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A CONTRAST PATTERN OF REEF FISH SPECIES DIVERSITY AND DISTRIBUTION USING ENVIRONMENTAL DNA (eDNA) METABARCODING IN LONGITUDINAL DISTANCE FROM JAKARTA BAY

POLA KEANEKARAGAMAN DAN DISTRIBUSI SPESIES IKAN TERUMBU MENGGUNAKAN ENVIRONMENTAL DNA (eDNA) METABARCODING PADA JARAK LONGITUDINAL DARI TELUK JAKARTA

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ABSTRACT

The existence of reef fish is certainly closely related to the existence of reefs coral because the ecosystem is a habitat for reef fish. Coral reefs are ecosystems that are commonly found on small islands in the tropics including the Seribu Islands. The Seribu Islands are a group of 110 islands located off the coast of Jakarta and up to 80 kilometers north of the Java Sea. In this study, we examined the species distribution and diversity of reef fish species on two different distance location in Jakarta Bay using environmental DNA (eDNA) metabarcoding analysis from two sites which are Untung Jawa Island and Harapan Island. The 4L eDNA seawater samples were collected at a depth of 8-9 meters at each site and then analysis using specific primer (MiFish U) of 12S rRNA. Overall, the higher species richness was found on Harapan Island (52 species) followed by Untung Jawa Island (11 species). The Shannon-Wiener Index also showed Harapan Island has higher reef fish diversity based on three taxonomic level (family, genus, and species). There were only five mutual reef fish species found in the two locations, namely *Atherinomorus aetholepis*, *Auxis thazard*, *Cephalopholis sexmaculata*, *Epinephelus chlorostigma*, and *Plectropomus areolatus*. The results of these findings in this current study are in line with anthropogenic pressure different where Untung Jawa Island is the closer one to Jakarta Bay than the Harapan Island that located relatively far from Jakarta Bay.

Keywords: anthropogenic, biodiversity, biomonitoring, coral reef, Next Generation Sequencing

ABSTRAK

Keberadaan ikan terumbu tentunya erat kaitannya dengan keberadaan terumbu karang karena ekosistem tersebut merupakan habitat bagi ikan karang. Terumbu karang merupakan ekosistem yang banyak terdapat di pulau-pulau kecil di daerah tropis termasuk Kepulauan Seribu. Kepulauan Seribu adalah sekelompok 110 pulau yang terletak di lepas pantai Jakarta dan hingga 80 kilometer sebelah utara Laut Jawa. Dalam studi ini, kami meneliti distribusi spesies dan keanekaragaman spesies ikan terumbu pada dua lokasi yaitu Pulau Untung Jawa dan Pulau Harapan yang berbeda jarak di Teluk Jakarta menggunakan analisis metabarcoding DNA lingkungan (eDNA). Sebanyak 4 liter sampel air laut dikoleksi pada kedalaman 8-9 meter per lokasi lalu dilakukan analisis menggunakan primer spesifik (MiFish U) dengan marka 12s rRNA. Secara keseluruhan, kekayaan spesies yang lebih tinggi ditemukan di Pulau Harapan (52 spesies) diikuti oleh Pulau Untung Jawa (11 spesies). Indeks Shanon-Wiener juga menunjukkan bahwa Pulau Harapan memiliki keanekaragaman ikan terumbu yang lebih tinggi berdasarkan tiga tingkatan taksonomi (famili, genus, dan spesies). Hanya ada lima spesies ikan terumbu mutual yang ditemukan di dua lokasi tersebut, yakni Atherinomorus aetholepis, Auxis thazard, Cephalopholis sexmaculata, Epinephelus chlorostigma, dan Plectropomus areolatus. Hasil temuan dalam penelitian ini sejalan dengan perbedaan antropogenik dimana Pulau Untung Jawa lebih dekat dengan Teluk Jakarta dibandingkan dengan Pulau Harapan yang letaknya relatif jauh dari Teluk Jakarta.

Kata kunci: antropogenik, biodiversitas, biomonitoring, terumbu karang, *Next Generation Sequencing*

I. INTRODUCTION

Reef fish are a group of individual fish that live in an area coral reef ecosystems (Choat & Bellwood, 1991). According to Nybakken (1993), reef fish are organisms in the sea that are associated with coral reefs in large numbers and settle in coral reef habitats. The majority of reef fish are small (less than 30 cm) almost all of his life has always lived on coral reefs (Sale, 1991). The existence of reef fish is certainly closely related to the existence of reefs coral because the ecosystem is a habitat for reef fish. The higher the live coral cover will impact the increase of reef fish abundance (Komyakova *et al*., 2013). Coral reefs are ecosystems that are commonly found on small islands in the tropics including the Seribu Islands (Darwin, 2020). The life cycle of reef fish could not be separated from coral reefs as their main habitat. Reef fish are closely associated with coral reefs (Paulangan *et al*., 2019). The reef fish community uses the niches on coral reefs as a feeding ground, a nursery ground, and a spawning ground. These factors cause the diversity and presence of reef fish to be strongly influenced by the conditions of the existing coral reef cover (Rumkorem *et al*., 2019). The presence and richness of reef fish species is closely related to coral reef cover (Graham & Nash, 2012). As the most species of vertebrate group, reef fish are frequently used as indicators of the health of marine ecosystems (Madduppa *et al*., 2013). The distribution and abundance of reef fish are essential factors to advise management and exploitation of these fish ecosystems, which generally support multi-species fisheries, especially in regions with significant fish biodiversity.

The Seribu Islands is one of the locations in Indonesia recognized for the state of the country's most small islands, particularly in the western region (Pratiwi *et al*., 2021). The Seribu Islands are a group of 110 islands located off the coast of Jakarta and up to 80 kilometers north of the Java Sea (Madduppa *et al*., 2013). These islands are under threat from a variety of human-caused pressures which greatly affects the health condition of coral reefs including the condition of reef fish (Farhan & Lim, 2012). One of the islands that exists near to Jakarta Bay is Untung Jawa Island. Untung Jawa Island is geographically adjacent to the mainland of Tanjung Pasir and Jakarta, so this area is a tourist attraction that is crowded with domestic tourists for beach recreation and marine tourism purposes and is supported by other facilities (Razak & Suprihardjo, 2013). Comparing to this island, Harapan Island is one of the islands included in the national park settlement zone which is administratively included in the North Seribu Islands District (Sumarno & Muryanto, 2014). One of the potentials for marine tourism that can invite tourists is the condition of a good coral reef ecosystem. Monitoring of coral reef ecosystems is needed to monitor the health of existing coral reefs, one of the monitoring methods is to identify the diversity of reef fish.

