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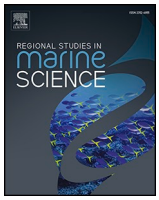

eDNA uncovers hidden fish diversity in the coral reef ecosystems of Karimunjawa National Park, Indonesia

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ABSTRACT

Environmental DNA (eDNA) has emerged as a powerful tool for swiftly assessing coral reef ecosystems, particularly for detecting fish diversity. This study focused on employing eDNA to investigate fish biodiversity and its functional traits in the Karimunjawa National Park (KNP), Indonesia. The use of eDNA and then validating with visual census results to reveal fish diversity was implemented across four management zones within the park (i.e., the core zone at Taka Malang, the protection zone at Menjangan Kecil Island, the tourism zone at Cilik Island, and an open access location at Genting Island). Sampling involved collecting one liter of seawater per site, filtering, and processing to target the 12S locus, and then sequencing using the MinION machine (Oxford nanopore). The eDNA results show higher species diversity in the tourism zone compared to the core, protection, and open access zones. However, beta diversity analysis revealed no significant differences in community composition between the zones. Moreover, this research revealed 147 species belonging to 31 families, with 60 % species and 30 % families identified solely through eDNA, that were not covered by the visual census. This research also reveals that eDNA is an excellent approach to detecting functional trait diversity, including environment preference and migratory and nocturnal behavior. This research underscores the potential of eDNA for evaluating fish diversity in KNP, proposing a combined eDNA and visual census approach to fill existing gaps in biodiversity assessment. Such integration promises to bolster conservation efforts within Marine Protection Areas like KNP.

1. Introduction

A Marine Protected Area (MPA) is an established area to protect ecosystem sustainability and ecological function (Edgar et al., 2014). However, ensuring the effectiveness of an MPA is needed to look after the healthy ecosystem, including monitoring activities (Dunham et al., 2020). MPAs in Indonesia have been monitored to see constituent ecosystem organisms like fish diversity and abundance as healthy ecosystem indicators, including monitoring within the coral reef ecosystems (Wilson and Green, 2009; Madduppa et al., 2013; Putra et al., 2015; Nadia et al., 2018; Yuliana et al., 2020; Ulfah et al., 2021). In addition, comprehensive and accurate management of MPAs is still an issue today.

One issue lies in the inability of current methods, like visual census, to detect all types of species to map the functional diversity (various fish lists to provide their role information on the ecosystems) of all these species (Aglieri et al., 2021). Monitoring and evaluation methods for coral reef ecosystems in Indonesia are still carried out conventionally

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using visual census methods (http://coremap.or.id/; Marwayana et al., 2022). However, the visual census has a weakness in detecting the complexity of limited taxa. For example, using a visual census requires expertise in identification, limited time to conduct a survey (needs to be sunny or daytime) and expensive cost for a survey (Boussarie et al., 2018; Gold et al., 2021). Alternatively, environmental DNA (eDNA) methods and technologies can be used to assess marine biodiversity (Thomsen and Willerslev, 2015), including in the MPA area (Kelly et al., 2014; Deiner et al., 2017; Gelis et al., 2021). In addition, sampling for eDNA could be implemented in any condition that may be difficult to detect by visual census, such as deep-sea (McClenaghan et al., 2020) and surfzone (Gold et al., 2023). Moreover, eDNA could be potentially used to give a new identification that was never detected from a visual census (Polanco Fernández et al., 2021; Muenzel et al., 2024).

eDNA is a method to detect various living organisms (prokaryotic or eukaryotic) that leave traces of their DNA through cells, skin, or any part of the living body itself that contains genetic material (Pilliod et al., 2013). These DNA traces are left in the environment, such as soil, water, and sediment (Williams et al., 2016; Ruppert et al., 2019). eDNA also does not cause environmental damage because it will only take environmental materials such as water without touching organisms contained in that environment (Sahu et al., 2022). eDNA has been widely used to identify the presence of various species (Hunter et al., 2018; Ragot and Villemur, 2022; Ariza et al., 2023), including reef fish (DiBattista et al., 2017; Gelis et al., 2021; Gold et al., 2021; Zamani et al., 2022). Then, several studies also implemented eDNA to survey biodiversity in MPA, such as Gold et al. (2021) MPA in the Scorpion State Marine Reserve off Santa Cruz Island, Gelis et al. (2021) MPA in Lombok Island, and Marwayana et al. (2022) MPA in Raja Ampat.

One of the MPAs in Indonesia with high coral reef diversity including reef fish is Karimunjawa National Park (KNP) (Yuliana et al., 2016). Karimunjawa National Park (KNP), situated in northern Java (Fig. 1), is renowned for its rich biodiversity, particularly in coral reef ecosystems (Campbell et al., 2013; Kennedy et al., 2020). Managed through a zoning system to promote sustainability (Yuliana et al., 2016), KNP's monitoring primarily relies on visual census assessments (2018; Wijayanto et al., 2021). However, to comprehensively evaluate the presence of taxa or species within KNP, a comparative method like eDNA is necessary. Therefore, this research seeks to evaluate environmental DNA (eDNA) use and compile a comprehensive taxonomic list for monitoring endeavors across KNP, mainly based on zoning systems. The study compares eDNA metabarcoding with visual surveys of fish diversity and their functional traits conducted within KNP.

