanced and non-invasive next-generation biomonitoring method for determining the presence of aquatic organisms such as fish. Therefore, this study aimed to assess the community structure of coral fish from three different marine protected area reef zones (utility zone, open access zone, core zone) around Lombok Island using eDNA metabarcoding. Biological community composition, richness, and diversity were evaluated based on reads from mid-column water and sediment samples. A total of 58 species were identified from the eDNA samples using the Multiplex Barcode Research And Visualization Environment (mBRAVE) pipeline. The Shannon–Wiener index (H') showed significantly higher species diversity in the core zone than the utility and open access zones. There was no significant between-zone difference in community structure (ANOSIM, $R = 0.11 < 0.25$). NMDS analysis using the Bray–Curtis test showed significant between-zone differences in species diversity and abundance (PERMANOVA Adonis Pr $(>F) = 0.001$, $p < 0.05$). Based on the high number of fish species detected in this study, eDNA can be recommended as an alternative tool or as a complement to visual survey methods for biological monitoring and diversity assessment of remote reefs, with less stringent requirements in terms of field conditions (e.g. visibility) and taxonomic expertise.

Keywords Fish diversity · High throughput sequencing · Lombok Island · Next-generation biomonitoring · Reef fish · Species detection

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s10641-021-01118-3.

E. R. E. Gelis · B. Subhan · H. Madduppa (✉)
Department of Marine Science and Technology, Faculty of Fisheries and Marine Science, IPB University, Bogor 16680, Indonesia
e-mail: hawis@apps.ipb.ac.id

M. M. Kamal
Department of Aquatic Resources Management, Faculty of Fisheries and Marine Science, IPB University, Bogor 16680, Indonesia

I. Bachtiar
Department of Biology Education, Faculty of Education, Universitas Mataram, Jl. Majapahit 62, Mataram 83125, Indonesia

L. M. I. Sani · H. Madduppa
Oceanogen Environmental Biotechnology Laboklinikum, Bogor 16112, Indonesia

Published online: 28 July 2021

Introduction

Biological diversity can be a major challenge in understanding ecological processes, or conducting biomonitoring for various taxonomic groups; this is especially true for fishes (Leray et al. 2013). The most species vertebrate group, fishes are commonly used as indicators of marine ecosystem health (Madduppa et al. 2013). Among the most diverse fish assemblages are those living in and associated with coral reef habitats (Madduppa et al. 2012). Many fish populations are overexploited, posing a threat to marine ecosystems (Collette et al. 2011; Hutchings 2000; Jackson et al. 2001; Pauly et al. 2002). In addition to their ecological roles, fishes living in a coral reef environment can be grouped into three categories based on their roles: target (mostly large fishes of economic value, consumed by humans), indicator (fishes associated with coral reef health), and major (other, mostly small fishes often present in high numbers) (Dewi and Sukandar 2018; English et al. 1997). Coral reef fishes are important for food security and as economic commodities, especially in fishing communities (Madduppa et al. 2014). The distribution and abundance of reef fish are important parameters, especially in areas with high fish biodiversity, to inform the management and utilization of these fish communities which typically support multi-species fisheries (Anderson et al. 2019; Friedlander et al. 2007; McCoy et al. 2010; Varkey et al. 2012).

The most diverse and abundant marine life in Indonesia can be found in the eastern region, at the heart of the Coral Triangle, which is home to more than 2000 species of fish (Allen and Adrim 2003). Lombok is one of several small islands in the Greater Sunda Islands chain, at the southern edge of the Coral Triangle, with narrow straits forming the main gateways for the flow of water masses from the Pacific to the Indian Ocean (Wahyudewantoro 2018). Underwater Visual Census (UVC) is the most frequently used methodology for biomonitoring in coral reef ecosystems and is based on the direct observation of marine organisms during an underwater survey (e.g. Cleary 2017; Madduppa et al. 2013; Prato et al. 2017). Research around Gili Lawang, Gili Sulat, and Gili Bidara in East Lombok has identified 53 species of reef fish belonging to 17 families (Arifin and Yulianda 2003). Another study by Wahyu et al (2018) found a total of 69 fish species belonging to 12 families in West Lombok. These two studies showed the Pomacentridae, Labridae, and Chaetodontidae as the most dominant fish families in these waters. These three families also dominate coral reef fish communities at other locations in Indonesia (Meekan et al. 1995; Green 1996; Madduppa et al. 2012).

2 Environmental DNA (eDNA) can be an effective method for detecting the presence of organisms, such as fish, amphibians, and other taxa without isolating the target organism (Laramie et al. 2015; Lodge et al. 2012). The genetic material is extracted easily through environmental samples such as soil and water (Barnes and Turner 2016; Tringe and Rubin 2005). eDNA comprises dormant organisms, dead cells, free molecules, or those adsorbed on the surface of different types of organic or mineral particles (Levy-Booth et al. 2007; Pietramellara et al. 2009; Taberlet et al. 2018). Analysis of eDNA from water samples is an effective method for determining the presence of aquatic organisms such as fish, because they release a lot of genetic material in the form of lysed cells or faeces; these break down into small fragments which can be retained in the water column or settle and become retained in the sediment (Goldberg et al. 2011; Jerde et al. 2011; Laramie et al. 2015; Taberlet et al. 2012; Takahara et al. 2013). According to Dell'Anno and Danovaro (2005), sediment is considered to be the largest DNA reservoir in the ocean at levels often exceeding 90% of the extracellular DNA present, although this depends on the condition of the aquatic environment. In the water column, DNA gradually degrades over several hours, days or even weeks depending on the biotic and abiotic conditions (Barnes et al. 2014; Corinaldesi 2004; Dejean et al. 2011; Dell'Anno and Green et al. 2011; Thomsen et al. 2012). The use of eDNA is considered as a viable alternative for monitoring fishes (Thomsen et al. 2012), in particular species diversity and richness (Cilleros et al. 2018), with more than 200 fish species identified through eDNA analysis (Jerde et al 2019; Murakami et al. 2019; Sard et al. 2019). Furthermore, it has been suggested that eDNA could be used as a tool to improve estimations of fish biodiversity in coral reef ecosystems (Nguyen et al. 2020), including in the context of marine protected areas with a lack of biodiversity data (Moore et al. 2021).

Marine Protected Areas (MPAs) are a management strategy that plays an important role in the preservation of biodiversity, including coral reef-associated organisms (Bonaldo et al. 2017). Located at the southern edge of the Coral Triangle, Lombok Island has a relatively long history of habitat protection through the creation of marine protected areas (MPAs) comprising all three main tropical coastal ecosystems (mangrove forests, seagrass beds, and coral reefs). Despite the lack of data, this area could be expected to have healthy coastal ecosystems, supporting abundant and diverse coral reef fish communities. There is therefore a need to evaluate the biodiversity of reef fish communities in the Lombok Island MPAs. The primary objective of this study was to evaluate fish diversity and species composition in the waters around Lombok Island using an eDNA method to make an inventory of genetic material retrieved from mid-column seawater and sediment samples. A secondary objective of this study was to disseminate the use of eDNA as a biomonitoring tool to address the lack of biodiversity data hampering the evaluation and management of coral reef fishes across Indonesia.

Materials and methods

Study sites

This research was conducted at 14 sites in the coastal waters around Lombok Island (Fig. 1). These sites were divided into three groups based on geography:

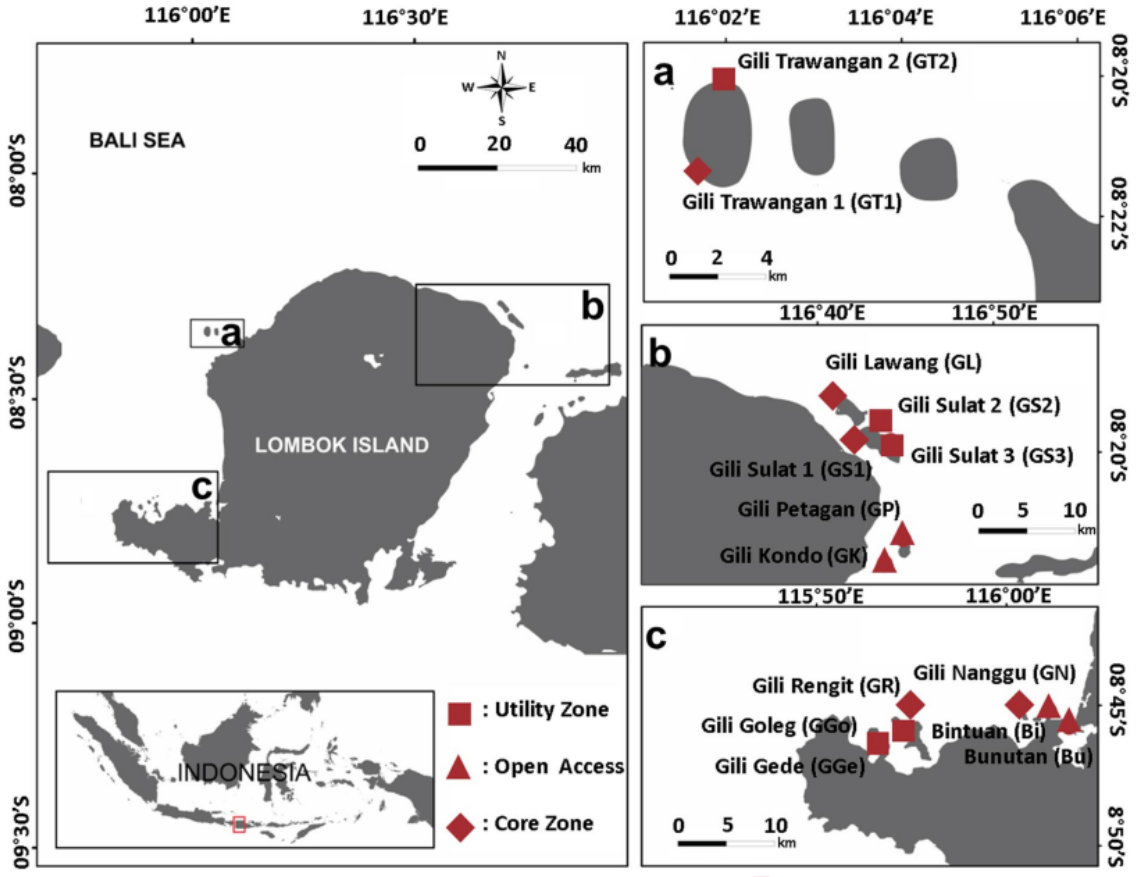

Fig. 1 Locations of eDNA biomonitoring sampling (mid-column water and sediment) around Lombok Island, Indonesia: (**a**) North Coast; (**b**) East Coast; and (**c**) West Coast

