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Environmental DNA metabarcoding reveals biodiversity marine fish diversity of a small island at Manokwari District, West Papua, Indonesia

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Abstract. *Pranata B, Kusuma AB, Sabariah V, Kim HW, Andriwiyono S. 2022. Environmental DNA metabarcoding reveals biodiversity marine fish diversity of a small island at Manokwari District, West Papua, Indonesia. Biodiversitas 23: 5982-5988.* The uniqueness of small island biodiversity becomes very important to study. Molecular approaches to collecting biodiversity information are currently quite developed. Manokwari district, which is located at the head of Papua Island, Indonesia, has very diversity of marine resources, especially coral reef fish. Currently, we successfully identified marine fish in the coral reef fish ecosystem of Lemon and Mansinam Island. Environmental DNA on Lemon Island and Mansinam Island was successfully carried out. This small island area is located in Manokwari District, Papua, which has a high biodiversity potential, including fishery resources. The MiFish pipeline is used in this Metabarcoding approach by combining water samples from Lemon Island and Mansinam Island. We filtered 1 liter of marine water and pool together from fourteen sampling sites for genomic DNA extraction. A total of 101,001 reads (88.31%) were assigned to 34 species. From the environmental DNA metabarcoding analysis results, the Pomacentridae (8 species), which are reef fish, dominate in this area. Besides, Acanthuridae, Carangidae, and Lutjanidae were identified in this area, which as coral reef fish associates. The results of this environmental DNA also identified 2 species from the Family Scombridae, namely *Gymnosarda unicolor* and *Thunnus obesus*. This information is expected to support the sustainable management of the Lemon Island and Mansinam Island Areas for tourism and conservation activities.

Keywords: Conservation, coral reef fish, diversity, environment, management, metabarcoding

INTRODUCTION

One indicator of the health of an aquatic ecosystem is the diversity of aquatic animals that live in the area, such as fish. This shows that these waters play an important role in providing habitat and primary productivity that supports life (Díaz-Pérez et al. 2016; Eugenia et al. 2019). Especially in coral reef ecosystems, the life of reef fish is an important part. It supports the symbiosis between the coral ecosystem and the biota that live around it, including reef fish. In addition, fish biodiversity is also very important in evaluating environmental conditions (Ahn et al. 2020). The condition of biodiversity found in an area is important information for understanding the ecological processes and interactions that are formed, including studies that are important in supporting the monitoring of environmental damage from its biological aspect (Leray et al. 2013). One of the important marine areas in the waters of Manokwari District is Lemon Island and Mansinam Island. Several reports on this island’s biodiversity were reported, such as microalgae diversity (Ayhuan et al. 2017), including coral reef composition between two areas (Sitanala et al. 2022).

The study of fish diversity in this area is still very limited. Currently, the coastal areas of these islands are only used as traditional fishing locations with the types of grouper and reef fish. Previous research has also revealed that the walking shark (*Hemiscyllium galei*) is also found in this area (Mangubhai et al. 2012). In addition to fishing activities, this area is still being visited by domestic tourists for spiritual tourism activities with historical relics that have received enough attention from the local community (Ihalauw et al. 2016). Domestic tourists who visit the two islands are due to the close access from the district capital. The impact of this activity is felt by the presence of domestic waste in the form of plastic waste and organic materials, which put pressure on the aquatic ecosystem of Mansinam Island and Lemon Island.

One of the studies on the impact of coral reef damage has been carried out previously. Coral reef ecosystems on both islands show very low-cover coral reefs and fall into the category of fairly severe damage (Dasmasela et al. 2019), and Lemon Island shows more degraded by several human activities (Sitanala et al. 2022). Thus, there is a need for other supportive studies to collect a database of the diversity of reef fish species that can still be found in this archipelago. Previous research has documented the types of fish in the Mansinam Island area and found 15 genera (unpublished). However, the study approach is still carried out with conventional studies such as underwater visual censuses and surveys of local fishermen's catches. Several approaches in collecting biodiversity data are by conducting surveys. In coral reef ecosystems, the most common approach is the underwater visual census (UVC) (Pinheiro et al. 2016; Polanco-Fernández et al. 2021). In addition, the use of fishing gear such as nets (Lodge et al. 2012), trawl (Thomsen et al. 2016), anglig (Thomsen et al. 2012), echo-sounding (Yamamoto et al. 2016) and other fishing gear can be used but has several disadvantages such as time and cost that are quite large. Another approach that is currently developing is the molecular approach. Research on the application of molecular approaches has been successfully carried out by many researchers by taking DNA samples from the environment, which is known as Environmental DNA (eDNA). Research on biodiversity in the areas of Lemon Island and Mansinam uses the approach eDNA application through metabarcoding has never been done.