Identification of reef fish has been carried out by many previous researchers using different methods such as the Underwater Visual Census (UVC) where researchers go directly to the data collection point and then identify reef fishes (Madduppa *et al*., 2012; Madduppa *et al*., 2013; Setiawan *et al*., 2013; Prabowo *et al*., 2019). However, this method has an several constraint procedure, and the accuracy subjective data such as unclear water conditions can affect the identification of fish, as well as the limited ability of observer in species identification (Dearden *et al*., 2010). This limitation in the identification process of the Underwater Visual Census (UVC) method supports research to be carried out using more effective methods and analyses such as environmental DNA (eDNA), targeting all genetic material stored in the waters. Environmental DNA (eDNA) metabarcoding method is the on of the current alternative and effective bio-

monitoring method for identifying reef fish species (DiBattista *et al*., 2017; Evans *et al*., 2017; Gelis *et al*., 2021; Wang *et al.,* 2021). Thomsen *et al.* (2012) stated that environmental DNA (eDNA) metabarcoding is a good method for identifying reef fish. Environmental DNA (eDNA) metabarcoding is an update method for estimating the richness and abundance of organisms such as fish and others that are difficult to identify and detect from the water column and sediments (Taberlet *et al*., 2012; Takahara *et al*., 2013; Gelis *et al*., 2021). Environmental DNA methods are also very effective for detecting the presence of organisms such as fish without isolating the target organisms (Lodge *et al*., 2012; Laramie *et al*., 2015). This is because organisms such as fish generally release a lot of genetic material in the form of cells or feces that can be broken down into small pieces that can be retained in the waters and then settle in the environment (Taberlet *et al*., 2012; Takahara *et al*., 2013). The genetic material is easily extracted from samples from soil, water and air (Tringe & Rubin, 2005; Barnes & Turner, 2016).

Here, we examined the species distribution and diversity of reef fish species on two different distance location in Jakarta Bay using eDNA metabarcoding analysis. The eDNA approach would like to bring an informative result as complimentary study to the diversity of reef fish species in this area. The study about biomonitoring of reef fish by the environmental gradient in North of Jakarta was conducted by Madduppa *et al*., (2013). By carrying out reef fish biomonitoring with eDNA metabarcoding, the species identified was expected to complement the previous study that implemented conventional survey in the areas. The eDNA metabarcoding analysis would detect reef fish species and community composition and structure of Harapan and Untung Jawa Island. This study aimed to identify reef fish species on Harapan Island and Untung Jawa Island, Seribu Islands using eDNA metabarcoding and uncover their species distribution and diversity pattern.

II. MATERIAL AND METHODS

2.1. eDNA Sampling

Sampling was carried out in the waters of the Thousand Islands, precisely on Harapan and Untung Jawa islands,which was held in August 2019 (Figure 1). Seawater samples were taken directly at the bottom of the water at a depth of 8-9 meters with total volume of 4L per sample (Madduppa *et al*., 2021). The sampling method used is the same as that used by some researchers such aslocation (Miya *et al*., 2015; Andruszkiewicz *et al*., 2017).

The water sample was put into a 5L water bottle which was collected by diving using SCUBA equipment. There was one sample collected for each location. The seawater samples were then filtered using a vaccum pump which had 0.45 µm filter paper installed (Gelis *et al*., 2021; Madduppa *et al*., 2021) After the filtering is complete, the filter paper is then put into a 2 mL cryotube which has been filled with 1 mL of DNA shield. To prevent any contamination, the 10% of commercial bleach was always used to sterilized equipment before seawater collecting and filtering was conducted. Sample processing and data analysis were carried out at the Oceanogen Research Center and Biodiversity and Marine Biosystematics laboratory, Faculty of Fisheries and Marine Sciences, IPB University.

2.2. DNA Extraction and Amplification

The DNA extraction process carried out in this study used the ZymoBIOMICS DNA extraction kit manufactured by Zymo Research Corporation in accordance with the manufacturer's guidelines provided (Verma & Satyanarayana, 2011; Li *et al*., 2018; Gelis *et al*., 2021).

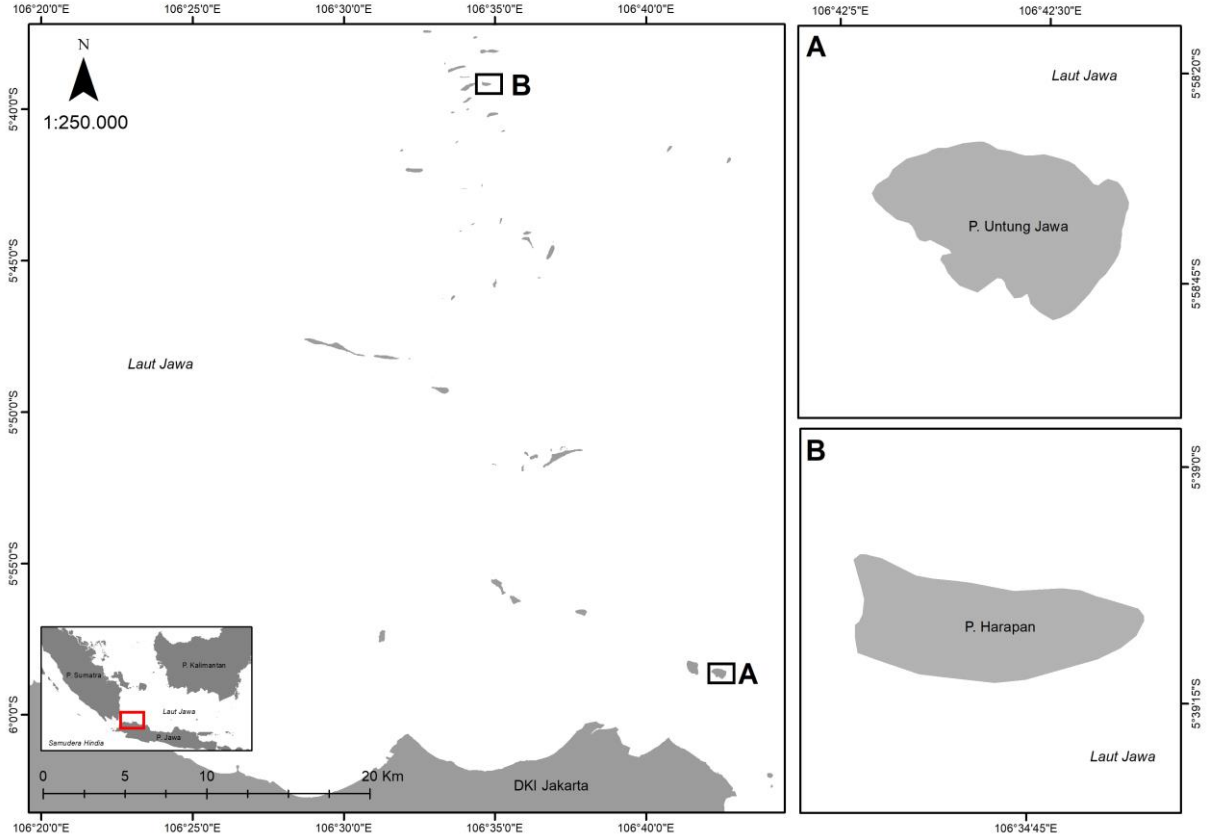

Figure 1. Map of eDNA sampling locations on Harapan Island (A) and Untung Jawa Island (B), Seribu Islands.