East (Gili Sulat 1, 2, and 3; Gili Lawang; Gili Petagan; Gili Kondo), North (Gili Trawangan 1 and 2), and West (Gili Nanggu; Bunutan; Bintuan; Gili Rengit; Gili Goleg; Gili Gede). Based on MPA zonation, the sites were divided into three zones: core zone (Gili Sulat 1; Gili Lawang; Gili Nanggu; Gili Rengit; Gili Trawangan 1), utility zone (Gili Sluat 2; Gili Sulat 3; Gili Goleg; Gili Gede; Gili Trawangan 2) and open access (Gili Petagan; Gili Kondo; Bunutan, and Bintuan). All samples were collected from 5th–12th August 2018. Sites were selected based on the variability in reef condition and current patterns as well as the zonation plan.

Ethical approval, conflict of interest statement and data availability.

The study did not involve human subjects and/or animals. Seawater samples were filtered following eDNA seawater sampling protocols (e.g. Goldberg and Strickler 2017). eDNA seawater sampling in this study was permitted within the framework of the United States Agency for International Development—Sustainable Higher Education Research Alliances (USAID-SHERA) program through the Centre for Collaborative Research Animal Biotechnology and Coral Reef Fisheries (CCR ANBIOCORE) of IPB University. The authors declare that there is no conflict of interest. The data that support the findings of this study are available at the public storage with the following link: ipb.link/ednalombok.

eDNA seawater collection

Environmental DNA samples were divided into two categories: mid-column water (n = 14) and sediment (n = 14). The two sample types were collected at each site using 4 L bottles by divers using Self-Contained Underwater Breathing Apparatus (SCUBA). Seawater samples, hereafter referred to as mid-column water samples, were collected from the water column at approximately half the water depth at each station. Sediment samples comprised about 50% sediment and 50% seawater collected immediately above the substrate; these were then shaken vigorously to ensure homogenous mixing prior to the filtering process. The contents of each 4 L sampling bottle were then pumped through a filter paper (0.4 μm pore size, 47 mm diameter) using a peristaltic filtering device (MASTERFLEX number 13–310-662) (Bakker et al. 2017; Deiner et al. 2018). After the filtering process was completed, the filter paper was then cut into two halves using sterilised scissors. Each half of the filter paper was placed into a 2 mL cryotube containing 1.5 mL DNA shield (ZymoBIOMICS DNA/RNA shield). To avoid contamination, all sampling equipment used at each stage of the sampling procedure was sterilised with a 10% solution of commercial bleach.

eDNA extraction, library preparation and high throughput sequencing

The extraction of eDNA retained in the filters was carried out using ZymoBIOMICS DNA extraction kits (Zymo Research, USA) following the manufacturer's protocol (Li et al. 2018; Verma and Satyanarayana 2011). In the first Polymerase Chain Reaction (PCR) stage, the primer pair used to amplify the target samples comprised the forward mlCOIintF (Leray et al. 2013) and reverse dgHCO2198 (Meyer 2003). This combination is considered suitable for detecting a wide variety of taxa based on a 313 bp target COI fragment (Leduc et al. 2019; Leray et al. 2013). Forward and reverse Illumina hangovers were added to the primer sequences. The first PCR process used a thermocycling profile of 95 °C for 5 min, then 35 cycles of 94 °C for 1 min, 48 °C for 45 s and 72 °C for 30 s, and a final elongation at 72 °C for 10 min (Leray et al. 2016). In the second PCR process, the dual indices and Illumina sequencing adapters from the TruSeq PCR-Free LT kit were added to the target amplicons, using Kapa HotStart HiFi 2 × ReadyMix DNA polymerase (Kapa Biosystems Ltd., London, UK) with the following profile: 95 °C for 3 min, then 9 cycles of 95 °C for 30 s, 55 °C for 30 s, and final elongation at 72 °C for 5 min. The length of PCR product or amplicon was determined through electrophoresis, and the product purified using AMPure XP beads (LABPLAN; Naas, Ireland). All libraries were quantified using a Qubit fluorometer with Qubit dsDNA HS Assay reagent (Invitrogen, CA, USA) then pooled at equal concentrations. The library pool was diluted and denatured according to the Illumina MiSeq library preparation guide. The amplicon library (10 pM) was spiked with 20% denatured and diluted PhiX Illumina control library version 3

(Ilumina Inc., San Diego, CA, USA). The sequencing was conducted on an Illumina MiSeq using the MiSeq paired end sequencing reagent kit V3 500 cycle (Ilumina Inc., San Diego, CA, USA).

Bioinformatics using mBRAVE pipeline

Initial quality filtering of reads was performed using the Multiplex Barcode Research and Visualization Environment (mBRAVE) online pipeline (www.mbrave.net) (Ratnasingham 2019). The forward and reverse primer sequences were removed, allowing three mismatches in the primer. Low-quality bases were removed by trimming the reads at the beginning of the first poor quality window, and defined as a 10 bp region with an average quality score less than 25 (Mirimin et al. 2020). Reads meeting these criteria were further filtered to retain those with a length between the 140 bp minimum and 313 bp maximum. Only paired-end reads that met the filtering criteria in both forward and reverse directions were included in further analyses. For taxonomic assignment of COI sequences, a custom database was created, consisting of a file associated with a reference sequence from the BOLD (Ratnasingham and Hebert 2007). The custom BOLD system database in this study comprised 9829 sequences belonging to 2197 BINs (Barcode Index Numbers) of the Class Actinopterygii. The taxonomic identification results were visualised as a map showing community structure distribution, based on fish families and genera. The relative abundance composition of fish species was compared between the mid-column water and sediment samples.

Data analysis

All statistical analyses were conducted and visualized in R v. 3.6.2 (R Core Team 2013). Taxonomic identification results by family and genus were visualised on maps using pie charts for each of the 14 sites. Species composition and relative abundance of the fish identified from the mid-column water and sediment samples respectively were analysed and visualized using the ggplot2 package (Wickham 2009). Species Accumulation Curve (SAC), Shannon–Wiener (H') and Simpson Index (D), and statistical analysis routines were implemented in the vegan package (Oksanen et al. 2019). Species Accumulation Curve (SAC) analysis was used to estimate the cumulative mean species richness based on all study sites combined. This was achieved by applying the rarefy function with site samples corresponding to the mean species richness at each site (Colwell et al. 2012). Shannon–Wiener (H') and Simpson Index (D) were calculated to evaluate species diversity and dominance at each sampling station, and for each of the three MPA zones (utility zone, open access zone, and core zone). Alpha diversity of each sample was assessed using the ANOSIM routine (Zubcoff 2012) to evaluate the difference in fish species composition (H' and D) and relative abundance between the two eDNA sample types (mid-column seawater and sediment). The PERMANOVA Adonis routine was used to assess the distance index and to determine the significance level of between-site and between-zone differences (McMurdie and Holmes 2013). The abundance table was transformed to presence-absence data and the binary Bray–Curtis index (between mid-column water and sediment samples) was calculated and analysed using the non-metric multidimensional scaling (NMDS) routine.

Results

A total of 2,813,411 original reads were generated from the high throughput sequencing of amplicons from 28 samples and filtered to 704,898 reads by the mBrave pipeline. On average, the mid-column water samples contributed 8% fewer filtered reads ($24{,}239.21 \pm 3349.52$) compared to sediment samples ($26{,}110.64 \pm 6413.33$)samples was of (Table 1), however the difference was not statistically significant (ANOVA, $p = 0.86357$). There was also no significant difference between the numbers of filtered reads obtained from the three MPA zones (utility zone, open access, and core zone) (ANOVA, $p = 0.44212$). The taxa identified from the eDNA samples comprised 36 genera (Fig. 2) from 26 families (Fig. 3), Serranidae was the most commonly identified fish family at all sites. Based on sample type, 58 species were identified, 38 species belonging to 16 genera from mid-column water samples and 48 species belonging to 29 genera from sediment samples (Fig. 4). High between-site variability in species composition was observed for both mid-column water and sediment samples. The species with the highest relative abundance and rates of occurrence were *Caesio*

Table 1 Summary table of sequencing depth based on sample type: mid-column water (n = 14); sediment (n = 14) and zone: utility zone (n = 10); open access (n = 8); core zone (n = 10)

Samples		Original reads	Post filter reads	Filtered %	Dereplicated %
Sample type	Mid-column water	94,810.78 ± 14,402.34	24,239.21 ± 3349.52	2.04 ± 0.62	26.89 ± 9.95
	Sediment	106,147.14 ± 25,149.27	26,110.64 ± 6413.33	1.24 ± 0.50	29.50 ± 7.15
Zone	Utility Zone	107,150.50 ± 19,751.65	26,914.90 ± 5160.25	1.66 ± 0.68	28.39 ± 10.71
	Open Access	96,002.00 ± 10,638.32	24,441.13 ± 3341.59	1.61 ± 0.63	30.80 ± 6.39
	Core Zone	97,389.00 ± 26,057.4	24,022.00 ± 5949.20	1.64 ± 0.75	25.92 ± 7.55