Environmental DNA has been widely applied in studies in collecting biodiversity data. The environmental DNA approach collects genetic material released from organisms into the environment, which is then analyzed more quickly and at a relatively low cost when compared to direct survey methods (Coulter et al. 2019; Harper et al. 2019; Bessey et al. 2020). This method has received considerable attention for ecological studies (Ahn et al. 2020). Environmental DNA applications in coral reef fish community monitoring studies have been carried out in previous studies (Andriyono et al. 2019; Andriyono et al. 2021; Gelis et al. 2021, Zuhdi et al. 2021). The data that can be collected includes species diversity and chordate composition in various taxa, including mollusks and echinoderms (Madduppa et al. 2021), as well as the diversity of reef fish species (Ahn et al. 2020). By looking at the advantages of this method, environmental DNA is a potential alternative for analyzing fish species diversity (Thomsen et al. 2012).

MATERIALS AND METHODS

Sample collection and environmental-DNA extraction

The eDNA water samples were collected from five stations in Pulau Lemon dan nine stations in Mansinam, Manokwari District, West Papua, Indonesia, on March-April 2021 (Figure 1).

Seawater was collected from a depth of about 1 to 17 meters (Govindarajan et al. 2021; Monuki et al. 2021). One liter of water was at each sampling site and each filter paper (Andriyono et al. 2019; Andriyono et al. 2021), then the genomic extraction from 14 samples were pooled (same volume for each sample) for further analysis. All the filters were immediately stored in ice until brought to the laboratory at Universitas Papua for filtration. Every single filter 0.45 µm pore-sized GN-6 membrane (PALL Life sciences, Mexico) for one liter of the water sample, then the filters were kept and mixed into 2.0mL tubes with DNA Shield for further analysis. Prevention, the cross-contamination in all steps was conducted by washing up with 10% commercial bleach and 70% ethanol for all filtration equipment. The membrane filters gSYNC™ Geneaid were used for the genomic DNA extraction process, according to the company's guidelines. Homogenizing of the membrane filters by TissueLyser II motorized homogenizer (QIAGEN, Hilden, Germany), the quantification of the extracted genomic DNA was measured by using ND-1000 NanoDrop (Thermo Scientific, Waltham, MA, USA). The water quality has been measured. Water temperature, pH value, and TDS were measured by a conductivity meter (CD-4307SD, LUTRON), then salinity was measured by a refractometer manual (ATAGO).

Construction of library and MiSeq sequencing

The MiFish universal primer sets were used to construct the amplicon libraries of partial 12S rRNA markers (Miya et al. 2015). Library concentrations were estimated using the Qubit dsDNA HS assay kit and Qubit fluorometer (Life Technologies). The double-stranded DNA concentration from the pooled library was adjusted to 4 nM (assuming one bp equals 660 g mol^{-1}) using Milli-Q water and 5L from the 4 nM library denatured with 5L NaOH 0.1 N. Including the HT1 buffer (provided by the Illumina MiSeq Reagent v. 2 kit for 2 × 150 bp PE), the denatured library (10L; 2 nM) was diluted to a final concentration of 12 m for sequencing on the MiSeq platform. 30L of control DNA spike PhiX (12 m) was added to improve the data quality of low-diversity samples, such as the single PCR amplicon used in this study.

Bioinformatics analysis of NGS data

Before uploading NGS raw data to the MiFish pipeline, Phyton27 (an open-source software) was used to make the pairing of both reverse and forward sequences with the specific script (Zhang 2015). In the MiFish pipeline, the raw reads by MiSeq sequencing run FASTQC, which will be trimmed for the low-quality tail of reads (QV≤20). After that, several steps include assembled paired-end reads and followed by removed N-containing reads, filtered reads by length (~229 bp), run the Usearch® (0.99 for clustering of identity, and 10 for minimum read size for filtering), BLASTN based on GenBank database, and then created multi-FASTA files for each sample. The next step is to run MAFFT, run the Morphy for each sample, run the Morphy against the merged sample, run BLASTN, and finalization of the last process by BLASTN. The entire sequences stipulated to representative genotype by compared to the GenBank database, then the sequences were ascertained as '*species*', '*genera*', and '*unknown or unidentified*' level if the sequence identity more than or similar to 99%, 97-98%, and less than 97%, respectively. The distribution for each species was confirmed by the FishBase (http://www.fishbase.org/) then taxonomic nomenclature was approved under the World Register of Marine Species, WORMS (http://www.marinespecies.org/).