This study used MiFish-U primers (Forward and Reverse) (Miya *et al*., 2015) with a base sequence for forward, namely 5'-TCG TCG GCA GCG TCA GAT GTG TAT AAG AGA CAG -3' and reverse 5'-GTC TCG TGG GCT CGG AGA TGT GTA TAA GAG ACA G-3. In addition to primers, other components used in this study were DNA templates and 2x MyTaq Hs Red Mix produced by Bioline Meridian Bioscience. PCR was carried out in stages, namely: (1) pre-denaturing DNA templates; (2) denaturation of DNA templates; (3) primer attachment to the template (annealing); (4) primary lengthening (extension) and (5) stabilization (post extension) (Handoyo & Rudiretna, 2001). Each of these processes was carried out at 95 C for 10 minutes, 94 C for 10 seconds, 67 C for 10 seconds, 72 C for 10 seconds, and 72 C for 5 minutes (Miya *et al*., 2015).

To confirm the amplicon product of PCR section, we used Electrophoresis to visualize the fragment length of the DNA (basepair). In principle, electrophoresis works under the influence of an electric field charge that makes particles and molecules move towards the electrode bearing which has the opposite charge (Westermeier, 2005). The electrophoresis process was running in 2% gel agarose with the 220 V for 35 minutes and then visualized through a Gel Doc machine.

2.4. High Throughput Sequencing (HTS)

The DNA sequencing stage was carried out using the Illumina iSeq Series machine which was carried out at the Oceanogen Research Center, Indonesia. For indexing, all PCR products that passed quality control on the electrophoresis step

underwent a second PCR. Using 12.5 l of Kapa HotStart HiFi 2 ReadyMix DNA polymerase and 2 l of PCR product, the target amplicon was amplified using the IDT double index and Illumina sequencing adaptor for Illumina - Nextera DNA Unique Dual Index, Set A (catalog number 20027213) (Illumina, San Diego, USA). To begin, the PCR was run at 95°C for 3 minutes to denaturate the DNA. Thereafter, the PCR was run nine times at 95°C for 30 seconds each, 55°C for 30 seconds, 72°C for 30 seconds, and 72°C for 5 minutes to extend the DNA. Prior to the following step, the first and second PCR products were purified using AMPure XP (Beckman Coulter, Inc.). The Illumina MiSeq 16S metagenomic sequencing library technique was used for the DNA sequencing on an Illumina iSeq 100 reagent kit cycle. In order to determine the concentration of the libraries, a Qubit fluorometer was used. To pool the amplicon barcode library, the stock solution was diluted to 10 nM. The library pool was prepared using the Illumina MiSeq library preparation instructions, which calls for diluting and denatureing the sample. The final output consisted of 16 L of the 40 pM amplicon library and 4 L of the 60 pM PhiX Illumina version 3 control library. The Illumina iSeq v.2 Reagent kit for 2150 bp PE was used with a run-time of around 18 hours and generated a Fastq file. For Illumina - Nextera DNA Unique Dual Index, the particular barcodes index of IDT double and Illumina sequencing adaptor were excluded.

III. DATA ANALYSIS

3.1. Species Identification

The data from DNA sequencing in the form of files with the fastq extension were then analyzed bioinformatically using MiFish Pipeline (Sato *et al*., 2018). DNA sequencing fastq files are uploaded on the MiFish Pipeline web (http://mitofish.aori.u-tokyo.ac.jp/mifish). MiFish Pipeline (Sato *et al*., 2018) intercepts low-quality sequence reads (QV 20), merges final paired reads, removes incorrect combined reads containing N-nucleotides, filters reads by length (~229 bp), runs Usearch (0.99 for identity grouping, and 10 for minimum read size for filtering), MiFish Pipeline will then generate data in the form of species that are read according to the database based on the fastq file by dividing the data at two levels of similarity, namely 80-97% and >97%. The identification data used is data with similarity >97%, data with high similarity has a level of confidence in accurate identification.

Fish living in a coral reef ecosystem are divided into three groups depending on their ecological roles: target (usually big fish of economic importance, devoured by humans), indicator (fish linked with the health of coral reefs), and major (other fish that mostly small fishes often present in high numbers) (English *et al*., 1997). An enlightening discovery in this study is the eDNA metabarcoding analysis turns out to be able to capture a wider spectrum of families

3.2. Diversity Analysis

All diversity analyses in this study required an incidence data. Shannon-Wiener Index (H') were calculated using manually using Microsoft Excel. The analysis of reef fish diversity using the formula from Shannon-Wiener (Magurran, 2004), as follows:

$$H' = -\Sigma\, pi \ln(pi) \quad \text{.....(1)}$$

Note :
H' : Shannon-Wiener Index
pi : The ratio of the number of species to-i (ni) to the total number of reef fish (N) $pi = niN$

According to Jørgensen *et al*. (2005) The categories for the range of values of the Shannon-Wiener Index are:

$H' < 1$: Low diversity
$1 < H' < 3$: Medium diversity
$H' > 3$: High diversity

A dataset's diversity index measures how many distinct categories (such as species) in a community. This Shannon-Wiener Index (H') could be used as indicators of statistical representations of biodiversity in the areas.

IV. RESULTS AND DISCUSSION

4.1. Species Identification and Distribution

The current study presented reef fish community on small island environment in two distances of Jakarta Bay. A total of ~100.000 paired-end reads sequence were obtained for each eDNA samples from Harapan dan Untung Jawa Island. This study succeeded in identifying 58 reef fish species with ≥97% similarity or percentage of identification in the Mifish Pipeline 12S rRNA database on Harapan Island and Untung Jawa Island, Seribu Islands (Table 1). The reef fish on Harapan Island have a higher number of species with total of 52 species when compared to the reef fish species on Untung Jawa Island which only have 11 species based on environmental DNA samples collected.

The species richness based on family category is showed on Figure 2. The highest species was found on Carangidae family followed by Scombridae and Atherinidae family. In comparison of total family found, Untung Jawa Island showed only seven reef fish families while Harapan Island had 20 families. In general, the types of species in family showed fewer results in species that fall into the major category. In example, the family of Labridae and Pomacentridae as the two of major category of reef fish that frequently occurred on coral reef ecosystems by using Underwater Visual Census (UVC) method (Madduppa *et al*., 2013) were only observed in low number in this study (Figure 2). In the other hand, species in economically family are observed on higher number (i.e Carangidae, Scrombidae, Serranidae, and Lutjanidae). A study by Marwayana *et al*., (2021) found that eDNA metabarcoding analysis was able to capture a spectrum of fish species that were rarely recorded when using the visual census method.

Based on the mutual reef fish species found in the two locations, there were 5 species of reef fish (Figure 3), namely *Atherinomorus aetholepis, Auxis thazard, Cephalopholis sexmaculata, Epinephelus chlorostigma,* and *Plectropomus areolatus*. This group of fish is generally a group of target fish or consumption fish from the Atherinidae, Serranidae, and Scombridae families. Harapan and Untung Jawa Island have different coral reef conditions. Harapan Island has a better coral reef condition than Untung Jawa Island. This is because Untung Jawa Island is located closer to Java Island than Harapan Island, so that anthropogenic activities on Java Island affect coral reefs on Untung Jawa Island more than Harapan Island. This is also supported by research conducted by Zaneveld & Verstappen (1952) and Giyanto & Sukarno (1997) that the closer the coral reefs are to the mainland of Java Island, the worse the condition will be. This indicates that human activities play an

Table 1. Species identification of reef fish based on MiFish Pipeline 12S rRNA database (only ≥97% percentage of identification that used to further analysis).