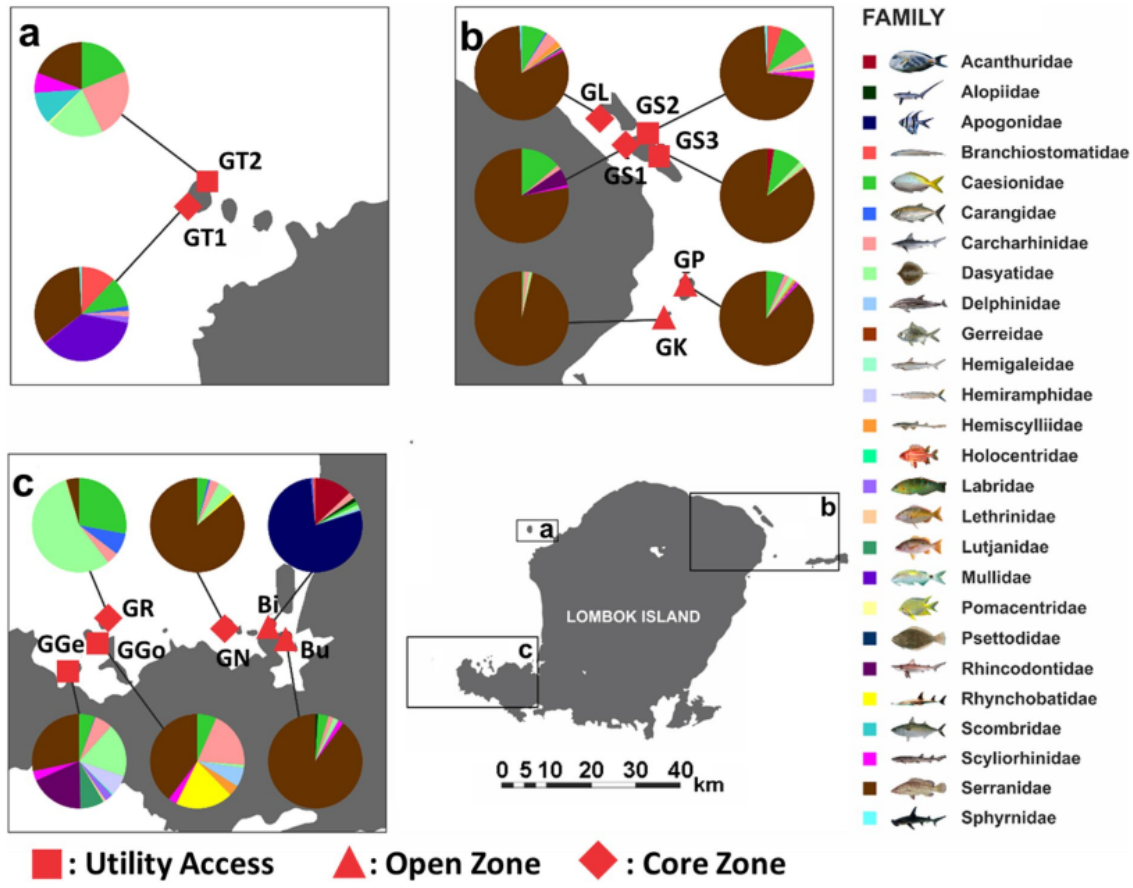

Fig. 2 Family-level fish community structure at 14 sites around Lombok Island: (**a**) North Coast; (**b**) East Coast; and (**c**) West Coast

cuning in mid-column water samples (Fig. 4a), and *Anyperodon leucogrammicus* in sediment samples (Fig. 4b). Both species composition and relative abundance detected from sediment samples varied significantly between the sites (ANOSIM on relative species abundance (%) by site: R = 0.66). The species accumulation curve (SAC) analysis (Fig. 5) shows that the increase in species richness between the stations was linear.

In general, the Shannon–Wiener diversity index (H') was positively correlated with species richness and negatively correlated with the Simpson

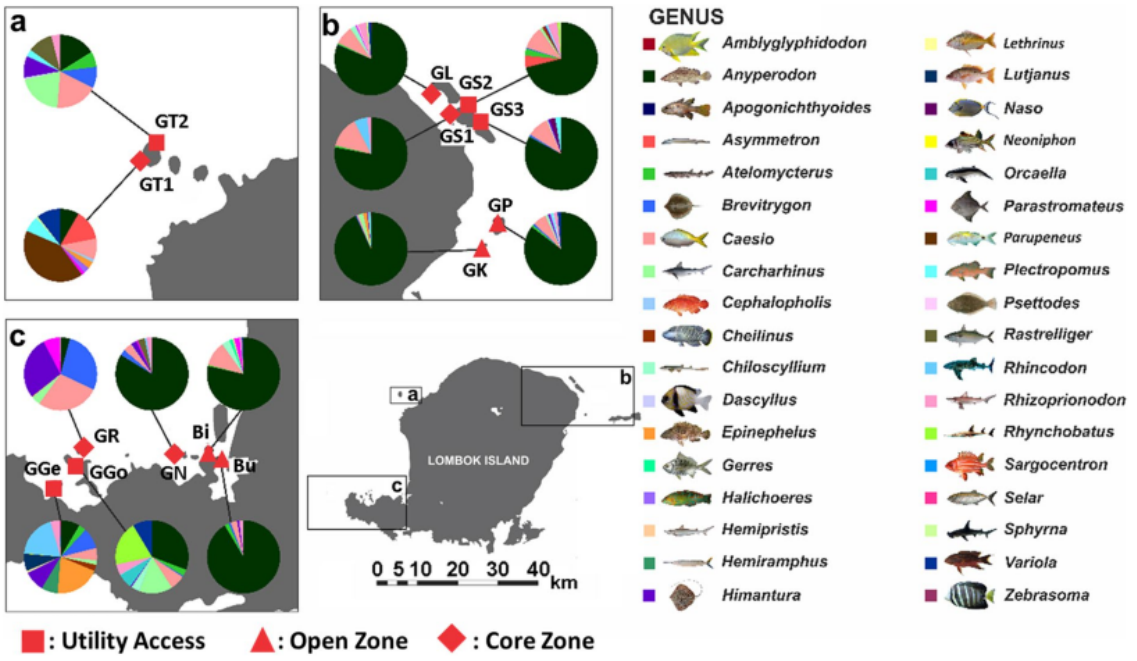

Fig. 3 Genus-level fish community structure at 14 sites around Lombok Island: (**a**) North Coast; (**b**) East Coast; and (**c**) West Coast

dominance index (D) (Fig. 6). The diversity index H' ranged from 0 to 2.45, in the low to moderate category, while the dominance index (D) ranged from 0 to 0.89, with sites categorized from low to high. The values of the community structure indices reflect the species composition at each site, with differing sequence reads, especially in the mid-column water samples. For both sample types, the sites with the highest diversity index values were in the utilization zone, while in the core zone (H') values were low to moderate (Fig. 6a). The highest diversity index (H') was 2.48, in the medium category, in the sediment sample from Gili Goleg site (GGo) in West Lombok. In both core and utility zones, several sites had high dominance index (D) values for both mid-column water and sediment samples (Fig. 6b). The uniformity and dominance index value (D) were always inversely correlated. The overall Simpson Dominance Index (D) was close to 0, indicating that no taxon was dominant across the whole study area. ANOSIM tests indicated the no differences between-site and zone according to Shannon–Wiener diversity index (H') and Simpson dominance index (D), as shown in Fig. 6 ($R = 0.04$).

There were differences in species composition in the three marine protected area zones (utility zone, open access zone and core zone) of MPAs in North, East and West Lombok (Fig. 7). Venn diagrams of the number of species in each area by sampling type (Fig. 7a) show considerable overlaps between the two sample types, although many species were only found in either the mid-column water of the sediment samples. The number and identity of species also varied between the MPA zones (Fig. 7b) with complex overlaps. The PERMANOVA test showed that for each site, the subsample from the mid-column water and sediment differed in both the number of species and their respective relative abundance. The amount of DNA accumulated was greater in sediment samples than in mid-column water samples. The Bray–Curtis distance index NMDS analysis (Fig. 8) indicates significant differences in fish species composition between mid-column water and sediment samples. Statistical significance of these differences was confirmed by the PERMANOVA Adonis $Pr(>F) = 0.001$ (<0.05).