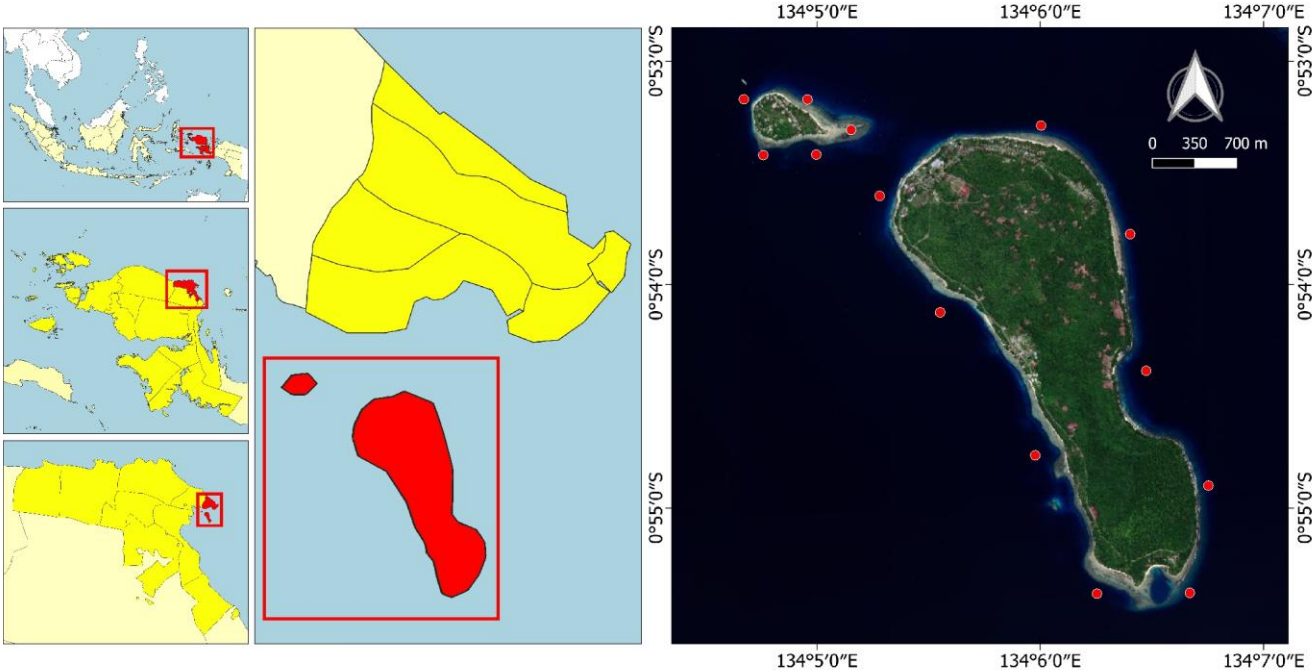

Figure 1. Distribution of sampling site at Pulau Lemon and Mansinam, Manokwari District, West Papua, Indonesia

Statistical analysis in biodiversity indices

The measurements of alpha biodiversity were carried out for the average read number data in each sampling location. Analyses of the index on alpha diversity include the index of Shannon-Wiener (H'), which informs of heterogeneity diversity or total species richness in certain areas (Magurran 1988; Gray 2000) and Margalef diversity index (d). The H' index, Margalef index (d), and Pielou's evenness index was calculated using PRIMER7® software v7 (Clarke and Gorley 2015a). The phylogenetic tree was performed by MEGAX (Kumar et al. 2018) and cluster based on Family.

RESULTS AND DISCUSSION

Physico-chemical parameters

Water salinity of the sample range from 31-32 PSU in both locations inland. No dilution happened from freshwater rivers and rain as well. The temperature of the samples ranges from 27-28°C, then the pH of the samples ranges from 7.9-8.0.

Analysis of read obtained by MiFish pipeline

After clustering and trimming the raw reads (142,601 OTU) from the MiFish platform, 117,833 OTU (82.63%) and remaing 24,768 reads (17.37%) were discharged. From the clean read, 96.55% (113,771 OTU), were assigned into 14 families, 34 species which have percent identity 97-100%. After the taxonomic assignment, 4062 OTU (3.44%) were discarded due to having a lower identity between 80-97% identity.

Marine fish biodiversity

The biodiversity indices in both island sampling sites have been analyzed by Primer v7 (Clarke and Gorley 2015b). The diversity index has been widely used for the measurement on quantitative of how many different species inhabit a certain community (Tucker et al. 2017). Here, the Shannon index was used in diversity studies in the ecological literature (Spellerberg 2003) for both islands (Island of Mansinam and Lemon).