2. Materials and methods

This research used one liter of seawater filtered using Sterivex (Gold et al., 2021) for an eDNA sample, with three samples as replicates for each site around the Karimunjawa National Park (KNP) as primary data. Meanwhile, the visual census data were used as secondary data for comparison. Visual census data were used based on the results report published by the Karimunjawa National Park Agency (BTNKJ) in 2022. However, there were differences in timing between eDNA sample collection and conducting visual census (data retrieved from a report published by BTNKJ). The eDNA sample collection was conducted in August 2022, while the visual census data were based on a report

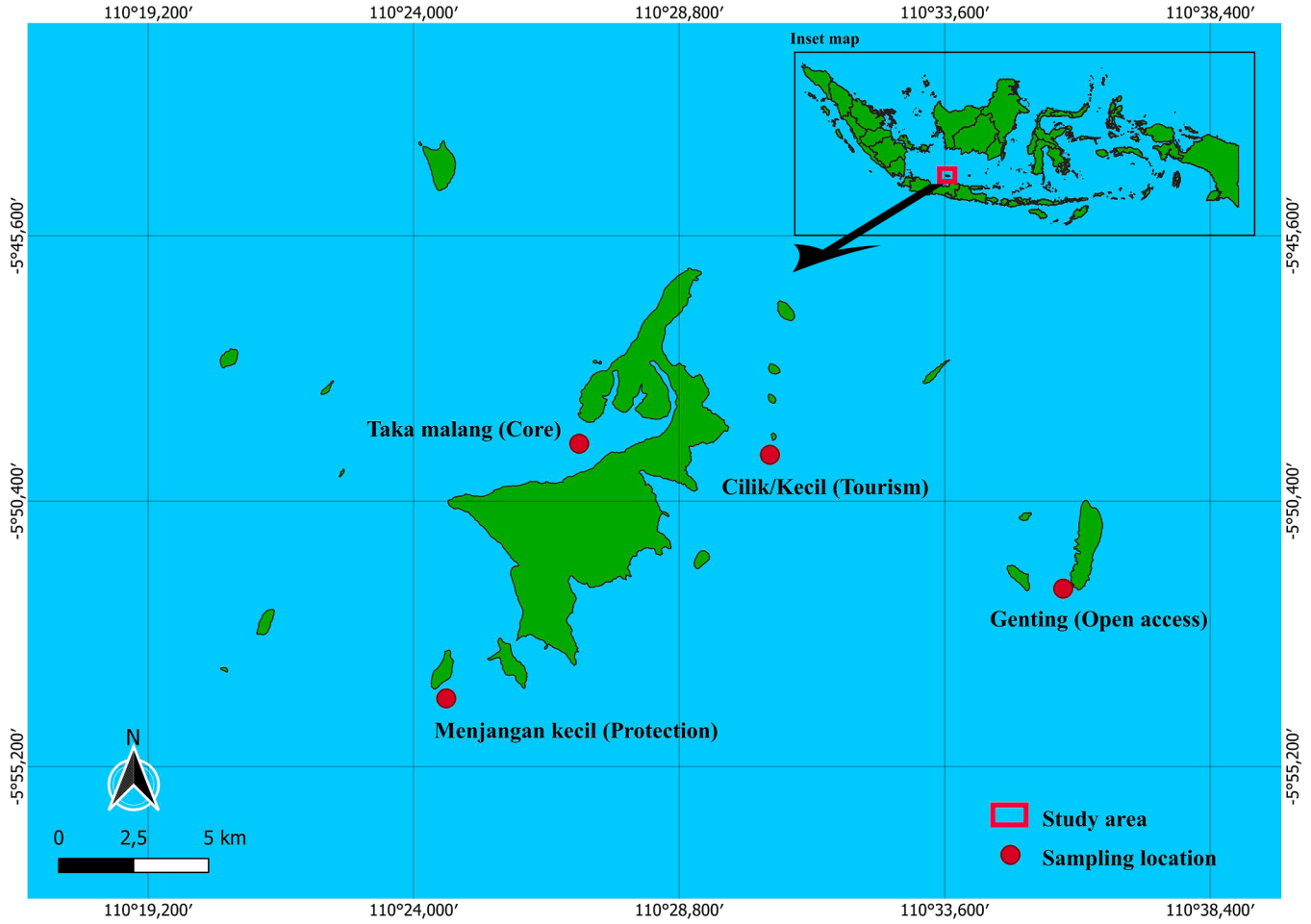

Fig. 1. Research location to take seawater samples using eDNA method with four locations (each location represents zonation see on map).

conducted in March 2022.

2.1. Sample collection

In total, four sampling sites consisting of three representative zones (one site each at the protecting, core, and tourism zones) and one site at the open access zone from KNP (Fig. 1) were used in this study. One liter of seawater was taken at the depth of 3–5 m (shallow) around the coral reef ecosystem by SCUBA diving, and then the seawater was captured using a water bag (gravity feeding bag). Sampling of one liter of water at each location was repeated three times. As a result, a total of twelve samples were collected for this research. The seawater was filtered using sterivex with pore size of 0.22 μm (MilliporeSigma, Burlington, MA, USA) (Gold et al., 2021) on the land (homestay) to keep it sterile. Then, the sterivex filter was pushed using a syringe to ensure the seawater in the sterivex was removed. After filtration, the sterivex filter was given 2 ml of ATL buffer as preservation and stored in the freezer at −20°C until further processing (laboratory process).

2.2. eDNA extraction

Sterivex was extracted using the DNAeasy Tissue and Blood Kit protocol (Qiagen Inc.) to obtain genomic DNA from the sample (gDNA). The extraction process involved the addition of 720 μL ATL buffer and 80 μL proteinase K into sterivex. Then, Sterivex was placed into the rotary shaking incubation machine for 12 hours at 56 °C, after which 200 μL AE buffer was added (Gold et al., 2021). After the incubation process was carried out, all the liquid in the sterivex was taken using a syringe into a 1.7 ml tube with a size of 450 μL. Then, the extraction process was continued by the Qiagen DNAeasy blood and tissue protocol.