Location	Family	Genus	Species	Per.Ident (%)
Harapan	Pomacentridae	*Abudefduf*	*Abudefduf vaigiensis*	100
Harapan	Monacanthidae	*Acreichthys*	*Acreichthys tomentosus*	99.41
Harapan	Carangidae	*Alectis*	*Alectis indica*	100
Harapan	Pomacentridae	*Amblyglyphidodon*	*Amblyglyphidodon orbicularis*	100
Harapan	Atherinidae	*Atherinomorus*	*Atherinomorus aetholepis*	100
Harapan	Atherinidae	*Atherinomorus*	*Atherinomorus cf*	98.82
Harapan	Atherinidae	*Atherinomorus*	*Atherinomorus endrachtensis*	99.41
Harapan	Atherinidae	*Atherinomorus*	*Atherinomorus lacunosus*	100

Location	Family	Genus	Species	Per.Ident (%)
Harapan	Atherinidae	*Atherinomorus*	*Atherinomorus sp*	98.82
Harapan	Carangidae	*Atule*	*Atule mate*	100
Harapan	Leiognathidae	*Aurigequula*	*Aurigequula fasciata*	100
Harapan	Scombridae	*Auxis*	*Auxis rochei*	100
Harapan	Scombridae	*Auxis*	*Auxis thazard*	98.82
Harapan	Carangidae	*Carangoides*	*Carangoides plagiotaenia*	98.82
Harapan	Carangidae	*Caranx*	*Caranx heberi*	100
Harapan	Carangidae	*Caranx*	*Caranx ignobilis*	100
Harapan	Carangidae	*Caranx*	*Caranx melampygus*	100
Harapan	Serranidae	*Cephalopholis*	*Cephalopholis sexmaculata*	99.41
Harapan	Carangidae	*Decapterus*	*Decapterus macarellus*	100
Harapan	Carangidae	*Decapterus*	*Decapterus russelli*	100
Harapan	Carangidae	*Decapterus*	*Decapterus tabl*	100
Harapan	Blenniidae	*Entomacrodus*	*Entomacrodus striatus*	100
Harapan	Serranidae	*Epinephelus*	*Epinephelus areolatus*	99.41
Harapan	Serranidae	*Epinephelus*	*Epinephelus chlorostigma*	99.41
Harapan	Scombridae	*Euthynnus*	*Euthynnus affinis*	100
Harapan	Scombridae	*Grammatorcynus*	*Grammatorcynus bilineatus*	100
Harapan	Tripterygiidae	*Helcogramma*	*Helcogramma striata*	97.65
Harapan	Hemiramphidae	*Hemiramphus*	*Hemiramphus far*	100
Harapan	Clupeidae	*Herklotsichthys*	*Herklotsichthys quadrimaculatus*	97.13
Harapan	Scombridae	*Katsuwonus*	*Katsuwonus pelamis*	100
Harapan	Lethrinidae	*Lethrinus*	*Lethrinus harak*	100
Harapan	Mugilidae	*Liza*	*Liza macrolepis*	100
Harapan	Lutjanidae	*Lutjanus*	*Lutjanus decussatus*	100
Harapan	Lutjanidae	*Lutjanus*	*Lutjanus erythropterus*	100
Harapan	Clupeidae	*Nematalosa*	*Nematalosa come*	98.83
Harapan	Ariidae	*Netuma*	*Netuma bilineata*	97.11
Harapan	Serranidae	*Plectropomus*	*Plectropomus areolatus*	100
Harapan	Plotosidae	*Plotosus*	*Plotosus lineatus*	100
Harapan	Pomacentridae	*Pomacentrus*	*Pomacentrus nigromanus*	100
Harapan	Lutjanidae	*Pterocaesio*	*Pterocaesio marri*	99.41
Harapan	Scombridae	*Rastrelliger*	*Rastrelliger kanagurta*	98.82
Harapan	Clupeidae	*Sardinella*	*Sardinella albella*	99.42
Harapan	Scombridae	*Scomberomorus*	*Scomberomorus sinensis*	99.41
Harapan	Carangidae	*Selar*	*Selar crumenophthalmus*	99.41
Harapan	Carangidae	*Selaroides*	*Selaroides leptolepis*	100
Harapan	Siganidae	*Siganus*	*Siganus fuscescens*	100
Harapan	Apogonidae	*Sphaeramia*	*Sphaeramia orbicularis*	98.82
Harapan	Clupeidae	*Spratelloides*	*Spratelloides gracilis*	99.42
Harapan	Engraulidae	*Stolephorus*	*Stolephorus indicus*	99.43
Harapan	Carangidae	*Trachinotus*	*Trachinotus ovatus*	100
Harapan	Belonidae	*Tylosurus*	*Tylosurus crocodilus*	99.41
Harapan	Apogonidae	*Zoramia*	*Zoramia leptacantha*	98.82
Untung Jawa	Pomacentridae	*Abudefduf*	*Abudefduf bengalensis*	100
Untung Jawa	Atherinidae	*Atherinomorus*	*Atherinomorus aetholepis*	100
Untung Jawa	Scombridae	*Auxis*	*Auxis thazard*	100
Untung Jawa	Serranidae	*Cephalopholis*	*Cephalopholis sexmaculata*	100
Untung Jawa	Syngnathidae	*Corythoichthys*	*Corythoichthys haematopterus*	99.4
Untung Jawa	Engraulidae	*Encrasicholina*	*Encrasicholina heteroloba*	100
Untung Jawa	Engraulidae	*Encrasicholina*	*Encrasicholina punctifer*	100
Untung Jawa	Serranidae	*Epinephelus*	*Epinephelus chlorostigma*	98.82
Untung Jawa	Serranidae	*Epinephelus*	*Epinephelus sexfasciatus*	100
Untung Jawa	Labridae	*Halichoeres*	*Halichoeres argus*	98.21
Untung Jawa	Serranidae	*Plectropomus*	*Plectropomus areolatus*	100