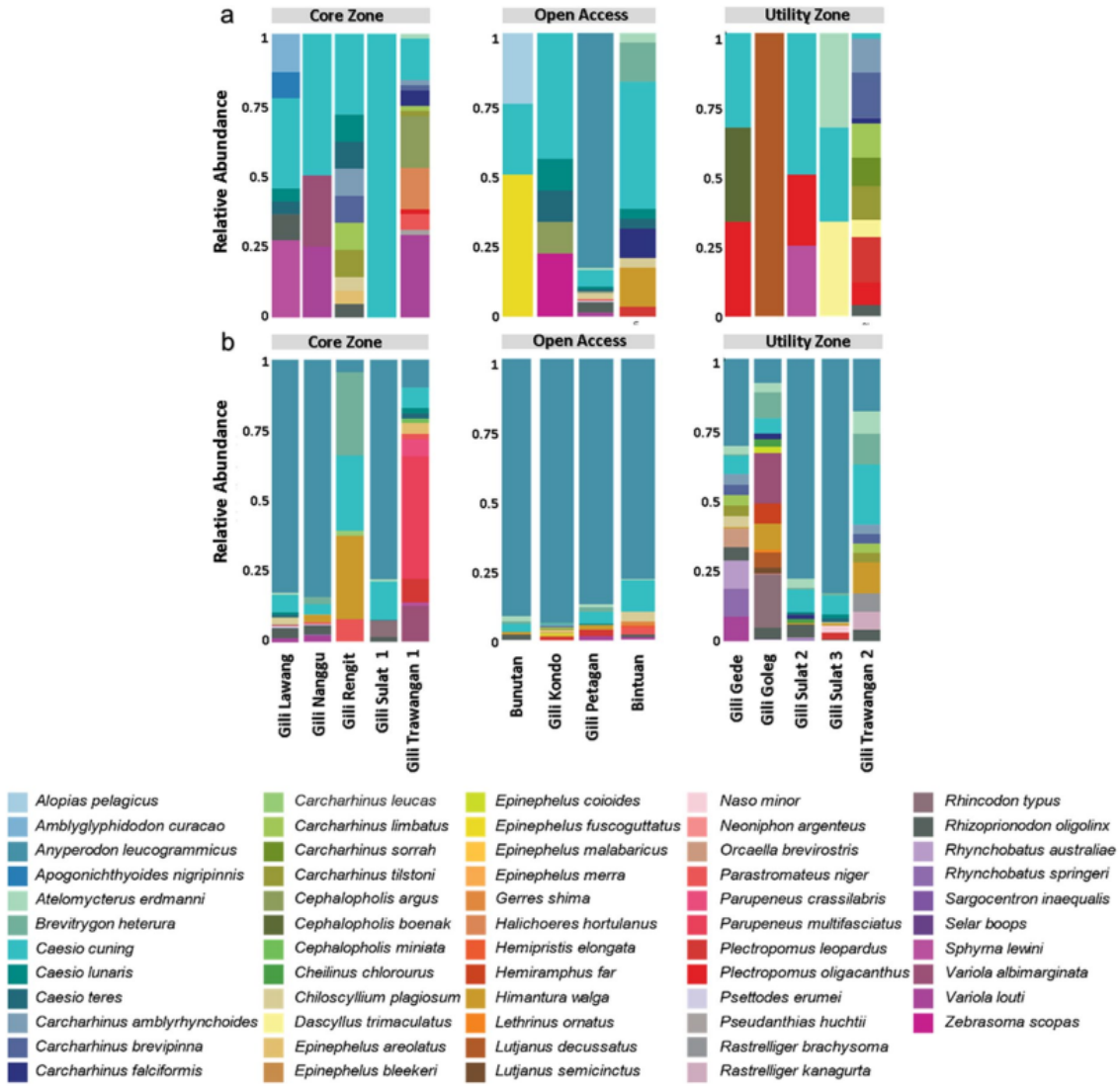

Fig. 4 Fish species composition and relative abundance based on two eDNA sample types: (**a**) mid-column water; (**b**) sediment

Discussion

Reef fish community structure revealed by eDNA biomonitoring

The patterns of fish abundance and growth are largely influenced by environment pattern and habitat of origin (Chabanet et al. 1997; Eschmeyer et al. 2010; Galzin et al. 1994). In particular, the percentage of coral cover can dramatically influence fish abundance and growth (Bell and Galzin 1984). Data on the coral reefs around Lombok are limited. However, a study by Ahyadi and Jufri (2008) in the Lombok MPAs with Taman Wisata Perairan (TWP) status reported significant damage to 75% of the coral reefs surveyed. More recently, surveys in northern Lombok Island found coral cover under 25% at 1–5 m depth and around 50% at 6–10 m depth (Wahyu et al. 2018). The eDNA

analysis identified 58 species, 38 in mid-column water and 48 in the sediments samples. It should be noted that many more sequences were produced, corresponding to species that were not identified due to technical issues (e.g. incomplete reference databases), a factor which must be considered when interpreting data for fisheries management and ecosystem monitoring (Gilbey et al. 2021). However, the number of fish families detected by this study (26) is greater than the 17 and 12 families reported by Arifin and Yulianda (2003) and Wahyu et al. (2018), respectively.

Each site had a different species composition and richness, based on each sample type alone and the combination of the two sample types. For example, while the diversity index was highest in the sediment sample from the Gili Goleg site (H' = 2.48, moderate category), only a three species were detected from the mid-column water sample. While the analyses showed that more genetic material was found in sediment samples than in samples taken from the water column, dominancy of specific species was more prevalent in sediment samples, although at two sampling stations only a single fish taxonomic unit (species) was identified from the mid-column seawater sample reads. However, a dominance index score of 0 indicates that no species were predominant in the study area (Muniaha et al. 2016). The observed patterns are consonant with the statement by Turner et al (2014) that sediments contain more DNA than mid-column waters, because they store genetic material for a longer period, but in contrast with the greater number of fish species identified in the water column compared to sediment by Shaw et al (2016). Another technical factor that can influence species detection in eDNA samples is the primer chosen to amplify a specific mtDNA region. for example, Zhang et al. (2020) found that eDNA primers targeting the 12S rRNA gene identified more fish diversity than those targeting 16S rRNA or COI genes.

A wide range of environmental factors, such as oceanographic, biological, and chemical parameters can lead to wide variability in eDNA particles present; for example, in the offshore environment, eDNA tends to degrade more slowly than in the coastal area (Collins et al. 2018; Hansen et al. 2018). Factors causing differences in eDNA abundance and diversity at the study site most likely included the topographic and oceanographic conditions affecting water transport processes, as eDNA retention and quality can be influenced by environmental factors including water temperature, organic matter, pH, UV radiation, water currents and seasonal stratification as well as by the type and amount of material used during sampling (Gilbey et al. 2021; Hansen et al. 2018; Lacoursiere-Roussel et al. 2018). Deiner and Altermatt (2014) found that environmental DNA from aquatic organisms could be transported over considerable distances; depending on sea conditions, both fish and invertebrate material were found up to around 10 km from the original habitat of the organisms studied. Thus, the observed differences in species abundance and composition between the mid-column seawater and sediment samples could be related to genetic material decomposition rates, and chemical-physical factors that affect DNA degradation rates and hence DNA persistence (Andruszkiewicz et al. 2019; Barnes et al. 2014; Li et al. 2019).

The Bray–Curtis NMDS analysis and PERMANOVA Adonis indicated significantly different results between the two sampling types, while PERMANOVA tests showed significant differences between the sampling types not only in terms of the species present but also their relative abundance. According to Shelton et al. (2016) and Thomsen et al. (2016), quantitative inferences from eDNA in marine environments can be obscured by many factors, making the direct application of eDNA read abundances somewhat speculative. However, seasonal qualitative data can provide valuable information on species occurrences, movements, and distributions. In the context of environmental change, eDNA sampling can be especially relevant as the approach is efficient and relatively easy to standardize across space, time, and personnel (Sigsgaard et al. 2017).

Potential limitations in this study include the potential failure to identify species present. DNA degradation, primer sensitivity, and a lack of template, can all play a role in failure to amplify DNA from the samples collected; moreover, for eDNA surveys such as this, it is difficult to determine the original DNA template concentration in the water column at the time of collection (DiBattista et al. 2017). Another challenge that can be encountered when applying eDNA monitoring methods in flowing waters, is that species DNA will be transported downstream, often through poorly known processes and over undetermined distances (Deiner and Altermatt 2014). It is increasingly recognised that eDNA sampling has many advantages, with

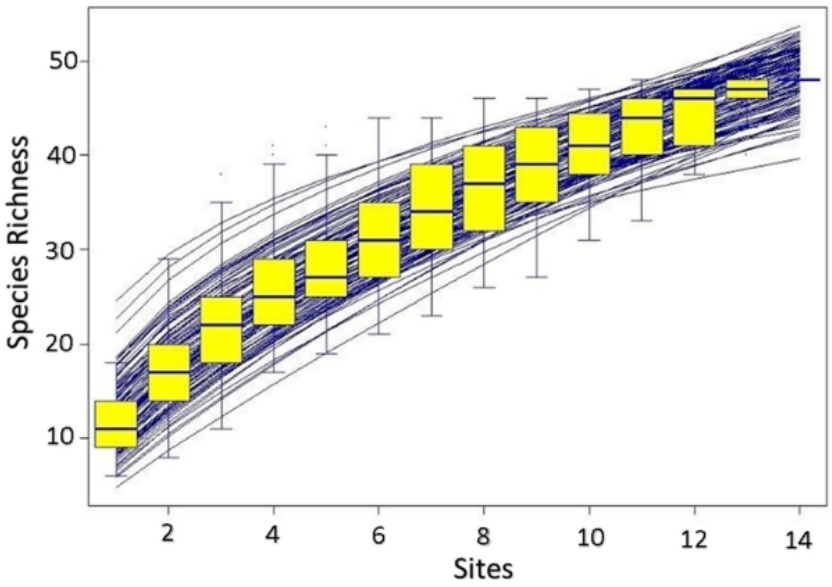

Fig. 5 Fish species accumulation curves based on species richness from 14 sites around MPAs in Lombok Island; error bars indicate between-site standard deviation

the ability to undertake sampling not (or minimally) affected by changing field conditions, visibility, or lack of taxonomic expertise (Thomsen and Willerslev 2015). Compared to underwater visual census (UVC), this method can be a relatively sensitive indicator and useful for monitoring population advances or retreats within coral reef fish communities, as well as for enabling rapid assessments of populations across greater number locations than would normally be logistically possible using UVC.