2.3. PCR amplification and MinION library preparation for sequencing

After gDNA was obtained from eDNA samples, PCR amplification was conducted to obtain a specific DNA locus target (12S) with MiFish-U for universal fish (Miya et al., 2015), which included a "tail" (short nucleotide has a function to attach the Native Barcoding Kit during nanopore sequencing process) in the locus-specific PCR. Forward (5' TTTCTGTTGGTGCTGATATTGCGCCGGTAAAACTCGTGCCAGC 3'), and reverse (5' ACTTGCCTGTCGCTCTATCTTCCATAGTGGGGTATCTA ATCCCGTTTG 3'). The amplification process was carried out for 25 cycles consisting of pre-denaturation at 95°C for 10 minutes, denaturation at 94°C for 30 seconds, annealing at 50°C for 30 seconds, and extension at 72°C for 1.5 minutes, and final extension at 72°C for 10 minutes.

The PCR amplicon product was sequenced using the Oxford Nanopore Technology (ONT) MinION sequencing template. The DNA library was prepared following the manufacturers' protocols for Native Barcoding Kit 96 V14 (SQK-LSK114). Sequencing was done using the R10.4 flow cell (FLO-MIN112; Oxford Nanopore Technologies) for a total of 24 hours.

2.4. Data analysis

The nanopore signals were basecalled using the MinKNOW software, provided by Oxford Nanopore Technologies (ONT) and embedded in the Guppy pipeline, employing a High-Accuracy model. Adapters and primers were trimmed using Porechop. Then, the process of quality-checking and filtering from the results of the nucleotide sequences generated by using NanoPlot 1.40.0 and Nanofilt 2.8.0 (De Coster et al., 2018). The Phred score of more than 12 or q-12 and read length with a minimum of 150 and maximum of 200 were filtered for further analysis. VSEARCH (Rognes et al., 2016) was used to dereplicate, clustered with de novo, and perform chimera detection. A value of 95 % was set for clustering the reads or Operational Taxonomic Units (OTUs), accounting for a suggested 5 % error rate (Santos et al., 2020).

The in-house reference database has been curated from FishCard (Gold et al., 2021) and combined with taxon lists from the Visual Census report from BTNKJ in 2022. However, there were thirteen species from the visual census report for which no genetic information could be found (both 12S or genome data or see: Supplementary Material Table 1). Taxon classification for sequence data identification used BLAST (blastn algorithm v2.10.1) with minimum threshold values for query coverage alignment to 90 % (van der Reis et al., 2023) and percentage identity to 90 % (Truelove et al., 2019; Munian et al., 2024). All taxon classification names were checked in the public database from FishBase and the World Register of Marine Species (WORM). The output data, including the identification of taxa for each sequence in the sample processed and read as a phyloseq object in R using phyloseq (McMurdie and Holmes, 2013). The Principal Coordinate Analysis (PCoA) plot using the Aitchison distance, and Permutational Analysis of variance (PERMANOVA) have been used to see differences in taxa composition between repeat samples in each zoning in KNP.

Furthermore, only list species from eDNA and visual census results have been filtered to see functional traits in the FishBase database using the 'rfishbase' package in R (Boettiger et al., 2012). Four data parameters extracted from the Fishbase database include Environment, Migratory, Nocturnal, and Tropic preferences (Supplementary Material Table 2).

3. Results

A total of 3881,590 reads were sequenced from the 12 distinct barcodes (12 samples) of the genomic library during a 24-hour run, using a high-accuracy basecalling model. Following the processes of barcode and primer trimming, filtering, dereplicating, clustering, and chimera detection, 193,404 reads remained with 23,643 Operational Taxonomic Units (OTUs) for 12 samples.

The eDNA data comprises 147 fish species, 70 fish genera, 31 fish families, 18 fish orders, and 2 fish classes after assignment with minimum 90 % identity from blastn. Fifteen families have been found from Taka Malang (core), and the three most abundant were Pomacentridae (53.9 %), Spratelloididae (30.3 %), and Apogonidae (7.8 %). There were seventeen families for Menjangan Kecil (protection), and the three most abundant were Pomacentridae (38.7 %), Chaetodontidae (38.6 %), and Spratelloididae (10 %). Then, there were nineteen families for Cilik (tourism), and the three most abundant were Pomacentridae (27.5 %), Spratelloididae (23.5 %), and Chaetodontidae (14.3 %). Moreover, sixteen families of Genting (open access), and the three most abundant were Spratelloididae (57.1 %), Pomacentridae (13.3 %), and Acanthuridae (5.9 %) (Fig. 2).

A comparison of the diversity based on observed and Shannon results between locations has a variety of results that indicate that Cilik (tourism) has the highest diversity, followed by Taka Malang (core) and Genting (open access); then, Menjangan Kecil (protection) has the lowest diversity (Fig. 3). The different test results from ANOVA of alpha diversity between locations indicate a significant difference from the Shannon result (ANOVA-Shannon $= 4.308$, p-value < 0.05). This difference was caused by the significant results (p-value < 0.05) from the Tukey test between Cilik (tourism) and Menjangan Kecil (protection), both from Shannon and also observed results (Table 1).

Beta diversity from fish community composition between locations by a Principal Coordinate Analysis (PCoA) plot using the Aitchison distanceindicated that between locations overlapping each other (Fig. 4). Moreover, the result from PERMANOVA and Dispersion homogeneity tests (Betadisper) indicated non-significant differences between locations (p-value > 0.05) (Table 2).

Table 1
Alpha diversity difference test from OTUs diversity for each zones using an Analysis of Variance (ANOVA) and Tukey HSD.