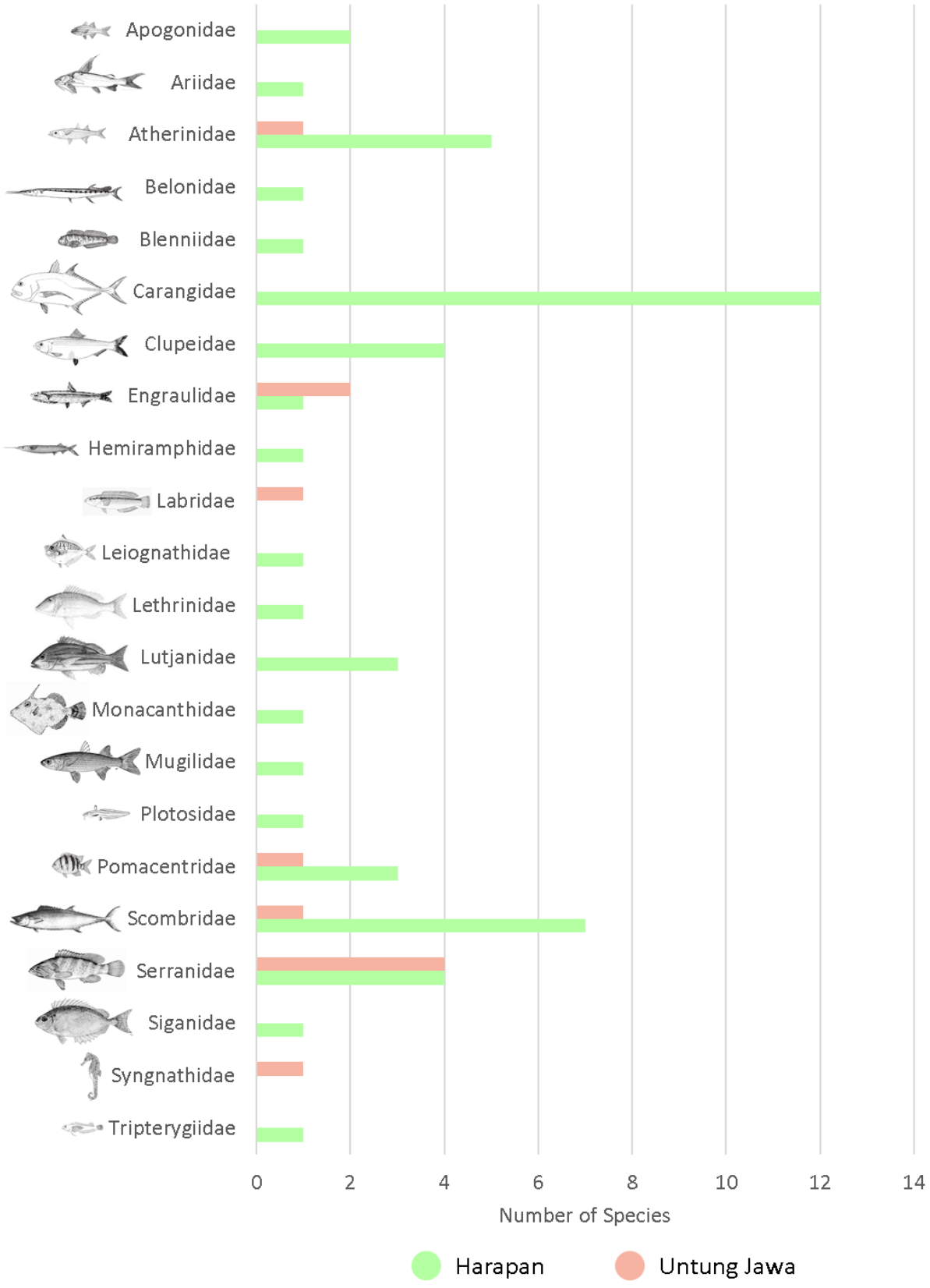

Figure 2. Bar chart showed species number of reef fish based on Family on Harapan Island (Green) and Untung Jawa Island (Red) in the Seribu Islands.

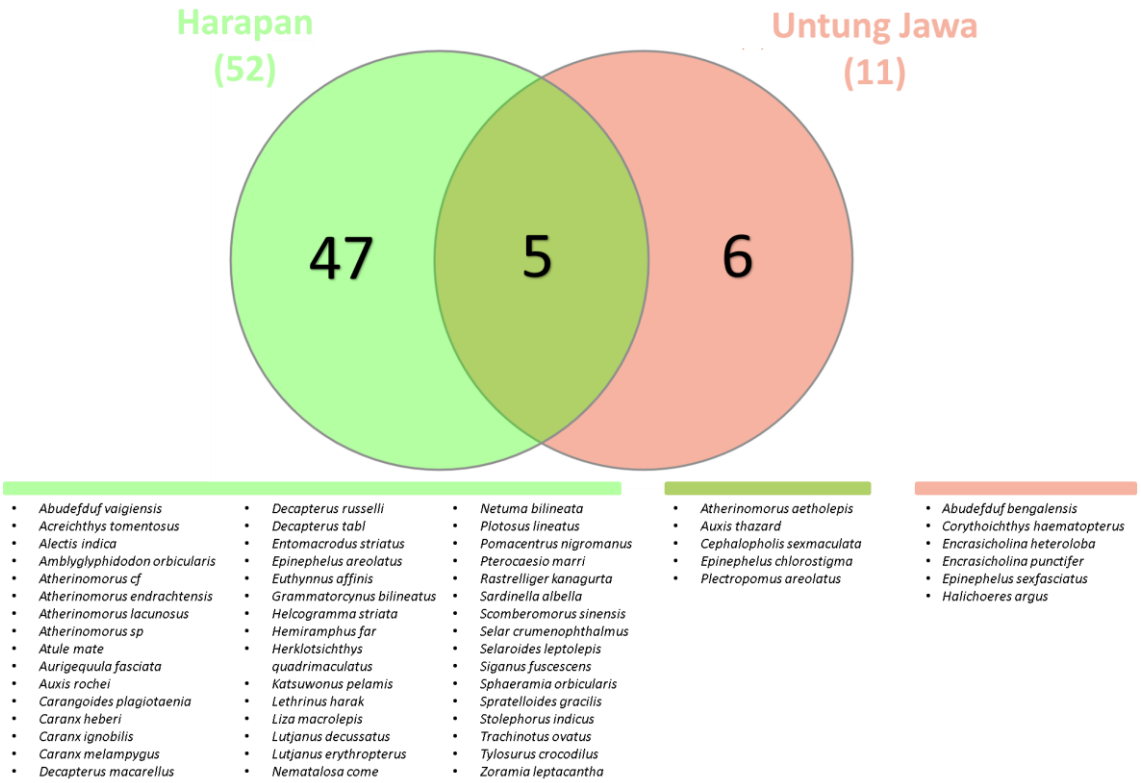

Figure 3. Diagram Venn showed species distribution of reef fish on Harapan Island (Green) and Untung Jawa Island (Red) in the Seribu Islands.

important role in the destruction of coral reef ecosystems. The reef fish communities have an unstable structure at the species level, meaning that species composition at a particular location may not revert to its previous condition following natural or human disturbances (Sale, 1977).

Based on this study, the eDNA metabarcoding analysis using seawater samples to determine the presence of reef fish species was considered effective with a total of 58 species in two locations which are Harapan and Untung Jawa Island. Environmental DNA becomes very effective because fish organisms generally release a lot of genetic material in the form of cells or feces that can be broken down into small pieces that can be retained in the waters and then settle in the environment (Taberlet *et al*., 2012; Takahara *et al*., 2013). Environmental DNA (eDNA) technique is a good alternative method for identifying reef fish (Thomsen *et al*., 2012; Miya *et al*., 2015). In recent years DNA metabarcoding has developed to solve many marine problems such as in the conservation and monitoring of marine organisms, especially reef fish, which were previously less well documented (Nguyen *et al*., 2019; Taberlet *et al*., 2012; Huhn *et al*., 2020; Madduppa *et al*. 2021). This method is very effective for detecting the presence of organisms such as fish without isolating the target organisms (Lodge *et al*., 2012) The genetic material is extracted easily on samples from soil, water, and air (Tringe & Rubin, 2005; Barnes & Turner, 2016).