Based on the analysis of the fish species diversity index using Primer V7 (Clarke and Gorley 2015b), the islands of Mansinam and Lemon have a value 3.52 (more than three points according to the Shannon-Wiener Index formula), which category is high in biodiversity (Ludwig et al. 1988). Besides, the species reach (Margalef Index) is 5.95, and the Pielou's evenness index of this location is 0.99.

Phylogenetic of marine fish species

From the results of the grouping, the Pomacentridae group, which is a reef fish, dominates the study results with a total of 8 species, followed by Carangidae (24,060 OTU), Lutjanidae (14,565 OTU), and Acanthuridae (8746 OTU) respectively having the number of species detected with environmental DNA as many as 4 species each Family. The results of this environmental DNA also identified 2 species from the Family Scombridae, namely *Gymnosarda unicolor* (13,874 OTU) and *Thunnus obesus* (1083 OTU).

Table 1. The average on water quality measurement on Lemon and Mansinam Island, Manokwari District, West Papua, Indonesia

Water quality parameter		Unit	Standart quality	Average measurement
Physical	Temperature	ºC	Natural	29.7
	Current	m/s	Natural	0.11
	Smell		Not smell	Not smell
	Color		30	Clear
	Brighness	m	>6	13.2
	TSS	m/L	80	131.5
Chemical	pH		7.75	8.18
	DO	mg/L	5.1	7.74
	BOD	mg/L	10	4.29
	Amonia	mg/L	0.3	0.05
	Nitrat	mg/L	0.008	0.027
	Phosphat	mg/L	0.015	0.021
	Salinity	PSU	Natural	29.75
	Oil and fat	mg/L	5	6.5
	Surfactan	mg/L	1	0.252
Heavy metal	Pb	mg/L	0.005	Not detected
	Cd	mg/L	0.002	Not detected
	Cu	mg/L	0.05	0.035
Bilogical	Total Coliform	MPN/100mL	1000	>2030
	Faecal Coliform	MPN/100mL	200	>609

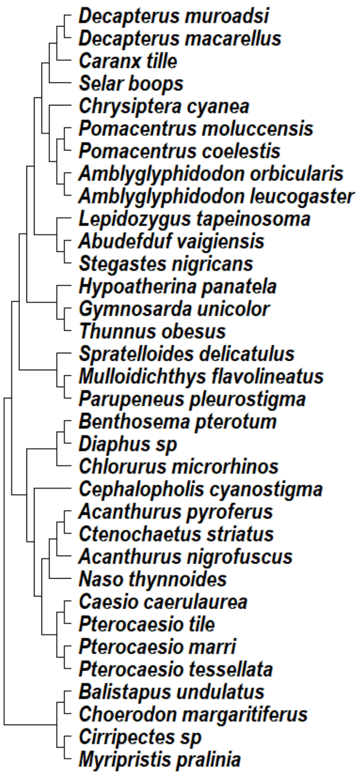

Figure 2. Phylogenetic tree of marine fish diversity generated from MEGAX using Neighbor-Joining Tree algoritm

Discussion

Small islands have become one of the centers of biodiversity and become an important distribution of coral reef ecosystems (Hafezi et al. 2020). Unfortunately, there is no definite data on the diversity of aquatic biota associated with coral reef ecosystems in the small islands of Manokwari. The data on reef fish species in Indonesia is still uncertain, with the finding of different data from a number of databases such as FishBase and research results. Several studies have been conducted to document the biodiversity of these reef fish from various regions in Indonesia (Andriyono et al. 2019; Andriyono et al. 2021). The types of fish obtained were shallow sea fish, and several species were found foraging fish in coral reef areas and also found migrating fish globally.

This biodiversity study is in line with conservation activities that are expected to be carried out to protect coral reef ecosystems, which are currently experiencing various pressures, especially from anthropogenic activities. Like the Lemon and Mansinam Island Regions, previous research on coral reef cover in this area was reported to have decreased significantly (Dasmasela et al. 2019). The estimated impact is the destruction of fish resources in this area which is the source of community life. In addition to meeting food needs, this area is also a destination for domestic tourists who do spiritual tourism. Apart from plastic waste (Rochman et al. 2016), fishery activities that are not environmentally friendly have also been reported to be the cause of the decline in coral reef cover due to bombing and the use of cyanide (Dasmasela et al. 2019).

In measuring water quality, all parameters indicate good conditions based on the Minister of Environment Regulation no. 51 of 2004 concerning Seawater Quality Standards (Tanjung and Hamuna 2019). Even though the water quality condition is quite good, there is still limited biodiversity study in this area. Previous studies recorded 15 genera of reef fish around the Mansinam Island Region which were not published. This is the basis for the importance of the reef fish resource database in the area of Mansinam Island and Lemon Island in Mnokwari District. In addition, support for routine survey activities to monitor the existence of these very valuable natural resources needs to be carried out as well as efforts to increase community capacity in carrying out independent conservation which is a local culture (Ihalauw et al. 2016).