ANOVA								
					F value			Pr (>F)
Observed					3.761			0.059
Shannon					4.308			0.044*
Tukey HSD								
Zonation	Observed				Shannon			
	Diff	Lwr	Upr	P adj	Diff	Lwr	Upr	P adj
Open access-Core	−1229.0	−6300.7	3842.7	0.863	−0.297	−1.471	0.876	0.847
Protection-Core	−3256.6	−8328.4	1815.1	0.245	−0.597	−1.771	0.576	0.415
Tourism-Core	1919.0	−3152.7	6990.7	0.637	0.660	−0.513	1.834	0.338
Protection-Open access	−2027.6	−7099.4	3044.1	0.598	−0.299	−1.473	0.874	0.844
Tourism-Open access	3148.0	−1923.7	8219.7	0.268	0.958	−0.215	2.132	0.114
Tourism-Protection	5175.6	103.9	10247.4	0.045*	1.258	0.084	2.431	0.036*

* Significant value (p-value < 0.05)

Table 2
Beta diversity difference test from community composition based on all ASVs for each zones using a PERMANOVA and BETADISPER.

adonis PERMANOVA					
	Df	Sum of Sqs	R2	F	Pr(>F)
Zone	3	62831	0.316	1.2346	0.087
Residual	8	135713	0.683		
Total	11	198544	1.000		
Multivariate homogeneity of group dispersions test (BETADISPER) with 999 permutations					
	Df	Sum Sq	Mean Sq	F	Pr(>F)
Groups	3	1424	4747	1.895	0.221
Residual	8	2003	2503		

3.1. Comparison between eDNA and visual census in detecting fish species and functional traits

In total eDNA could detect 147 fish species and visual census 153 species from four sites (Fig. 5a or see the detail in Supplementary Material Table 3). Visual census had higher species detection than eDNA in three locations such as Menjangan Kecil (protection zone) with 56 species for eDNA and 81 species for visual census, Cilik (tourism zone) with 82 species for eDNA and 93 species for visual census and Genting (open access) with 71 species for eDNA and 82 species for visual census. Only sites from Taka Malang (core zone) eDNA could detect more species (with 71 species) than visual census (with 70 species). Overall, eDNA could detect 61 species (40 %), the same as the visual census in all locations. However, both eDNA and visual census can detect unique species that cannot be detected by each other (85 species for eDNA and 93 species for visual census). Furthermore, eDNA could detect up to 70 % of the family same with visual census in all four locations from this study (see: Supplementary Material Fig. 1).

The functional traits information from the FishBase database revealed that this result revealed species of fish from eDNA detection could get a variety of environment preferences compared to visual censuses that only detected reef-associated fish. Overall, demersal, benthopelagic, and pelagic-neritic fish are underrepresented in visual census data. eDNA could detect two species from Taka Malang (core) and two species from Cilik (tourism) that have a preferred habitat as demersal. One species from Cilik (tourism) has a preference for benthopelagic. One species from Taka Malang (Core), one species from Menjangan Kecil (protection), two species from Cilik (tourism), and two species from Genting (open access) have been detected as pelagic-neritic species. In addition, one species from Menjangan Kecil (protection) has detected bathypelagic fish (Fig. 6).

Species for migratory preference eDNA could not only detect species that have non-migratory category but also could detect species oceanodromous with 3 species from Taka Malang (core), four species from Menjangan Kecil (protection), six species from Cilik (tourism) and two species from Genting (open access), and also amphidromous with one species from Taka Malang (core). Moreover, the visual census also got migratory preference besides the non-migratory category, which was oceanodromous: one species from Menjangan Kecil (protection), three species from Cilik (tourism), and one species from Genting (open access). Then, the trophic preference result got both eDNA and visual census results equal to that of the preferred trophic with three variances: carnivore, herbivore, and omnivore. However, the unknown preferred trophic was the highest from eDNA data and visual census data (Fig. 6).

The eDNA results revealed a higher domination of nocturnal fish than visual census results from all locations except Cilik (tourism). Taka Malang (core) has seven nocturnal fishes from eDNA and three nocturnal fishes from the visual census. Menjangan Kecil (protection) has three nocturnal fishes from eDNA and one nocturnal fish from the visual census. Cilik (tourism) has only one nocturnal fish from eDNA and four nocturnal species from the visual census. Furthermore, Genting (open access) has six nocturnal species from eDNA and one nocturnal species from the visual census (Fig. 6).

4. Discussion

This study revealed that eDNA has potentially explored comprehensive fish biodiversity in coral reef ecosystems. Understanding species diversity is essential in gaining effective conservation practices, and management strategies within coral reef ecosystems (Madduppa et al., 2021). The use of eDNA represents a valuable modern approach to studying biodiversity across diverse habitats. Several studies have provided evidence of how the eDNA successfully detects fish diversity in several coastal ecosystems, such as Coral reef ecosystems in Lombok Island (Gelis et al., 2021) and Tidung Kecil Island (Zamani et al., 2022), Mangrove ecosystems in Peninsular Malaysia (Zainal Abidin et al., 2022), and Seagrass beds in Southern California (Waters et al., 2023). This study detected 147 fish species from 31 families from four locations of different representative zones in the Karimunjawa islands through eDNA in shallow waters from coral reef ecosystems. The result in this study was higher than that of a dry season in Tidung Island, with only 27 species of 17 families. However, this result was lower than the rainy season in Tidung Island, with 209 species of 56 families (Zamani et al., 2022).

Two fish families have always been found in all locations and dominate the top three relative abundance groups of this eDNA result: Pomacentridae and Spratelloididae. Damselfishes (Pomacentridae) are a fish that always inhabit coral reef ecosystems, and this fish presence could play a significant role in coral reef ecosystems (Ormond et al., 1996). The presence of damselfish can enhance coral growth by providing cleaning services and nutrient input caused by feeding algae (herbivores) that maintain coral reef health from competitors (Holbrook et al., 2008). The coral reef ecosystems are also beneficial for damselfish for refuge to avoid their predators (Holbrook and Schmitt, 2002). The