4.2. Species Diversity

The Shannon-Wiener Index (H') has been used to calculate alpha diversity, as shown as a plot in Figure 4. Shannon-Wiener Index (H') shows species diversity of reef fish on Harapan and Untung Jawa Island by the the family, genus, and species. Overall,

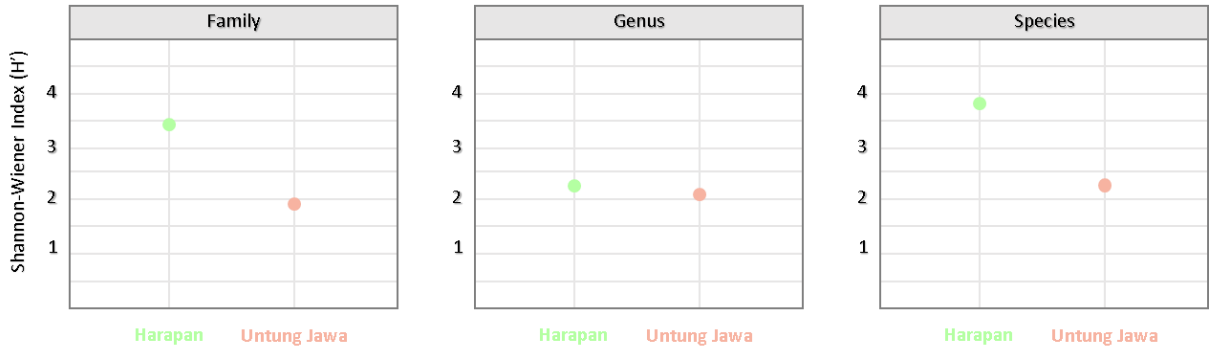

Figure 4. Alpha diversity represents Shannon-Wiener Index of reef fish based on family, genus, and species on Harapan Island (Green) and Untung Jawa Island (Red) in the Seribu Islands.

the higher Shannon-Wiener Index (H') was found on Harapan Island in these three taxonomic levels. On the family level, the Shannon-Wiener Index (H') of Harapan Island was 2.99 (medium diversity) followed by 1.94 on Untung Jawa Island (medium diversity). The medium diversity also found in genus level where Harapan Island showed 2.39 and Untung Jawa Island showed 2.19 of Shannon-Wiener Index (H') value. On species level, high diversity was found on Harapan Island (3.95) meanwhile the Untung Jawa Island showed medium diversity (2.39). This study showed there was a positive correlation between species richness and the Shannon–Wiener Index (H'). This finding was also showed by Gelis *et al*. (2021) that implemented reef fish biomonitoring using eDNA metabarcoding was in another region of Indonesia. The finding with eDNA that found more diversity of reef fish in Harapan than in Untung Jawa related to anthropogenic stress was also shown by Bekker *et al*. (2017) and also Madduppa *et al*. (2021).

As a group, the Seribu Islands off the coast of Jakarta Bay provide an ideal case study region for examining the impacts of various human-caused stresses on coral reef ecosystems and coastal livelihoods. Local anthropogenic influences have generated substantial alterations in the coral reef ecosystems here (e.g. Van der Meij *et al*., 2010). Jakarta Bay, which is home to the Jakarta Metropolitan Area's approximately 30 million people, is suffering from severe pollution. Marine resources are over-exploited, yet local coral reefs nevertheless support millions of people's livelihoods despite this (Baum *et al*., 2016). Untung Jawa Island as a small island that close to Jakarta Bay has suffering from the anthropogenic impacts. The anthropogenic aspect is widely known as a factor that responsible for coral reef damage (Dollar *et al*., 2004; Wielgus *et al*., 2004; Tilot *et al*., 2008; Kelly *et al*., 2014). The life cycle of reef fish could not be separated from coral reefs as their main habitat. Reef fish are closely associated with coral reefs (Paulangan *et al*., 2019). The results of the findings made in this current study are in line with this condition. Untung Jawa Island has lower number on species richness and diversity comparing to Harapan Island that located relatively far from Jakarta Bay. Harapan Island is located on the Kepulauan Seribu Marine National Park, which in this area, the anthropogenic pressure is still not as big as that in Untung Jawa Island. Madduppa *et al*. (2012) revealed that many damaging activities are threatening coral reefs in the Kepulauan Seribu Marine National Park, which is near to the Indonesian capital Jakarta, resulting in habitat loss and species diversity. Our results revealed that the archipelago's islands appeared to be linked to

environmental variables such as sedimentation, pollution, and other human activities in Jakarta Bay and Seribu Islands (Rees *et al*., 1999; Rachello-Dolmen & Cleary 2007; Madduppa *et al*., 2012).

V. CONCLUSION

eDNA metabarcoding analysis on biomonitoring reef fish species on Harapan and Untung Jawa Island shows different community by distribution and diversity. The results show Harapan Island has higher in species richness and diversity than Untung Jawa Island which the closer one to Jakarta Bay as the high anthropogenic pressure in the areas. The further studies of eDNA metabarcoding analysis of reef fish biomonitoring need to be conducted on more complete spectrum of time and location points to support more complete results.

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REFERENCES

Andruszkiewicz, E.A., H.A. Starks, F.P. Chavez, L.M. Sassoubre, B.A. Block, & A.B. Boehm. 2017. Biomonitoring of marine vertebrates in Monterey Bay using eDNA metabarcoding. *PLoS One*, 12(4): e0176343. https://doi.org/10.1371/journal.pone.0176343

Bakker, J., O.S. Wangensteen, D.D. Chapman, G. Boussarie, D. Buddo, T.L. Guttridge, H. Hertler, D. Mouillot, L. Vigliola, & S. Mariani. 2017. Environmental DNA reveals tropical shark diversity in contrasting levels of anthropogenic impact. *Scientific reports*, 7(1): 1-11. https://doi.org/10.1038/s41598-017-17150-2

Barnes, M.A. & C.R. Turner. 2016. The ecology of environmental DNA and implications for conservation genetics. *Conservation genetics*, 17(1): 1-17. https://doi.org/10.1007/s10592-015-0775-4

Baum, G., I. Kusumanti, A. Breckwoldt, S.C. Ferse, M. Glaser, L. Adrianto, S. van der Wulp, & A. Kunzmann. 2016. Under pressure: Investigating marine resource-based livelihoods in Jakarta Bay and the Thousand Islands. *Marine pollution bulletin*, 110(2): 778-789. https://doi.org/10.1016/j.marpolbul.2016.05.032

Choat, J.H. & D.R. Bellwood. 1991. *Reef Fishes: Their History and Evolution.* In The ecology of fishes on coral reefs. Academic press. pp. 39-66.

Darwin, C. 2020. *The Structure and Distribution of Coral Reefs.* University of California Press. https://doi.org/10.1525/9780520327337

Dearden, P., M. Theberge, & M. Yasué. 2010. Using underwater cameras to assess the effects of snorkeler and SCUBA diver presence on coral reef fish abundance, family richness, and species composition. *Environmental monitoring and assessment*, 163(1): 531-538.

https://doi.org/10.1007/s10661-009-0855-3

DiBattista, J.D., D.J. Coker, T.H. Sinclair-Taylor, M. Stat, M.L. Berumen, & M. Bunce. 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

Dollar, S.J. & R.W. Grigg. 2004. Anthropogenic and natural stresses on selected coral reefs in Hawai'i: A multidecade synthesis of impact and recovery. *Pacific Science*, 58(2): 281-304. https://doi.org/10.1353/psc.2004.0016.