Implications for environmental monitoring and conservation of coral reefs

Environmental DNA-based monitoring can be a huge boon for the often underfunded public institutions required to comply with data-hungry regulations. In particular, eDNA metabarcoding can be useful for monitoring communities where there are many species of conservation concern, especially endangered species. Improved estimates of vulnerable species distribution can facilitate policy development and allow efficient targeting of cross-habitat management of fishes (Kelly et al. 2014; Thomsen and Willerslev 2015). For example, documenting the presence of a threatened species in a habitat has been known to trigger a series of actions under laws relating to the conservation of biodiversity (Deiner et al. 2017). If surveys are conducted at regular intervals (e.g. every 6 months), it will be possible to react faster if newly introduced or invasive species are found (Huhn et al. 2019). However, environmental DNA cannot be used to differentiate between living and dead organisms, or to estimate demographic parameters of importance in population viability analyses (Deiner et al. 2017; Taberlet et al. 2018).

In this study, we found that sites in the utility zone had the highest diversity index (H') value, in contrast to the core region where (H') ranged from low to moderate with high dominance index (D) values at each observation site for both the mid-column water and sediment samples. In general, one key measure of MPA implementation effectiveness in ecological terms is biotic community composition, and in particular fish diversity (Blowes et al. 2020; Kruschel et al. 2012; Ramírez-Ortiz et al. 2020). The higher diversity in the utility zone compare to the core zone could be related to how the MPA is implemented. A major factor influencing the success of MPA implementation is stakeholder engagement (Giakoumi et al. 2018). Indonesia has more than 17 million hectares of marine conservation areas, many of which still lack effective management, and could be considered as so called "paper parks" (Agardy et al. 2011; Park et al. 2006; Scianna et al. 2015). There is a need to improve the management effectiveness of the Lombok Island MPAs, ensuring a high level of buy in and compliance with regulations through stakeholder involvement as well as strong

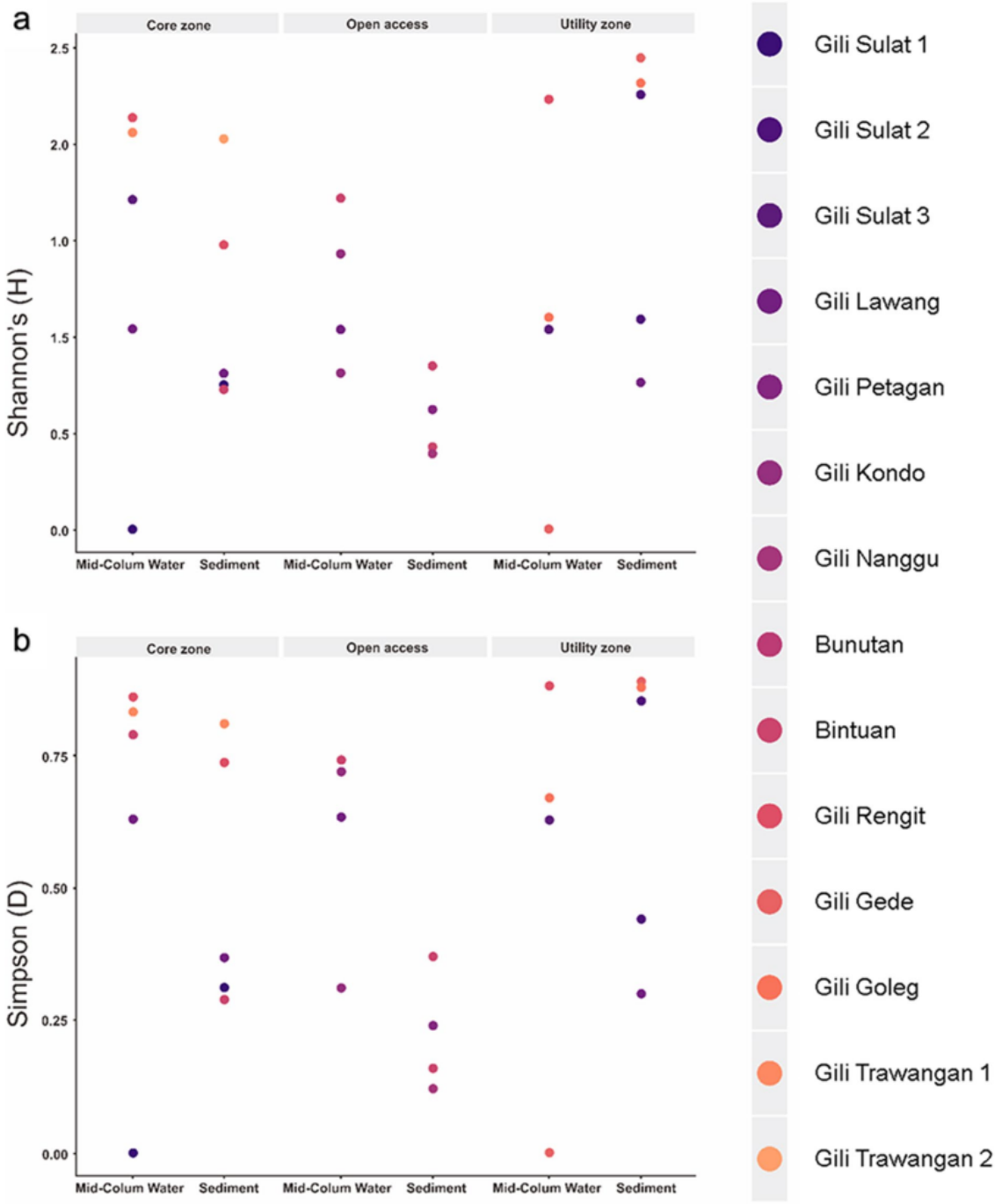

Fig. 6 Scatter plots for fish species identified using two eDNA sample types for 14 sites within three MPA zones around Lombok Island showing: (**a**) Shannon–Wiener Diversity Index (H'); (**b**) Simpson Dominance Index (D) values

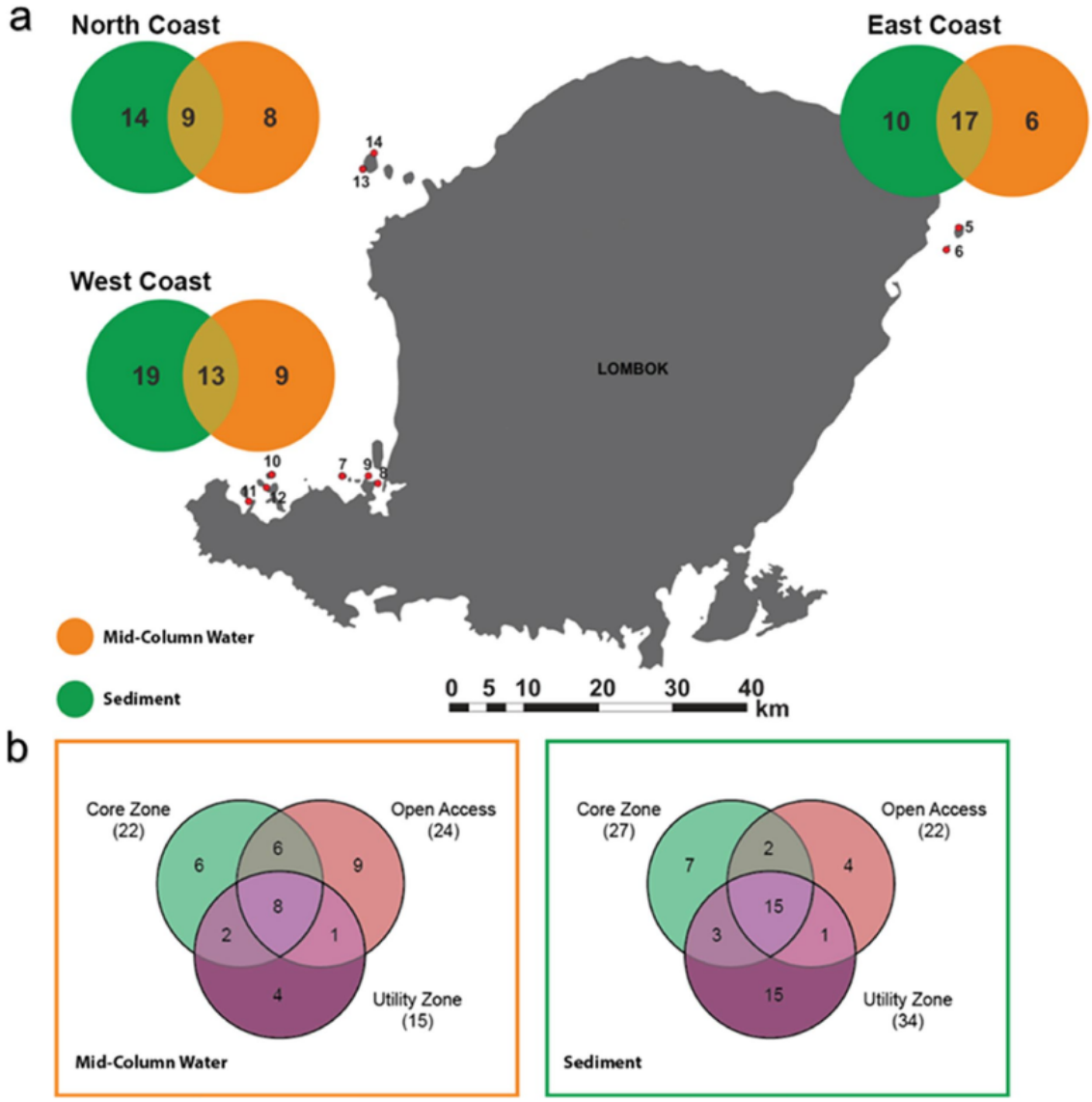

Fig. 7 Venn diagrams of fish species identified from Lombok protected reefs using two eDNA sample types (green: sediment; orange: mid-column water) by: (**a**) geographical area; (**b**) MPA zone

surveillance and enforcement in order to truly protect the ecosystems and resources within these areas. In addition, knowledge regarding reef fish distribution and abundance is important as a basis for improving management effectiveness and to guide resource utilization, especially in high biodiversity areas (Anderson et al. 2019; Friedlander et al. 2007; McCoy et al. 2010; Varkey et al. 2012). The lack of data on reef fish communities around Lombok has been an obstacle to fisheries and MPA management and to the evaluation of MPA effectiveness in this area. The data on reefs fish distribution and abundance from this study is a snapshot in time, describing the current situation, and can be used to inform management of the coral reef