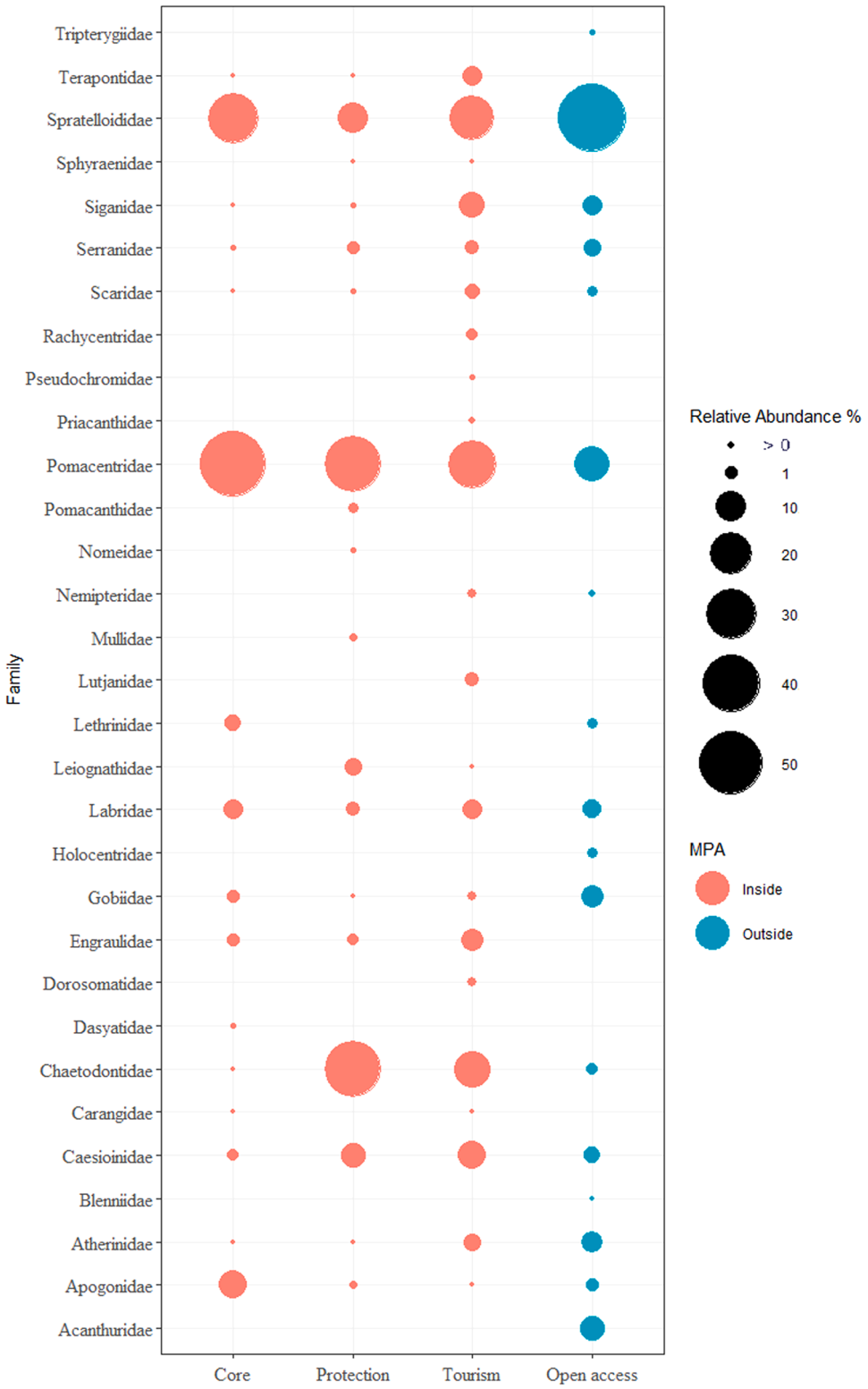

Fig. 2. Bubble plot based on Family for four zones based on the number of reads. The bubble plot was constructed based on family contributing data of the relative abundance reads at each location, and the color indicated is for locations within Marine Protection Area (MPA) and non-MPA.

family from Pomacentridae has also been found in metabarcoding eDNA conducted by Andriyono et al. (2021) in Palabuhanratu Bay. Furthermore, Spratelloididae is a reef-associated fish (Esmaeili and Echreshavi, 2023), and has been reported to be found in several locations in Indonesia, such as South Sulawesi (Erftemeijer and Allen, 1993), Lombok (Mahrus et al., 2022), and Seribu Islands (Simanjuntak et al., 2020). This fish can be used as seafood (Nasution et al., 2019) or as fishing bait for tuna (Milton et al., 1991).

eDNA has been known as a tool for comprehensive biodiversity assessment to support conservation efforts (Nguyen et al., 2020) in Marine Protected Areas (MPAs), especially in this study that focused on one of the MPA in Indonesia from Karimunjawa National Park (KNP).