Evans, N.T., Y. Li, M.A. Renshaw, B.P. Olds, K. Deiner, C.R. Turner, C.L. Jerde, D.M. Lodge, G.A. Lamberti, & M.E. Pfrender. 2017. Fish community assessment with eDNA metabarcoding: effects of sampling design and bioinformatic filtering. *Canadian Journal of Fisheries and Aquatic Sciences*, 74(9): 1362-1374. https://doi.org/10.1139/cjfas-2016-0306

Farhan, A.R. & Lim S. 2012. Vulnerability assessment of ecological conditions in Seribu Islands, Indonesia. *Ocean & coastal management*, 1(65): 1-4. https://doi.org/10.1016/j.ocecoaman.2012.04.015

Gelis, E.R.E., M.M. Kamal, B. Subhan, I. Bachtiar, L.M.I Sani, & H. Madduppa. 2021. Environmental biomonitoring of reef fish community structure with eDNA metabarcoding in the Coral Triangle. *Environmental Biology of Fishes*, 1-17. https://doi.org/10.1007/s10641-021-01118-3

Graham, N.A.J. & K.L. Nash. 2013. The importance of structural complexity in coral reef ecosystems. *Coral reefs*, 32(2): 315-326. https://doi.org/10.1007/s00338-012-0984-y

Huhn, M., H.H. Madduppa, M. Khair, A. Sabrian, Y. Irawati, N.P. Anggraini, S.P. Wilkinson, T. Simpson, K. Iwasaki, D.H. Setiamarga, & P.J. Dias. 2020. Keeping up with introduced marine species at a remote biodiversity hotspot: awareness, training and collaboration across different sectors is key. *Biological Invasions*, 22(2): 749-771. https://doi.org/10.1007/s10530-019-02126-2

Hutami, R., N. Idzni, R. Ranasasmita, & M. Suprayatmi. 2017. Metode ekstraksi dna untuk deteksi molekuler. *Jurnal Pertanian*, 8(2): 106-112. https://doi.org/10.30997/jp.v8i2.1056

Jeníková, G., J. Pazlarová, & K. Demnerová. 2000. Detection of Salmonella in food samples by the combination of immunomagnetic separation and PCR assay. *International Microbiology*, 3(4): 225-229. https://pubmed.ncbi.nlm.nih.gov/11334305/

Jørgensen, S.E., F.L. Xu, F. Salas, & J.C. Marques. 2005. Application of indicators for the assessment of ecosystem health. *Handbook of ecological indicators for assessment of ecosystem health*, 2: 5-65. https://archive.epa.gov/solec/web/pdf/ecological_indicators.pdf

Kelly, L.W., G.J. Williams, K.L. Barott, C.A. Carlson, E.A. Dinsdale, R.A. Edwards, A.F. Haas, M. Haynes, Y.W. Lim, T. McDole, & C.E. Nelson. 2014. Local genomic adaptation of coral reef-associated microbiomes to gradients of natural variability and anthropogenic stressors. *Proceedings of the National Academy of Sciences*, 111(28): 10227-10232. https://doi.org/10.1073/pnas.1403319111

Komyakova, V., P.L. Munday, & G.P. Jones. 2013. Relative importance of coral cover, habitat complexity and diversity in determining the structure of reef fish communities. *PloS one*, 8(12): p.e83178. https://doi.org/10.1371/journal.pone.0083178

Li, Y., N.T. Evans, M.A. Renshaw, C.L. Jerde, B.P. Olds, A.J. Shogren, K. Deiner, D.M. Lodge, G.A. Lamberti, & M.E. Pfrender. 2018. Estimating fish alpha-and beta-diversity along a small stream with environmental DNA metabarcoding. *Metabarcoding and Metagenomics*, 2: p.e24262. https://doi.org/10.3897/mbmg.2.24262

Laramie, M.B., D.S. Pilliod, & C.S. Goldberg. 2015. Characterizing the distribution of an endangered salmonid using environmental DNA analysis. *Biological Conservation*, 183: 29-37. https://doi.org/10.1016/j.biocon.2014.11.025

Lodge, D.M., C.R. Turner, C.L. Jerde, M.A. Barnes, L. Chadderton, S.P. Egan, J.L. Feder, A.R. Mahon, & M.E. Pfrender. 2012. Conservation in a cup of water: estimating biodiversity and population abundance from environmental DNA. *Molecular ecology*, 21(11): 2555-2558. https://doi.org/10.1111/j.1365-294X.2012.05600.x

Madduppa, H. 2013. *Bioekologi dan Biosistematika Ikan Terumbu, Teknik Sampling Genetika & Monitoring Ikan Studi Kasus Kepulauan Seribu Petunujuk Identifikasi Ikan di Indonesia*. First Edition. IPB Press. 31 p.

Madduppa, H.H., B. Subhan, E. Suparyani, A.M. Siregar, D. Arafat, S.A. Tarigan, A. Alimuddin, D. Khairudi, F. Rahmawati, & A. Brahmandito. 2013. Dynamics of fish diversity across an environmental gradient in the Seribu Islands reefs off Jakarta. *Biodiversitas Journal of Biological Diversity*, 14(1): 17-24. https://doi.org/10.13057/biodiv/d140103

Madduppa, H.H., K. von Juterzenka, M. Syakir, & M. Kochzius. 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 & coastal management*, 100: 41-50. https://doi.org/10.1016/j.ocecoaman.2014.07.013

Madduppa, H., N.K.D. Cahyani, A.W. Anggoro, B. Subhan, E. Jefri, L.M.I Sani, D. Arafat, N. Akbar, & D.G. Bengen. 2021. eDNA metabarcoding illuminates species diversity and composition of three phyla (chordata, mollusca and echinodermata) across Indonesian coral reefs. *Biodiversity and Conservation*, 3087-3114. https://doi.org/10.1007/s10531-021-02237-0

Magurran, A.E. 2013. *Measuring biological diversity*. John Wiley & Sons, ltd. Oxford. 50 p.

Marwayana, O.N., Z. Gold, C.P. Meyer, & P.H. Barber. 2021. Environmental DNA in a global biodiversity hotspot: Lessons from coral reef fish diversity across the Indonesian archipelago. *Environmental DNA*, 00: 1-17. https://doi.org/10.1002/edn3.257

Miya, M., R.O. Gotoh, & T. Sado. 2020. MiFish metabarcoding: a high-throughput approach for simultaneous detection of multiple fish species from environmental DNA and other samples. *Fisheries Science*, 1-32. https://doi.org/10.1007/s12562-020-01461-x

Miya, M., Y. Sato, T. Fukunaga, T. Sado, J.Y. Poulsen, K. Sato, T. Minamoto,

S. Yamamoto, H. Yamanaka, H. Araki, & M. Kondoh. 2015. MiFish, a set of universal PCR primers for metabarcoding environmental DNA from fishes: detection of more than 230 subtropical marine species. *Royal Society open science*, 2(7): p.150088. https://doi.org/10.1098/rsos.150088

Nguyen, B.N., E.W. Shen, J. Seemann, A.M. Correa, J.L. O'Donnell, A.H. Altieri, N. Knowlton, K.A. Crandall, S.P. Egan, W.O. McMillan, & M. Leray. 2020. Environmental DNA survey captures patterns of fish and invertebrate diversity across a tropical seascape. *Scientific reports*, 10(1): 1-14. https://doi.org/10.1038/s41598-020-63565-9

Nybakken, J.W. 2001. *Marine biology: an ecological approach (Vol. 5)*. Benjamin Cummings. San Francisco.