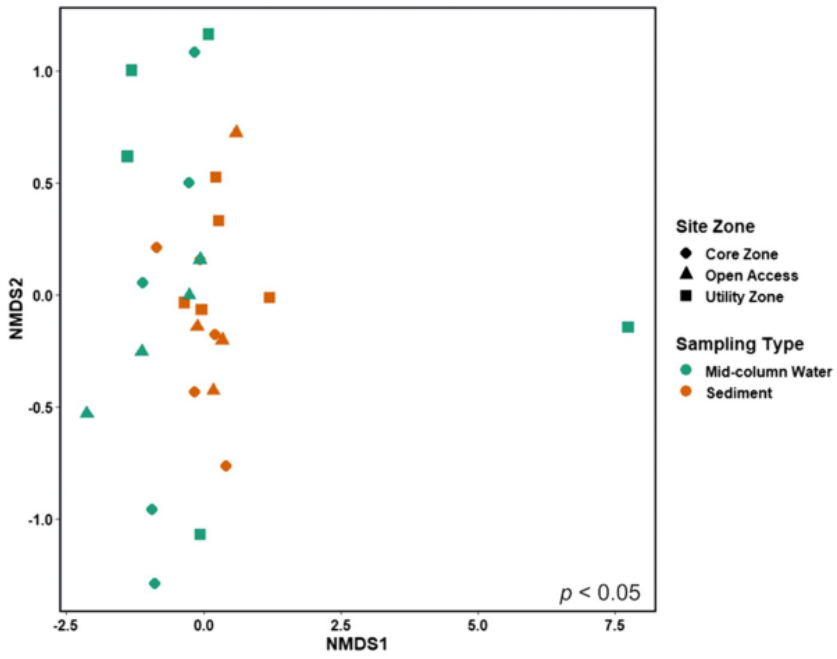

Fig. 8 Nonmetric multidimensional scaling (NMDS) analysis of fish abundance and sampling type based on Bray–Curtis distance between mid-column seawater and sediment samples with stress 0.154

ecosystems and the multi-species fisheries they support. Furthermore, these data can serve as a baseline for regular monitoring using eDNA as a tool to support MPA and fisheries management in the coastal waters of Lombok, and as a model for replication at other data-poor sites facing similar challenges.

Conclusions

The well-marked community structure of coral reef fishes observed in this study illustrates the promise of eDNA for 'next generation' biomonitoring. There were significant differences in species composition between mid-column water and sediment samples, as well as between zones with different levels of protection. Moreover, this study illustrates that eDNA metabarcoding methods can be successfully applied as a fish biomonitoring method in tropical marine waters. Expanding the use of eDNA metabarcoding has the potential to assist conservation and monitoring programs in the tropics by increasing the capacity to record and map species diversity even in remote areas with minimal underwater survey and taxonomic resources.

Acknowledgements This research was supported by USAID through the Sustainable Higher Education Research Alliances (SHERA) Program – Centre for Collaborative Research on Animal Biotechnology and Coral Reef Fisheries (CCR ANBIOCORE).

Author contribution Ester Restiana and Hawis Madduppa conceived the research idea; Ester Restiana, Hawis Madduppa, Beginer Subhan, and Imam Bachtiar collected and processed eDNA samples; Ester Restiana performed laboratory work and data analysis; Ester Restiana and Lalu M Iqbal Sani produced the graphics; Hawis Madduppa and M Mukhlis Kamal led the writing of the manuscript then edited and improved the manuscript together with Lalu M Iqbal Sani and Ester Restina.

References

Ahyadi H, Jufri A (2008) Analisis perubahan ekosistem terumbu karang untuk menunjang pengelolaan kawasan TWAL Gili Indah yang berkelanjutan. Laporan kegiatan riset dan pengembangan daerah. Badan Perencanaan Pembangunan Daerah, Nusa Tenggara Barat, Mataram

Allen GR, Adrim M (2003) Coral reef fishes of Indonesia. Zoological Studies-Taipei 42(1):1–72

Anderson AB, Batista MB, Gibran FZ, Félix-Hackradt FC, Hackradt CW, García-Charton JA, Floeter SR (2019) Habitat use of five key species of reef fish in rocky reef

systems of southern Brazil: evidences of MPA effectiveness. Mar Biodivers 49(2):1027–1036
Andruszkiewicz EA, Koseff JR, Fringer OB, Ouellette NT, Lowe AB, Edwards CA, Boehm AB (2019) Modeling environmental DNA transport in the coastal ocean using Lagrangian particle tracking. Front Mar Sci 6:1–14. https://doi.org/10.3389/fmars.2019.00477
Agardy T, Di Sciara GN, Christie P (2011) Mind the gap: addressing the shortcomings of marine protected areas through large scale marine spatial planning. Mar Pol 2:226–232
Arifin MA, Yulianda F (2003) Coral reef diversity in East Lombok, Nusa Tenggara Barat. Jurnal Ikhtiologi 3:1–8. https://doi.org/10.32491/jii.v3i1.268
Bakker J, Wangensteen OS, Chapman DD, Boussarie G, Buddo D, Guttridge TL, Hertler H, Mouillot D, Vigliola L, Mariani S (2017) Environmental DNA reveals tropical shark diversity in contrasting levels of anthropogenic impact. Sci Rep 1:1–11. https://doi.org/10.1038/s41598-017-17150-2
Barnes MA, Turner CR, Jerde CL, Renshaw MA, Chadderton WL, Lodge DM (2014) Environmental conditions influence eDNA persistence in aquatic systems. Environ Sci Technol 48:819–1827
Barnes MA, Turner CR (2016) The ecology of environmental DNA and implications for conservation genetics. Conserv Gen 17(1):1–17. https://doi.org/10.1007/s10592-015-0775-4
Bell J, Galzin R (1984) Influence of live coral cover on coral-reef fish communities. Mar Ecol Prog Ser 15:265–274. https://doi.org/10.3354/meps015265
Blowes SA, Chase JM, Di Franco A, Frid O, Gotelli NJ, Guidetti P, Knight TM, May F, McGlinn DJ, Micheli F, Sala E (2020) Mediterranean marine protected areas have higher biodiversity via increased evenness, not abundance. J Appl Ecol 57(3):578–589
Bonaldo RM, Pires MM, Guimarães PR, Hoey AS, Hay ME (2017) Small marine protected areas in Fiji provide refuge for reef fish assemblages, feeding groups, and corals. PloS one 12(1): e0170638
Cilleros K, Valentini A, Allard L, Dejean T, Etienne R, Grenouillet G, Iribar A, Taberlet P, Vigouroux R, Brosse S (2018) Unlocking biodiversity and conservation studies in high-diversity environments using environmental DNA (eDNA): a test with Guianese freshwater fishes. Mol Ecol Resour 19(1):27–46. https://doi.org/10.1111/1755-0998.12900
Chabanet P, Ralambondrainy H, Amanieu M, Faure G, Galzin R (1997) Relationships between coral reef substrata and fish. Coral Reefs 16(2):93–102. https://doi.org/10.1007/s003380050063
Cleary DF (2017) Linking fish species traits to environmental conditions in the Jakarta Bay-Pulau Seribu coral reef system. Mar Pollut Bull 122(1-2):259–62
Collette BB, Carpenter KE, Polidoro BA, Boustany A, Die DJ, Elfes C, Fox W, Graves J, Nelson R et al (2011) High value and long life-double jeopardy for tunas and billfishes. Sci J 333:291–292. https://doi.org/10.1126/science.1208730
Collins RA, Wangensteen OS, O'Gorman EJ et al (2018) Persistence of environmental DNA in marine systems. Comm Biol 1:1–11. https://doi.org/10.1038/s42003-018-0192-6
Colwell RK, Chao A, Gotelli NJ, Lin S, Mao CX, Chazdon RL, Longino JT (2012) Models and estimators linking rarefaction, extrapolation and comparison of assemblages. J Plant Ecol 5(1):3–21. https://doi.org/10.1093/jpe/rtr044
Deiner K, Altermatt F (2014) Transport distance of invertebrate environmental DNA in a natural river. PLoS One 9(2):e88786. https://doi.org/10.1371/journal.pone.0088786
Deiner K, Bik HM, Elvira M, Seymour M, Creer S, Bista I, Pfrender ME, Bernatchez L, Altermatt F, Lodge DM et al (2017) Environmental DNA metabarcoding : transforming how we survey animal and plant communities. Mol Ecol 26:5872–5895. https://doi.org/10.1111/mec.14350
Deiner K, Lopez J, Bourne S et al (2018) Optimising the detection of marine taxonomic richness using environmental DNA metabarcoding : the effects of filter material, pore size and extraction method. Metabarcod Metagen 2:1–15. https://doi.org/10.3897/mbmg.2.28963
Dell'Anno AD, Corinaldesi C (2004) Degradation and turnover of extracellular DNA in marine sediments : ecological and methodological considerations. Appl Environ Microbiol 70(7):4384–4386. https://doi.org/10.1128/AEM.70.7.4384
Dell'Anno A, Danovaro R (2005) Extracellular DNA plays a key role in deep-sea ecosystem functioning. Science 309(5744):2179
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 6:8–11. https://doi.org/10.1371/journal.pone.0023398
Dewi CSU, Sukandar HCJ (2018) Karang dan Ikan Terumbu Pulau Bawean. UB Press, Malang (ID)
DiBattista JD, Coker DJ, Sinclair-Taylor TH, Stat M, Berumen ML, Bunce M (2017) Assessing the utility of eDNA as a tool to survey reef-fish communities in the Red Sea. Coral Reefs 36(4):1245–1252. https://doi.org/10.1007/s00338-017-1618-1
English S, Wilkinson C, Baker V (1997) Survey Manual for Tropical Marine Resources. Australia Institute of Marine Science, Townsville (AU)
Eschmeyer WN, Fricke R, Fong JD, Polack DA (2010) Marine fish diversity: history of knowledge and discovery (Pisces). Zootaxa 2525:19–50. https://doi.org/10.11646/zootaxa.2525.1.2
Friedlander AM, Brown E, Monaco ME (2007) Defining reef fish habitat utilization patterns in Hawaii: comparisons between marine protected areas and areas open to fishing. Mar Ecol Prog Ser 6(351):221–233
Galzin R, Planes S, Dufour V, Salvat B (1994) Variation in diversity of coral reef fish between French Polynesian atolls. Coral Reefs 13(3):175–180. https://doi.org/10.1007/BF00301196
Giakoumi S, McGowan J, Mills M, Beger M, Bustamante RH, Charles A, Christie P, Fox M, Garcia-Borboroglu P, Gelcich S, Guidetti P (2018) Revisiting "success" and "failure" of marine protected areas: a conservation scientist perspective. Front Mar Sci 5:p223
Gimmler A, Korn R, Vargas C De, Audic S, Stoeck T (2016) The Tara Oceans voyage reveals global diversity and