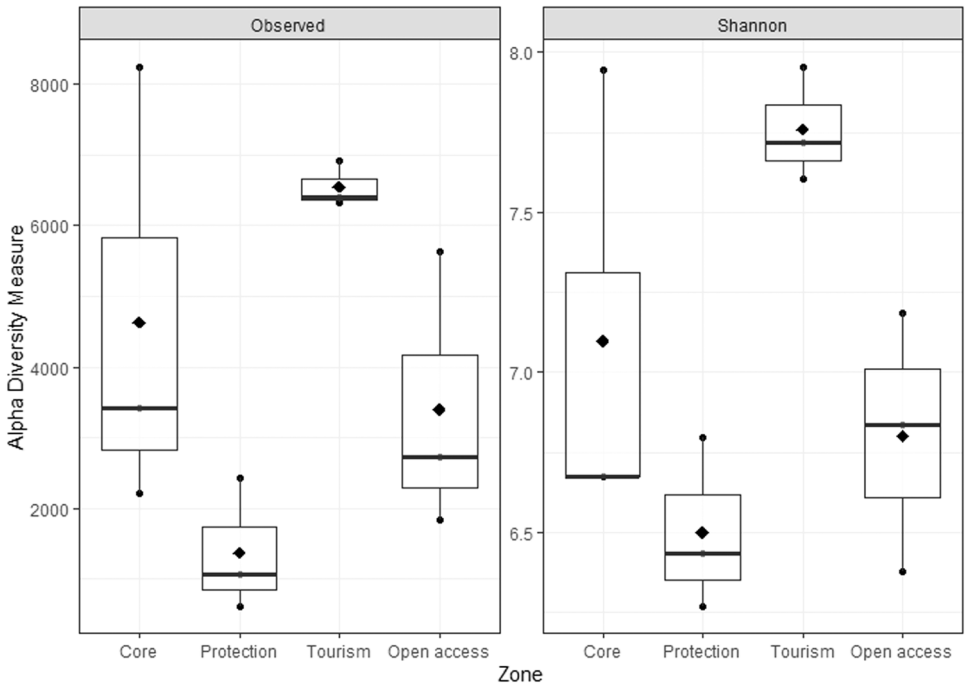

Fig. 3. Operational Taxonomic Units (OTU) richness and diversity from using the 12S rRNA gene in each zonation. The median is shown by the solid line across the box while the mean is indicated by the black diamond.

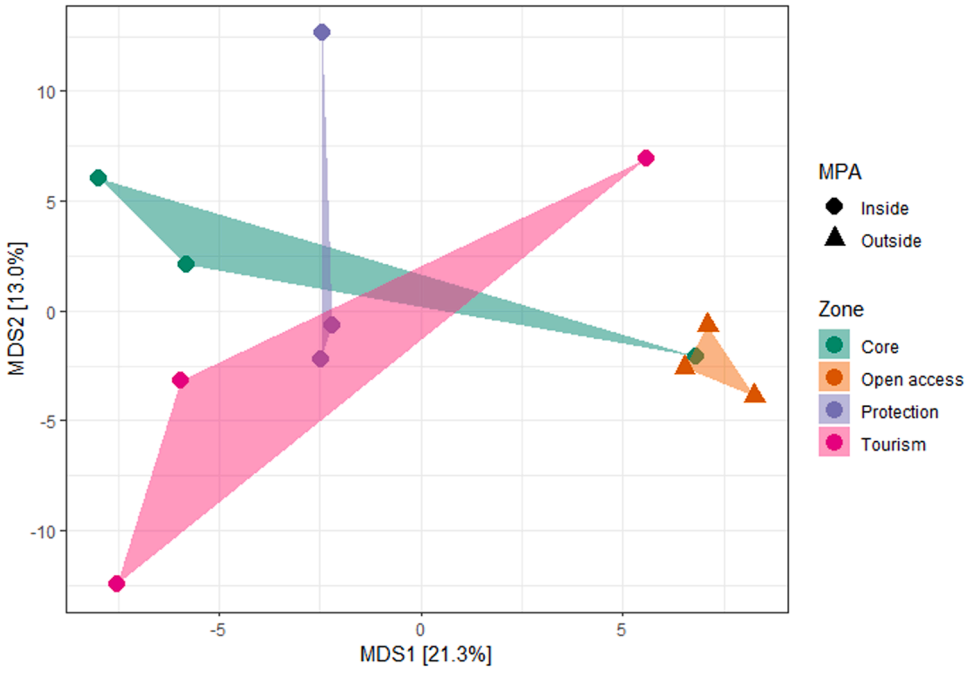

Fig. 4. Principal Coordinate Analysis (PCoA) plot based on the data from Indonesian OTUs only.

Although this result has a significant difference in alpha-diversity (Observed and Shannon) between the tourism zone and protection zone, this result indicated that the tourism zone (Cilik) has a higher abundance of OTUs than other zones in this study, such as the core zone (Taka Malang), protection zone (Menjangan Kecil), and open access (Genting). However, a study from Gelis et al. (2021) in coral reef ecosystems showed that the core zone has significantly higher reef fish diversity than the utility and open access zone in Lombok.

According to a survey by Hartati et al. (2018) and Muhidin et al. (2022), the highest abundance of invertebrates in the Tourism zone (including Cilik Island) was Sea Urchins (Family: Diadematidae). The presence of sea urchins, with their balancing numbers, would be beneficial for healthy coral reef ecosystems due to their behavior of feeding on algae, thereby reducing potential coral reef competitors and promoting coral growth (McClanahan et al., 1996). The balanced growth of sea urchins is also affected by the presence of their predators in this area, such as Labridae (Figueiredo et al., 2005) found in both eDNA and Visual census, and also Balistidae (Tebbett and Bellwood, 2018) only found in visual census. This ecological stability in the area has resulted in coral cover in this region reaching around 60 % in the Tourism area

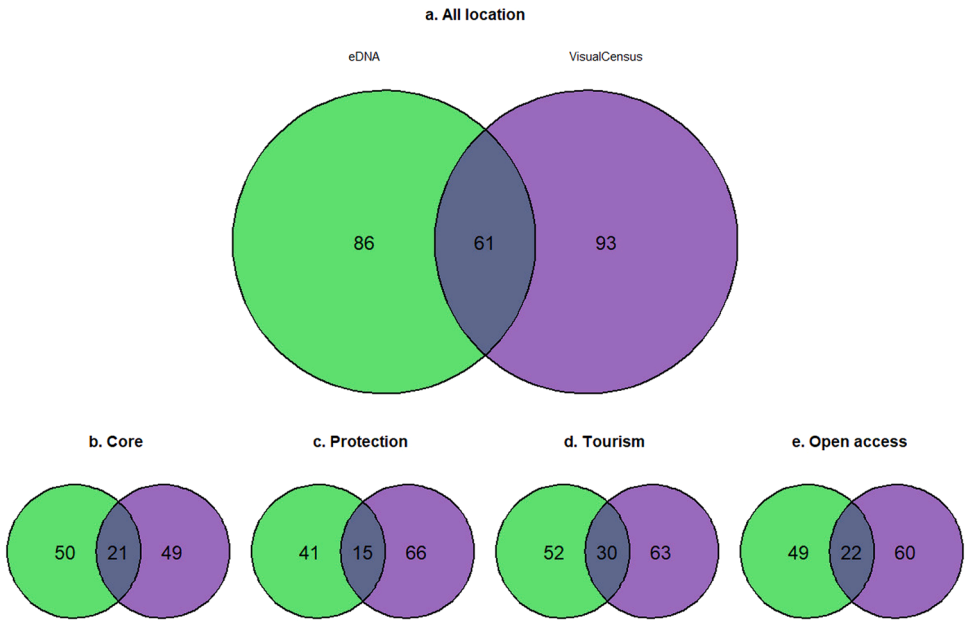

Fig. 5. Species from eDNA versus Visual census, (A) all locations, (B) Core, (C) Protection, (D) Tourism, (E) Open acces.