Paulangan, Y.P., A. Fahrudin, D. Sutrisno, & D.G. Bengen. 2019. Keanekaragaman dan kemiripan bentuk profil terumbu berdasarkan ikan karang dan lifeform karang di Teluk Depapre Jayapura, Provinsi Papua, Indonesia. *Jurnal Ilmu dan Teknologi Kelautan Tropis*, 11(2): 249-262. https://doi.org/10.29244/jitkt.v11i2.24140

Prabowo, B., K. Fahlevy, N.F.D. Putra, M. Rizqydiani, B.M.K. Rahman, A. Habibie, B. Subhan, & H. Madduppa. 2019. Trophic structure of reef fishes and relationship of corallivore fishes with hard coral in Kepulauan Seribu, Jakarta. *In IOP Conference Series: Earth and Environmental Science*, 278(1): p. 012059. https://doi.org/10.1088/1755-1315/278/1/012059

Pratiwi, W.N., T. Nurlambang, & M.H.D. Susilowati. 2021. Households electricity consumption patterns in small island–case study: Kepulauan Seribu regency, DKI Jakarta. *In IOP Conference Series: Earth and Environmental Science* 649(1): p. 012010. https://doi.org/10.1088/1755-1315/649/1/012010

Rachello-Dolmen, P.G. & D.F.R. Cleary. 2007. Relating coral species traits to environmental conditions in the Jakarta Bay/Pulau Seribu reef system, Indonesia. *Estuarine, Coastal and Shelf Science*, 73(3-4): 816-826. https://doi.org/10.1016/j.ecss.2007.03.017

Razak, A. & R. Suprihardjo. 2013. Pengembangan kawasan pariwisata terpadu di Kepulauan Seribu. *Jurnal Teknik ITS*, 2(1): C14-C19. https://doi.org/10.12962/j23373539.v2i1.2461

Rees, J.G., D. Setiapermana, V.A. Sharp, J.M. Weeks, & T.M. Williams. 1999. Evaluation of the impacts of land-based contaminants on the benthic faunas of Jakarta Bay, Indonesia. *Oceanologica Acta*, 22(6): 627-640. https://doi.org/10.1016/S0399-1784(00)88954-9

Rumkorem, O.L.Y., R. Kurnia, & F. Yulianda. 2019. Asosiasi antara tutupan komunitas karang dengan komunitas ikan terumbu karang di Pesisir Timur Pulau Biak, Kabupaten Biak Numfor. *Jurnal Ilmu dan Teknologi Kelautan Tropis*, 11(3): 615-625. https://doi.org/10.29244/jitkt.v11i3.23375

Setiawan, F., J.D. Kusen, & G.J. Kaligis. 2013. Community changes of coral reef fishes in Bunaken National Park, North Sulawesi, Indonesia. *Aquatic Science & Management*, 1(2): 117-123. https://doi.org/10.35800/jasm.1.2.2013.7273

Sale, P.F. 1991. Reef fish communities: open nonequilibrial systems. *The ecology of fishes on coral reefs*, 564-598.

http://www.vliz.be/en/imis?refid=9169

Sale, P.F. 1977. Maintenance of high diversity in coral reef fish communities. *The American Naturalist*, 111(978): 337-359. https://doi.org/10.1086/283164

Sato, Y., M. Miya, T. Fukunaga, T. Sado, & W. Iwasaki. 2018. MitoFish and MiFish pipeline: a mitochondrial genome database of fish with an analysis pipeline for environmental DNA metabarcoding. *Molecular biology and evolution*, 35(6): 1553-1555. https://doi.org/10.1093/molbev/msy074

Sumarno, D. & T. Muryanto. 2016. Kadar salinitas, oksigen terlarut, dan suhu air di unit terumbu karang buatan (TKB) Pulau Kotok Kecil dan Pulau Harapan Kepulauan Seribu–Provinsi DKI Jakarta. *Buletin Teknik Litkayasa Sumber Daya dan Penangkapan*, 12(2): 121-126. https://doi.org/10.15578/btl.12.2.2014.121-126

Taberlet, P., E. Coissac, M. Hajibabaei, & L.H. Rieseberg. 2012. Environmental DNA. molecular ecology. *Molecular Ecology*, 21(8): 1789-1793. https://doi.org/10.1111/j.1365-294X.2012.05542.x

Takahara, T., T. Minamoto, H. Yamanaka, H. Doi, & Z.I. Kawabata. 2012. Estimation of fish biomass using environmental DNA. *PloS one*, 7(4): p.e35868. https://doi.org/10.1371/journal.pone.0035868

Thomsen, P.F., J. Kielgast, L.L. Iversen, P.R. Møller, M. Rasmussen, & E. Willerslev. 2012. Detection of a diverse marine fish fauna using environmental DNA from seawater samples. *PLoS one*, 7(8): e41732. https://doi.org/10.1371/journal.pone.0041732

Tilot, V., W. Leujak, R.F.G. Ormond, J.A. Ashworth, & A. Mabrouk. 2008. Monitoring of South Sinai coral reefs: influence of natural and anthropogenic factors. *Aquatic Conservation: Marine and Freshwater Ecosystems*, 18(7): 1109-1126. https://doi.org/10.1002/aqc.942

Tringe, S.G. & E.M. Rubin. 2005. Metagenomics: DNA sequencing of environmental samples. *Nature reviews genetics*, 6(11): 805-814. https://doi.org/10.1038/nrg1709

van der Wulp, S.A., A. Damar, N. Ladwig, & K.J. Hesse. 2016. Numerical simulations of river discharges, nutrient flux and nutrient dispersal in Jakarta Bay, Indonesia. *Marine pollution bulletin*, 110(2): 675-685. https://doi.org/10.1016/j.marpolbul.2016.05.015

Verma, D. & T. Satyanarayana. 2011. An improved protocol for DNA extraction from alkaline soil and sediment samples for constructing metagenomic libraries. *Applied biochemistry and biotechnology*, 165(2): 454-464. https://doi.org/10.1007/s12010-011-9264-5

Wang, S., Z. Yan, B. Hänfling, X. Zheng, P. Wang, J. Fan, & J. Li. 2021. Methodology of fish eDNA and its applications in ecology and environment. *Science of the Total Environment*, 755: 142622. https://doi.org/10.1016/j.scitotenv.2020.142622

Westermeier, R. 2016. *Electrophoresis in practice: a guide to methods and applications of DNA and protein separations*. John Wiley & Sons, ltd. Oxford.

Wielgus, J., N.E. Chadwick-Furman, & Z. Dubinsky. 2004. Coral cover and partial mortality on anthropogenically impacted coral reefs at Eilat, northern

Red Sea. *Marine Pollution Bulletin*, 48(3-4): 248-253. https://doi.org/10.1016/j.marpolbul.2003.08.008

Zaneveld, J.S. & H.T. Verstappen. 1952. *A Recent Investigation on the Geomorphology and Flora of Some Coral Islands in the Bay of Djakarta*. Kementerian Pertahanan, Direktorat Topografi Angkatan Darat, Dinas Geografi.

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