distribution patterns of marine planktonic ciliates. Nature 6:33555. https://doi.org/10.1038/srep33555

Gilbey J, Carvalho G, Castilho R, Coscia I, Coulson MW, Dahle G, Derycke S, Francisco SM, Helyar SJ, Johansen T, Junge C (2021) Life in a drop: sampling environmental DNA for marine fishery management and ecosystem monitoring. Mar Pol 124:104331. https://doi.org/10.1016/j.marpol.2020.104331

Goldberg CS, Pilliod DS, Arkle RS, Waits LP (2011) Molecular detection of vertebrates in stream water: a demonstration using rocky mountain tailed frogs and Idaho giant salamanders. PLoS one 6(7): https://doi.org/10.1371/journal.pone.0022746

Goldberg C, Strickler K (2017) eDNA Protocol Sample Collection. Adapted from Protocol Version 04/12/2012 (D.S. Pilliod, R.S. Arkle, and M.B. Laramie) USGS Snake River Field Station. Washington State University. https://s3.wp.wsu.edu/uploads/sites/686/2017/01/WSU-eDNA-sampling-protocol-Jan2017.pdf. Accessed 30 May 2017

Green AL (1996) Spatial, temporal and ontogenetic patterns of habitat use by coral reef fishes (Family Labridae). Mar Ecol Prog Ser 133:1–1

Green HC, Shanks OC, Sivaganesan M, Haugland RA, Field KG (2011) Differential decay of human faecal Bacteroides in marine and freshwater. Environ Microbiol 13:3235–3249

Hadi S, Andayani N, Muttaqin E, Simeon BM, Ichsan M, Subhan B, Madduppa H (2020) Genetic connectivity of the scalloped hammerhead shark *Sphyrna lewini* across Indonesia and the Western Indian Ocean. PLoS One 0230763:1–14. https://doi.org/10.1371/journal.pone.0230763

Hansen BK, Bekkevold D, Clausen LW, Nielsen EE (2018) The sceptical optimist: challenges and perspectives for the application of environmental DNA in marine fisheries. Fish Fish 19(5):1–18. https://doi.org/10.1111/faf.12286

Huhn M, Madduppa HH, Khair M, Sabrian A, Irawati Y, Anggraini NP, Wilkinson SP, Simpson T, Iwasaki K, Setiamarga DH, Dias PJ (2019) Keeping up with introduced marine species at a remote biodiversity hotspot: awareness, training and collaboration across different sectors is key. Biol Inv. https://doi.org/10.1007/s10530-019-02126-2

Hutchings JA (2000) Collapse and recovery of marine fish. Nature 406:882–885. https://doi.org/10.1038/35022565

Jackson JBC, Kirby MX, Berger WH, Bjorndal KA, Botsford LW, Bourque BJ, Bradbury RH, Cooke R, Erlandson J, Warner RR et al (2001) Historical overfishing and the recent collapse of coastal ecosystems. Science 293(5530):629–637. https://doi.org/10.1126/science.1059199

Jerde CL, Mahon AR, Chadderton WL, Lodge DM (2011) "Sight-unseen" detection of rare aquatic species using environmental DNA. Conserv Lett 4(2):150–157. https://doi.org/10.1111/j.1755-263X.2010.00158.x

Jerde CL, Wilson EA, Dressler TL (2019) Measuring global fish species richness with eDNA metabarcoding. Mol Ecol 19:19–22. https://doi.org/10.1111/1755-0998.12929

Kelly RP, Port JA, Yamahara KM, Martone RG, Lowell N, Thomsen PF, Mach ME, Bennett M, Prahler E, Caldwell MR et al (2014) Harnessing DNA to improve environmental management. Science 344:1455–1456. https://doi.org/10.1126/science.1251156

Kruschel C, Schultz ST, Bakran-Petricioli T, Petricioli D (2012) Comparing predator abundance and fish diversity in MPA sites (Kornati NP, Croatia) and adjacent sites exploited by fisheries. Croatian J Fish: 65–78

Lacoursière-Roussel A, Howland K, Normandeau E, Grey EK, Archambault P, Deiner K, Lodge DM, Hernandez C, Leduc N, Bernatchez L (2018) eDNA metabarcoding as a new surveillance approach for coastal Arctic biodiversity. Ecol Evol 8:7763–7777. https://doi.org/10.1002/ece3.4213

Laramie MB, Pilliod DS, Goldberg CS (2015) Characterizing the distribution of an endangered salmonid using environmental DNA analysis. Biol Conserv 183:29–37. https://doi.org/10.1016/j.biocon.2014.11.025

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. Environ DNA 1:1–17. https://doi.org/10.1002/edn3.35

Leray M, Yang JY, Meyer CP, Mills SC, Agudelo N, Ranwez V, Boehm JT, Machida RJ (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. Front Zool 10(1):34. https://doi.org/10.1186/1742-9994-10-34

Leray M, Haenel Q, Bourlat SJ (2016) Preparation of amplicon libraries for metabarcoding of marine eukaryotes using Illumina Miseq: the adapter ligation method. Mar Genom 1452(14):1–10. https://doi.org/10.1007/978-1-4939-3774-5

Levy-Booth DJ, Campbell RG, Gulden RH, Hart MM, Powell JR, Klironomos JN, Pauls KP, Swanton CJ, Trevors JT, Dunfield KE (2007) Cycling of extracellular DNA in the soil environment. Soil Biol Biochem 39:2977–2991. https://doi.org/10.1016/j.soilbio.2007.06.020

Li J, Handley LJL, Harper LR, Brys R, Watson HV, Di C, Xiang M, Bernd Z (2019) Limited dispersion and quick degradation of environmental DNA in fish ponds inferred by metabarcoding. Environ DNA 1:238–250. https://doi.org/10.1002/edn3.24

Li Y, Evans NT, Renshaw MA, Jerde CL, Olds BP, Shogren AJ, Pfrender ME (2018) Estimating fish alpha and beta diversity along a small stream with environmental DNA metabarcoding. Metabarcod Metagen 2:e24262. https://doi.org/10.3897/mbmg.2.24262

Lodge DM, Turner CR, Jerde CL, Barnes MA, Chadderton L, Egan SP, Feder JL, Mahon AR, Pfrender ME (2012) Conservation in a cup of water: estimating biodiversity and population abundance from environmental DNA. Mol Ecol 21:2555–2558. https://doi.org/10.1111/j.1365-294X.2012.05600.x

Madduppa H, Ayuningtyas RU, Subhan B, Arafat DP (2016) Exploited but unevaluated: DNA barcoding reveals skates and stingrays (Chordata, Chondrichthyes) species landed in the Indonesian fish market. IJMS 21:77–84. https://doi.org/10.14710/ik.ijms.0.0

Madduppa HH, Ferse SCA, Aktani U, Palm HW (2012) Seasonal trends and fish-habitat associations around Pari Island, Indonesia: setting a baseline for environmental monitoring. Environ Biol Fish 95(3):383–398

Madduppa HH, Subhan B, Suparyani E, Siregar AM, Arafat D, Tarigan SA, Alimuddin KD, Rahmawati F, Bramandito A (2013) Â Dynamics of fish diversity across an environmental gradient in the Seribu Islands reefs off Jakarta. Biodiversitas 14:17–24

Madduppa HH, von Juterzenka K, Syakir M, Kochzius M (2014) Socio-economy of marine ornamental fishery and its impact on the population structure of the clown anemonefish *Amphiprion ocellaris* and its host anemones in Spermonde Archipelago, Indonesia. Ocean Coast Manag 100:41–50

McCoy CM, Dromard CR, Turner JR (2010) An evaluation of Grand Cayman MPA performance: a comparative study of coral reef fish communities. Proc 62nd Gulf Caribb Fish Ins 337–345

McMurdie PJ, Holmes S (2013) Phyloseq: an R package for reproducible interactive analysis and graphics of microbiome census data. PLoS one. 8:e61217. https://doi.org/10.1371/journal.pone.0061217

Meekan MG, Steven AD, Fortin MJ (1995) Spatial patterns in the distribution of damselfishes on a fringing coral reef. Coral Reefs 14(3):151–61