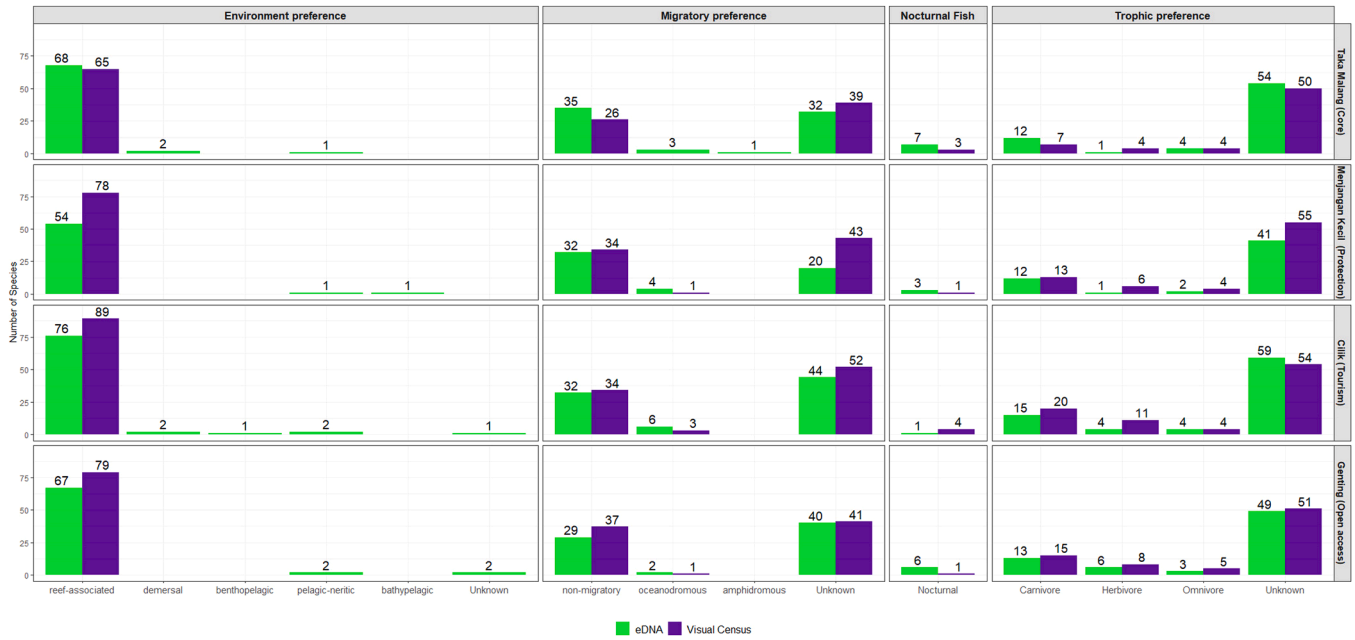

Fig. 6. Number of fish from Four functional traits (Environment, Migratory, Nocturnal, and Tropic preferences) between eDNA and visual Census.

(including Cilik Island) under good conditions (Muhidin et al., 2022), and the good condition of the coral reef ecosystem will sustain biodiversity within the region.

The 12S locus to detect fish has been widely used to detect fish diversity (Stoeckle et al., 2017; Marwayana et al., 2022; Munian et al., 2024). However, the in-house reference database information from this research showed that 12 species from the visual census report in Karimunjawa islands have no sequence information either from 12S or whole genome information. The quality of eDNA results will be limited when an inadequate database is possessed (Pascher et al., 2022). In addition, uncertainties exist concerning sensitivity and accuracy in detecting individual species using metabarcoding eDNA, which may be hindered by incomplete reference databases, marker resolution, and amplification biases, particularly in complex samples from diverse communities (Briski et al.,2016; Hatzenbuhler et al., 2017; Leray and Knowlton, 2017). As a result, limited reference database coverage can decrease the taxonomic resolution of eDNA studies.

4.1. eDNA as a complement data for detecting variety species and functional traits diversity

These findings reveal that eDNA could be a valuable solution for detecting a broader range of fish species that visual censuses might miss due to their procedural limitations related to time and conditions. Visual censuses can only detect species physically observed by human observers, and they are often conducted during daylight hours. In contrast, eDNA detects DNA traces left by animals in their surroundings (Deiner et al., 2017), which can persist for several hours to days and are

detectable in water samples (Thomsen et al., 2012; Collins et al., 2019). Therefore, even though eDNA sampling was conducted only during daylight in the coral reef ecosystem and in shallow water, it successfully detected a greater variety of trait diversity compared to visual censuses. Then, even comparing results from different times in resulting data (eDNA in August 2022 and visual census in March 2022), both eDNA and visual census have the same dominance in detecting reef-associated fish.