This study of reef fish biodiversity on Lemon and Mansinam Island is expected to be basic data in the management of the area, which has become a tourist area (Ihalauw et al. 2016) so that it can still provide a source of income and at the same time maintain the wisdom of the local community. From this environmental DNA approach, at least 34 species of fish have been recorded, which are an important commercial fish group. These fish are grouped into several classes, which are an important part of capture fisheries management in the Manokwari District in particular.

The first group of fish is reef fish, which make coral reef ecosystems the main habitat for these types of fish. Fish species in the Pomacentridae group dominate the reef fish species identified in this study. In addition, several species of fish in the Labridae group (*Chlorurus microrhinos* and *Chaerodon marginatiferus*). Almost all reef fish are economically important fish (Indrawati et al. 2020), both as consumption fish and ornamental fish, whose prices are much more economical than consumption fish. This group of fish makes coral reefs the main habitat, which shows the condition of coral reefs that is still good (Falah et al. 2020). However, the Chaetodontidae fish group was not identified in this study. Furthermore, Pomacetridae can also be used as an indicator of the condition of coral reefs. The more abundant Pomacetridae in an area, it is possible that Pomacetridae will also be able to dominate in this region (Allen et al. 2013). The interest of this fish group is because they are planktivorous and abundant microalgae in the coral reef ecosystem (Santana et al. 2021). Thus, the reef fish identified in this study indicate that they are an important chain in the coral ecosystem that connects the ecological chain at the health level of coral reef ecosystems.

Furthermore, the fish identified were also fish associated with coral reefs at an early stage in their reproductive cycle. Juvenile young fish will use coral reefs as a feeding ground and nursery group that provides a place of protection and resting place because the structure of coral reefs is quite diverse from massive, foliose and branching forms. For example, the fish Cephalopholis cyanostigma hunts in coral areas (Nanami 2021). This fish is included in Serranidae (grouper species), which is an economically important fish species (Rimmer and Glamuzina 2019). Fish belonging to the Serranidae make coral reef areas a potential habitat, so fishermen can easily find this type of grouper in coral areas. Among the 76 species in the Family Serranidae (Zgliczynski et al. 2013), *Cephalopholis cyanostigma* is rarely found in traditional markets. A common type of grouper is from the genus *Epinephelus* (Tapilatu et al. 2021). Nevertheless, the species *Cephalopholis cyanostigma* is very popular for coastal communities in eastern Indonesia, even though its status in the IUCN is still in the least Concern (LC) condition, which is considered stable in its natural habitat (Zgliczynski et al. 2013).

In addition, other species, such as snapper fish, have become coral reef areas as important habitats. Some of the species identified include *Caesio caerulaurea*, *Pterocaesio marri*, *Pterocaesio tessellata*, and *Pterocaesio tile*, which is included in the Family Caesionidae (Guo et al. 2016) are economically marine fish species in Indonesia (Huliselan et al. 2018). This fish family is distributed almost throughout the Indo-West Pacific Regio (Froese 2009). This type of fish is also an important economic fish species in shallow sea waters that are sometimes found in schools. This group hunts zooplankton in shallow sea areas (less than 60 meters) (Carpenter and Niem 2001) and is almost always found in coral reef areas (Rosdianto 2021). The importance of conservation activities is also related to the types of fish that are economically important and become the target of catching fishermen with various fishing gear. The snapper group has become quite a favorite fish for fishing tourism activities and is served in restaurants in the form of various culinary seafood offerings that attract tourists.

The value of diversity which is quite high in the Mansinam Island and Lemon Island areas, needs to be maintained and improvements are made in terms of habitats that support reef fish in this area. Government support in the conservation of the area is also very important. Several areas in Indonesia have been designated as conservation areas based on local regulations. It is hoped that the diversity in these two small island areas in Manokwari can be maintained with the support of improving the quality of the surrounding coral reef habitat.

In conclusion, biodiversity in Lemon and Mansinam Islands is an important resource in the marine waters of Manokwari District. Biodiversity estimation has been successfully carried out using the environmental DNA approach. At least 34 species of coral reef fish have been identified, almost all of which are symbiotic fish in the coral reef area during their life cycle. Further research to document biodiversity in coral reef areas on the small islands of Lemon and Mansinam Islands needs to be done to obtain data accuracy through a combination of other method approaches.

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