Meyer CP (2003) Molecular systematics of cowries (Gastropoda: Cypraeidae) and diversification patterns in the tropics. Biol J Linn Soc 79:401–459

Mirimin L, Hickey A, Barrett D, et al (2020) Environmental DNA detection of Arctic char (*Salvelinus alpinus*) in Irish lakes : development and application of a species-specific molecular assay. Environ DNA 221–233. https://doi.org/10.1002/edn3.60

Moore AM, Jompa J, Tassaka ACMAR, Yasir I, Ndobe S, Umar W, Gold Z, Barber PH (2021) Sharks and rays (Chondrichthyes) around Banggai Island, Banggai MPA, Indonesia: biodiversity data from an environmental DNA pilot study. AACL Bioflux 14:725–745

Muniaha H, Andi IN, Ramadhani (2016) Studi kelimpahan ikan karang berdasarkan kondisi terumbu karang di Desa Tanjung Tiram Kabupaten Konawe Selatan. Jurnal Manajemen Sumber Daya Perairan 2(1): 9-19

Murakami H, Yoon S, Kasai A, et al (2019) Dispersion and degradation of environmental DNA from caged fish in a marine environment. Fish Sci https://doi.org/10.1007/s12562-018-1282-6

Mustaruddin LRTS, Pandu S (2013) Penurunan hasil tangkap ikan karang akibat kegiatan industri pariwisata di kawasan Gili Sulat dan Gili Lawang, Kabupaten Lombok Timur. Jurnal Ilmu Dan Teknologi Kelautan Tropis 2(2337):113–120

Nguyen BN, Shen EW, Seemann J, Correa AM, O'Donnell JL, Altieri AH, Knowlton N, Crandall KA, Egan SP, McMillan WO, Leray M (2020) Environmental DNA survey captures patterns of fish and invertebrate diversity across a tropical seascape. Sci Rep 10(1):1–4

Oksanen J, Blanchet FG, Kindt R, Legendre P, Minchin PR, O'Hara RB, Simpson GL, Solymos P, Stevens MH, Wagner H, Oksanen MJ (2013) Package 'vegan.' Comm Ecol Pack 10:631–637

Parks JE, Pomeroy RS, Philibotte J (2006) Experiences and lessons learned from evaluating the management effectiveness of marine protected areas in Southeast Asia and the Pacific Islands. Invited Paper Presentation from the CBD/IUCN International Workshop for Better Management of Protected Areas, Jeju Island, Korea

Pauly D, Christensen V, Guénette S, Pitcher TJ, Sumaila UR, Walters CJ, Zeller D (2002) Towards sustainability in world fisheries. Nature 418(6898):689–695. https://doi.org/10.1038/nature01017

Pesant S, Not F, Picheral M, Kandels-Lewis S, Le Bescot N, Gorsky G, Iudicone D, Karsenti E, Speich S, Troublé R, Dimier C (2015) Open science resources for the discovery and analysis of Tara Oceans data. Nature 2:150023. https://doi.org/10.1038/sdata.2015.23

Pietramellara G, Ascher J, Borgogni F, Ceccherini MT, Guerri G, Nannipieri P (2009) Extracellular DNA in soil and sediment : fate and ecological relevance. Biol Fertil Soils 219–235. https://doi.org/10.1007/s00374-008-0345-8

Prato G, Thiriet P, Di Franco A, Francour P (2017) Enhancing fish Underwater Visual Census to move forward assessment of fish assemblages: An application in three mediterranean marine protected areas. PLoS One 12(6):e0178511

R Core Team (2013). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. http://www.R-project.org/. Accessed 28 Sept 2017

Ramírez-Ortiz G, Reyes-Bonilla H, Balart EF, Olivier D, Huato-Soberanis L, Micheli F, Edgar GJ (2020) Reduced fish diversity despite increased fish biomass in a Gulf of California Marine Protected Area. Peer J 8: p.e8885

Ratnasingham S, Hebert PDN (2007) BOLD: the barcode of life datasystem. Mol Ecol 7:355–364

Sard NM, Herbst SJ, Nathan L, Uhrig G, Kanefsky J, Robinson JD, Scribner KT (2019) Comparison of fish detections, community diversity , and relative abundance using environmental DNA metabarcoding and traditional gears. Environ DNA 368–384. https://doi.org/10.1002/edn3.38

Scianna C, Niccolini F, Gaines SD, Guidetti P (2015) 'Organization Science': a new prospective to assess marine protected areas effectiveness. Ocean Coast Manag 116:443–448

Searle D, Sible E, Cooper A, Putonti C (2016) 18s rDNA dataset profiling microeukaryotic populations within Chicago area nearshore waters. Data in Brief 6:526–529

Setiawan F, Muttaqin A, Tarigan SA, Sabil A, Pinkan J (2017) Pemutihan karang akibat pemanasan global tahun 2016 terhadap ekosistem terumbu karang: studi kasus di TWP Gili Matra (Gili Air, Gili Meno dan Gili Trawangan) Provinsi NTB. J Fish Mar Sci 1:39–54. https://doi.org/10.21776/ub.jfmr.2017.001.02.1

Shaw JLA, Clarke LJ, Wedderburn SD, Barnes TC, Weyrich LS, Cooper A (2016) Comparison of environmental DNA metabarcoding and conventional fish survey methods in a river system. Biol Conserv 197:131–138. https://doi.org/10.1016/j.biocon.2016.03.010

Shelton AO, O'Donnell JL, Samhouri JF, Lowell N, Williams GD, Kelly RP (2016) A framework for inferring biological communities from environmental DNA. Ecol Appl 26:1645–1659

Sigsgaard EE, Broman I, Henrik N, Anders M, Steen K, Knudsen W, Xing Y, Hejl T, Hansen H, Rask P, et al (2017) Seawater environmental DNA reflects seasonality of a coastal fish community. Mar Biol. https://doi.org/10.1007/s00227-017-3147-4

Taberlet P, Coissac E, Hajibabaei M, Rieseberg LH (2012) Environmental DNA. Mol Ecol 21(8):1789–1793. https://doi.org/10.1111/j.1365-294X.2012.05542.x

Taberlet P, Bonin A, Lucie Z, Coissac E (2018) Environmental DNA: For Biodiversity Research and Monitoring. Oxford Press, UK

Takahara T, Minamoto T, Doi H (2013) Using environmental DNA to estimate the distribution of an invasive fish species in ponds. PLoS one 8(2):e56584. https://doi.org/10.1371/journal.pone.0056584

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. https://doi.org/10.1371/journal.pone.0041732

Thomsen PF, Willerslev E (2015) Environmental DNA – an emerging tool in conservation for monitoring past and present biodiversity. Biol Conserv 183:4–18. https://doi.org/10.1016/j.biocon.2014.11.019

Thomsen PF, Møller PR, Sigsgaard EE, Knudsen SW, Jørgensen OA, Willerslev E (2016) Environmental DNA from seawater samples correlate with trawl catches of subarctic, deepwater fishes. PLoS One 11:e0165252. 10.1371/ journal.pone.0165252

Turner CR, Uy KL, Everhart RC (2014) Fish environmental DNA is more concentrated in aquatic sediments than surface water. Biol Conserv 183:93–102. https://doi.org/10.1016/j.biocon.2014.11.017

Tringe SG, Rubin EM (2005) Metagenomics: DNA sequencing of environmental samples. Nature 6(11):805–814. https://doi.org/10.1038/nrg1709

Varkey D, Ainsworth CH, Pitcher TJ (2012) Modelling reef fish population responses to fisheries restrictions in marine protected areas in the coral triangle. J Mar Biol https://doi.org/10.1155/2012/721483

Verma D, Satyanarayana T (2011) An improved protocol for DNA extraction from alkaline soil and sediment samples for constructing metagenomic libraries. Appl Biochem Biotechnol 165:454–464. https://doi.org/10.1007/s12010-011-9264-5

Wahyu TAP, Mubarak H, Rolian DL, Pardede HG, Safitri DR, Putri KZ, Kenedi MI, Shanti AG, Afrizal SN, Santoso NL, Maharani K (2018) Associated of Coral Reef with reef fish in Northern and Southern Gili Air Island and Gili Trawangan Island Lombok. SCiFiMaS 47: p04003 EDP Sciences

Wahyudewantoro G (2018) The fish diversity of mangrove waters in Lombok Island, West Nusa Tenggara Indonesia. Biodivers J Biol Divers 19(1):71–76

Wickham H (2009) ggplot2: Elegant graphics for data analysis. Springer, New York

Zhang S, Zhao J, Yao M (2020) A comprehensive and comparative evaluation of primers for metabarcoding eDNA from fish. Methods Ecol Evol 11(12):1609–1625

Zubcoff J (2012) ANOSIM ANalysis Of SIMilarities. J Nat Lang Process 18(4):367–381

C7. Imam Bachtiar

ORIGINALITY REPORT

5%	2%	5%	2%
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

PRIMARY SOURCES

1	Arief Pratomo, Dietriech G. Bengen, Neviaty P. Zamani, Christopher Lane et al. "Diversity and distribution of Symbiodiniaceae detected on coral reefs of Lombok, Indonesia using environmental DNA metabarcoding", PeerJ, 2022 Publication	2%
2	repository.unja.ac.id Internet Source	2%
3	Submitted to Leiden University Student Paper	2%

Exclude quotes	On	Exclude matches	< 2%
Exclude bibliography	On		

C7. Imam Bachtiar

GRADEMARK REPORT

FINAL GRADE

/0

GENERAL COMMENTS

Instructor

PAGE 1

PAGE 2

PAGE 3

PAGE 4

PAGE 5

PAGE 6

PAGE 7

PAGE 8

PAGE 9

PAGE 10

PAGE 11

PAGE 12

PAGE 13

PAGE 14

PAGE 15

PAGE 16

PAGE 17