This result found that although eDNA has less detecting number of fish species than visual census in almost all four locations (Fig. 5), except Taka Malang (core), eDNA could give a new spectrum of species variety that is not detected yet by the visual census. In this study, at least 60 % or 86 of the species and 30 % or 12 of the families were produced from eDNA, which is new and has not yet been listed in the visual census of four locations in the Karimunjawa islands. eDNA could provide new data for families (6 of 12; Atherinidae, Carangidae, Engraulidae, Leiognathidae, Nomeidae, and Rachycentridae) living as pelagic fish (Shaffer and Nakamura, 1989; Zaragoza et al., 2004; Potier et al., 2008; Seah et al., 2009; de Morais et al., 2016) and families (3 of 11; Spratelloididae, Gobiidae and Tripterygiidae) living as small and cryptic (Milton et al., 1991; Tornabene et al., 2013; Esmaeili et al., 2022) that could be difficult to detect from visual census.

The comparison study between eDNA and visual census has been implemented under several locations. A study by Marwayana et al. (2022) compared eDNA and visual censuses to detect fish diversity in coral reef ecosystems in Raja Ampat. The study revealed that eDNA could capture new data up to 57.1 % (mean value) of species list that is not present in visual census results. Another result from detecting fish species by Munian et al. (2024) that was conducted in freshwater from Malaysia revealed that eDNA could bring a new species data list to 54 % that was never listed with the visual census. A higher percentage of the same detection from eDNA and visual census occurred by Gold et al. (2021) in the Scorpion State Marine Reserve off Santa Cruz Island, who found that eDNA could capture 76 % of species and 95 % of genera, the same as with the visual census. Then, a study conducted by Lee et al. (2022) showed that eDNA could detect 80 % of the same species with visual census results in Korea. Nonetheless, this study and the studies referenced above (Gold et al., 2021; Lee et al., 2022; Marwayana et al., 2022; Munian et al., 2024) also confirm that eDNA cannot capture the entire taxon list obtained through visual census. This finding underscores the necessity of combining eDNA with visual census to maximize the inventory or representation of biodiversity (Muenzel et al., 2024) from various locations, including conservation areas.

Although eDNA could potentially detect new data sets that have never been recorded through visual censuses. It also has the capability to identify a variety of functional traits and diversity. This study found that eDNA could detect fish with a preference for more varied that not only reef-associated fish but also pelagic fish, such as demersal, benthopelagic, pelagic-neritic, and bathypelagic habitats. This contrasts with visual censuses, which only detected reef-associated fish species. Additionally, eDNA identified a greater variety of migratory fish and a higher number of nocturnal fish than visual censuses. A study from Aglieri et al. (2021) from the subtidal rocky zone in the Central and the Western Mediterranean Sea demonstrated a comparable outcome, indicating that eDNA detection could identify a diverse range of functional traits in fish than other visual methods (underwater visual census strip transects, baited underwater videos, and small-scale fishery catches, including those that are facultative or obligate schoolers and pelagic fish.

4.2. Conservation implication

eDNA analysis has emerged as a valuable tool for conducting comprehensive biodiversity assessments, particularly for fish. Accurate taxonomic assignments are crucial because they directly influence the biotic indices generated and the overall ecological assessments (Cahyani et al., 2024). These assessments are vital for devising effective management strategies in conservation areas. The use of eDNA enables researchers to detect a wide array of species, including those that are typically challenging to observe due to factors such as elusiveness, crypticity, nocturnal habits, or migratory behaviors. Integrating eDNA analysis and visual census can enhance data accuracy, ensuring a more exhaustive biodiversity inventory within Marine Protected Areas (MPAs) like Karimunjawa National Park (KNP) in Indonesia, managed through a zonation system. Visual censuses have traditionally served as the primary tool for monitoring marine biodiversity and evaluating management strategies across different zones within KNP. However, this study represents the first comparison of eDNA analysis with visual censuses within the KNP, highlighting the potential of combining these methodologies to gain a more nuanced understanding of biodiversity and inform conservation management practices. Moreover, the cost-effectiveness of eDNA analysis (Munian et al., 2024) enables more frequent and extensive monitoring endeavors, with the integration of portable machine sequencing technologies such as Oxford Nanopore facilitating rapid and scalable data processing.

5. Conclusion

The utilization of eDNA in Karimunjawa National Park (KNP) has proven to detect 147 fish species across 31 families, showcasing diversity and functional traits across four zones: the core zone at Taka Malang, the protection zone at Menjangan Kecil Island, the tourism zone at Cilik Island, and open access at Genting Island. Moreover, eDNA analysis covered 40 % of the same species and 70 % of the same families as visual census methods. Notably, eDNA analysis revealed a wider array of functional traits compared to visual census methods, including environmental preferences, migratory patterns, and nocturnal behaviors. Overall, the integration of eDNA analysis alongside traditional visual censuses offers a promising approach for enhancing biodiversity assessments within Marine Protection Areas like KNP, providing practical advantages in terms of data processing capabilities, and holding significant potential for bolstering the effectiveness of conservation efforts.

CRediT authorship contribution statement

Aji Wahyu Anggoro: Writing – review & editing. **Rian Prasetia:** Writing – review & editing. **Eka Maya Kurniasih:** Writing – review & editing, Data curation. **Ni Kadek Dita Cahyani:** Writing – review & editing, Validation, Resources, Project administration, Methodology, Funding acquisition, Data curation, Conceptualization. **Muhammad Danie Al Malik:** Writing – original draft, Visualization, Methodology, Formal analysis, Data curation, Conceptualization. **Yuliana Syamsyuni:** Writing – review & editing, Data curation. **Fauzi Muh:** Writing – review & editing, Methodology. **Nining Nursalim:** Writing – review & editing, Methodology, Data curation. **Nenik Kholilah:** Writing – review & editing, Methodology, Data curation. **Ambariyanto Ambariyanto:** Writing – review & editing, Supervision. **Retno Hartati:** Writing – review & editing, Supervision.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.rsma.2024.103945.

Data availability

All data used have been submitted to public databases such as NCBI and GitHub Repository. The accession number and link have been attached to the data availability section of this manuscript.

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